Relationship of Growth Media and Drying and of Age of Sclerotia to Eruptive Germination and Infection by *Sclerotinia minor*

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ABSTRACT

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The relationship of growth media and drying and of age of sclerotia to eruptive germination and infection by *Sclerotinia minor* was determined. Drying of sclerotia for about 24 hr was required for eruptive germination. Germination was highest when sclerotia were produced on autoclaved potato or carrot. Little or no germination occurred when sclerotia were produced on oats, sorghum, or agar media. Eruptive germination by sclerotia produced on some media increased with age but decreased after 6 mo of storage. There was a very high correlation between eruptive germination by sclerotia of *S. minor* and infection of lettuce.

Sclerotinia minor Jagger has a wide host range that includes species in 64 plant families (5). The fungus is soilborne and produces small (0.5–2.0 mm), black, irregularly shaped sclerotia. The sclerotia germinate eruptively (3) by producing a plug of vegetative mycelium through the rind (4). A single eruptively germinating sclerotium can infect and kill a plant without prior colonization of a senescent food base (3). Thus, at least in the incidence of lettuce (*Lactuca sativa* L.) drop, the number of soilborne sclerotia of *S. minor* is highly correlated with the amount of disease likely to occur (2).

The percent germination of sclerotia of *S. minor* extracted from soil or produced on infected plant residue usually is low. Further, sclerotia cultured on artificial media also germinate at a low rate or not at all. Because large quantities of sclerotia are required to compensate for poor germination, in vitro studies on sclerotial behavior are difficult to conduct. The purpose of this project, therefore, was: 1) to develop a method for producing sclerotia of *S. minor* that consistently germinate eruptively, 2) to determine the effect of sclerotial age on germination, and 3) to correlate eruptive germination with incidence of infection.

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MATERIALS AND METHODS

Production of sclerotia and germination tests. The identification and geographic location of the *S. minor* isolates tested are given in Table 1. All isolates were obtained from lettuce. Stock cultures were obtained from sclerotia produced on infected host tissue collected in the field or extracted from soil. Sclerotia were surface-sterilized in a vacuum flask

Table 1. Geographic location of the *Sclerotinia minor* isolates tested[a]

Isolate	Geographic location
AZ1	Yuma, Arizona
AZ3	Yuma, Arizona
NY1	New York
NY3	New York
SV1	Salinas Valley, California
SV3	Salinas Valley, California
SV7	Salinas Valley, California
BF1	Bakersfield, California
IV1	Imperial Valley, California

[a]All isolates were collected from lettuce.

containing 0.5% NaOCl for 30 sec and rinsed in sterile distilled water. They were placed on 10 ml of acidified potato-dextrose agar (PDA) (1), adjusted to pH 4.5 with 25% lactic acid (APDA), in 9.0-cm petri dishes. Mycelium was hyphal-tipped and transferred to 15-ml slants of PDA (1) in 12 × 200 mm tubes. After growth and sclerotial development, stock cultures were stored at 2 C.

Growth media (Table 2) were autoclaved (121 C; 1.05 kg/cm^2) 20 min and tested in 9.0-cm petri dishes (15 ml/dish). V-8 agar, nutrient agar (NA), King's medium B (KMB), yeast extract-dextrose-carbonate agar (YDC), and Czapek-Dox agar (CDA) were prepared as described elsewhere (1). Water agar (WA) was prepared by autoclaving 15 g of Difco Bacto agar in 1.0 L of water. The basal salts medium (BSM) contained (per liter of distilled water): 680 mg KH_2PO_4, 180 mg $MgSO_4$, 149 mg KCl, 14.2 mg $ZnSO_4$, 4.0 mg $CuSO_4$, 4.0 mg $FeCl_3$, 500 mg Difco yeast extract, 1.0 g NH_4NO_3, and 15.0 g Difco Bacto agar. Potato agar (PA), acidified potato agar (APA), potato-sucrose agar (PSA), and acidified potato-sucrose agar were prepared by the method for PDA (1) and APDA; the glucose, however, was deleted or replaced with sucrose. Carrots were sliced diagonally in 50-mm thick disks, and potatoes (skins removed) were cut in 1.0-cm cubes; 10 pieces of each were autoclaved in 500-ml Erlenmeyer flasks twice in 24-hr intervals. Celery petioles were washed and cut in 7.0-cm lengths; four petioles were autoclaved in 500-ml Erlenmeyer flasks for 20 min. Oats or sorghum were autoclaved 20 min with water, WA, NA, PDA, or PSA (2:1, v/v) in 500-ml Erlenmeyer flasks twice in 24-hr intervals.

Agar media were inoculated with a 3.0-mm actively growing mycelial disk of a *S. minor* isolate from PDA stock cultures and incubated at 18 C. Media containing carrot, celery, potato, oats, or sorghum were inoculated with three mycelial disks and hand-shaken intermittently during incubation to dislodge mycelia and sclerotia.

