Diurnal Release of Ascospores by *Gibberella zeae* in Inoculated Wheat Plots

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ABSTRACT

Paulitz, T. C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Dis. 80:674-678.

The daily pattern of ascospore release by *Gibberella zeae (= Fusarium graminearum)*, the causal agent of Fusarium head blight of wheat, was investigated in artificially inoculated wheat plots. Mature perithecia and ascospores appeared on corn colonized by *G. zeae* 2 to 3 weeks after being placed in the plots (mid June). Ascospores over the plots were sampled with a Burkard continuous 7-day spore sampler. Temperature, relative humidity (RH), leaf wetness, and rainfall were also recorded in the plots on an hourly basis. Ascospores were released during the first three weeks of July in 1992 and 1993, with hourly concentrations of 600 to 9,000 ascospores/m^3. Ascospore release typically showed a diurnal pattern. Release began around 1600 to 1800 hours, reached a peak usually before midnight, and declined to low levels by 0900 hours the following morning. The beginning of ascospore release was correlated with a rise in RH during early evening hours. Ascospore release occurred before leaf wetness was detected and was not correlated with rainfall or continuous high RH during the preceding daylight hours. Peak ascospore releases occurred 2 to 4 days after major rainfalls. Ascospore release was diminished on days with continuous RH >80% or rainfall >5 mm. Light rain during a spore release event temporarily washed ascospores from the air; however, heavy rain (>5 mm) stopped spore release. This data suggests rainfall may be needed for perithecial and ascospore formation and maturity on crop debris, but not to trigger the actual release of ascospores. Perithecial drying during the day, followed by sharp increases in RH, may provide the stimulus for release of ascospores.

Fusarium head blight of wheat and ear rot of corn, caused by *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) is the most important disease of wheat and corn in eastern Canada. The fungus produces mycotoxins in the grain, most notably deoxynivalenol (DON; a trichothecene) and zearalenone. These mycotoxins can cause feeding refusal and reproductive disorders in cattle, swine, and poultry fed with contaminated grain. Tolerance limits for DON in grain for human consumption has been set at 2 mg/kg by Agriculture Canada (14). Significant economic losses occur from lower grain yield and quality, poor animal performance, and the cost of disease monitoring and regulation. This disease is endemic in eastern Canada and the northeast U.S., but epidemics occur periodically, as in 1980 on winter and spring wheat in Ontario, Quebec, and the Maritime provinces, and in 1990 on corn in eastern Canada and the northeast U.S. (4). The disease is almost absent from the drier wheat-growing areas of the upper midwest U.S. and prairie provinces of Canada, but reached epidemic proportions in parts of North Dakota and the Red River Valley of Manitoba in 1993 and 1994, due to the unusually wet conditions. At present, there is no complete resistance in any commercial corn or wheat cultivars.

Fusarium graminearum Group 2 is most prevalent in eastern Canada. Isolates readily form perithecia, and cause head blight or scab of wheat and barley. In the drier climates of Australia and western North America, Group 1 predominates. These do not form a sexual stage and primarily cause crown and root rots. The disease cycle and epidemiology of Fusarium head scab has been summarized by Sutton (23) and Parry et al. (16). *G. zeae* overwinters on wheat or corn debris in the field. The following spring, purplish black perithecia arise from stromata on the host tissue and forcibly discharge 3-septate ascospores in late June to July. Macroconidia (3- to 7-septate) are also produced in sporodochia on crop debris. Ascospores or macroconidia are deposited on the heads of wheat at anthesis, which usually takes place in early July in Quebec. The fungus colonizes the developing head, infecting the seeds, glumes, and rachis. Mycotoxins produced by the fungus can also be translocated into parts of the head. Two to three weeks after infection, infected spikelets turn brown prematurely, causing the scab symptom. Under conditions of high relative humidity (RH) or rain, infected heads may produce pinkish mycelia and sporodochia, and macroconidia may infect secondary tillers later in the season. Severely infected seeds (tombstones) are smaller, shriveled, and white. However, significant seed infection and mycotoxin production may be present with little symptom development.

