Germination of *Sclerotinia minor* and *S. sclerotiorum* Sclerotia Under Various Soil Moisture and Temperature Combinations

J. J. Hao, K. V. Subbarao, and J. M. Duniway

First and third authors: Department of Plant Pathology, University of California, One Shields Ave., Davis 95616; and second author: Department of Plant Pathology, University of California, Davis, c/o U.S. Agricultural Research Station, 1636 E. Alisal St., Salinas 93905. Accepted for publication 19 November 2002.

ABSTRACT

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Sclerotial germination of three isolates each of *Sclerotinia minor* and *S. sclerotiorum* was compared under various soil moisture and temperature combinations in soils from Huron and Salinas, CA. Sclerotia from each isolate in soil disks equilibrated at 0, -0.03, -0.07, -0.1, -0.15, and -0.3 MPa were transferred into petri plates and incubated at 5, 10, 15, 20, 25, and 30°C. Types and levels of germination in the two species were recorded. Petri plates in which apothecia were observed were transferred into a growth chamber at 15°C with a 12-h light-dark regime. All retrievable sclerotia were recovered 3 months later and tested for viability. Soil type did not affect either the type or level of germination of sclerotia. Mycelial germination was the predominant mode in sclerotia of *S. minor*, and it occurred between -0.03 and -0.3 MPa and 5 and 25°C, with an optimum at -0.1 MPa and 15°C. No germination occurred at 30°C or

0 MPa. Soil temperature, moisture, or soil type did not affect the viability of sclerotia of either species. Carpogenic germination of *S. sclerotiorum* sclerotia, measured as the number of sclerotia producing stipes and apothecia, was the predominant mode that was affected significantly by soil moisture and temperature. Myceliogenic germination in this species under the experimental conditions was infrequent. The optimum conditions for carpogenic germination were 15°C and -0.03 or -0.07 MPa. To study the effect of sclerotial size on carpogenic germination in both *S. minor* and *S. sclerotiorum*, sclerotia of three distinct size classes for each species were placed in soil disks equilibrated at -0.03 MPa and incubated at 15°C. After 6 weeks, number of stipes and apothecia produced by sclerotia were counted. Solitary *S. minor* sclerotia did not form apothecia, but aggregates of attached sclerotia readily formed apothecia. The number of stipes produced by both *S. minor* and *S. sclerotiorum* was highly correlated with sclerotial size. These results suggest there is a threshold of sclerotial size below which apothecia are not produced, and explains, in part, why production of apothecia in *S. minor* seldom occurs in nature.

Sclerotinia minor Jagger and *S. sclerotiorum* (Lib.) de Bary are two pathogens that cause lettuce (*Lactuca sativa* L.) drop (1,6,30). The symptoms caused by the two species are similar in many ways but differ depending on the inoculum source. Regardless of the species causing the disease, symptomatic plants turn brown and exhibit soft watery decay and the whole plant eventually collapses as a result of the destroyed crown tissue. White mycelial mats form on the infected plant parts that ultimately develop into sclerotia. Sclerotia are formed primarily on the root, stem, and crown tissues of plants infected by *S. minor*, whereas sclerotia are produced on the aerial foliar tissues of lettuce infected by *S. sclerotiorum*.

Mycelia from eruptive germination of *S. minor* sclerotia come in contact with stems and leaves of lettuce plants to initiate infection (16,24). *S. sclerotiorum* also infects lettuce plants occasionally by this mode, but a majority of infections by this species are initiated by airborne ascospores (1,14). Even though both *Sclerotinia* spp. cause lettuce drop in California, their distribution differs within the lettuce production areas (30). In the Salinas and Santa Maria Valleys, *S. minor* is the predominant pathogen causing lettuce drop. Although lettuce drop caused by *S. sclerotiorum* has been observed occasionally in these same locations, those infections are caused primarily by the mycelial germination of sclerotia. In the San Joaquin Valley, *S. sclerotiorum* is the major cause of lettuce drop on winter lettuce and the infections are invariably caused by airborne inoculum. Why the two species occur in different niches within California is currently under investigation,

and the results are likely to determine the risks associated with each species outside their niche.

Previous research has shown that *S. sclerotiorum* rarely infects hosts by direct germination of sclerotia due to lack of exogenous energy sources (1,15). In contrast, *S. minor* commonly infects plants directly following eruptive germination of sclerotia (7,8). Production of apothecia by *S. minor* has been observed in New Zealand (21,22), but it seldom occurs in the United States (2,6,30). Factors that affect survival and germination of sclerotia may contribute to the differential distribution of the two *Sclerotinia* spp. in California.

