Potential for Integrated Control of *Sclerotinia sclerotiorum* in Glasshouse Lettuce Using *Coniothyrium minitans* and Reduced Fungicide Application

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Accepted for publication 20 November 2000.

ABSTRACT

Budge, S. P., and Whipps, J. M. 2001. Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. *Phytopathology* 91:221-227.

All pesticides used in United Kingdom glasshouse lettuce production (six fungicides, four insecticides, and one herbicide) were evaluated for their effects on *Coniothyrium minitans* mycelial growth and spore germination in vitro agar plate tests. Only the fungicides had a significant effect with all three strains of *C. minitans* tested, being highly sensitive to iprodione (50% effective concentration [EC₅₀] 7 to 18 µg a.i. ml⁻¹), moderately sensitive to thiram (EC₅₀ 52 to 106 µg a.i. ml⁻¹), but less sensitive to the remaining fungicides (EC₅₀ over 200 µg a.i. ml⁻¹). Subsequently, all pesticides were assessed for their effect on the ability of *C. minitans* applied as a solid substrate inoculum to infect sclerotia of *Sclerotinia sclerotiorum* in soil tray tests. Despite weekly applications of pesticides at twice their recommended concentrations, *C. minitans* survived in the soil and infected sclerotia equally in all pesticide-treated and untreated control soil trays. This demonstrated the importance of assessing pesticide compatibility in environmentally relevant tests. Based on

these results, solid substrate inoculum of a standard and an iprodione-tolerant strain of *C. minitans* were applied individually to *S. sclerotiorum*-infested soil in a glasshouse before planting lettuce crops. The effect of a single spray application of iprodione on disease control in the *C. minitans* treatments was assessed. Disease caused by *S. sclerotiorum* was significantly reduced by *C. minitans* and was enhanced by a single application of iprodione, regardless of whether the biocontrol agent was iprodione-tolerant. In a second experiment, disease control achieved by a combination of *C. minitans* and a single application of iprodione was shown to be equivalent to that of prophylactic sprays with iprodione every 2 weeks. The fungicide did not affect the ability of *C. minitans* to spread into plots where only the fungicide was applied and to infect sclerotia. These results indicate that integrated control of *S. sclerotiorum* with soil applications of *C. minitans* and reduced foliar iprodione applications was feasible, did not require a fungicide tolerant isolate, and that suppression of *Sclerotinia* disease by *C. minitans* under existing chemical control regimes has credence.

Additional keywords: mycoparasite, protected lettuce.

The sclerotial mycoparasite, *Coniothyrium minitans* Campbell, is an effective antagonist and biocontrol agent of the plant pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary (18,30,32,34,52). As with other potential biocontrol agents, however, the ability of *C. minitans* to control *Sclerotinia* disease can diminish when disease pressure increases. In previous glasshouse lettuce trials, *C. minitans* consistently achieved over 60% control of *S. sclerotiorum* when disease incidence approached 50%, but this level of control was quickly lost as the amount of disease increased (5,6,26,50). To overcome such problems, one approach is to search for more efficient isolates of *C. minitans*. From a worldwide collection of *C. minitans* isolates, however, little difference in ability to prevent infection of plant tissue and to reduce sclerotial production and infection has been found (17). Improving activity by manipulating inoculum production and formulation is another strategy (15), but a range of *C. minitans* isolates produced in a variety of solid substrates or in liquid culture, and incorporated into soil, showed little difference in ability to infect sclerotia of *S. sclerotiorum* and provide disease control in glasshouse lettuce (32,34).

Another approach to enhance the activity of *C. minitans* is to develop integrated control strategies (37,48). Considerable research has been done on integrating biocontrol agents with fungicides. Most studies have considered integration of fungal

biocontrol agents with fungicides in foliar (6,12,13,20,40,41, 42,43,56,59,60), soil and seed (1,8,9,35,36,38), or postharvest (7,10,39) environments. Integration of bacterial biocontrol agents with fungicides has been examined but to a lesser extent, in foliar (24), soil and seed (4,27,29,47,58), and postharvest (39) environments. In many of these studies increased disease control was achieved from combinations of the biocontrol agent with the fungicide applied at the full rate. Nevertheless, the use of fungicides at normal rates and application frequencies in such integrated systems seems to negate the principle of using biological agents to reduce pesticide use. There has been some work, however, involving the use of lower amounts of fungicide in integrated systems, either through a combination of a lower dose applied simultaneously with a biocontrol agent (7,8,10,20,28,43), or by using alternating treatments of chemicals and biocontrol agents (13,20,40,41). This approach reduces the environmental risks pressure. To achieve a fully integrated control program, however, it is important to ascertain if selected natural biocontrol agents or those trained or mutagenized to be more resistant to specific fungicides, are more tolerant of other fungicides, insecticides, selective herbicides, and antibiotics used within the same crop. This is gradually becoming more appreciated (1,11,12,14,21, 25,41,57).

