Factors Affecting Myceliogenic Germination of Sclerotia of *Sclerotinia sclerotiorum*

H. C. Huang

Plant pathologist, Agriculture Canada Research Station, Lethbridge, Alberta, Canada T1J 4B1.
The author thanks G. C. Kozub for assistance in statistical analyses.
Accepted for publication 15 November 1984.

ABSTRACT

Huang, H. C. 1985. Factors affecting myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Phytopathology 75:433-437.

Sclerotia of *Sclerotinia sclerotiorum* collected from different fields varied in ability to undergo myceliogenic germination without addition of exogenous nutrients. Of three samples tested from sunflower, mungbean and safflower, the frequency of germinated sclerotia was 8, 79, and 98%, respectively. These differences were not due to the existence of distinct strains because germination of daughter sclerotia produced on potato-dextrose agar was similar. Light-colored sclerotia, resulting from incomplete melanization of the rind, were found in 14 of the 15 samples collected from five hosts in western Canada and comprised from 0.5 to 85% of the sclerotia in each sample. Germination occurred readily in intact light-colored sclerotia incubated on autoclaved moist sand, but it occurred in black sclerotia only when the rind was injured. The incompletely melanized, light-colored sclerotia were more frequently contaminated with microorganisms than were the completely blackened ones. It is concluded that myceliogenic germination of sclerotia is affected by the extent of melanization of the rind and that the black, melanized rind prevents germination in the absence of exogenous nutrients and also protects sclerotia from invasion by microorganisms.

Additional key words: *Sclerotinia minor*.

Sclerotinia sclerotiorum (Lib.) de Bary and *S. minor* Jagger attack many crops and may cause severe losses (24). The former is particularly important on oilseed and pulse crops (27) and the latter on lettuce (22) and peanut (6). Both species can occur on the same host such as on lettuce in the United States (2) and New Zealand (9) and on sunflower in Australia (23,30). On the Canadian prairies, however, *S. sclerotiorum* is the species that attacks sunflower (*Helianthus annuus* L.) (16), Jerusalem artichoke (*H. tuberosus* L.) (19), rapeseed (*Brassica napus* L. and *B. campestris* L.) (7), safflower (*Carthamus tinctorius* L.) (25), mungbean (*Phaseolus mungo* L.), and bean (*P. vulgaris* L.). *S. minor* has not been reported on these crops in the field.

Sclerotia of *S. sclerotiorum* and *S. minor* germinate myceliogenically to produce vegetative hyphae or carpogenically to produce airborne ascospores (28). Adams and Tate (4) further described the myceliogenic germination of sclerotia as the "hyphal" type in *S. sclerotiorum*, in which individual hyphae emerged through the rind, and as the "eruptive" type in *S. minor*, in which a mycelial plug developed in the medullary region and emerged through the ruptured rind. Abawi and Grogan (1,2) reported that myceliogenic germination of sclerotia of *S. sclerotiorum* on beans occurred only when exogenous nutrients were present. They proposed that, under field conditions, *S. sclerotiorum* is only epidemiologically important through carpogenic germination. Conversely, *S. minor* is significant primarily through myceliogenic germination. It was suggested that the different modes of infection exhibited by the two species probably result from continued selection for adaptation to their ecological niches (2).

Sclerotinia wilt of sunflower is caused by infection following hyphal germination of sclerotia of *S. sclerotiorum* (17). This disease is more important than head rot caused by ascospore infections following carpogenic germination of sclerotia in Manitoba where the crop is grown under dryland conditions (10). Infection of Jerusalem artichoke by *S. sclerotiorum* was found primarily on roots and basal stems following myceliogenic germination of sclerotia in an unirrigated field (19), and on aboveground stems and leaves from carpogenic germination of sclerotia in an irrigated field at Morden, Manitoba. Therefore, both myceliogenic and carpogenic germination of sclerotia occur on the Canadian prairies and the importance of each type depends upon the crops and growth conditions.