A number of factors affect the survival and germination of sclerotia (13). Soil moisture and temperature (5,12,14,17,26,29), sclerotial position and duration in soil (4,5,9,13,25), sclerotial shape (23), soil gases or chemicals (6,24), activities of other microorganisms (3,11,28), nutrition (12), and other factors (23,25,31) are known to affect survival and germination of sclerotia. Decreasing water potential in the soil increases survival, and sclerotia only survive short periods in saturated soils at 0 MPa (3). Temperatures higher than 30°C greatly reduce survival (4,5). On culture media, sclerotia can germinate well between 6 and 30°C, although the optimum for *S. minor* is in the range of 18 to 25°C (16,26) and, for *S. sclerotiorum*, the optimum is 10°C (1). Sclerotia of *S. minor* can germinate directly at soil moisture levels between -0.03 and -1.5 MPa (26), while sclerotia of *S. sclerotiorum* germinate between 0 and -0.6 MPa (17,18). With the exception of saturation, high soil moisture tends to promote sclerotial germination. The fraction of sclerotia that germinate and the numbers of apothecia produced per sclerotium increase with sclerotial size in *S. sclerotiorum* (15).

Most previous studies (1,16,18) on survival and germination of *S. minor* and *S. sclerotiorum* sclerotia as affected by soil moisture

Corresponding author: K. V. Subbarao; E-mail address: kvsubbarao@ucdavis.edu

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and temperature have been conducted on culture media. In addition, the effects of temperature or moisture on survival and germination of sclerotia were studied either on individual species (3–5) or in a limited combination of temperature and moisture (26). Results from studies on culture media are not likely to represent responses in soil environments. A comparison of the responses of the two *Sclerotinia* spp. in soil environments at various temperature and moisture combinations is likely to provide reliable information on the ecology of the two species. Furthermore, carpogenic germination of *S. minor* sclerotia rarely has been observed in nature (10,22,27), and it is important to identify factors that either prevent or trigger this mode of germination in a soil medium.

The objectives of this study were to determine the effects of soil type and combinations of soil temperature and moisture on mycelial and carpogenic germination of sclerotia from the two species, whether the geographical isolation of the two species observed within California is related to their differential survival, and the effect of sclerotial size on carpogenic germination. A preliminary report of the results obtained on *S. sclerotiorum* has been published (20).

MATERIALS AND METHODS

Isolate collection and production of sclerotia. Isolates of *S. minor* and *S. sclerotiorum* used in this study were collected from diseased lettuce plants in commercial fields in California during 1995. The isolates of *S. minor* were from Salinas (SM01), Gonzales (SM02), and Santa Maria (SM03), and the isolates of *S. sclerotiorum* were from Huron (SS01), Salinas (SS02), and Lompoc (SS04). Sclerotia collected from plants were surface-sterilized for 3 min with 10% bleach (Clorox, Oakland, CA), and a single sclerotium was placed on each potato dextrose agar (PDA) plate at 20°C. Three days later, a block of agar with mycelium from the edge of the culture was transferred onto fresh PDA plates to obtain pure cultures. The process was repeated for each isolate.

Sclerotia of each isolate needed for experiments were produced on pieces of potato tubers. Freshly cut pieces (45 g of 1-cm³ pieces) of potato were placed in 250-ml conical flasks and autoclaved for 1 h at 121°C twice within 24 h. The flasks were inoculated with three mycelial plugs from 3-day-old cultures of each isolate. After 3 weeks of incubation on laboratory benches, sclerotia were harvested on a sieve under running water and air dried for 2 weeks. Sclerotia of *S. minor* were grouped by sight into three size classes: large (>2.0 mm), medium (1 to 2.0 mm), and small (<1 mm). Similarly, *S. sclerotiorum* sclerotia were sized into large (>4.75 mm), medium (2.0 to 4.75 mm), and small (<2.0 mm) groups. Aggregated sclerotia were considered a single

sclerotium piece. Medium-sized sclerotia of *S. minor* and large-sized sclerotia of *S. sclerotiorum* were used to determine the effects of combinations of soil temperature and moisture on germination and viability. Air-dried sclerotia were stored at 5°C until they were used in the experiments.

Water uptake and loss by sclerotia. Air-dried sclerotia from each isolate were used to determine rates of water uptake. Approximately 3 g of *S. minor* sclerotia and 7 g of *S. sclerotiorum* sclerotia of each isolate were placed in a small copper mesh cup. The cup with sclerotia was fully submerged in distilled water for 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 24 h. At the end of each wetting period, sclerotia were blotted dry on paper towels, weighed, and submerged in distilled water for the next cycle. Following the final weighing, sclerotia were returned to the cups, placed in a vented chemical hood at room temperature (23 ± 1°C), and weighed periodically to determine the rate of drying. Sclerotia were finally dried in an oven at 90°C for 48 h and weighed to determine dry weight.