Consequently, the aim of this study was to evaluate the sensitivity of *C. minitans* to all pesticides used in the production of glasshouse lettuce in the United Kingdom and to determine whether effective integrated control of *S. sclerotiorum* could be achieved with this agent with reduced application of the fungicide iprodione.

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Publication no. P-2000-1218-02R

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MATERIALS AND METHODS

Fungi and inoculum production. An isolate of *S. sclerotiorum* was originally obtained from diseased glasshouse lettuce and was maintained on potato dextrose agar (PDA) (Unipath Ltd., Basingstoke, U.K.). Sclerotia used for the soil tray experiments were produced by autoclaving 25 g of wheat grain (cv. Armada) and 50 ml of water in 250-ml conical flasks for 15 min at 121°C, then inoculating the grain with three 10-mm diameter agar plugs cut from *S. sclerotiorum* plate cultures. After 3 weeks of incubation at 20°C, sclerotia were removed and graded to use those between 2- to 4-mm diameter.

One standard isolate of *C. minitans* (Conio; IMI 134523) was used in all experiments and was originally isolated from a sclerotium of *S. sclerotiorum* (45). Two other isolates of *C. minitans* were employed in in vitro tests: IVT-1, isolated from a sclerotium of *S. sclerotiorum* (17), and CH1, isolated from soil (51). An iprodione-tolerant strain of *C. minitans* (derived from Conio) used in the first glasshouse trial was obtained during the in vitro plate tests; restricted colonies of *C. minitans* growing on PDA containing high concentrations of iprodione (500 mg liter⁻¹) frequently sectoried to give mycelium growing at normal rates. Single spore isolates from the fast-growing sectors were made and the most stable isolate was maintained alternatively on PDA and PDA incorporating 500 mg liter⁻¹ of iprodione. Stability of fungicide resistance was tested by subculturing eight times on PDA and transferring onto iprodione-containing media.

Maizemeal-perlite inoculum of *C. minitans* used in the tray tests and glasshouse trials was prepared by adding 1.7 liters of perlite (horticultural grade; Silvaperl, Gainsborough, U.K.), 300 ml of ground maize (2- to 5-mm diameter; Bartholomews, Chichester, U.K.) and 400 ml of tap water into mushroom spawn bags (22.5 × 56 cm; Van Leer, Poole, UK) and autoclaving for 15 min at 121°C. Bags were inoculated with 100 ml of a spore suspension of *C. minitans* (approximately 10⁶ conidia per ml) made by flooding 14-day-old PDA plate cultures with sterile water and gently scraping the colony surface with a spatula. Inoculated bags were incubated at 18°C for 21 days. Previous studies (5,6) have demonstrated that this inoculum consistently contains between 7 × 10⁷ to 4 × 10⁸ CFU/cm³ fresh material.

Pesticides. All pesticides were commercial formulations approved for use in glasshouse lettuce in the United Kingdom (53). Fungicides used in the in vitro plate tests were: iprodione (Rovral WP, 50% a.i.; maximum recommended application rate (MRAR) 500 g ha⁻¹); mancozeb (Karamate Dry Flo, 75% a.i.; MRAR 3.9 kg ha⁻¹); metalaxyl plus thiram (Favour 600 SC, 10% plus 50% a.i.; MRAR 3.0 liter ha⁻¹); thiram (Unicrop Thiosan DG, 80% a.i.; MRAR 4 g liter⁻¹ sprayed to run-off); tolclofos-methyl (Basilex 50% a.i.; MRAR 20 kg ha⁻¹); and zineb (Unicrop Zineb, 70% a.i.; MRAR 2 g liter⁻¹ sprayed to run-off). Insecticides used in in vitro plate tests were: cypermethrin (Ambush C, 10% a.i.; MRAR 250 ml ha⁻¹); dimethoate (BASF Dimethoate 40, 40% a.i.; MRAR 850 ml ha⁻¹); malathion (Malathion 60, 60% a.i.; MRAR 2.1 liters ha⁻¹); and pirimicarb (Standon Pirimicarb H, 50% a.i.; MRAR 0.5 g liter⁻¹ sprayed to run-off). The herbicide chlorpropham with cetrime (Croxtex Pewter, 8% plus 8% a.i.; MRAR 11 liters ha⁻¹) was used for in vitro plate tests. For soil tray tests, all the above chemicals were used as well as the fumigant fungicide, dicloran (Fumite Dicloran Smoke, Hortichem, 40% a.i.; MRAR 90 mg a.i. per m³) and the fumigant insecticide, pirimiphos-methyl (Fumite Pirimiphos Methyl Smoke, Hortichem, 22.5% a.i.; 50 mg a.i. per m³).

