Environmental Influences on the Release of *Ophiosphaerella agrostis* Ascospores Under Controlled and Field Conditions

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Accepted for publication on 25 July 2005.

ABSTRACT

Kaminski, J. E., Dernoeden, P. H., and O'Neill, N. R. 2005. Environmental influences on the release of *Ophiosphaerella agrostis* ascospores under controlled and field conditions. Phytopathology 95:1356-1362.

Ophiosphaerella agrostis, the causal agent of dead spot of creeping bentgrass (*Agrostis stolonifera*), can produce prodigious numbers of pseudothecia and ascospores throughout the summer. The environmental conditions and seasonal timings associated with *O. agrostis* ascospore release are unknown. The objectives of this research were to (i) determine the influence of light and relative humidity on ascospore release in a controlled environment, (ii) document the seasonal and daily discharge patterns of ascospores in the field, and (iii) elucidate environmental conditions that promote ascospore release under field conditions. In a growth chamber, a sharp decrease (100 to ≈50%; 25°C) in relative humidity resulted in a rapid (1- to 3-h) discharge of ascospores, regardless of whether pseudothecia were incubated in constant light or dark. In the field, daily ascospore release increased between 1900 and 2300 h and again between 0700 and 1000 h local time. The release of ascospores occurred primarily during the early morning hours when relative humidity was decreasing and the canopy began to dry, or during evening hours when relative humidity was low and dew began to form. Few ascospores were released between 1100 and 1800 h when the bentgrass canopy was dry. The release of ascospores also was triggered by precipitation. Of the ascospores collected during precipitation events, 87% occurred within 10 h of the beginning of each event.

Additional keyword: turfgrass.

Ophiosphaerella agrostis Dernoeden, M. P. S. Câmara, N. R. O'Neill, van Berkum, et M. E. Palm is an ascomycete capable of producing prodigious numbers of pseudothecia and viable ascospores (4,16). This pathogen causes dead spot of both creeping bentgrass (*Agrostis stolonifera* L.) and hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy) golf putting greens throughout the eastern and southern regions of the United States (7,15,17).

Unlike other *Ophiosphaerella* spp. associated with turfgrass diseases, *O. agrostis* commonly produces pseudothecia in nature (7). In the mid-Atlantic region of the United States, pseudothecia generally develop within necrotic bentgrass tissues between late June and mid-August (14). Ascospores serve as the sole source of infectious propagules throughout the growing season and play an integral role in the dead spot disease cycle (16). In nature, the pathogen is believed to exist solely as a teleomorph, and attempts to detect an anamorph have been unsuccessful (4,15,16).

Although ascospores rarely are found in nature for other *Ophiosphaerella* spp., ascospore production and the influence of environmental factors on ascospore release have been examined for other plant pathogens (13), for which rain, moisture, and humidity were shown to play a major role in the release of ascospores (9, 12,18–20,24). Temperature also can impact ascospore release (12).

Previous studies with *O. agrostis* revealed that pseudothecia can be produced in vitro on a tall fescue (*Festuca arundinacae* Schreb.) seed and wheat (*Triticum aestivum* L.) bran mix (16). Under constant light, pseudothecia developed in as few as 4 days, but no pseudothecia were produced in the dark. The environmental conditions associated with the release of *O. agrostis* ascospores are unknown, as are their daily and seasonal release timings. Elucidation of these conditions will lead to a better understanding of the epidemiology of dead spot and biology of this pathogen.

This project was designed to investigate the timing of and environmental conditions that influence the release of *O. agrostis* ascospores. The specific objectives were to (i) determine the influence of light and relative humidity on ascospore release in a controlled environment, (ii) document the seasonal and daily discharge patterns of ascospores in the field, and (iii) elucidate the impact of environmental conditions that promote ascospore release under field conditions.

MATERIALS AND METHODS

Ascospore sampler. Ascospore release in the experiments described below was monitored using a 7-day recording volumetric spore sampler (Burkard Manufacturing Co. Ltd., Rickmansworth Hertfordshire, UK). The Burkard trap samples 0.6 m^3 of air h^{-1} through an orifice (14 by 2 mm) located 45 cm above the ground. *O. agrostis* ascospores are long and slender, and range from 70 to 190 by 2.0 to 2.5 μm, making them suitable for collection using the volumetric spore sampler (11,14,16). Ascospores in the sampled air were trapped on melinex tape, which had been coated with a silicone (high vacuum grease; Dow Corning) and hexane mixture (5:1 wt/vol) that remained tacky, but did not become too viscous during the heat of the summer. The tape rotates at a rate of 2 mm h^{-1}; therefore, each 2- by 14-mm section of tape represents 1 h of spore collection. Because the orifice is 2 mm wide, the total number of ascospores captured within any given hour may have been deposited during a 2-h period (4-mm section). Spores deposited along a narrow (100 μm) linear traverse across the 14-mm wide opening will have been deposited within a single hour. Therefore, ascospores quantified along the narrow linear traverse are regarded as an estimate of the total number of spores

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DOI: 10.1094/PHYTO-95-1356

collected for 1 h. In this study, ascospore release was determined by counting all ascospores deposited within a 100-µm by 14-mm traverse. Ascospores counted along the traverse represent 1/20 of the total ascospores estimated for the hour.

Ascospore release in controlled conditions. The effect of relative humidity (RH) and light on ascospore release were monitored in a walk-in growth chamber (Environmental Growth Chambers, Chagrin Falls, OH). Due to large fluctuations in RH within the growth chamber, a boxed-in enclosure (≈1.5 m^3) was constructed using 4-mil clear plastic. RH was maintained using a Model M3DH30B2A dehumidifier (Maytag, Newton, IA). Air temperature (T_a) within the enclosure was maintained at 25°C with a standard deviation of 1.9°C.