Soil preparation and moisture adjustment. Soils were collected to a depth of 10 cm from commercial lettuce fields in Huron (HUR) in the San Joaquin Valley and Salinas (SAL) in the Salinas Valley. Both fields had a history of severe lettuce drop. The HUR soil was a sandy loam, pH 6.6, and SAL soil was a silty clay loam, pH 6.6. The other characteristics of the soils are summarized in Table 1. Soils from both locations were air dried in a greenhouse, ground into fine particles using a soil grinder, and sieved (No. 10 sieve, 2-mm openings; Soiltest Inc., Evanston, IL).

To establish various soil moisture treatments, rubber rings 5 cm in diameter by 1 cm high were filled with soil on a pressure plate and saturated with distilled water for 12 h. In all, 20 sclerotia from each isolate of either *S. minor* or *S. sclerotiorum* were pressed approximately 2 mm from the top into two soil disks with 10 sclerotia per disk, 4 h after initial wetting. The soil disks on the ceramic plates were equilibrated to soil matric potentials (ψ_m) of 0, -0.03, -0.07, -0.1, -0.15, and -0.3 MPa over 24 h at room temperature using a gas pressure extractor (Model 1500; Soil Moisture Equipment Corp., Santa Barbara, CA). After the soil disks were equilibrated to the desired matric potentials, they were placed individually in plastic petri dishes (9 cm in diameter) with 2-cm-tall lids and tightly sealed with parafilm. Petri plates with soil disks at different ψ_m values were randomly placed in crisper (22.5 by 30 by 10 cm; Pioneer Plastics, KY), and incubated at 5, 10, 15, 20, 25, and 30°C in the dark. The water content of soil disks at different ψ_m was determined by taking the weight of soil immediately after moisture equilibration and again after oven drying at 90°C for 24 h.

Sclerotial germination and viability. After 4 weeks of incubation, or when the first stipe from sclerotia was observed, the plates with sclerotia were transferred to a growth chamber with a 12-h light–dark regime at the same temperature, while the plates of *S. minor* were retained in the incubator. Light intensity from a mixture of fluorescent and incandescent lamps in the chamber was 40.3 W s⁻¹. Four plates were put in a transparent plastic bag (26.8 by 27.9 mm) horizontally, and the bags were placed at the same level on the rack of a growth chamber. Temperature inside the plates was measured using a Hobo data logger (Onset Computer Corp., Pocasset, MA) and the chamber temperature was reduced when lights were on to eliminate possible effects of heating from the lamps and to keep the chamber temperature constant. Mycelial germination of *S. minor* and both mycelial and carpogenic germination in *S. sclerotiorum* were examined visually after 1 week. The number of germinated sclerotia and the type of germination were recorded weekly for 6 weeks. After 3 months, all sclerotia were retrieved using the wet-sieving method and their viability tested on water agar. The sclerotia were considered viable if they produced typical mycelium on water agar. For *S. sclerotiorum*, the number of stipes, apothecial initials, and mature apothecia were recorded on each sclerotium. Total viable sclerotia

TABLE 1. Physical properties of soils from Huron (HUR) and Salinas (SAL)^a

Variable	HUR	SAL
pH ^b	6.6	6.6
Ca (meq/liter)	5.8	2.5
Mg (meq/liter)	6.2	1.7
Na (meq/liter)	2.8	1.3
CEC (meq/100 g)	19.4	29.3
P (ppm) ^c	29.1	101
NH ₄ (ppm)	10.7	3.7
NO ₃ N (ppm)	42.7	12.9
Organic matter (%)	0.2	2.2
Sand (%)	67	19
Silt (%)	18	51
Clay (%)	15	30

^a Measurements by University of California Division of Agriculture and Natural Resources Analytical Laboratory.

^b Soil pH was analyzed on a saturation paste extract.

^c P in soil was determined using extractable phosphate based on alkaline extraction by 0.5 N NaHCO₃ and measuring P by spectrophotometry.

for *S. sclerotiorum* was calculated as the sum of sclerotia with carpogenic germination and those without stipes that remained viable at the termination of the experiment.

