Biological Control of Sclerotinia Wilt of Sunflower with *Talaromyces flavus* and *Coniothyrium minitans*

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ABSTRACT

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Six field experiments were conducted in Manitoba and Alberta during 1982–1987 to evaluate the hyperparasites *Talaromyces flavus* and *Coniothyrium minitans* for the control of Sclerotinia wilt, caused by *Sclerotinia sclerotiorum*, of sunflower. Application of *T. flavus* and *C. minitans* to soil at seeding time reduced disease incidence and subsequent loss in seed yield. Plots treated with *T. flavus* and/or *C. minitans* for two consecutive years developed a suppressive effect to *S. sclerotiorum* in the third year but not in the fourth year. The application of combinations of *T. flavus* and *C. minitans* was as effective as the application of *C. minitans* alone.

Additional keywords: *Helianthus annuus*, mycoparasites

Sclerotinia sclerotiorum (Lib.) de Bary, causal agent of Sclerotinia wilt and head rot of sunflower (*Helianthus annuus* L.), is a destructive pathogen and can cause serious losses in yield and quality of the crop (7,12). Sclerotia in soil germinate myceliogenically to cause wilt (19) or carpogenically to cause head and stalk rot (36). Sclerotia are subject to attack by soil microorganisms such as *Coniothyrium minitans* Campbell (6,8,11,32–35), *Talaromyces flavus* (Klöcker) A.C. Stolk & R.A. Samson (27,31) (teleomorph of *Penicillium vermiculatum* Dangeard), *Sporidesmium sclerotivorum* Uecker, Ayers, & Adams (2), *Trichoderma viride* Pers.:Fr. (17,25), and *Gliocladium catenulatum* Gilman & Abbott (16,17).

Hyphae and sclerotia of *S. sclerotiorum* are parasitized and killed by *C. minitans* (17,20,22,23) and *T. flavus* (26,27,31). *C. minitans* parasitizes sclerotia of *S. sclerotiorum* in the field (15,18) and is effective in controlling Sclerotinia wilt of sunflower in fields infested naturally or artificially with sclerotia of *S. sclerotiorum* (5,17). Although *T. flavus* has shown promise as a biological control agent for Verticillium wilt of potato, caused by *Verticillium dahliae* Kleb., in the field (10), no information is available on the control of *S. sclerotiorum* by this hyperparasite under field conditions.

The objectives of this study were to investigate the effect of *T. flavus* and *C. minitans* on the incidence of Sclerotinia wilt and yield of sunflower in Alberta and Manitoba during and following the years when the hyperparasites were applied.

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MATERIALS AND METHODS

Source of sclerotia and seed. Sclerotia of *S. sclerotiorum* used for artificial infestation of field soil in field experiments from 1982 to 1987 were collected from diseased plants of sunflower, bean (*Phaseolus vulgaris* L.), and rapeseed/canola (*Brassica napus* L. and *B. rapa* L.), obtained from seed-cleaning companies. Sclerotia from sunflower, bean, and canola were from 8–10, 5–10, and 2 mm in diameter, respectively. Whole sclerotia were used in all experiments except in 1982 and 1983, when large sclerotia from sunflower were chopped up with a grinder and screened for pieces measuring 4–6 mm in diameter. Sclerotia from pure cultures grown on a bean substrate (homogenized red kidney or baked beans) at 20 C for 12 days were also used in a 1985 field experiment. All sclerotia were stored at 4–5 C under air-dried conditions until used. Prior to use in the field, randomly selected sclerotia were surface-sterilized, placed on potato-dextrose agar (PDA) or PDA amended with streptomycin sulfate, and incubated at room temperature for 1 wk to determine viability. All samples of sclerotia had a viability greater than 90%. The sunflower cultivars used during 1982–1985, 1986, and 1987 were Hybrid 894, Cargill 204, and Dahlgren 131, respectively.

Inoculum of the hyperparasites. Dry wheat bran was moistened with water (850 ml/kg of dry bran), placed in aluminium foil containers, autoclaved at 121 C for 30 min each day for two consecutive days, and inoculated with a spore suspension from 10-day-old PDA cultures of *T. flavus* (DAOM 172557) (26) or *C. minitans* (DAOM 149432) (13). The inoculated substrate was incubated at 20 C for 21–28 days, air-dried for 3–4 days, and stored at 5 C until used in the field. All inoculum was prepared as described above except in 1982, when *T. flavus* was grown on bran in glass jars for 9 days and then air-dried. For control treatments, wheat bran with or without colonization of *C. minitans* was autoclaved and then air-dried. In 1987, a bran/limestone mixture (1:24, w/w) also was utilized for production of *C. minitans*, using the procedures as described for the bran substrate.

