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Effects of Soil Temperature, Moisture, and Burial Depths on Carpogenic Germination of *Sclerotinia sclerotiorum* and *S. minor*

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ABSTRACT

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Extensive studies have been conducted on the carpogenic germination of *Sclerotinia sclerotiorum,* but carpogenic germination in *S. minor* has not been studied adequately. It remains unclear why apothecia of this pathogen have seldom been observed in nature. In this study, a new method was developed to produce apothecia in the absence of soil or sand, and carpogenic germination without preconditioning was recorded for 95 of the 96 *S. sclerotiorum* isolates tested. Carpogenic germination of the two species was compared under a variety of temperature, soil moisture, burial depths, and short periods of high temperature and low soil moisture. The optimal temperatures for rapid germination and for maximum germination rates were both lower for *S. minor* than for *S. sclerotiorum*. The temperature range for carpogenic germination was also narrower for *S. minor* than for *S. sclerotiorum*. A 5-day period at 30°C, either starting on the 10th or 20th day of incubation, did not significantly affect carpogenic germination of *S. sclerotiorum*. For both *S. minor* and *S. sclerotiorum*, the percentage of carpogenically germinated sclerotia increased as soil water potential increased from –0.3 to –0.01 MPa. In the greenhouse, a 10- or 20-day dry period completely arrested carpogenic germination of *S. sclerotiorum*, and new apothecia appeared after an interval of 35 days following rewetting, similar to the initial carpogenic germination regardless of when the dry period was imposed. In naturally infested fields, the number of sclerotia in 100 cc of soil decreased as depth increased from 0 to 10 cm before tillage, but became uniform between 0 and 10 cm after conventional tillage for both species. Most apothecia of *S. minor* were, however, produced from sclerotia located at a depth shallower than 0.5 cm while some apothecia of *S. sclerotiorum* were produced from sclerotia located as deep as 4 to 5 cm. These results provide the much needed information to assess the epidemiological roles of inoculum from sexual reproduction in diseases caused by the two *Sclerotinia* species in different geographical regions. However, more studies on effects of shorter and incompletely dry periods are still needed to predict production of apothecia of *S. sclerotiorum* in commercial fields under fluctuating soil temperature and moisture.

Additional keywords: fungal biology.

Sclerotinia sclerotiorum (Lib) de Bary and *S. minor* Jagger are two closely related plant pathogens that cause significant losses worldwide in many commercial crops including lettuce, beans, sunflower, peanut, and oilseed rape (8,28,36). Both fungi survive mainly as sclerotia in soil. Sclerotia of both species can germinate either carpogenically to form apothecia that in turn release ascospores for infection, or myceliogenically (eruptively for *S. minor*) to infect host plants directly. *S. sclerotiorum* has a host range of more than 400 species (8) and a worldwide distribution. *S. minor* has a narrow host range relative to *S. sclerotiorum* (28), and lettuce and peanut are important commercial hosts of this pathogen. For most diseases caused by *S. sclerotiorum*, ascospores produced through carpogenic germination are the primary source of inoculum (1,2,3,31), although the pathogen infects plants occasionally by direct germination of sclerotia (1,2,32,33). Concentrations of airborne ascospores have been related to subsequent infection and disease development in both sunflower and oilseed rape (26,27,44). Airborne ascospore concentration is therefore used as a major predictor of disease incidence. However, carpogenic germination by *S. minor* has been rarely observed in nature, and the fungus mainly infects plants via eruptive germination of sclerotia (4,18,32,33).

Since ascospores play a critical role in the epidemics caused by *S. sclerotiorum*, factors affecting carpogenic germination have been extensively studied to predict the production of apothecia and release of ascospores (12,13,15,21–23,29,34,35,39,43,44). While carpogenic germination by *S. sclerotiorum* has been studied extensively, very little work on the carpogenic germination by *S. minor* has been published (16–20,32*)*. It was presumed that many isolates of *S. minor* could not germinate carpogenically because they were heterothallic (32). It is unclear why carpogenic germination of *S. minor* seldom occurs in nature.

Several factors were found to affect the production of apothecia. Light with wavelength <390 nm is required for stipes to develop into fully expanded apothecia, but not for sclerotia to form apothecium stipes (43). Continuously high soil moisture is required for apothecial development, and even a slight moisture tension prevents apothecial formation (2). Favorable temperature for carpogenic germination of *S. sclerotiorum* is mostly between 10 and 20°C, but the results on the specific temperature requirements differed among laboratories (15,21,24,29,34,37,38, 41). Sclerotia of *S. sclerotiorum* were widely reported to require a preconditioning period under wet and low temperature conditions (24,38), but the preconditioning treatment varied widely. Some reported preconditioning at 0 to 5°C (24,29,34,38,41), others at 8 to 16°C (15,21), and yet others found chilling does not increase carpogenic germination (37). Phillips (34) also found that preconditioning was more effective for sclerotia produced on carrots than for sclerotia formed on natural hosts. Temperature requirements for carpogenic germination of *S. sclerotiorum* are also dependent on the origin of isolates as well as the temperature at which the sclerotia were produced (21). The time required for apothecium production varied widely in different studies, ranging from about 1 to 6 weeks depending on preconditioning treatments (15,34,41).

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Burial depth is another factor affecting the carpogenic germination of *S. sclerotiorum*. Different studies concluded that sclerotia buried at depth 0 to 2 cm produce earlier and more apothecia than those buried deeper (7,30). One reason for this may be that stipes produced from sclerotia deep in the soil cannot reach the soil surface for sunlight that is required for development and expansion of apothecia. It was observed previously during field experiments that sclerotia of *S. sclerotiorum* formed stipes even when they were buried deep in the soil, but no apothecia resulted from those stipes (B. M. Wu and K. V. Subbarao, *unpublished data*). Similar observations were also made by Cook et al. (14) who found that sclerotia at depths of 5 and 10 cm formed apothecium stipes of lengths up to 6.4 cm. Soil aeration (oxygen supply) instead of exhaustion of food reserves was assumed to be responsible for low or no apothecium production from sclerotia deep in the soil (35). Although extensive studies have been done on effects of burial depth on carpogenic germination of *S. sclerotiorum*, it has not been studied how burial depth affects the carpogenic germination of *S. minor*. How sclerotia of *S. sclerotiorum* and *S. minor* are vertically distributed in the commercial fields is also unknown.