Microclimatic conditions, including T_a (°C) and RH (%), were monitored within the plastic enclosure with a thermistor and a Vaisala capacitive sensor (model HMP35C; Campbell Scientific, Logan, UT), respectively. A pyranometer (LI200X; LI-COR, Lincoln, NE) also was placed in the enclosure to monitor light and dark treatments (described below). All instruments were connected to a CR-10 datalogger (Campbell Scientific) encased in an aluminum box. Environmental instruments were programmed to measure variables at 1-min intervals, and data were averaged each hour.

Eight individual treatments were evaluated as follows: A, 6 h of light (120 W m^{-2}; fluorescent and incandescent) followed by 6 h of dark at ≈50% RH; B, 6 h of dark followed by 6 h of light at ≈50% RH; C, 6 h of light followed by 6 h of dark at 100% RH; D, 6 h of dark followed by 6 h of light at 100% RH; E, 6 h of ≈50% RH followed by 6 h of 100% RH in constant light; F, 6 h of 100% RH followed by 6 h of ≈50% RH in constant light; G, 6 h of ≈50% RH followed by 6 h of 100% RH in constant darkness; and H, 6 h of 100% RH followed by 6 h of ≈50% RH in constant darkness (Table 1).

Pseudothecia were produced on a mixture of tall fescue seed and wheat bran (50:50, vol:vol) using the method previously described (16), with the following modifications. To minimize the impact of potential biological differences among varying strains of *O. agrostis*, three separate mixtures were prepared using isolates OpMD-4, OpMD-9, and OpOH-1. Despite differences in colony morphology when grown on potato dextrose agar (PDA), the three isolates all rapidly developed pseudothecia (14). All isolates had been collected from creeping bentgrass putting greens in Maryland or Ohio in 1998 and were maintained on PDA slants or on PDA plugs within sterile distilled water at 4°C. Infested mixtures were incubated in a dark growth chamber at 25°C for at least 16 days. Approximately 0.2 g of each infested mix then was placed on sterile, moist filter paper (Qualitative 415; VWR Scientific, West Chester, PA) in a 90-by-15-mm plastic petri dish. Petri dishes containing infested media were set in a growth chamber at 25°C in constant light (72 µmol m^{-2} s^{-1}). The inoculum and filter paper initially were moistened with 1 ml of sterile water and periodically were remoistened after the filter paper dried. After 7 days, the mixture contained pseudothecia with mature ascospores. Prior to use of the pseudothecia-infested mixture, ascospore release for each isolate was confirmed visually under a stereo microscope. Although the tall fescue–bran mixture contained varying numbers of pseudothecia, only mixtures in which ascospores were forcibly ejected from ostioles were used. Pseudothecia-infested mixtures (12 to 34 days old) of each isolate were combined (1:1:1; 0.6 g total) in a single petri dish and placed in the growth chamber under the initial starting conditions (Table 1) 6 h prior to treatment initiation. The mixture containing mature pseudothecia was set on a Styrofoam box placed directly below the spore trap collection orifice and was replaced with a fresh mixture of pseudothecia prior to each treatment. After the mixture was removed from the growth chamber, pseudothecia were examined microscopically for the presence of ascospores as described previously.

Only one volumetric spore sampler was available; therefore, treatments were conducted in the same growth chamber. The experiment was conducted three times. Prior to analyses, ascospore counts were square root transformed. The total number of ascospores collected over the 12-h period was determined for each of the three replications and data were subjected to analyses of variance using the MIXED procedure in SAS. Data also were subjected to repeated-measures analyses using the REPEATED statement within the MIXED procedure in SAS. The covariance structure of the repeated measures procedure was selected based on Akaike's Information Criterion (AIC).

Field ascospore release. The release of ascospores under field conditions was monitored at the University of Maryland Paint Branch Turfgrass Research Facility in College Park in 2001 and 2002. In August 1999, a research putting green was constructed to United States Golf Association specifications (26). Soil was a modified sand mix (97% sand, 1% silt, and 2% clay) with a pH of 6.9 and 10 mg of organic matter g^{-1} of soil. During the study period, soil P (79 kg ha^{-1}) and K (16 kg ha^{-1}) levels were in the low to moderate range.

A 465-m^2 area was seeded with 'Providence' creeping bentgrass (50 kg of seed ha^{-1}, 10 September 1999) and irrigated to maintain adequate moisture. Bentgrass seedling emergence occurred after 5 days, and the turf was maintained under putting green conditions thereafter. Inoculum (OpVA-1 and OpMD-9) was prepared using the tall fescue–wheat bran mixture described above, and a full description of the procedure was described previously (15). The site was inoculated by placing 0.5 g of inoculum at the soil surface in a grid pattern spaced approximately every 1.5 m on center on 12 March 2001. Due to relatively low disease levels in 2001, an area adjacent to the study site was prepared and fumigated for the 2002 study year. On 23 August 2001, a 465-m^2 area was vertical mowed to a depth of 1 cm, covered, and fumigated with methyl bromide (98% methyl bromide + 2% chloropicrin). The area was seeded to 'L-93' creeping bentgrass on 30 August 2001 and, after seedling emergence, the turf was maintained under putting green conditions. On 21 March 2002, the area was inoculated with a mixture of isolates OpOH-1 and OpVA-1 using the same technique as in 2001. Field release of ascospores was monitored hourly between 10 June and 31 October 2001 and 14 May and 31 October 2002 using the Burkard volumetric spore trap. Development of pseudothecia within the tall fescue–bran inoculum as well as bentgrass tissue also was monitored.

