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Production, survival and evaluation of solid-substrate inocula of *Coniothyrium minitans* against *Sclerotinia sclerotiorum*

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Abstract

Coniothyrium minitans grew on all ten solid-substrates (barley, barley-rye-sunflower, bran-vermiculite, bran-sand, maizemeal-perlite, millet, oats, peat-bran, rice and wheat) tested, producing high numbers of germinable pycnidiospores ($1.9–9.3 \times 10^8$ g^{-1} air dry inocula). All solid substrate inocula survived better in the laboratory at 5 and 15 °C than at 30 °C for at least 64 weeks.

In pot bioassays carried out in the glasshouse and field, soil incorporations of each inoculum almost completely inhibited carpogenic germination of *S. sclerotiorum*. In the field bioassay, no sclerotia were recovered after 38 weeks from *C. minitans*-treated pots compared to 56% from control pots. In the glasshouse bioassay, 9–30% of sclerotia were recovered after 20 weeks from *C. minitans*-treated pots, but 88–100% of these were infected by the antagonist. The antagonist also spread to infect sclerotia in control pots.

In larger scale glasshouse trials, single preplanting soil-incorporations of five inocula (barley-rye-sunflower, maizemeal-perlite, peat-bran, rice and wheat) controlled Sclerotinia disease in a sequence of lettuce crops, with only small differences between the types of inocula tested. At harvest, *C. minitans* reduced sclerotial populations on the soil surface and over 74% of sclerotia recovered from *C. minitans*-treated plots were infected by the antagonist. *C. minitans* survived in soil in all solid-substrate inocula-treated plots for at least 39 weeks at levels of $10^4–10^5$ colony forming units cm^{-3} soil and spread to infect over 36% of sclerotia recovered from control plots.

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a widespread soil-borne plant pathogen affecting more than 360 plant species [Purdy, 1979]. In Europe, it regularly causes severe crop losses in beans, caraway, carrots, celery, chicory, lettuce, oilseed rape, potatoes and sunflowers. The pathogen survives between crops as sclerotia in the soil [Merriman, 1976; Coley-Smith, 1979]. These may germinate either myceliogenically to infect plants directly, or carpogenically to produce apothecia which release ascospores that can infect aerial plant parts. Traditionally, crop losses due to ascospore infections have been reduced by prophylactic spraying with fungicides. If the disease builds up in glasshouse soils, sclerotia of the pathogen can be eliminated by steam sterilisation or fumigation with chemicals such as methyl bromide. However, the number of effective fungicides is gradually decreasing, soil fumigation is becoming environmentally unacceptable and steam sterilisation is expensive. Consequently, alternative disease control methods, such as biological control, are urgently required for *S. sclerotiorum*.

Coniothyrium minitans Campbell is a promising biological control agent of *S. sclerotiorum*, and has

been shown to control the pathogen at low disease levels in both field and glasshouse experiments [Huang, 1980; Zazzerini and Tosi, 1985; Lynch and Ebben, 1986; Whipps et al., 1989; Budge and Whipps, 1991; Şesan and Cśep, 1991a, b; Whipps and Budge, 1992]. Biological control of *S. sclerotiorum* with this antagonist has generally been obtained following soil incorporation of solid-substrate inocula prior to sowing or planting. However, the range of solid-substrate inocula used has been limited, consisting of barley-rye-sunflower [Huang, 1980], maizemeal-perlite [Whipps et al., 1989; Budge and Whipps, 1991; Whipps and Budge, 1992] or wheat grain [Lynch and Ebben, 1986; Ebben, 1987]. A direct comparison of the biological control efficacy of these solid-substrate inocula against *S. sclerotiorum* has not been made. In view of these observations, it is clear that the production of additional solid-substrate inocula preparations and a comparison of their efficacy is required. Many other solid-substrate inocula preparations have been used for delivering other potential fungal biological control agents, including wheat bran [Ruppell et al., 1983], peat-bran [Sivan et al., 1984], milled rice [Ahmed and Tribe, 1977] and a range of grains [Wilson et al., 1988; McQuilken et al., 1992].

In the present investigation, one isolate of *C. minitans* antagonistic against *S. sclerotiorum* [Turner and Tribe, 1976; Whipps and Budge, 1990; Whipps and Gerlagh, 1992 (and references therein)], was examined for its ability to grow on a range of solid-substrates. Subsequently, survival of air-dried solid-substrate inocula was examined in the laboratory and soil incorporations tested for ability to infect and inhibit carpogenic germination of sclerotia of two isolates of *S. sclerotiorum*, using simple glasshouse and field pot bioassays. Finally, single soil incorporations of five different solid-substrate inocula were evaluated against *S. sclerotiorum* in a sequence of glasshouse lettuce crops, with the aim of improving the biological control of Sclerotinia disease.

