Environmental Factors Affecting Survival of Ascospores of Sclerotinia sclerotiorum

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ABSTRACT


Laboratory studies showed that temperatures of 25 C or greater combined with relative humidities in excess of 35% were most detrimental to Sclerotinia sclerotiorum ascospore survival. Generally, ascospore mortality increased as temperature and relative humidity increased. Temperature correlated most closely with ascospore mortality on the topmost leaves of the bean (Phaseolus vulgaris) canopy in field studies. Ascospore mortality on the topmost leaves during a 48-hr period was significantly positively correlated with the number of hours at or above 21.1 C during the same period ($r = 0.678$ in 1980 and $r = 0.766$ in 1981). Ascospore survival on shaded leaves $\sim12-15$ cm above ground within a dense canopy averaged 21.5% greater than on topmost leaves. Few ascospores survived on topmost leaves after 6 days in the field, with ascospore viability ranging from 7 to 0% after 6 and 14 days, respectively. Shelters of plastic films differentially transmissive of ultraviolet (UV) radiation significantly reduced ascospore mortality on bean leaves in the field. After 224 hr of irradiation with two FS-40 Sunlamp tubes in the laboratory, ascospore survival was 49.0 and 13.4% under plastic films that transmitted 10 and 50-60% UV radiation in the 300-400 nm range, respectively.

White mold, incited by Sclerotinia sclerotiorum (Lib.) DeBary, is a major disease of snap beans in the state of New York, where severe losses have been reported in some years (22). Investigations by Abawi and others clarified basic aspects of the epidemiology of white mold in New York (1,2,9) and elsewhere (5,6,29,30). However, additional quantitative data, especially collected under field conditions, is needed on the effects of various environmental parameters on important phases of the pathogen cycle (3,8) of S. sclerotiorum. Such data might be useful in developing a disease forecast.

Ascospores of S. sclerotiorum are the primary infective propagules of this fungus on beans in New York (1). Bean blossoms are required for infection of the bean plant by ascospores in the absence of wounds or necrotic tissue. After infection and colonization of blossoms, the fungus invades green tissue in contact with the colonized blossom. Should conditions for ascospore production, release, and deposition occur before bloom, it would be useful to predict the percentage of ascospores that are likely to survive until bloom. The present study was undertaken to assess the duration of ascospore survival on bean plants in the field and the principal environmental factors affecting ascospore mortality.

MATERIALS AND METHODS

In vitro studies on the effect of temperature and relative humidity on the survival of ascospores. The interaction of temperature and relative humidity (RH) on ascospore survival was studied by utilizing the temperature-controlled water bath of a differential respirometer (B. Braun Co., West Germany) (7). Reaction vessels of the differential respirometer contained reagent grade saturated salt solutions (35) to maintain a range of RH from 10 to 98% at each of six temperatures studied (5, 10, 15, 20, 25, and 30 C, each $\pm 0.5$ C). Glass-distilled water was used to maintain 100% RH at all temperatures, although condensation frequently formed at low temperatures. Temperatures from 5 to 20 C were obtained by placing the respirometer in a cold room ($\sim5$ C).

Sclerotia of S. sclerotiorum were collected in the spring from bean fields and stored at 2-4 C until used. Ascospores were collected from apothecia produced from these sclerotia by the method of Grogan and Abawi (9). Triangular slivers of glass coverslips were placed on a piece of plastic netting and held over apothecia that ejected ascospores onto the slivers. The glass slivers were then suspended over salt solutions or glass-distilled water in reaction vessels mentioned above and described by Dubin (7). Ascospores were exposed at each temperature and RH for durations ranging from 24 hr to 21 days. At the end of the exposure period, ascospore survival was determined by placing slivers on potato-dextrose agar (PDA) for ascospore germination. A range of RHs at one temperature was considered an experiment and was repeated twice.