Environmental conditions were monitored between 1 May and 31 October in 2001 and 2002. Variables T_a and RH were measured with the previously described sensors (model HMP35C) placed 30 cm above the turf near the center of each study area. Sensors were housed in a 12-plate radiation shield to protect them from sunlight and rain. Soil temperature (T_s) was measured by averaging data from two thermistors (model 107; Campbell Scientific) placed 2.5 cm below the soil surface and spaced approximately 10 m apart. All temperature (T_a and T_s) and RH

TABLE 1. Growth chamber conditions during a 12-h period for eight treatments designed to assess the impact of changing levels of relative humidity and light on *Ophiosphaerella agrostis* ascospore release from pseudothecia

	Relative humidity (%)		Light[a]	
Treatment	0–6 h	6–12 h	0–6 h	6–12 h
A	≈50	≈50	Light	Dark
B	≈50	≈50	Dark	Light
C	100	100	Light	Dark
D	100	100	Dark	Light
E	≈50	100	Light	Light
F	100	≈50	Light	Light
G	≈50	100	Dark	Dark
H	100	≈50	Dark	Dark

[a] Light = 120 W m^{-2}.

instruments were programmed to measure environmental conditions at 2-min intervals, and the mean, maximum, and minimum values were recorded every 60 min.

Leaf wetness duration (LWD) was estimated by placing two electrical impedance grids (model 237; Campbell Scientific) horizontally on the turf canopy. The sensors were coated with flat-white latex paint to improve their accuracy (10). The electrical resistance for the sensors at the transition between wet and dry was 150 kOhms. Sensors for LWD were programmed to record readings every 15 min, and resistance values for each sensor were recorded as either 0 for dry or 0.25 for wet to represent each quarter hour.

Hourly precipitation was determined using a tipping rain bucket (Texas Electronics Inc., Dallas) situated 30 cm above the turf. Precipitation included both natural rainfall and irrigation applied using an automatic overhead irrigation system. Solar irradiance (SI) and wind speed (WS) data were collected at the United States Department of Agriculture Beltsville Agricultural Research Station, located approximately 0.3 km from the study site. The SI sensor (LI200X; LI-COR) consisted of a silicon photocell pyranometer mounted 3 m above the ground on a tripod. Wind speed measurements were recorded at the same location using a Model 5103 Wind Monitor (R. M. Young Company, Traverse City, MI).

The percentage of the total number of ascospores collected during each hour in the day (0000 to 2300 h) was determined for both years to reveal daily patterns of ascospore release. Daily means for measured environmental variables were obtained by averaging hourly data. Additionally, the number of hours with precipitation (natural rainfall and irrigation), maximum intensity of precipitation (mm h^{-1}), and maximum hourly increase and decrease in RH were tabulated for each day. Correlation analyses were performed between daily ascospore release and measured environmental variables. To account for intercorrelation of related variables, a Bonferroni correction factor (i.e., type I error adjustment) was used to compare the 17 variables at the appropriate $P < 0.05$ level.

Associations between precipitation, leaf wetness, and *O. agrostis* ascospore release were determined each year. The percentage of ascospores collected each hour under varying conditions of leaf wetness or precipitation was determined. To examine the effect of the duration of a precipitation event on ascospore capture, the number of ascospores deposited during each hour since the beginning of each precipitation event was determined. To account for intermittent breaks in precipitation, a precipitation event was defined, as described by Pinkerton et al. (22), as a continuous series of hours during which the individual hours in the event met one of the following criteria: rain occurred; the turfgrass canopy was wet and rain occurred during the following hour; or the turfgrass canopy was wet and rain occurred during at least one of the two preceding hours. Hourly means of the number of ascospores captured and the percentage of the total ascospores collected during all rain events were determined for each hour since the beginning of a precipitation event.

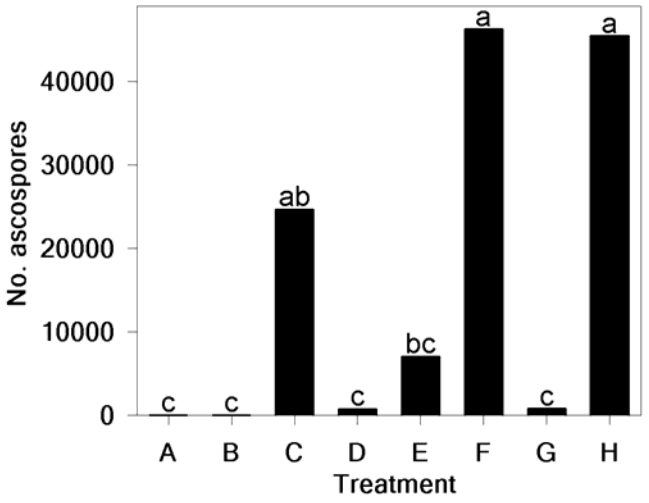

Fig. 1. Total number of *Ophiosphaerella agrostis* ascospores collected during 12 h of incubation at 25°C under changing relative humidity (RH) or light treatments. Treatments were as follows: A, ≈50% RH, light to dark; B, ≈50% RH, dark to light; C, 100% RH, light to dark; D, 100% RH, dark to light; E, ≈50 to 100% RH, light; F, 100 to ≈50% RH, light; G, ≈50 to 100% RH, dark; and H, 100 to ≈50% RH, dark. Total ascospores collected for each treatment were square root transformed prior to analyses, but back-transformed data are shown. Treatment means followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's test ($n = 3$).