In vitro pesticide tests. The effect of each pesticide on radial growth rate and conidial germination of the three isolates of *C. minitans* was tested on PDA amended with a range of seven concentrations determined from preliminary experiments up to a maximum of twice the manufacturers' application dosage. Pesticides were added to the agar at 50°C before adding 15 ml of the

medium to each petri dish. Radial growth was determined by placing 5-mm disks cut from the growing edge of 7-day-old PDA cultures upside down in the center of the test plate. Growth was measured on four set radii every 48 h for 12 days at 20°C. Conidial germination was tested by spraying approximately 0.05 ml of spore suspension (2 × 10⁶ spores per ml) onto the plate surface with an atomizer. After the plates were incubated for 32 h, germination was assessed by flooding the plates with Cotton Blue in lactophenol and counting the number of germinated conidia out of 100 in three set areas of the plates. For all treatments there were 20 replicate plates, and all experiments were conducted three times.

Soil-tray experiments. The aim of these experiments was to evaluate the toxicity of pesticides to *C. minitans* and the effects of pesticides on colonization of sclerotia by the biocontrol agent in soil. Plastic trays, 53 × 38 × 7.5 cm, with small holes in the bottom for drainage, were first filled with 2 cm of gravel, then a 2-cm layer of unsieved soil (brickearth; silt loam, Hamble Series), and finally a 3-cm layer of the same sieved (5.6 mm) soil. The soil was moistened to 75% field capacity. Maizemeal-perlite inoculum of *C. minitans* (120 ml per tray = 0.6 liters m⁻²) was added to appropriate trays and lightly worked into the layer of sieved soil. This rate provided reproducible disease control and infection of sclerotia in previous experiments (5,6). Fifty sclerotia of *S. sclerotiorum* were lightly pressed into the soil surface of each tray so they were evenly spaced and remained just visible. For each pesticide, there were four treatments: first, sclerotia only; second, sclerotia plus pesticide; third, sclerotia plus *C. minitans*; and fourth, sclerotia plus *C. minitans* plus pesticide. Pesticides were applied at double the manufacturer's recommended rate immediately after setting up the trays and three times thereafter at weekly intervals. Applications were made by spraying the two treatment (second and fourth) trays placed within a 1-m² area with the appropriate volume of pesticide. Volumes were calculated from those applied to glasshouse lettuce until run-off, for a given area. For the two fumigant pesticides, the trays were placed within a glasshouse that was half the volume of that appropriate for the fumigant generating unit to double the application rate. All trays were covered with a second empty tray placed upside down on top of the filled tray and incubated at 18°C for 4 weeks. Soil moisture was maintained by spraying water onto the soil surface with a hand sprayer.

Soil samples (5 ml) were taken from three points in each tray immediately after addition of inoculum and each week thereafter. Because *C. minitans* does not grow through soil (54,55), *C. minitans* survival was assessed by soil dilution plating using PDA amended with Triton X-100 (2 ml liter⁻¹) and chlortetracycline (20 mg liter⁻¹). After 4 weeks, all sclerotia were removed, surface sterilized, bisected, and placed onto 15-mm PDA disks containing chlortetracycline using a modification to the original technique (49). In the modified system, after surface sterilization, sclerotia were individually rinsed in 2 ml of sterile water contained within a compartmentalized square petri dish (Bibby Sterilin, Stone, U.K.). After shaking the plate gently on a small platform shaker, sclerotia were individually transferred to a second segmented petri dish and shaken again. This modification eliminated the potential problem of spores of *C. minitans* being disseminated from a single heavily infected sclerotium to contaminate those uninfected by the antagonist that may occur during batch washing of sclerotia. Numbers of viable sclerotia and those infected by *C. minitans* were recorded after 14 days. Three replicate runs of the complete set of pesticide treatments were carried out with each treatment comprising three trays of 50 sclerotia.

First glasshouse trial: Use of iprodione-tolerant mutant of *C. minitans*. A single glasshouse (Cambridge Alumabrite) chamber (13 × 9 m) used for previous trials of *S. sclerotiorum* on lettuce (5), was sheet steamed and planted with a crop of lettuce (cv. Hudson) following standard horticultural practices. Steaming is a standard procedure for glasshouse crops in the United Kingdom and is becoming more common following the constraints on

the use of soil sterilant methyl bromide. Glasshouse temperatures were maintained at 10°C minimum day temperature, with vents opening at 18°C and with a 5°C minimum night temperature. Sclerotia were established in the soil by artificially inoculating the lettuce with 0.5-cm PDA mycelial disks of *S. sclerotiorum* onto wounds made on leaf petioles. Following harvest of healthy plants, diseased material was allowed to dry on the soil surface. Sclerotia that formed were evenly spread throughout the glasshouse chamber and the soil rotovated to a depth of 15 cm. Twenty plots (1.8 × 1.6 m) separated by 0.5-m paths were marked out, to give four plots across and five plots down the north-south orientated chamber. The number of sclerotia present on the soil surface within the plots were assessed by counting those in five randomly selected quadrats (500 cm²) in each plot. Mean numbers of sclerotia ranged between 0.4 and 2.6 per quadrat.

