Cyclic Occurrence of Sclerotinia Wilt of Sunflower in Western Canada

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ABSTRACT

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Field studies in Manitoba and Alberta revealed that *Sclerotinia sclerotiorum* may cause wilt of sunflower (*Helianthus annuus*) at any stage of growth but that the disease occurs mainly in two cycles: the first during seed germination and seedling establishment and the second from bud formation through seed development. Increase of wilt at the vegetative growth stage between the two disease cycles is low. In greenhouse and field experiments using artificial inoculation of sunflower with light brown, brown, black, or black, injured sclerotia, the first cycle of wilt at the establishment stage was attributed to myceliogenic germination of incompletely melanized or injured sclerotia. The second cycle of wilt may possibly be attributable to myceliogenic germination of black sclerotia, induced by exogenous nutrients.

Sclerotinia wilt caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is the most important disease of sunflower (*Helianthus annuus* L.) in Manitoba (4) and Alberta (11,12). Seed yield of plants infected by *S. sclerotiorum* and wilted at any stage from flowering to near maturity is significantly reduced (3). Seed quality as measured by test weight, oil content, and protein content was significantly reduced if plants wilted within 6 wk after flowering (3). Sclerotinia head rot due to infection by ascospores of *S. sclerotiorum* occurred on sunflower in Manitoba and Alberta but was generally less severe than the Sclerotinia wilt (4,11,12).

Previous studies indicated that Sclerotinia wilt of sunflower is caused by infection of the roots by mycelia from myceliogenic germination of sclerotia of *S. sclerotiorum* (7,8). Although some wilt has been observed during sunflower establishment (7), most wilt symptoms occurred from the budding to seed-development stages in Manitoba (1,3,5) and Montana (16). Wilt incidence is generally low during the vegetative stage of sunflower growth (5).

Huang (6) reported that myceliogenic germination of sclerotia of *S. sclerotiorum* was related to the degree of melanization of the sclerotial rind. Only the brown sclerotia (6,10) or black, injured sclerotia (6) were capable of undergoing myceliogenic germination in the absence of exogenous nutrients. Huang and Kokko (9) further reported that myceliogenic germination of sclerotia was controlled by the black pigment, not by the brown pigment. Thus, a speedy completion of the process of melanization prevented further development of the mycelia of a germinated brown sclerotium or a germinated, black, injured sclerotium (6). Since light-colored sclerotia are common in samples collected in Manitoba and Alberta (6), a study was conducted to determine the relationship of sclerotial type to development of wilt during the different growth stages of sunflower at two locations in western Canada.

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MATERIALS AND METHODS

Indoor experiments. Three experiments were conducted to compare the ability of different types of sclerotia of *S. sclerotiorum* to infect sunflower seedlings. Sunflower seeds, hybrid 894 for experiment 1 and Cargill 204 for experiment 2 and 3, were surface-disinfested in 70% ethanol for 90 sec, then wrapped in wet paper towels and kept at room temperature (22 ± 1 C) for 3–5 days. Germinated seeds were planted approximately 1–2 cm deep in Cornell peat-lite mix (2) in Rootrainer Books (Spencer Lemaire Industry Ltd., 11413 - 120 St., Edmonton, Alberta), with one seed per cell, 4 cells per book, and 8 books per basket.

Sclerotia of *S. sclerotiorum* collected from diseased bean (*Phaseolus vulgaris* L.) (experiment 1) and canola (*Brassica napus* L.) tissues (experiments 2 and 3) were buried beside the germinated seeds at 1 sclerotium per seed. Plants were kept in a growth cabinet at 22 C during the day and 18 C during the night, with 16 hr of light per day. Each experiment was set up using a completely randomized design with 5 treatments × 2 replicates (baskets) for experiment 1, and 5 treatments × 3 replicates for experiments 2 and 3. The treatments were: uninoculated control; black, uninjured sclerotia; black, injured sclerotia; brown sclerotia; light brown sclerotia. Injured sclerotia were obtained by cutting individual black sclerotia into two halves. The black sclerotia with large dark brown patches were used as brown sclerotia and the greyish black or dark brown sclerotia with large light brown patches were used as light brown sclerotia (6). Seedlings were examined for symptoms of Sclerotinia wilt (7) weekly for 4 wk. The proportion of plants that became diseased each week and the total proportion diseased after 4 wk were