Despite what is known about the disease cycle, very little is known about the epidemiology of the *G. zeae* stage, especially the environmental conditions and diurnal periodicity of ascospore formation and release. Most of the studies on this subject have been done in controlled environments in the laboratory, with very few performed under field conditions. Ayers et al. (2) sampled ascospores from April to October in corn and wheat fields in Pennsylvania, with most spores sampled from 2100 to 0600 hours. Tschanz et al. (24,25) found that light and perithecial dehydration were required for ascospore maturation and release in controlled environment chambers. Maximum perithecia production occurred at 28°C, but maximal ascospore discharge occurred at 16°C with no discharge above 26°C (25). Release of ascospores in the field in Brazil and China was related to rainy conditions (5,19).

The purpose of this research was to examine the periodicity of ascospore release in a wheat field, and correlate these events with temperature, RH, rainfall, and leaf wetness. Ascospores were sampled with a Burkard 7-day spore sampler (Burkard Scientific Sales, Ltd., Rickmansworth, UK). To ensure a large number of ascospores, a method was developed to artificially inoculate field plots in mid June with corn kernels colonized by *G. zeae*. After 3 weeks, mature perithecia and ascospores were produced. A preliminary report has been published (17).

MATERIALS AND METHODS

Fungal inoculum. Inoculum was prepared according to the methods of Fauzi and Paulitz (6). Macroconidia of *Fusarium graminearum* DAOM isolate 178148, from the Biosystematics Research Centre, Ottawa, were prepared in carboxymethylcellulose medium (CMC) (3). A 4-mm-diameter plug from a potato dextrose agar culture was placed in 500 ml of CMC medium, and incubated for 7 days at 24°C (100 rpm, Lab-Line Orbital Shaker, Lab-Line Instruments, Inc., Melrose Park, IL) with 16 h of light per day. Conidia were harvested and filtered through two layers of cheesecloth. Ten milliliters of macroconidia inoculum (approximately 1×10^4 spores/ml) was placed on 500 g of corn

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Accepted for publication 9 March 1996.

Publication no. D-1996-0415-04R

(*Zea mays* L.), which had been previously autoclaved for 45 min on two consecutive days in 1-liter glass jars. The jars were sealed with canning lids containing two 6-mm-diameter holes. A 70-mm-diameter filter disk (Fungi Perfecti, Olympia, WA) was placed on the inside of the lid to prevent contamination while allowing air exchange. Jars were incubated for 6 to 8 weeks at 20°C, under 16 h per day fluorescent lighting. Jars were shaken every few days to break up clumps of kernels.

Inoculation of field plots. In 1992 and 1993, at the Horticulture Farm of Macdonald Campus of McGill University, a 10 × 10 m plot was planted with a susceptible cultivar of wheat (*Triticum aestivum* L. cv. Max). Rows were seeded 10 cm apart, with a density of approximately 450 plants per m^2. The plot had not been previously planted with corn or wheat, and there was no corn or wheat within 300 m of the plot. Apple orchards were over 200 m away on the windward side of the plots, with a row of pine trees and fallow land on the leeward side. The plots were planted on 13 May in 1992 and 19 May in 1993. Five liters of corn inoculum was spread in the center 3 × 3 m area of the plot on 14 June 1992 and 16 June 1993. Perithecia were visible by 4 July 1992 and 5 July 1993, with mature ascospores by 15 July 1992 and 14 July 1993. Anthesis began 10 July 1992 and 18 July 1993.

Spore sampling and measurement of environmental parameters. A Burkard 7-day spore sampler was set up downwind 1 m away from the corner of the inoculated area, in a direction 20° from the center of the plot. The sampler was powered by a 12 volt automobile battery and was adjusted to sample 10 liters of air per min, with a tape speed of 2 mm/h. The sampler orifice was at the level of the heads at 80 cm. In 1992, temperature, RH, rainfall, and leaf wetness were monitored with an Agriscribe RSS-413 environmental monitor (Reuter-Stokes, Geneq Inc., Montreal, Quebec). In 1993, data were recorded with a Campbell CR 10 datalogger (Campbell Scientific, Logan, UT). Temperature and RH were monitored with a sensor (model HMP35A, Vaisala, Helsinki, Finland) located 0.5 m above the ground in a Stevenson shelter. Leaf wetness was monitored with a sensor (model 237) installed under the canopy 20 cm above the ground. All weather data were saved as 1-h averages. Rainfall, wind direction, and wind velocity data in 1993 were obtained from a meteorological station at the Emile Lods Seed Farm, approximately 1 km away. Spore sampling began on 9 July 1992 and 5 July 1993 and continued until 24 July 1992 and 14 August 1993, respectively. The sticky tape was removed from the spore sampler at weekly intervals, cut into 2-cm sections, and mounted in lactoglycerin-trypan blue on glass slides. The slide was scanned under a compound microscope (400×). The number of ascospores in each longitudinal section, (corresponding to 15 min of exposure) was counted. Because of their distinct shape and septation, ascospores of *G. zeae* could be distinguished from other Fusarium macroconidia. The sampled ascospores were assumed to be from the inoculated site, since the spore sampler was almost over the inoculated site, and the amount of perithecia present (5 liters of infected corn kernels) would yield ascospores many times greater than background levels. Similar trends were seen in both years, but the results of 1993 are presented, because of the more complete data set.

