Control of Apothecial Production of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* and *Talaromyces flavus*

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ABSTRACT

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In seven field experiments conducted in Alberta from 1984 to 1987, the application of *Coniothyrium minitans* to soil at seeding time reduced apothecial production of sclerotia of *Sclerotinia sclerotiorum* under the canopies of bean, canola, wheat, and barley. Application of *Talaromyces flavus* was ineffective, and combinations of *T. flavus* and *C. minitans* were as effective or less effective than *C. minitans* alone, indicating that no synergism occurred between these hyperparasites. Application of *C. minitans* to soil in the spring reduced apothecial production from sclerotia of *S. sclerotiorum* buried in the soil, and increased parasitism on sclerotia produced on diseased bean plants.

Additional keywords: *Brassica napus, B. rapa,* carpogenic germination, *Hordeum vulgare, Phaseolus vulgaris, Triticum aestivum,* white mold

Sclerotinia sclerotiorum (Lib.) de Bary is an important pathogen of bean (*Phaseolus vulgaris* L.) (20) and canola (*Brassica rapa* L., syn. *B. campestris* L. and *B. napus* L.) (27) in Canada. It can cause losses in yield (9,28,30) and contaminate harvested seed with sclerotia (1,29). Under suitable environmental conditions, sclerotia germinate carpogenically to produce apothecia. Ascospores originating from apothecia of *S. sclerotiorum* are the primary source of inoculum on bean (1) and canola (27).

Sclerotia of *S. sclerotiorum* in soil may be attacked by hyperparasites such as *Coniothyrium minitans* Campbell (7,10,12,15, 36,39), *Talaromyces flavus* (Klöcker) A.C. Stolk & R.A. Samson (25,26,35), *Trichoderma viride* Pers.:Fr. (17,22), *Gliocladium catenulatum* Gilman & E. Abbott (16,17), *G. virens* J.H. Miller, J.E. Giddens, & A.A. Foster (38), and *Sporidesmium sclerotivorum* Uecker, Ayers, & Adams (2). *C. minitans* was reported to be an effective biocontrol agent of *S. sclerotiorum* in sunflower (*Helianthus annuus* L.) (5,17), bean (37), and lettuce (*Lactuca sativa* L.) (6), and of *Sclerotium cepivorum* Berk. in onion (3). *T. flavus* has shown promise as a biological control agent for Verticillium wilt of potato (11) and Sclerotinia wilt of sunflower (24).

Crops that are not hosts of *S. sclerotiorum,* such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), are recommended for rotation with oilseeds and pulse crops in western Canada. No information is available on the control of apothecial production of *S. sclerotiorum* by hyperparasites under the canopies of nonhost crops. The objective of this study was to investigate the control of apothecial production of *S. sclerotiorum* by *C. minitans* and *T. flavus* under the canopies of the host crops, bean and canola, as well as the nonhost crops, wheat and barley.

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MATERIALS AND METHODS

Source of sclerotia and seed. Sclerotia of *S. sclerotiorum* (SS) used for artificial infestation of soil in field experiments from 1984 to 1987 were collected from field-grown diseased sunflower and bean plants. Sclerotia from sunflower and bean ranged in size from 8 to 10 mm and from 5 to 10 mm in length, respectively. All sclerotia were stored air-dry at 5°C for up to 8 months before use. Prior to use in the field, arbitrarily selected sclerotia were surface-sterilized in 95% ethanol for 90 s, placed on potato-dextrose agar (PDA) or PDA amended with streptomycin sulfate (200 ppm), and incubated at room temperature (20 ± 2°C) for 1 week to determine viability by mycelial germination. All samples of sclerotia had viability greater than 90%. Cultivars of bean (cv. Great Northern US 1140), canola (cv. Westar), wheat (cv. Fielder), and barley (cv. Sampson) were used for the experiments.

Hyperparasite inoculum. *T. flavus* (DAOM 172557) (25) and *C. minitans* (DAOM 149432) (14) were grown on wheat bran, dead sclerotia, or a limestone–bran mixture for 21 days, then air-dried and stored at 5°C until field application, as described by McLaren et al. (24).

To prepare the sclerotial substrate, sclerotia of *S. sclerotiorum* were ground into pieces (approximately 5 mm long), soaked in water for 2 h, drained, dried for 2 to 3 h, placed in foil containers, and autoclaved (24). The limestone–wheat bran substrate (24:1 wt/wt) was moistened with water (80 ml kg^{-1} of dry substrate), placed in foil containers, and autoclaved. Autoclaved wheat bran substrate was used as a control and was either uninoculated or inoculated with the hyperparasite and killed by autoclaving after incubation for 21 days.

Field experiments. During 1984 to 1987, seven field experiments were conducted at Lethbridge and Bow Island, Alberta (Table 1). With the exception of experiment 5, all fields were artificially infested with sclerotia of *S. sclerotiorum.* Sclerotia from diseased bean plants were used in experiments 1, 2, and 3; and sclerotia from diseased sunflower plants were used in experiments 4, 6, and 7.