Materials and methods

Source and maintenance of fungi. Two isolates of *S. sclerotiorum* originally isolated from glasshouse lettuce [SB; Whipps and Budge, 1990] and winter oilseed rape [JN1; Mylchreest and Wheeler, 1987] were used. *Coniothyrium minitans* (IMI 134523) was isolated from a sclerotium of *S. sclerotiorum* [Turner and Tribe, 1976]. Both fungi were maintained on Oxoid potato dextrose agar (PDA) at 18 °C.

Sclerotial production of S. sclerotiorum. Cultured sclerotia of each isolate of *S. sclerotiorum* were produced on sterilised wheat grain, cv. Armada, following inoculation and incubation at 20 °C for 3 weeks [Mylchreest and Wheeler, 1987]. Batches of twenty washed sclerotia (*c.* 2–4 mm diameter) of each isolate were placed in Terylene net bags (*c.* 5 × 5 cm, mesh < 2 mm) for immediate use in glasshouse and field pot bioassays.

Production and shelf life of solid-substrate inocula of C. minitans. Ten solid-substrate media were tested for producing inocula of *C. minitans*: (i) barley (1 l cleaned barley grain and 450 ml tap water); (ii) barley-rye-sunflower [Huang, 1980] (modified to contain 11 barley grain + rye grain + sunflower seeds (1:1:1 v/v) and 500 ml tap water); (iii) bran-vermiculite [Keinath et al., 1991] (modified to contain 1.3 l wheat bran (particle size < 2 mm), 500 ml graded horticultural vermiculite (Vermiperl; Silvaperl products, Sinclair Horticultural & Leisure Ltd., Lincoln, UK) and 600 ml tap water); (iv) bran-sand [Lewis and Papavizas, 1984] (modified to contain 1.6 l wheat bran, 150 ml silver sand and 300 ml tap water); (v) maizemeal-perlite [Whipps and Budge, 1990; Budge and Whipps, 1991] (2 l of micronised flaked maizemeal (2–5 cm diameter)/horticultural grade perlite (Silvaperl; Silvaperl Ltd., Gainsborough, Lincs., UK) (15:75% v/v) and 400 ml tap water); (vi) millet (1.5 l millet grain and 400 ml tap water); (vii) oats (1 l oat grain and 450 ml tap water); (vii) peat-bran [Sivan et al., 1984] (modified to contain 1 l Irish sphagnum moss peat (particle size 500 μm–2 mm, 1 l wheat bran and 900 ml tap water); (ix) rice [Ahmed and Tribe, 1977] (modified to contain 600 ml American long grain rice and 400 ml tap water); (x) wheat (1 l wheat grain and 450 ml tap water).

Mixtures of each medium in spawn bags (22.5 × 56 cm with a microporous strip 15 cm wide; Van Leer UK Ltd., Poole, Dorset) were well combined and autoclaved twice on two consecutive days

at 121 °C for 15 min. Four replicate bags of each solid-substrate were each inoculated with a 100 ml spore suspension containing 2×10^6 spores ml^{-1} in distilled water. The spore suspension was prepared by flooding 14 day-old PDA Petri dish cultures of *C. minitans* with sterile distilled water and gently scraping the colony surfaces with a spatula. Bags were incubated at 18–20 °C for 28 days during which time they were shaken periodically.

Following incubation, inocula were removed from spawn bags and air-dried for 7 days in a stream of sterile air at 18–22 °C. Four replicate 1 g samples of air-dried 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. Suspensions were passed through two layers of muslin to remove debris and appropriate dilutions made so that pycnidiospores could be counted in a haemacytometer. Number of pycnidiospores g^{-1} air-dry weight of preparation were then calculated. Serial dilutions were also made onto Petri dishes of PDA containing Triton X-100 (2 ml l^{-1}) and Aureomycin (0.32 g l^{-1} of powder containing 5.5% chlortetracycline hydrochloride; Cyanamid, Gosport, Hants., UK). Following incubation at 20–22 °C for 14 days, colonies of *C. minitans* were counted and colony forming units (CFUs) g^{-1} air-dry inoculum calculated. By this time there was no further increase in CFUs g^{-1} air-dry inoculum.