After maturation, sclerotia were removed from the medium, washed in running tap water for 24 hr, and tested immediately or dried (24 C under forced air) for 24 hr. Eruptive germination by 100 sclerotia of each isolate from each medium was tested by placing 25 sclerotia on 20 ml of moist quartz sand in 9.0-cm petri dishes at 18 C for 14 days. Each test was replicated four times.

Percent germination was recorded daily and expressed as an average of all isolates tested. Data were analyzed by a split-plot-factorial analysis of variance (ANOVA); the factors included drying or no drying of sclerotia (main plot), media tested (subplot), isolates tested, and number of replications. Differences between treatments were determined by Fisher's protected LSD (PLSD) test at the $P = 0.01$ level.

Eruptive germination in relation to age of sclerotia. Sclerotia were produced on the different media, washed, dried, and stored in 7.5 × 15.0 cm seed packets at 18 C; 400 sclerotia of each isolate were tested for eruptive germination monthly for 12 mo.

Data were recorded as percent germination and expressed as an average of all isolates. Data were analyzed by a three-way factorial ANOVA; the factors included isolate, media tested, and date of germination test. Linear increase in germination with age was tested by regression analysis; differences between treatments were determined by a PLSD at the $P = 0.01$ level.

Correlation of in vitro eruptive germination by sclerotia and infection of lettuce. Lettuce (cv. Salinas) was tested to correlate in vitro eruptive germination by sclerotia of *S. minor* with infection. Lettuce seeds were planted in 5 × 5 × 5 cm (125 cm^3) pots containing Yolo fine sandy loam soil and thinned to one plant per pot. When the plants had four true leaves they were inoculated with a sclerotium of an individual isolate of *S. minor* from the different growth media

Table 2. Effect of growth media and drying on eruptive germination by sclerotia of *Sclerotinia minor*[a]

Medium	Percent germination[b]	
	Dried	Not dried
PA	2	0
APA	0	0
PDA	0	0
APDA	1	0
PSA	0	1
APSA	1	0
V-8 agar	0	0
NA	3	0
WA	1	0
KMB	1	0
BSM	0	0
YDC	0	0
CDA	0	0
Oats + water	7	0
Oats + WA	6	0
Oats + NA	4	0
Oats + PDA	0	0
Oats + PSA	2	0
Sorghum + water	5	0
Sorghum + WA	4	0
Sorghum + NA	6	0
Sorghum + PDA	6	0
Sorghum + PSA	0	0
Celery	0	0
Carrot	72	0
Potato		
Red la soda	94	0
Russet Burbank	97	2
White rose	95	0

[a]Sclerotia were produced, dried or not dried, placed on 20 ml of moist sand in 9.0-cm petri dishes (25 sclerotia/dish), and observed for germination daily for 14 days.
[b]Average of 100 sclerotia from nine isolates, replicated four times. PLSD (0.01) = 15.87.

Table 3. Effect of age on eruptive germination by sclerotia of *Sclerotinia minor*

Medium	Percent germination[a]												
	0	1	2	3	4	5	6	7	8	9	10	11	12
PA	1	3	2	0	0	0	1	2	0	0	0	1	0
APA	0	0	1	1	0	0	0	0	0	3	0	0	0
PDA	1	1	0	0	2	4	0	2	0	0	5	0	0
APDA	0	0	4	3	0	0	0	0	1	0	0	0	5
PSA	4	0	2	4	0	0	0	1	0	0	0	0	0
APSA	0	0	0	0	0	3	0	0	0	1	0	0	4
V-8 agar	0	0	0	0	0	0	4	4	0	0	0	0	0
NA	1	1	4	0	2	0	0	0	4	0	2	2	0
WA	0	4	3	5	0	8	0	0	0	3	0	0	0
KMB	0	0	0	0	0	3	0	0	1	0	0	0	0
BSM	4	0	0	0	0	2	0	0	0	0	0	0	0
YDC	0	0	0	0	0	0	0	0	0	0	0	0	0
CDA	0	0	1	0	0	0	2	0	0	0	0	0	0
Oats + water	1	4	12	16	22	30	42	12	4	0	2	0	0
Oats + WA	4	6	16	23	27	35	41	9	2	0	0	0	0
Oats + NA	0	1	1	4	0	0	0	0	0	3	0	0	1
Oats + PDA	2	2	0	0	3	0	0	0	0	0	0	0	0
Oats + PSA	0	0	5	0	0	0	0	2	0	0	0	1	0
Sorghum + water	6	10	18	24	30	38	45	15	6	0	2	0	1
Sorghum + WA	8	14	22	28	37	44	52	12	3	1	0	0	0
Sorghum + NA	0	0	0	4	0	2	0	0	0	0	1	0	0
Sorghum + PDA	1	1	0	0	0	0	0	2	0	0	0	3	0
Sorghum + PSA	0	0	0	0	0	1	1	0	0	0	0	0	0
Celery	2	8	14	22	30	38	47	51	13	6	0	0	1
Carrot	69	71	70	67	73	70	71	75	32	17	9	0	0
Potato													
Red la soda	93	93	98	92	89	91	90	93	89	55	19	7	5
Russet Burbank	90	97	96	90	91	93	92	92	94	50	22	10	4
White rose	93	93	89	97	95	95	90	88	92	52	17	9	8

