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BIOLOGICAL CONTROL OF SCLEROTINIA WILT OF SUNFLOWER BY HYPERPARASITES

By

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Abstract

The biological control of Sclerotinia wilt of sunflower by the hyperparasites, Coniothyrium minitans, Gliocladium catenulatum and Trichoderma viride, was studied in the greenhouse as well as in the field. All three hyperparasites were capable of destroying sclerotia of Sclerotinia sclerotiorum in soil under greenhouse conditions. Results from two years of field trials showed that the introduction of hyperparasites, particularly C. minitans, into the Sclerotinia-infested soil at planting was effective in decreasing the rate of wilt incidence, and consequently, increasing the yield of sunflowers. In addition, the number of plants killed by the primary infection loci or sclerotia was significantly lower in the hyperparasite-treated plots than in the untreated control. However, the rate of disease spread from these loci was not different significantly. This suggests that the reduction of Sclerotinia wilt of sunflower is mainly due to the effective control of primary inoculum or sclerotia by the hyperparasites.

In North America, the Sclerotinia wilt of sunflower caused by Sclerotinia sclerotiorum (Lib.) de Bary, was first recorded in 1912 (11) but it received attention until 1920 when the disease was reported as common and destructive to cultivated sunflowers in Canada (1) and in the United States (13). Due to the rapid increase of sunflower acreage in the past two decades, this disease has become one of the limiting factors in sunflower production in western Canada (6).

Sclerotinia sclerotiorum survives in soil mainly as black, resting bodies or sclerotia. The sclerotia may germinate to produce mycelia which penetrate into sunflower roots resulting in root rot, basal stem canker and wilt of the plant; or under certain environmental conditions, they may germinate to produce airborne ascospores which attack sunflower heads causing head rots. In Manitoba, root rot and wilt is generally more destructive to the crop than the head rot (6).

Survival of S. sclerotiorum in the soil is affected not only by environmental factors (3,16,18) but also by soil micro-organisms including hyperparasites such as Coniothyrium minitans Campbell (2,4,7,8,10,14,17), Gliocladium catenulatum Gilman & Abbott (9), Gliocladium roseum (Link.) Bainier (12), and Trichoderma viride Pers. ex Fr. (10,12). Some of the hyperparasites have been frequently isolated from sunflower fields in Manitoba (5.8). This study is to investigate the control of Sclerotinia wilt of sunflower by the hyperparasites under greenhouse and field conditions.

Materials and Methods

Source and Increase of Hyperparasites

Coniothyrium minitans (DAOM 149432), G. catenulatum (DAOM 149586) and T. viride were isolated from sclerotia of S. sclerotiorum collected from sunflower fields near Morden (5). Stock cultures of these hyperparasites were maintained on sclerotia of S. slcerotiorum by the dual culture technique (6).

The hyperparasites were increased for greenhouse and field application on a substrate composed of barley, rye and sunflower seeds (1:1:1 v/v/v). The mixture of seeds was soaked in water for approximately 2 hours in autoclavable polypropylene bags (Bel-Art Products, Pequannock, N.J. 07440, USA). They were autoclaved at 121 C, 15 minutes for three times at daily intervals. The seeds were inoculated with spore suspension of the hyperparasites harvested from cultures growing on potato dextrose agar and incubated at room temperature for 4 to 6 weeks. They were then applied to the soil as moist inoculum or they were air-dried for 2 to 4 weeks prior to application.

Greenhouse Experiments

Efficacy of the hyperparasites against S. sclerotiorum was assessed by burying 1000 large sclerotia (> 4 mm) in pot soil treated with 50 g of hyperparasites grown on autoclaved seeds. The treatments were: (a) S. sclerotiorum alone, (b) S. slcerotiorum and C. minitans, (c) S. sclerotiorum and G. catenulatum, (d) S. sclerotiorum and T. viride, and (e) S. slcerotiorum and the three hyperparasites. Each treatment contained four pots; two of them were planted with four sunflowers of the cultivar Krasnodarets in each pot. Sunflowers were watered daily to maintain the normal growth of the plants, while the pots without sunflowers were watered periodically to maintain a soil moisture content similar to field capacity. After an incubation period of 100 days, the amount of sclerotia remaining in the soil of each treatment was recovered by wet sieving technique (5).