Since soil temperature near the soil surface is affected more by the weather conditions, agricultural practices, and other factors than deep in the soil, it fluctuates daily in the soils and this fluctuation is especially large near the soil surface. Similarly, soil moisture near the soil surface also fluctuates daily, and particularly varies according to irrigation cycles. Workneh and Yang (*unpublished data* in reference 29) observed that soil temperature and moisture fluctuated more at the soil surface than at 5-cm depth in soybean fields. Fluctuation of soil temperature and moisture was also observed in carrot (25), peanut (10), and lettuce (B. M. Wu and K. V. Subbarao, *unpublished data*) fields. Although effects of constant soil moisture and temperature on apothecium production have been widely studied, effects of fluctuating soil temperature and moisture have rarely been investigated (29). This information is particularly needed to assess apothecium production for disease forecast since only those sclerotia located in the topsoil produce apothecia in commercial fields.

Comparison of the requirements for carpogenic germination in the two species will not only develop techniques to produce apothecia from sclerotia, but also establish a foundation for further genetic analyses. It potentially will also improve our understanding of the epidemiology of diseases caused by the two species, their geographical distribution worldwide, and the evolutionary processes of fungi in general and thus improve our ability to predict the epidemics caused by the two *Sclerotinia* species. It was hypothesized by the authors that the different geographical distributions of the two species in the world were, in part, due to the different requirements for carpogenic germination. Thus, the objectives of this research were to compare carpogenic germination of the two species under a variety of soil temperature and moisture conditions, to determine the vertical distribution of their sclerotia in the soils before and after conventional tillage and how burial depths of sclerotia affect production of apothecia, and to determine the effects of brief periods of high temperature and low soil moisture on carpogenic germination of *S. sclerotiorum*.

MATERIALS AND METHODS

Production of sclerotia. Two isolates of *S. minor*, Bm005 collected from lettuce in Salinas and Sc2 collected from peanut in Oklahoma (provided by J. P. Damicone), were used in all experiments. Isolates of *S. sclerotiorum* used were BS001, collected from lettuce in Salinas, and BS014, collected from cauliflower in Santa Maria (provided by F. Laemmlen). Potato tubers were peeled and sliced into 1-cm cubic pieces, filled into 500-ml flasks up to 300 ml, and autoclaved twice at 121°C within a 24-h interval. Two or three agar disks from the leading edges of cultures of each isolate on potato dextrose agar (PDA) were transferred and placed on potato pieces in each flask. Flasks were incubated at room temperature (20 ± 3°C) for 3 weeks and then the sclerotia of each isolate were harvested by washing off the potato debris, mycelia, and immature sclerotia. Sclerotia were air-dried, and stored at room temperature for use in experiments within 6 months. Because the size of sclerotia affects carpogenic germination of *S. minor* (18) and only sclerotia of large size germinate carpogenically, to prevent experimental error resulting from the differences in the sclerotium sizes, sclerotia of similar sizes were used in this study. Sclerotia of *S. minor* and *S. sclerotiorum* were sieved with a set of sieves, US No. 10 and No. 8 sieves for *S. minor*, and US No. 6 and No. 4 sieves for *S. sclerotiorum,* to eliminate very small and very large sclerotia. The diameter of sclerotia used in the following experiments ranged from 2.0 to 2.4 mm for *S. minor*, and 3.4 to 4.8 mm for *S. sclerotiorum*.

Effects of constant temperature on carpogenic germination of *S. sclerotiorum* and *S. minor*. Fifty sclerotia of two isolates each of *S. sclerotiorum* and *S. minor* were placed on a 2.54-cm-thick and 7.62-cm-diamter polyurethane foam block (Foamex Intl., San Leandro, CA; density = 16.82 kg/m^3, indentation load deflection = 2.39 kg/cm^2) in a Styrofoam cup (6 cm in height, and diameters at the bottom and top was 7 and 8.5 cm, respectively) with four 2-mm-diameter holes punctured at 1 cm height from the bottom. The cups were covered with 100-mm petri dish lids, placed in a plastic box, and incubated in darkness at 5, 10, 15, 20, and 25°C. Moisture was maintained by misting the sclerotia with sterilized double-distilled water twice a week, and excessive water was removed from time to time from the plastic boxes. Carpogenic germination of sclerotia was evaluated every 2 to 3 days after the first appearance of stipes. The sclerotia with visible stipes were considered germinated carpogenically. The experiments were conducted three times. For each species × replication × temperature combination, a logistic model $g = a/[1 + \exp(-bt + c)]$ (where g is percentage of germinated sclerotia, t is days of incubation, and a, b, and c are parameters) was fitted to the temporal progress of carpogenic germination using nonlinear regression procedure in SAS (version 9.10, SAS Institute Inc., Cary, NC). Then the effects of incubation temperature and *Sclerotinia* species on the estimated parameters a, b, and c were analyzed by analysis of variance using the mixed procedure in SAS.

Validation of the above technique using multiple isolates of *S. sclerotiorum*. Ninety-six isolates of *S. sclerotiorum* collected from the three major lettuce production areas in California, the Salinas, San Joaquin, and Santa Maria Valleys were used. These isolates belonged to 51 different mycelial compatibility groups (MCGs), and among them 16, 7, 6, and 5 isolates belonged to the four most common MCGs A, B, C, and D (45). Fifty sclerotia from each isolate were placed on a polyurethane block as described previously, and incubated at 18°C as this was determined to be the optimal temperature from the above experiments. The cups were monitored for carpogenic germination every 2 to 3 days after the first appearance of apothecium initials. The sclerotia with visible apothecium stipes were considered germinated carpogenically. Repeated measures analysis of variance (ANOVA) was performed using general linear model in SAS to determine differences in carpogenic germination rates among the isolates from the Salinas and San Joaquin Valleys, and among isolates belonging to the four mycelial compatibility groups.

Effects of interrupted temperature on carpogenic germination of *S. sclerotiorum*. Fifty sclerotia from isolates BS001 and BS014 of *S. sclerotiorum* were placed on a polyurethane block in a Styrofoam cup and incubated at 15°C as described previously. Three temperature treatments were included: (i) incubation at 15°C throughout the experiment, (ii) initial incubation at 15°C for 10 days, and then at 30°C for 5 days before returning them for incubation to 15°C for the remainder of the experiment, (iii)

initial incubation at 15°C for 20 days, then at 30°C for 5 days before returning them for incubation to 15°C for the remainder of the experiment. Saturated moisture was maintained by misting sterilized double-distilled water twice a week on the polyurethane blocks. Carpogenic germination of sclerotia was evaluated as described previously. Logistic models were fitted to the progress curves as described previously for each treatment × replication × isolate combination, and ANOVAs were conducted on the estimated parameters as described previously.