determined for each basket. Analyses of variance (14) were carried out within and over experiments to study the fixed effects of sclerotium type and experiment and their interaction on disease incidence. At the end of each experiment, the books were unfolded to examine the change of sclerotial color. Seedlings killed at the preemergence stage were surface-disinfested in 70% ethanol for 60 sec and then placed on potato-dextrose agar to verify infection by *S. sclerotiorum*.

Field experiments. Field experiments were conducted in 1979 in Morden, Manitoba, and in 1985 and 1986 in Lethbridge, Alberta. In 1979, seeds of the sunflower cultivar Peredovik were sown at a rate of 15/m of row on May 25 in a field naturally infested with *S. sclerotiorum*. There were 4 plots (replicates), each consisting of 8 rows; rows were 6 m long and 0.9 m apart. Thinning was done at the end of the establishment stage on June 13 to leave approximately 41 plants in each row with a 15-cm spacing between plants. Plants were examined weekly for stage of growth and incidence of wilt from June 1 to September 4. The five stages of sunflower growth designated by Siddiqui et al (13) were used in this study.

For each plot and for the total over plots, the cumulative number of wilted plants at each sampling date was determined. For each growth stage, the rate of wilting was estimated for each plot in this way: the number of plants wilting during the stage was divided by the number of days the plants were in that stage. An adjustment was made to the number of wilted plants to take into account the thinning that occurred following the establishment stage (Fig. 1). Analyses of variance were carried out on the rate of wilting variable with variation due to replicates and stages included in the statistical model. A log transformation was applied to the data because it was evident that the magnitudes of the means were not independent of their standard deviations. Although statistical comparisons were made on this scale, results are presented using the original scale to simplify interpretation.

For the 1985 and 1986 tests in the artificially infested fields in Lethbridge, seed of the sunflower hybrid Dalgren 704 XL was sown to the depth of 5 cm on May 16, 1985, and May 1, 1986, in plots of 8 rows with 6-m row length, 0.6-m row spacing, and 0.15-m within-row plant spacing. Each test consisted of five treatments (untreated control; black sclerotia; black, injured sclerotia; brown sclerotia; and light brown sclerotia) arranged in a randomized complete block design with 4 replicates (plots) for each treatment. Sclerotia of *S. sclerotiorum* collected from a diseased canola crop in 1984 were used for the 1985 experiment, and sclerotia collected from a diseased sunflower crop in 1985 were used for the 1986 experiment. Sclerotia were sorted according to type. Two sclerotia were buried near each seed at the time of seeding. Plants were examined weekly for growth and incidence of Sclerotinia wilt throughout the entire growing season. By the end of the establishment stage (June 10, 1985, and June 2, 1986), the areas where plants failed to emerge were dug up and the cause determined by the same agar plating method used in the indoor experiments. The presence or absence of the two sclerotia buried with the seed, and any changes in their color, were determined. The number of daughter sclerotia, recognizable by their smaller size, was also recorded.