There were five treatments: control, no treatment; standard *C. minitans* only; standard *C. minitans* plus single Rovral spray; iprodione-tolerant *C. minitans* only; and iprodione-tolerant *C. minitans* plus single Rovral spray. The treatments were randomized within the 20 plots in an incomplete Latin square arrangement, with four replicate plots per treatment each containing 72 plants. Three crops of lettuce were grown sequentially: the first was planted in mid-July and harvested in late August; the second was planted in mid-September and harvested in mid-November; and the third was planted in early December and harvested in early March. Immediately before planting each crop, 1.7 liters (0.6 liters m⁻²) of *C. minitans* maize meal-perlite inoculum was evenly applied to each appropriate plot and raked into the soil surface to a depth of approximately 3 cm. The single application of Rovral WP (0.5 kg/ha) was applied during each lettuce crop to the appropriate plots until run-off as disease symptoms first began to proliferate (26).

Each crop was harvested and the number of *S. sclerotiorum*-infected plants assessed, leaving diseased material on the plot surface. The number of sclerotia present on the soil surface in each plot were then counted using the quadrat system described earlier. From three of the quadrats in each plot, 20 sclerotia were sampled, and if there were insufficient numbers within a quadrat, the nearest adjacent sclerotia were selected for evaluation. All sampled sclerotia were evaluated for viability and infection by *C. minitans* as described above. In preliminary tests, attempts were made to distinguish the iprodione-tolerant strain from the standard strain of *C. minitans* in infected sclerotia. Due to the frequent appearance of iprodione tolerance in the standard *C. minitans* strain, however, sclerotia that were heavily infected with the standard strain of *C. minitans* repeatedly gave rise to colonies able to grow out on the fungicide-amended agar. Consequently, differentiation between infection by the standard isolate and iprodione-tolerant strain in sclerotia was not possible.

Throughout each crop, soil samples were taken from the top 5 cm in each plot immediately after planting, at 3-week intervals thereafter, and again at harvest. *C. minitans* populations were assessed as described earlier. Again, initial efforts to differentiate between standard and iprodione-tolerant strains of *C. minitans* proved unsuccessful and, therefore, results are given for total numbers only.

After all sampling, plots were individually dug and raked to a depth of approximately 15 cm before application of *C. minitans* inoculum and planting of the next crop.

Second glasshouse trial: Use of standard *C. minitans* and single iprodione spray versus standard iprodione spraying regime. The same glasshouse chamber was used for the second glasshouse trial after thorough steam-sterilization of the soil. Disease inoculum was re-established as described for the first glasshouse trial and the same experimental design was implemented. Sclerotial numbers were assessed as before and ranged between 0.2 and 3.2 per quadrat.

There were five treatments: control (no treatment), single Rovral spray, Rovral spray applications every 2 weeks, standard

C. minitans only, and standard *C. minitans* plus single Rovral spray. The treatments were rerandomized to differentiate this second glasshouse trial from the first trial. Three crops were grown sequentially with the planting and harvest times for each crop similar to the first glasshouse trial. Plant disease, sclerotial numbers, sclerotial viability and infection, and survival of *C. minitans* in the soil were all assessed and recorded as for the first trial.

Statistical analysis. For the in vitro pesticide tests, data from all three repeat runs were analyzed separately by analysis of variance (ANOVA) and as the variances were found to be similar, the three complete data sets were combined. Inhibition of radial growth and spore germination were calculated as a percentage of the corresponding control, and pesticide concentrations required to inhibit growth or spore germination by 50% (EC₅₀) were estimated from regression analysis of percentages against the common logarithm of percentage concentration.

A formal analysis on the sclerotial data from the soil tray experiments was considered unnecessary as every replicate run provided consistently large differences in viability, i.e., where *C. minitans* was applied, sclerotial viability was always below 20%, whereas in control treatments without *C. minitans* the sclerotial viability was always over 88%.