Effects of soil moisture on carpogenic germination of *S. sclerotiorum* and *S. minor*. Soils collected from five lettuce fields in the San Joaquin Valley were pooled, pulverized, sieved, and autoclaved. Twenty sclerotia from two isolates each of *S. sclerotiorum* and *S. minor* were mixed with 50 g of soil in a petri dish. Sterilized distilled water was added into the petri dish to achieve water potentials of –0.01, –0.05, –0.1, –0.3, and –3.9 MPa based on a standard moisture curve developed for the same soil sample in a preliminary experiment. The petri dishes were sealed and incubated in darkness at 15°C for 8 weeks, and then carpogenic germination of sclerotia was evaluated based on the criteria described previously. The experiment was conducted three times. The germination percentages were first transformed using $Y = \ln[(y + 0.0001)/(1 - y)]$, and then ANOVA was performed to determine the effects of different soil moisture levels, species, and isolates within species using mixed procedure in SAS. Because isolates within species were not a significant source of variation, germination data from two isolates within each species were combined in the analysis of the effects of soil moisture, species, and their interaction.

Effects of interrupted soil moisture on carpogenic germination of *S. sclerotiorum*. Forty sclerotia each from two isolates of *S. sclerotiorum* were buried 1 cm deep in a 200-cell tray with one sclerotium per cell. The trays were maintained in the greenhouse and watered daily for 10, 20, 30, and 40 days followed by a 10-, 20-, or 40-day dry period during which no water or moisture was provided, soil moisture dropped quickly to –0.2 MPa within 2 days, and reached –0.8 MPa within 4 days. Two replications were included for each treatment in the experiment. After the

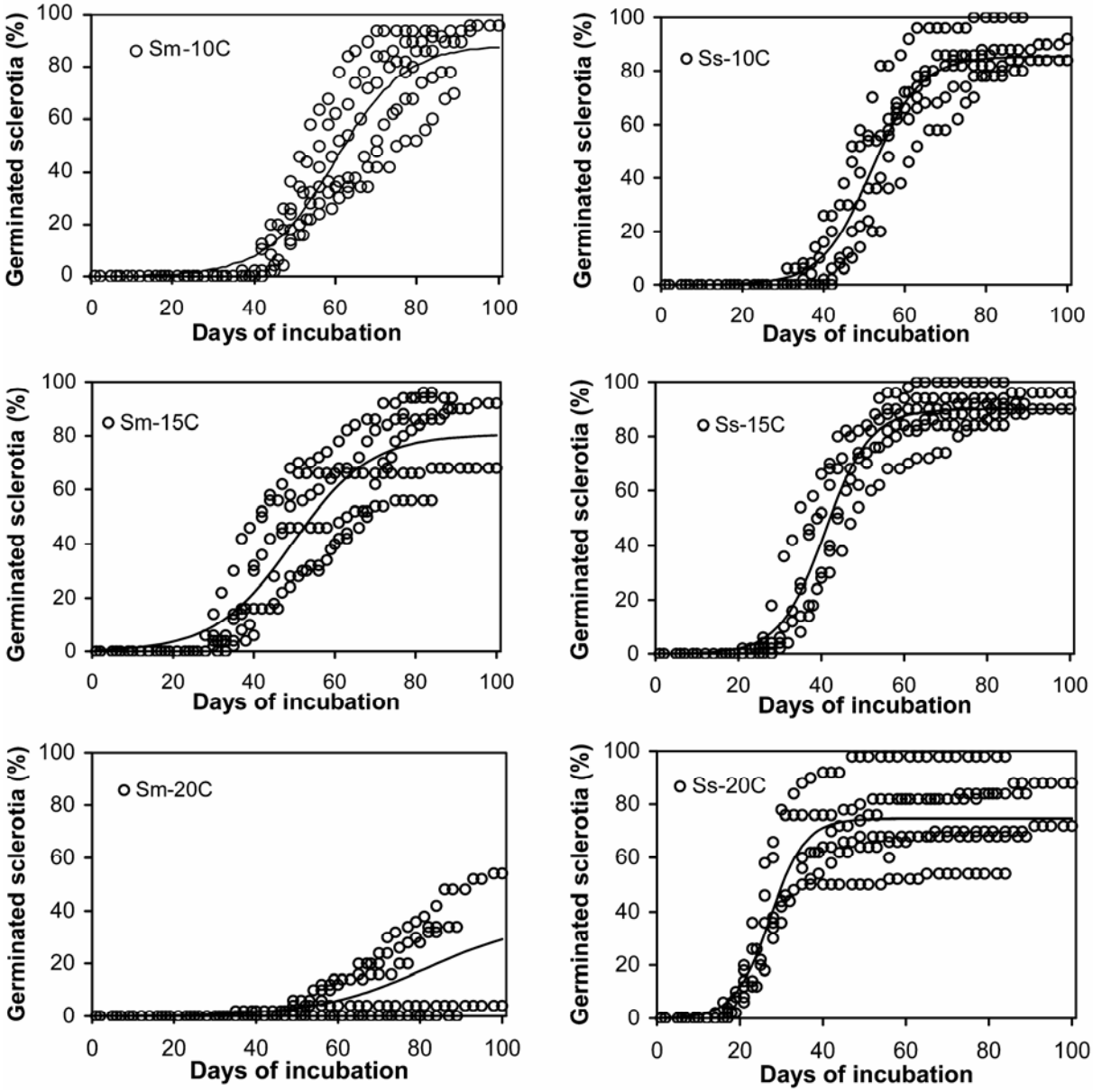

Fig. 1. Carpogenic germination of *Sclerotinia sclerotiorum* (Ss) and *S. minor* (Sm) incubated in darkness at saturated moisture and constant temperature (10 to 20°C) without preconditioning treatment. Sclerotia that formed visible stipes were considered germinated. The dots represent observations, and the solid lines are nonlinear regression lines using logistic model $g = a/[1 + \exp(-bt + c)]$, where g is the percent sclerotia germinated, t is the days of incubation, and a, b, and c are estimated parameters.

corresponding dry-periods, the trays were re-watered daily. Apothecia produced in each cell were counted and recorded three times on 77, 91, and 108 days after the initial watering. Since no apothecia were produced during the dry period, and production of apothecia resumed about 3 weeks after rewetting and increased with time, the average number of apothecia produced from each sclerotium was regressed against the number of days after rewetting in SAS using the NLIN Procedure with a logistic model $g = a/[1 + \exp(-bt + c)]$, where g is number of apothecia produced per sclerotium, t is the number of days since rewetting, and a, b, and c are estimated parameters. The residuals, which were calculated as the observed number minus the predicted number of apothecia per sclerotia, were then analyzed for the effects of starting date, length of dry period, and reading date.

