Effect of Sclerotial Water Content on Carpogenic Germination of *Sclerotinia sclerotiorum*

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Abstract

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The relationship between moisture content and carpogenic germination (CG) of *Sclerotinia sclerotiorum* sclerotia and the dynamics of sclerotial water imbibition were studied in a controlled environment. The study was conducted using laboratory-produced sclerotia from seven *S. sclerotiorum* isolates. The quantity and rate of water imbibition by three sizes of sclerotia was determined gravimetrically in silty clay, sandy loam, and sandy soils maintained at 100, 75, 50, and 25% of soil saturation and in distilled water. Smaller sclerotia imbibed water at a significantly faster rate ($P = 0.05$) than larger sclerotia in water and in soil at all saturation percentages. When buried in soil, small, medium,

and large sclerotia were fully saturated within 5, 15, and 25 h, respectively, in all three soil types and moisture percentages. The effect of sclerotia moisture content on CG was evaluated on sclerotia maintained at 95 to 100, 70 to 80, 40 to 50, and 20 to 30% of their water saturation capacity using cool mist humidifiers. Sclerotial moisture content significantly influenced CG ($P = 0.05$). Maximum CG was observed on fully saturated sclerotia, while no CG was observed below 70 to 80% of saturation. These findings help explain *S. sclerotiorum*'s ability to produce apothecia in soils with relatively low moisture levels.

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most successful fungal plant pathogens, with more than 400 known plant species from 64 botanical families as hosts (4,8). Losses caused by this pathogen are not restricted to reduced yields (13) but also result from reductions in quality of the produce due to contamination of seed with sclerotia (3) and postharvest attacks in storage facilities (22).

Carpogenic germination (CG) of *S. sclerotiorum* sclerotia is a crucial event in the life cycle of this pathogen because it allows for sexual recombination (5,21,31) and for production of ascospores, which are considered the primary inoculum for many of the diseases it causes (1,2,30). A thorough understanding of this process, which is heavily influenced by soil temperature and moisture content (2,3,17,23,34), among other factors, could lead to the development of improved disease management strategies and more accurate disease-warning systems.

Numerous studies have been conducted to evaluate the effect of moisture on CG. Sun and Yang (32) evaluated sand moisture contents "at saturation, very close to saturation, and at 50% of the second moisture level." They concluded that CG was higher when moisture content was closer to saturation. Mila and Yang (23) evaluated the effect of sand moisture content fluctuations on CG; in their study, saturated sand (approximately -0.1 kPa) was allowed to dry to -30 to -40, -60 to -70, or -90 to -100 kPa and then brought back to saturation. This cycle was repeated for the duration of the study. They concluded that moisture fluctuations reduced CG and higher fluctuations were more detrimental than lower fluctuations. A similar result was obtained by Nepal (24), who used soils with textures ranging from sandy to silty clay in a controlled-environment study. In her study, Nepal (24) also observed CG in silty clay soils kept at -412 kPa. Clarkson et al. (12) also studied the effect of soil texture and moisture contents on CG in a controlled environment. In their study, they used peat and silty

clay loam soils set at moisture contents of -0.2 to -412 kPa. Clarkson et al. (12) did not observe CG when moisture contents were less than -100 kPa. In contrast, field studies (1,7,9,15) have reported CG to occur mostly under very wet conditions (i.e., more than -20 kPa). Variations in CG under these conditions has been attributed to the natural variability among isolates without further investigating the effect of sclerotial moisture content on CG or the relationship between sclerotial moisture content and soil moisture content.

Soils act as moisture reservoirs and protect sclerotia from both the sun and weather. Because sclerotia do not have a plant-like vascular system to absorb water, water absorption occurs by imbibition. Therefore, soil texture, structure, and organic matter contents, which are known to affect the soils' ability to retain water (10), could affect the transfer of moisture from soil to sclerotia and, in turn, affect CG. In spite of its importance, the ability of sclerotia to imbibe water from soil and the relationship between sclerotial water content and CG has largely been ignored by researchers. Elucidation of these processes would improve our understanding of why CG also occurs in soils with moisture levels well below saturation and could lead to the development of more accurate models for management of diseases caused by *S. sclerotiorum* (23). The objectives of this study were to determine (i) the relationship between sclerotial and soil moisture content and (ii) the effect of sclerotial moisture content on CG of sclerotia of *S. sclerotiorum*.