Field Experiments

Experiments were conducted in fields naturally infested with sclerotia of S. sclerotiorum. The field was divided into 16 plots of 9.1 m x 6.1 m each, with 4 replicates of 4 treatments in a randomized complete block design. Each plot contained 10 rows of "Krasnodarets" sunflower with 0.91 m row spacing and 0.13 m within-row plant spacing. The treatments for 1976 were: (a) control; (b) C. minitans; (c) mixture of three hyperparasites; (d) mixture of three hyperparasites, 2 applications; whereas the treatments for 1977 were: (a) control; (b) C. minitans; (c) mixture of three hyperparasites; and (d) C. minitans, air-dried. All the hyperparasites applied to the soil were in a moist condition except the fourth treatment of 1977 which was air-dried. Also, all the inoculum was buried at seed level (approximately 5 cm deep) in a band approximately 8 cm wide during seeding except the fourth treatment of 1976 which received an additional inoculation one month after seeding. The additional inoculum was applied to the base of sunflower stems and then covered with soil. Approximately 1 kg of moist inoculum was applied to each row. For the treatments of three hyperparasites, approximately 0.4 kg of inoculum from each was mixed prior to application.

Plots were examined for symptoms of Sclerotinia wilt on sunflowers weekly from the vegetative to the late seed development stages (15). Plants that wilted independently within the row were considered as the result of primary infection by the primary inoculum or sclerotia in the soil. Thus, each dead plant bounded by healthy ones was regarded as a primary infection locus. Assuming a plant killed next to a primary infection was due to spread or secondary infection, then the rate of spread in each infection locus was calculated by: number of diseased plants/number of primary infection loci.

Shortly after seeding, 25 soil samples of approximately 15 cm^3 each, were collected from randomized spots along rows to determine the inoculum density of S. sclerotiorum in each plot. Soil samples were air-dried, weighed, and then processed by wet-sieving to recover the sclerotia in each sample.

Sunflowers were harvested at the late seed development stage and seed yield determined.

Results

Biological control of Sclerotinia sclerotiorum under greenhouse conditions

When 1000 large sclerotia were buried in soil without sunflowers for 100 days, the amount of sclerotia was reduced to 3, 67, 42 and 5% of the original, respectively, in treatments with C. minitans, G. catenulatum, T. veride and the combination of three hyperparasites; whereas the amount of sclerotia in the control pots increased to 114% (Table 1). Similarly, the amount of sclerotia in pots with sunflowers was 4, 40, 84 and 7% respectively, in the treatments of C. minitans, G. catenulatum, T. viride and the combination of three hyperparasites as compared to the 151% in the control pots (Table 1). Small, secondary sclerotia (< 4 mm) were found in all treatments, however, the amount of such sclerotia was consistently lower in the hyperparasite-treated soil than in that of the untreated controls (Table 1).

TABLE 1. Control of sclerotia of Sclerotinia sclerotiorum by hyperparasites

Treatment[a]	Sclerotia recovered after 100 days[b]							
	No sunflower				Sunflower			
	Large	Small	Total	(%)	Large	Small	Total	(%)
Control	770	367	1137	(114)	794	720	1514	(151)
C	18	7	25	(3)	25	11	36	(4)
G	472	193	665	(67)	255	146	401	(40)
T	310	110	420	(42)	568	275	843	(84)
C+G+T	33	15	48	(5)	49	20	69	(7)

[a] C, Coniothyrium minitans; G, Gliocladium catenulatum; T, Trichoderma veride.

[b] 1000 large sclerotia were buried in each pot; sclerotia recovered were the average of two pots in each treatment.

