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Environmental Factors for Germination of *Sclerotinia sclerotiorum* Sclerotia

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Abstract

Basal stalk rot of sunflower is an economically important and rather unique disease among crops that are susceptible to *Sclerotinia sclerotiorum*. This disease is the result of myceliogenic germination of sclerotia whereby the vegetative hyphae infect the sunflower below the soil level. In contrast, sunflower head rot and similar diseases of susceptible crops result from carpogenic germination to produce airborne ascospores that infect above ground senescent or wounded tissues. Research was initiated on several factors reported to affect sclerotia germination as a prelude to genomic investigations of myceliogenic and carpogenic germination. Specifically, the effects of inoculum development temperature, sclerotia development temperature, conditioning temperature, conditioning of hydrated and desiccated sclerotia, and the duration of sclerotia desiccation on germination strain Sun-87 sclerotia were reevaluated. As reported previously, we were not able to use conditioning temperature from -20°C to 30°C to differentiate myceliogenic and carpogenic germination for either hydrated or desiccated sclerotia. Besides conditioning temperature, inoculum production temperature, sclerotia formation period and temperature, and desiccation failed to distinguish the two forms of germination. The high level of variability for sclerotia germination between experiments indicates the critical nature of repeating all experiments aimed at understanding factors that influence sclerotia germination. Thus, other methods will be required to discover a reliable and non-confounded method that clearly differentiates myceliogenic and carpogenic germination of *S. sclerotiorum*.

Keywords: Ascospore; Carpogenic; Conditioning; Germination; Head rot; Myceliogenic; Sclerotinia; Stalk rot; Temperature

Introduction

Sclerotinia sclerotiorum is a necrotropic fungal plant pathogen with a broad crop and non-crop host range that produces hard, asexual resting structures called sclerotia [1,2]. Sclerotia are a means by which a quiescent state in the soil can be maintained in the absence of suitable host or conditions favorable for germination and active growth [3]. Myceliogenic germination of sclerotia follow by root invasion of hyphae leads to basal stalk rot disease of sunflower, whereas carpogenic germination leads to head rot [2]. Some information exists on conditions for carpogenic germination; less information exists related to myceliogenic germination.

Huang [4] reported a method that changes the germination behavior from carpogenic to myceliogenic based on the temperature during conditioning of mature sclerotia of strain Sun87. Carpogenic germination occurred exclusively for sclerotia conditioned for 4 weeks at 0.5°C to 25°C; whereas, only myceliogenic germination occurred if conditioned at -20°C to -10°C. Additionally, Huang and Kozub [5] and Huang et al. [6] examined the effects of inoculum (mycelial mat) production temperature and desiccation of sclerotia after development, respectively on germination. Huang's group reported that the favorable inoculum production temperature for carpogenic germination of Sun87 was 10°C for daughter sclerotia formed at 20°C, and that myceliogenic germination of Sun87 occurred most readily when the sclerotia were formed at 20°C to 25°C and were desiccated prior to germination.

We sought a reliable and non-confounded method to obtain exclusively myceliogenic or carpogenic germination to investigate the germination transcriptome regulating the two forms of sclerotial germination. Therefore, the objective of these experiments was to verify that the conditioning temperature treatments [4] would clearly distinguish carpogenic and myceliogenic germination.

Materials and Methods

Sclerotia for *Sclerotinia sclerotiorum* (Lib.) de Bary strains Sun87 (also known as 2148) originating from Canada [7] was provided by Dr. K.Y. Rashid, Agriculture and Agri-Food Canada. Strain 1980 originating from Nebraska [8] was provided by Dr. J. Rollins, University of Florida.

Effect of sclerotia formation period and temperature and conditioning temperature on germination of Sun87

Two experiments were conducted as described below.

Experiment 1: Sclerotia were formed by transferring plugs of mycelial mat to the center of 100 × 15 mm Petri dish containing full strength potato dextrose agar (PDA). These dishes were incubated in the dark at 10°C and 20°C for 4 and 8 weeks to facilitate growth of mycelia and formation of sclerotia (Figure 1). The sclerotia were then transferred to an open Petri dish in a laminar flow hood for a 1 day desiccation treatment or remained hydrated on the PDA. The desiccated and hydrated sclerotia were conditioned at -20°C, -10°C, 0.5°C, 10°C, or 30°C for 4 weeks as per Huang [4]. Thereafter, sclerotia were placed in glass 100 × 15 mm Petri dishes containing moist, sterile sand, sealed with parafilm, and carpogenic or myceliogenic germination (Figure 2) was determined after 4 weeks at 16°C in low continuous light. Each treatment had 6 biological replications with 20 sclerotia per replicate petri dish.