As with the 1979 experiments, the cumulative number of wilted plants was

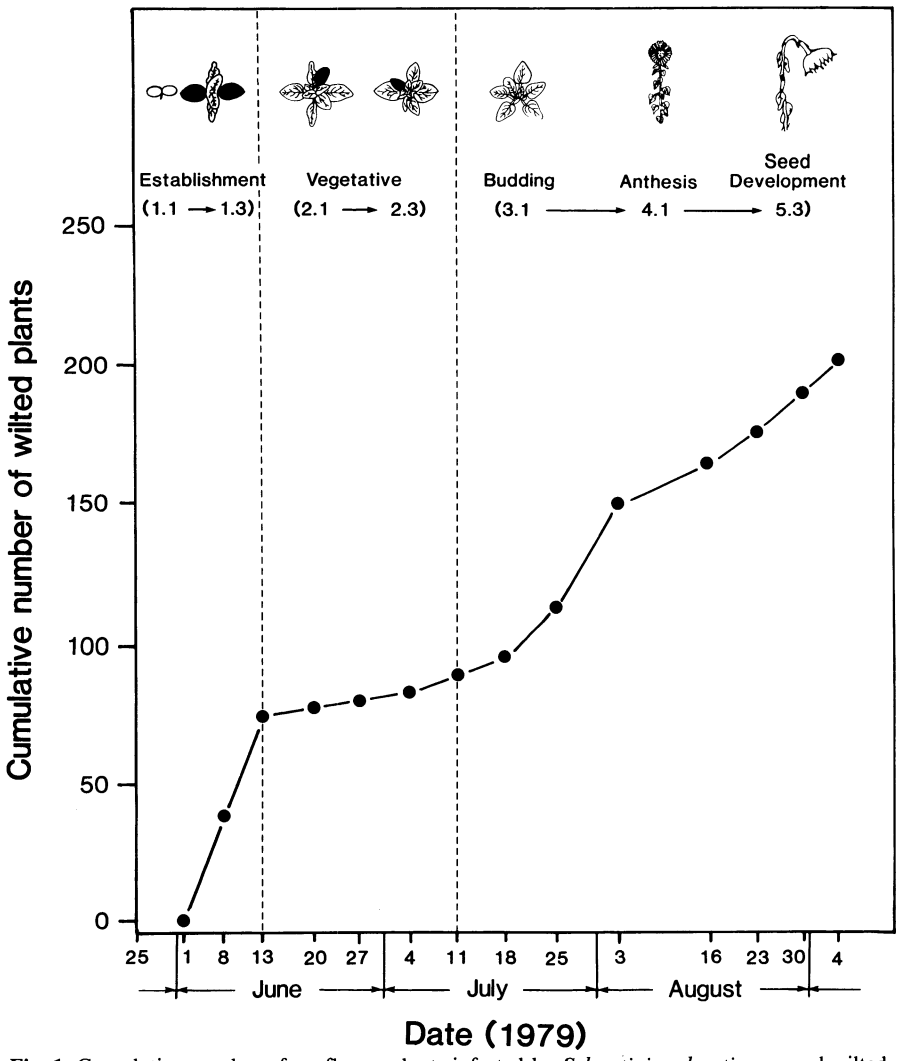

Fig. 1. Cumulative number of sunflower plants infected by *Sclerotinia sclerotiorum* and wilted at various stages of plant development in 1979 at Morden, Manitoba.

Table 1. Effect of color or injury of sclerotia of *Sclerotinia sclerotiorum* on wilt of sunflower at the establishment stage (indoor experiments)[a]

Sclerotial type	Percentage of plants with Sclerotinia wilt				
	Week 1	Week 2	Week 3	Week 4	Total
Black	6.3 b[b]	6.6 b	2.1	1.8	16.8 b
Black, injured	12.2 b	8.5 ab	1.8	0.7	23.2 b
Brown	28.7 a	10.4 a	0.7	0.7	40.5 a
Light brown	32.0 a	5.0 b	0.4	1.0	38.4 a
SE[c] (20 df)	3.7	1.2	...	...	3.9

[a] Based on the results of three indoor experiments.
[b] Means within each column followed by the same letter are not significantly different ($P > 0.05$) according to the protected LSD test; the control treatment had no plants with Sclerotinia wilt.
[c] Standard error of a mean (SE) not determined for weeks 3 and 4.