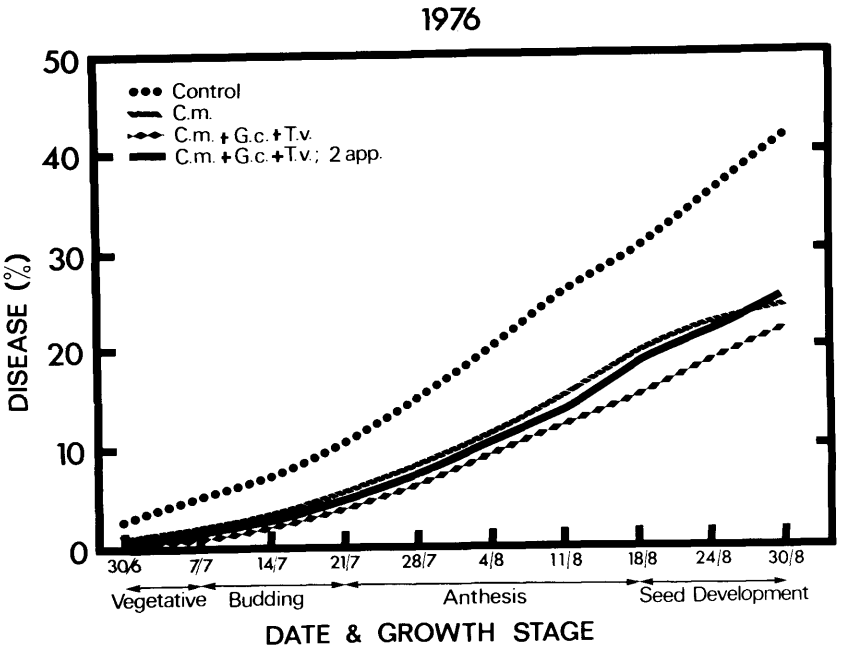

Figure A
1976 field experiment

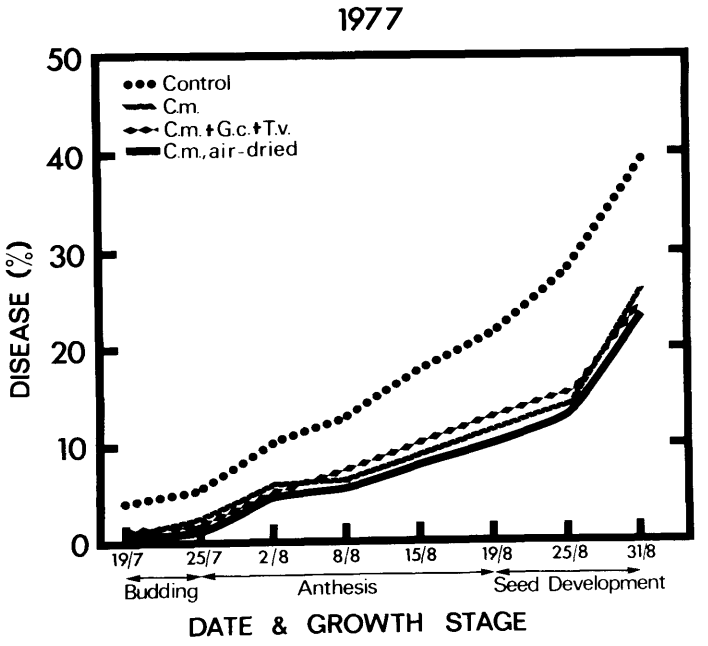

Figure B
1977 field experiment

Fig. 1 (A-B). Biological control of Sclerotinia wilt of sunflower by hyperparasites. The hyperparasites are: C.m., Coniothyrium minitans; G.c., Gliocladium catenulatum; T.v., Trichoderma viride.

Biological Control of Sclerotinia Wilt of Sunflower Under Field Conditions.

Results of the two-year field trials showed that the hyperparasites were effective in controlling S. sclerotiorum and, consequently, reducing wilt incidence in control plots remained low during vegetative and budding stages of growth. It increased steadily at a rate of approximately 5% every week throughout anthesis and the seed development stages (Figure 1). A similar increase in disease was also observed in hyperparasite-treated plots but the number of wilted plants at each growth stage was proportionally lower than that of the untreated controls (Figure 1). By the late seed development stage, the disease was over 40% in the untreated controls but it remained under 25% in the hyperparasite-treated plots (Table 2). Among the treatments with hyperparasites, C. minitans alone, either in a moist state or as air-dried inoculum, was just as effective as the combined three hyperparasites. Also, the additional application of three hyperparasites to the soil did not increase the efficacy in their control of Sclerotinia wilt of sunflower as compared to the single application of the same hyperparasites (Table 2).

Application of hyperparasites to the soil also resulted in the reduction in yield losses in sunflower due to Sclerotinia wilt. The two-year field trials showed that seed yield was significantly higher in the hyperparasite-treated plots than that of the untreated controls (Table 2). No significant difference in seed yield was found between the treatment of C. minitans alone and that of the three hyperparasites together.

TABLE 2. Effect of hyperparasites on Sclerotinia wilt and seed yield in sunflower.

Treatment[a]	Disease (%)		Yield (kg/ha)	
	1976	1977	1976	1977
1. (Control)	43	40	1213	902
2. (C)	25 b[c]	24 b	1495 b	1121 b
3. (C+G+T)	21 b	23 b	1473 b	1085 b
4.[b]	25 b	23 b	1539 b	1103 b

[a] C, Coniothyrium minitans; G, Gliocladium catenulatum; T, Trichoderma veride.

[b] The fourth treatment for 1976 was C+G+T with two applications, and for 1977 was C, air-dried.