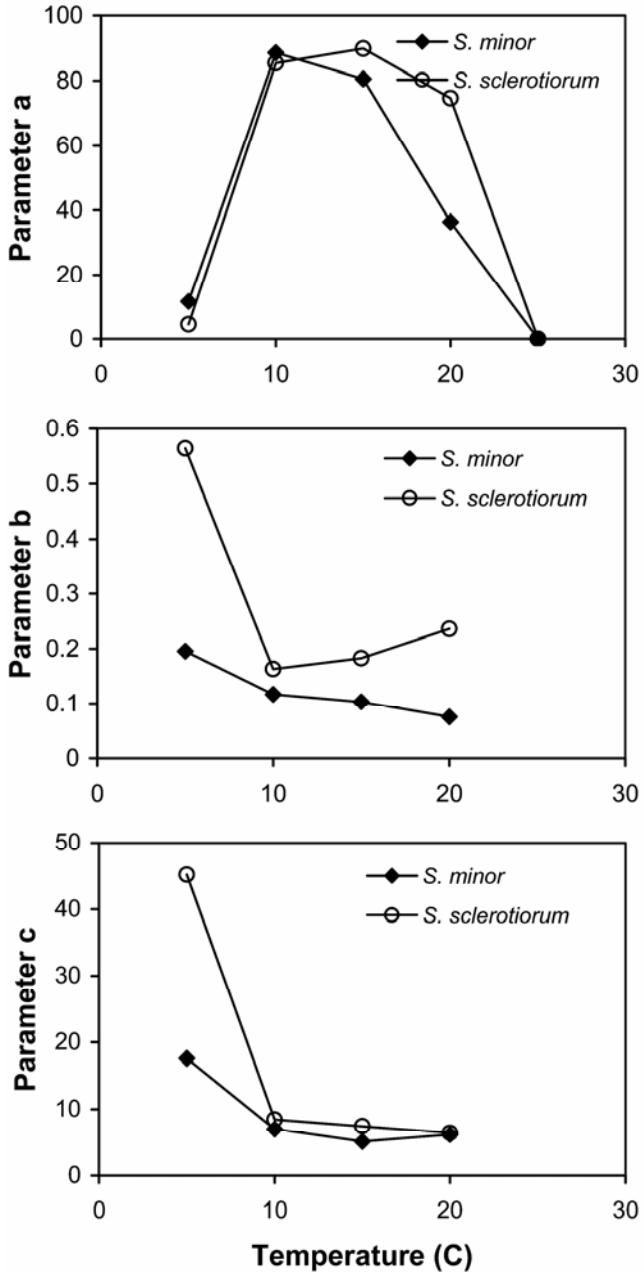

Fig. 2. Effects of temperature on parameters of logistic model $g = a/[1 + \exp(-bt + c)]$, where g is germination percentage of sclerotia, t is the days of incubation at the specified temperature and saturated moisture, and a, b, and c are estimated parameters.

Relationship between the vertical distribution of sclerotia in the field and carpogenic germination. In a lettuce field infested with *S. minor*, and a coriander (also called cilantro, *Coriandum sativum*) field infested with *S. sclerotiorum*, a 500-cc soil sample was collected at 10 random sites at 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 8, and 8 to 10 cm depths before and after conventional tillage. The number of sclerotia of the two *Sclerotinia* species in the sampled soil was assayed using wet sieving (40). ANOVA was conducted on the effects of tillage and depth on the number of sclerotia by using a general linear model in SAS. During the rainy winter, apothecia of *S. sclerotiorum* were randomly selected from the same coriander field, a block of soil around each apothecium was dug out, soil was then carefully removed to locate the sclerotia attached to the apothecia keeping a portion of the soil block intact, and the depth of each sclerotium location was measured as if it was buried in the field.

Because carpogenic germination of *S. minor* in fields has not been observed in commercial fields, the effects of burial depth on carpogenic germination were investigated in a greenhouse experiment. Sclerotia of *S. minor* were uniformly mixed in soil and filled into 12-oz foam cups. The cups were maintained in the greenhouse and watered daily from November through January. Apothecia were randomly identified and the depths of the corresponding sclerotia from which apothecia emerged were determined using the technique described for *S. sclerotiorum*. For *S. sclerotiorum* and *S. minor*, depths of sclerotia from which apothecia emerged were measured for 105 and 50 apothecia, respectively. The apothecia and parent sclerotia were allocated to different groups based on the measured depths, and the frequency of each depth group was calculated as the number of sclerotia in the group/total number of sclerotia measured, and plotted against the burial depth. A negative exponential model was fitted to the relationship between sclerotia germinated and the depth of sclerotia.

RESULTS

Effects of constant temperature on carpogenic germination. Sclerotia of both *S. minor* and *S. sclerotiorum* did not germinate carpogenically at 25°C and less than 5% sclerotia germinated at 5°C. At 10, 15, and 20°C, percentage of sclerotia germinated carpogenically in both species could be estimated with a high degree of confidence using the logistic model $g = a/(1 + e^{-bt + c})$ (Fig. 1). For *S. minor*, the maximum percentage of sclerotia that germinated carpogenically (reflected by the asymptote parameter

TABLE 1. Analysis of variance for the effects of constant temperature (5 to 25°C in 5°C intervals) on the estimated parameters a, b, and c of logistic models, $g = a/[1 + \exp(-bt + c)]$, fitted to the temporal progress curves of carpogenic germination of *Sclerotinia minor* and *S. sclerotiorum*