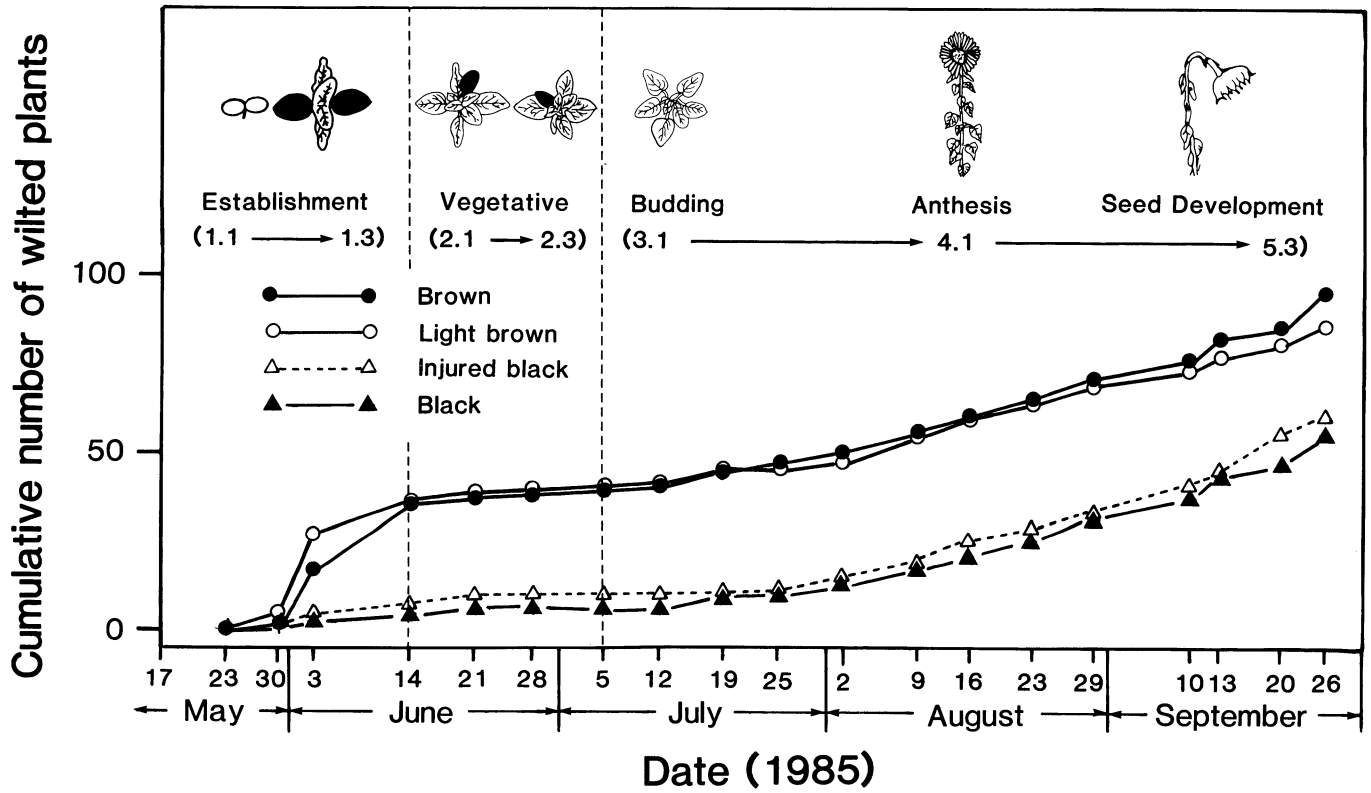

Fig. 2. Cumulative number of wilted sunflower plants caused by different types of sclerotia of *Sclerotinia sclerotiorum* in 1985 at Lethbridge, Alberta.