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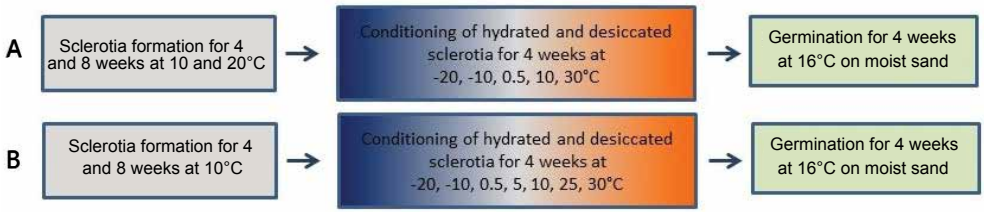

Figure 1: Experimental design to determine the effects of sclerotia formation period, development temperature, and conditioning temperature on germination of *Sclerotinia sclerotiorum* strain Sun87. (A) Experiment 1, (B) Experiment 2.

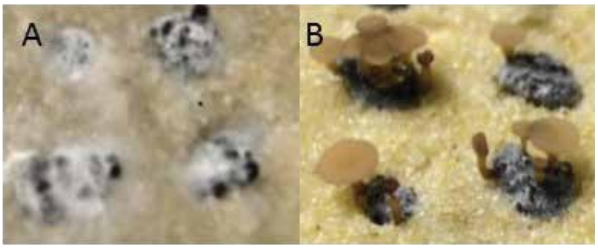

Figure 2: Myceliogenic (A) and carpogenic (B) germination on sand after 4 weeks incubation of *Sclerotinia sclerotiorum* strain Sun87 sclerotia.

Figure 3: Experimental design to determine the effects of sclerotia inoculum production temperature, formation temperature, and desiccation time on germination of *Sclerotinia sclerotiorum* strain Sun87 and 1980 for experiments 3 and 4.

Experiment 2: Experiment 1 was repeated with slight modification; that is, elimination of the 20°C sclerotia formation temperature and addition of 5°C and 25°C conditioning temperature.

Effect of inoculum production temperature, sclerotia formation temperature, and duration of sclerotia desiccation on germination of Sun87 and 1980

Two experiments with identical designs were conducted as described below.

Experiment 3: Inoculum for procuring plugs of mycelia mat for subsequent sclerotia formation was produced at 10 and 25°C (8 h light/16 h dark) over a 7 to 10 day period in Petri dishes containing ½ strength PDA (Figure 3). Plugs were then transferred to the center of 100 × 15 mm Petri dish containing full strength PDA. These dishes were incubated in the dark at 10°C and 20°C for 8 weeks to facilitate growth of mycelia and formation of sclerotia. The sclerotia were then transferred to a Petri dish for desiccation treatments of 1 to 21 days or remained hydrated on the PDA; however, only 0 and 1 day data are presented. Thereafter, sclerotia were placed in glass 100 × 15 mm Petri dishes containing moist, sterile sand, sealed with parafilm, and germination was determined at 16°C after 4 and 8 weeks of incubation under continuous low light. Each treatment had 4 biological replicates with most replicate petri dishes having 20 sclerotia but some having as few as 7. In this experiment several petri dishes for Sun87 (the plugs of mycelial mat for sclerotia formation produced at 25°C) were contaminated; therefore, the data was excluded.

Experiment 4: Experimental design was identical to Experiment 3 (Figure 3).

Statistical analysis

Data collected from the experiments revolved around counting the number of sclerotia germinating out of a set number of sclerotia exposed to a treatment condition. Therefore, experiments were evaluated by fitting generalized linear mixed models in order to test the factors of interest in the experiment. These models fit binomial proportions to treatments with a logit link. Proportions of sclerotia that germinated were compared among treatment conditions using Likelihood Ratio, Chi-Square or Z statistics.