Effect of sclerotium size on germination. To determine if the size of sclerotia affects carpogenic germination, 20 sclerotia from each size class for *S. minor* or *S. sclerotiorum* were placed in soil disks equilibrated to -0.03 MPa. The soil disks then were placed in petri dishes, sealed, and incubated at 15°C by the methods given above. In a separate experiment, petri dishes (9 cm in diameter) were filled with 30 g of soil and saturated with distilled water. Depending on the size of sclerotia, 30 to 100 sclerotia were placed on the soil surface in each dish and a total of 12 disks with mixed-size sclerotia for each species were used. The disks were incubated at 15°C as described above and all sclerotia were recovered 6 weeks later. The number of stipes and the diameter of apothecia were measured for all sclerotia. The relationship between the number of stipes and size of sclerotia was determined by correlation analyses.

Statistical analyses. All data analyses were performed using SAS (version 7.0; SAS Institute, Inc., Cary, NC). The main experiment was considered to be a split factorial design, and a mixed model was used to determine the fixed effects of soil moisture, temperature, and soil types on germination. Isolate and temperature-isolate terms were considered to be random effects. Linear and quadratic effects of soil temperature and moisture, and interactions between quadratic terms and other individual factors, were tested. The higher order effects that were not significant ($P = 0.15$) were deleted stepwise.

RESULTS

Water release and uptake characterization of soils and sclerotia. At each ψ_m tested, SAL soil had significantly higher water content than HUR soil (Fig. 1). Although the larger sclerotia of *S. sclerotiorum* took longer to hydrate fully, air-dried sclerotia of both species took up water rapidly when placed in water (Fig. 2). Fully hydrated sclerotia dried quickly in air, losing half their water content in 6 to 7 h (Fig. 2).

Fig. 1. Relationship between water content (grams of H_2O per 100 g of dry soil) and matric potential for soils collected in Huron and Salinas, CA. Data were collected from subsamples in each experiment on sclerotial germination when soil disks were equilibrated to specific matric potentials on a pressure plate. Vertical bars are standard errors of the mean water contents at individual matric potentials.

Mycelial germination of *S. minor*. The mycelial germination of three *S. minor* isolates responded to the combined factors similarly, and sclerotia germinated eruptively by mycelial growth in all permissive treatment combinations. Analysis of variance showed that soil temperature significantly affected the germination of *S. minor* sclerotia. Both linear and quadratic effects of soil temperature and quadratic effects of soil moisture were significant, while soil type did not affect mycelial germination of *S. minor* (Table 2). In HUR soil, sclerotia of *S. minor* germinated at -0.03 to -0.3 MPa, across all temperatures, between 5 and 25°C (Fig. 3). No germination occurred at 30°C or at 0 MPa. Typically, germination was the highest at 15°C , and decreased as the temperature increased or decreased. Germination was optimal at -0.15 MPa, intermediate at -0.03 MPa, and less in drier soil treatments regardless of the temperature. In SAL soil, optimal temperature for mycelial germination was also 15°C , and germination decreased as the temperature increased or decreased from 15°C (Fig. 3). At 0 MPa, sclerotia germinated only at 15°C not at other tempera-

Fig. 2. Rate of water uptake and drying of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*. Air-dried sclerotia were placed in water to measure water uptake and fully hydrated sclerotia were dried in air at ambient laboratory temperatures to measure rate of drying. Water content of each species was measured with three isolates as replications. Water content is given as a percentage of the water content when fully hydrated. Vertical bars are standard errors for each data point.

tures. Optimal matric potentials for germination in SAL soil were -0.03 and -0.07 MPa, and both -0.1 and -0.15 MPa were nearly optimal at all temperatures (Fig. 3). Germination was diminished at the dry (-0.3 MPa) and wet (0 MPa) extremes tested (Fig. 3).

Viability of *S. minor* sclerotia. Soil temperature, moisture, and type did not affect sclerotial viability significantly; however, the temperature-moisture interaction was significant, indicating that viability at specific temperatures was dependent on moisture treatment (Table 2). In HUR soil, viability of sclerotia varied from 30 to 80%: viability was lowest at 0 MPa and 30°C , and at -0.03 MPa and 10°C , and highest at -0.01 MPa and 15°C (Table 3). In SAL soil, viability of sclerotia varied between 33 and 90%: viability was lowest at 0 MPa and 10°C , and highest at -0.07 MPa and 20°C (Table 3).

Carpogenic germination of *S. sclerotiorum*. The carpogenic germination of three *S. sclerotiorum* isolates responded to the combined factors similarly, and germination occurred in both soil types equally well (Table 4). Analysis of variance showed that soil temperature and moisture significantly affected carpogenic germination; both linear and quadratic effects of soil temperature and moisture were significant, as were soil-moisture and temperature-moisture-soil interactions (Table 4). Temperature and quadratic effects of soil moisture affected the number of stipes and apothecia produced per sclerotium significantly (Table 4). Other combined effects, such as temperature-moisture, were not significant.

