Effect of Environmental Factors on *Sclerotinia minor* and Sclerotinia Blight of Peanut

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ABSTRACT

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Myceliogenic sclerotial germination of *Sclerotinia minor* and growth as well as infection and colonization of peanut (*Arachis hypogaea*) tissue was optimum at 20–25 C. Ninety-five to 100% relative humidity (RH) for more than 12 hr was necessary for germination of sclerotia. Germination exceeding 80% was obtained when sclerotia were incubated at 100% RH for varying periods of time. Lateral branch and main branch tissues were colonized by *S. minor* similarly. The infection rate of young, juvenile tissues was significantly greater than that of maturing plant tissues.

Additional keywords: epidemiology, sclerotial germination, temperature.

Sclerotinia blight of peanut caused by *Sclerotinia minor* Jagger was first discovered in the United States in 1971 in Virginia (14). Since then, this disease has been reported in North Carolina (15), Oklahoma (18), and Texas (D. L. Smith, *personal communication*). Peanut yield losses in Virginia due to Sclerotinia blight can exceed 15% and can cost growers millions of dollars annually (17).

Sclerotinia blight is usually worse during cool, wet weather (8). However, despite cool, wet weather, disease development in the field is low when plants are small and without a dense canopy or complete ground cover. Temperature and moisture are commonly mentioned as significant factors affecting development of diseases caused by species of *Sclerotinia* (1,11,20). *Sclerotinia minor* has been reported to grow from 0 to 35 C on agar media with optimum growth between 15–25 C (10,12,13,16,20). Myceliogenic germination of sclerotinia of lettuce isolates of *S. minor* was best at soil water potential near −0.3 bars; however, considerable germination occurred at a soil water potential of −2 bars (2). Lettuce drop caused by *S. minor* was more severe when the soil moisture fluctuated between 30–40% field capacity and 100% than when it fluctuated between 80–90% and 100% field capacity (5). Survival of lettuce isolates of *S. minor* was best in soil where matric potential varied weekly between 0 and −430 bars.

The objectives of this research were to determine the effects of temperature on myceliogenic germination of sclerotia, mycelial growth, and infection potential of *S. minor* to peanuts; the effects of relative humidity (RH) on myceliogenic germination of sclerotia; and the effect of inoculation site and age of the peanut plant on disease development under laboratory and greenhouse conditions. An abstract of the temperature studies has been published (9).

MATERIALS AND METHODS

Effects of temperature on myceliogenic germination of sclerotia and mycelial growth. Three isolates of *S. minor* (Sm1, Sm2, and Sm3) obtained from infected horsenettle (*Solanum carolinense* L.), soybean (*Glycine max* L. Merr.), and peanut (*Arachis hypogaea* L.), respectively, were used. Isolates were grown on Difco potato dextrose agar (PDA) at 20 or 25 C and stored in a refrigerator.

Unwashed sclerotia scraped from 2-wk-old PDA cultures were dried in a Microvoid transfer hood (Air Control Inc., Huntington, PA) with constant filtered air flow for 24 hr. This was done to provide a drying period for the sclerotia and to decrease the risk of growth of surface hyphae. Seven sclerotia per isolate were placed on PDA in 9-cm-diameter plates. Five plates of each isolate were incubated at 10, 15, 20, 25, and 30 C. Myceliogenic sclerotial germination (production of numerous hyphae in an eruptive tuft of growth or production of a single or few strands of hyphae) (6) and radial growth were measured after 1, 2, and 3 days.

In other experiments, sclerotia of the Sm3 isolate were produced at 20 C on a sterile medium of soil-cornmeal (50 g soil-cornmeal [50% w/w] + 20 ml distilled water in 9-cm-diameter petri dishes). Sclerotia were scraped from the surface of 2-wk-old cultures, vigorously washed under high-pressure tap water for 10 min, and dried on a screen in the transfer hood with constant air flow for 24 hr. Germination at 15, 20, 25, and 30 C was studied on sclerotia incubated on moistened filter paper in petri dishes or acid-washed glass slides supported on glass rods above water in petri dishes.