Experiments 1 to 3 were established during 1984 to 1987 to study the control of apothecial production of *S. sclerotiorum* by *C. minitans, T. flavus,* or both in bean fields. The treatments for experiment 1 were (1) SS, (2) SS and autoclaved bran (AB), (3) SS and *C. minitans* on bran (CMB), (4) SS and *T. flavus* on bran (TFB), (5) SS and CMB–TFB (2:1 wt/wt), (6) SS and CMB–TFB (1:1 wt/wt), and (7) SS and CMB–TFB (1:2 wt/wt). Each plot in experiment 1 consisted of eight rows of bean 2.0 m long with row spacing of 0.5 m. Plots were seeded on 18 May 1984, with one burial area (BA) of 2.16 m^2 (1.8 × 1.2 m) per plot established during 25 to 29 June. In treatment 1, sclerotia were buried 2 cm deep, 1,200 sclerotia per BA. In treatments 2 to 7, 360 g of bran or air-dried inoculum was mixed with approximately 12 liters of field soil and applied to each BA. One-half of the soil mixture was spread at a depth of approximately 4 cm, followed by application of 1,200 sclerotia, and then covered with the remaining soil

mixture. The area was packed with a hoe. The same treatments as in 1984 were established on 7 to 8 May 1985, with 300 sclerotia and 30 g of the hyperparasite per burial area of 0.18 m^2. The methods for application of the hyperparasites and burial of sclerotia were the same as in 1984. All plots were seeded with bean on 18 to 19 May 1985.

Experiments 2 and 3 examined different rates of *C. minitans*. The treatments in experiment 2 were (1) SS, (2) SS and 60 g of AB, (3) SS and 120 g of AB, (4) SS and 180 g of AB, (5) SS and 200 g of autoclaved sclerotia substrate (AS), (6) SS and 400 g of AS, (7) SS and 800 g of AS, (8) SS and 60 g of CMB, (9) SS and 120 g of CMB, (10) SS and 180 g of CMB, (11) SS and 200 g of *C. minitans* grown on autoclaved sclerotia substrate (CMS), (12) SS and 400 g of CMS, and (13) SS and 800 g of CMS. The treatments for experiment 3 were (1) SS, (2) SS and 60 g of *C. minitans* grown on bran for 21 days and then autoclaved (CMBA), (3) SS and 120 g of CMBA, (4) SS and 180 g of CMBA, (5) SS and 60 g of CMB, (6) SS and 120 g of CMB, (7) SS and 180 g of CMB, (8) SS and 450 g of *C. minitans* grown on a limestone–bran substrate (CML), (9) SS and 900 g of CML, and (10) SS and 1,800 g of CML.

Each plot for experiment 2 consisted of four rows of bean 2.0 m long with a 0.5-m row spacing, and contained one BA of 0.72 m^2 (0.6 × 1.2 m). Plots were seeded with bean during 27 to 28 May 1986, and the treatments were applied on 29 May. Each plot of experiment 3 consisted of eight rows of bean, with the same row length and spacing and BA size as experiment 2. Beans were seeded on 22 May 1987, and treatments were applied on 10 to 11 June. For experiments 2 and 3, the air-dried inoculum or substrate was mixed with 4 liters of soil and distributed below and above 400 sclerotia in each BA. Sclerotia were buried at a depth of approximately 2.5 cm.

Experiments 4 and 5 were established to evaluate *C. minitans* for control of *S. sclerotiorum* in canola fields. The treatments for experiment 4 were (1) SS, (2) SS and 60 g of CMBA, (3) SS and 120 g of CMBA, (4) SS and 180 g of CMBA, (5) SS and 60 g of CMB, (6) SS and 120 g of CMB, and (7) SS and 180 g of CMB. The treatments for experiment 5 were the same as for experiment 4 except that the field for experiment 5 was naturally infested with *S. sclerotiorum*. For experiment 4, each plot had seven rows of canola with a 7.0-m row length and a 0.18-m row spacing. For experiment 5, there were 16 rows per plot with a 3.7-m row length and a 0.18-m row spacing. Experiments 4 and 5 were seeded on 29 April 1987 and 1 May 1987, respectively. There was one BA per plot in experiment 4, 0.72 m^2, and in experiment 5, 2.1 m^2. The treatments were applied on 8 to 9 June 1987 for experiment 4 and on 30 April 1987 for experiment 5. The establishment of BA and the number of sclerotia buried in experiment 4 were the same as in experiments 2 and 3. In the naturally infested field (experiment 5), the hyperparasite inoculum or uninoculated substrate was raked into the soil to a depth of approximately 2.5 cm.

Experiments 6 and 7 were conducted to investigate the effect of *C. minitans* on apothecial production of *S. sclerotiorum* under the canopy of wheat (experiment 6) and barley (experiment 7). The treatments

Table 1. Locations used for experiments in control of apothecia of *Sclerotinia sclerotiorum* by hyperparasites in host and nonhost crops

Exp.	Location	Year	Crop[x]	SS[y]	Hyperparasite[z]
1	Lethbridge	1984-85	Bean	A	C and T
2	Lethbridge	1986	Bean	A	C
3	Lethbridge	1987	Bean	A	C
4	Lethbridge	1987	Canola	A	C
5	Bow Island	1987	Canola	N	C
6	Lethbridge	1987	Wheat	A	C
7	Lethbridge	1987	Barley	A	C

[x] Bean and canola are host crops for *S. sclerotiorum*; wheat and barley are nonhost crops.
[y] SS = fields infested artificially (A) or naturally (N) with sclerotia of *S. sclerotiorum*.
[z] C = *Coniothyrium minitans*; T = *Talaromyces flavus*.