Experimental design. During 1982–1987, six field experiments were conducted in Alberta and Manitoba (Table 1). All fields were artificially inoculated with sclerotia of *S. sclerotiorum*. The treatments in each experiment were arranged in a randomized complete block design (30). Unless indicated otherwise, each treatment was replicated four times and each plot had four 6-m rows of sunflower spaced 0.9 m apart. At seeding time in May, 90 sunflower seeds, 250 sclerotia of *S. sclerotiorum*, and inoculum of *C. minitans* or *T. flavus*, or both, were applied to each row. Sclerotia and inoculum were distributed uniformly in a trench 6.5 cm deep, 8.0 cm wide, and 6.0 m long. Seeds were distributed evenly along the row length, and all material was covered with soil and packed. Plant spacing was adjusted by thinning to 0.15 m between plants at the establishment stage (29).

Experiments 1 and 2 were established at Winnipeg and Portage la Prairie, Manitoba, respectively, to assess disease control with *T. flavus*. The treatments for both experiments were: 1) untreated control, 2) sclerotia of *S. sclerotiorum* (SS), and 3) SS and *T. flavus* on bran. In these experiments, 100 g of bran infested with the hyperparasite was applied to each row. Sclerotia from sunflower were used in both experiments.

Experiments 3 and 4 were established to assess disease control with *T. flavus* and *C. minitans*. Experiment 3 was conducted for 4 yr (1983–1986) at Lethbridge, Alberta, and experiment 4 was conducted for 3 yr (1983–1985) at Winnipeg, Manitoba. The treatments of experi-

ment 3 were: 1) untreated control, 2) bran control, 3) SS, 4) SS and noninoculated bran, 5) SS and *T. flavus* on bran, 6) SS and *C. minitans* on bran, and 7) SS and a combination of *T. flavus* and *C. minitans* (1:1, w/w). In experiment 3, 45 and 50 g of hyperparasite-infested or noninfested bran was applied to each row in 1983 and 1984, respectively. The experiment was continued at the same site in 1985 and 1986 by application of sclerotia to each row without addition of hyperparasites or bran. The same treatments were used in experiment 4 as in experiment 3 except that number 7 was omitted. During 1983 and 1984, 23 and 100 g, respectively, of hyperparasite-infested or noninfested bran was applied to each row. In 1985, the experiment was continued in the same site by inoculation with sclerotia without addition of the hyperparasites or bran. Sclerotia from sunflower were used for the 1983 and 1986 tests and from bean for the 1984 and 1985 tests.

Experiment 5 was established in the fall of 1984 to study the effect of a fall (F) compared with a spring (SP) application of *T. flavus* (T) and *C. minitans* (C) on disease incidence in 1985. All sclerotia were applied in the spring. The treatments were: 1) bran (F), 2) bran (SP), 3) SS, 4) bran (F) and SS, 5) bran (SP) and SS, 6) T (F) and SS, 7) T (SP) and SS, 8) C (F) and SS, 9) C (SP) and SS, 10) C/T (1:1, w/w) (F) and SS, 11) C/T (1:1, w/w) (SP) and SS, 12) C/T (2:1, w/w) (F) and SS, 13) C/T (2:1, w/w) (SP) and SS, 14) C/T (1:2, w/w) (F) and SS, and 15) C/T (1:2, w/w) (SP) and SS. In this experiment, 100 g of hyperparasite-infested or noninfested bran was applied. Each plot in the randomized block design consisted of three 6- m rows of sunflower 0.6 m apart.