To assess pycnidiospore germinability, spore suspensions were concentrated by centrifugation at 38,000 g for 3 min and resuspended in sterile distilled water to give a final concentration of *c.* 2.0×10^6 spores ml^{-1}. Suspensions (0.5 ml) were spread over the surface of PDA (3 mm thick: in three 9 cm diameter Petri dishes) with a glass spreader. Dishes were placed in sealed plastic bags and incubated at 18 °C in the dark for 36 h, after which time germination of at least 250 pycnidiospores on each plate was scored. A pycnidiospore was considered to have germinated when the germ tube length was equal to or greater than the length of the spore.

To monitor shelf-life, batches of each air-dried solid-substrate inoculum were divided into fifteen 100 cm^3 samples and each placed in a plastic screw cap bottle (500 ml). Bottles were capped loosely and stored at 5, 15 and 30 °C, with four replicates for each temperature. After 4, 8, 16, 32 and 64 weeks, a 1 g sample was removed from each replicate bottle, macerated in sterile 0.01% agar and CFUs g^{-1} air-dry inoculum determined as before.

Glasshouse and field pot bioassays. Glasshouse soil (brickearth; silt-loam, Hamble series) was collected to a depth of 15 cm, sieved (10 mm mesh) and adjusted to *c.* 20% moisture content (–0.1 MPa). Each solid-substrate inoculum of *C. minitans* was mixed thoroughly with soil (3% v/v) prior to filling ten replicate rectangular plastic pots (11 × 10 cm; Cookson Plantpak Ltd., Maldon, Essex, UK). Twenty sclerotia (isolate SB), enclosed in Terylene net bags (*c.* 5 × 5 cm, mesh < 2 mm), were buried in each replicate pot of soil-solid-substrate mix *c.* 1 cm beneath the surface. Pots were buried in soil in a glasshouse chamber (4 × 3 m; 10 °C minimum day temperature, vents opening at 18 °C, with a 5 °C minimum night temperature) with the soil surface in the pot at the same level as the surrounding glasshouse soil. Control treatments consisted of pots containing bags of sclerotia, but lacking solid-substrate inocula. Treatment pots were arranged in a randomized complete block design and watered twice weekly to maintain the moisture content at *c.* 20%.

Numbers of apothecia produced from sclerotia in each pot were counted at fortnightly intervals for 20 weeks. Apothecia were not removed after counting. After 20 weeks, bags of sclerotia were removed from each replicate pot and the number of sclerotia recovered counted by washing opened bags under a running cold water-tap through a 1 mm mesh screen. Recovery of sclerotia was calculated as a percentage of the total number buried in each bag. All sclerotia recovered were surface sterilised, bisected and placed on 15 mm diameter PDA plugs containing Aureomycin [Whipps and Budge, 1990]. Numbers of sclerotia infected by *C. minitans* were recorded after 10–14 days incubation at 18 °C.

The experiment was also conducted in the field using the same soil and sclerotia of isolate JN1. Treatment pots were arranged in a randomized complete block design in the soil outside at HRI,

Littlehampton in late September 1992. Numbers of apothecia produced from the sclerotia in each pot were counted at fortnightly intervals during May and June 1993. Recovery and infection of sclerotia by *C. minitans* was assessed 38 weeks after the start of the experiment as described earlier.

Glasshouse lettuce trial

Five solid-substrates (barley-rye-sunflower, maizemeal-perlite, peat-bran, rice and wheat) which yielded high pycnidiospore counts were evaluated against Sclerotinia disease in a sequence of glasshouse lettuce crops. The experiment was carried out in a single Frampton Ferguson glasshouse chamber (15 × 13 m), previously used for trials involving *S. sclerotiorum* [Budge et al., in preparation]. Following sheet steaming, a crop of lettuce (cv. Hudson) was planted and artificially inoculated with *S. sclerotiorum* (isolate SB), to produce subsequently a natural population of pathogen sclerotia in the soil [Budge and Whipps, 1991]. Twenty-four plots (1.4 × 2.8 m) separated by 0.6 m paths, were marked out, to give an arrangement of four plots across and six plots down the West-East orientated chamber. The numbers of sclerotia on the soil surface within five random quadrats (each 500 cm^2) were counted in each plot. Mean numbers of sclerotia ranged between 0.4 and 2.0 per 500 cm^2 quadrat. For each crop, pelleted lettuce seeds (cv. Hudson) were sown into peat blocks (5 × 5 × 5 cm). Approximately 2–3 weeks later, after normal propagation, blocks were planted to half their depth at 20 × 20 cm spacing (84 blocks per plot). Glasshouse temperatures were maintained at 10 °C minimum day temperature, with vents opening at 18 °C and with a 5 °C minimum night temperature. Insecticides were applied when necessary to control aphids and caterpillars.