	Source	Num df	Den df	*F* value	*P* > *F*
Parameter *a*	Species	1	18	32.75	<0.0001
	Temperature	4	18	319.15	<0.0001
	Species × temperature	4	18	21.84	<0.0001
Parameter *b*	Species	1	12	4.00	0.0687
	Temperature	3	12	3.72	0.0423
	Species × temperature	3	12	0.44	0.7254
Parameter *c*	Species	1	12	1.67	0.2209
	Temperature	3	12	6.69	0.0066
	Species × temperature	3	12	0.81	0.5119

a in the logistic model) increased from 5 to 10°C, then declined slightly at 15°C but precipitously at 20°C while the maximum germination percentage for *S. sclerotiorum* peaked at 15°C and declined only slightly at 20°C (Figs. 1 and 2A). The maximum daily germination rate (the biggest increase in germination rate of the curves) occurred earlier in *S. minor* as temperature increased from 10 to 15°C, and was delayed when temperature increased further to 20°C. In contrast, the daily maximum germination rate occurred earliest at 20°C for *S. sclerotiorum* (Fig. 1). This difference between the two species was also reflected by the estimated parameters *b* and *c* of the logistic models (Fig. 2B and C). Parameter *b*, the logistic rate of carpogenic germination, increased when temperature increased from 10 to 20°C for *S. sclerotiorum*, but decreased for *S. minor* (Fig. 2B). Parameter *c*, which in combination with *b* determines the time for increase in germination percentage, showed little change from 10 to 20°C for both species (Fig. 2C). ANOVA demonstrated that incubation temperature significantly affected all three parameters, and the two *Sclerotinia* species showed significant differences in the value of parameters *a* (Table 1).

Variation among isolates. Of the 96 *S. sclerotiorum* isolates tested for germination at 18°C using methods developed in this study, 88.5% germinated carpogenically within 37 days, 99% by 89 days (Fig. 3). The 50 isolates collected from the Salinas Valley on average had significantly higher germination rates than the 38 isolates collected from the San Joaquin Valley (Fig. 4A). When the isolates were grouped based on their MCG, germination rates of isolates in MCG-D were significantly lower than those of isolates in MCG-A, MCG-B, and MCG-C (Fig. 4B). ANOVA revealed significant differences in carpogenic germination rate among the Valleys and MCGs (data not shown).

Effects of interrupted temperature on carpogenic germination. Incubation at 30°C for 5 days, beginning the 10th or 20th day of incubation, did not significantly affect carpogenic germination of *S. sclerotiorum*. Even though germination progress curves for isolate BS001 (Fig. 5A) showed treatment differences with rapid carpogenic germination in treatments with a 5-day 30°C interruption compared to those maintained at constant 15°C, the differences in estimated parameters were not statistically significant (data not shown). The carpogenic germination progress curves of isolate BS014 among the three treatments were nearly identical (Fig. 5B). The three parameters *a*, *b*, and *c*, also were not significantly different between the two isolates (data not shown).

Effects of soil moisture. For both *S. minor* and *S. sclerotiorum*, no sclerotia germinated during the 8-week incubation at 15°C and –3.9 MPa, and the percentage of carpogenically germinated sclerotia increased as soil water potential increased from –0.3 to –0.01 MPa (Fig. 6). The percentages of carpogenic germination were higher for *S. minor* than for *S. sclerotiorum* at water potentials between –0.3 to –0.01 MPa (Fig. 6). ANOVA demonstrated that carpogenic germination of sclerotia was significantly affected by soil water potential for both species, and that the two species significantly differed in their response to soil water potential and was consistent across the soil water potential treatments tested (Table 2).

Effects of interrupted soil moisture. In greenhouse experiments, a 10- or 20-day dry period completely arrested carpogenic germination of *S. sclerotiorum*, with no new apothecia during the dry period or the period immediately after termination of the dry period. The duration between rewetting and the appearance of new apothecia was about 35 days regardless of when the dry period started and how long it lasted (Fig. 7). The relationship between the number of apothecia produced per sclerotium and the days since rewetting could be fitted well with a logistic model. ANOVA on the residuals showed insignificant influence of reading date, beginning of dry period, and the length of dry period (Table 3).

Vertical distribution of sclerotia in the field and carpogenic germination of sclerotia at different depths. At the end of the

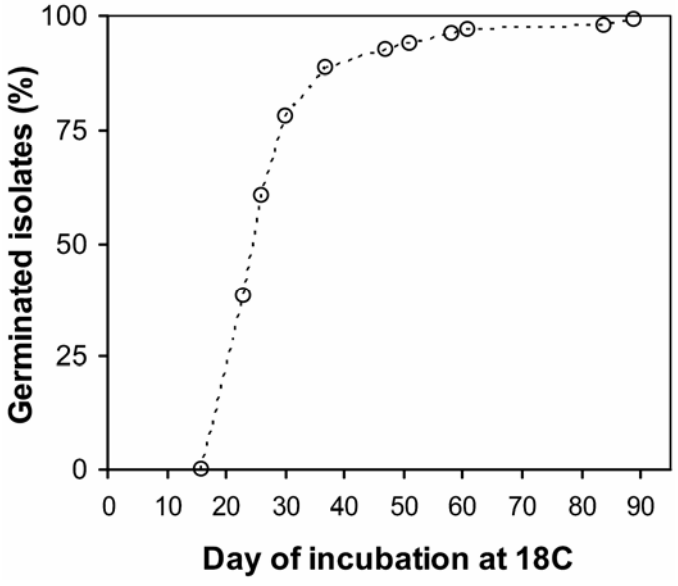

Fig. 3. Percentage of isolates that germinated carpogenically (96 California isolates were tested) during incubation at constant temperature 18°C using the new method without preconditioning (chilling) treatment. An isolate was considered germinated if any of the sclerotia germinated carpogenically.