Effects of temperature on myceliogenic germination, infection, and colonization of peanut. Young peanut leaflets that had just fully expanded were randomly selected from plants and placed in petri dishes on glass slides supported on glass rods above distilled water (100% RH). Five sclerotia were spaced evenly on each of the leaflets. Four plates of leaflets were incubated at each of four temperatures (15, 20, 25, and 30 C). Myceliogenic germination and infection were monitored daily for 1 wk.

Six-wk-old peanut plants (cultivar Florigiant) were inoculated with a 7-mm-diameter mycelial disk from an actively growing Sm3 isolate on PDA. The inoculum was placed on the lower internodes of the branch and two lateral branches of each plant. Sterile, moist gauze that remained moist throughout the experiment was held in place over the inoculum with masking tape. Five well-watered plants were placed in an inflated, clear plastic bag in a 12-hr light cycle growth chamber set to establish temperatures in the bags of 15, 20, 25, or 30 C. Plants were watered heavily before treatment began to assure high relative humidities within the enclosed bag. Observations on infection, based on symptom expression and lesion measurements, were made after 8 days.

Effects of tissue age on colonization and comparative susceptibility of lateral versus main branch tissue. The main branch and two lateral branches of 8-wk-old Florigiant peanut plants (without flowers) and 16-wk-old Florigiant peanut plants (with flowers) were inoculated with a PDA disk of Sm3 mycelial inoculum and incubated at 20 C in a growth chamber as previously described. Observations of disease development were made after 1 wk. This experiment was repeated once as described and similarly conducted with 6-wk-old and 13-wk-old plants.

Effects of relative humidity on myceliogenic germination of sclerotia of *S. minor*. Two glass slides containing 20 Sm3 sclerotia each were placed on tubing above saturated solutions in storage dishes (80 mm height × 100 mm diameter) sealed with petroleum jelly. Various relative humidities (52, 55, 65, 70, 75, 80, 85, 92, and 95% RH) were established using saturated chemical solutions (21). Two dishes, used for each relative humidity, were submerged in water and placed in an incubator maintained at 20 C. In an additional experiment, dishes were maintained at 25 C at relative humidities of 50, 55, 62, 72, 75, 78, 85, 92, or 97%. Following a 1-wk incubation at each relative humidity, myceliogenic germination of sclerotia was determined. Sclerotia were then subjected to a saturated environment (100% RH) for an additional week and reobserved for evidence of germination.

Time for germination and effects of fluctuating relative humidity on myceliogenic germination of sclerotia. Four hundred Sm3 sclerotia were placed on glass slides with 20 sclerotia per slide. The slides of sclerotia were supported on glass tubing above distilled deionized water (100% RH) in storage dishes sealed with petroleum jelly (two slides per dish) and incubated at 20 C. Germination of 40 sclerotia was counted every 12 hr; the slides bearing these sclerotia were then transferred to dishes containing a solution establishing 80% RH (chosen because germination had not occurred at this relative humidity) and incubated at 20 C. After 2 days in this drier environment, germinated sclerotia were counted and the slides were replaced in 100% RH at 20 C and recounted every 12 hr. This experiment was repeated twice.

All experiments were repeated at least three times, unless otherwise noted. Results were analyzed with analysis of variance and Duncan's multiple range test ($P = 0.05$).

RESULTS

Effects of temperature on myceliogenic germination of sclerotia and mycelial growth. Figure 1 shows the results of myceliogenic germination of sclerotia of isolate Sm3 on PDA at five temperatures for 3 days. The germination of sclerotia of SM1 and Sm2 was similar to Sm3. Myceliogenic germination of all isolates increased from day 1 to day 3 at all temperatures. Optimum germination after 2 days on PDA occurred at 20–25 C for isolate Sm3. After 3 days on PDA, germination was equally good at 20 or 25 C for all the isolates.