The formation of secondary sclerotia by *S. sclerotiorum* (5,11,29) and *S. minor* (3) has provided further evidence for the occurrence of myceliogenic germination in soil even when the pathogen is not associated with hosts. Nevertheless, information on mechanisms that control myceliogenic germination and the production of secondary sclerotia is meager (3,21). Adams (3) investigated the effect of soil amendments, temperature, and moisture on the production of secondary sclerotia of *S. minor* in soil in the absence of host plants but obtained no conclusive results.

The objective of this report is to describe some of the factors that influence myceliogenic germination of sclerotia of *S. sclerotiorum* in the absence of exogenous nutrients. This information is important to understanding the epidemiology of sclerotinia wilt of sunflower and perhaps other crops grown under dryland conditions.

MATERIALS AND METHODS

Sclerotia of *S. sclerotiorum* were collected from diseased sunflowers, Jerusalem artichokes, mungbeans, safflowers, and beans grown in fields in Manitoba and Alberta (Table 1). They were separated from plant debris by hand and stored in paper bags at room temperature. To test myceliogenic germination of sclerotia, samples from these sunflower (Sun-2), mungbean (Mung-1) and safflower (Saf-1) plants were used as parent material. Daughter sclerotia from each sample were produced from single-sclerotium isolates grown on potato-dextrose agar (PDA) at room temperature for 3 wk. They were air-dried and stored for 3 days by the technique described by Huang (12). Three parental and three daughter sclerotial samples were placed on autoclaved, moist sand in petri dishes (14-cm diameter) with 80 sclerotia for each sample at 10 sclerotia per dish. The sclerotia were incubated at room temperature and examined daily for myceliogenic germination during the 1-wk incubation period.

To test relationships of sclerotial color to myceliogenic germination, six samples of sclerotia collected in Manitoba and Alberta, three from sunflower (Sun-2, 3, and 5) and one each from mungbean (Mung-1), dry bean (Bn-1), and safflower (Saf-1) (Table

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

1), were used. Sclerotia of each sample were separated into three color groups: black, black with brown patches, and light brown. Eighty sclerotia of each color group were washed in running tap water for 1 hr except for one group of black sclerotia from bean (Bn-1) which was unwashed. The sclerotia were not artificially injured except for one group of black sclerotia from sunflower (Sun-5) in which each sclerotium was cut at both ends. The sclerotia were surface sterilized in 70% ethanol for 90 sec, placed on autoclaved, moist sand in petri dishes at 20 sclerotia per dish for each color group, and examined for myceliogenic germination and microbial contamination after incubation for 1 wk at room temperature.

Twenty black sclerotia (>6 mm) from sunflower were used to study the process of re-melanization of the injured rind. Each sclerotium was surface-sterilized in 70% ethanol for 90 sec; dissected; placed on autoclaved, moist sand in petri dishes; and incubated at room temperature. Each day for 2 wk, sclerotia were examined for myceliogenic germination and re-melanization of tissue at the injured surface.

RESULTS

Sclerotia collected from naturally infected sunflower (Sun-2), mungbean (Mung-1), and safflower (Saf-1) germinated myceliogenically when incubated on moist sand without added nutrients but germination varied among samples (Fig. 1). The frequency of germinated sclerotia in Sun-2, Mung-1, and Saf-1 was 8, 79, and 98%, respectively. However, daughter sclerotia of respective samples from PDA cultures remained dormant and the frequency of germinated sclerotia was below 4% in all samples.

Whereas the daughter sclerotia from cultures on PDA were black, many of those produced on naturally infected plants varied in color from black with brown patches to light brown (Fig. 2A) or white. The white sclerotia were apparently immature as they were soft and spongy. The number of sclerotia of each color varied among the 15 samples collected from five hosts (Table 1). The frequency of sclerotia with brown or light brown patches was high (>50%) in three samples (Mung-1 and Saf-1,2), and low (<10%) in seven samples (Sun-1, 2, 4, 5, 6, Ja-1, and Bn-1). Shape of the sclerotia was often associated with the type of host; cone-shaped from safflower heads but cylindrical from mungbean stems (Fig. 1). Color changes were observed most frequently at the tip of sclerotia from safflower and at the ends of sclerotia from mungbean.