CFU data were subjected to ANOVA after log₁₀ transformation and treatment means compared using the least significant difference (LSD) at a level of $P < 0.05$, where appropriate, based on the *F* test in ANOVA. Complete data sets from both glasshouse experiments were subjected to ANOVA after angular transformation of percentage data and square-root transformation of sclerotial number data. The models incorporated row and column effects to account for the incomplete Latin Square structure and the sequential harvests were treated as a split-plot. Significant differences between treatments were assessed using least significant difference (LSD) ($P < 0.05$), where appropriate, based on the *F* test in ANOVA. These fully replicated glasshouse experiments were not repeated as numerous previous experiments using the same experimental design and basic procedures (5,6,32,34,50) demonstrated the reproducibility of the type of results obtained in this system.

RESULTS

In vitro pesticide tests. Of the 11 pesticides tested, all fungicides and 2 insecticides had some effect on radial growth and spore germination of the three isolates of *C. minitans* (Table 1). Insecticides malathion and pirimicarb only reduced growth rate and spore germination at concentrations above their maximum recommended application rates. The most toxic fungicides were iprodione (EC₅₀ 7 to 18 µg a.i. ml⁻¹) followed by thiram (EC₅₀ 52 to 106 µg a.i. ml⁻¹). Sensitivity of *C. minitans* to the remaining fungicides was relatively less, when effects were only found at concentrations over 200 µg a.i. ml⁻¹. There were no consistent differences in sensitivity between the isolates of *C. minitans*, or between their responses of growth rate and spore germination. Sectoring of *C. minitans* was frequently observed during growth on iprodione-amended PDA plates.

Soil-tray experiments. When all the pesticides tested were applied weekly at twice their recommended dose rate, none affected either the survival of the three strains of *C. minitans*, or their capacity to infect sclerotia relative to the untreated controls (data not shown). Briefly, the number of CFU remained constant over the 4-week period of the tests between 0.2 to 5.6 × 10⁶ CFU/cm³ of soil. The level of infection of sclerotia by all isolates of *C. minitans* was always greater than 80% and was normally over 95%. Sclerotial viability in *C. minitans* treatments was reduced to less than 20% in comparison with control treatments lacking *C. minitans* where sclerotial viability was always over 88%.

First glasshouse trial: Use of iprodione-tolerant mutant of *C. minitans*. Very little disease was present at harvest of the first

crop, when only 3% of control plants became infected with *S. sclerotiorum* (Table 2). In the second crop, disease incidence increased with 63% of control lettuce infected at harvest. All treatments significantly controlled *S. sclerotiorum* in the second crop but no differences in effect between individual treatments were found from the LSD test. However, in a separate analysis when the treatment pairs were considered together, the combined treatments (fourth and fifth) of both *C. minitans* and single Rovral sprays gave significantly better control than *C. minitans* when no Rovral (treatments two and three) was applied. In the third crop, although the level of control was reduced, all treatments except the iprodione-tolerant *C. minitans* (third treatment) again significantly controlled *S. sclerotiorum* disease, when 55% of control lettuce became infected. In this crop, the combined *C. minitans* and Rovral treatments gave significantly more disease control than the *C. minitans*-only treatments. There were no differences between the standard and iprodione-tolerant strains of *C. minitans* in their ability to control disease in all crops.

The numbers of sclerotia counted on the soil surface after harvest of the first crop were small due to the low level of disease present (Table 2). All treatments significantly reduced the number of sclerotia in the second and third crops. There was a significant reduction in sclerotial viability in the second and third crops by all treatments with the exception of the iprodione-tolerant *C. minitans* in the third crop. Over 90% of sclerotia recovered were infected by *C. minitans* in all treated plots. Infection of sclerotia by *C. minitans* in control plots was significantly less than in other plots. Nevertheless, 58% became infected in the second crop and 73% were infected in the third crop.

Populations of *C. minitans* in the soil were the same in all *C. minitans* treatments. Following solid substrate incorporation before the first crop was planted, *C. minitans* populations were between 0.8 to 3.4×10^7 CFU/cm³ of soil (data not shown). By the end of the first crop numbers ranged between 2.7 to 8.2×10^6 CFU/cm³ of soil. In the second crop, CFU were initially between 0.9 to 4.6×10^7 , then declined to between 0.6 to 1.2×10^7 CFU/cm³ of soil at harvest. During the third crop, numbers of *C. minitans* were initially at 1.1 to 3.8×10^7 , then declined to between 4.8 to 9.2×10^6 CFU/cm³ of soil at harvest.

Second glasshouse trial: Use of standard *C. minitans* and single iprodione spray versus standard iprodione spraying regime. Disease severity in the first crop was low with 12% of control plants infected (Table 3). All treatments significantly reduced disease incidence in the first crop. In the second crop, 32% of control plants became infected by *S. sclerotiorum*. Again, all

treatments significantly reduced disease, with over 50% control achieved by the *C. minitans* plus single Rovral spray treatment and 85% control obtained by sprays of Rovral every 2 weeks. In the third crop disease increased to 84% in the control plots. The single Rovral spray no longer gave control, but both *C. minitans* treatments and the Rovral spray every 2 weeks significantly controlled disease. *C. minitans* provided nearly 50% control, but a significantly greater reduction in disease was obtained when *C. minitans* was combined with a single Rovral spray, and this combined treatment was not significantly different to the level of control achieved by Rovral spray every 2 weeks.