Materials and Methods

The ability of *S. sclerotiorum* sclerotia to imbibe water and the effect of sclerotial moisture content on CG were studied in controlled conditions. Water imbibition by sclerotia was measured both in free water and in soils of three different textures maintained at different soil moisture percentages, while the effect of sclerotial moisture content on CG was evaluated in mist chambers.

Isolate preparation. Seven *S. sclerotiorum* isolates (WM031, WM032, WM080, WM081, WM091, WM107, and WM173) collected from North Dakota fields were used in the study. Isolates were cultured on potato dextrose agar (PDA) in polystyrene petri dishes (9 cm in diameter) at 21°C under continuous light. Multiple agar plugs containing hyphal tips from 5-cm-diameter colonies were transferred into 1-liter conical flasks containing corn meal sand medium (60 g of white sand, 3 g of yellow corn meal [Quaker, Chicago], and 16 ml of 0.1 M phosphate buffer, pH 5.8)

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(18). The cultures were incubated for 30 to 40 days at 21°C. To facilitate colonization and promote sclerotia production, the medium was stirred weekly using a sterile spatula. Sclerotia were harvested by wet sieving and air dried at 21°C for 24 h before being classified by size using U.S. Standard Brass Test Sieve numbers 4, 8, and 18 (Fisherbrand, Pittsburgh); these sieves have nominal openings of 4.75, 2.36, and 1 mm, respectively. The sieves were stacked with sieve number 8 sandwiched between 4 and 18, and air-dried sclerotia were poured on them. Sclerotia retained in each sieve were considered large, medium, or small, respectively. After sizing, sclerotia were stored at -5°C in petri dishes sealed to avoid contamination and prevent further water loss. At the start of this study, viability of sclerotia was estimated by plating 15 sclerotia from each isolate on PDA and quantifying the percentage of sclerotia that germinated myceliogenically after 3 days of incubation at 21°C. One month prior to CG experiments, sclerotia were conditioned for apothecia formation by alternately freezing and thawing them in 24-h cycles for 8 days at temperatures of -5° and 21°C, respectively (18,25). Sclerotia conditioned in this way were used in all studies described below.

Soil mixture preparation and characterization of physical soil properties. Soil collected from the upper 5 in. of a Fargo silty clay and an Aylmer-Bantry fine sand were air dried for 1 week in a greenhouse at approximately 30°C and passed through U.S. Standard Brass Test Sieve number 10 (2-mm nominal opening) to screen out plant debris. The soils were then autoclaved at 120°C and 103.4 kPa for 45 min. Sterilized soils were mixed in proportions of 1:1 by volume to create an intermediate soil texture, and the three soils were stored for later use. Mechanical analyses of the three soils (soil texture and percentage of silt, sand, and clay contents) were conducted by the North Dakota State University Soil Testing Laboratory in Fargo, ND using the Hydrometer Method (16).

Volumetric water content of all soil mixtures when at 100, 75, 50, and 25% of saturation were estimated using the van Genuchten equation $\theta(h) = \theta_r + ([\theta_s - \theta_r] / [1 + (\alpha h)^\eta]^{1-1/\eta})$, where $\theta(h)$ represents the volumetric water content (cm^3/cm^3) of the soil as a function of h ; h represents the water pressure head at which water is extracted from the soil (cm); θ_r and θ_s represent residual and saturated water contents (cm^3/cm^3), respectively; and α (expressed in $1/\text{cm}$ units) and η are empirical shape parameters (33). These parameters were calculated using the Rosetta software (28,29). Sand, silt, and clay contents (as percentages); bulk density; and water content at 33 and 1,500 kPa were used as inputs for parameter estimation. Soil bulk density was calculated in the lab by oven drying 50 g of soil at 110°C for 24 h. The volume and mass of oven-dried soil was measured and the bulk density was calculated as a ratio of mass per volume of soil (6). Water content at 33 and 1,500 kPa was estimated using the Pressure Plates Method (27) using a 15-bar Ceramic Plate Extractor (model number 1500; Soil Moisture Equipment Corporation, Santa Barbara, CA) through the services of Agvise Laboratories, Fargo, ND. The gravimetric water content, which is expressed in grams of water per gram of soil, was

calculated for each soil sample, dividing its volumetric water content, estimated by the van Genuchten equation, by the sample's bulk density (6). This allowed the estimation of water contents at 25, 50, 75, and 100% saturation in a sample by weight.