RESULTS

Ascospore release in controlled conditions. Due to a large variation in the numbers of ascospores released within each treatment, ascospore data were square root transformed prior to analyses. However, all ascospore data presented have been back transformed. Treatment regimes (A to H) are shown in Table 1. The total number of ascospores collected throughout the 12-h period was significantly different ($P = 0.0037$) among treatments. The greatest release of ascospores occurred from those treatments (F = 100 to ≈50%, light; and H = 100 to ≈50%, dark) in which RH was lowered from 100 to ≈50%, regardless of light treatment (Fig. 1). Similar numbers of ascospores also were released in treatment C (100% RH; light to dark). When compared with treatments F and H, fewer ascospores were released from treatment E, in which RH was increased from ≈50% to 100% in constant light. Very few ascospores were collected within treatments D (100% RH; dark to light) and G (≈50 to 100% RH; dark). Ascospores were not released or were released in extremely low numbers (≤60 ascospores per 12 h) in treatments A (≈50% RH; light to dark) and B (≈50% RH; dark to light), in which RH remained at ≈50% for the entire 12-h period.

To assess the impact of changing environmental conditions (light and RH), hourly ascospore means across the three replicates were plotted for the 12-h period (Fig. 2). Repeated measures analysis revealed a significant treatment–time interaction ($P < 0.0001$). Due to this interaction, the release of ascospores during the 12-h incubation period was assessed individually for each treatment. Based on the total release of ascospores throughout the 12-h period (Fig. 1) and the distribution of the ascospore release (Fig. 2), only treatments in which the mean was ≥1,000 ascospores per 12 h (treatments C, E, F, and H) were subjected to repeated-measures analyses. Based on AIC values, the best covariance structure was determined to be first-order autoregressive. There were no differences in the number of ascospores released each hour for treatments C (100% RH; light to dark) and E (≈50% to 100% RH; light) (Fig. 2). The influence of decreasing RH, however, was apparent in treatments F and H. Regardless of light, both treatments in which RH was lowered from 100 to ≈50% resulted in a considerable increase in the release of ascospores. The release of ascospores in treatments F and H began immediately following the drop in RH, and a significant increase in the number of ascospores collected continued only for a short period (1 to 2 h). In both treatment F and H, peak ascospore release occurred at hour 7.

In summary, ascospores generally were not released in treatments where the RH was low (≈50%) throughout the 12-h period. During these periods of low RH, the tall fescue seed–wheat bran mixture generally appeared dry. The release of ascospores from other fungal ascocarps also occurs only after hydration by free moisture or RH (1–3). Varying numbers of ascospores were released when the RH was adjusted to or maintained at 100%. Only a sharp drop in RH stimulated the greatest and most rapid release of ascospores in constant light or darkness. Hong and Michailides

(12) unexpectedly found a similar increase in the release of ascospores of *Monilinia fructicola* due to rapid decreases in RH. Ascospore release of various plant pathogens has been shown to be influenced by temperature (1,12,19,24) and light (24). Despite some fluctuations in T_a within the growth chamber (standard deviation of 1.9°C), results and observations from this experiment indicated that decreasing RH, and not changing light or varying temperature, resulted in the greatest release of ascospores.

Field ascospore release. In 2001, dead spot symptoms first appeared on 1 June. Pseudothecia first were observed within necrotic bentgrass tissue on 4 June. Ascospore collection had begun by the time the volumetric spore trap was placed in the field on 10 June. The total number of ascospores counted between 10 June and the final rating date (31 October) was 42,340. Based on the percent total ascospores collected each year and counted in a single day, a total of 11 major (≥847 ascospores day^{-1}, ≥2% of seasonal capture) and 16 moderate (≥423 and <847 ascospores day^{-1}, 1 to 2% of seasonal capture) ascospore release events were recorded in 2001 (Fig. 3A). The first major discharge of ascospores occurred on 13 June and accounted for 2.2% of the total number of ascospores counted. Ten major events occurred between June and July, with the final event occurring on 30 August. Moderate ascospore release events generally occurred until late August, with low numbers of ascospores released in September and October. Although 180 ascospores were collected on 26 October, no new infection centers appeared after 5 October.

Following fumigation of the area in 2001, dead spot symptoms first appeared within inoculated spots on 12 May 2002. Ascospore counts began on 14 May. Pseudothecia first were observed within the inoculum on 18 April 2002 and a total of 34,800 (25.8% of seasonal total) ascospores were collected prior to the development of pseudothecia within necrotic bentgrass tissue on 10 June. Hence, fruiting bodies developing within the infested inoculum likely were responsible for the major discharge events (≥2,700 ascospores day^{-1}, ≥2% of seasonal capture) occurring on 16, 18, 19, and 27 May and 6 June. In 2002, dead spot was severe and, in all, 135,020 ascospores were collected. Throughout the season, 13 major and 16 moderate (≥1,350 and <2,700 ascospores day^{-1}, 1 to 2% of seasonal capture) ascospore release events were observed (Fig. 3B). Similar to 2001, low levels of ascospores were collected until early autumn (21 October); however, no new infection centers developed after 9 October.

Temperature likely plays an important role in the infection process by ascospores. Although ascospores were released throughout May 2002, mean air and soil temperature during the period in which ascospores were released was 10 and 13°C, respectively. An increase in the number of dead spot infection centers, however, did not occur until mid-June, when both air and soil temperatures averaged 24°C. Temperatures during the early release events likely were too low for infection to occur.

