Mycelial Growth and Infection Without a Food Base by Eruptively Germinating Sclerotia of *Sclerotium rolfsii*

Z. K. Punja and R. G. Grogan

Graduate research assistant and professor, Department of Plant Pathology, University of California, Davis 95616.
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ABSTRACT

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Sclerotia of *Sclerotium rolfsii*, which were induced to germinate eruptively by drying for 10 hr at 15–20% relative humidity, infected bean and sugar beet plants at 25 or 30 C without an exogenous food base of nonliving organic material. On unsterilized field soil, eruptively germinating sclerotia infected sugar beet leaf petioles from a distance of 3.5 cm; on acid-washed and sterilized quartz sand, this distance was reduced to 1.5 cm. The maximum distance from which undried sclerotia could infect host tissue both on field soil and on quartz sand was 0.5 cm. Volatiles from various dried and remoistened plant tissues (hay) triggered eruptive germination of undried sclerotia and increased the competence distance on field soil and quartz sand from 0.5 cm to 3.5 cm and 1.0 cm, respectively. A directional growth of mycelium toward the source of volatiles was observed on all substrates tested. Infection of host tissue by dried sclerotia was greatly increased in the presence of moistened hay, and infection from distances up to 3.0 cm on quartz sand and 6.0 cm on field soil were recorded. The epidemiological significance of mycelial growth from eruptively germinating sclerotia of *S. rolfsii* is discussed.

Numerous reports indicate that sclerotia of *Sclerotium rolfsii* (the resistant propagules by which the organism survives in soil) are not capable of infecting susceptible hosts without an exogenous energy source or food base such as that provided by nonliving organic material; sclerotia were reported to have insufficient endogenous energy to overcome host defense mechanisms (5–7). Several reports have shown that volatiles from dried and remoistened plant tissues (hay) stimulate germination of sclerotia and thus potentially influence the level of disease under field conditions (3,4,10–13). We reported that eruptively germinating sclerotia of *S. rolfsii* were capable of infecting susceptible hosts without a food base (14). However, information on the distance that mycelium from germinating sclerotia can traverse is lacking

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and the epidemiological significance of eruptive germination has not been determined.

The objectives of this study were to establish the precise role of nonliving organic material and of dried and remoistened plant tissues (hay) for infection of susceptible hosts by germinating sclerotia of *S. rolfsii*, and to determine the epidemiological significance of mycelial growth from eruptively germinating sclerotia.

MATERIALS AND METHODS

Source of isolates and production of sclerotia. Four isolates of *S. rolfsii* described previously (15) were used in addition to isolates 120 and 122 from blue lupine and peanut, respectively. Sclerotia used in all experiments were produced in oat cultures as previously described (15).

Requirement of food base for infection of susceptible hosts by sclerotia. Two-week-old bean (Red Kidney) and 2-mo-old sugar beet (US H10) plants were used in this experiment. All plants were grown in pasteurized UC-mix in 10-cm-diameter pots (two plants in each pot) in the greenhouse. All senescent cotyledons or leaves were removed prior to inoculation and the upper 2.5 cm (1 inch) of the soil in each pot was replaced with sterilized quartz sand; each plant was inoculated with a single sclerotium. The following five inoculation methods were compared: sclerotia from 2-mo-old oat culture in vitro were dried for 10 hr at 15–20% relative humidity (RH) prior to placement on moistened quartz sand adjacent to the stems of the plants; similarly dried sclerotia were placed against the stems of the plants on an exogenous food base of nonliving leaf debris from either bean or sugar beet plants; undried sclerotia from oat culture were used without a food base; undried sclerotia were provided with a food base; and uninoculated controls. For each of the five inoculation methods, two sets each of 20 bean or 20 sugar beet plants were used. The pots were covered with plastic bags following inoculation and one set of plants was placed at 25 C and the other at 30 C; all plants were maintained with a 14-hr photoperiod. Plants were recorded as diseased if lesions were evident 9 days after inoculation. The experiment was repeated five times.