In all crops, the number of sclerotia on the soil surface was significantly reduced by all treatments except the single Rovral spray, in comparison to the control (Table 3). In addition, the Rovral spray every 2 weeks reduced sclerotial numbers more than the *C. minitans* only treatment in all three crops, but similar levels of sclerotial reduction were also achieved by *C. minitans* plus single Rovral spray in the first and third crops. The viability of recovered sclerotia was significantly reduced by both *C. minitans* treatments in all three crops (Table 3). There was also a general decline in viability of sclerotia retrieved from control and Rovral sprayed plots between the first and third crops. The percentage of recovered sclerotia that were infected with *C. minitans* was significantly greater in those plots treated with the antagonist. *C. minitans*, however, spread to infect sclerotia in other plots from up to 12% in the first crop to nearly 74% by the third crop. There was no difference in the degree that *C. minitans* had spread to infect sclerotia in the control plots or the Rovral treated plots in any crop.

The numbers of CFU of *C. minitans* per cm³ of soil were the same in all *C. minitans* treatments and ranged between 1.6 to 4.2×10^7 at the start of the first crop and between 0.7 to 3.8×10^7 at harvest (data not shown). In the second crop, populations of *C. minitans* ranged from 2.4 to 4.8×10^7 at planting to 0.5 to 2.6×10^7 CFU/cm³ of soil at harvest. In the final crop, CFU ranged between 2.7 to 5.4×10^7 at planting to 0.4 to 3.1×10^7 per cm³ of soil at harvest.

DISCUSSION

In the in vitro pesticide tests, radial growth and spore germination of all three isolates of *C. minitans* were inhibited similarly by fungicides, with greatest sensitivity toward iprodione and thiram. In soil tray experiments, however, even when the same chemicals were applied at twice the concentration and frequency of application recommended by the manufacturer, no effect on the ability of

TABLE 1. Effect of pesticides used in the production of glasshouse lettuce in the United Kingdom on radial growth rate and spore germination of three isolates (Conio, IVT-1, and CH1) of *Coniothyrium minitans* in vitro^z

		Pesticide sensitivity: EC ₅₀ (µg a.i. ml ⁻¹)					
		Conio		IVT-1		CH1	
		Radial growth	Spore germination	Radial growth	Spore germination	Radial growth	Spore germination
Pesticide	Maximum recommended application rate (µg a.i. ml ⁻¹)						
Fungicides							
Iprodione	500	11	9	14	18	7	10
Mancozeb	450	320	300	340	350	290	300
Metalaxyl + thiram	400 + 2,000	240	320	190	230	220	280
Thiram	3,200	84	68	106	87	92	52
Tolclofos-methyl	700	290	310	330	280	300	280
Zineb	1,400	420	360	390	370	340	350
Insecticides							
Cypermethrin	70	—	—	—	—	—	—
Dimethoate	300	—	—	—	—	—	—
Malathion	1,920	3,200	2,600	3,000	3,200	3,700	—
Pirimicarb	250	400	—	300	3,60	280	—
Herbicide							
Chlorpropham + cetrimide	260 + 260	—	—	—	—	—	—

^z Data are from the pooled results of three repeat runs. Dashes indicate no reduction in radial growth or spore germination at pesticide concentrations of up to twice the maximum recommended application rate. Pesticides added as the commercial formulation.

C. minitans to infect and destroy sclerotia was observed. In vitro agar plate or nutrient-broth based experiments are often used as test systems to determine potential tolerance of fungi to pesticides (2,14,20,38,43), but as the results of this study show such systems may not reflect tests conducted in natural environments. A similar difference between pesticide tests carried out in vitro and in soil was found for infection of *S. minor* sclerotia by the mycoparasite *Sporidesmium sclerotivorum* (1), but these authors did not extend their work to plant- or crop-based experiments.

In vitro agar plate tests may be relevant to application of bio-control agents to the phylloplane, but additional factors come into play when using inocula applied to soil. For example, there is the dilution factor when pesticides are applied to a volume of soil, particularly if low-volume sprays are involved when most active ingredient will remain near the soil surface. Furthermore, most pesticides in use today are quickly broken down in soil and thus their potential effects on the ability of a biocontrol agent to infect sclerotia may only be short lived. In addition, any biocontrol agent such as *C. minitans* applied as solid substrate inoculum may be less susceptible to the effects of pesticides due to physical protection afforded by the inoculum matrix itself. Thus, the soil tray experiments used in this work appear to provide a more realistic test of pesticide compatibility than agar-incorporation tests for a biocontrol agent aimed for use against the sclerotial stage of *S. sclerotiorum* in soil. In general, assessing compatibility between a biocontrol agent and pesticides in an environment that reflects the planned formulation and application of the biocontrol agent in the field should provide a more reliable measure of compatibility.