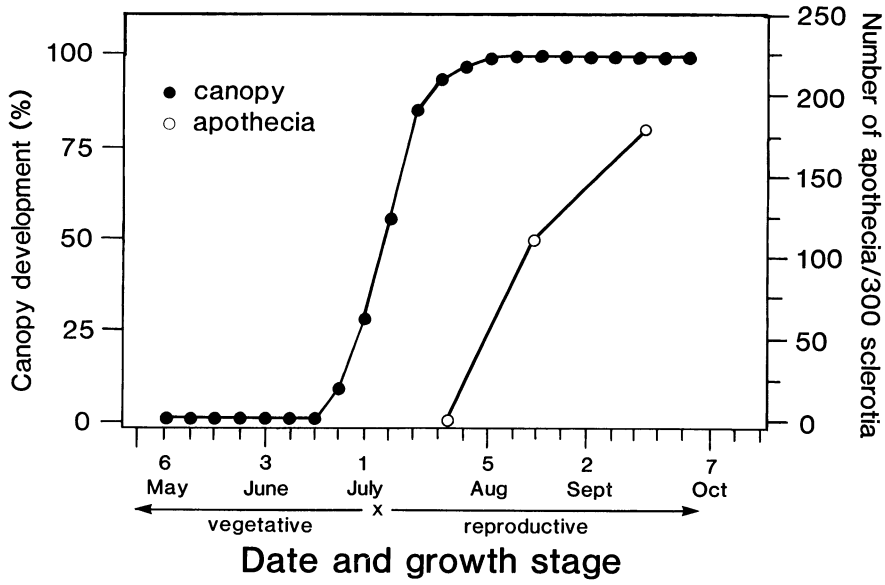

Fig. 1. Canopy development of dry bean and production of apothecia of *Sclerotinia sclerotiorum* in a Lethbridge field in 1985. Canopy development was estimated from coverage of interrow spaces.

Table 2. Effect of *Talaromyces flavus* and *Coniothyrium minitans* on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* (SS) in beans in 1984 and 1985 (experiment 1)

	1984[y]			1985[y]		
Treatment[x]	GN/BA (%)	AP/BA (no.)	AP/GS (no.)	GN/BA (%)	AP/BA (no.)	AP/GS (no.)
S. sclerotiorum	5.0 ab[z]	188.5 ab	3.3 a	6.8 a	366.6 ab	4.5 ab
SS + bran (AB)	6.4 a	287.1 a	3.7 a	6.6 a	435.7 a	5.5 a
SS + TFB	3.3 bc	125.1 abc	3.4 a	4.2 b	218.9 b	4.4 ab
SS + CMB–TFB (1:2)	1.6 cd	74.8 bcd	4.1 a	0.7 c	34.8 c	4.5 ab
SS + CMB–TFB (1:1)	1.3 d	46.7 cd	3.7 a	0.8 c	26.3 c	2.9 bc
SS + CMB–TFB (2:1)	0.8 d	27.0 d	3.0 a	0.4 c	11.2 d	2.1 c
SS + CMB	0.5 d	21.4 d	3.1 a	0.3 c	17.5 cd	4.9 a

[x] AB = autoclaved bran, TFB = *Talaromyces flavus* grown on bran, CMB = *Coniothyrium minitans* grown on bran. Rates of AB, TFB, CMB/TFB, and CMB were 360 g per burial area (BA) of 2.16 m^2 in 1984 and 30 g per BA of 0.18 m^2 in 1985.
[y] Values averaged over four replicates; based on 1,200 (1984) and 300 (1985) sclerotia per BA. GN/BA = percentage of buried sclerotia that germinated per BA, AP/BA = no. of apothecia per BA, AP/GS = no. of apothecia per germinated sclerotium.
[z] Means within columns followed by same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test.

for both experiments were (1) SS, (2) SS and 120 g of CMB, and (3) SS and 120 g of CMBA. All plots were seeded 29 April 1987 and had six rows of barley or wheat with a 7.6-m row length and a 17.8-cm row spacing. Each plot contained one BA, 0.72 m^2, and treatments were applied to the BA during 28 to 29 May 1987. The hyperparasite inoculum or uninoculated substrate was mixed with 8 liters of field soil and distributed below and above 400 sclerotia buried at a depth of 2.5 cm.

Apothecia produced from sclerotia of *S. sclerotiorum* in each BA were counted twice in all experiments except experiment 1 (1984), where they were counted three times, and experiments 3 and 4, where they were counted once. Wooden sticks were used to mark apothecial production per sclerotium. The crops were irrigated regularly using sprinklers. At the time of the apothecial counts, the growth stage of bean (31), canola (13), and wheat and barley (21) was assessed, and canopy coverage was determined by the percent coverage of the interrow spaces.