Mycelial growth and infection of host tissue from various distances by eruptively germinating sclerotia. The distances on moistened unsterilized field soil (F-1) and on quartz sand from which germinating sclerotia infected susceptible host tissue were determined both in the absence and presence of volatiles from various dried and remoistened plant tissues (hay). The characterisitics of the field soil and quartz sand employed in this study are as follows: the field soil was a Tyndall very-fine sandy loam with pH 6.8, organic matter content $<1\%$, conductivity 330 mmhos, and a moisture-holding capacity at saturation of 250 ml/kg of dry soil. The quartz sand (Ottawa Silica Co., Ottawa, IL) was sieved through a 0.71-mm (24-mesh) screen, acid-washed, and sterilized. Approximately 25 cm^3 was added to each 100 × 25-mm glass petri dish. The pH of the sand (2:1 mixture of sand and 0.01 M $CaCl_2$) was reduced from 8.6 to 5.5 by three washes with 0.1 N HCl and three rinses with distilled water. Sclerotia were produced in sterile oat culture, and either used without drying or after drying for 10 hr at 15–20% RH (15).

To determine the effects of hay on sclerotial germination, mycelial growth, and infection, 0.05 g of each of four different hays (alfalfa, peanut, sugar beet, and bentgrass [15]) was placed in a single 1-cm-wide × 0.5-cm-high glass vial and placed either in the center of a 60 × 15-mm petri dish containing sclerotia incubated on 1% Noble water agar, moistened quartz sand or unsterilized field soil, or placed adjacent to the host tissue in 100 × 25-mm petri dishes containing either quartz sand or field soil. The sclerotia were exposed to volatiles from the hays for the 5-day duration of the experiment. Sugar beet (US H10) was the host tissue used in determining the distances from which mycelium from sclerotia could infect. Leaf petioles were cut into 6-cm sections, rinsed in water, blotted dry, and placed on the surface of moistened F-1 soil or quartz sand in 100 × 25-mm petri dishes, one petiole per plate. Eight sclerotia were placed at distances from 0 to 6 cm away from the petiole at 0.5-cm increments. Sclerotial germination and infection of the petioles were recorded after 5 days of incubation at 27 C. Each distance was replicated 10 times, and the experiment was repeated twice. In a subsequent experiment to determine whether volatiles from hay acted directly upon sclerotia or indirectly by affecting microorganisms in the soil, F-1 field soil previously kept moistened for 48 hr was exposed to the hay for an additional 48 hr, after which the vials containing hay were removed, and 25 sclerotia, either undried or dried, were incubated on the soil surface in each petri dish. Percent germination and infection by sclerotia at different distances were recorded after 5 days of incubation at 27 C.

TABLE 1. Infection of bean and sugar beet plants by single undried or dried sclerotia of *Sclerotium rolfsii* at two temperatures in the absence or presence of a food base

Sclerotial treatment[x]	Food base provided[y]	Infection (%)[w]			
		Bean		Sugar beet	
		25 C	30 C	25 C	30 C
None	−	0 c[z]	8 c	0 c	4 c
None	+	57 b	69 b	40 b	42 b
Dried	−	95 a	97 a	82 a	88 a
Dried	+	85 a	91 a	75 a	81 a
LSD ($P = 0.01$)		18.3	7.1	11.7	12.4

[w] Plants were rated as infected if lesions were evident 9 days after inoculation. Data are the means of two replications of 20 plants each; the experiment was repeated five times.

[x] Sclerotia from 2-mo-old oat cultures were used either before or after drying for 10 hr at 15–20% relative humidity.

[y] Food base was comprised of a layer of either nonliving bean or sugar beet leaf debris upon which a single sclerotium was placed adjacent to the stem of each plant.