Myceliogenic germination was related to sclerotial color (Figs. 2A and B). Black sclerotia germinated the least readily (Table 2). One sample of black sclerotia from bean was tested without washing but the percentage of germinated sclerotia was still low and not significantly different from the washed black sclerotia (Table 2).

Myceliogenic germination was affected by injury. In one sample from sunflower (Sun-5), 96% of black sclerotia with cuts germinated but only 10% of the uninjured sclerotia germinated (Table 2). Although the field-collected black sclerotia used in the germination tests showed no apparent injury when examined

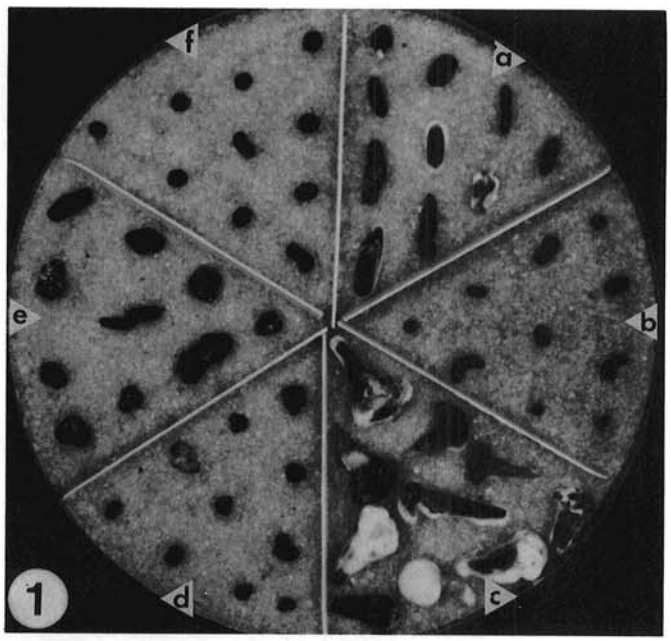


Fig. 1. Sclerotia of *Sclerotinia sclerotiorum* from mungbean (a and b), safflower (c and d), and sunflower (e and f) incubated on moist sand at room temperature for 4 days. Note the difference in myceliogenic germination among samples from natural hosts (a, c, and e) but not from their daughter sclerotia produced on potato-dextrose agar (b, d, and f).

TABLE 1. Frequency of discolored sclerotia of *Sclerotinia sclerotiorum* from various hosts in western Canada

Source of sclerotia			Sclerotia (%) in each color group		
Isolate	Host tissue	Year and location[z]	Black	Black with brown patch	Light brown
Sun-1	Sunflower stem	1977, Morden, Manitoba	99.5	0.5	0
Sun-2	Sunflower head	1978, Morden, Manitoba	95.1	0.7	4.1
Sun-3	Sunflower head	1979, Altona, Manitoba	67.2	21.6	11.2
Sun-4	Sunflower head	1980, Morden, Manitoba	93.0	4.0	3.0
Sun-5	Sunflower head and stem	1981, Bow Island, Alberta	98.8	0.3	0.8
Sun-6	Sunflower stem	1981, Morden, Manitoba	100.0	0	0
Sun-7	Sunflower head	1983, Bow Island, Alberta	88.4	7.1	4.5
Mung-1	Mungbean stem and pod	1979, Portage La Prairie, Manitoba	43.0	21.0	36.0
Ja-1	Jerusalem artichoke stem	1981, Morden, Manitoba	95.8	1.2	3.0
Saf-1	Safflower head	1981, Bow Island, Alberta	15.0	25.0	60.0
Saf-2	Safflower head	1982, Lethbridge, Alberta	46.2	42.2	11.6
Bn-1	Dry bean stem and pod	1981, Bow Island, Alberta	91.7	4.5	3.8
Bn-2	Dry bean stem and pod	1982, Bow Island, Alberta	78.6	14.3	7.1
Bn-3	Dry bean stem and pod	1983, Bow Island, Alberta	85.5	11.3	3.2
Bn-4	Dry bean stem and pod	1983, Grassy Lake, Alberta	87.9	7.8	4.3