Field studies: Instrumentation and inoculation. Phaseolus vulgaris L. ‘Bush Blue Lake 274’ was planted in field plots at the rate of 10-12 seeds per 30 cm with 91 cm (36 in) row spacing during 1980 and 1981. A hygrothermograph equipped with a leaf wetness detector (31) was placed centrally in the field plot to continuously record ambient temperature, RH, and hours of leaf wetness. Rainfall data (daily totals) were obtained from an official weather station (no. 3184) 0.8 km from field plots or from an accumulating rain gauge in the field plot. In addition, during 1981 data were obtained from a recording rain gauge 0.08 km from the field plot. A CR-21 micro-data logger (Campbell Scientific, Inc., Logan, UT 84321) was used to record temperature at different positions in bean canopies or under radiation shelters.

Ascospores were collected and applied dry to bean leaves in the field to simulate natural deposition. It is possible that an extended exposure to water, as would occur if ascospores were atomized onto leaves, might alter their survivability by affecting metabolism or nutrient status (24) or by causing removal or the mucilaginous coating around the spore (23). In vitro studies were conducted using dry ascospores for the same reason. Saran plastic wrap was stretched over the mouth of a 12-cm funnel to form a broad, flat surface. An electrostatic charge was imparted to the plastic wrap by rubbing it on a piece of cloth. The plastic surface was held over apothecia at a distance of 2-3 cm, and ejected ascospores were distributed in a single layer over its surface. Ascospores were
applied to leaves by lightly touching the spore-bearing surface of the plastic wrap to the bean leaves. In later studies, greater numbers of ascospores were transferred to leaves by lightly misting the plastic surface with water before transferring spores to bean leaves.

Quantification of ascospore viability on leaf surfaces. A fluorescent stain consisting of 8 mg europium chelate (europium [III] thienyltrifluoroacetate, Eastman Organic Chemicals, Rochester, NY 14650) and 5 mg Calcifluor White M2R (disodium salt of 4,4'-bis[4-anilino-6-bis(2-hydroxyethylamino)-5-triazin-2-ylamino]-2,2'-stilbene disulfonic acid) dissolved in 5 ml of a 50% solution of absolute ethanol and distilled water was used to assess spore viability. The formula was slightly modified from one provided by J. P. Hubbard (13). Differential fluorescent stain (DFS) (4) stains living cells fluorescent orange and dead cells fluorescent blue. A 13-mm-diameter cork borer was used to punch disks from bean leaves onto which ascospores had been placed.

Each ascospore survival datum point from field studies was usually the mean of 16 leaf disks (four per leaf from four leaves). Leaf disks, transferred from the field to the laboratory in plastic petri dishes packed in ice, were stained with DFS by inverting them in a small droplet of the stain on a glass slide for 4 min. Excess stain was removed from the leaf disks by rinsing them with 3–4 drops of a 50% ethanol-water solution (28). A Zeiss photomicroscope equipped for epifluorescence microscopy, including an ultraviolet (UV) light source, dichroic exciter filters (Zeiss Equipment nos. 48 7702 and 48 7705), and barrier filters LP417 and LP418, was used to view fluorescing ascospores.

The reliability of the europium-based DFS to assess ascospore viability was determined by exposing ascospores on bean leaves in the field for various time periods. Eight to 12 leaf disks were removed from each leaf; half were stained with DFS, and half were atomized with potato-dextrose broth (PDB). The percent orange-

![Graph showing effect of temperature and relative humidity on the survival of ascospores of Sclerotinia sclerotiorum ejected onto glass coverslips and held over saturated salt solutions with different equilibrium humidities. Each line represents one relative humidity treatment and is the mean of three experiments, replicated four times.](image-url)
fluorescing ascospores was determined immediately after leaves were brought into the laboratory. Disks sprayed with PDB were held 36 hr at 100% RH and 20–25 C. The percent germination was determined on these disks by staining them with lactophenol-cotton blue, clearing over a flame, and viewing with the light microscope.