The lack of effect of iprodione on *C. minitans* in the tray experiments was reflected in the first glasshouse trial. When combined with a single Rovral spray, there was no difference in efficacy between iprodione-tolerant and standard *C. minitans* isolates in controlling Sclerotinia disease or in decreasing sclerotial numbers and viability, or in infecting sclerotia. Exposure of *C. minitans* to this fungicide application would have been relatively minimal, as at the time of application lettuce plants completely covered the soil surface, and only the recommended concentration

was used. Far smaller amounts of fungicide would consequently have reached the soil than those used in the soil-tray experiments.

The design of the glasshouse trials means that in untreated plots, greater numbers of sclerotia are returned to the soil than in those with fungicide or *C. minitans* treatments, potentially magnifying the efficacy of these control treatments. However, this reflects the natural situation in glasshouse lettuce cropping in the United Kingdom if no control measures are applied. The rapid spread of *C. minitans* to untreated control and fungicide treated plots, which has been reported before (54,55), further compounds data interpretation. Thus, *C. minitans* may have suppressed the level of disease developing in these plots, potentially lessening the magnitude of the impact of *C. minitans* observed. Nevertheless, despite these potential problems, reproducible disease control was found using *C. minitans* in both glasshouse trials, demonstrating the robustness of this experimental system, which has been demonstrated previously (5,6,32,34,50).

In both glasshouse trials, significant disease control was achieved using *C. minitans* alone when disease levels built up to over 50% in control plots. This improved control over previous experiments (32,33) is probably due to the repeated application of *C. minitans* before each crop rather than a single application to the first crop only. The repeated application of *C. minitans* allows the mycoparasite to come into contact with new sclerotia on the surface of the soil at the start of each crop as well as enabling previously applied inocula to continue to attack sclerotia buried in the soil. Thus the whole sclerotial population is being subjected to a high level of the antagonist. Nevertheless, potentially, with additional introduction of *C. minitans* during sclerotia formation on the crop, perhaps as a foliar spray, the disease could be controlled further. This type of repeated exposure of sclerotial populations of *S. sclerotiorum* to *C. minitans* by foliar sprays has provided long-term field control of Sclerotinia disease in a 5-year trial (16).

The level of control achieved by *C. minitans* was enhanced further in both series of lettuce crops by the single application of a Rovral spray. The level of control achieved with this integrated treatment was statistically as good as that obtained by the use of

TABLE 2. First glasshouse trial: effect of standard and iprodione-tolerant isolates of *Coniothyrium minitans* applied alone and in combination with single Rovral sprays on the percentage of *Sclerotinia sclerotiorum*-diseased plants, and the number, viability, and infection of sclerotia of *S. sclerotiorum* by *C. minitans* after harvest of sequential lettuce crops^z

Treatment	% Diseased plants			Mean number of sclerotia per 500 cm ² of soil			% Viability of sclerotia			% Sclerotia infected by <i>C. minitans</i>		
	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3
Control (nil)	3 a	63 a	55 a	3.0 a	33.0 a	47.2 a	n.d.	81.3 a	68.2 a	n.d.	57.5 a	72.8 a
Standard <i>C. minitans</i>	1 a	24 b	31 b	1.7 a	11.9 b	23.3 b	n.d.	47.5 b	51.7 b,c	n.d.	96.3 b	90.4 b
Iprodione-tolerant <i>C. minitans</i>	0 a	30 b	37 a,b	0.0 a	17.6 b	29.1 b	n.d.	55.8 b	59.3 a,b	n.d.	94.2 b	97.1 b
Standard <i>C. minitans</i> + Rovral	1 a	17 b	22 c	1.5 a	9.8 b	18.8 b	n.d.	35.0 b	44.9 c	n.d.	95.4 b	89.6 b
Iprodione-tolerant <i>C. minitans</i> + Rovral	2 a	19 b	24 c	1.3 a	14.4 b	24.7 b	n.d.	47.9 b	56.2 b	n.d.	92.5 b	94.5 b

^z *C. minitans* applied as maize meal-perlite solid substrate inoculum (0.6 liters m⁻²). Single Rovral spray applied at onset of disease (0.5 kg ha⁻¹). Numbers in the same columns followed by the same letters are not significantly different according to least significant difference ($P < 0.05$) based on the *F* test in analysis of variance. Not determined due to lack of sclerotia.