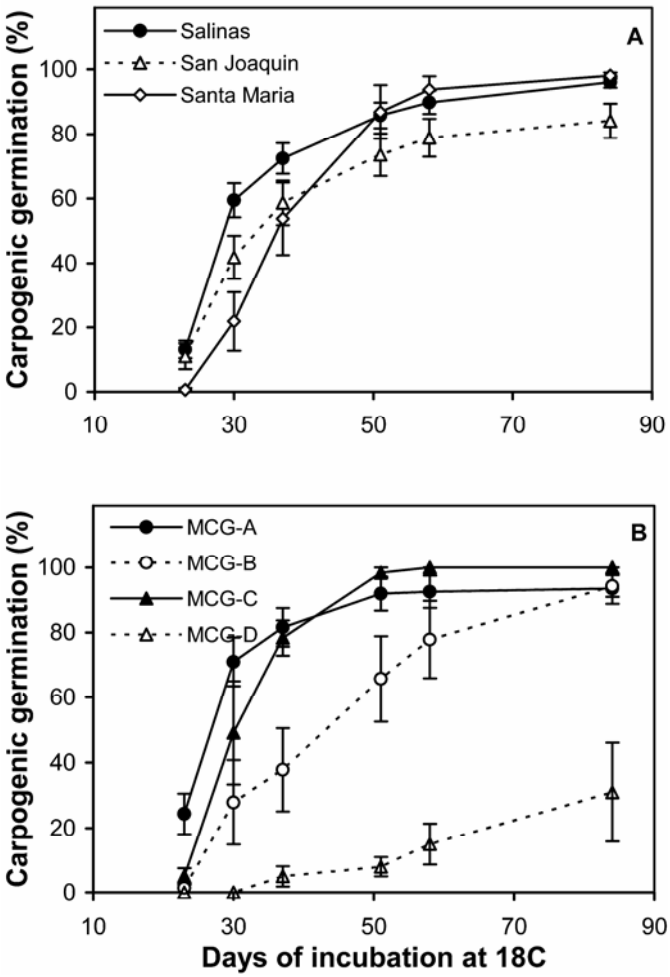

Fig. 4. Difference in percentage of sclerotia germinated carpogenically among **A,** isolates collected from different locations in California, and **B,** those belonging to different mycelium compatibility groups (MCG-A to MCG-D).

fall lettuce crop but before disking the residue, the top soil at 0 to 1 cm and 1 to 2 cm depths contained significantly more sclerotia of *S. minor* than at deeper depths, and this difference disappeared after conventional tillage (Fig. 8A). In a coriander field infested with *S. sclerotiorum*, the number of sclerotia in soil also decreased with depth before tillage, and showed almost uniform distribution across the depths after conventional tillage (Fig. 8A). ANOVA revealed significant difference in the density of sclerotia in the soil among different depths before tillage, but the number of sclerotia at different depths after tillage was not significantly different (data not shown). Given the near-uniform distribution of sclerotia in the soil (*S. minor* in greenhouse, and *S. sclerotiorum* in the field), most apothecia of *S. minor* were produced from sclerotia located at <0.5 cm depth while many apothecia of *S. sclerotiorum* were produced from sclerotia buried at 2 cm or deeper (Fig. 8B). The maximum depth from which a sclerotium of *S. minor* and *S. sclerotiorum* produced apothecia was 0.8 and 4.0 cm, respectively. Negative exponential model fitted the curves well for both species with a much greater decline rate for *S. minor* than for *S. sclerotiorum* (Fig. 8B).

DISCUSSION

Using a simple but novel method, high percentages of carpogenic germination were achieved for both *S. sclerotiorum* and *S. minor* sclerotia regardless of the isolates or their origin. Unlike previous studies that reported the requirement of a preconditioning treatment for carpogenic germination of *S. sclerotiorum* (15,21,24,29,34,38,41), 95 out of 96 isolates tested germinated carpogenically without a chilling period. The new method is different from other methods in that it successfully integrated all factors reported to be necessary to promote carpogenic germination of sclerotia. High moisture, optimal temperature, and oxygen were provided, and potential inhibitors, if any, as reported (11), removed by the sterilized water sprayed repeatedly on the

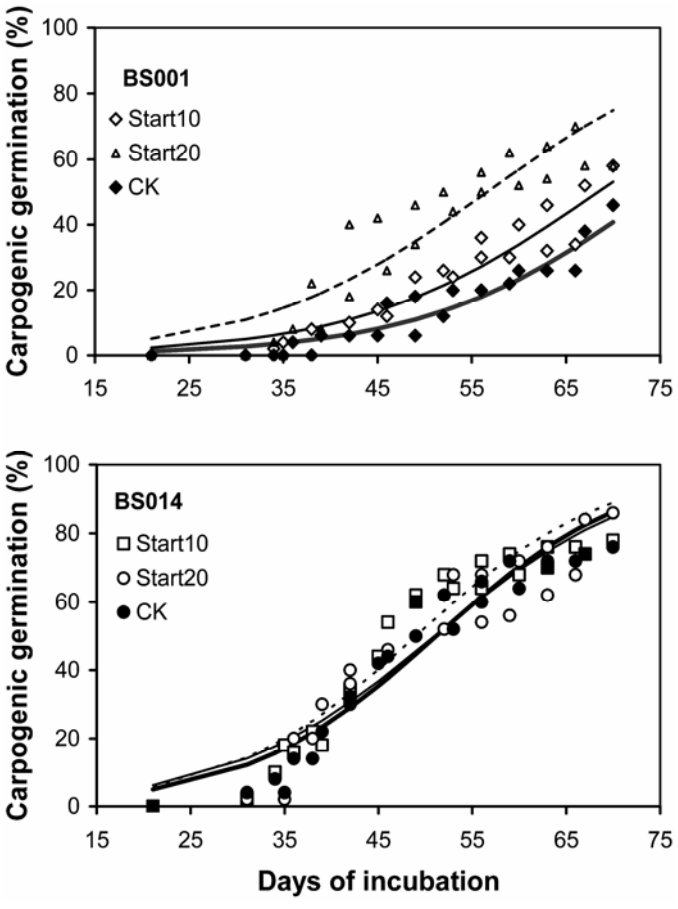

Fig. 5. Carpogenic germination of sclerotia incubated in darkness at 15°C without interruption (CK) or with a 5-day period at 30°C begun on the 10th and 20th day of incubation. Sclerotia that formed visible stipes were considered germinated. The lines are predicted data from the nonlinear regression using a logistic model, $g = a/[1 + \exp(-bt + c)]$, where g is the percent sclerotia germinated, t is the days of incubation, and a, b, and c are estimated parameters.