The percentages of *S. sclerotiorum* sclerotia producing stipes at various levels of soil moisture and temperature are shown in Figure 4. Mycelial germination occasionally occurred before stipes grew out of the soil, but all sclerotia producing mycelium also produced stipes. A majority of stipes produced subsequently formed apothecia. Germination responses to temperature and moisture followed similar trends in both HUR and SAL soils (Fig. 4). Temperatures of 10 to 20°C were optimal or nearly so, while germination was limited at 5°C and did not occur at 25 and 30°C . Soil matric potentials of -0.03 and -0.07 MPa were highly conducive to germination and, although intermediate levels of germination occurred at 0, -0.1 , and -0.15 MPa, little or no germination occurred at -0.3 MPa (Fig. 4).

The number of stipes produced per germinated sclerotium was highly correlated with the number of germinated sclerotia. The number of stipes produced by one sclerotium varied from 1.5 to 16, and the optimal temperature was 10 or 15°C , depending on the moisture (data not shown). Apothecium formation was somewhat more limited by variation in soil temperature and moisture than was germination (Figs. 4 and 5). The optimum temperature for apothecia was 15°C , numbers of apothecia decreased at 10 and 20°C , and none were formed at 5, 25, or 30°C in both soils. Optimum matric potentials for apothecial formation were -0.03 MPa

in HUR soil and -0.07 and -0.03 MPa in SAL soil. No apothecia were produced at -0.3 MPa and production was intermediate at 0, -0.1 , and -0.15 MPa.

Viability of *S. sclerotiorum* sclerotia. The viability of sclerotia recovered from the soils at the termination of the experiment was not affected by any one variable, but moisture-temperature interaction affected sclerotial viability significantly (Table 4). Viability of recovered sclerotia was close to 90% in most treatment combinations, but decreased with temperature at -0.07 MPa in HUR soil and at 0 MPa in SAL soil (Fig. 6).

Relationship between sclerotia size and carpogenic germination. Normal-sized *S. minor* sclerotia (diameter < 0.02 cm, or length $\times$ width < 1.2 cm²) used in the experiments above (Fig. 3) did not produce apothecia. Apothecia were produced frequently, however, when aggregates of attached sclerotia were placed in

Fig. 3. Mycelial germination of *Sclerotinia minor* sclerotia incubated at various moisture and temperature combinations in Huron (HUR) and Salinas (SAL) soils. Each line represents germination at one matric potential after 12 weeks of incubation. Each data point is the mean of three isolates as the response of the three isolates to the treatments was similar. Vertical bars are standard errors.

TABLE 2. Analysis of variance on effects of soil temperature, moisture, and type on sclerotial germination and viability of *Sclerotinia minor*

Variable, source ^a	df ^b	F	P > F ^c
Germination			
Temp	13	89.81	0.0001
Temp $\times$ Temp	12	86.97	0.0001
Moist	279	2.59	0.1090
Moist $\times$ Moist	279	3.98	0.0469
Temp $\times$ Moist	279	2.80	0.0956
Soil	279	0.68	0.4092
Temp $\times$ Soil	279	2.70	0.1013
Temp $\times$ Moist $\times$ Soil	279	2.97	0.0857
Viability			
Temp	40	0.79	0.3784
Moist	367	2.28	0.1315
Soil	365	3.56	0.0601
Temp $\times$ Moist	366	4.17	0.0418

^a Moist = soil moisture, Temp = soil temperature.

^b Degrees of freedom.

^c Probability associated with F value.

soil. In the experiment where aggregates of sclerotia were implanted in HUR and SAL soils and then equilibrated to -0.03 MPa, stipes, apothecia, or both were observed in 34 of 48 plates, and the average carpogenic germination rate of *S. minor* sclerotial aggregate was 10.7%. Normal-sized sclerotia of *S. sclerotiorum* (<5 mm²) in both germination methods used produced stipes and apothecia (averaged germination 98.6 and 72.8%, respectively, in soil at -0.03 MPa or on top of saturated soil). In *S. sclerotiorum*, germination of larger sclerotia was 100% and that of the smaller sclerotia was 30%. There was a strong correlation between the number of stipes produced per sclerotium and the size of sclerotia for both *S. minor* and *S. sclerotiorum* (Fig. 7). The coefficients of determination (R^2) for linear regression between sclerotial size and production of apothecia were 0.82 ($P = 0.0001$) and 0.87 ($P = 0.0001$) for *S. minor* and *S. sclerotiorum*, respectively.