Hyperparasitism of sclerotia formed on diseased bean plants. Sclerotia of *S. sclerotiorum* produced on infected bean pods or stems were collected in the fall of 1985 (experiment 1) and 1987 (experiment 3) to determine their viability and parasitism by *T. flavus* or *C. minitans*. Twenty sclerotia were collected from each plot of the seven treatments of experiment 1. In experiment 3, only four of the 10 treatments were chosen as collection sites, and these were (1) CMB (180 g BA^{-1}), (2) CML (1,800 g BA^{-1}), (3) CMBA (180 g BA^{-1}), and (4) control. All sclerotia were surface-disinfested for 90 s in 95% ethanol, air-dried, and plated onto PDA amended with streptomycin sulfate (200 ppm). After incubation at room temperature for 7 days, sclerotia were examined for viability and infection by the hyperparasites.

Density of natural sclerotia in the fields. The natural inoculum of *S. sclerotiorum* was determined at all field locations. Soil samples averaging approximately 3.2 kg were collected arbitrarily from the experimental sites. The numbers of samples collected at the experiment 1 to 7 field sites were 28, 25, 20, 10, 28, 10, and 10, respectively. All samples were air-dried, and sclerotia were separated using a wet sieving technique (14).

Experimental design and statistical analyses. The treatments in each experiment were arranged in a randomized complete block design (RCBD), and each treatment, unless otherwise indicated, was replicated four times. Analyses of variance (34) for an RCBD were carried out separately for each experiment. A logistic transformation was used for the data on percent germinated sclerotia, number of apothecia per BA, and number of apothecia per germinated sclerotium in the analyses. Means of treatments were converted back to raw form for presentation.

RESULTS

Inoculum density of *S. sclerotiorum* varied from field to field. In the naturally infested field (experiment 5), the level of natural inoculum was high (3.6 sclerotia kg^{-1} of soil), but the level was low (<0.18 sclerotia kg^{-1} of soil) in the artificially infested fields (experiments 1 to 4, 6, and 7).

Apothecia of *S. sclerotiorum* were first observed when crop canopies were well developed. In bean plots, apothecia were first sighted in late July, when the bean growth stage reached R4 to R5 and the canopy coverage was approximately 95% (Fig. 1). Apothecia production increased through August and into September, when canopy coverage of the interrow spaces was approximately 100%. The crop growth stage at the time of the apothecial counts was 11.2 to 11.4 in wheat and barley, and 4.2 to 5.3 in canola.

C. minitans applied at seeding time reduced carpogenic germination of sclerotia of *S. sclerotiorum* (Table 2). In 1984 (experiment 1), only 0.5% of the sclerotia per BA germinated carpogenically in the *C. minitans*–treated plots, compared with 5% germination in the untreated control plots. The numbers of apothecia were 21.4 and 188.5 per BA in the *C. minitans*–treated and control plots, respectively. Similar levels of control of apothecia by *C. minitans* were observed in beans in 1985. *T.*

Table 3. Effect of *Coniothyrium minitans* on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* (SS) buried in a bean field in 1986 (experiment 2)

Treatment[x]	Germinated sclerotia per BA (%)[y]	Apothecia per BA	Apothecia per germinated sclerotium
S. sclerotiorum	1.5 a-c[z]	18.3 ab	2.2 bc
SS + AB			
60 g	2.4 ab	21.2 ab	2.2 bc
120 g	1.3 a-d	19.7 ab	2.6 bc
180 g	2.5 ab	42.4 a	7.0 a
SS + AS			
200 g	3.3 a	37.1 a	2.2 bc
400 g	2.3 ab	27.1 ab	2.9 b
800 g	0.7 b-e	7.9 bc	2.6 bc
SS + CMB			
60 g	0 e	0.5 c	0.5 bc
120 g	0 e	0.2 c	0.2 c
180 g	0 e	0 c	0 c
SS + CMS			
200 g	0.1 de	0.7 c	0.5 bc
400 g	0 e	0 c	0 c
800 g	0.2 c-e	2.1 c	1.5 bc

[x] AB = autoclaved bran, AS = autoclaved sclerotia, CMB = *C. minitans* grown on bran, CMS = *C. minitans* grown on autoclaved sclerotia. Rates of AB, AS, CMB, and CMS refer to the amount of material per burial area (BA) of 0.72 m^2.

[y] Values averaged over four replicates with 400 sclerotia per BA.

[z] Means within columns followed by same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test.

Table 4. Effect of *Coniothyrium minitans* on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* (SS) buried in a bean field in 1987 (experiment 3)

Treatment[x]	Germinated sclerotia (%)[y]	Apothecia per BA	Apothecia per germinated sclerotium
S. sclerotiorum	7.6 ab[z]	130.5 a	4.0 ab
SS + CMBA			
60 g	11.8 c	130.5 a	4.0 ab
120 g	7.6 ab	141.3 a	4.5 ab
180 g	5.2 b	120.3 a	6.4 a
SS + CMB			
60 g	0.2 cd	11.8 c	1.0 d
120 g	0.1 d	11.0 c	1.0 d
180 g	0.1 d	11.4 c	1.4 cd
SS + CML			
450 g	1.6 c	26.3 b	2.5 b-d
900 g	0.7 cd	18.4 bc	3.1 bc
1800 g	0.9 cd	19.7 bc	2.7 b-d

[x] CMBA = *C. minitans* grown on bran for 21 days and then autoclaved, CMB = *C. minitans* grown on bran, CML = *C. minitans* grown on mixture of limestone and bran (24:1 wt/wt). Rates of CMBA, CMB, and CML refer to the amount of material per burial area of 0.72 m^2.