[a]Presented as number of months after production; average of 400 sclerotia from each of nine isolates. PLSD (0.01) = 6.73; $Y = 0.86$

Table 4. Correlation of eruptive germination and infection by sclerotia of *Sclerotinia minor*

Medium	Correlation[a]	
	Percent germination[b]	Percent infection[c]
PA	0	1
APA	0	0
PDA	0	0
APDA	1	1
PSA	0	0
APSA	2	1
V-8 agar	1	0
NA	0	2
WA	3	1
KMB	0	0
YDC	2	1
CDA	0	0
Oats + water	3	1
Oats + WA	5	6
Oats + NA	3	3
Oats + PDA	0	1
Oats + PSA	0	0
Sorghum + water	3	4
Sorghum + WA	2	2
Sorghum + NA	1	0
Sorghum + PDA	7	5
Sorghum + PSA	5	5
Celery	0	0
Carrot	69	72
Potato		
Red la soda	93	91
Russet Burbank	96	100
White rose	93	94

[a] $r^2 = 0.89$.
[b] Determined on 20 ml of Yolo fine sandy loam in 9.0-cm petri dishes (25 sclerotia/dish) replicated four times. Percentages are expressed as an average of nine isolates.
[c] Determined by inoculating 100 lettuce plants each grown in Yolo fine sandy loam with a single sclerotium. Percentages are expressed as an average of nine isolates.

(100 plants/isolate/medium). Sclerotia were placed 1.0 cm below the soil surface and adjacent to the root. In addition, 100 sclerotia of each isolate were tested for in vitro eruptive germination on 20 ml of Yolo fine sandy loam as described previously.

Germination of sclerotia and lettuce infection (determined by wilting of plants and development of mycelium and sclerotia on the roots) were observed daily for 28 days. Data were recorded as percent germination and wilt, respectively, and presented as an average of all isolates tested. Data were analyzed by a two-way factorial ANOVA; the factors included the isolate tested and percent germination or infection. Differences between treatments were determined by a PLSD at the $P = 0.01$ level. The r^2 value was derived by correlation of determination between percent sclerotial germination and infection.

RESULTS

Growth media and eruptive germination. There were no significant differences ($P = 0.01$) between the number of experiments or isolates of *S. minor* tested. Drying of sclerotia was required for eruptive germination to occur (Table 2). After drying, germination by sclerotia produced on agar, celery, oats, or sorghum media was very low or nil. Sclerotia from potato media germinated at a rate of 90% or greater; there were no significant differences between the different potato cultivars tested ($P = 0.01$). Germination by sclerotia produced on carrot was 72% and was significantly lower ($P = 0.01$) than germination on autoclaved potato. (Although data were not reported, sclerotia of *S. minor* extracted from soil collected in fields with a cropping history of potato [a host of *S. minor*] usually eruptively germinated at a high rate.)

Germination and age of sclerotia. There were no significant differences ($P = 0.01$) between isolates of *S. minor* tested. There was a linear increase ($Y = 0.86$) in germination by sclerotia produced on some media during the first 6 mo of storage; germination, however, decreased thereafter (Table 3). These media included celery and oats and sorghum supplemented with water or WA. Maximal germination by sclerotia produced on these media was significantly lower ($P = 0.01$) than germination by sclerotia produced on potato. Germination by sclerotia produced on potato did not increase with age and significantly decreased ($P = 0.01$) after 8 mo.

Correlation between sclerotial germination and infection. There were no significant differences ($P = 0.01$) between the isolates of *S. minor* tested. There was an excellent correlation ($r^2 = 0.89$) between percent eruptive germination by sclerotia and percent infection of lettuce (Table 4). Sclerotia produced on potato media resulted in the highest percentage of infection, followed by carrot. Sclerotia produced on media that did not result in eruptive germination caused very little or no infection.

DISCUSSION

The results from these tests confirmed that drying was required for eruptive germination by sclerotia of *S. minor* and that autoclaved potato was the most effective medium for producing sclerotia that germinate eruptively (Table 2). Sclerotia produced on potato agar medium, however, did not germinate at a high rate, indicating that insoluble materials in potato may have been responsible for eruptive germination.

Age influenced eruptive germination of sclerotia produced on some media (Table 3), but for all isolates germination decreased after 6 mo. Thus, long-term storage of sclerotia is not recommended.

The correlation ($r^2 = 0.89$) between eruptive germination by sclerotia and infection of lettuce was very high (Table 4), confirming other reports (3,4) that eruptive germination was requisite for infection.

It is important to conduct pathogenicity tests, in vitro germination experiments, and inoculum density relationship studies with sclerotia of *S. minor* capable of eruptive germination. Actively growing mycelia from agar media, or other types of inocula, do not represent the mechanism of infection by *S. minor* and may produce biologically indifferent results.

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