[z]Sclerotia were collected during crop harvest, separated from crop tissues by hand and stored in paper bags at room temperature until use.

macroscopically, many had numerous fine cracks which were visible on the rind when examined microscopically (Fig. 3).

Myceliogenic germination occurred within 1–2 days on moist sand by development of sparse hyphae on the rind of each sclerotium (Fig. 3). The hyphae continued to grow and finally formed colonies with dense, white mycelia (Fig. 2B). Secondary sclerotia, smaller than parental ones, were frequently observed on the colonies. Colonies that developed from brown or light-brown sclerotia were generally larger than those that developed from black sclerotia because of more vigorous mycelial growth (Fig. 2B). The hyphae that developed from many black sclerotia were sparse (Fig. 3) and failed to develop into dense colonies during the 1-wk period of incubation.

Under moist conditions, the process of melanization resumed on both the light brown, uninjured sclerotia (Fig. 2A and B) and on the black, injured sclerotia (Fig. 5). All of 20 severely injured, black sclerotia germinated myceliogenically; six produced secondary sclerotia (Fig. 4), 11 showed signs of re-melanization of the injured tissue (Fig. 5), and the remaining three had dense mycelial mats on the cut surface. No further hyphal development was observed after melanization of the injured surfaces was completed.

The percentage of sclerotia contaminated with microorganisms varied with samples collected from different fields (Table 3). Several fungi including *Rhizopus* sp., *Fusarium* spp., and *Alternaria* sp. frequently grew from sclerotia collected from sunflower plants. Within each sample, the frequency of fungal contaminants was significantly higher for the light-brown sclerotia than for the black ones (Table 3 and Fig. 2B).