[y] Values averaged over four replicates with 400 sclerotia per burial area (BA).

[z] Means within columns followed by same letter are not significantly different ($P > 0.05$) using the least significant different (LSD) test.

flavus applied at seeding time was less effective than *C. minitans* in the control of carpogenic germination of sclerotia and production of apothecia (Table 2). Treatment of *T. flavus* resulted in a significant reduction in carpogenic germination of sclerotia in 1985, but it did not cause a significant reduction in number of apothecia produced per BA in either 1984 or 1985 experiments. In 1984 and 1985, combinations of *C. minitans* and *T. flavus* were as effective as the application of *C. minitans* alone (Table 2). The difference in number of apothecia produced from germinated sclerotia between the control and the treatment with *C. minitans* or *T. flavus* was not significant.

Application of *C. minitans* on different substrates at different rates significantly reduced the production of apothecia from sclerotia of *S. sclerotiorum* under bean (Tables 3 and 4) and canola (Table 5). For example, in a canola field in Lethbridge, less than 0.2% of buried sclerotia per BA germinated in plots treated with *C. minitans* compared with 6.8% germination in the untreated control plots (Table 5, experiment 4). Fewer than 2.9 apothecia per BA were observed in the *C. minitans*–treated plots compared with 77.2 apothecia per BA in the control plots. All rates of *C. minitans* on bran, from 60 to 180 g per 0.72 m^2, were effective. For example, in 1987, less than 12 apothecia per BA were observed in all *C. minitans*–treated plots, compared with 130.5 apothecia per BA in the untreated control and 120.3 to 141.3 apothecia per BA in the plots treated with CMBA (*C. minitans* grown on bran and then autoclaved) (Table 4). Results also suggest that a limestone–bran mixture (Table 4) or dead sclerotia (Table 3) as substrates would be effective, although each of these formulations was tested in only one year. In the field infested naturally with *S. sclerotiorum* (Table 5, experiment 5), the application of *C. minitans* on bran at 120 g per 0.72 m^2 resulted in the production of approximately half the 1,067.8 apothecia produced per BA in the untreated control (Table 5). A reduction in number of apothecia per germinated sclerotium was again not consistently observed (Tables 3 to 5).

Carpogenic germination of sclerotia of *S. sclerotiorum* occurred under the canopy of the nonhost crops, wheat and barley. Application of *C. minitans* caused a significant reduction in apothecial production (Table 6). Under a canopy of wheat, no buried sclerotia germinated in the *C. minitans*–treated plots compared with 24.3% in control plots, which produced 555.4 apothecia per BA (Table 6, experiment 6). Similar effects were observed in *C. minitans*–treated plots in a barley field (Table 6, experiment 7). The average number of apothecia produced per germinated sclerotium was reduced by the treatment of *C. minitans* under both wheat and barley.

Numerous sclerotia collected from diseased bean pods and stems in the late fall of 1985 (experiment 1) and 1987 (experiment 3) were infected by *C. minitans* (Table 7). Infection by this hyperparasite was observed in sclerotia collected from both the *C. minitans*–treated plots and the untreated control plots. Among sclerotia collected from the *C. minitans*–treated plots, 23.4 to 39.9% were infected by *C. minitans*, compared with 28.8% in the control (Table 7, experiment 1). Similar results were observed in experiment 3 (Table 7). The percentage of sclerotia infected by *T. flavus* was extremely low (less than 0.4%) in all plots (Table 7, experiment 1).

Table 5. Effect of *Coniothyrium minitans* on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* (SS) in a canola field in 1987 (experiments 4 and 5)

	Lethbridge (experiment 4)[x]			Bow Island (experiment 5)[y]		
Treatment[w]	GN/BA (%)	AP/BA (no.)	AP/GS (no.)	GN/m^2 (no.)	AP/m^2 (no.)	AP/GS (no.)
S. sclerotiorum	6.8 b[z]	77.2 b	3.0 ab	558.5 a	1,067.8 a	1.9 ab
SS + CMBA						
60 g	13.3 ab	210.0 a	4.1 a	480.5 a	891.5 ab	1.9 ab
120 g	16.2 a	240.8 a	3.6 ab	454.3 a	906.3 ab	2.0 ab
180 g	7.5 b	96.4 ab	3.4 ab	416.8 a	860.3 ab	2.1 ab
SS + CMB						
60 g	0.2 c	2.9 c	1.6 bc	433.0 a	794.0 ab	1.8 b
120 g	0.1 c	0.2 c	0.2 c	248.8 b	591.0 bc	2.4 a
180 g	0.1 c	0.5 c	0.5 c	229.0 b	439.8 c	1.9 ab

[w] CMBA = *C. minitans* grown on bran for 21 days and then autoclaved, CMB = *C. minitans* grown on bran. Rates of CMBA and CMB refer to the amount of material per burial area (BA) of 0.72 m^2.
[x] Values averaged over four replicates; based on 400 sclerotia per BA. GN/BA = percentage of buried sclerotia that germinated; AP = no. of apothecia; AP/GS = no. of apothecia per germinated sclerotium. One BA is 0.72 m^2.
[y] This field was naturally infested with sclerotia of *S. sclerotiorum*. GN/m^2 = number of germinated sclerotia per m^2. The area of each treated plot is 2.1 m^2.
[z] Means within columns followed by same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test.