DISCUSSION

Eruptive germination of *S. minor* sclerotia by growth of mycelium and the carpogenic germination of *S. sclerotiorum* sclerotia were affected significantly by soil moisture and temperature. Sclerotial germination by both species, however, was similar in the two soils even though one (SAL) was collected from the Salinas Valley where *S. minor* is the predominant cause of lettuce drop and the other (HUR) from the San Joaquin Valley where *S. sclerotiorum* is predominant. At equivalent matric potentials, the HUR soil had a lower water content than the SAL soil, yet sclerotial germination responses to matric potential were similar in both soils. This suggests that soil matric potential is a more important variable for sclerotial germination than soil water content. The restricted germination of *S. minor* at saturation (0 MPa), however, may be due to limited gas exchange with the air at saturated water content. Although rates of sclerotial equilibration with soil moisture were not measured directly, the rapid rates of water uptake and loss by sclerotia in water and air, respectively, suggested that sclerotia equilibrated with soil matric potential within approximately 1 day.

Stipes from the sclerotia of *S. sclerotiorum* were observed at approximately 4 weeks, reached a peak between 7 and 8 weeks, and then declined after incubation in temperature and moisture combinations that were favorable. Apothecia from the expanded tips of the stipes formed approximately a week after the stipes were observed. In contrast, eruptive germination of a majority of *S. minor* sclerotia occurred within 1 to 2 weeks of incubation at optimal treatment combinations; only a few additional sclerotia

germinated over the following 2 weeks. At the termination of the experiments, no additional sclerotia of either *S. sclerotiorum* or *S. minor* germinated. Thus, the time course of events associated with both the carpogenic and eruptive germination was characterized accurately in this study.

Previous studies showed that temperature and soil moisture greatly affect sclerotial germination in both *S. minor* and *S.*

TABLE 4. Analysis of variance on effects of soil temperature (Temp), moisture (Moist), and soil type on sclerotial germination and viability of *Sclerotinia sclerotiorum*^a

Variable, source ^b	df ^c	F	P > F ^d
Germination			
Temp	7	73.03	0.0001
Temp × Temp	7	60.41	0.0001
Moist	270	4.98	0.0265
Moist × Moist	270	40.18	0.0001
Soil	270	1.06	0.3044
Temp × Soil	270	3.18	0.0756
Moist × Soil	270	7.51	0.0066
Temp × Moist × Soil	1270	5.16	0.0239
Apothecia			
Temp	10	18.13	0.0018
Moist	203	0.08	0.7830
Soil	199	0.21	0.6471
Temp × Temp	9	17.97	0.0019
Moist × Moist	201	9.69	0.0021
Temp × Moist	202	0.58	0.4476
Stipe			
Temp	7	29.26	0.0010
Temp × Temp	7	24.36	0.0017
Moist	273	0.18	0.6687
Moist × Moist	273	7.09	0.0082
Soil	273	0.33	0.5680
Viability			
Temp	38	3.20	0.0818
Moist	662	1.54	0.2115
Temp × Moist	662	4.90	0.0271
Soil	654	3.57	0.0593

^a Data were derived from all 20 sclerotia per isolate, including those that did not germinate.

^b Germination = percentage of germinated sclerotia in soil. Apothecia = number of apothecia produced per sclerotium. Stipe = number of stipes produced per sclerotium. Viability = viability of the recovered sclerotia that did not germinate in soil during the experiment, calculated as the percentage of the ratio between the number of germinated sclerotia on water agar over the total numbers of sclerotia tested.

^c Degrees of freedom.

^d Probability associated with the F test.

TABLE 3. Viability of *Sclerotinia minor* sclerotia exposed for 12 weeks to various moisture and temperature combinations in Huron (HUR) and Salinas (SAL) soils

Soil, T (°C) ^b	Viability (%) ± standard error of the mean at various soil moisture levels (MPa) ^a					
	0.00	-0.03	-0.07	-0.10	-0.15	-0.30
HUR						
5	68.33 ± 15.00	66.02 ± 12.10	62.90 ± 12.46	44.58 ± 2.84	54.83 ± 8.12	57.33 ± 7.25
10	74.44 ± 9.30	31.52 ± 3.08	64.00 ± 7.62	63.33 ± 9.80	39.80 ± 13.45	62.51 ± 8.21
15	69.43 ± 12.70	55.28 ± 5.59	53.30 ± 8.43	82.08 ± 8.12	56.94 ± 7.10	...
20	64.37 ± 9.23	59.72 ± 8.72	78.10 ± 6.62	77.50 ± 9.47	67.22 ± 8.71	...
25	76.16 ± 8.37	66.45 ± 8.75	72.80 ± 5.93	73.33 ± 5.96	78.73 ± 8.17	...
30	29.17 ± 17.10	67.67 ± 8.74	58.50 ± 8.33	73.71 ± 6.23	75.33 ± 10.75	82.59 ± 6.37
SAL						
5	75.00 ± 12.91	53.15 ± 5.00	62.50 ± 5.74	45.56 ± 8.29	84.01 ± 3.52	58.52 ± 7.47
10	33.33 ± 16.67	76.40 ± 8.08	69.94 ± 8.94	60.14 ± 5.23	72.92 ± 9.36	50.10 ± 6.25
15	69.44 ± 10.34	66.89 ± 5.77	80.28 ± 6.84	54.17 ± 13.81	72.22 ± 6.69	...
20	68.61 ± 4.91	76.25 ± 12.21	90.00 ± 6.12	64.00 ± 18.60	64.72 ± 10.46	...
25	...	77.50 ± 7.04	89.13 ± 3.54	72.22 ± 16.48	73.33 ± 19.44	80.56 ± 12.49
30	58.33 ± 22.05	50.00 ± 6.09	80.36 ± 6.82	85.42 ± 5.24	80.56 ± 12.49	66.50 ± 7.83