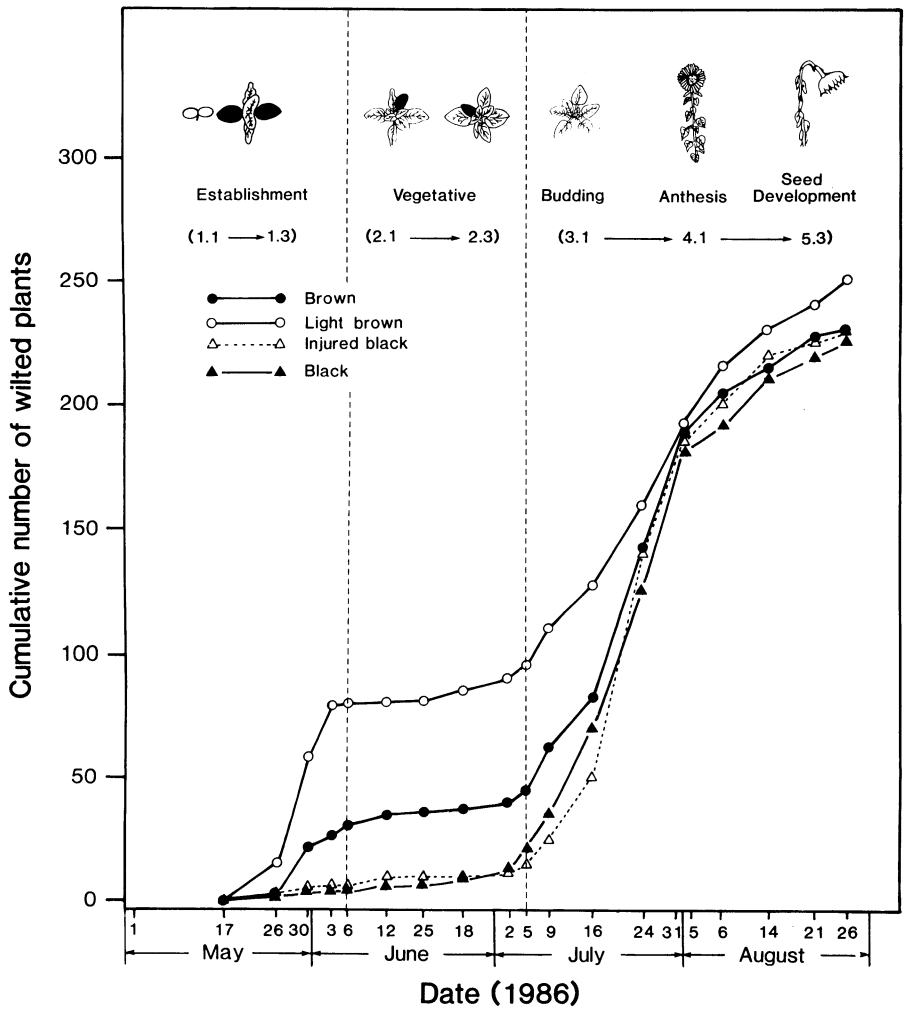

Fig. 3. Cumulative number of wilted sunflower plants caused by different types of sclerotia of *Sclerotinia sclerotiorum* in 1986 at Lethbridge, Alberta.

obtained for each sclerotial type, and analyses of variance were carried out to examine the effect of sclerotial type on the log of the rate of wilting variable for each stage of growth. Repeated-measures analyses of variance (15) were also carried out over the different growth stages with the stage and the sclerotium type × stage interaction being regarded as subplot effects.

From the data collected at the end of the establishment stage in each year, the numbers of parent sclerotia recovered and daughter sclerotia produced per site where plants were killed at preemergence and postemergence were determined for each plot, and randomized-block analyses of variance were carried out to compare the sclerotial type treatments within each year. The average number of sites examined per plot was 47 and 66 in 1985 and 1986, respectively. Analyses were also carried out over the years to determine if there was a sclerotial type × year interaction.

RESULTS

Sclerotial type and seedling wilt of sunflower. In the three indoor experiments using pregerminated sunflower seeds, infection and wilt of sunflower seedlings were caused by myceliogenic germination of the sclerotium buried near the seed. The effect of sclerotial type was consistent in all three experiments; the interaction for sclerotial type × experiment was not significant ($P > 0.05$). The rate of infection was different ($P < 0.01$) between sclerotial types: high in light

brown and brown sclerotia but low in black and black, injured sclerotia (Table 1). Within each treatment, most seedlings wilted during the first 2 wk after inoculation when the plants were at the cotyledon (1.1) and first pair of true leaves (1.2) stages. Few plants wilted in the third and fourth week after inoculation (Table 1).