At the end of 8 days, myceliogenic germination of the Sm3 isolate on either moist filter paper or acid-washed glass slides was significantly higher ($P = 0.05$) at 20 and 25 C than at 15 or 30 C. In all germination experiments, with or without nutrient medium, germination of 70% or better was obtained at the optimum temperature. Myceliogenic germination was less common at 10 and 15 C than at 20 or 25 C. Hyphal myceliogenic germination (single or few stands) was more characteristic at 30 C, at which sclerotia exhibited less than 5% germination, than at the other temperatures tested.

Mycelial growth after 3 days on PDA was optimum ($P = 0.05$) at 20–25 C for all isolates (Fig. 2). Radial growth was greatest ($P = 0.05$) after 3 days for all isolates. Sm2 grew most rapidly and Sm1 least rapidly. Little growth occurred for Sm1 and Sm3 at 10 C or Sm1 and Sm2 at 30 C.

Effects of temperature on myceliogenic germination, infections, and colonization of peanut tissues. Germination of sclerotia of Sm3 on detached peanut leaflets was better at 20 and 25 C than at 15 or 30 C (Fig. 3). Infection was optimum at 20 C. There was no difference in percent infection between 15 and 25 C. Negligible germination and infection occurred at 30 C.

Lesion development was greatest at 20 C (Fig. 4) on lower internodes of both main and lateral branch tissue of intact peanut

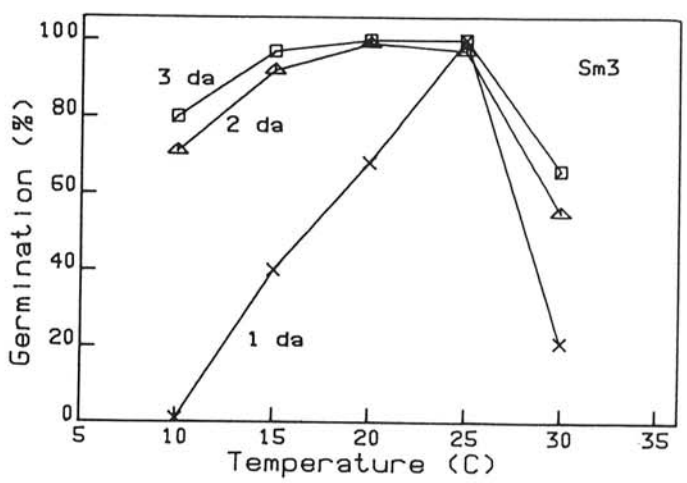

Fig. 1. Myceliogenic germination of *Sclerotinia minor* (isolate Sm3) on potato dextrose agar at five temperatures after 1, 2, and 3 days.

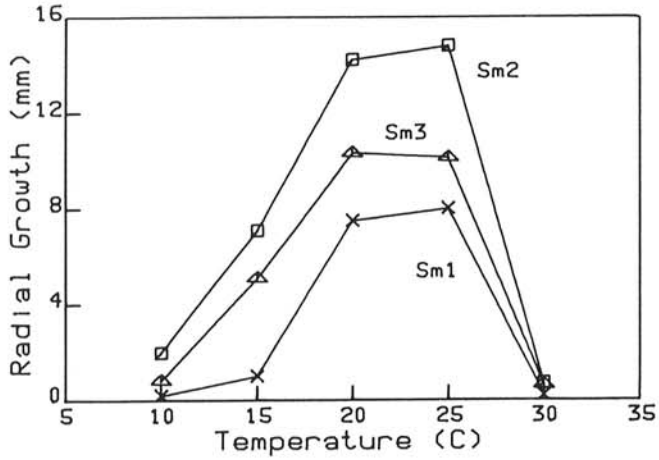

Fig. 2. Radial growth of three isolates of *Sclerotinia minor* (Sm1, Sm2, and Sm3) on potato dextrose agar at five temperatures after 3 days.