There were six treatments: (i) control, with no inoculum applied; (ii) barley-rye-sunflower; (iii) maizemeal-perlite; (iv) peat-bran; (v) rice; (vi) wheat. One day before planting the first of three successive crops, 1.64 l of each inoculum was evenly applied to each plot (2.7 m^2) to give 0.6 l inoculum m^{-2} and raked into the soil surface to a depth of *c.* 2–5 cm. Four replicates of each of the six treatments were randomised throughout the chamber in an incomplete Latin square arrangement. No more inocula were applied before successive crops.

Soil samples were taken from the top 3 cm in every plot at regular intervals and the survival of the antagonist assessed by soil dilution plating on PDA containing Triton X-100 and Aureomycin [Whipps et al., 1989]. At planting, plots treated with barley-rye-sunflower, maizemeal-perlite,

Table 1. Effect of difference solid-substrate media on pycnidiospore production by *Coniothyrium minitans* in spawn bags at 18–30 °C for 28 days

Solid-substrate	Air-dry inocula				Pycnidiospore germination[a] (% at 36 h)
	Pycnidiospore counts $(\times 10^8)g^{-1}$		Colony forming units (CFUs; $\times 10^{10})g^{-1}$		
Maizemeal-perlite	9.32	(8.97)[b]	125.01	(12.08)[c]	91–97
Peat-bran	8.18	(8.92)	10.91	(11.04)	95–98
Oats	7.49	(8.87)	6.28	(10.79)	93–96
Barley-rye-sunflower	5.88	(8.77)	6.69	(10.82)	94–96
Wheat	5.48	(8.74)	5.25	(10.72)	91–97
Rice	5.37	(8.73)	6.91	(10.84)	96–98
Barley	4.83	(8.68)	6.62	(10.82)	92–98
Millet	4.00	(8.60)	7.44	(10.87)	90–98
Bran-vermiculite	3.43	(8.53)	0.52	(9.70)	90–97
Bran-sand	1.91	(8.28)	0.24	(9.38)	92–95
LSD (30 df)[c]		(0.049)		(0.053)	

[a] Germination of at least 250 pycnidiospores on three replicate plates was scored after incubation at 18 °C in the dark for 36 h

[b] Values in parentheses are mean pycnidiospore counts or CFUs calculated after $\log_{10}$ transformation of replicate counts

[c] LSD is the least significant difference at a probability of 5% (P = 0.05). A significant difference between two means at the P = 0.05 level is given by LSD (LSD = $t_v \times$ SED), where t = critical value (P = 0.05) of Student's **t** distribution for *v* degrees of freedom (df) and SED = standard error of the difference between two treatment means.

peat-bran, rice and wheat inocula of *C. minitans* contained 0.53–0.72, 1.14–2.32, 1.76–2.58, 0.92–1.44 and 0.47–0.64 × 10^6 CFUs cm^{-3} soil, respectively.

Each crop was harvested and assessed for the number of diseased plants, leaving diseased material on plots. Numbers of sclerotia present in each plot were assessed using the quadrat system described earlier. From each of five quadrats in each plot, 10 sclerotia were removed. If sufficient numbers of sclerotia were not present within a quadrat, the nearest were chosen. Infection of recovered sclerotia by *C. minitans* was assessed as described earlier.

Statistics. Colony and pycnidiospore counts were transformed to $\log_{10}$ values before analysis of variance. Apothecial production, sclerotial recovery and infection data are presented as means ± standard errors (SE) of counts or percentages. More formal analysis of this data (e.g. generalized linear model methods) was made difficult by the number of null or complete responses. The glasshouse trial was not analyzable by standard analysis of variance methods due to columns and treatments not being orthogonal. To overcome this, the results were analyzed using the regression module of Genstat which allows for row and column effects to be removed prior to treatments. Percentages were transformed to logits and sclerotial numbers were transformed to $\log_{10}$ values before analysis. Treatment means were compared with the least significant difference (LSD) at a probability of 5% (P = 0.05).

Results

Pycnidiospore production on solid-substrate media and subsequent germination. High numbers (1.91–9.32 × 10^8 g^{-1} air-dry inocula) of pycnidiospores were produced on all of the solid-substrates tested (Table 1). Production of pycnidiospores was particularly high on maizemeal-perlite (9.32 × 10^8 g^{-1}), peat bran (8.18 × 10^8 g^{-1}) and oats (7.49 × 10^8 g^{-1}), but the greatest numbers were produced on maizemeal-perlite. High numbers (0.24–125.01 × 10^{10} g^{-1} air-dry inocula) of colony forming units (CFUs) were also obtained with all of the solid-substrates tested. Colony counts generally increased with an increase in the number of pycnidiospores. Indeed, the highest CFUs g^{-1} were obtained with maizemeal-perlite (125.01 × 10^{10} g^{-1}), which produced the greatest number of pycnidiospores. Pycnidiospores from all solid-substrate media were capable of germination when spread onto the surface of PDA and ranged between 90–98% after 36 h incubation at 18 °C (Table 1).