Results

Effects of sclerotia formation period, development temperature, and conditioning temperature on germination of Sun87

We observed 88% and 98% carpogenic germination in the first experiment when sclerotia were developed for 4 and 8 weeks respectively at the 10°C formation temperature followed by conditioning them in a hydrated state at 10°C (Figure 4). Comparatively, carpogenic germination at 8 weeks was 85% and 51% for sclerotia conditioned at 0.5°C and 30°C, respectively. Conversely, carpogenic germination for sclerotia formed for 4 and 8 weeks at the 20°C formation temperature and conditioned in a hydrated state at 10°C was observed to be significantly ($P<0.01$) lower at 17% and 23% respectively when compared to sclerotia formed at the 10°C temperature. However, when sclerotia were desiccated prior to germination, carpogenic germination was limited to between 0% and 21% for either formation period (4 or 8 weeks) or for either development temperature (10°C or 20°C) and for all conditioning temperatures (Figure 4). Moreover, no more than 6% myceliogenic germination was observed among treatment means

in experiment 1 regardless of the sclerotia formation period and temperature, and conditioning temperature (Supplemental Figure 1a).

Because germination levels in experiment 1 for Sun87 sclerotia formed at 20°C were decreased when compared to those formed at 10°C (Figure 4), we eliminated the 20°C sclerotia formation temperature and added two additional conditioning temperatures (5°C and 25°C) to be evaluated in experiment 2. The conditioning temperatures in experiment 2 that were most efficacious for carpogenic germination were similar to those in experiment 1, i.e., 0.5 and 10°C. In both experiments for all conditioning temperatures and sclerotia formed at 10°C, germination was generally greater for sclerotia formed for 8 weeks relative to 4 weeks (Figures 4 and 5). Overall carpogenic germination ranged from 1% to 98% in experiment 1 and from 5% to 99% in experiment 2 for sclerotia formed at 4 and 8 weeks at 10°C and conditioned in a hydrated state between -20°C to 30°C (Figures 4 and 5). When sclerotia were desiccated and conditioned at -20°C to 30°C, carpogenic germination was limited (0% to 40%) regardless of sclerotia formation period (Figure 5); these results are similar to those from experiment 1. For myceliogenic germination, treatments in experiment 2 resulted in 0% to 34% germination (Supplemental Figure 1b).

Effect of inoculum production temperature, sclerotia formation temperature, and duration of sclerotia desiccation on germination of Sun87 and 1980

Observations taken from experiments 1 and 2 did not lead to a high certainty of treatment conditions for obtaining strictly myceliogenic or carpogenic germination using the range of treatments, including conditioning temperatures as reported by Huang [4]. Therefore, we further utilized sclerotinia strains Sun87 and 1980 to examine two additional factors that could possibly separate carpogenic and myceliogenic germination, but were not explored in experiments 1 and 2 (Figure 3). These factors were: 1) the temperature for inoculum production (the material used to obtain mycelial mats for sclerotia formation), and 2) the duration of sclerotia desiccation. Strain 1980 was specifically chosen for experiments 3 and 4 to evaluate its germination since its genome has been sequenced, making it ideal for further genomics studies [9].

Evaluation of Strain Sun87

In experiment 3, the 8 weeks germination evaluation revealed that hydrated Sun87 sclerotia had 84% and 61% carpogenic germination in response to the 10°C and 25°C inoculum production temperatures, respectively, when the sclerotia formation temperature was 10°C (Figure 6a). Germination was 85% and 79% for the same conditions in experiment 4 (Figure 6b). When the sclerotia formation temperature was 20°C (Figure 6a) carpogenic germination at 8 weeks was observed to be 66% and 10% in response to the 10°C and 25°C inoculum production temperatures, respectively, in experiment 3. However, there was no carpogenic germination in experiment 4 with these treatments (Figure 6b).

Eight week germination rates for sclerotia strain Sun87 under the

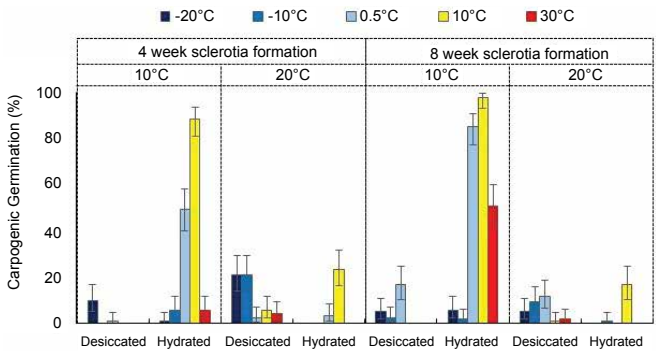

Figure 4: Carpogenic germination of *Sclerotinia sclerotiorum* strain Sun87 sclerotia in experiment 1. Sclerotia were formed for 4 and 8 weeks at 10°C and 20°C followed by conditioning under desiccated and hydrated conditions between -20°C to 30°C (see Figure 1A).