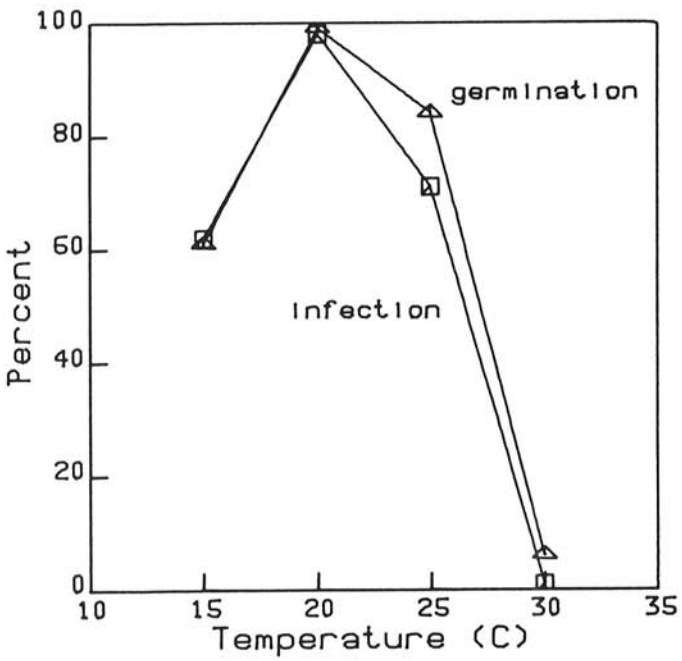

Fig. 3. Myceliogenic germination of *Sclerotinia minor* (isolate Sm3) and infection of detached peanut leaflets (cultivar Florigiant) at four temperatures after 1 wk.

plants. Infection of main and lateral lower internode tissue at 30 C resulted in brown lesions measuring 0.5 to 2 mm in diameter. Analysis of colonization of main branch and lateral branch tissues at all temperatures showed no significant difference between the two tissues.

Effects of tissue age on colonization and comparison of susceptibility of lateral versus main branch tissue. Infection by *S. minor* was greater in 8-wk-old plants than in 16-wk-old plants. The 16-wk-old plants had no infection, while the 8-wk-old plants had 67% infection. Infection of the 6-wk-old plants was 100%, and infection of the 13-wk-old plants was 67% in the second experiment. Once infection occurred, lesion development (colonization) as measured by lesion length was not significantly different in different age plants. There was no difference in infection and colonization of lateral versus main branch tissue on the plants.

Effects of relative humidity on myceliogenic germination of sclerotia of *S. minor*. Myceliogenic germination of sclerotia of *S. minor* did not occur following incubation at 20 or 25 C for 1 wk at relative humidities less than 95%. However, when sclerotia from

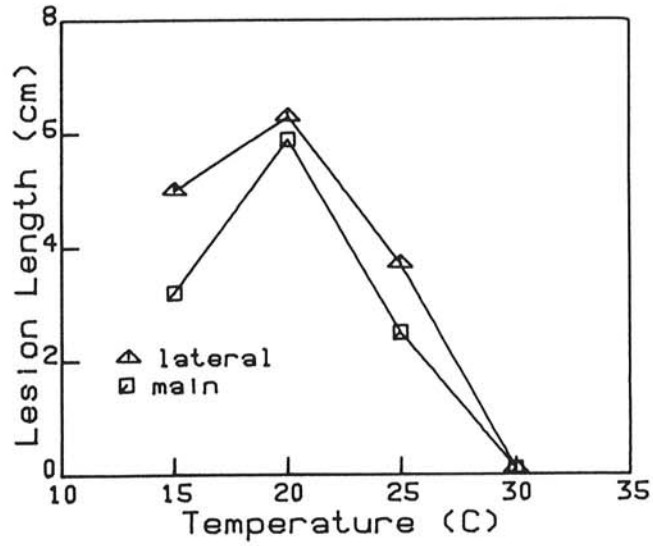

Fig. 4. Lesion length of *Sclerotinia minor* on inoculated main and lateral branches of peanut plants (cultivar Florigiant) maintained at four temperatures for 8 days after inoculation with a mycelial disk.