Experiment 6 was established in 1987 to study the effectiveness of *C. minitans* grown on different substrates and applied at different rates. The treatments were: 1) control, 2) SS, 3) C at 25 g per row and SS, 4) C at 50 g per row and SS, 5) C at 100 g per row and SS, 6) C autoclaved and at 25 g per row and SS, 7) C autoclaved and at 50 g per row and SS, 8) C autoclaved and at 100 g per row and SS, 9) C at 250 g per row and SS, 10) C at 500 g per row and SS, and 11) C at 1,000 g per row and SS. *C. minitans* was grown on wheat bran in all treatments except 9–11, in which the hyperparasite was produced on a limestone/bran substrate. There were five replicate blocks with four 6.1-m rows per plot and row spacing of 0.6 m. Sclerotia of *S. sclerotiorum* from diseased sunflower were used.

All sunflower plots in all experiments were examined weekly, from the vegetative to the seed development stages, for the incidence of Sclerotinia wilt characterized by wilt, root rot, and basal stem canker (21). Disease percentages are based on the total number of diseased plants that occurred in a plot during the vegetative to seed development stages of growth. Approximately 160 plants per plot were examined for disease, except in experiments 3 and 5, where 180 and 135 plants per plot, respectively, were used to calculate disease percentages. Sunflower seed yield was determined in all but experiment 5. For experiments 1 and 2, sunflower heads in the center two rows of each plot were bagged with cotton sacks at early to mid-anthesis to prevent bird damage. These heads were hand-harvested at maturity, dried, and threshed, and the seed were cleaned and weighed.

Table 1. Experimental sites in Canada for biological control of Sclerotinia wilt of sunflower with *Talaromyces flavus* (T) and *Coniothyrium flavus* (C)[z]

Expt.	Location	Years	Hyperparasite
1	Winnipeg, Man.	1982	T
2	Portage, Man.	1982	T
3	Lethbridge, Alta.	1983–86	T + C
4	Winnipeg	1983–85	T + C
5	Lethbridge	1984–85	T + C
6	Lethbridge	1987	C

[z] Fields were infested artificially with sclerotia of *Sclerotinia sclerotiorum*.

Table 2. Effect of *Talaromyces flavus* (T) on incidence of Sclerotinia wilt caused by *Sclerotinia sclerotiorum* (SS), and seed yield of sunflower in 1982 at Winnipeg (experiment 1) and Portage la Prairie (experiment 2)[w]

Treatment	Percent wilted plants[x]		Yield (kg/ha)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Control	3.4 a[y]	30.0 a	2,897 a	1,926 a
SS	47.3 b	83.5 b	2,350 b	1,430 b
SS + T	3.4 a	5.7 a	2,870 a	2,140 a
SE[z] (6 df)	0.11	0.10	150	143

[w] Both fields were infested artificially with sclerotia of *S. sclerotiorum*.
[x] Percent wilted plants are back-transformed means following a $\log_{10}[W/(100 - W)]$ transformation, where W is the percent wilted plants.
[y] Means within columns followed by the same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test.
[z] Standard error of a mean in $\log_{10}$ units for percent wilted plants.

Table 3. Effect of *Talaromyces flavus* (T) and *Coniothyrium minitans* (C) on incidence of Sclerotinia wilt, caused by *Sclerotinia sclerotiorum* (SS), and seed yield of sunflower in Lethbridge (experiment 3, 1983–86) and Winnipeg (experiment 4, 1983–85)

Treatment[u]	Percent wilted plants[v]							Yield[w] (kg/ha)				
	Experiment 3				Experiment 4			Experiment 3			Experiment 4	
	1983	1984	1985	1986	1983	1984	1985	1984	1985	1986	1984	1985
None	0.0	0.0	0.0	0.1 b[x]	0.5 c	1.3 c	0.3 b	1,260 abc	1,960 ab	1,490 a	1,930 a	1,230 a
Wheat bran	1.0 c	0.4 b	0.6 cd	0.0 b	0.5 c	1.6 c	1.0 b	1,390 a	2,370 a	1,490 a	2,040 a	1,310 a
SS	54.2 a	47.5 a	36.7 a	54.1 a	65.2 a	79.0 a	19.1 a	950 c	870 c	300 b	660 d	990 a
SS + bran	16.7 ab	17.3 a	14.8 ab	51.8 a	54.6 a	60.1 ab	24.5 a	990 bc	1,190 bc	330 b	1,120 cd	1,090 a
SS + T	0.0	0.7 b	3.8 bc	51.0 a	14.0 b	32.6 b	20.3 a	1,430 a	1,910 ab	340 b	1,460 bc	1,210 a
SS + C	3.3 bc	1.1 b	1.2 cd	41.7 a	2.5 c	0.9 c	0.5 b	1,330 abc	1,730 ab	590 b	1,860 ab	1,220 a
SS + C/T	1.4 bc	0.0	0.3 d	45.9 a	...[y]	...	...	1,360 ab	2,100 a	330 b	...	...
SE[z] (df)	0.38(12)	0.37(12)	0.32(15)	0.35(15)	0.22(15)	0.26(15)	0.43(15)	130(18)	263(18)	155(18)	155(15)	116(15)