RESULTS

Mature ascospores were initially detected on 9 July 1992 and 7 July 1993, approximately 3 weeks after colonized corn was placed in the field. A typical diurnal pattern of ascospore release was seen on day 198 (17 July 1993) (Fig. 1). Each point represents the number of ascospores sampled in a 15-min period (150 liters of air sampled). Spore numbers from 0800 to 1600 hours were very low, with <20 spores sampled in a 15-min period, an hourly ascospore concentration of 133 ascospores/m^3. Spore release began at approximately 1800 hours, and reached a peak at 2300 hours, with peak hourly concentrations of 4,333 ascospores/m^3. In general, higher numbers of ascospores were released before midnight than after. The highest peak concentration recorded in 1993 was 9,333 ascospores/m^3. Ascospore release continued throughout the night until 0400 to 0800 hours. Ascospore release began before leaf wetness was detected in the lower canopy, which generally occurred from 2200 to 2400 hours. Ascospore release occurred at temperatures from 11 to 30°C and RH from 60 to 95%. During evening release periods, wind velocity ranged from 2 to 10 km/h and was generally equal to or less than velocities during the afternoon period. Wind direction during the afternoon and evening was generally from the west or southwest, so the spore sampler was downwind from the inoculated area. No major shift in wind direction between the afternoon and evening was measured. The beginning of ascospore release was highly correlated with the time that RH increased in the late afternoon, due to falling temperatures (Fig. 2). The start of an ascospore release event was counted from the time when ascospore counts exceeded 100/15 min, an hourly concentration of 666/m^3. Peak ascospore releases were not directly associated with rain events or continuous RH. On days with rainfall >5 mm during the day, ascospore release the subsequent night was inhibited (Fig. 3). Ascospore release was also inhibited on days with continuous RH >80% (Fig. 4) or high RH accompanied by intermittent rainfall throughout the day (Fig. 5). When the amplitude of nightly ascospore release was plotted over time (Fig. 6), peak release events did not exactly coincide with rainfall >5 mm (solid arrows) or days with RH >80% (broken arrow), but occurred 1 to 4 days later. Small rainfall events during evening release temporarily washed ascospores from the air (Fig. 7; Fig. 8, evening). However, larger rainfall events during ascospore release (>5 mm) completely washed ascospores from the air (Fig. 8, early morning).

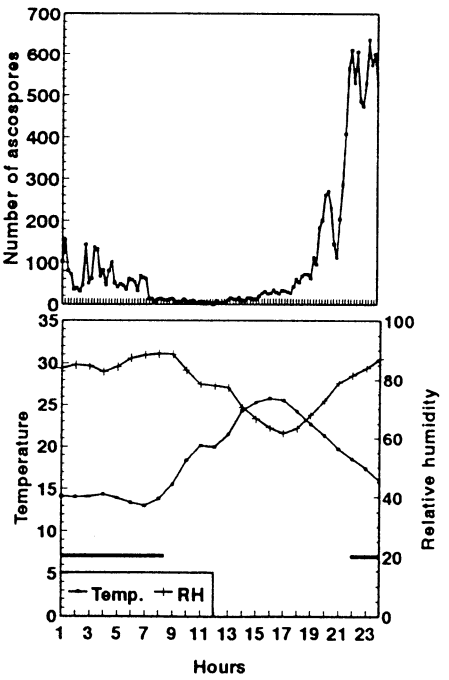

Fig. 1. Number of ascospores sampled, temperature, and relative humidity in an inoculated wheat plot, day 198 (17 July 1993). Solid horizontal lines denote periods of leaf wetness.