Table 6. Effect of *Coniothyrium minitans* on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* (SS) buried in wheat (experiment 6) and barley (experiment 7) fields in 1987 at Lethbridge

	Wheat (experiment 6)[y]			Barley (experiment 7)[y]		
Treatment[x]	GN/BA (%)	AP/BA (no.)	#AP/GS (no.)	GN/BA (%)	AP/BA (no.)	AP/GS (no.)
S. sclerotiorum	24.3 a[z]	555.4 a	5.5 a	29.9 a	738.3 a	5.3 a
SS + CMBA (120 g)	17.0 a	349.8 a	5.7 a	29.8 a	729.8 a	5.5 a
SS + CMB (120 g)	0 b	0 b	0 b	0 b	0 b	0 b

[x] CMBA = *C. minitans* grown on bran for 21 days and then autoclaved, CMB = *C. minitans* grown on bran. Rate of CMBA and CMB refer to the amount of material per burial area (BA) of 0.72 m^2.
[y] Values averaged over four replicates; based on 400 sclerotia per BA. GN/BA = percentage of buried sclerotia that germinated; AP = no. of apothecia; AP/GS = no. of apothecia per germinated sclerotium.
[z] Means within columns followed by same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test.

DISCUSSION

This study indicates that soil amendment with *C. minitans* is effective in controlling the production of apothecia of *S. sclerotiorum* under the canopy of host and nonhost crops. These findings support previous reports on the effectiveness of *C. minitans* as a hyperparasite against sclerotia of *S. sclerotiorum* (17,18,36,37). Huang (17) reported that the reduction of Sclerotinia wilt of sunflower in the field was due mainly to the destruction by *C. minitans* of sclerotia, which are the main source of inoculum for root infection of sunflower in Canada. Under the canopies of bean and canola, ascospores are the primary source of inoculum (1,28). The present study indicates that viability and subsequent apothecial production of the sclerotia buried in the soil are reduced by *C. minitans*, thereby reducing the inoculum potential of *S. sclerotiorum* under the canopies of host and nonhost crops. At the one site naturally infested with *S. sclerotiorum*, *C. minitans* did not have as effective control of the pathogen. This reduced efficiency of control of apothecial production of *S. sclerotiorum* by *C. minitans* in the naturally infested soil may be due to natural distribution of sclerotia, which reduced the chance of contact with the hyperparasite applied to the topsoil zone of up to 2.5 cm in depth.

McLaren (23) observed a reduction in Sclerotinia wilt of sunflower due to the destruction of sclerotia by *T. flavus* under field conditions. However, in the present study, *T. flavus* was not effective in reducing apothecial production. The sunflower

study was conducted at Winnipeg, Manitoba, and Lethbridge, Alberta, under dryland conditions (24); whereas the present study on bean, canola, wheat, and barley crops was carried out under irrigated conditions to maintain a microclimate suitable for carpogenic germination of sclerotia and production of apothecia. Biocontrol agents have specific environmental requirements for optimal biological control activity (4,32). The lack of control of *S. sclerotiorum* by *T. flavus* in this study may be associated with the irrigation conditions for the crops. Further investigation of the ecological factors affecting the survival and activity of *T. flavus* under different cropping systems is warranted.

A synergistic effect between *C. minitans* and *T. flavus* did not occur. Use of combinations of these hyperparasites was no more effective in reducing carpogenic germination and apothecial production of sclerotia of *S. sclerotiorum* than was the use of *C. minitans* alone.

Wheat and barley are not hosts of *S. sclerotiorum* and are often used in rotation with susceptible crops such as canola or bean to reduce disease pressure. Morrall and Dueck (27) reported that a 3-year rotation to barley did not appreciably decrease numbers of germinated sclerotia in a field subsequently sown to rapeseed for two consecutive years. We observed that numerous apothecia were produced under wheat and barley canopies, thus providing a potential source of airborne inoculum for neighboring fields of susceptible crops. The present study also indicates that spring application of *C. minitans* to fields of barley and wheat artificially infested with *S. sclerotiorum* significantly reduced sclerotial germination and apothecial production. This suggests that *C. minitans* can be used for soil treatment to reduce the inoculum potential of *S. sclerotiorum* in fields sown to nonhost crops such as cereals.