Water imbibition by sclerotia. The rate of sclerotial water imbibition (water uptake) was measured in free water and in soil using a replicated completely randomized design with a factorial arrangement of treatments. Experiments in both environments were conducted at 21°C with 10 sclerotia/replication and the experiments were repeated once.

Imbibition in free water was studied using four isolates (WM031, WM080, WM081, and WM091) and three sclerotial sizes (large, medium, and small). Treatments were replicated four times and the entire experiment was conducted twice. The weight of each group of 10 sclerotia was recorded at the beginning of the experiment. Sclerotia were then immersed in 25 ml of distilled water and weighed at 2-min intervals for the first 10 min, every 10 min for the next 50 min, and then at hourly intervals until the weight remained constant. At that time, sclerotia were considered to be saturated. Prior to each weighing, sclerotia were lightly tapped in a paper towel to eliminate water from their surface. The maximum amount of water a sclerotium can absorb was calculated by subtracting the dry weight of sclerotia from its final constant weight and dividing that by 10.

Water imbibition in soil was also evaluated. Because the isolate-sclerotial size interaction in free water was not significant ($P > 0.05$), only large, medium, and small sclerotia of isolate WM031 were used for this study. Water uptake was measured on 200 g of Fargo silty clay, Aylmer Bantry fine sand, and a 1:1 mix of both that had a loamy sand texture. Treatments were replicated three times and the entire experiment was repeated once. Approximately 200 g of soil from each type was placed in plastic containers with a lid. Dry weight of 10 sclerotia was measured and then the sclerotia were covered with a single layer of cheesecloth and buried between two layers of soil in the center of each dish. Soils were then moistened to 100, 75, 50, and 25% of their respective saturation capacity by carefully pouring the appropriate amount of water uniformly round the edges of each container and avoiding depositing water directly on sclerotia. Containers were closed to prevent loss of moisture. Sclerotia were taken off the soil to be weighed every hour until a constant weight was obtained. Care was exerted to free sclerotia of soil particles when taking their weight. The process of retrieving, weighing, and returning sclerotia to each container took less than 1 min and the containers were kept closed during the weighing process.

CG in relation to sclerotial moisture content. The effect of sclerotial moisture content on CG was evaluated in moist chambers created using Ziploc plastic boxes (25 by 15 by 8 cm; SC Johnson & Son, Inc., Racine, WI) and cool mist humidifiers (Vicks Ultrasonic Humidifier, model V5100NS; Hudson, NY) (Fig. 1). Several preliminary trials were conducted to identify misting schedules that would maintain sclerotia at 95 to 100, 70 to 80, 40 to 50, and 20 to 30% of saturation. During these trials, the temperature of the chamber was kept at 18 and 14°C, day and night, respectively, and sclerotia were weighed prior to the start of each run and before and after each misting period for 2 consecutive weeks to confirm that they remained within the desired moisture level. Setting the humidifiers at minimum volume discharge and operating them for 15 min every 6 h kept sclerotia at 95 to 100% saturation, whereas operating it for 30 min every 12, 24, or 36 h kept sclerotia at 70 to 80, 40 to 50, and 20 to 30% of saturation, respectively. Once these settings were identified, the experiment was conducted three times. The experiment was organized as a split-split plot design, where moisture levels were considered main plots, isolates were considered subplots, and sclerotial sizes were sub-subplots. During the first two trials, isolates WM031, WM080, WM081, and WM091 were used. In the third trial, isolates WM032, WM107, and WM173 were used. Each experimental unit consisted of 15 sclerotia. Sclerotia were visually inspected every 5 days until the first stipe primordium was observed; then, data on number of sclerotia

Fig. 1. Diagram of the mist chambers used to study the influence of sclerotial moisture content on carpogenic germination of *Sclerotinia sclerotiorum*. Cool mist machines were activated periodically to keep sclerotia at moisture percentages required by experimental design. Sclerotia were placed on petri dish bottoms. Moist air flowed from the machine into the box via a clear plastic tube and exited the box on the opposite side through three ventilation openings. Misting chambers were placed in growth chambers for incubation. Diagram was not drawn to scale.

germinated and number of apothecia per sclerotium were recorded every 2 days. A sclerotium was considered to be germinated when stipes were at least 1 mm in length.