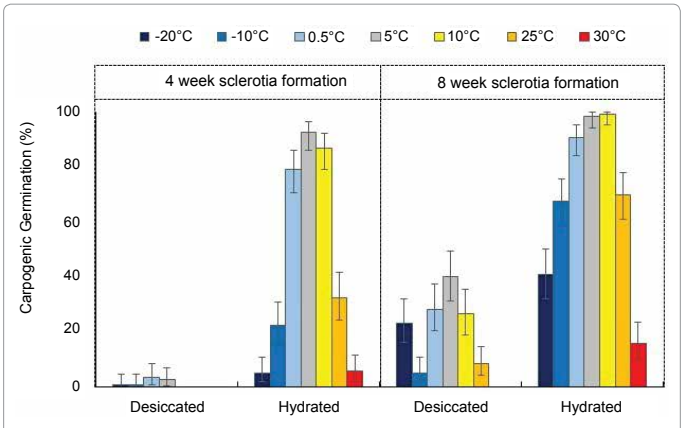

Figure 5: Carpogenic germination of *Sclerotinia sclerotiorum* strain Sun87 sclerotia in experiment 2. Sclerotia were formed for 4 and 8 weeks at 10°C followed by conditioning under desiccated and hydrated conditions between -20°C to 30°C (see Figure 1B).

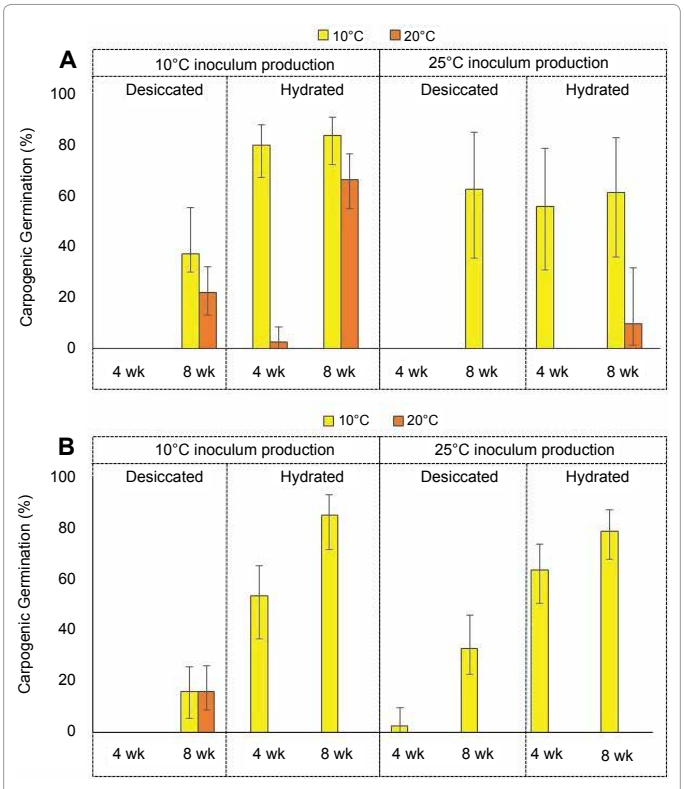

Figure 6: Carpogenic germination of *Sclerotinia sclerotiorum* strain Sun87 sclerotia in experiment 3A and experiment 4B. Inoculum production temperature for sclerotia was 10°C and 25°C, the sclerotia formation temperature was 10°C and 20°C, and germination was evaluated at 4 and 8 weeks. Prior to germination sclerotia were kept hydrated or desiccated for 1 day (see Figure 3).