[c] Means within columns followed by the same letter are not significantly different at the 0.05 level (Duncan's multiple range test). Raw percentage disease data are converted to arc sin square root percent.

The inoculum density of S. sclerotiorum surveyed at seeding time varied greatly among soil samples, ranging from 0 to 1.74 sclerotia/kg soil in the field of 1976 and from 0 to 2.32 sclerotia/kg soil in the field of 1977 (Table 3). However, there was no significant difference in inoculum density between hyperparasite-treated plots and untreated ones in both fields (Table 3).

TABLE 3. Inoculum density of Sclerotinia sclerotiorum in the biological control fields.

Treatment[a]	Inoculum density (No. sclerotia/kg soil)			
	1976		1977	
	Range	Average	Range	Average
1. Control	0.00 - 0.92	0.50 a[c]	0.00 - 0.84	0.26 a
2. C	0.12 - 0.92	0.52 a	0.00 - 2.32	0.58 a
3. C+G+T	0.00 - 1.74	0.56 a	0.00 - 0.97	0.36 a
4.[b]	0.11 - 1.72	0.68 a	0.00 - 1.50	0.57 a

[a] C, Coniothyrium minitans; G, Gliocladium catenulatum; T, Trichoderma veride.

[b] The fourth treatment for 1976 was C+G+T with two applications and for 1977 was C, air-dried.

[c] Means within columns followed by the same letter are not significantly different at the 0.05 level (Duncan's multiple range test).

The field trials showed that the number of primary infection loci differed significantly between hyperparasite-treated plots and untreated controls. Compared with controls, the frequency of primary infection loci in the treatment of C. minitans alone was reduced to 58% and 69%, respectively, in 1976 and 1977 (Table 4). When the rate of secondary infection from each primary infection locus was examined, no significant difference was found between the hyperparasite-treated plots and the untreated ones (Table 5). The average number of plants killed by each primary infection locus ranged from 1.63 to 1.92 and 1.61 to 1.83 in 1976 and 1977 experiments, respectively (Table 5).

TABLE 4. Effect of hyperparasites on primary infection of sunflower by Sclerotinia sclerotiorum

Treatment[a]	Average no. of primary infection loci/plot	
	1976	1977
1. Control	105	89
2. C	61 b[c]	61 b
3. C+G+T	55 b	59 b
4.[b]	61 b	57 b

[a] C, Coniothyrium minitans; G, Gliocladium catenulatum; T, Trichoderma viride.

[b] The fourth treatment for 1976 was C+G+T with two applications, and for 1977 was C, air-dried.

[c] Means within columns followed by the same letter are not significantly different at the 0.05 level (Duncan's multiple range test).

TABLE 5. Effect of hyperparasites on the secondary infection of Sclerotinia wilt of sunflower.

Treatment[a]	Average no. of plants killed by each infection locus	
	1976	1977
1. Control	1.92 a[c]	1.83 a
2. C	1.86 a	1.61 a
3. C+G+T	1.63 a	1.76 a
4.[b]	1.88 a	1.68 a

[a] C, Coniothyrium minitans; G, Gliocladium catenulatum; T, Trichoderma veride.

[b] The fourth treatment for 1976 was C+G+T with two applications and for 1977 was C, air-dried.

[c] Means within columns followed by the same letter are not significantly different at the 0.05 level (Duncan's multiple range test).

Discussion

This study indicates that mass introduction of hyperparasites to the soil at seeding time is effective in controlling Sclerotinia wilt and reducing yield losses in sunflower. It further suggests that C. minitans is the most promising biological control agent for S. sclerotiorum among the three hyperparasites tested. Although G. catenulatum and T. viride are effective in destroying sclerotia of S. sclerotiorum under greenhouse conditions, they do not enhance the efficacy of C. minitans in the control of Sclerotinia wilt of sunflower. Both G. catenulatum (9) and T. viride (12) are reported to be hyperparasites with wide host range. It is possible that, in addition to the hyperparasitic effect on S. sclerotiorum, these two hyperparasites may also affect the growth of C. minitans in soil.

The significant reduction in the number of primary infection loci (Table 4) reveals that the reduction of wilt incidence in sunflower is the result of the control of primary inoculum or sclerotia of S. sclerotiorum by hyperparasites. However, the hyperparasites appear to be ineffective in controlling S. sclerotiorum in an actively growing state as there was no significant reduction in rate of spread of the disease in hyperparasite-treated plots (Table 5). Actively growing hyphae of S. sclerotiorum are readily killed by C. minitans (7), and G. catenulatum (9) under laboratory conditions. Nevertheless, the fast-growing nature of S. sclerotiorum in soil will make it impossible for the hyperparasites to destroy them and prevent them from penetrating into other sunflower plants.