Data analyses. To calculate the rate of water imbibition by sclerotia in water or soil, the amount of water imbibed by sclerotia during different incubation periods was calculated by subtracting the weight of sclerotia at a given time from its initial dry weight. These data were fitted to a modified monomolecular growth model (11). The linearized form of the monomolecular model [$dy/dt = r(1 - y)$] is expressed as $\ln[1/(1 - y)] = \ln[1/(1 - y_0)] + r_M t$, where 1 is a correction factor that represents the upper limit of y ; y_0 and y represent the amounts of water imbibed at any given period of time with length t and the total amount of water at saturation, respectively, and r_M represents the rate of water imbibition in milligrams of water per hour. The treatments were then compared using the general linear model procedure (PROC GLM) of SAS (version 9.2; SAS Institute, Inc., Cary, NC) in these studies and the CG studies. Treatment means were compared using Fisher's protected least significance difference ($P = 0.05$). The Brown-Forsythe test was conducted to determine whether variances between data sets were homogeneous. If variances of the data sets were homogeneous and the interactions between treatments and trials were not statistically significant ($P > 0.05$), a combined analysis was conducted.

Results

Physical soil properties. The soils used in this study had large differences in their physical properties as well as in their water-holding capacity (Table 1). The soil with the highest content of clay and silt, the Fargo silty clay, had the lowest bulk density and the highest water retention capacity. Further, at saturation (0 kPa), the silty clay soil retained 103 g of water, which is 1.9 times more water than what was retained by the sandy loam mix (53.3 g) and three times more water than the sandy soil (33.3 g). The differences in water-holding capacity among soils were even larger when measured at the permanent wilting point (−1,500 kPa). At wilting point, the silty clay soil retained 34.7 g of water, which is 14 and 2.5 times the amount of water retained by the sandy (2.5 g) and sandy loam soils (14.0 g), respectively. The matric potential in the silty clay soil increased from −22 to −460 kPa, a 21-fold increase, when soil moisture was reduced from 75 to 25% saturation. A similar reduction in soil moisture resulted in seven- and fourfold increases in matric potential in the sandy loam and sandy soils, respectively (Table 1). Further, when the moisture content of the silty clay soil was at 50% of saturation, its matric potential was −81 kPa. At the same moisture content, the matric potential of the sandy loam and sandy soils was −24 and −19 kPa, respectively. At 25% moisture saturation, the matric potential of the Fargo silty clay soil was −460 kPa, which was 6.5 and 11.8 times larger than that of the sandy loam (−71 kPa) and sandy soils (−39 kPa), respectively.

Sclerotial imbibition in free water. Water imbibition by sclerotia was influenced by their size ($P = 0.05$) but not by their origin (isolate). The monomolecular growth model provided a good fit to the imbibition data, with statistically significant models ($P <$

0.0003) that explained greater than 93% of the variability observed ($r^2 > 0.93$). The rates (r_M) of water imbibition were 0.75, 0.48, and 0.40 mg/h for small, medium, and large sclerotia, respectively. In all three size categories, water imbibition proceeded at a high rate during the first 2 to 4 h, then declined asymptotically as the sclerotia achieved 100% of their maximum moisture uptake. Small sclerotia were 100% saturated in less than 5 h, whereas medium and large sclerotia were saturated in approximately 10 and 15 h, respectively (Fig. 2). By the time sclerotia were saturated, they had doubled in weight regardless of size. Water imbibition was not significantly ($P > 0.05$) affected by isolate–sclerotial size interactions.

Sclerotial imbibition in soil. Sclerotia imbibed water from soil in a manner similar to that in free water; the rate of water imbibition was fast at the beginning when sclerotia were drier, and decreased as sclerotial moisture content increased. The rate of imbibition was unaffected by soil moisture percentage (Fig. 3). In all experiments, sclerotia absorbed more than 70% of their fully saturated water content within 5 h of being buried in soil. In all soil types and moisture contents, small, medium, and large sclerotia became fully saturated in ≤ 5, 15, or 25 h, respectively. The monomolecular model provided a good fit to the imbibition data, with statistically significant models ($P < 0.0001$). The rate (r_M) of water imbibition by sclerotia was not significantly affected by soil mois-

Fig. 2. Effect of size of *Sclerotinia sclerotiorum* sclerotia on its rate of water imbibition in water. Numbers at the beginning of each curve indicate air-dried weight of sclerotia and numbers at the end indicate the weight of fully imbibed sclerotia. Each data point represents the mean weight averaged across four replications and 10 sclerotia in each replication. Error bars correspond to mean standard error.