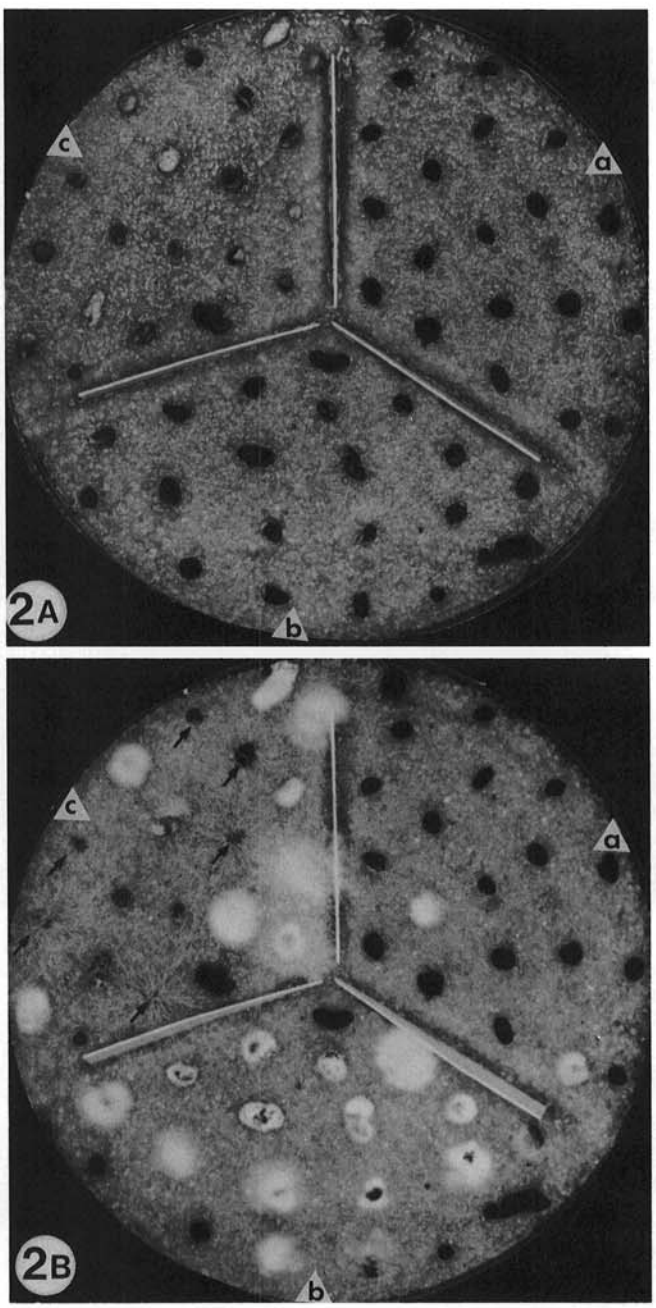


Fig. 2. A sample from sunflower containing black (a), black with brown patches (b), and light-brown (c) sclerotia. The sclerotia were incubated on moist sand at room temperature for **A,** 2 hr and **B,** 4 days. Compare the difference in number of germinated sclerotia between black (a) and light-colored sclerotia (b and c). Note that some light-brown sclerotia (c in Fig. 2A) became darker after 4 days of incubation (c in Fig. 2B). Six light-brown sclerotia (arrows) did not germinate and were colonized by a *Rhizopus* sp.

DISCUSSION

Results of this study suggest that the dark pigments of the rind are key components for controlling myceliogenic germination of

TABLE 2. Myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum* from various hosts as related to pigmentation, injury, and washing

Sclerotia		Sclerotial color, condition, and germination (%)[x]		
Isolate	Host	Black, washed	Black with brown patch, washed	Light-brown, washed
Sun-2	Sunflower	9.7 b[y]	66.5 a	47.5 a
Sun-3	Sunflower	4.0 b	62.6 a	50.0 a
Sun-5	Sunflower	9.7 b (96.1a)[z]	90.4 a	81.1 a
Mung-1	Mungbean	55.2 b	96.1 a	82.1 ab
Saf-1	Safflower	5.0 c	87.5 a	55.0 b
Bn-1	Dry bean	5.3 b (9.7 b)[z]	94.4 a	93.6 a

[x]Data are means based on 80 sclerotia in each sample.
[y]Means within each isolate followed by the same letter are not significantly different ($P \leq 0.05$) according to Tukey's multiple comparisons test.
[z]Value in parentheses for isolate Sun-5 represents germination of black, washed sclereotia that were each injured by cutting at both ends. Value in parentheses for isolate Bn-1 represents germination of unwashed black sclerotia.