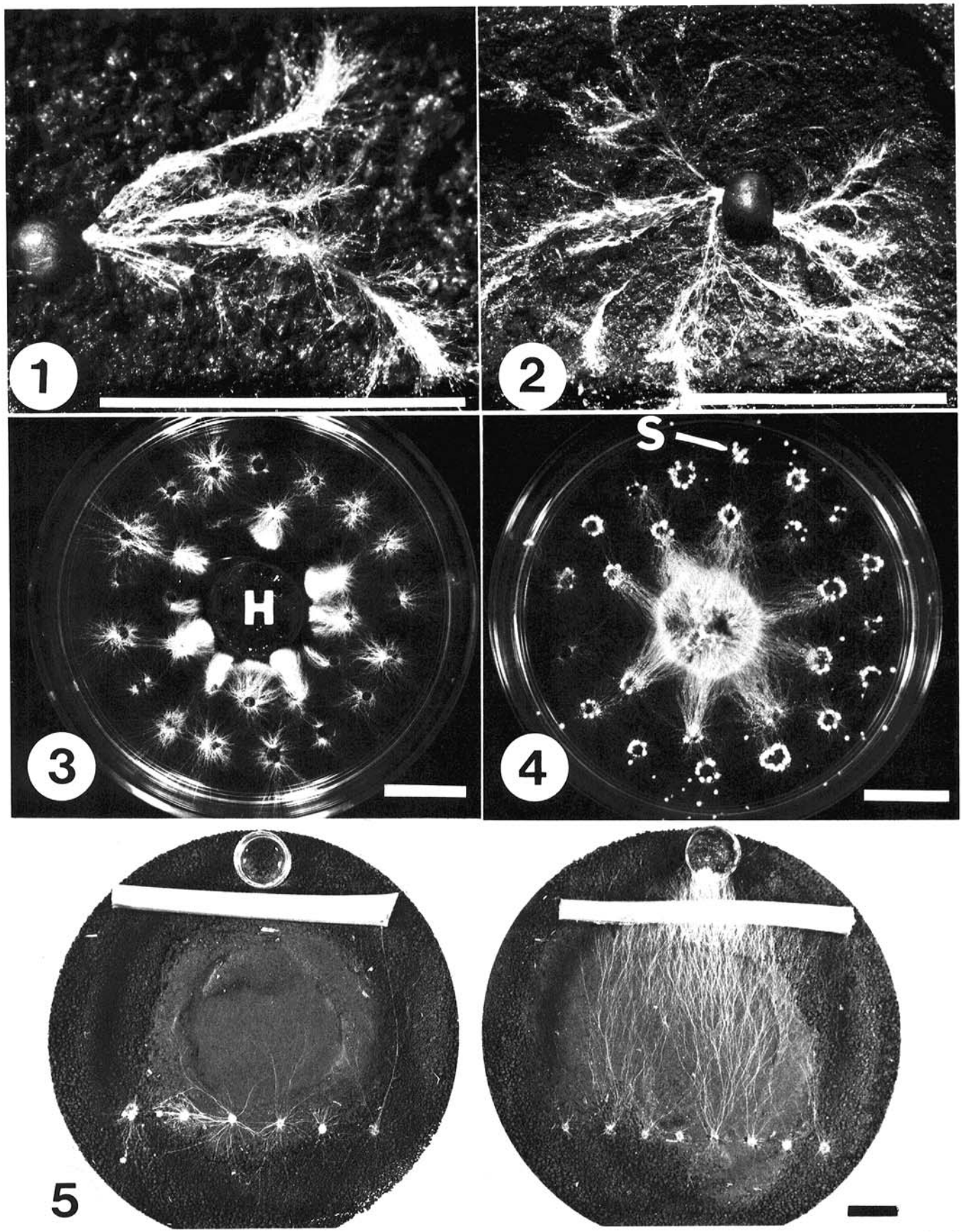
[z] Means in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's mutiple range test.

RESULTS

Requirement of food base for infection of susceptible hosts by sclerotia. Infection of bean and sugar beet plants at either 25 or at 30 C was not significantly different; however, slightly more bean than sugar beet plants were infected at both temperatures (Table 1). Sclerotia that had not been dried caused only about 8% infection in the absence of a food base; when a food base was provided, infection was increased to 69%. Dried sclerotia were capable of infecting up to 97% of the plants in the absence of a food base, and the addition of a food base did not significantly increase the percent of infection (Table 1).

Mycelial growth and infection of host tissue from various distances by eruptively germinating sclerotia. Sclerotia of *S. rolfsii* dried for 10 hr at 15–20% RH germinated eruptively when incubated on moistened field soil for 72 hr (Figs. 1 and 2). Sclerotia of all isolates incubated in 60 × 15-mm petri dishes containing all hay types also were stimulated to germinate eruptively on the three substrates tested, and a directional growth toward the hays was observed (Figs. 3 and 4). The initial plugs of mycelium erupting through the rinds of the germinating sclerotia were not oriented toward the hay, but occurred apparently at random; only subsequent growth of mycelium was directional.