Daily and hourly patterns. The release of ascospores was monitored over 7,120 h between mid-May (2001) or mid-June (2002) and 31 October of each year. To determine any patterns in ascospore release during the day, the percentage of the total number of ascospores collected each hour (0000 to 2300 h) was examined. The percent ascospores collected during each hour

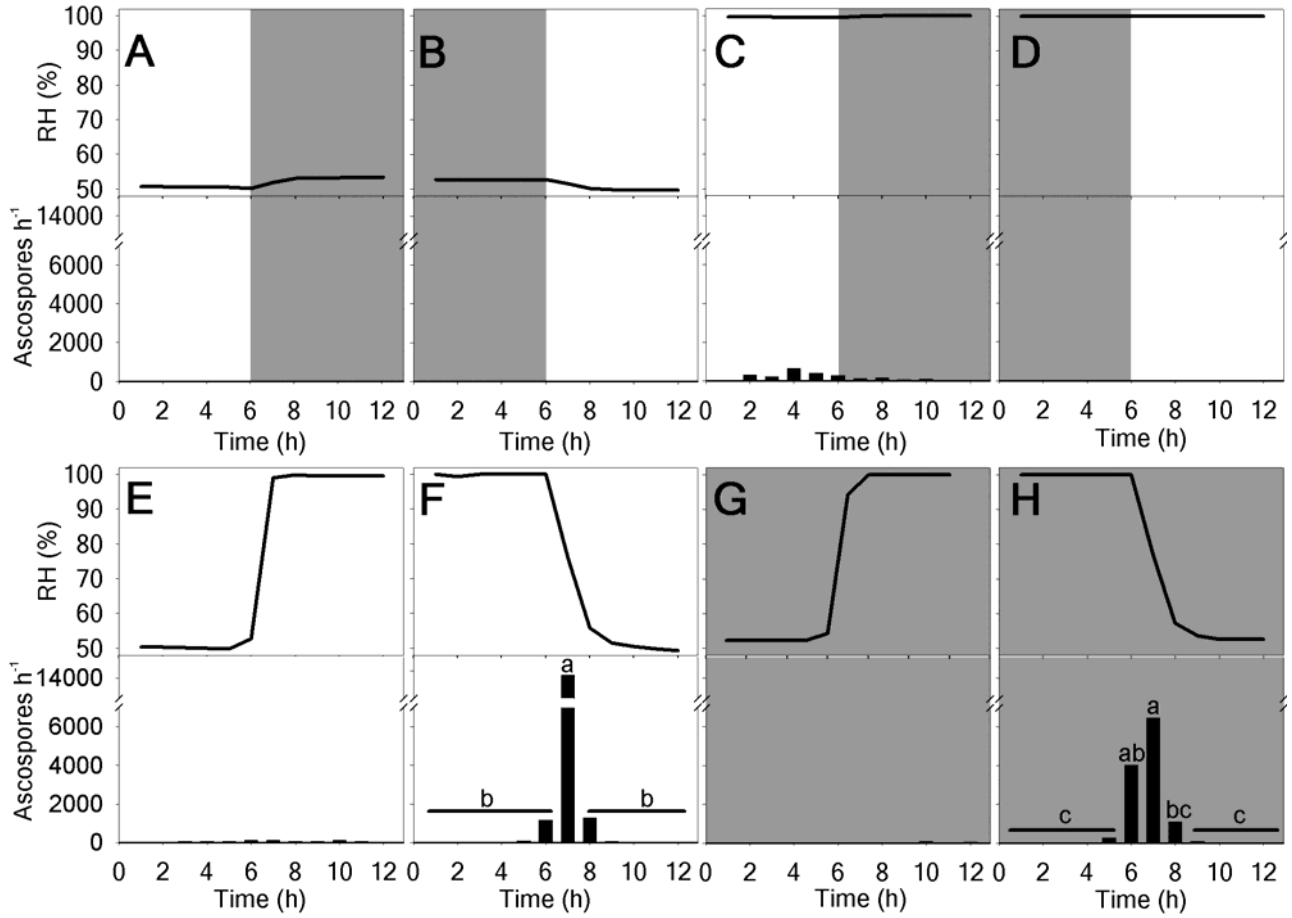

Fig. 2. *Ophiosphaerella agrostis* ascospore release under varying controlled conditions (A to H) in which relative humidity (RH) (solid line), light (white background) or dark (gray background) treatments were imposed at hour 6 of the 12-h incubation period at 25°C. Treatments were as follows: **A,** ≈50% RH, light to dark; **B,** ≈50% RH, dark to light; **C,** 100% RH, light to dark; **D,** 100% RH, dark to light; **E,** ≈50 to 100% RH, light; **F,** 100 to ≈50% RH, light; **G,** ≈50 to 100% RH, dark; and **H,** 100 to ≈50% RH, dark. Data are the mean number of ascospores collected throughout the 12-h period of three replications of the experiment. Hourly data were square root transformed prior to analyses, but back-transformed data are shown. For each treatment, significant differences in the number of ascospores captured during each hour are indicated by different letters.

occurred in a cyclic pattern and ranged from 1.9 to 7.8% and 1.4 to 7.0% in 2001 and 2002, respectively (Fig. 4). In 2001, the percentage of ascospores collected decreased after peaking between 2100 and 2200 h and then peaked again during the morning hours (0800 h). In 2002, large numbers of ascospores were collected throughout the night, but ascospore release again peaked during the morning hours (0700 h). In 2001 and 2002, only 15 and 23% of the total ascospores collected, respectively, were captured between 1100 and 1800 h. It is likely that moisture changes within pseudothecia stimulated the discharge of ascospores. In the field, the bentgrass canopy became wet in the evening hours while RH was still low (mean RH = 60% at 1900 h). Ascospore discharge was stimulated again as RH decreased and when pseudothecia began to dry during the early morning hours (0700 to 1000 h). Low numbers of ascospores (10 to 12%) were collected when the canopy was dry (Table 2).