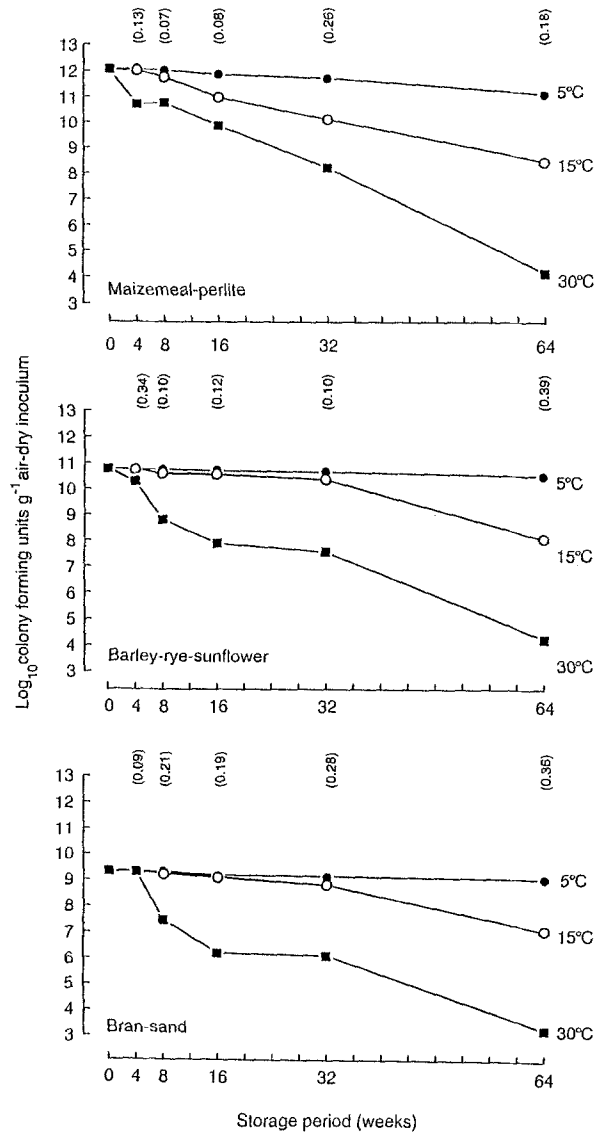

Fig. 1. Shelf life (survival) of solid-substrate inocula of *Coniothyrium minitans* in the laboratory at 5 (–●–), 15 (–○–) and 30 °C (–■–). Each point is the mean of four replicates. The value in brackets above each series of points is the LSD (least significant difference) at a probability of 5% (P = 0.05) and 6 degrees of freedom (df).

Shelf life of solid-substrate inocula. There was a general decline in CFUs g^{-1} air-dry inoculum with time for all solid-substrates stored in the laboratory (Fig. 1; data shown only for maizemeal-perlite, barley-rye-sunflower and bran-sand). This decline was more pronounced at 30 °C than at either 15 or 5 °C over the 64 week storage period. The best temperature for storage was at 5 °C, with levels of CFUs g^{-1} air-dry inocula only decreasing by up to 1.0 $\log_{10}$ units after 64 weeks storage. At 30 °C, levels of CFUs g^{-1} decreased by 3–4 $\log_{10}$ units after 32 weeks storage. By 64 weeks, counts had declined to 10^3–10^4 CFUs g^{-1}, an overall decrease of 6–8 $\log_{10}$ units.

Effect of soil incorporations of solid-substrate inocula on carpogenic germination of sclerotia of S. sclerotiorum. In the pot-bioassay carried out in the glasshouse, soil incorporations of each solid-substrate inoculum of *C. minitans* significantly delayed and reduced the numbers of apothecia produced from sclerotia of *S. sclerotiorum* (isolate SB) (Table 2). However, there was no differences between the different solid-substrates of the antagonist. Twenty weeks after burying sclerotia, high numbers (88–100%) of sclerotia recovered from *C. minitans*-treated pots were infected by the antagonist. The antagonist also spread to control pots, infecting 2% of the sclerotia recovered. In the pot-bioassay carried out in the field, soil incorporation of each solid-substrate inoculum either inhibited carpogenic germination completely or significantly reduced the numbers of apothecia produced from sclerotia of isolate JN1 (Table 3). No sclerotia were recovered from pots treated with the antagonist compared to 56% from control pots, 38 weeks after the start of the experiment.