Field and laboratory studies on the effect of UV radiation on ascospore survival. Wooden frames covered with plastic film having different UV transmission characteristics were set over bean plants in the field. The top section of plastic film on these shelters could be retracted to allow dew formation and rainfall on the plants. Side sections hung freely so that airflow was only slightly impeded. Ascospores of *S. sclerotiurn* were applied to leaves at the top of bean canopies under these shelters and outside the shelters by the method described. Ascospore viability was assessed at 48-hr intervals. In 1980, shelters were covered with 0.127-mm type A Mylar (Mylar-A) (du Pont de Nemours & Co., Wilmington, DE 19989). In 1981, shelters were covered with 0.127-mm Mylar-A and 0.127-mm type S Mylar (Mylar-S). The UV transmission of the former is approximately 10% over the 300–400-nm range of the solar spectrum, whereas the latter transmits an average of 50–60% over the same interval. Neither material is transmissive of solar UV radiation below 300 nm.

In vitro studies on the effect of UV radiation on ascospore survival were conducted in a growth chamber maintained at 15 ± 1 C and equipped with two FS-40 Sunlamp fluorescent tubes (Westinghouse Corp., Bloomfield, NJ 07003) along with two Vita-Lite fluorescent tube (Duro-Test Corp., North Bergen, NJ 07047) to provide simultaneous radiation of photoreactivating wavelengths (≥313 nm) (14). Ascospores discharged onto pieces of glass coverslips were placed on 10 × 14 cm aluminum platforms (painted white to reduce UV reflection) under three different plastic filters: 0.127-mm cellulose acetate, 0.127-mm Mylar-A, and 0.0254-mm Mylar-S. Cellulose acetate is 1–2% transmissive of UV between 260 and 280 nm and averages 87.1% transmission from 300 to 400 nm. Ascospores were subjected to 16 hr of light and 8 hr of darkness in each 24-hr period. Approximate UV dosages were estimated using published data (16). Estimated UV dosage under the cellulose acetate filter summed over 250–279 nm and 280–369 nm was 19.2 J/m² and 3.2 × 10⁵ J/m² per 32 hr, respectively. Surface temperature measurements were obtained using thermistors or glass thermometers placed in contact with the aluminum surface 20 cm from the light source. At the end of a given exposure period, ascospores on pieces of coverslip were inverted on PDA and the percent germination determined after 24 hr.

**RESULTS**

In vitro studies on the effect of temperature and RH. Ascospores survived for 2 wk or more at low temperatures (5–10 C) with RHs up to 80% (Fig. 1). An increase in temperature from 5 to 10 C was associated with increased mortality of ascospores at all humidities and most particularly at 98 and 100% RH. Ascospore survival profiles were nearly equal at 15 and 20 C. Relative humidities in the 34–80% range (most relevant to field conditions) were most detrimental when combined with temperatures at or above 25 C. A rapid decline in germination was observed at 30 C, and complete mortality was reached sooner for all RHs at this temperature than at lower temperatures. The decline in percent ascospore germination at 98 and 100% RH was rapid at all temperatures above 5 C.

Field studies on ascospore survival. There was a high correlation between the percentage of ascospores that fluoresced bright orange when stained with DAF and ascospore germination on PDA of a concurrent sample of the same size and exposure time under field conditions (r = 0.984). The experiment was repeated twice with similar results and correlation coefficients (r = 0.995 and 0.950). Based on these data, the DAF technique was used to determine ascospore viability on bean leaves from the field.

Ascospores placed on the adaxial surface of leaves uppermost in the bean canopy had average survival rates of 51% after 2 days and 22% after 4 days of exposure to field conditions for studies conducted during June to October of 1980 and 1981. During the same exposure period, mean survival after 6 and 8 days was 7% and 4%, respectively. Few viable ascospores were observed after 12 days of exposure in the field. Temperatures above 29.4 C were associated with a rapid decline in viability; eg, the first 2 days, represented by Fig. 2, curve C, had a mean maximum temperature of 32.7 C with temperature moderating thereafter. Moderately warm temperatures (~26 C) were also associated with a steady, rapid decline in ascospore viability (Fig. 2, curve B). Under cooler conditions (~23 C) ascospores survived the longest and in highest numbers (Fig. 2, curve A).