To determine the environmental conditions that influence ascospore release, correlation analyses were performed between the number of ascospores collected and daily environmental variables. Because fewer ascospores were released in 2001 (42,340 ascospores) when compared with 2002 (135,020 ascospores), the percentages of ascospores collected on each day relative to the seasonal total were used in the analyses. Correlation analyses of all data revealed only weak associations between a select set of environmental variables and percent daily ascospore collection (Table 3). In 2001 and 2002, significant positive correlations occurred between percent ascospores collected and total precipitation (r = 0.25 and 0.26, respectively) and precipitation duration (r = 0.38 and 0.29, respectively). Percent seasonal ascospores collected daily between 0000 and 2300 h also was negatively correlated with the maximum hourly decrease in RH day^{-1} (r = –0.20 and –0.34, respectively), indicating a relationship between abrupt decreases in RH and increasing release of ascospores. Various correlations existed between ascospore collection and several other environmental variables, but the significance was not consistent between years.

Impact of precipitation on ascospore release. Precipitation (rain and irrigation) and leaf wetness greatly influenced the re-

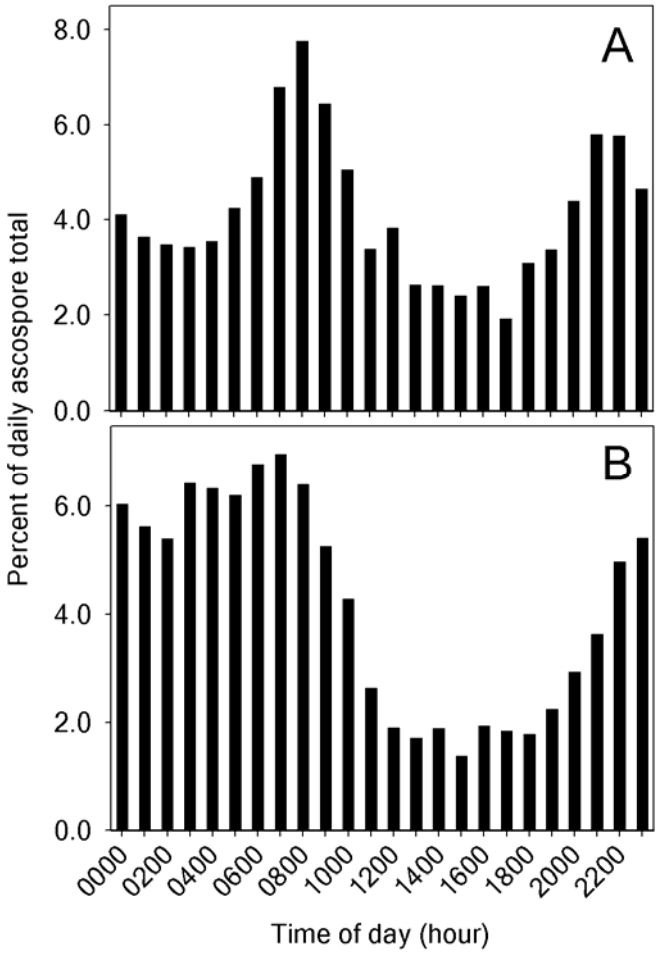

Fig. 4. Hourly percentage of the total number of *Ophiosphaerella agrostis* ascospores collected from creeping bentgrass in **A,** 2001 and **B,** 2002. Ascospores were collected hourly with a volumetric spore sampler between 10 June and 31 October 2001 and 1 May and 31 October in 2002.