Effect of soil incorporations of solid-substrate inocula on Sclerotinia *disease in successive lettuce crops.* There was no disease present in the first lettuce crop. In the second crop, all solid-substrate inocula treatments of *C. minitans* reduced the numbers of diseased plants when 44% of plants in control plots were diseased (Table 4). There were no significant differences in disease control between any of the five different solid-substrate inocula of the antagonist tested. In the third crop, all solid-substrate inocula reduced the number of diseased plants when 82% of plants in control plots were diseased. The number of diseased

Table 2. Glasshouse pot bioassay. Effect of different soil incorporations of solid-substrate inocula of *Coniothyrium minitans* on apothecial production, percentage recovery and infection of sclerotia of *Sclerotinia sclerotiorum* (isolate SB)[a] by the antagonist

Treatment	Number of apothecia						% recovery[b]	% infection by *C. minitans*[c]
	Weeks after burying sclerotia/soil incorporation					Sum of fortnightly totals		
	10	12	14	16	18			
Control (nil)	8.2 ± 1.74[d]	20.0 ± 1.51	32.2 ± 1.24	29.0 ± 0.70	15.8 ± 1.16	105.2 ± 3.24	88.0 ± 4.05	2.2 ± 2.22
Barley	0.0	0.0	1.0 ± 0.32	2.0 ± 0.45	0.45 ± 0.24	3.2 ± 0.66	9.0 ± 3.31	100.0
Barley-rye-sunflower	0.0	0.0	2.6 ± 0.24	2.4 ± 0.24	0.8 ± 0.20	5.8 ± 0.49	17.0 ± 2.99	100.0
Bran-sand	0.0	0.0	1.2 ± 0.37	2.2 ± 0.58	0.4 ± 0.24	3.8 ± 0.80	14.0 ± 4.84	100.0
Bran-vermiculite	0.0	0.0	2.8 ± 0.80	3.0 ± 0.84	0.4 ± 0.40	6.2 ± 1.90	18.0 ± 5.38	96.0 ± 3.99
Maizemeal-perlite	0.0	0.0	2.2 ± 0.58	2.4 ± 0.75	0.8 ± 0.37	5.4 ± 1.69	29.0 ± 6.77	98.0 ± 2.00
Millet	0.0	0.0	1.8 ± 0.49	0.8 ± 0.40	0.4 ± 0.24	4.6 ± 0.98	15.0 ± 4.18	100.0
Oats	0.0	0.0	2.0 ± 0.45	2.2 ± 0.58	0.4 ± 0.24	4.6 ± 1.09	17.0 ± 3.38	88.0 ± 7.98
Peat-bran	0.0	0.0	1.6 ± 0.40	1.6 ± 0.40	0.2 ± 0.20	3.4 ± 0.68	12.0 ± 2.99	100.0
Rice	0.0	0.0	2.0 ± 0.32	2.4 ± 0.51	0.6 ± 0.24	5.0 ± 0.00	22.0 ± 4.89	90.0
Wheat	0.0	0.0	1.8 ± 0.58	2.4 ± 0.51	0.8 ± 0.22	5.0 ± 1.18	9.0 ± 3.31	100.0

[a] Twenty sclerotia (*c.* 2–4 mm diameter) were buried in each of ten replicate pots of soil-solid-substrate mix *c.* 1 cm beneath the surface.
[b] Recovery of sclerotia 20 wk after burying sclerotia/soil incorporation.
[c] Infection of sclerotia by *C. minitans* was calculated as a percentage of the total number of sclerotia recovered.
[d] Value are means ± sample standard error, based on observation of ten replicate treatment pots.