Sclerotia are the primary survival structures of *S. sclerotiorum* (8,40) and can be produced in abundance on infected plant tissue (17). Trutmann et al. (37) reported that spraying bean plants with spores of *C. minitans* at flowering time reduced the number of sclerotia produced on infected bean plants. The present study indicates that *C. minitans*, applied to soil at seeding time, not only reduces apothecial production from buried sclerotia during the growing season but also infects sclerotia formed on diseased plants, potentially reducing the overwintering inoculum of *S. sclerotiorum*. Infection of sclerotia produced in bean tissue indicates the ability of the hyperparasite to move from the soil to aboveground tissue. Pathogens may be spread through irrigation practices (33), and the presence of *C. minitans*–infected sclerotia in both *C. minitans*–treated and control plots suggests the hyperparasite may have spread from one plot to another by irrigation water. The ability of *C. minitans* to spread to areas removed from the site of application improves the attractiveness of this hyperparasite as a biological control agent.

C. minitans has proven effective as a hyperparasite of sclerotia of *S. sclerotiorum* (18,19) and as a biocontrol agent for control of Sclerotinia wilt of sunflower in the field (5,17). The present study indicates that *C. minitans* is effective in controlling apothecial production in soil under host and nonhost crops and is able to colonize sclerotia produced in infected bean tissue. Consequently, *C. minitans* definitely has potential as a biocontrol agent for the management of *S. sclerotiorum* in various crops, including both host and nonhost species.

Table 7. Effect of *Coniothyrium minitans* and *Talaromyces flavus* on the viability of sclerotia of *Sclerotinia sclerotiorum* (SS) collected from the aboveground parts of diseased bean plants (experiments 1 and 3, Lethbridge)

	Experiment 1 (1985)[x]			Experiment 3 (1987)[x]	
Treatment[w]	Viability (%)	C (%)[y]	T (%)[y]	Viability (%)	C (%)[y]
S. sclerotiorum	10.3 b[z]	28.8 a	0 a	9.7 a	0.4 c
SS + bran (AB)	22.2 ab	35.8 a	0 a	–	–
SS + TFB	26.1 a	38.6 a	0 a	–	–
SS + CMB–TFB (1:2)	15.4 ab	35.8 a	0.4 a	–	–
SS + CMB–TFB (1:1)	23.1 ab	23.4 a	0 a	–	–
SS + CMB–TFB (2:1)	25.9 a	28.1 a	0 a	–	–
SS + CMB	14.2 ab	39.9 a	–	9.7 a	22.4 a
SS + CML	–	–	–	6.5 a	11.4 ab
SS + CMBA	–	–	–	2.6 a	6.4 bc

[w] AB = autoclaved bran, TFB = *Talaromyces flavus* grown on bran, CMB = *C. minitans* grown on bran, CML = *C. minitans* grown on mixture of limestone and bran (24:1 wt/wt), CMBA = *C. minitans* grown on bran for 21 days and then autoclaved. Rate of AB, TFB, CMB/TFB, and CMB was 360 g per burial area (BA) (2.16 m^2). For experiment 3, rates of CMB, CML, and CMBA were 180 g, 1,800 g, and 180 g per BA (0.72 m^2), respectively.

[x] Values averaged over four replicates; represent the percentage of collected sclerotia that were viable or infected by a hyperparasite; 20 sclerotia were collected from each replicate in the fall.

[y] Represents the percentage of sclerotia infected by *C. minitans* (C) or *T. flavus* (T).

[z] Means within columns followed by the same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test.

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LITERATURE CITED

1. Abawi, G. S., and Grogan, R. G. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. Phytopathology 65:300-309.
2. Adams, P. B., and Ayers, W. A. 1980. Factors affecting parasitic activity of *Sporidesmium sclerotivorum* on sclerotia of *Sclerotinia minor* in soil. Phytopathology 70:366-368.
3. Ahmed, A. H. M., and Tribe, H. T. 1977. Biological control of white rot of onion (*Sclerotium cepivorum*) by *Coniothyrium minitans*. Plant Pathol. 26:75-78.
4. Baker, R. 1987. Mycoparasitism: Ecology and physiology. Can. J. Plant Pathol. 9:370-379.
5. Bogdanova, V. N., Karadzhova, L. V., and Klimenko, R. F. 1986. Using *Coniothyrium minitans* Campbell as a hyperparasite in controlling the pathogen of watery soft rot of sunflower. Skh. Biol. 5:80-84.
6. Budge, S. P., and Whipps, J. M. 1991. Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. Plant Pathol. 40:59-66.
7. Campbell, W. A. 1947. A new species of *Coniothyrium* parasitic on sclerotia. Mycologia 39:190-195.
8. Coley-Smith, J. R., and Cooke, R. C. 1971. Survival and germination of sclerotial fungi. Annu. Rev. Phytopathol. 9:65-92.
9. Dueck, J. 1977. Sclerotinia in rapeseed. Agric. Can. 22(3):7-9.
10. Fedulova, T. Yu. 1983. Activity of the fungus *Coniothyrium minitans*, hyperparasite of soft rot. Dokl. Vses. Akad. Skh. Nauk 10:44-45.
11. Fravel, D. R., Davis J. R., and Sorensen, L. H. 1986. Effect of *Talaromyces flavus* and metham on verticillium wilt incidence and potato yield 1984-1985. Biol. Cult. Tests Control Plant Dis. 1:17.
12. Ghaffar, A. 1972. Some observations on the parasitism of *Coniothyrium minitans* on the sclerotia of *Sclerotinia sclerotiorum*. Pak. J. Bot. 4:85-87.
13. Harper, F. R., and Berkenkamp, B. 1975. Revised growth-stage key for *Brassica campestris* and *B. napus*. Can. J. Plant Sci. 55:657-658.
14. Hoes, J. A., and Huang, H. C. 1975. *Sclerotinia sclerotiorum*: Viability and separation of sclerotia from soil. Phytopathology 65:1431-1432.
15. Huang, H. C. 1977. Importance of *Coniothyrium minitans* in survival of sclerotia of *Sclerotinia sclerotiorum* in wilted sunflower. Can. J. Bot. 55:289-295.
16. Huang, H. C. 1978. *Gliocladium catenulatum*: Hyperparasite of *Sclerotinia sclerotiorum* and *Fusarium* species. Can. J. Bot. 56:2243-2246.
17. Huang, H. C. 1980. Control of sclerotinia wilt of sunflower by hyperparasites. Can. J. Plant Pathol. 2:26-32.
18. Huang, H. C., and Hoes, J. A. 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. Can. J. Bot. 54:406-410.
19. Huang, H. C., and Kokko, E. G. 1987. Ultrastructure of hyperparasitism of *Coniothyrium minitans* on sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 65:2483-2489.
20. Huang, H. C., Kokko, M. J., and Phillippe, L.