TABLE 1. Myceliogenic germination of sclerotia of *Sclerotinia minor* incubated at 20 or 25 C and maintained at various relative humidities (RH)[a] for 1 wk and then transferred to a saturated environment (100% RH) for an additional week

Temperature, incubation time, and relative humidity	Germination[b] (%) at 85% RH	92% RH	95% RH	97% RH	100% RH
20 C, 1 wk at established RH	0	0	90	ND[c]	90
Additional week at 100% RH	83	84	90	ND	95
25 C, 1 wk at established RH	0	0	ND	95	95
Additional week at 100% RH	86	79	ND	95	95

[a] Relative humidities established using saturated chemical solutions or distilled, deionized water.
[b] Means of four experiments.
[c] Not determined.

each of the relative humidity treatments (50, 52, 55, 62, 66, 70, 72, 75, 78, 80, 85, 92, 95, and 97) were subjected to an additional week in a saturated environment (100% RH) at 20 or 25 C, myceliogenic germination ranged from 76 to 94%. Following incubation for 1 wk at 20 C and 95% RH, sclerotia germinated at a frequency of 90% (Table 1). At 25 C and 97% RH, sclerotia germinated at a frequency of 95% after 1 wk incubation. Germination frequency was slightly higher at 25 C than at 20 C.

Time for germination and effects of fluctuating relative humidity on myceliogenic germination of sclerotia. The results of two tests varying the duration of exposure at 100% RH showed that more than 12 hr initial exposure at 100% RH was necessary for germination (Table 2). Ninety-nine percent germination occurred after 96 hr. Sclerotia, initially exposed for varying periods of time in 100% RH and then transferred to 80% RH for 2 days, germinated equally well after replacement in 100% RH conditions. After the 2-day drying period, 81% or better germination was achieved in all treatments.

DISCUSSION

Temperature, relative humidity, and soil moisture, either singularly or in combination, play a vital role in the infection process and colonization of plant tissues by *S. minor* (1,5,9,20). In this work, the optimum temperature for myceliogenic germination of *S. minor* sclerotia, infection, and colonization of peanut (cultivar Florigiant) tissues appears to be 20–25 C. Similar data have been reported for other species of *Sclerotinia* (1,19).

TABLE 2. Myceliogenic germination of sclerotia of *Sclerotinia minor* after initial exposure (first) to 100% relative humidity (RH) for 12–96 hr and after a 4-day reexposure (second) to 100% RH following a 2-day drying (80% RH) period[a]

Exposure	Germination (%) at 100% RH
First (hours)	
12	0
24	45
36	46
48	55
60	83
72	92
84	93
96	99
Second (hours)	
12	81
24	92
36	92
48	92
60	94
72	89
84	93
96	ND[c]

[a]Relative humidity established using distilled saturated chemical solution.
[b]Means of two experiments. Sclerotia incubated at 20 C.
[c]Not determined.

Optimum sclerotial germination and mycelial growth of lettuce isolates of *S. minor* was 18 C (11). Possibly this lower optimum temperature represents an adaption of *S. minor* to the cooler growing conditions normally associated with lettuce production. Because nighttime temperatures in Virginia often drop to 20 C and below during the later part of the growing season, temperature would not be a limiting factor to disease development. However, in other peanut-producing areas where Sclerotinia blight has not been observed, nighttime temperatures rarely drop below 25 C. This may account in part for the lack of disease in these areas.

The duration of optimum temperatures favorable for disease development also might become a limiting factor. When temperatures become unfavorable (above 30 C) (20) during sclerotial germination, mycelial growth apparently stops. In this study, a 45% germination frequency occurred after 24 hr at 20 and 25 C and at relative humidities of at least 95%. Sclerotia did not germinate in these conditions after only 12 hr incubation. With the lower nighttime temperatures normally associated with September in Virginia, temperatures more favorable to the growth of *S. minor* prevail and help explain why disease severity increases during this month. More importantly, however, is the influence of the dense plant canopy on the plant microclimate near the soil surface. The primary infection sites of *S. minor* usually occur in or near the soil-plant interface zone. The shading effect of the canopy as well as the denseness of the canopy (which allows minimum air movement) apparently have a direct influence on prolonging the effects of the nighttime temperatures and relative humidities that would be conducive to the continued growth of the mycelium of germinating sclerotia. Infection of plant tissues in this zone usually originates from germinating sclerotia on or in the top 1.5 cm soil layer.