desiccated study conditions showed consistency between experiments 3 and 4 although the levels of germination differed. Analysis of germination rates for both experiments showed a significant interaction between inoculum production and sclerotia formation temperatures with significance of P<0.01 for both. Evaluation of germination showed an increase from 37% carpogenic germination for the 10°C inoculum production temperature (N=4 reps, 64 sclerotia tested) to 63% carpogenic germination for the 25°C inoculum production temperature (N=1 rep, 16 sclerotia tested) when the sclerotia formation temperature was 10°C in experiment 3 (Figure 6a), although this was not found to be highly statistically significant compare to 25°C inoculum production temperature (P=0.0755, Z=-1.436). We qualify this result by noting that experiment 3 encountered contaminations for strain Sun87 in 3 of the 4 replicates when inoculum production occurred at 25°C, and do not consider this convincing evidence that increasing sclerotia formation temperature from 10°C to 20°C increases carpogenic germination. However for the same treatment conditions in experiment 4 (Figure 6b) , the increase from 10°C to 25°C inoculum production temperature resulted in an increase in germination from 16% to 33% (P<0.01, Z=-2.489) for 10°C sclerotia formation temperature. For sclerotia formed at 20°C in experiment 3 (Figure 6a), the 8 weeks germination evaluation revealed 22% (N=4 reps, 79 sclerotia tested) and 0% (N=1 rep, 20 sclerotia tested) carpogenic germination in response to the 10°C and 25°C inoculum production temperatures, respectively. A similar decrease in germination was observed for carpogenic germination with 16% (N=4 reps, 80 sclerotia) for the 10°C inoculum production temperature and 0% (N=4 reps, 80 sclerotia) or the 25°C inoculum production temperatures in experiment 4 (Figure 6b). However, neither of these decreases were found to be statistically significant (P>0.25)

Evaluation of Strain 1980

Both experiments showed a statistically significant effect of sclerotia formation temperature on 8 week germination for hydrated sclerotia (P<0.001 for both experiments), but also statistically significant interaction between inoculum production temperature and sclerotia formation temperature on 8 weeks germination for hydrated sclerotia (P=0.074 for experiment 3 and P=0.044 for experiment 4). In experiment 3, the 8 weeks germination evaluation revealed that hydrated 1980 sclerotia increased (P=0.073) from 26% (N=4 reps, 66 sclerotia) to 35% (N=4 reps, 76 sclerotia) carpogenic germination in response to the 10°C and 25°C inoculum production temperatures, respectively, when sclerotia formation temperature was 10°C (Figure 7a). However the 8 weeks evaluation revealed no significant difference (P=0.297) for carpogenic germination in response to the 10°C and 25°C inoculum production temperatures, which was 71% (N=4 reps, 77 sclerotia) and 65% (N=4 reps, 80 sclerotia) respectively, when sclerotia formation temperature was 20°C (Figure 7a). For experiment 4 (Figure 7b), carpogenic germination was 23% (N=4 reps, 71 sclerotia) and 25% (N=4 reps, 70 sclerotia) after 8 weeks for the 10°C and 25°C inoculum production temperature, respectively, in combination with the 10°C sclerotia formation temperature and represented no significant difference. However, germination was 66% (N=4 reps, 80 sclerotia) and 48% (N=4 reps, 80 sclerotia) after 8 weeks for the 10°C and 25°C inoculum production temperature in combination with the 20°C sclerotia formation temperature (Figure 7b) representing a significant decrease (P<0.01). Significant increases were observed for carpogenic germination in both experiments when the sclerotia formation temperature was increased from 10°C to 20°C with germination increasing from 30% (N=8 reps, 142 sclerotia) to 68% (N=8 reps, 157 sclerotia) in experiment 3 and from 24% (N=8 reps, 141 sclerotia) to 57% (N=8 reps, 160 sclerotia) in experiment 4. For experiments 3 and 4, there was essentially no carpogenic germination after 8 weeks for desiccated 1980 sclerotia with any treatment combinations (Figures 7a and 7b).

Finally, for both Sun87 and 1980, there was no more than 5% myceliogenic germination (Supplemental Figures 2 and 3) for any combination of treatments. However, significant myceliogenic germination (P<0.0001), although very low, occurred only for the desiccated treatments. Additionally, no consistent differences in germination were observed between sclerotia desiccated beyond 1 day for either strain (data not presented).