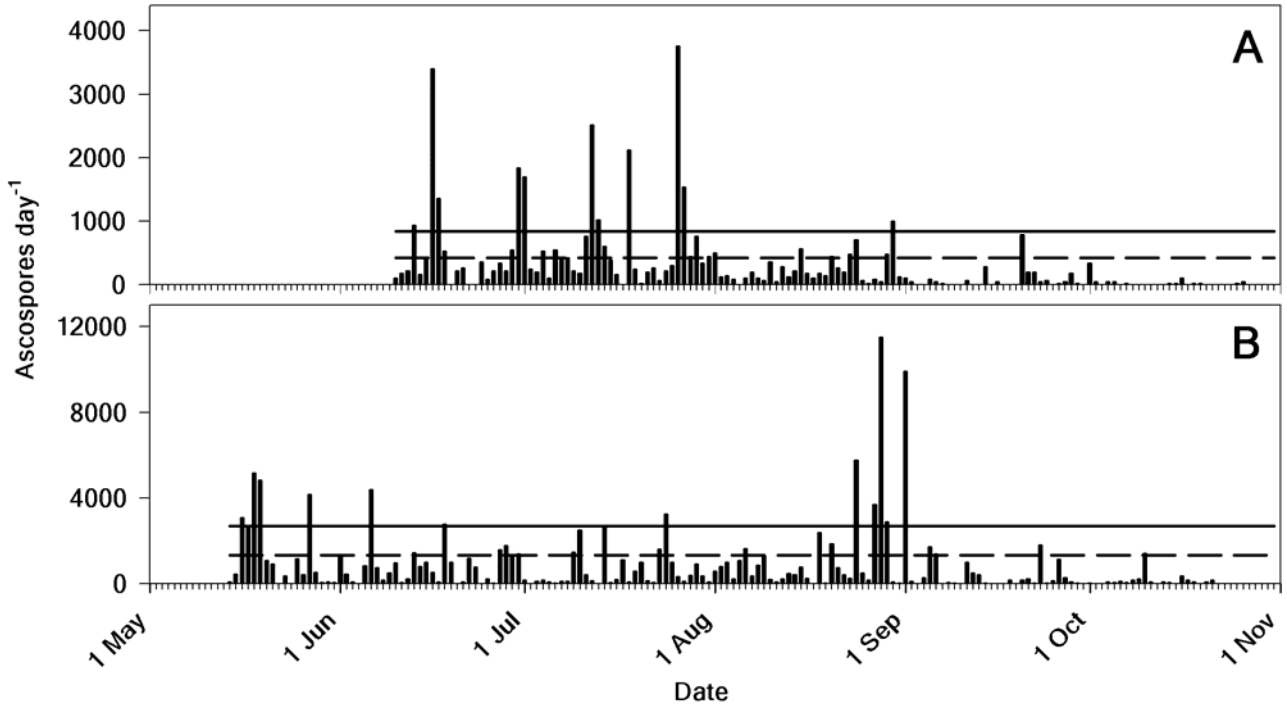

Fig. 3. Daily capture of *Ophiosphaerella agrostis* ascospores from a creeping bentgrass putting green in **A,** 2001 and **B,** 2002. Days with a major (≥2% of total season capture) or moderate (1.0 to 2%) ascospore release event are indicated by the solid and dashed lines, respectively. Ascospores were collected between 10 June and 31 October 2001 and 14 May and 31 October 2002.

lease of ascospores in the field. A large percentage of ascospores were released when the canopy was wet (Table 2). Precipitation events accounted for 16 and 20% (2001 and 2002, respectively) of the total number of hours monitored in this study, but the percentage of ascospores collected each year during precipitation events was 32.9 and 41.9% in 2001 and 2002, respectively (Table 2). Ascospore release patterns were more apparent when data were summarized relative to hours since the beginning of each precipitation event (Fig. 5). The number of ascospores collected increased during the first 5 h of precipitation and ascospores continued to be collected throughout much of each event (Fig. 5B). Because the average precipitation event lasted ≤7 h, the greatest percentages of ascospores were collected during this period (Fig. 5C). Additionally, examination of ascospore data collected during precipitation events indicated that ascospore release often declined as precipitation rate increased and vice versa (data not shown). This supports previous observations in which ascospores were observed to exude from pseudothecia in the presence of free water (16). These data indicate that, although ascospore release may continue for the duration of a rain event, pseudothecia begin ejecting large numbers of ascospores immediately after the initiation of a precipitation event.

DISCUSSION

Dead spot is a polycyclic disease, and the importance of *O. agrostis* ascospores in the spread and development of the disease was examined. Ascospores of *O. agrostis* are discharged

TABLE 2. Associations between precipitation (rain and irrigation) and leaf wetness duration and the release of *Ophiosphaerella agrostis* ascospores on a creeping bentgrass putting green in 2001 and 2002[a]

Environmental factor	2001	2002
Total hours of precipitation	508	789
Total hours of leaf wetness	2,390	2,560
Percentage of ascospores collected		
With leaf wetness[b]	86.8	85.8
Without leaf wetness[b]	12.1	9.6
During a precipitation event	32.9	41.9
With leaf wetness and no precipitation	54.1	44.0
Number of hours when ascospore capture was		
>100 to 400 ascospores h^{-1} with precipitation	44	137
>100 to 400 ascospores h^{-1} without precipitation	47	103
>400 ascospores h^{-1} with precipitation	6	50
>400 ascospores h^{-1} without precipitation	3	11

[a] Ascospores were collected between 10 June and 31 October 2001 and 14 May and 31 October 2002.
[b] Percentages not totaling 100% indicate missing data.

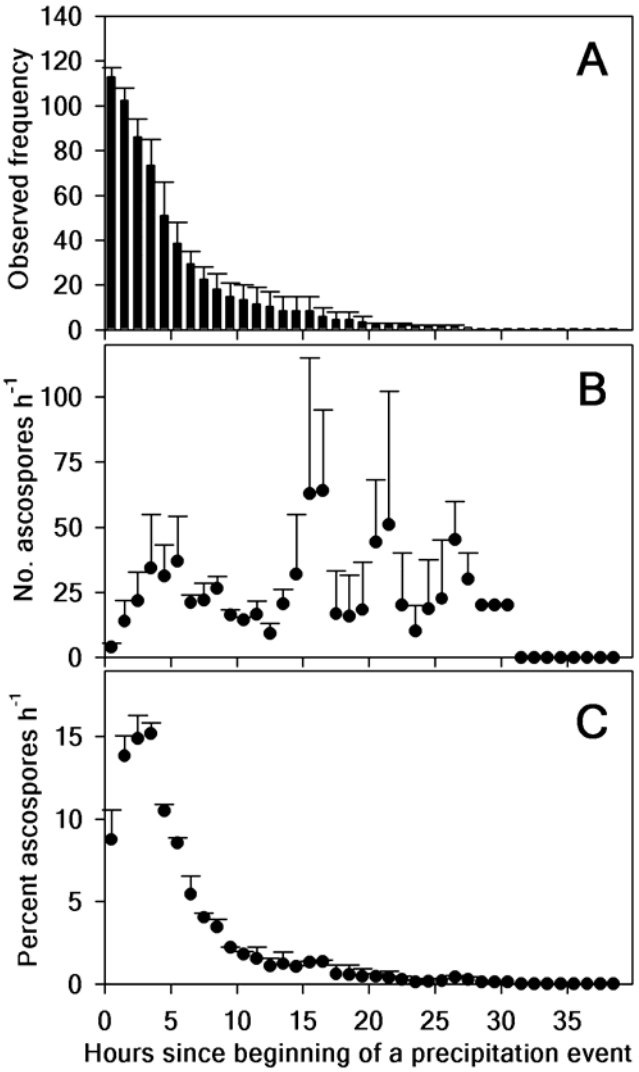

Fig. 5. Relationship between the number and percentage of *Ophiosphaerella agrostis* ascospores collected each hour since the beginning of a precipitation event. Data are mean values of 227 precipitation (rain and irrigation) events on a creeping bentgrass putting green from 10 June to 31 October in 2001 and 14 May to 31 October 2002. **A,** Observed frequency of the number of hours for each rain event; **B,** mean number of ascospores collected each hour since the beginning of the precipitation event; and **C,** percent of the total ascospores collected from 227 precipitation events during each hour of the event. Error bars indicate standard error of the means between 2001 and 2002 ($n = 2$).