[u] Sclerotia from diseased sunflower heads (1983 and 1986) or diseased bean plants (1984) were applied at 250 per row, excluding the wheat bran treatment. Application rates for T, C, C/T, and bran per 6-m row were: 45 g (1983) and 50 g (1984) in Lethbridge and 23 g (1983) and 100 g (1984) in Winnipeg. At both sites, sclerotia and hyperparasites were applied in 1983–84; only sclerotia were applied in 1985 and 1986.
[v] Percent wilted plants are back-transformed means following a $\log_{10}[W/(100 - W)]$ transformation, where W is the percent wilted plants.
[w] Estimated yields based on a regression of yield on head size using 40 heads per treatment.
[x] Means within columns followed by the same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test. Treatments with no wilted plants in all replicates were excluded from the statistical analysis.
[y] Data unavailable.
[z] Standard error of a mean in $\log_{10}$ units for percent wilted plants.

In 1983, severe bird damage prevented the determination of yield in experiment 3 at Lethbridge and experiment 4 at Winnipeg. During 1984–1987, when experiments were too large to bag individual heads, seed yield was estimated from the regression of yield on head size (W. O. Chubb, *personal communication*) as follows: The largest and smallest diameters of heads in the center two rows of each plot were measured late in the season when seeds were filled but heads were still green. Diameters of 40 heads per treatment (10 heads per replicate) that had been bagged previously were also measured. At maturity, the bagged heads were harvested and individual head yields were determined. A linear regression equation (30) relating yield and the product of the two head diameters (size) was obtained for each treatment and used to estimate yields for the center two rows of each plot containing that treatment.

Statistical analysis. Analyses of variance (30) for a randomized block design were carried out on the disease and yield data for each year for each experiment. A logistic transformation (4) was used for the percent wilted plants data because it was evident that the treatment means and variances were not independent. Observed 0% wilted plants were replaced by $25/N$ (N = number of plants examined) prior to transforming the data, and treatments were omitted from the analysis of variance when there were 0% wilted plants in all replicates of an experiment. Except for experiment 6, which had treatments with quantitative rates, multiple comparisons were carried out using the least significant difference (LSD). The comparisons were carried out on the transformed scale for the percent wilted plants. When subsets of treatments were factorial in nature or other nonpairwise comparisons were of interest, contrasts were calculated.

RESULTS

Data from 6 yr of field trials indicated that the application of *T. flavus* and *C. minitans* at seeding can reduce the incidence of Sclerotinia wilt of sunflower (Tables 2–5). Treatment differences were first noticeable during the vegetative and budding stages of growth, with fewer plants becoming infected in the hyperparasite-treated plots than in the control (SS) and SS and bran-treated plots (Fig. 1). By the late seed development stage, the incidence of disease in the treatments with sclerotia only or sclerotia and bran was significantly higher than in the treatments with *C. minitans* or *T. flavus*, or both.

T. flavus significantly reduced wilt incidence at the Winnipeg site in 1982, with 3.4% disease in the *T. flavus*-treated plots and 47.3% disease in the control (SS) plots (Table 2). Similar results were observed in Portage la Prairie in 1982 (Table 2) and in Lethbridge and Winnipeg during 1983–1984 (Table 3). For example, at Lethbridge in 1983, 0.7 and 54.2% disease occurred in the *T. flavus*-treated (with or without *C. minitans*) and control (SS) plots, respectively ($P < 0.01$).