^a Viability of the recovered sclerotia that did not germinate during the experiment. Viability was calculated as the number sclerotia germinated on water agar over the total numbers of sclerotia tested. Data are the mean of three isolates ± the standard error of the mean in both soils; ... = data unavailable because no sclerotia were retrieved.

^b T = soil temperature.

sclerotiorum (2). For *S. minor*, sclerotia germinated on agar media at temperatures between 6 and 30°C, with an optimum of 18°C and nearly zero germination at 30°C (26). The moisture range for the mycelial germination of *S. minor* in soil was 0 to -1.5 MPa, with an optimum at -0.03 MPa (2,26). Although the previous studies were conducted in culture and this study was conducted in soil, the results obtained were similar. For *S. sclerotiorum*, sclerotia germinated in water at temperatures between 10 and 25°C, with the optimum at 10°C (1). Carpogenic germination occurred at solute potentials higher than -0.6 MPa (18). In soil, optimum matric potentials for carpogenic germination were between -0.008 and -0.024 MPa, depending on the isolate (17). In the present study, stipe production occurred at 5 to 20°C, with both the lowest and highest temperature for germination being

lower than those of previous reports. At 5°C, the rate of carpogenic germination was low and the time of incubation required was much longer than that at other temperatures in our study, and very slow rates of germination at 5°C may not have been observed in the previous studies (1). Apothecium production by *S. sclerotiorum* was largely confined to 10 and 15°C, with a clear optimum at 15°C, which is higher than the 10°C optimum reported previously (1). Differences in media, soil, or the isolates used may have contributed to the differences in the studies on sclerotial germination (17).

Even though sclerotial germination by *Sclerotinia* spp. can vary with the isolates, media, and other experimental conditions used (1,2,17,18,26), we found that mycelial germination by *S. minor* and carpogenic germination by *S. sclerotiorum* had generally simi-

Fig. 4. Carpogenic germination of *Sclerotinia sclerotiorum* sclerotia incubated at various moisture and temperature combinations in Huron (HUR) and Salinas (SAL) soils. Each line represents germination at one matric potential after 12 weeks of incubation. Each data point is the mean of three isolates as the response of the three isolates to the treatments was similar. Vertical bars are standard errors.

Fig. 5. Number of apothecia produced per sclerotium of *Sclerotinia sclerotiorum* incubated at various moisture and temperature combinations in Huron (HUR) and Salinas (SAL) soils. Apothecia were counted only for the sclerotia that had carpogenic germination. Each line represents germination at one matric potential after 12 weeks of incubation. Each data point represents the mean of three isolates. Vertical bars are standard errors.

lar responses to soil moisture and temperature. The major exceptions were that sclerotia of *S. minor* germinated more at 25°C and at -0.3 MPa and less at 0 MPa than did sclerotia of *S. sclerotiorum*.

Although germination was the main focus of this study, we also examined the viability of sclerotia that were retrieved after imposing the treatment combinations for 12 weeks in soil. When the moisture was optimal for germination, viability of remaining sclerotia at temperatures >30°C was greatly reduced. Studies evaluating viability of sclerotia are generally of a long-term nature (9). However, results in this study provided information on whether the sclerotia that failed to germinate during the experiments were nonviable. Although the survival of ungerminated sclerotia finally recovered from the soil varied between moisture and temperature treatments, viability did not vary with any one variable systematically. These results are in general agreement with earlier research (3), which showed that sclerotia survive longer periods

than maintained in this study at temperatures <35°C and soil matric potentials between 0 and -43 MPa. The temperature and moisture treatments used here are more likely to have indirect effects on viability because of changes in the microorganisms that may affect sclerotial survival.