Percent infection of detached sugar beet leaf petioles by germinating sclerotia on field soil and on quartz sand are presented in Table 2. The few undried sclerotia that germinated eruptively on field soil were capable of infecting from a distance of only 0.5 cm; drying sclerotia increased the percent germination and the competence distance also was increased to 3.5 cm. In the presence of hay, these distances were increased to 3.5 and 6.0 cm for undried and dried sclerotia, respectively, apparently as a result of directional growth toward the hay (Fig. 5). Undried sclerotia in the presence of hay behaved similarly to dried sclerotia without hay; however, the percent germination of undried sclerotia was increased only when sclerotia were located within 3.5 cm of the



Figs. 1–5. Eruptive germination and mycelial growth of *Sclerotium rolfsii* on the absence and presence of volatiles from alfalfa hay. **1–2,** Eruptive germination of dried sclerotia on moistened unsterilized field soil, showing plugs of mycelium and extensive mycelial growth in the absence of volatiles. **3,** Eruptive germination of undried sclerotia on 1% Noble water agar after incubation for 3 days in the presence of moistened alfalfa hay (H) placed in a glass vial in the center of the petri dish. Note directional growth of mycelium toward the hay. Similar observations were made on quartz sand and field soil. **4,** Same as Fig. 3, but taken after 8 days of incubation. Note colonization of hay and secondary sclerotial (S) production. **5,** Infection of detached sugar beet leaf petioles by dried sclerotia in the absence (left) and presence (right) of alfalfa hay placed in a vial adjacent to the petiole. Sclerotia were placed on moistened unsterilized field soil 5 cm from the petiole; photograph was taken 5 days after incubation at 27 C. Note directional growth toward the hay. Scale bars represent 1 cm.

source of volatiles; at greater distances, percent germination was not significantly increased. Germination of dried sclerotia and growth of mycelium, however, were increased in the presence of hay even from a distance of 6.0 cm (Table 2). On autoclaved field soil, although germination of dried sclerotia was higher than on nonautoclaved soil in the absence of hay, the distance from which sclerotia could infect was not increased over the 3.5 cm observed for nonautoclaved soil (*unpublished*).

On quartz sand, germination and infection data followed a trend similar to that observed on field soil; however, the competence distances for infection were much less. Undried sclerotia could not infect from a distance greater than 0.5 cm, and exposure to volatiles from hay increased this to 1.5 cm; dried sclerotia infected from 1.5 cm and exposure to hay increased this to 3.0 cm (Table 2). Pre-exposure for 48 hr of moistened field soil to volatiles from hay did not affect germination of sclerotia subsequently placed on the soil surface; dried and undried sclerotia germinated about 79 and 39%, respectively, on both untreated and treated soils.

DISCUSSION

Infection of susceptible hosts by germinating sclerotia of *S. rolfsii* is reported to require an exogenous food base (5–7). We reported for the first time that eruptively germinating sclerotia have sufficient energy to infect without a food base (14). This form of germination is characterized by plug(s) of mycelium erupting through the sclerotial rind and utilization of internal stored materials, leaving an empty sclerotial rind (15). Therefore, the energy required by the mycelium for infection is derived from the sclerotium itself. In contrast, hyphal germination was characterized by growth of individual hyphal strands from the surface of the sclerotium; unless provided with an extraneous nutrient source, growth from hyphal germination is barely perceptible (15). Therefore, hyphally germinating sclerotia would probably have insufficient energy to infect in the absence of an organic substrate. In attempting to understand claims by various workers that exogenous energy sources are required, we can only assume that sclerotia incapable of eruptive germination were used in their experiments. We have shown that sclerotia capable only of hyphal germination do indeed require prior colonization of organic matter for successful infection. The conclusion made by Beckman and Finch (2), however, that 1-mo-old fresh sclerotia, but not 6-mo-old dried sclerotia, were capable of infecting without a food base is difficult to interpret. Only about 8% of undried 1-mo-old sclerotia from PDA cultures germinate eruptively (15); therefore most of them would not be able to cause infection unless provided with a food base. Thus, the 1-mo-old sclerotia used by Beckman and Finch must have had greater capability for eruptive germination. Storage of sclerotia under dry conditions for prolonged periods reduced eruptive germination drastically (15), although they are still viable and germinate hyphally on PDA; a food base is requisite for infection, however (*unpublished*).