TABLE 3. Second glasshouse trial: effect of *Coniothyrium minitans* alone and in combination with a single Rovral spray in comparison with single and Rovral sprays every 2 weeks on the percentage of *Sclerotinia sclerotiorum*-diseased plants, and the number, viability and infection of sclerotia of *S. sclerotiorum* by *C. minitans* after harvest of sequential lettuce crops^z

Treatment	% Diseased plants			Mean number of sclerotia per 500 cm ² of soil			% Viability of sclerotia			% Sclerotia infected by <i>C. minitans</i>		
	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3
Control (nil)	12 a	32 a	84 a	19.6 a	34.9 a	71.5 a	96.9 a	83.6 a	79.4 a	12.7 a	29.3 a	63.3 a
Single Rovral spray	7 b	20 b	72 a	12.1 a,b	24.5 a,b	67.1 a	92.1 a	90.5 a	86.1 a	16.9 a	35.7 a	73.9 a,b
Rovral spray every 2 weeks	5 b	5 b	21 c	5.8 c	6.2 c	23.2 c	95.8 a	88.9 a	80.5 a	9.5 a	27.8 a	68.7 a
<i>C. minitans</i>	7 b	19 b	44 b	11.1 b	19.7 b	39.3 b	59.3 b	60.7 b	62.6 b	90.2 b	98.2 b	91.5 b
<i>C. minitans</i> + single Rovral spray	1 b	15 b	29 c	3.4 c	15.9 b	28.4 b,c	62.7 b	63.1 b	58.9 b	97.6 b	89.1 b	95.9 b

^z *C. minitans* applied as maize meal-perlite solid substrate inoculum (0.6 liters m⁻²). Single Rovral spray applied at onset of disease (0.5 kg ha⁻¹). Numbers in the same columns followed by the same letters are not significantly different according to least significant difference ($P < 0.05$) based on the *F* test in analysis of variance.

prophylactic spraying of Rovral. The ability of *C. minitans* to infect sclerotia found on the diseased lettuce was not inhibited by the single Rovral spray, nor by the standard Rovral spray regime. *C. minitans* spread into the plots treated with Rovral every 2 weeks just as efficiently as in the control plots. Therefore, Rovral sprays appear to have no adverse influence on the capacity of *C. minitans* to spread to nontreated plots, infect sclerotia, or reduce disease and the subsequent number of new sclerotia formed. As *C. minitans* cannot grow through soil (54,55), spread of *C. minitans* by water splash or mesofauna (54,55) may be important in developing field-scale suppression of Sclerotinia disease, which has been associated with *C. minitans* previously (23,30).

These trials show the potential for the use of *C. minitans* in an integrated program to control Sclerotinia disease in glasshouse lettuce. The timing of application of fungicide is, however, very important. Through the experience of running many trials involving *S. sclerotiorum* on lettuce we were able to apply Rovral at the optimum time that a single application would have the most effect, just as the disease was beginning to proliferate (26). There is no reason, however, why the onset of disease could not be predicted more accurately in glasshouse-grown lettuce, if more research was carried out on environmental effects on sclerotial germination and lettuce infection in this cropping system. Such studies have proven successful recently for Sclerotinia stem rot in spring-sown oilseed rape (46). With targeted application of fungicides to lettuce or other crops at the most susceptible stages of the pathogen life cycle, fewer fungicide applications might be required, and they might be more effective. Evidence from a 4-year series of field trials comparing routine benomyl sprays with foliar applications of microbial antagonists, including *C. minitans*, for control of *S. sclerotiorum* on bean, indicated that benomyl provided consistent, reproducible control but the microbial antagonists gave reproducible control in certain years only (3). This may be due to environmental factors influencing the ability of microbial antagonists to control ascospore infection of petals by *S. sclerotiorum* (19). Thus, fungicide sprays may provide a more stable control of *S. sclerotiorum* than microbial antagonists at this stage of the life cycle of the pathogen (3). Mycoparasitic biocontrol agents such as *C. minitans*, however, may be more valuable to decrease production of sclerotia on the plant or to attack sclerotia formed on plant tissue later in the life cycle (18,44) particularly if the strains are tolerant of the pesticides in use. In addition, *C. minitans* may be valuable in attacking sclerotia already in the soil that are not so susceptible to attack by chemicals (6,22,30,31) and to clean-up *S. sclerotiorum*-infested seed lots by eliminating sclerotia that fungicide treatments alone cannot eliminate (33).

ACKNOWLEDGMENTS

This work was funded by the Ministry of Agriculture, Fisheries, and Food (MAFF) for England and Wales. We thank A. Jackson for help with the statistical design and analysis, J. Fenlon for further biometrics advice, and the nursery staff for their assistance with glasshouse experiments at Horticulture Research International, Littlehampton, where all work was carried out.

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