M. 1988. White mold of dry bean (*Phaseolus vulgaris* L.) in southern Alberta, 1983-87. Can. Plant Dis. Surv. 68:11-13.
21. James, W. C. 1971. A Manual of Assessment Keys for Plant Disease. Can. Dep. Agric. Publ. 1458.
22. Lee, Y.-A., and Wu, W.-S. 1986. Chemical and biological controls of sunflower sclerotinia disease. Plant Prot. Bull. (Taiwan) 28:101-109.
23. McLaren, D. L. 1989. Biocontrol of sclerotinia disease (*Sclerotinia sclerotiorum*) of sunflower and bean by *Talaromyces flavus* and *Coniothyrium minitans*. Ph.D. thesis. University of Manitoba, Winnipeg, Manitoba.
24. McLaren, D. L., Huang, H. C., Kozub, G. C., and Rimmer, S. R. 1994. Biological control of sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. Plant Dis. 78:231-235.
25. McLaren, D. L., Huang, H. C., and Rimmer, S. R. 1986. Hyperparasitism of *Sclerotinia sclerotiorum* by *Talaromyces flavus*. Can. J. Plant Pathol. 8:43-48.
26. McLaren, D. L., Huang, H. C., Rimmer, S. R., and Kokko, E. G. 1989. Ultrastructure of the infection of sclerotia of *Sclerotinia sclerotiorum* by *Talaromyces flavus*. Can. J. Bot. 67:2199-2205.
27. Morrall, R. A. A., and Dueck, J. 1982. Epidemiology of sclerotinia stem rot of rapeseed in Saskatchewan. Can. J. Plant Pathol. 4:161-168.
28. Morrall, R. A. A., Dueck, J., McKenzie, D. L., and McGee, D. C. 1976. Some aspects of *Sclerotinia sclerotiorum* (Lib.) de Bary in Saskatchewan from 1970 to 1975. Can. Plant Dis. Surv. 56:56-62.
29. Morrall, R. A. A., Loew, F. M., and Hayes, M. A. 1978. Subacute toxicologic evaluation of sclerotia of *Sclerotinia sclerotiorum* in rats. Can. J. Comp. Med. 42:473-477.
30. Natti, J. J. 1971. Epidemiology and control of bean white mold. Phytopathology 61:669-674.
31. NDSU. 1981. Dry Bean Production Handbook. Circ. A602. Cooperative Extension Service, North Dakota State University, Fargo.
32. Phillips, A. J. L. 1986. Factors affecting the parasitic activity of *Gliocladium virens* on sclerotia of *Sclerotinia sclerotiorum* and a note on its host range. J. Phytopathol. 116:212-220.
33. Steadman, J. R. 1983. White mold - A serious yield-limiting disease of bean. Plant Dis. 67:346-350.
34. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, Toronto, Ontario.
35. Su, S. J., and Leu, L. S. 1980. Three parasitic fungi on *Sclerotinia sclerotiorum* (Lib.) de Bary. Plant Prot. Bull. (Taiwan) 22:253-262.
36. Trutmann, P., Keane, P. J., and Merriman, P. R. 1980. Reduction of sclerotial inoculum of *Sclerotinia sclerotiorum* with *Coniothyrium minitans*. Soil Biol. Biochem. 12:461-465.
37. Trutmann, P., Keane, P. J., and Merriman, P. R. 1982. Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyperparasite *Coniothyrium minitans*. Trans. Br. Mycol. Soc. 78:521-529.
38. Tu, J. C. 1980. *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotiorum*. Phytopathology 70:670-674.
39. Turner, G. J., and Tribe, H. T. 1976. On Coniothyrium minitans and its parasitism of Sclerotinia species. Trans. Br. Mycol. Soc. 66:97-105.
40. Willetts, H. J., and Wong, J. A.-L. 1980. The biology of *Sclerotinia sclerotiorum, S. trifoliorum* and *S. minor* with emphasis on specific nomenclature. Bot. Rev. 46:101-165.