Ascospore mortality was correlated with various environmental factors. Percent mortality was derived by subtracting the percent ascospore viability at any given time from the percent viability 48 hr earlier for a population of ascospores placed on bean leaves in the field at the same time. There was no significant correlation of temperature, RH, hours of leaf wetness, rainfall, or any combination thereof with ascospore mortality after 6 days or generally when ascospore viability was 10% or less. Therefore, correlations of mortality with environmental conditions were calculated by using the first three 48-hr intervals that ascospores were in the field. In 1980 and 1981, ascospore mortality correlated most closely with temperature. Total hours at or above 21.1 C over a 48-hr period correlated most closely with mortality in 1980 (r = 0.678) and in 1981 (r = 0.766). The mean of the two temperature maxima occurring during the 48 hr that ascospores were exposed in the field also gave significant correlations (r = 0.735 and 0.757). Total hours at or above 60% RH over 48 hr was only weakly correlated with mortality. In 1980 and 1981 the total hours of leaf wetness during a 48-hr period was inversely correlated with mortality. The correlation of total rainfall over 48 hr with mortality was weak in both years.

Data from 1980 and 1981 were combined for two temperature factors, the mean of two maxima over 48 hr (Fig. 3) and the number of hours at or above 21.1 C (Fig. 4), and were used to construct two regression models to predict ascospore mortality on topmost leaves of bean plants. One equation,

\[
Y = 14.9 - 1.65X + 0.998X^2 \quad (R^2 = 0.655)
\]

where \(Y\) is percent ascospore mortality and \(X\) is mean of two temperature maxima over 48 hr, described best the relationship between mortality and the mean of two temperature maxima.
Another equation,
\[ \hat{Y} = 18.3 + 1.10X \quad (R^2 = 0.537) \] (2)
described the line giving the best fit for the regression of the number of hours at or above 21.1 °C over 48 hr (X) on the percent ascospore mortality over the same 48-hr period (Y).

Ascospores applied to shaded bean leaves near the ground under a well-developed canopy usually survived better than ascospores on unshaded leaves at the top of the canopy, regardless of the exposure period. For example, survival of ascospores on leaves low in a dense canopy was 13% higher than survival on topmost leaves after 2 days of field exposure and 28% higher after 10 days. Generally, warmer intervals led to smaller canopy effects on ascospore viability (Fig. 5). In general, the position of ascospores in the bean canopy could mean a difference of about 20% in viability up to 10 days after deposition.

Thermistors were used to record ambient temperature at high and low positions in the bean canopy in the field. Large differences in temperature were observed between the two positions (Fig. 6). Thus, it is apparent that temperature is a contributing factor in differences in ascospore survival on leaves high and low in the canopy.

Field and laboratory studies on the effect of UV radiation on ascospore survival. Differential screening of UV radiation with plastic filters that reduced total irradiance and cut off irradiance at various wavelengths significantly affected ascospore survival in field and laboratory studies. In field studies there was greater survival of ascospores under UV-reducing filters compared to those unsheltered up to 14 days of exposure (Fig. 7A-B). Survival of ascospores under the more transmissive Mylar-S UV shelter treatment was intermediate between the Mylar-A and unsheltered treatments. Thermocouples were used to record leaf temperature in 1980, and the greatest difference in leaf temperature among all the UV shelter treatments was 1.5 °C.

Studies on ascospore survival in growth chambers equipped with two FS-40 sunlamp lights and one Vita-Lite supported field findings on the effect of UV radiation. Complete mortality of ascospores under the cellulose acetate filter occurred within 48 hr, whereas survival of ascospores after 224 hr of irradiation was 13.4 and 49% under Mylar-S and Mylar-A, respectively (Fig. 8).