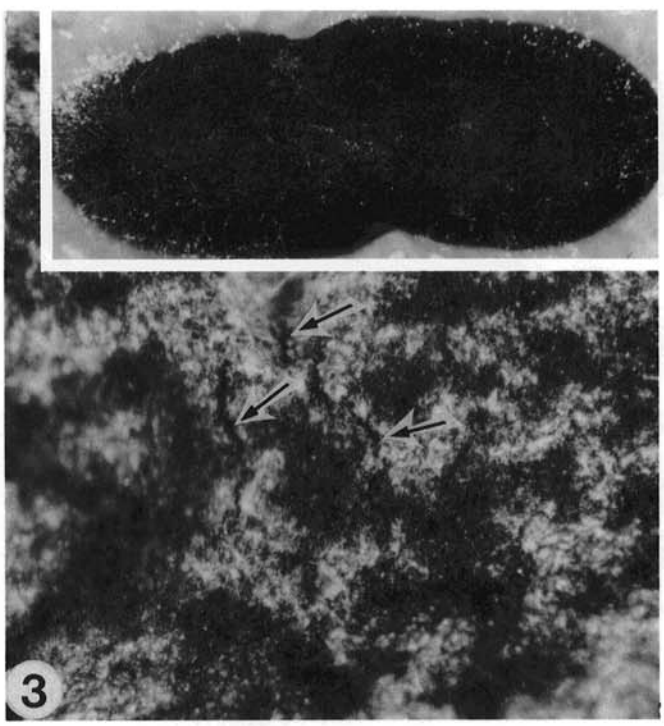
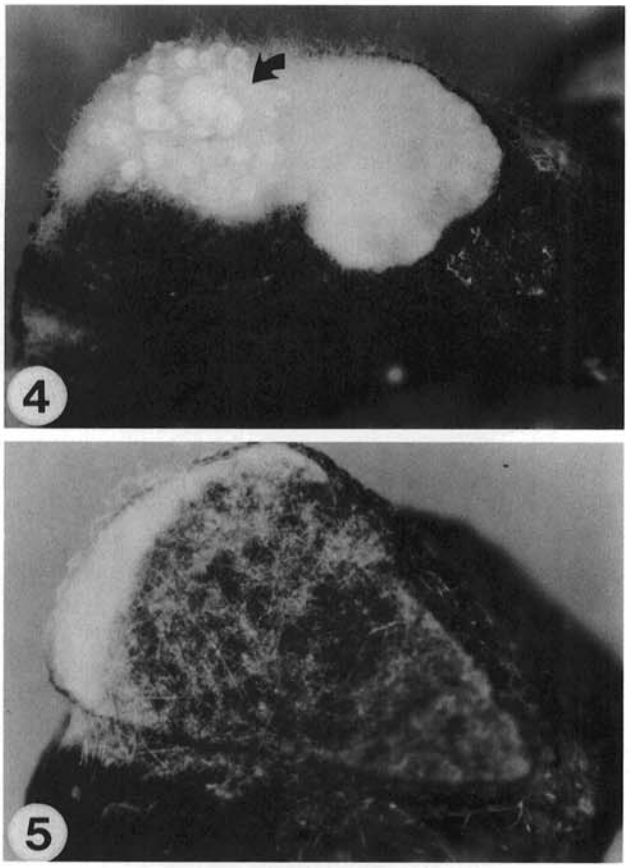


Fig. 3. A black sclerotium from mungbean germinated on moist sand and produced sparse hyphae (Fig. 3, inset). Note numerous fine cracks (arrows) on the magnified view of the sclerotial surface (×11 and ×56, respectively).



Figs. 4–5. Germination of two injured sclerotia incubated on moist sand for 4 days. **4,** Myceliogenic germination resulting in the formation of a secondary sclerotial primordium (arrow) (×9) and **5,** darkening of the injured surface (×15).

sclerotia and protecting them from invasion by microorganisms. Jones (20) reported that the dark pigments in the rind cells of *S. sclerotiorum* are melanins. Sclerotia with an incompletely melanized rind are either black with brown patches or light brown. In this study, the incompletely melanized sclerotia differed from the black ones by lack of dormancy and by increased contamination by microorganisms. The lack of dormancy and susceptibility to potential parasites of the light-colored sclerotia collected from naturally infected plants correspond to observations of tan-colored sclerotia produced by aberrant strains of *S. sclerotiorum* from sunflower (12,14) and lettuce (8). Therefore, the melanized rind appears to serve both as a dormancy-controlling site that prevents myceliogenic germination and as a protective layer. The ability of the sclerotium to function as a dormant propagule is altered if the sclerotium fails to become completely melanized during maturation, or if it is completely melanized but the rind layer is naturally or artificially injured.