Relative humidities approaching saturation favor the development of Sclerotinia blight in peanut. This was also one of the conclusions of Abawi and Grogan (1) who studied the effects of many environmental parameters on the epidemiology of *Whetzelinia sclerotiorum* (syn. *Sclerotinia sclerotiorum*). The results of this study indicate that relative humidity of 95% or greater promoted myceliogenic germination of sclerotia of *S. minor*. Lesion length was also positively correlated with relative humidity. Similar observations have been recorded (1). Although the minimum number of hours needed for myceliogenic germination of sclerotia of *S. minor* is greater than 12 hr at relative humidities of 95% or greater, a few infection sites occurred in this study when these conditions were not prevalent. Therefore, the microclimate at the soil-plant interface underneath the plant canopy must have provided environments favorable for germination and colonization by *S. minor*. Rainfall during this period would extend periods of high relative humidity and increase soil moisture. These results also suggest that the microclimate beneath the plant canopy might become more favorable for Sclerotinia blight disease development as the plants approach maturity (September and October) because of the cool nighttime temperatures and high relative humidities normally associated with autumn. This, in fact, agrees with the observations of disease development in the field and supports data provided by others (1,11) of the necessity of free water at the infection site before infection can occur.

Only germinating sclerotia located on or near the soil surface are thought to cause infection. These sclerotia are especially vulnerable to weather factors. However, drying sclerotia of *S. minor* under the hood or using saturated solutions did not adversely affect their ability to germinate when temperature and humidity conditions were favorable for germination. Adams and Tate (4) suggested that drying sclerotia stimulated germination of *S. minor* when soil moisture conditions returned to −0.3 bars. Periodic wetting and drying might result in repeated sclerotial germination which would ultimately exhaust the energy reserves of the sclerotium. Periodic wetting and drying of the upper soil in the field is common during the early part of the plant growing season. However, the wetting and drying cycles would decrease as the plants matured and the canopy thickened. Moisture at the plant-soil interface (soil moisture in the uppermost portion of the soil profile and existing relative humidity) would continue to fluctuate but the cycles would be shortened especially during periods following rainfall. Abawi et al (3) showed that fluctuating soil moisture (up to −430 bars) promoted the survival of sclerotia of *S. minor*.

In Virginia, symptoms of Sclerotinia blight are generally first observed during mid-July. Experiments conducted to determine if plant age affected susceptibility demonstrated that younger plants are more prone to infection by *S. minor* than older plants. However, under field conditions, older plant parts are more prone to attack than younger plant parts. This apparent reversal in tissue susceptibility to *S. minor* can be partially explained by the presence of a more favorable microclimate as plants mature, more plant tissues available for infection at the plant-soil interface, the presence of an increased supply of host exudates (1), increased plant injury as a result of dense plant growth within the plant's microclimate, and a combination of any of these factors.

In the field, lateral branches are more commonly infected, and lesion development is more extensive than on main stems. However, the results presented here indicate no differences in susceptibility of main stems versus lateral branches. This suggests that the laterals are more often infected and colonized because there are more laterals present for infection, more soil contact points, and more favorable microclimatic conditions. The significance of microclimate is supported by results of screening for resistance in peanuts. Coffelt and Porter (7) found canopy structure to be an important consideration for resistance genotypes. All resistance genotypes identified had plant canopy structures different from the dense, spreading canopy of most Virginia-type cultivars. Resistance seemed to be associated with both morphological escape and physiological resistance. If the microclimate that exists at the plant-soil interface is as important in the development of Sclerotinia blight of peanut caused by *S. minor* as the data presented suggest, then this factor should be considered in future disease control strategies.

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