C. minitans, applied annually in 1983 and 1984, effectively reduced the incidence of Sclerotinia wilt of sunflower (Tables 3 and 4). In 1983 at Lethbridge (experiment 3), 2.4% disease occurred in the *C. minitans*-treated (with or without *T. flavus*) plots compared with 54.2% disease in the control (SS) plots ($P < 0.01$). In 1984, the *C. minitans*-treated plots and the control showed 0.6 and 47.5% disease, respectively ($P < 0.01$). Results were similar with the *C. minitans*-treated and control (SS) plots at Winnipeg (experiment 4) over the 2-yr period (1983–1985) (Table 3). Except when grown on bran that was then autoclaved, *C. minitans* significantly reduced ($P < 0.01$) the incidence of Sclerotinia wilt of sunflower regardless of the rate of application (Table 4, experiment 6). All rates of *C. minitans* grown on bran (25–100 g per row) and on a limestone/bran mixture (250–1,000 g per row) resulted in less than 8.0% diseased plants in the treated plots compared with 47.9% in the SS control. The effect of rate of application was not significant ($P > 0.05$) except for *C. minitans* produced on a limestone/bran mixture, where the percent wilted plants decreased linearly with increasing rate of application ($P < 0.05$).

The application of *C. minitans* and *T. flavus* in various ratios resulted in less disease than in the SS control plots (Table 5). The SS control plots averaged 16.3% disease, whereas plots with both *C. minitans* and *T. flavus* had less than 1% disease ($P < 0.01$). The application of the hyperparasite combinations produced results similar to the application of *C. minitans* alone (Tables 3 and 5). The time of application (fall 1984 and spring 1985) and the time of application × hyperparasite interaction were not significant ($P > 0.05$).

The tests in Lethbridge and Winnipeg also showed that the application of hyperparasites to field soil for two consecutive seasons (1983 and 1984) could be effective in reducing the incidence of disease in the third season (1985), when sclerotia, but no hyperparasites, were applied (Table 3). In the plots treated with *T. flavus* (T), *C. minitans* (C), and C/T at Lethbridge (experiment 3), the mean percentage of disease was 1.5% compared with 36.7% in the SS control ($P < 0.01$). At the Winnipeg site (experiment 4), the incidence of disease was reduced significantly in the *C. minitans*-treated plots but not in the *T. flavus*-treated plots, with 0.5 and 20.3% disease, respectively, and 19.1% disease in the SS control (Table 3). In 1986, the experiment in Lethbridge was continued for the fourth season by the application of sclerotia to the seed rows. The suppressive effect observed in 1985 was no longer evident in the hyperparasite-treated plots.

Table 4. Effect of application rates of *Coniothyrium minitans* (C) on incidence of Sclerotinia wilt, caused by *Sclerotinia sclerotiorum* (SS), and seed yield of sunflower in Lethbridge (experiment 6, 1987)

Treatment[x]	Percent wilted plants[y]	Yield (kg/ha)
None	0.2	1,360
SS	47.9	800
SS + C		
25 g bran	0.5	1,200
50 g bran	0.2	1,260
100 g bran	0.3	1,240
SS + C, auto		
25 g bran	38.1	890
50 g bran	32.2	1,040
100 g bran	35.8	980
SS + C		
250 g ls/br	8.0	1,330
500 g ls/br	7.1	1,040
1,000 g ls/br	2.4	1,670
SE[z] (40 df)	0.17	127

[x] *C. minitans* was grown on bran or a mixture of limestone and bran (ls/br). C, auto = *C. minitans* grown on bran and then autoclaved.
[y] Percent wilted plants are back-transformed means following a $\log_{10}[W/(100 - W)]$ transformation, where W is the percent wilted plants.
[z] Standard error of a mean in $\log_{10}$ units for percent wilted plants.

Table 5. Effect of application of *Talaromyces flavus* (T) and *Coniothyrium minitans* (C) in fall (1984) or spring (1985) on incidence of Sclerotinia wilt, caused by *Sclerotinia sclerotiorum* (SS), of sunflower in Lethbridge (experiment 5)

Treatment[w]	Percent disease[x]
Wheat bran	0
SS	16.3 a[y]
SS + bran	12.1 ab
SS + T	4.1 b
SS + C/T 1:2	0.5 c
SS + C/T 1:1	0.7 c
SS + C/T 2:1	0
SS + C	0
SE[z] (27 df)	0.20

[w] Application rate for bran or hyperparasites was 100 g per 6-m row. All sclerotia (250 per 6-m row) were applied in the spring of 1985.
[x] Percent wilted plants are back-transformed means following a $\log_{10}[W/(100 - W)]$ transformation, where W is the percent wilted plants.
[y] Means followed by the same letter are not significantly different ($P > 0.05$) using the least significant difference (LSD) test. Treatments with no wilted plants in all replicates were excluded from the statistical analysis.
[z] Standard error of a mean in $\log_{10}$ units.