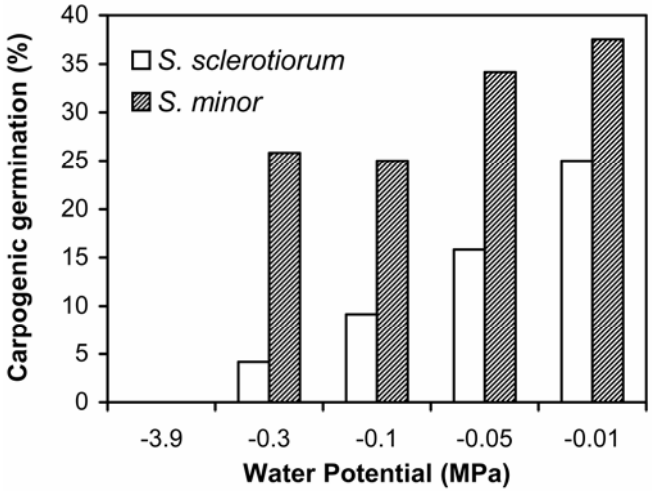

Fig. 6. Carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor* after 8 weeks incubation in darkness at 15°C and different soil moisture conditions. Sclerotia that formed visible stipes were considered germinated.

TABLE 2. Analysis of variance for the effects of constant soil water potential ranging from –3.9 to –0.1 MPa, on carpogenic germination[a] of *Sclerotinia minor* and *S. sclerotiorum*

Source	Num df	Den df	*F* value	*P* > *F*
Species	1	18	10.44	0.0046
Soil moisture	4	18	20.73	<0.0001
Species × soil moisture	4	18	2.45	0.0838

[a] Germination data from two isolates (each consisted of 20 sclerotia per treatment and replication) within each species were combined and transformed ($\ln[(y + 0.0001)/(1 - y)]$) for the analysis.

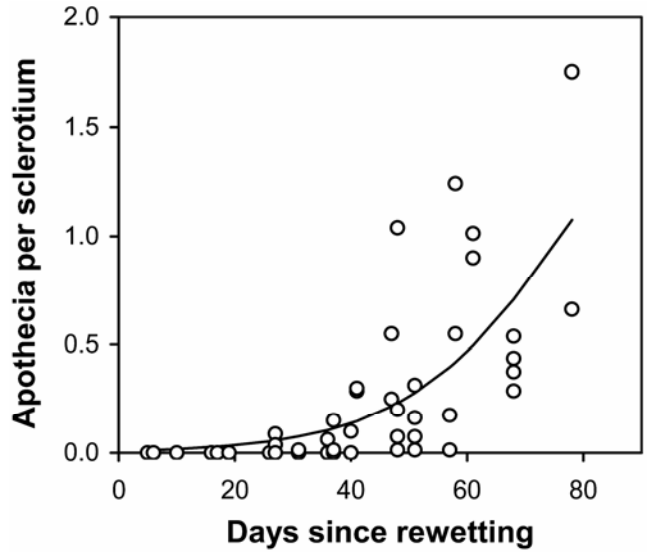

Fig. 7. Apothecium production by *Sclerotinia sclerotiorum* in greenhouse after rewetting that was preceded by a 10- to 40-day dry period that began 20- to 40-days post initial watering. The dots represent observations, and the solid lines are predicted data generated from nonlinear regression using a logistic model $g = a/[1+\exp(-bt + c)]$, where g is the number of apothecia per sclerotium, t is the days of incubation, and a, b, and c are estimated parameters.

sclerotia. This method significantly reduced the time required to produce ascospores of *S. sclerotiorum*, from about 2 months or longer for most reported studies (15,21,29,34,41) to about 35 days at 18 to 20°C for most isolates tested. Apothecium stipes were observed in about 25 days and mature apothecia in about 35 days under optimal conditions. This soilless method also contributed to few or no contamination problems while significantly shortening the time required for ascospore production, and facilitated the observation and handling of apothecia during their development. This technique is therefore useful in studies that require reliable production of apothecia, especially for genetic studies that require sexual progenies produced under axenic conditions from a small number of sclerotia.

All *S. sclerotiorum* isolates evaluated in this study were from California and did not require preconditioning for carpogenic germination using the new method. Whether this method is universally applicable to isolates from other regions as well is, however, unclear at this stage, and more isolates from other geographical regions including isolates from areas reported to require preconditioning need to be evaluated to arrive at similar conclusions. Carpogenic germination has been reported to be dependent on the origin of isolates (21). Even though the agroclimatic profiles of the Salinas Valley are very different from those in the San Joaquin Valley, 95 of the 96 isolates of *S. sclerotiorum* collected from the two Valleys germinated carpogenically using the newly developed method. While differences in the ability of isolates from the two valleys to germinate carpogenically were limited, significant differences in the average carpogenic germination rates were detected between isolates from the two valleys. Carpogenic germination also varied among different batches of sclerotia produced over time, and this is consistent with the previous reports that conditions of sclerotium production also is a factor affecting carpogenic germination of the sclerotia (6,9,22,38).

Although carpogenic germination of *S. sclerotiorum* has been studied extensively, this is the first study comparing the responses of two plant pathogenic *Sclerotinia* species to soil temperature, moisture, and burial depth. These comparative results offer a better understanding of the differential geographical distribution of the two species and the relative epidemiological roles of ascospores in the epidemics caused by the two pathogens. While the two species responded similarly to temperature and soil moisture, they also exhibited differences relative to the optimal temperature and soil moisture. The required temperature was lower for rapid germination and maximum carpogenic germination rate, and optimal temperature range narrower for *S. minor* (10 to 15°C) than for *S. sclerotiorum* (10 to 20°C). The temperature for highest germination rate was 10°C for *S. minor* and 15°C for *S. sclerotiorum*, and the most rapid germination by sclerotia of *S. minor* occurred at 15°C compared with 20°C for sclerotia of *S. sclerotiorum*. The results on *S. minor* from this study were consistent with those obtained by Hawthorne (20), who found this species formed stipes only between 11 to 17°C (20). For both species, the higher the soil moisture, the higher was the rate of carpogenic germination. Results from these studies also revealed that under optimal soil moisture and temperature, *S. minor* took longer to germinate carpogenically than *S. sclerotiorum*.

This is also the first study on effects of short periods of low soil moisture or high temperature on carpogenic germination by *S. sclerotiorum*. The results demonstrated that a 10- to 20-day period of low soil moisture can completely arrest carpogenic germination of *S. sclerotiorum*, and it takes about the same time as the initial watering to resume production of apothecia. However, a 5-day period of high temperature at 30°C had little effect on carpogenic germination of *S. sclerotiorum*. These results provide helpful information for predicting production of apothecia/ascospores in commercial fields where soil temperature fluctuates daily and soil moisture varies between irrigation and rainfall events.