TABLE 3. Correlation of percent daily capture of *Ophiosphaerella agrostis* ascospores on a bentgrass putting green with the environmental variables measured

	2001		2002	
Environmental variable	Correlation coefficient	*P*	Correlation coefficient	*P*
Mean relative humidity (%)	0.31	0.0002	0.09	0.2499
Mean air temperature (°C)	0.38	<0.0001	0.06	0.4479
Mean soil temperature (°C)	0.40	<0.0001	0.10	0.1960
Mean solar radiation (W m^{-2})	0.10	0.2341	0.09	0.2231
Mean wind speed (m s^{-1})	0.13	0.1349	0.10	0.1941
Total precipitation (mm day^{-1})	0.25	0.0028	0.26	0.0005
Leaf wetness duration (hours day^{-1})	0.05	0.5307	0.05	0.4920
Total daily hours of precipitation	0.38	<0.0001	0.29	0.0002
Minimum relative humidity (%)	0.35	<0.0001	0.05	0.4877
Minimum air temperature (°C)	0.46	<0.0001	0.15	0.0466
Minimum soil temperature (°C)	0.47	<0.0001	0.14	0.0780
Maximum relative humidity (%)	0.12	0.1553	0.03	0.7023
Maximum air temperature (°C)	0.29	0.0005	0.00	0.9619
Maximum soil temperature (°C)	0.36	<0.0001	0.07	0.3910
Maximum decrease in relative humidity h^{-1}	–0.34	<0.0001	–0.20	0.0110
Maximum increase in relative humidity h^{-1}	–0.01	0.9014	0.03	0.6950
Maximum precipitation intensity (mm h^{-1})	0.16	0.0604	0.12	0.1141

from pseudothecia in large numbers throughout the year and serve as an important source of inoculum. Although no conidial state has been found, the pathogen is capable of producing multiple cycles of fruiting bodies and viable ascospores within a short time period. This is in contrast to many other plant-pathogenic fungi that produce ascocarps as an overwintering mechanism and whose ascospores generally serve as a primary source of inoculum the following spring (6,12,21,22).

Ascospores of *O. agrostis* were observed to be forcefully ejected through ostioles of pseudothecia or exuded *en masse* in the presence of water (16). In growth chamber and field studies, ascospores rarely were released when pseudothecia were dry. In the field, ascospores were released in large numbers at dawn and dusk, and also during precipitation events. Ascospore release events occurred when periods of low RH coincided with periods of leaf wetness. During the morning hours in the field study, RH was observed to decrease sharply while the canopy remained wet. Conversely, as dew and guttation fluid began to form in the evening hours, RH remained low (≈60%). Although it is clear that some level of moisture is needed for ascospore release, it appears that lower levels of RH in conjunction with moist or saturated pseudothecia are essential for forceful ascospore release. It is possible, however, that high levels of RH which saturate pseudothecia may result in an oozing rather than ejection of ascospores into the air.

Regardless of whether ascospores are forcefully discharged or ooze from pseudothecia, the initiation of their release is rapid (≤1 h) and may continue as long as free moisture is present. In the laboratory, ascospores were observed to germinate in as little as 2 h (16). During the early hours of incubation, ascospores generally germinated in larger numbers in the presence of light and bentgrass leaves or roots. Using an ascospore suspension, ascospore germination and infection were observed to occur within 24 h, but the infection process may occur more rapidly (16). It is likely that ascospores released in nature coincide with an extended period of leaf wetness and are more likely to complete the infection process. In this study, large numbers of ascospores were collected between 0700 and 1000 h and 1900 and 2300 h. The bentgrass canopy, however, generally was dry by 1000 or 1100 h, leaving only a short period for germination and infection during morning hours. Therefore, only small numbers of ascospores released in the morning or during short irrigation cycles (15 to 30 min) are likely to cause infection due to the rapid drying (1 to 2 h) of the bentgrass canopy. A majority of successful *O. agrostis* infections likely occur following the release of ascospores at dusk, prior to extended periods of leaf wetness during the evening hours.

Although unknown, the field environmental conditions necessary for ascospore germination and penetration of bentgrass leaves likely include prolonged periods of leaf wetness and average daily air temperatures ≥22°C. The importance of LWD and temperature for the incidence and severity of plant pathogens has been widely studied (5,8,23,25,27). The requirement for some minimum temperature for infection by *O. agrostis* to occur is supported by the observation that the large numbers of ascospores released in May and again in September did not coincide with the appearance of similarly large numbers of new infection centers. Although average monthly air temperatures in May and September (2001 to 2002) ranged from 17 to 21°C, air temperatures between June and August ranged between 22 and 26°C. The aforementioned temperatures, as well as extended periods of leaf wetness, are common at dusk and early evening hours on creeping bentgrass putting greens in Maryland throughout the summer.

ACKNOWLEDGMENTS

We thank the United States Golf Association for financial assistance in support of this study, E. C. Synkowski for graphic and statistical assistance, and A. Grybauskas for helpful comments.

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