S. minor apothecia have not been observed in lettuce production fields in California, although they have been reported in other areas (21,22). Results from this study clearly established that size of sclerotia is a major determinant of whether or not a sclerotium produces apothecia. Even *S. sclerotiorum* sclerotia were unable to produce apothecia if their size was very small, consistent with previous research (15,23). This is the first study, however, that has identified sclerotial size as a major factor for the production of apothecia by *S. minor*. Even though we observed the production of apothecia by this species in sclerotial aggregates, occurrence of sclerotial aggregates in field soil is rare. Repeated sampling of soil both from commercial and experimental fields in California over the years and assaying for *S. minor* sclerotia has mostly yielded solitary sclerotia (K. V. Subbarao, unpublished data). Even though

Fig. 6. Viability of *Sclerotinia sclerotiorum* sclerotia at various moisture and temperature combinations in Huron (HUR) and Salinas (SAL) soils. The viability was tested only on the sclerotia that did not produce stipes during the experiment. Each line represents viability at one matric potential. Each data point represents the mean of three isolates. Vertical bars are standard errors.

Fig. 7. Relationship between the number of stipes produced per sclerotium and size of *Sclerotinia minor* and *S. sclerotiorum* sclerotia. Size of sclerotia was determined by multiplying the measured length and width. Isolates SM01 of *S. minor* and SS01, SS03, and SS04 of *S. sclerotiorum* were used in the experiment. Sclerotia with different sizes were incubated at -0.03 MPa in soil disks (Salinas, CA [SAL]) at 15°C under a 12-h light-dark regime in a growth chamber. At the end of experiment (approximately 6 weeks), sclerotia with stipes and the number of stipes in each germinated sclerotium were counted, and the sclerotia size was measured. Size of aggregated sclerotia was measured as a single sclerotium piece.

sclerotia in aggregates might form on infected plants in the field, tillage operations, during both residue incorporation and subsequent field preparation, have the potential to fragment these aggregates. The smaller fragments may not possess sufficient reserves to support the production of apothecia. Instead, they follow the eruptive mycelial germination mode when appropriate soil conditions are encountered (2). Factors other than sclerotial size also may have a role in triggering the production of apothecia.

Factors affecting sclerotial germination and survival are also important for subsequent infection of lettuce by *S. minor* and *S. sclerotiorum*. Soil temperature and moisture during the winter months at the two locations from which the soils were taken were within the range of temperature and moisture treatments included in this study (*unpublished data*). Winter soil temperatures in the two locations ranged between 10 and 18°C. Thus, they were not limiting for germination or survival of sclerotia. However, soil and cropping conditions are different in the two locations during spring and summer, which may affect survival and germination of sclerotia in both species. In the Salinas Valley, at least two lettuce crops per year are grown in a majority of fields, and lettuce is usually rotated with cauliflower, also a host of *S. minor*. This continuous cropping, especially with hosts of the pathogen, can lead to rapid accumulation of *S. minor* sclerotia. However, in the San Joaquin Valley, lettuce is grown only during the winter months and, owing to high temperatures during the summer months, the fields are planted with alfalfa or other nonhosts of *S. minor*. Soil temperatures in the San Joaquin Valley can approach 35°C for several days during the summer. In contrast, temperatures rarely exceed 25°C in the Salinas Valley. Thus, higher temperatures in the San Joaquin Valley can be especially damaging to smaller sclerotia produced by *S. minor*.

Following carpogenic germination, several factors affect ascospore release, spread, germination, and infection. Even in the San Joaquin Valley, ascospore infection of lettuce by *S. sclerotiorum* was observed only in certain seasons (19), when intermittent rainfall caused soil saturation and extended leaf wetness. Another factor possibly limiting ascospore infection in the Salinas Valley is leaf wetness. Long periods of leaf wetness are required for ascospores of *S. sclerotiorum* to infect (2,29), and factors affecting air movement in the plant canopy also are involved in infection. Infection of bean occurs when air circulation is limited (2). The wind speed during the lettuce season in Salinas is almost double that of Huron (CIMMIS database, California), which could influence the leaf wetness as well as ascospore release, deposition, survival, and germination on lettuce plants. Thus, environmental conditions other than soil temperature and moisture may explain the limited infection of lettuce by *S. sclerotiorum* in the Salinas Valley and *S. minor* in the San Joaquin Valley.

In summary, soil temperature and moisture are critical factors that affect the sclerotial germination and survival of *S. minor* and *S. sclerotiorum*. Carpogenic germination occurs in sclerotia large enough to support the production of apothecia. Along with some other environmental and agricultural factors, they also play an important role limiting the geographic distribution of the two *Sclerotinia* spp. in California.

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