Sclerotia induced to germinate eruptively by volatiles from hay can infect susceptible hosts directly (3); a directional growth of hyphae toward the source of volatiles also has been reported (10). A single sclerotium germinating eruptively can initiate an infection without requirement of an extraneous food base of nonliving organic material (14) and the probability of infection is influenced by the distance from the host that the sclerotium is located. Our results show that dried sclerotia on field soil can infect from 3.5 cm, whereas exposure to volatiles from hay increases this competence distance to 6.0 cm. Mycelium from eruptively germinating sclerotia therefore can traverse a great distance without physical contact with nonliving organic material. In contrast, undried sclerotia on field soil can infect from only 0.5 cm away, and exposure to volatiles from hay increased this distance to 3.5 cm. On quartz sand, the distances from which undried and dried sclerotia can infect in the absence and presence of hay are much less than for field soil.

Dried sclerotia were more sensitive to volatiles than undried sclerotia both on field soil and on quartz sand, and the effect on sclerotia of drying followed by exposure to volatiles appeared to be additive. However, the increased distance from which sclerotia can infect in the presence of volatiles from hay may be the result either of directional growth of mycelium toward the hay or of direct or indirect utilization of some component of the volatiles as an energy source. This increase in distance from which sclerotia can infect is important epidemiologically. Inoculum density-disease incidence relationships are altered by increases in distance from which sclerotia can infect because the competence volume of soil is increased in proportion to the cube of the increase in competence distance (8). This distance may be affected by soil type, moisture content, and microbial activity, in addition to fungal isolate (*unpublished*).

Under field conditions, sufficient drying of sclerotia probably would be achieved most frequently by sclerotia on the soil surface; a minimum moisture tension of about −84 bars (94% RH) is necessary (15). Germination and infection by these dried sclerotia

TABLE 2. Eruptive germination (G) and infection (I) of sugar beet leaf petioles by undried and dried sclerotia of *Sclerotium rolfsii* from various distances on field soil and on quartz sand in the presence and absence of volatiles from moistened alfalfa hay

Substrate	Sclerotial treatment[z]		Percent eruptive germination and infection from various distances (cm)[y]												
			0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Natural field soil (F-1)	Undried	G	19	17	16	13	11	15	11	16	19	18	14	12	15
		I	12	3	–	–	–	–	–	–	–	–	–	–	–
	Undried + hay	G	90	82	73	78	67	61	51	43	17	15	14	18	9
		I	90	82	71	70	54	46	39	31	–	–	–	–	–
	Dried	G	78	68	75	78	71	78	63	70	74	78	68	74	73
		I	78	67	75	78	60	78	56	20	–	–	–	–	–
	Dried + hay	G	100	100	100	100	96	100	95	100	100	100	98	94	87
		I	100	100	100	100	91	100	87	100	100	89	82	41	16
Quartz sand	Undried	G	28	23	18	24	26	28	21	22	25	27	26	28	24
		I	22	11	–	–	–	–	–	–	–	–	–	–	–
	Undried + hay	G	87	87	83	71	76	64	42	21	9	13	19	11	6
		I	87	40	4	–	–	–	–	–	–	–	–	–	–
	Dried	G	100	100	100	100	99	100	100	99	100	100	100	100	100
		I	100	50	13	–	–	–	–	–	–	–	–	–	–
	Dried + hay	G	100	100	100	98	100	98	100	99	100	100	98	100	96
		I	100	100	96	84	92	78	29	–	–	–	–	–	–

[y]Percent germination and infection of detached sugar beet leaf petioles were recorded for each distance after 5 days of incubation at 27 C. Data are the means of 10 replications; the experiment was repeated twice.

[z]Sclerotia of four isolates from 2-mo-old oat cultures were either undried or dried for 20 hr at 15–20% RH. Data presented are for alfalfa hay, but peanut, sugar beet, and bentgrass hays were almost as effective; sclerotia were exposed to the volatiles for the entire 5 days of the experiment.

may be the reason, as suggested by Smith (16), that infections occur most commonly at the soil surface (1) and perhaps why disease incidence frequently is more severe after a protracted dry spell (17). Undried sclerotia retrieved from field soil germinate eruptively about 19–28% (15). By growing on nonliving organic matter, however, sclerotia that germinate either eruptively or hyphally could infect; thus, disease severity can be increased by the presence of a nonliving food base as has been frequently reported in the literature (1,5,6,9). In the absence of a susceptible host, mycelium from eruptively germinating sclerotia could be maintained either by growth on available organic matter or by the production of secondary sclerotia (15). Under these circumstances, drying of sclerotia followed by germination when remoistened probably would not be an effective biological control measure as suggested by Smith (16).

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