Although sclerotia collected from fields with different crops differed in ability to germinate myceliogenically (Fig. 1), this does not necessarily imply the existence of distinct strains of *S. sclerotiorum* as the difference does not appear in daughter sclerotia of the respective isolates. The fact that the process of melanization resumed in light-colored sclerotia under moist conditions suggests that the light color may result from rapid desiccation of the infected host tissue, which prevents the sclerotia from completing the darkening process during maturation. Therefore, the environmental conditions, moisture in particular, may affect the frequency of light-colored sclerotia produced from a crop in the field.

Myceliogenic germination can also result in the formation of secondary sclerotia. Production of secondary sclerotia in soil was reported in *S. sclerotiorum* (5,11,13,29) and *S. minor* (3) even when host plants were absent. However, the factors favorable for the production of secondary sclerotia remain unclear (3). Results of the

TABLE 3. Fungal contaminants on sclerotia of *Sclerotinia sclerotiorum* from various hosts

Sclerotia		Color, condition, and contamination of sclerotia (%)[x]		
Isolate	Host	Black, washed	Black with brown patch, washed	Light-brown, washed
Sun-2	Sunflower	5.7 b[y]	35.7 a	52.6 a
Sun-3	Sunflower	14.7 b	27.2 b	52.7 a
Sun-5	Sunflower	3.9 b (3.9b)[z]	5.7 ab	19.0 a
Mung-1	Mungbean	0.0	9.7 b	34.5 a
Saf-1	Safflower	9.6 c	30.5 b	50.0 a
Bn-1	Dry bean	0.0 (0)[z]	0.0	1.3

[x] Based on the same sclerotia used for germination tests in Table 2. Common fungal contaminants are *Rhizopus* sp., *Fusarium* sp., and *Alternaria* sp.
[y] Means within each isolate followed by the same letter are not significantly different ($P \leq 0.05$) according to Tukey's multiple comparisons test.
[z] Value in parentheses for isolate Sun-5 represents the percentage fungal contamination on black, washed sclerotia that were each injured by cutting at both ends. Value in parentheses for isolate Bn-1 represents percent fungal contamination relative to that of unwashed black sclerotia.

present study indicate that the melanized rind layer is important in governing the myceliogenic germination and, subsequently, the formation of secondary sclerotia. However, myceliogenic germination may not necessarily lead to the production of secondary sclerotia because the wounded rind may become re-melanized under moist conditions. Once the wound is healed, the sclerotium presumably would become dormant and behave like those freshly harvested from pure cultures. The sclerotium with an intact, black rind is likely to remain dormant in the soil until exogenous nutrients, such as root exudates, are present or until the rind is injured by wounding (15,26) or by microbial degradation.

Adams and Tate (4) reported myceliogenic germination of sclerotia in natural soil by *S. minor* but not by *S. sclerotiorum*. Moreover, they observed that freshly produced sclerotia of *S. minor* were dormant and that the dormancy period varied with the particular isolate of the pathogen. This dormancy in *S. minor* is comparable to that of *S. sclerotiorum* reported in the present study. The question of whether or not the melanized rind also plays a significant role in governing the dormancy of sclerotia of *S. minor* warrants further investigation.

Abawi and Grogan (2) suggested that sclerotia of *S. sclerotiorum* function essentially to produce airborne ascospores for infection on beans, and that this mode of infection probably results from continued selection for adaptation of this species to its ecological niche. On the Canadian prairies, however, sclerotia of *S. sclerotiorum* may germinate myceliogenically or carpogenically. Which type of germination is of epidemiological significance depends on the crops and their growth conditions. In Manitoba, infection of below-ground tissues originating from myceliogenic germination of sclerotia of *S. sclerotiorum* is important on dryland sunflower (10,18), wild sunflower (*H. annuus*), marigold, *Physostegia* sp., and burdock (*Arcticum* sp.), whereas infection of aboveground tissue resulting from carpogenic germination of sclerotia is important on processing beans and mungbeans.