Discussion

Our objective was to confirm previous reports outlining a reliable method to absolutely distinguish myceliogenic and carpogenic germination as a prelude to investigating the transcriptomes associated with the two forms of *S. sclerotiorum* germination. We procured strain Sun87 and repeated a study by Huang [4], which indicated different conditioning temperatures clearly distinguished carpogenic from myceliogenic germination. Our experiments were done in nearly the same manner as previously reported [4]. However, we conditioned sclerotia in our experiments in both the hydrated and desiccated states due to ambiguity in the methods, and a report by Huang et al. [6] indicating that desiccation of sclerotia was an important factor for myceliogenic germination. Huang [4] reported 100% myceliogenic germination for Sun87 sclerotia following conditioning at -20°C. However, we observed ≤5% myceliogenic germination of Sun87 sclerotia that were conditioned at -20°C in both the hydrated and

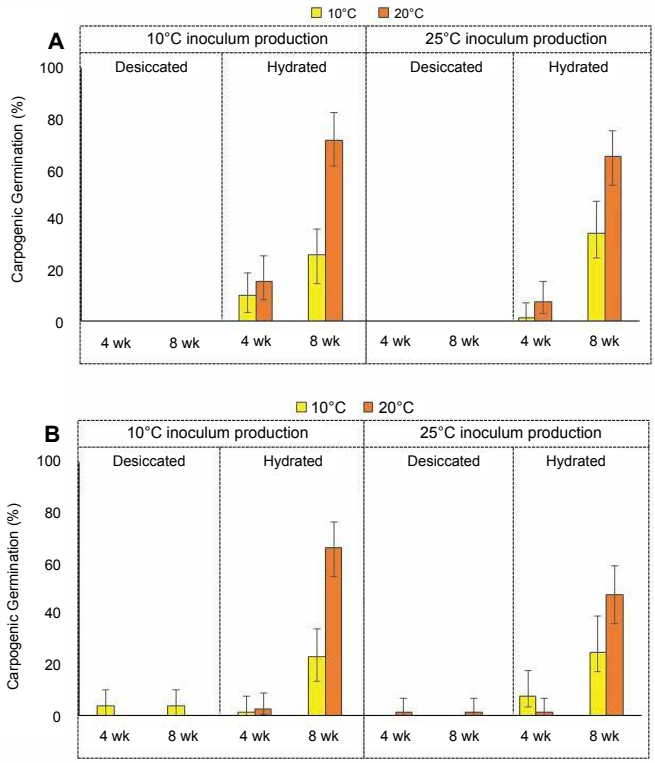

Figure 7: Carpogenic germination of *Sclerotinia sclerotiorum* strain 1980 sclerotia in experiment 3A and experiment 4B. Inoculum production temperature for sclerotia was 10°C and 25°C, the sclerotia formation temperature was 10°C and 20°C, and germination was evaluated at 4 and 8 weeks. Prior to germination sclerotia were kept hydrated or desiccated for 1 day (see Figure 3).

desiccated state (Supplemental Figure 1). We now assume that Huang [4] conditioned Sun87 sclerotia in a hydrated state because he obtained 90% to 100% carpogenic germination at conditioning temperatures of 0.5°C to 30°C for 8-week old cultures. Similarly, we obtained levels of carpogenic germination approaching 100% at conditioning temperatures of 0.5°C to 10°C for 8-week old cultures. Unfortunately, in our experiments distinct separation of the two forms of germination was not apparent. For example, in experiments 1 and 2, in a hydrated state, a conditioning temperature, such as -10°C that resulted in 2% to 34% myceliogenic germination, also resulted in 2% to 68% carpogenic germination. Beyond that, we obtain inconsistent results when we repeated the experiment 1 (i.e., experiment 2) to examine the effect of sclerotia formation period and temperature, and conditioning temperature on germination. Thus, the use of conditioning temperature alone to clearly differentiate myceliogenic and carpogenic germination for Sun87 sclerotia did not meet our objective.

We examined several other factors that might affect germination such as inoculum production temperature and duration of sclerotia desiccation. Huang and Kozub [5] examined inoculum production temperatures of 10°C, 25°C, and 30°C. They reported 84% carpogenic germination for Sun87 sclerotia whose inoculum was produced at 10°C and the sclerotia formed at 20°C; no more than 35% germination was observed for the 25°C and 30°C inoculum production temperatures for sclerotia formed at 20°C. In contrast, our results indicated that the 10°C and 25°C inoculum production temperatures for Sun87 resulted in 61% to 85% carpogenic germination, particularly when the sclerotia were formed at 10°C and they were kept hydrated (Figures 6a and 6b). Conversely, for strain Sun87 carpogenic germination was significantly lower ($P<0.0001$) if the sclerotia were produced at 20°C relative to 10°C when hydrated. However, for strain 1980 carpogenic germination was significantly higher ($P<0.0001$) if the sclerotia were produced at 20°C relative to 10°C (Figures 7a and 7b) when hydrated. It is known that carpogenic germination depends on the geographic origin of the strain and the temperature at which sclerotia are produced [7]. The different results from our two strains may be due to different geographic origins and thus temperature requirements. Ultimately, we were not able to verify that a low inoculum production temperature was the main factor affecting carpogenic germination of Sun87. Rather, a low temperature (10°C) during sclerotia formation seems to be the more important factor to enhance carpogenic germination of Sun87 and 1980.