The regression of yield on head size was significant ($P < 0.01$), and the proportion of the total variance accounted for (R^2) ranged from 79 to 92% in the control group. This indicates that the seed yield estimates for 1984–1987 are a good approximation to the unobserved yields in those years. Observed yields in experiments 1 and 2 (Table 2) and estimated yields in experiments 3, 4, and 6 (Tables 3 and 4) showed a reduction in Sclerotinia wilt due to the application of *T. flavus* (Tables 2 and 3) and *C. minitans* (Tables 3 and 4) also resulted in an increase in seed yield. In experiment 1, yield from the *T. flavus*-treated plots was 2,870 kg/ha compared with 2,350 kg/ha in the control (SS) plots (Table 2). Experiment 2 also showed a reduction in yield loss due to the application of *T. flavus* with yields of 2,140 and 1,430 kg/ha in the *T. flavus*-treated and SS control plots, respectively. In *C. minitans*-treated (with or without *T. flavus*) plots, a yield of 1,345 kg/ha was obtained, compared with 950 kg/ha in the SS control plots of experiment 3 (Table 3). Similar results were obtained in experiment 4 (Table 3), with a yield of 1,860 kg/ha in the *C. minitans*-treated plots compared with 660 kg/ha in the SS control plots. Experiment 6 also showed a reduction in yield loss due to the application of *C. minitans*, with yields of 1,233 and 800 kg/ha in the *C. minitans*-treated and the SS control plots, respectively (Table 4). Plots with *C. minitans* grown on bran (25–100 g per row) and then autoclaved had lower ($P < 0.05$) yields than corresponding plots that were not autoclaved (970 vs. 1,233 kg/ha, respectively), and there was no effect of rate of application or interaction with the autoclaving treatment in these plots ($P > 0.05$).

Plots treated with hyperparasites and sclerotia in 1983 and 1984 and with sclerotia only in 1985 showed a suppression of disease in 1985 (Table 3, experiment 3). The 1985 yield data from these plots reflected this suppression of disease. A seed yield of 870 kg/ha was obtained in the control plots compared with a mean of 1,913 kg/ha in the *T. flavus, C. minitans*, and C/T-treated plots (Table 3). In 1986, no yield difference between the hyperparasite-treated plots and the SS controls was observed because of high levels of disease in all treatments, including those that had shown a suppressive effect in 1985.

Considering all years for both locations, plots with no SS had higher yields than plots with SS but no parasites, and this effect was generally significant ($P < 0.05$).

DISCUSSION

This study demonstrated that *T. flavus* and *C. minitans* have potential as effective biocontrol agents of *S. sclerotiorum* in the Canadian prairies. Our findings support previous reports on the effectiveness of *C. minitans* (5,17) as a biological control agent for Sclerotinia wilt of sunflower in the field. Huang (17) reported that *C. minitans* reduced the incidence of Sclerotinia wilt of sunflower but did not reduce the secondary spread of the disease caused by direct contact with diseased roots. He attributed the reduction in disease mainly to the control of sclerotia of *S. sclerotiorum* by *C. minitans*. Since sclerotia are the source of inoculum for primary infection, the control of Sclerotinia wilt of sunflower by *C. minitans* and *T. flavus* in the present study also may be caused by the destruction of sclerotia by these hyperparasites.

Bogdanova et al (5) reported that when *C. minitans* was applied to soil in the fall, its hyperparasitic activity was not apparent until the spring, when temperatures increased. Sclerotia remained viable during the winter period. In the present study, the application of *C. minitans* and *T. flavus* was equally effective in controlling Sclerotinia wilt of sunflower in the fall or the spring. This indicates that *C. minitans* and *T. flavus* were able to overwinter under the freezing conditions of the Canadian prairies and subsequently to parasitize sclerotia of *S. sclerotiorum* in the soil.