Table 3. Field pot bioassay. Effect of different soil incorporations of solid-substrate inocula of *Coniothyrium minitans* on apothecial production, percentage recovery and infection of sclerotia of *Sclerotinia sclerotiorum* (isolate JN1)[a] by the antagonist

Treatment	Number of apothecia					% recovery	% infection by *C. minitans*[b]
	Date; weeks after burying sclerotia/soil incorporation				Sum of fortnightly totals		
	7 May; 31	21 May; 33	4 June; 35	18 June; 37			
Control (nil)	20.6 ± 1.80[c]	28.0 ± 2.48	26.0 ± 5.13	3.2 ± 0.58	77.8 ± 4.04	56.0 ± 6.39	0.0
Barley	0.0	0.4 ± 0.40	0.0	0.0	0.4 ± 0.40	0.0	–
Barley-rye-sunflower	0.0	0.0	0.4 ± 0.40	0.0	0.4 ± 0.40	0.0	–
Bran-sand	0.4 ± 0.40	1.2 ± 1.20	0.4 ± 0.40	0.0	2.0 ± 1.55	0.0	–
Bran-vermiculite	2.6 ± 1.77	4.2 ± 2.80	2.4 ± 1.50	0.0	9.2 ± 5.34	0.0	–
Maizemeal-perlite	0.0	2.6 ± 2.60	1.2 ± 1.2	0.0	3.8 ± 3.79	0.0	–
Millet	0.0	0.2 ± 0.20	0.0	0.0	0.2 ± 0.2	0.0	–
Oats	0.0	0.4 ± 0.40	0.0	0.0	0.4 ± 0.40	0.0	–
Peat-bran	0.0	0.0	0.4 ± 0.40	0.0	0.4 ± 0.40	0.0	–
Rice	0.0	0.0	0.0	0.0	0.0	0.0	–
Wheat	0.0	0.0	0.6 ± 0.6	0.0	0.6 ± 0.60	0.0	–

[a] Twenty sclerotia (*c.* 2–4 mm diameter) were buried on 29 September 1992 in each of ten replicate posts of soil-solid-substrate mix *c.* 1 cm beneath the surface.
[b] Infection of sclerotia by *C. minitans* was calculated as a percentage of the total number of sclerotia recovered.
[c] Values are means ± sample standard error, based on observation of ten replicate treatment pots.

Table 4. Effect of different solid-substrate inocula[a] of *Coniothyrium minitans* on the percentage of *Sclerotinia sclerotiorum*-diseased plants in successive lettuce crops

Treatment	2nd crop % diseased	3rd crop % diseased
Control	44.4 (–0.28)[b]	81.5 (1.51)
Barley-rye-sunflower	17.6 (–1.53)	33.4 (–0.72)
Maizemeal-perlite	15.2 (–1.85)	41.4 (–0.31)
Peat-bran	14.3 (–2.02)	35.4 (–0.69)
Rice	16.1 (–1.95)	31.3 (–0.98)
Wheat	14.0 (–1.86)	25.0 (–0.95)
LSD (10 df)[c]	(0.568)	(0.312)

[a] Treatments were applied to soil as solid-substrate preparations at a rate of 0.61 m^{-2} before planting the first lettuce crop only.
[b] Values in parentheses are logit transformations of percentage means from four plots each containing 120 plants.
[c] See footnote to Table 1 for definition.

plants (25–35%) was equal in plots treated with barley-rye-sunflower, peat-bran, rice or wheat, but significantly lower than in those treated with maizemeal-perlite (41%).

All solid-substrate inocula of *C. minitans* significantly reduced the number of sclerotia recovered at harvest in both the second and third lettuce crops (Table 5), but there were no significant differences in sclerotial numbers between any of the solid-substrate inocula at harvest of the second crop. However, in the third crop sclerotial numbers on plots treated with barley-rye-sunflower were sunflower were significantly lower than numbers on plots treated with maizemeal-perlite or peat-bran. The number of sclerotia recovered increased in all treatment between the two harvests. *C. minitans*-infected sclerotia were recovered from all plots, indicating spread of the antagonist from plots treated with the antagonist to the controls (Table 5). At harvest of either the second or third crops, levels of infection were always significantly higher in sclerotia recovered from *C. minitans*-treated plots. However, there were no significant differences in infection of sclerotia between different solid-substrate inocula of the antagonist. Infection of sclerotia increased with time for both control and antagonist treatments.

The antagonist exhibited a general decline in colony forming units (CFUs) cm^{-3} soil with time in all plots treated with solid-substrate inocula (Table 6). Sixteen weeks after soil incorporation and at subsequent samplings, CFUs cm^{-3} soil were the lowest in plots treated with rice inocula. The antagonist could still be detected within all treatment plots at levels of 2.2–15.5 × 10^4 CFUs cm^{-3} soil 39 weeks after soil incorporation.