Our first experiment indicated that drying sclerotia prior to conditioning had little effect on subsequent myceliogenic germination (Supplemental Figure 1). However, Huang et al. [6] reported that desiccation of strain S9 sclerotia at 25°C and near 0% relative humidity for 3 weeks significantly increased myceliogenic germination and hyphal growth at 25°C. We had data that air drying of Sun87 sclerotia for 1 day in a laminar flow hood facilitated complete desiccation (data not presented). Nevertheless, based on Huang et al. [6] we reasoned that prolonged desiccation may facilitate additional benefits for sclerotia germination similar to that which occurs when dormant seeds are subjected to afterripening under warm, dry conditions [10]. However, there was no benefit to a period of desiccation beyond 1 day for either strain 1980 or Sun87. In fact, desiccation still resulted in greater carpogenic than myceliogenic germination, even though the carpogenic germination levels were lower than if they were hydrated.

We did not identify a treatment that clearly distinguished myceliogenic from carpogenic germination for strain Sun87 based on inoculum production temperature, sclerotia formation period and temperature, conditioning temperatures, and desiccation. An important outcome of our experiments was the high level of variability for sclerotia germination indicating the critical nature of repeating experiments aimed at understanding factors that influence sclerotia germination, which may not have been done in past studies. In any event, other methods, such as injuring the sclerotia rind [11], manipulating the quantity and quality of light [12,13] and manipulating the type of germination using chemical treatments [14] are possible solutions to discovering a reliable and non-confounded method that clearly differentiate myceliogenic and carpogenic germination of *S. sclerotiorum*.

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References

1. Boland GJ, Hall R (1994) Index of plant hosts of *Sclerotinia sclerotiorum*. Can J Plant Path 16: 93-108.
2. Bolton MD, Thomma BP, Nelson BD (2006) *Sclerotinia sclerotiorum* (Lib.) De Bary: biology and molecular traits of a cosmopolitan pathogen. Mol Plant Pathol 7: 1-16.
3. Adams PB, Ayres WA (1979) Ecology of *Sclerotinia* species. Phytopath 69: 896-899.
4. Huang HC (1991) Induction of myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum* by exposure to sub-freezing temperatures. Plant Path 40: 621-625.
5. Huang HC, Kozub GC (1993) Influence of inoculum production temperature on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Can J Microbiol 39: 548-550.
6. Huang HC, Chang C, Kozub GC (1998) Effect of temperature during sclerotial formation, sclerotial dryness, and relative humidity on myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Can J Bot 76: 494-499.
7. Huang HC, Kozub GC (1991) Temperature requirements for carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* isolates of different geographic origin. Bot Bull Acad Sinica 32: 279-286.
8. Steadman, JR, Marcinkowska J, Rytledge S (1993) A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. Can J Plant Path 16: 68-70.
9. Amselem J, Cuomo CA, Van Kan JAL, Viaud M, Benito EP, et al. (2011) Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genet 7: e1002230.
10. Foley ME (1994) Temperature and water status of seed affect after ripening in wild oat (*Avena fatua*). Weed Sci 42: 200-204.
11. Huang, HC (1985) Factor affecting myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Phytopath 75: 433-437.
12. Huang HC, Kokko EG (1989) Effect of temperature on melanization and myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Can J Bot 67: 1387-1394.
13. Sun P, Yang XB (2000) Light, temperature, moisture effects on apothecium production of *Sclerotinia sclerotiorum*. Plant Dis 84: 1287-1293.
14. Steadman JR, Nickerson KW (1975) Differential inhibition of sclerotial germination in *Whetzelinia sclerotiorum*. Mycopathologia 57: 165-170.