Combinations of *T. flavus* and *C. minitans* were as effective as the application of *C. minitans* alone. The data also indicate that *C. minitans* may be more effective than *T. flavus* in controlling Sclerotinia wilt of sunflower. This may be related to the difference in ability of the two hyperparasites to destroy sclerotia as observed by Adams (1), who reported that more sclerotia of *S. sclerotiorum* were killed by *C. minitans* than by *T. flavus*. Moreover, biocontrol agents have specific environmental requirements for optimal biological control activity (3,28). It is possible that environmental conditions in Manitoba and Alberta may be more favorable for the survival of *C. minitans* than of *T. flavus*. Further investigations on the ecological factors affecting survival and activity of *C. minitans* and *T. flavus* in different soils are warranted.

This study provides evidence that annual application of *C. minitans* and *T. flavus* for 2 yr may induce the soil to become suppressive to *S. sclerotiorum* in the third year. In the Alberta study, the suppressive effect by *C. minitans* and *T. flavus* lasted for 1 yr. In a study in Manitoba, Huang and Kozub (24) observed a similar suppressive effect of *S. sclerotiorum* by *C. minitans* that lasted for 2 yr. The suppressive phenomenon may enhance the economic feasibility of practical application of these organisms for control of Sclerotinia disease of sunflower and other crops.

Huang and Hoes (21) found that sclerotia located adjacent to the seed were more important as a source of inoculum for Sclerotinia wilt of sunflower than sclerotia separated from the seed by a vertical distance of 4–5 cm or a horizontal distance of 10 cm. The high level of disease control obtained in the present study following row application of *T. flavus* and *C. minitans* may be caused by the elimination of those sclerotia close to the seed. Thus, row application of hyperparasites may be feasible for control of wilt in row crops such as sunflower.

C. minitans grown on bran was more

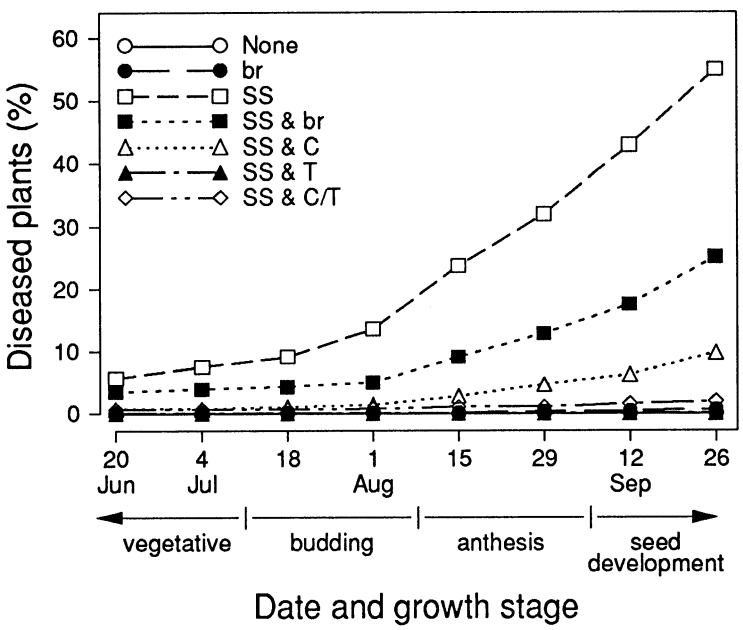

Fig. 1. Effect of *Talaromyces flavus* (T) and *Coniothyrium minitans* (C) on the development of Sclerotinia wilt, caused by *Sclerotinia sclerotiorum* (SS), of sunflower in 1983 at Lethbridge (experiment 3); br = bran.

effective than that grown on a limestone/bran mixture. Although high rates of the limestone/bran mixture may be as effective as the *C. minitans* grown on bran, the volume of material required for control precludes practical application of this substrate. On a large-scale basis, a formulation suitable for application of hyperparasites with conventional farm equipment still remains to be developed. *T. flavus* has been produced in alginate pellets by an inexpensive and versatile process (9). It would be of interest to evaluate the use of alginate pellets for application of *C. minitans* and *T. flavus* for control of *S. sclerotiorum* in fields artificially or naturally infested with sclerotia.

Sclerotinia wilt of sunflower has not been effectively controlled through the use of chemicals or resistant cultivars (14). We have demonstrated the potential for biological control of Sclerotinia disease by the hyperparasites *T. flavus* and *C. minitans*, especially in conjunction with recommended management procedures.

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