![Fig. 3. Mortality of ascospores of Sclerotinia sclerotiorum in the field on the topmost bean leaves over a 48-hr period expressed as a function of the mean of two temperature maxima occurring over the same 48-hr period. Predicted percent mortality, represented by the solid line is based on the best-fit regression equation \( \hat{Y} = 14.9 - 1.65X + 0.0998X^2 \) where \( \hat{Y} \) = percent mortality and \( X \) = mean of two temperature maxima over a 48-hr period.](image)

![Fig. 4. Mortality of ascospores of Sclerotinia sclerotiorum in the field on the topmost bean leaves over a 48-hr period expressed as a function of the number of hours at or above 21.1 °C over the same period. Predicted mortality, represented by the solid line, is based on the best-fit regression equation \( \hat{Y} = 18.3 + 1.10X \), where \( \hat{Y} \) = percent mortality and \( X \) = the number of hours at or above 21.1 °C over the same period.](image)

![Fig. 5. Survival of ascospores of Sclerotinia sclerotiorum on bean leaves at the top of the plant canopy and on leaves deep in the canopy. A) Mean daily maximum temperature, 29.9 °C. B) Mean daily maximum temperature, 24.3 °C. Small letters indicate significant differences (\( P \leq 0.05 \)) in ascospore viability at each observation time. A and B analyzed separately.](image)
DISCUSSION

Previous studies with *S. sclerotiorum* revealed that mortality of ascospores increased as temperature and RH increased, and decreased as temperature and RH decreased (9, 27), a trend reported for ascospores and conidia of other fungi (20, 26). Our studies further suggest that above a temperature threshold, mortality of ascospores increases significantly at most relative humidities. The significant correlation of ascospore mortality with hours at or above 21.1°C in the field was confirmed by in vitro studies where mortality was higher when temperatures were above 20°C and RH was greater than 35%.

Relative humidity was weakly correlated with ascospore mortality in field studies during 1980 and not at all in 1981. In vitro studies indicated that RH in the 34–80% range was most detrimental at or above 25°C. Temperatures above 25°C seemed to override any effect of RH on ascospore survival because exposure to these elevated temperatures promoted rapid mortality at most RHs. This observation was confirmed in field studies in 1981 when ascospore mortality was highest during periods of low RH and high temperature. In contrast, the effect of RH on ascospore mortality was significant in 1980 when low RH occurred during cool periods near the end of the season. RH seldom drops below 40% in the field during the summer months in New York, so it is usually within a range that, combined with all but the lowest possible temperatures, assures rapid ascospore mortality.

The influence of canopy density on the disease cycle of white mold was noted in New York (J. E. Hunter, New York State Agricultural Experiment Station, Geneva, personal communication), Nebraska (5, 30, 33), and elsewhere (10). A dense bean canopy is frequently cooler and wetter than the ambient environment (5) with temperature and moisture conditions favorable for infection, disease development (33, 34), and production of apothecia (29). Temperature and canopy density influenced ascospore survival at different positions in the bean canopy. We found a 20% increase in survival of ascospores on leaves low in the canopy compared to topmost leaves. Survival of ascospores deep in a dense canopy despite higher moisture levels therein, indicated the overriding effect of temperature.

The influence of temperature was further demonstrated by the significant, albeit low, negative correlation of hours of leaf wetness with ascospore mortality. Time intervals with temperature maxima ~29°C had minimal hours of leaf wetness but high ascospore

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**Fig. 6.** Recordings of air temperature under the topmost leaves and at the base of the plant in a dense bean canopy during September 1980.