Table 1. Gravimetric water content, soil matric potential, and soil textural and physical properties of the two soil types and soil mixture used as the soil substrate for sclerotia imbibition experiments

Soil ^y	Content (%)			Density (g/cm ³) ^z	Soil texture	Water content (g) per matric potential (kPa) ^w			Matric potential (kPa) per saturation (%) ^x			
	Sand	Silt	Clay			0	−33	−1,500	100	75	50	25
Silty clay	3.8	52.3	44.0	0.77	Silty clay	103.0	44.2	34.7	0	−22	−81	−460
Fine sand	91.9	6.9	1.3	1.27	Sandy	33.3	4.2	2.5	0	−10	−19	−39
Soil mixture	75.8	16.1	8.2	1.12	Sandy loam	53.8	17.3	14.0	0	−10	−24	−71

^w Gravimetric water content in soils at three different matric potentials per 100 g of oven-dried soil = volumetric water content/bulk density. Volumetric water content estimated at saturation (0 kPa), field capacity (−33 kPa), and permanent wilting point (−1,500 kPa) using pressure plates.

^x Soil matric potential of soils at four different percentages of moisture saturations was calculated using Rosetta software on soils kept at 25, 50, 75, and 100% of their water content at saturation.

^y Soil types = Fargo silty clay, Aylmer Bantry fine sand, and soil mixture produced by mixing the Fargo and Aylmer Bantry soils at a 1:1 (vol/vol) ratio.

^z Bulk density.

ture content ($P > 0.2757$) and soil texture ($P > 0.2869$) but it was by sclerotial size ($P < 0.001$). The rates of water imbibition by sclerotia across all soil moistures and textures were 0.34 ($r^2 = 0.79$, $P < 0.0001$), 0.72 ($r^2 = 0.80$, $P < 0.0001$), and 0.83 ($r^2 = 0.80$, $P < 0.0001$) milligram of water per hour for large, medium, and small sclerotia, respectively. Similarly, significant interactions were detected between soil moisture and sclerotial size ($P < 0.0001$); soil moisture and soil texture ($P = 0.053$); soil texture and sclerotial size ($P = 0.0013$); and soil moisture, soil texture, and sclerotial size ($P < 0.0001$). The rates of water imbibition by small and large sclerotia were relatively insensitive to changes in soil moisture content, whereas the rate of medium sclerotia tended to increase with increased soil moisture content (Fig. 4A). The rate of water imbibition by small and medium sclerotia tended to increase when the soil texture turned sandier but the rate of imbibition by large sclerotia tended to decrease (Fig. 4B).

CG in relation to sclerotial moisture content. The variances of the data sets in first two trials were homogeneous ($P = 0.6368$).

The interactions trial-moisture, trial-size, and trial-moisture-size were not statistically significant ($P = 0.4807$, 0.0741, and 0.2925, respectively) in the combined analyses. Isolates and sclerotial moisture contents were the driving forces behind CG. Their main effect and interactions were statistically significant ($P < 0.0046$) in the third run, where multiple isolates were evaluated. In general, isolateWM032 had the highest mean percent CG, with 37.8% (Table 2). The highest mean CG was obtained when sclerotia were kept at 95 to 100% moisture saturation in all three runs of the study, followed by 70 to 80% moisture saturation. CG did not occur when sclerotia were kept at <70% of moisture saturation (Table 2). Neither the main effect of sclerotial size nor its interaction with sclerotial moisture were significant in any of the trials ($P > 0.2215$), although the latter was significant in the combined analysis ($P = 0.0073$).

The interactions between isolates and sclerotial moisture contents had the strongest influence on CG. Three of the seven isolates (WM080, WM081, and WM091) used in this study did not germi-

Fig. 3. Effect of soil moisture content on water imbibition by air-dried *Sclerotinia sclerotiorum* sclerotia. Soil at A, 25%; B, 50%; C, 75%; and D, 100% of saturation. Each data point represents the average of three replications, 10 sclerotia, and three soil textures. Error bars correspond to mean standard error.

nate carpogenically under any sclerotial saturation percentage. However, when CG occurred, it happened on sclerotia that were kept at $\geq 70\%$ saturation regardless of the isolate (Fig. 5A and B). Four isolates (WM031, WM032, WM173, and WM107) were responsive to increments in sclerotial moisture contents. However, the level of their response differed. Isolate WM032 was the most responsive, with CG increments going from 0 to 60% when the level of moisture saturation of sclerotia increased from the 40 to 50% range to the 70 to 80% range (Fig. 5B). CG of the other three isolates increased from 0 to an average of 10% when sclerotial moisture saturation increased from the 40 to 50% range to the 70 to 80% range. The largest increase in CG occurred when sclerotial moisture saturation increased from the 70 to 80% range to the 95 to 100% range in all isolates but WM107.