Sclerotial type and effect of growth stage on wilt development. In the field experiments in Manitoba in 1979 and in Alberta in 1985 and 1986, the time required for growth of sunflower was approximately 3 wk for the establishment stage (1.1–1.3), 4 wk for the vegetative stages (2.1–2.3), and 8 wk for the budding (3.1–3.4), anthesis (4.1–4.5), and seed-development (5.1–5.3) stages (Figs. 1–3). These tests in both the naturally infested soil in Manitoba and in the artificially infested soil in Alberta indicated that the development of Sclerotinia wilt was affected by the growth stage of sunflower and type of sclerotia of *S. sclerotiorum* (Table 2). The rate of wilting for the vegetative stage was significantly lower ($P < 0.01$) than for the establishment stage and the budding and seed-development stages for each year. In 1979, 205 plants, representing 19.9% of the total population from four plots, wilted during the entire season: 6.5% wilted during the establishment stage in early to mid-June, 11.8% during budding and seed-development stages in mid-July to early September, and only 1.6% during the vegetative stage in mid-June to mid-July (Fig. 1). The rate of wilting was greatest in the establishment stage (Table 2). In 1985 and 1986, the effect of growth stage on wilt development was similar to that in 1979, although the level of disease was different between the 2 years (Figs. 2 and 3, Table 2).

There was a significant interaction ($P < 0.05$) between sclerotial type and growth stage in 1985 and 1986. The rate of Sclerotinia wilt differed significantly between treatments with different types of sclerotia (Table 2). It was high in the treatments with light brown and brown sclerotia, but low for black and black, injured sclerotia at the establishment stage (Figs. 2 and 3, Table 2). The differences were more pronounced in 1986 than in 1985. The effect of sclerotial type was not pronounced in the vegetative and in the budding, anthesis, and seed-development stages, particularly in 1985 ($P > 0.05$). Results of the 1985 and 1986 tests averaged over treatments showed, as in 1979, that the rate of Sclerotinia wilt was lowest in the vegetative stage; the difference among growth stages was greatest in the treatments with light-colored sclerotia (Figs. 2 and 3, Table 2).

Melanization of parental sclerotia in soil. The number and color of the parental sclerotia and the formation of daughter sclerotia at sites where seedlings were killed or failed to emerge were determined at the end of the establishment stage of sunflower growth in 1985 and 1986. The number of sclerotia buried at seeding time was reduced from 2 per site to an average of 1.2 per site after burial in soil for 3–4 wk (Table 3). There were no differences ($P > 0.05$) in number of parental sclerotia recovered among treatments. Melanization of the sclerotial rind occurred during the 3–4 wk of burial in soil and all the black injured, brown, and light brown sclerotia became black.

Daughter sclerotia produced in the soil during the establishment stage of sunflower were black. They were found in all treatments, but the number produced from the light brown and brown sclerotia was significantly higher ($P < 0.01$) than from black and black, injured sclerotia in 1986 (Table 3). In 1985, only the light brown sclerotia produced significantly more ($P < 0.05$) daughter sclerotia than the other sclerotial types.

DISCUSSION

This study indicates that Sclerotinia wilt of sunflower occurs in two discrete cycles within a single growing season in southern parts of Manitoba and Alberta. The first cycle occurs during the establishment stage in May and the second during the budding and seed-development stages in July-August. There is a low increase of wilt during the vegetative stage in June. Previous reports indicated that the first appearance of Sclerotinia wilt of sunflower is during mid-July to early August in Manitoba (1) and during early July in Montana (16). These are probably the second wave of the disease because sunflower growth has reached the budding stage in July in southern parts of the Canadian Prairies by that time. Although Sclerotinia wilt may occur on young seedlings in May (7), it is rarely reported in commercial sunflower fields because of the sporadic nature of the disease at the seedling stage and the rapid disappearance of infected seedlings in the field.