The field trial of 1977 indicates that the air-dried C. minitans is as effective as the freshly prepared, moist inoculum in the control of Sclerotinia wilt of sunflower. The use of such air-dried inoculum is highly desirable because it will facilitate the mechanical incorporation of the biological control agent into the soil.

Sclerotia of S. sclerotiorum may be persistent in soil because of their ability to produce new, secondary sclerotia even without the presence of sun-

flowers (Table 1). However, regeneration of secondary sclerotia from the old ones is effectively controlled by the presence of hyperparasites. Therefore, the application of hyperparasites to the soil has not only reduced wilt incidence in sunflower but also controlled the survival of sclerotia which serve as primary inoculum for the next crop season. The biological control of Sclerotinia wilt of sunflower by hyperparasites, particularly C. minitans, may have great potential as a supplement to the cultural practices used for the control of this disease.

Literature Cited

1. BISBY, G.R., 1921. Stem-rot of sunflowers in Manitoba. Sci. Agr. 2:58-61.

2. CAMPBELL, W.A., 1947. A new species of Coniothyrium parasitic on sclerotia. Mycologia 39:190-195.

3. COOK, G.E., J.R. STEADMAN, and M.G. BOOSALIS, 1975. Survival of Whetzelinia sclerotiorum and initial infection of dry edible beans in western Nebraska. Phytopathology 65:250-255.

4. GHAFFAR, A., 1972. Some observations on the parasitism of Coniothyrium minitans on the sclerotia of Sclerotinia sclerotiorum. Pak. J. Bot. 4:85-87.

5. HOES, J.A., and H.C. HUANG, 1975. Sclerotinia sclerotiorum: Viability and separation of sclerotia from soil. Phytopathology 65:1431-1432.

6. HOES, J.A. and H.C. HUANG, 1976. Importance of disease to sunflower in Manitoba in 1975. Can. Plant Dis. Surv. 56:75-76.

7. HUANG, H.C., and J.A. HOES, 1976. Penetration and infection of sclerotinia sclerotiorum by Coniothyrium minitans. Can. J. Bot. 54:406-410.

8. HUANG, H.C., 1977. Importance of Coniothyrium minitans in survival of sclerotia of Sclerotinia sclerotiorum in wilted sunflower. Can. J. Bot. 55:289-295.

9. HUANG, H.C., 1978. Gliocladium catenulatum: Hyperparasite of Sclerotinia sclerotiorum and Fusarium species. Can. J. Bot. (In press)

10. JONES, D., and D. WATSON, 1969. Parasitism and lysis by soil fungi of sclerotinia sclerotiorum (Lib.) de Bary, a photopathogenic fungus. Nature 224:287-288.

11. LAWRENCE, W.H., 1912. Plant diseases induced by Sclerotinia perlexa Nov. sp. Wash. Agr. Exp. Sta. Bull. No. 107. pp. 22.

12. MAKKONEN, R., and O. POHJAKALLIO, 1960. On the parasites attacking the sclerotia of some fingi pathogenic to higher plants and on the resistance of these sclerotia to their parasites. Acta Agr. Scand. 10:105-126.

13. MORRIS, H.E., and D.B. SWINGLE, 1921. An important new disease of cultivated sunflowers. Phytopathology 11:50.

14. SCHMIDT, H.H., 1970. Untersuchungen uber die Lebensdauer der Sklerotien von Sclerotinia sclerotiorum (Lib.) de Bary im Boden unter dem EinfluB verschiedener Pflanzenarten und nach Infektion mit Coniothyrium minitans Campb. Arch Pflanzenschutz Bd. 6, H.4, S321-334.

15. SIDDIQUI, M.Q., J.F. BROWN, and S.J. ALLEN, 1975. Growth stages of sunflower and intensity indices for white blister and rust. Plant Dis. Reptr. 59:7-11.

16. SMITH, A.M., 1972. Biological control of fungal sclerotia in soil. Soil Biol. Biochem. 4:131-134.

17. TURNER, G.J., and H.T. TRIBE, 1976. On Coniothyrium minitans and its parasitism of Sclerotinia species. Trans. Br. Mycol. Soc. 66:97-105.

18. WILLIAMS, G.H., and J.H. WESTERN, 1965. The biology of Sclerotinia trifoliorum Erikss, and other species of sclerotium-forming fungi. II. The survival of sclerotia in soil. Ann. Appl. Biol. 56:261-268.