Probably due to the significantly smaller size of sclerotia of *S. minor* relative to those of *S. sclerotiorum*, few sclerotia of *S. minor* buried at depths greater than 0.5 cm germinated carpogenically to form apothecia, while sclerotia of *S. sclerotiorum* germinated carpogenically even when they were buried at a depth of 4 cm. Considering that the top 0.5-cm of soil rarely remains moist over a long period of time except beneath dense plant canopies with adequate irrigation or frequent rainfalls, and soil temperature seldom remains stable around 10°C for prolonged periods, the different requirements found in this study explains why apothecia and ascospores of *S. minor* have been rarely observed in nature. In contrast, apothecia and ascospores are the primary inoculum source for many economically important

TABLE 3. Analysis of variance for the effects of starting date of dry period, length of the dry period, and reading date on the residuals of a logistic model[a] fitted for the relationship between apothecia produced per sclerotium and the days since rewetting date after a 10- to 40-day dry period starting on 20 to 40 days post initial watering

Source	Num df	Den df	*F* value	*P* > *F*
Reading date	2	51	0.09	0.9101
Starting date	2	51	3.12	0.0529
Length	3	51	2.66	0.0581

[a] The model used was $g = a/[1 + \exp(-bt + c)]$, where g is the number of apothecia per sclerotium, t is the days since rewetting, and a, b, and c are estimated parameters.

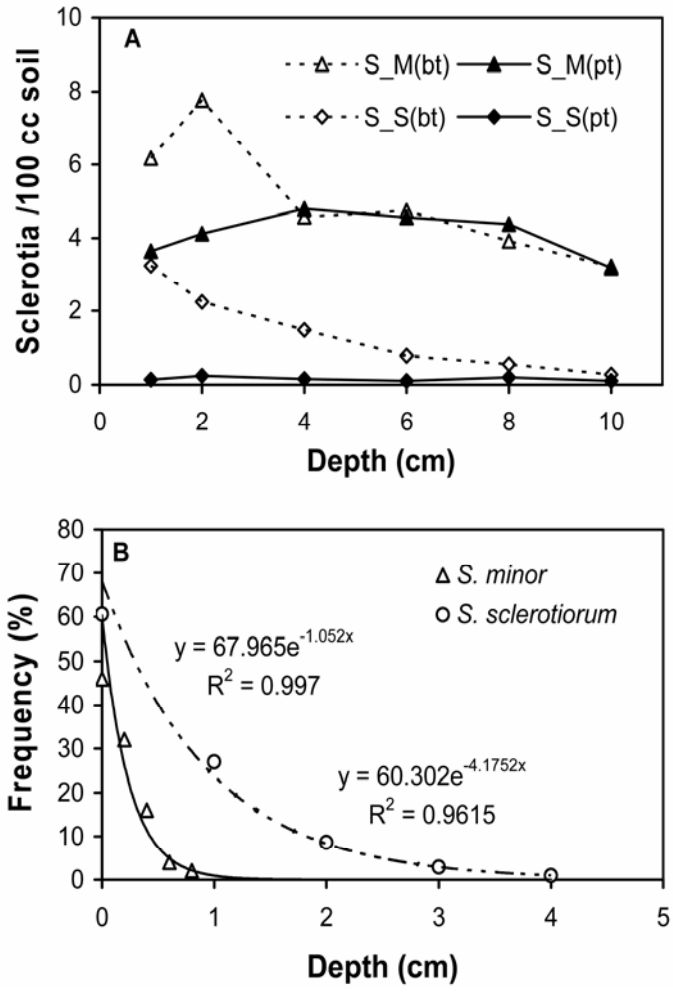

Fig. 8. A, Vertical distribution of total *Sclerotinia minor* and *S. sclerotiorum* sclerotia in field soil before (bt) and post (pt) conventional tillage, and **B,** frequency distribution of sclerotia that formed apothecia over different depths in the soil.

diseases caused by *S. sclerotiorum*. Ascospores from this species generally infect floral tissues such as in head blight of sunflower, white mold of beans and soybeans, and stem rot of canola, while only vegetative tissues are infected in lettuce.

The results of this study also explain in part, why infections of lettuce by airborne ascospore of *S. sclerotiorum* so far have played a minor role in epidemics of lettuce drop in the Salinas Valley, the most important lettuce production area in United States. Sclerotia of *S. sclerotiorum* requires about 35 days of high soil moisture in the top soil to germinate carpogenically to produce apothecia and ascospores. This requirement could hardly be met in commercial lettuce fields during the summer or fall when lettuce is grown in the Salinas Valley, unless canopy density becomes high close to harvest and/or the field is frequently irrigated. Although the requirements for carpogenic germination can be met during the rainy season from winter to early spring and apothecia were observed repeatedly in fields with cover crops in the Salinas Valley in the winter, lettuce usually is not grown in the winter, and is still at early stages of growth in early spring in commercial fields due to the lettuce-free period. This mismatch between production of ascospores and availability of lettuce at the appropriate growth stage may have contributed to the limited aerial infections of lettuce drop over years. Furthermore, any prolonged (>10 days) dry period resets the clock for carpogenic germination and thus, ascospore infections in lettuce in coastal California rarely occur during the spring and summer lettuce production seasons.

In this study, carpogenic germination rates achieved in the experiment on soil moisture when sclerotia were sealed in petri dishes with soil were much lower than in the experiments on temperature using the new method. One possible explanation for this is that sealing petri dishes helped maintain the soil moisture, but also limited the supply of oxygen which is believed to be required for carpogenic germination (5,42). The restricted supply of oxygen seemed to have a greater effect on *S. sclerotiorum* than on *S. minor*, which is consistent with our observations that effect of ventilation on production of sclerotia is greater for *S. sclerotiorum* than for *S. minor*, and that most sclerotia produced above ground for *S. sclerotiorum* whereas a large number of sclerotia are produced on infected plant parts below the ground by *S. minor* (B. M. Wu and K. V. Subbarao, *unpublished data*), suggesting a greater effect of oxygen on *S. sclerotiorum* than on *S. minor*.

The results of this study provide the much needed information on the epidemiological role of inoculum from sexual reproduction in diseases caused by the two *Sclerotinia* species in different geographical regions. However, because only the effects of longer than 10-day completely dry periods were investigated in this study, additional studies with shorter and incompletely dry periods are still needed to predict production of *S. sclerotiorum* apothecia in commercial fields under fluctuating soil temperature and moisture.

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