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. Abawi, G. S., and Grogan, R. G. 1979. Epidemiology of diseases caused by *Sclerotinia* species. Phytopathology 69:899-904.
3. Adams, P. B. 1975. Factors affecting survival of *Sclerotinia sclerotiorum* in soil. Plant Dis. Rep. 59:599-603.
4. Adams, P. B., and Tate, C. J. 1976. Mycelial germination of sclerotia of *Sclerotinia sclerotiorum* in soil. Plant Dis. Rep. 60:515-518.
5. Cook, G. E., Steadman, J. R., and Boosalis, M. G. 1975. Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans in western Nebraska. Phytopathology 65:250-255.
6. Dougherty, D. E., Sarojak, D. J., and Löcher, F. 1983. Fungicides for

reducing losses caused by Sclerotinia blight on peanut. Plant Dis. 67:312-314.
7. Dueck, J. 1977. Sclerotinia in rapeseed. Can. Agric. 22:7-9.
8. Garrabrandt, L. E., Johnston, S. A., and Peterson, J. L. 1983. Tan sclerotia of *Sclerotinia sclerotiorum* from lettuce. Mycologia 75:451-456.
9. Hawthorne, B. T. 1974. *Sclerotinia minor* on lettuce: Effect of plant growth on susceptibility to infection. N.Z. J. Agric. Res. 17:387-392.
10. Hoes, J. A., and Huang, H. C. 1976. Importance of diseases to sunflower in Manitoba in 1975. Can. Plant Dis. Surv. 56:75-76.
11. Huang, H. C. 1980. Control of Sclerotinia wilt of sunflower by hyperparasites. Can. J. Plant Pathol. 2:26-32.
12. Huang, H. C. 1981. Tan sclerotia of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 3:136-138.
13. Huang, H. C. 1982. Morphologically abnormal sclerotia of *Sclerotinia sclerotiorum*. Can. J. Microbiol. 28:87-91.
14. Huang, H. C. 1983. Pathogenicity and survival of the tan-sclerotial strain of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 5:245-247.
15. Huang, H. C. 1983. Histology, amino acid leakage, and chemical composition of normal and abnormal sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 61:1443-1447.
16. Huang, H. C. 1983. Sclerotinia wilt and head rot of sunflower. Canadex 632.145.
17. 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.
18. 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.
19. Huang, H. C., and Stauffer, M. D. 1979. Sclerotinia wilt on Jerusalem artichoke. Canadex 164.630.
20. Jones, D. 1970. Ultrastructure and composition of the cell walls of *Sclerotinia sclerotiorum*. Trans. Br. Mycol. Soc. 54:351-360.
21. LeTourneau, D. 1979. Morphology, cytology, and physiology of *Sclerotinia* species in culture. Phytopathology 69:887-890.
22. Marcum, D. B., Grogan, R. G., and Greathead, A. S. 1977. Fungicide control of lettuce drop caused by *Sclerotinia sclerotiorum* 'minor.' Plant Dis. Rep. 61:555-559.
23. Merriman, P. R., and Heathcote, R. 1978. Screening of sunflower seed for *Sclerotinia* spp. Aust. Plant Pathol. 7:43.
24. Purdy, L. H. 1979. *Sclerotinia sclerotiorum*: History, diseases and symptomatology, host range, geographic distribution, and impact. Phytopathology 69:875-880.
25. Sackston, W. E. 1960. *Botrytis cinerea* and *Sclerotinia sclerotiorum* in seeds of safflower and sunflower. Plant Dis. Rep. 44:664-668.
26. Smith, A. M. 1972. Biological control of fungal sclerotia in soil. Soil Biol. Biochem. 4:131-134.
27. Steadman, J. R. 1983. White mold—A serious yield-limiting disease of bean. Plant Dis. 67:346-350.
28. 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. 46:101-164.
29. Williams, G. H., and Western, J. H. 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.
30. Wong, A. L., and Willetts, H. J. 1975. Electrophoretic studies of Australian, North American, and European isolates of *Sclerotinia sclerotiorum* and related species. J. Gen. Microbiol. 90:355-359.