**Fig. 7.** Effect of solar radiation on survival of ascospores of *Sclerotinia sclerotiorum* in the field under various plastic films with different ultraviolet transmission properties. A) Ascospores on topmost leaves of bean plants unsheltered or sheltered with type A Mylar in 1980. B) Ascospores on topmost leaves of bean plants unsheltered or sheltered with type S Mylar or type A Mylar in 1981. Small letters indicate significant differences (P ≤0.05) in ascospore viability at each observation time, A and B analyzed separately.
mortality rates. Time intervals with temperature maxima of \( \approx 14^\circ C \) had long periods of leaf wetness but low ascospore mortality rates. This led to a false conclusion that total hours of leaf wetness was inversely correlated with mortality (ie, that leaf wetness promoted survival). Outside these two leaf wetness–mortality extremes, the data indicated a random association of leaf wetness with mortality in 1980 and 1981.

Of the two ascospore mortality prediction models presented, the simpler is equation 1, which utilizes the mean of two temperature maxima over a 48-h period. The two models are related. Daily temperature maxima were highly correlated with the number of hours at or above 21.1 C. For practical application, equation 1 is more useful because a maximum-minimum thermometer can be used instead of a recording thermometer as required by equation 2.

Temperature data in 1980 indicated that the maximum difference between air and leaves or leaves and leaves within or without radiation shelters was 1.5 C. Leaf temperatures of unbrushed plants in full sunshine tend to be near that of air (18, 19, 21). A study on soybeans (32), a crop similar to snap beans in canopy architecture and leaf size, found that temperature differences between leaves and air were never greater than 3 C for unbrushed plants. Bean plants in our field plots showed no visible signs of stress over periods during which significant differences in survival were seen between UV-sheltered and nonsheltered treatments.

Studies with artificial UV radiation simulated natural conditions in which the estimated dosage of UV radiation per 10-hr irradiation with two FS-40 sunlamp tubes was similar to average daily amounts expected at the Geneva, NY, latitude, 42° N, during June, July, August, and September. The average daily solar UV dosage over the 285–340 nm wavelength range during August at 40° N latitude is \( 4.04 \times 10^3 \) J/m² (15), with a 14-day total of \( 5.6 \times 10^5 \) J/m². Approximately the same dosage is achieved over the 280–369 nm interval with 560 hr of irradiance under two FS-40 sunlamp tubes: \( 5.76 \times 10^5 \) J/m². Differences in survival with differential filtration of UV over the 250–369 nm range markedly increased after 176 hr of FS-40 irradiance. Estimated total dosage at this time, \( 1.76 \times 10^5 \) J/m², is similar to that expected after 6 days in the field in August at 40° N, \( 2.42 \times 10^5 \) J/m², a point at which significant differences in survival were most obvious (Fig. 7A–B).

Rapid mortality of ascospores under the cellulose acetate filter in growth chamber studies (Fig. 8) was not surprising since it was 1–2% transmissive of UV radiation in the 250–290 nm range. Estimated dosage per 32-hr irradiance over 250–279 nm under two FS-40 sunlamp tubes was \( 1.92 \) J/m² under cellulose acetate (16). Estimated LD₀₉₈ of S. sclerotiorum ascospores under shortwave or UV-C radiation (250–280 nm) as transmitted by cellulose acetate was 9.6 J/m². Nearly complete mortality (99%) of ascospores under cellulose acetate was seen after 32 hr of irradiance. Lethality of shortwave or UV-C (250–280 nm) irradiation is well established for Neurospora crassa (12), Ustilago zeae (17), and Trichophyton mentagrophytes (11), as well as other fungi (25). These results provide evidence for a role of the UV-B (280–320 nm) and UV-A (320–400 nm) portions of the solar spectrum in the survival of ascospores of S. sclerotiorum. Quantitative data are needed on the effects of different levels of solar UV radiation on the survival of ascospores of S. sclerotiorum. Such data might improve the accuracy of temperature models for ascospore mortality prediction and could explain, in addition to temperature, disparities in ascospore survival and leaves at the top and deeper within the bean canopy.

**LITERATURE CITED**