Previous research has demonstrated that Sclerotinia wilt of sunflower was caused by myceliogenic germination of sclerotia of *S. sclerotiorum* (7) and that this type of germination was triggered by incomplete melanization and/or injury of the sclerotial rind (6). In the 1985 and 1986 field studies, incidence of seedling wilt in sunflowers was high after inoculation with light-colored sclerotia, but was low after inoculation with black or black, injured sclerotia. This suggests that sclerotia with incompletely

Table 2. Means and standard errors for rate of wilting[a] for each sclerotial type and growth stage combination in 1979, 1985, and 1986 field experiments

Year[b]	Sclerotial type	Growth stage of sunflower		
		Establishment	Vegetative	Budding/anthesis seed development
1979	...	1.56 ± 0.44	0.07 ± 0.02	0.55 ± 0.09
1985	Black	0.06 ± 0.03 b[c]	0.01 ± 0.01 a	0.14 ± 0.02 a
	Black, injured	0.15 ± 0.08 ab	0.01 ± 0.01 a	0.15 ± 0.04 a
	Brown	0.44 ± 0.19 ab	0.04 ± 0.03 a	0.18 ± 0.04 a
	Light brown	0.75 ± 0.13 a	0.01 ± 0.01 a	0.15 ± 0.04 a
1986	Black	0.46 ± 0.04 d	0.16 ± 0.01 a	0.91 ± 0.03 a
	Black, injured	1.24 ± 0.15 c	0.07 ± 0.05 b	0.95 ± 0.10 a
	Brown	2.34 ± 0.26 b	0.16 ± 0.02 a	0.81 ± 0.10 ab
	Light brown	5.32 ± 0.31 a	0.18 ± 0.05 a	0.67 ± 0.06 b

[a] Number of plants wilting per day.
[b] Sclerotial inoculum = natural infestation in the 1979 test and artificial infestation in the 1985 and 1986 tests.
[c] Letters that are the same within a year and growth stage indicate color treatments that are not significantly different ($P > 0.05$) according to the protected LSD test on log-transformed data.

Table 3. Means and standard errors for survival of parental sclerotia and production of daughter sclerotia of *Sclerotinia sclerotiorum* during the establishment stage of sunflower growth

Sclerotial type	Number of parent sclerotia[a] recovered per site		Number of daughter sclerotia[b] produced per site	
	1985	1986	1985	1986
Black	1.24 ± 0.03 a[c]	1.00 ± 0.20 a	0.03 ± 0.03 b	0.03 ± 0.02 c
Black, injured	0.96 ± 0.17 a	1.21 ± 0.14 a	0.03 ± 0.02 b	0.12 ± 0.07 bc
Brown	0.91 ± 0.33 a	1.27 ± 0.11 a	0.04 ± 0.02 b	0.23 ± 0.04 b
Light brown	1.21 ± 0.18 a	1.53 ± 0.05 a	0.24 ± 0.11 a	0.69 ± 0.09 a

[a] Two sclerotia were buried near each seed at the time of seeding.
[b] Daughter sclerotia are round in shape and are much smaller than the parent.
[c] Means within each column followed by the same letters are not significantly different ($P > 0.05$) according to the protected LSD test; the control treatment had no parent or daughter sclerotia recovered.

melanized rinds that are capable of undergoing myceliogenic germination at 4 C under moist conditions (H. C. Huang, *unpublished*) are responsible for the first cycle of wilt in commercial sunflower fields. Huang (6) reported that incompletely melanized sclerotia of *S. sclerotiorum* were common in samples collected from various diseased crops in late fall in southern parts of Manitoba and Alberta. These sclerotia in the field are likely to remain inactive during the winter months of below-freezing temperatures and become active in May when the soil temperature is above freezing and the soil is moist. When mycelia from the germinated sclerotia invade roots or the hypocotyl of sunflower, seedling wilt can occur.