Table 5. Effect of different solid-substrate inocula of *Coniothyrium minitans* on the number of sclerotia[a] of *Sclerotinia sclerotiorum* in soil after successive lettuce crops and the percentage infection of the sclerotia by the antagonist

Treatment	Number of sclerotia		% infection of sclerotia	
	2nd crop	3rd crop	2nd crop	3rd crop
Control	47.8 (1.61)[b]	107.8 (1.99)	36.0 (–0.44)[c]	53.5 (–0.06)
Barley-rye-sunflower	11.8 (1.11)	22.2 (1.23)	74.5 (1.50)	83.8 (1.83)
Maizemeal-perlite	20.4 (1.15)	37.5 (1.60)	74.8 (0.92)	98.0 (2.54)
Peat-bran	13.7 (1.02)	35.8 (1.53)	85.5 (2.10)	91.2 (2.81)
Rice	32.1 (1.19)	35.0 (1.46)	70.5 (1.29)	91.5 (2.48)
Wheat	23.0 (1.20)	23.5 (1.42)	89.0 (1.95)	87.8 (2.37)
LSD (10 df)[d]	(0.434)	(0.238)	(0.951)	(1.089)

[a] Sclerotia were sampled immediately after harvest.
[b] Values are mean number of sclerotia per 500 cm^2 from five random quadrats in each of four plots. Values in parentheses are $\log_{10}$ transformed.
[c] Values in parentheses are logit transformations of percentage data based on observation of 50 sclerotia sampled from each of four replicate plots.
[d] See footnote to Table 1 for definition.

Table 6. Survival ($\log_{10}$ colony forming units (CFUs) cm^{-3} soil) of *Coniothyrium minitans* in glasshouse soil following the single incorporation of different solid-substrate inocula[a] of the antagonist, one day before planting the first experimental crop

Treatment	Weeks after soil incorporation[a]				
	0	8	16	30	39
Barley-rye-sunflower	5.80[b]	5.41	5.51	5.16	4.91
Maizemeal-perlite	6.19	5.55	5.24	5.28	5.14
Peat-bran	6.31	5.31	5.37	5.23	4.98
Rice	6.05	5.36	4.95	4.59	4.35
Wheat	5.77	5.71	5.96	5.30	5.19
LSD (10 df)[c]	0.073	0.213	0.187	0.122	0.209

[a] First crop was harvested 7 weeks after soil incorporation. Second crop was planted and harvested 10 and 24 weeks after soil incorporation, respectively. Third crop was planted and harvested 28 and 39 weeks after soil incorporation, respectively.
[b] Values are the means from four replicate plots.
[c] See footnote to Table 1 for definition.

Discussion

Coniothyrium minitans grew on all the solid-substrates tested to produce high numbers (> 10^8 g^{-1} air dry inocula) of germinable pycnidiospores. Overall, there was little difference between the inocula in ability to infect sclerotia, reduce apothecial production or decrease Sclerotinia disease in lettuce. This suggests that any of these substrates could be suitable as basic materials for the development of commercial inocula of *C. minitans*. The only proviso must be that the application rate required is not excessive [Adams and Fravel, 1990]. On cost grounds, it has also been suggested [Churchill, 1982] that industry would prefer to use inoculum produced by liquid fermentation which could then be formulated to provide a material with high viability, long shelf-life and which would be easy to apply [Lumsden and Lewis, 1989; Knudsen et al., 1991a, b]. Work on this approach with *C. minitans* is currently underway.

All the inocula had a long shelf-life (> 64 weeks) if stored at 5 °C but viability decreased rapidly at 30 °C. Similar, negative effects of high temperatures on shelf-life have been noted before for a range of inocula of *Trichoderma* spp. and *Gliocladium virens*, the latter being particularly

sensitive [Jackson et al., 1991]. Nevertheless, Jones [1993] has demonstrated that maizemeal-sand inoculum of *C. minitans* stored at 4 °C can remain viable for at least 15 years, illustrating the potential for long term storage of this antagonist. In addition, in the glasshouse trial, all the solid-substrate inocula survived at levels of 10^4–10^5 CFU g^{-1} soil for 39 weeks, decreasing by only 1–2 $\log_{10}$ CFUs g^{-1} soil over the whole period, further demonstrating survival potential under glasshouse soil conditions.

In the pot bioassays where the inoculum was in close contact with the sclerotia, all inocula were extremely efficient at infecting sclerotia and reducing apothecial production; in some cases no apothecia were produced. This complete control was not translated into disease control in the glasshouse trial, possibly because newly formed sclerotia did not contact inocula of *C. minitans* quickly enough or at a high enough level to prevent apothecial production. Nevertheless, all inocula reduced sclerotinia disease in comparison with the controls after only a single treatment and spread to infect sclerotia in the control plots. This confirms the potential of *C. minitans* to survive and decrease Sclerotinia disease in the long-term [Budge and Whipps, 1991].

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