The effect of the interactions between sclerotial size and sclerotial moisture saturation levels and between sclerotial size and isolates on CG were significant in the combined analysis of trials 1 and 2 but not in trial 3. In trials 1 and 2, the interaction between sclerotial size and moisture contents was one of magnitude rather than direction. In general, large sclerotia were more responsive to increases in moisture content than the medium and small sclerotia

Fig. 4. Effect of the interaction between A, sclerotial size and soil moisture content and B, sclerotial size and soil texture on the rate of water imbibition by *Sclerotinia sclerotiorum* sclerotia. Error bars correspond to mean standard error.

(Fig. 6A). When kept at 95 to 100% saturation, CG of large sclerotia was almost twice that of the other two sizes. The response of medium sclerotia to increments in sclerotial moisture content was almost identical to that of smaller sclerotia. In the third trial, all sclerotial sizes reacted in a similar fashion to increases in sclerotial moisture content (Fig. 6B). In all three trials, no germination was observed on sclerotia kept at $<70\%$ of moisture saturation. Large sclerotia from isolate WM031 tended to have higher CG than smaller sclerotia (Fig. 7A) but that interaction was not significant ($P = 0.0817$) in the third trial (Fig. 7B).

Discussion

Imbibition of water by *S. sclerotiorum* sclerotia was evaluated in free water and in soils kept at different levels of water saturation. In both environments, $>80\%$ of the water imbibed by sclerotia was taken up within the first 5 h of exposure independent of sclerotial size, soil texture, or soil moisture content. Water temperature, while important, does not have an impact large enough as to keep sclerotia from becoming fully imbibed in ≤ 24 h of exposure (*data not shown*). Sclerotia became fully imbibed even when buried in soils kept at 25% of water saturation, a condition that in this study ranged between -460 and -39 kPa. Water imbibition by sclerotia in soils with moisture at less than 25% were not evaluated in this study, although it would be reasonable to believe that the threshold for water imbibition would not be far below this moisture percentage. The monomolecular growth model (11) provided a good fit to sclerotial water imbibition data in both environments (water and soil), with r^2 values > 0.95 . The rate of imbibition was initially high and then declined asymptotically as sclerotia achieved 100% of their maximum moisture uptake. The rates (r_M) of imbibition calculated in this study were inversely related to the size of sclerotia, a relationship that could be explained, in part, by their differences in surface/volume ratios. If one assumes that sclerotia were perfectly spherical, the surface/volume ratio of a small sclerotium would have been approximately 2.4 and 4.8 times that of a medium

Table 2. Effects of isolate, sclerotial size, and moisture content on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*

Variables ^y	Germination (%) ^x	
	Trials 1 and 2	Trial 3
Isolates		
WM031	11.9	—
WM032	—	37.8 a
WM107	—	6.7 b
WM173	—	11.1 b
$P > F$	—	0.0004
LSD	ND	13.8
Size		
Small	9.4	19.4
Medium	9.7	18.9
Large	16.4	17.2
$P > F$	0.2215	0.8102
LSD	NS	NS
Moisture (%) ^z		
20–30	0.0 b	0.0 c
40–50	0.0 b	0.0 c
70–80	7.0 b	27.4 b
95–100	40.4 a	46.7 a
$P > F$	0.0035	0.0008
LSD	11.1	15.6

^x Dash (—) = isolate not used in this trial, ND = no data, and NS = not significant. The third trial was conducted with a different set of isolates. Data for sclerotial size for trials 1 and 2 were combined for analysis because variances were homogeneous ($P = 0.6368$), no interactions were present ($P = 0.0741$), and trials were not significantly different ($P = 0.1327$). Data for sclerotial moisture content for trials 1 and 2 were combined for analysis because variances were homogeneous ($P = 0.6368$) and no interactions were present ($P = 0.4807$).

^y $P > F$ = the probability associated with the F value and LSD = Fischer's protected least significant difference at $\alpha = 0.05$.