Huang (6) observed that a remelanization process occurs frequently on the surface of black, injured sclerotia, and there is no further hyphal development after completion of the remelanization. This rapid healing process may be responsible for the low number of daughter sclerotia as well as the low incidence of wilt in sunflower seedlings inoculated with black, injured sclerotia in this study. The high incidence of seedling wilt in the treatments with brown and light brown sclerotia suggests that the remelanization process in these sclerotia does not occur as rapidly, thus the germinated hyphae from the light-colored sclerotia may have a greater chance of developing into a colony for infection of sunflower seedlings.

The infection at the establishment stage is of a sporadic nature because the 15-cm plant spacing effectively prevents the secondary spread of the disease by root contact between young seedlings. Therefore, in Western Canada, the incidence of Sclerotinia wilt at the establishment stage of sunflower growth will be high only when the number of light-colored or injured sclerotia in the soil is high and when the germination of these sclerotia coincides with the time of sunflower seeding.

The reason for the outbreak of the second cycle of the disease in the budding and seed-development stages remains unknown. The fact that the wilt incidence is low at the vegetative stage but high at the budding and seed-development stages suggests that germination of black sclerotia may be triggered by exogenous nutrients, such as root exudates of sunflower plants. Further studies are warranted, particularly in quantitative and qualitative differences of root exudates between sunflower plants at different growth stages and the effect of root exudates on germination of black sclerotia of *S. sclerotiorum*.

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LITERATURE CITED

1. Bisby, G. R. 1921. Stem-rot of sunflowers in Manitoba. Sci. Agric. (Ottawa) 2:58-61.
2. Boodley, J. W., and Sheldrake, R., Jr. 1982. Cornell peat-lite mixes for commercial plant growing. Cornell Univ. Info. Bull. 43. 8 pp. Revised.
3. Dorrell, D. G., and Huang, H. C. 1978. Influence of Sclerotinia wilt on seed yield and quality of sunflower wilted at different stages of development. Crop Sci. 18:974-976.
4. Hoes, J. A., and Huang, H. C. 1976. Importance of disease to sunflower in Manitoba in 1975. Can. Plant Dis. Surv. 56:75-76.
5. Huang, H. C. 1980. Control of Sclerotinia wilt of sunflower by hyperparasites. Can. J. Plant Pathol. 2:26-32.
6. Huang, H. C. 1985. Factors affecting myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Phytopathology 75:433-437.
7. Huang, H. C., and Dueck, J. 1980. Wilt of sunflower from infection by mycelial-germinating sclerotia of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 2:47-52.
8. Huang, H. C., and Hoes, J. A. 1980. Importance of plant spacing and sclerotial position to development of Sclerotinia wilt of sunflower. Plant Dis. 64:81-84.
9. Huang, H. C., and Kokko, E. G. 1989. Effect of temperature on melanization and myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 67:1387-1394.
10. Huang, H. C., and Sun, S. K. 1989. Comparative studies on myceliogenic germination of tan sclerotia of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Can. J. Bot. 67:1395-1401.
11. McLaren, D. L., Rimmer, S. R., and Huang, H. C. 1988. Survey of Sclerotinia wilt and head rot of sunflower in southern Alberta. 1986. Can. Plant Dis. Surv. 68:125.
12. McLaren, D. L., Rimmer, S. R., and Huang, H. C. 1988. Survey of Sclerotinia wilt and head rot of sunflower in southern Alberta, 1987. Can. Plant Dis. Surv. 68:126-127.
13. Siddiqui, M. Q., Brown, J. F., and Allen, S. J. 1975. Growth stages of sunflower and intensity indices for white blister and rust. Plant Dis. Rep. 59:7-11.
14. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames. 507 pp.
15. Winer, B. J. 1962. Statistical Principles in Experimental Design. McGraw-Hill, New York. 672 pp.
16. Young, P. A., and Morris, H. E. 1927. Sclerotinia wilt of sunflowers. Agric. Exp. Stn. Bull. 208. University of Montana, Bozeman. 32 pp.