^z Sclerotial moisture content from saturation.

and large sclerotium, respectively. Similarly, a large sclerotium had a roughly 2.7 and 10.7 times larger mass than a medium and small sclerotium, respectively, and could hold almost that much water.

The highest CG was observed on fully saturated sclerotia, which is a result that is in agreement with field observations of apothecia occurring in very wet fields (1,7,9,15) and results of laboratory studies in which sclerotia were incubated in water-saturated substrates (32) or soil-less environments (34). This is the first time, however, that the association between sclerotial moisture content and CG has been studied. No CG was observed on sclerotia kept below 70 to 80% moisture saturation. This saturation percentage could be considered the threshold for CG. Myceliogenic germination (MG) of *S. sclerotiorum* sclerotia, which is an asexual form of reproduction of this pathogen, is also influenced by sclerotial moisture content although no direct evidence, to our knowledge, has been published. Huang et al. (19) observed that MG was highest when sclerotia were incubated in environments with 100% relative humidity (RH), whereas only marginal MG levels were observed when sclerotia were incubated in an 85% RH environment. Although it may be logical to assume that sclerotia kept in 100% RH would eventually reach saturation, incubation at 85% RH does not necessarily imply sclerotia would imbibe water up to 85% of saturation.

Fig. 5. Effect of the interaction between *Sclerotinia sclerotiorum* isolates A, WM031 and B, WM107, WM032, and WM173 and four sclerotial moisture percentages on carpogenic germination. Error bars correspond to mean standard error.

Three of the four isolates used in the first two trials failed to germinate carpogenically at any level of sclerotial moisture saturation. Sclerotia used in these studies had >96% viability and had been conditioned for CG using a methodology successfully applied to produce ascospores of >50 other *S. sclerotiorum* isolates collected from North Dakota (L. E. del Río, *personal communication*). The lack of uniform response to induction of CG, which is characteristic of *S. sclerotiorum*, has been attributed by other researchers to several factors, including temperature, length and type of conditioning period (14,23), geographical origin of the isolates (20), and nutritional level of the sclerotia (25), among others. Nevertheless, whenever CG was observed in this study, it occurred only when sclerotia were kept at >70% of moisture saturation.

The ability of sclerotia to reach and maintain moisture saturation even in soils kept at 25% saturation helps explain why CG could occur in soils with relatively "low" moisture content (12,24,34). We hypothesize that any amount of water in excess of the 25% soil saturation would facilitate the germination process by removing potential inhibitors that may be present in sclerotia (14). Thus, the differences in the amount of CG observed between soils with constant and fluctuating moisture conditions (23,24) would be caused

Fig. 6. Effect of the interaction between three sclerotia sizes and four levels of sclerotial moisture percentages on carpogenic germination of *Sclerotinia sclerotiorum* sclerotia. A, Each data point represents the average germination of isolate WM031; B, each data point represents the average of three other isolates. Error bars correspond to mean standard error.

by differences in the rate at which these inhibitors may be removed, especially in cases when soil moisture fluctuations do not go below 25% of saturation. It is possible that sclerotia could maintain high moisture percentages for short periods of time even if soils dry below the 25% saturation. In such situations, the length of the periods with “low” moisture would be as important or perhaps more important than the degree of dryness reached. Keeping soils at ≥ -80 kPa for 10 to 20 days “arrested CG and reset the clock of germination” (34), whereas allowing soils to dry down to -460 kPa and rewetting them immediately reduces CG but does not arrest it or reset its clock.

The ability of *S. sclerotiorum* sclerotia to undergo CG even when hydrated only up to 70 to 80% of its moisture saturation capacity suggests that CG could take place in soils with less than 25% of water saturation. Detection of *S. sclerotiorum* ascospores in fields under dry conditions supports this conclusion (26). The ability to germinate even in low moisture conditions is a survival trait that could help perpetuate the species; after all, a single *S. sclerotiorum* sclerotium is capable of releasing millions of ascospores (30) and a few of them could succeed in infecting a host. However, the CG levels observed under such conditions might not cause significant economic losses to growers or justify the use of fungicides. Results of this study explain, in part, the ability of *S. sclerotiorum* to germinate carpogenically in rather dry conditions; however, the

physiological mechanisms associated with the germination process are still not clear and deserve additional attention.

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Fig. 7. Effect of the interaction between *Sclerotinia sclerotiorum* isolates A, WM031 and B, WM107, WM032, and WM173 and sclerotial size on carpogenic germination. Error bars correspond to mean standard error.

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