DISCUSSION

This work provides a detailed look at the dynamics of ascospore release on an hourly basis. The hourly ascospore concentration during the day (<133 ascospores/m^3) was

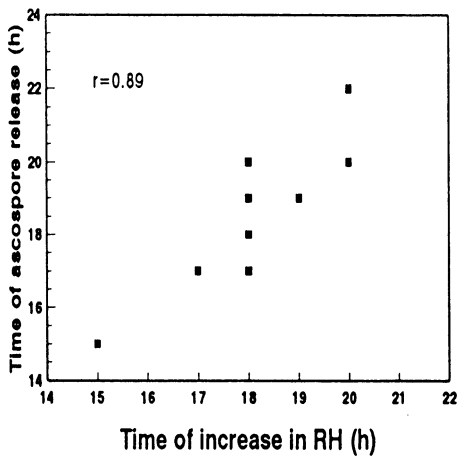

Fig. 2. Relationship between time of initiation of ascospore release and time of increase in relative humidity for major release events, 1993.

similar to that reported by Ayers et al. (2) (107 ascospores/m^3). However, in our study, the peak hourly releases in an inoculated field (600 to 9,000 ascospores/m^3) were much higher than Ayers et al. (2) reported in a naturally infested wheat field (107 to 1,428 ascospores/m^3). As shown by Ayers et al. (2), ascospore release was mainly nocturnal. Sanderson (20), using a Hirst spore sampler with hourly readings, found that ascospores of *Calonectria nivalis* on oats were released in a diurnal pattern, beginning between 1800 and 2400 hours, with a peak release around 2200 hours. This is similar to the pattern of two other perithecial pathogens that infect the floral parts of grasses, ergot (*Claviceps purpurea* (Fr.:Fr.) Tul. (1) and *Epichloe typhina* (Pers.:Fr.) Tul. (18). Nocturnal release may provide the pathogen with an adaptive advantage, since spores would be released during a period of high RH and dew. Ascospores are sensitive to drying and ascospore germination after 8 h was inhibited by water potentials less than –30 bars (–3 MPa) (22). Wheat plants are most susceptible to infection at anthesis when ascospores may infect the exposed anthers (23). Flowering occurs during the day and the anthers probably remain susceptible to infection for less than 24 h until the anthers dehisce. Anthers contain compounds that stimulate germination of the spores (21).

Our data showed that rainfall or high RH are not precisely correlated with ascospore release events under our field conditions. This is contrary to previous reports that show an association between ascospore release and high RH or rainfall (5,19). However, only daily rainfall totals were reported, so it is unclear when the rainfall occurred in relation to spore release. Our study looked at ascospore release on an hourly basis, a finer time scale than most studies. Our results agree with Sanderson (20), who also observed ascospore release on evenings with no rainfall during the day. However, he did report ascospore releases on an evening with 8 mm rain during the afternoon. High moisture conditions are probably required for perithecial formation and ascospore formation and maturation, but not for the actual release event. Sung and Cook showed that perithecial formation was maximum at –15 bars (–1.5 MPa), but almost completely inhibited below –50 bars (–5 MPa) (22). If the perithecia were not fully hydrated prior to the rain in the other studies, the moisture may have stimulated formation and maturation of asci and ascospores. In our experiments under the wheat canopy, the soil remained moist and did not dry out during the period when ascospores were sampled, so the perithecia were adequately hydrated throughout the release period. Rain or high RH occurred every 3 to 5 days in both years. In the study by Reis (19), moisture may have been more limiting, since the average rainless period (<1 mm/day) was more than 6 days.

The inhibition of ascospore release by heavy rainfall events before and during the release period may be caused by a layer of water covering the perithecia, so that the ascospores are oozed into a cirrus or gelatinous matrix around the ostiole instead of being explosively ejected (13). Sanderson (20) made similar observations with *Calonectria nivalis*. When leaf sheaths were covered by a thin layer of water, the energy of the discharged ascospores was insufficient to overcome the surface tension of the water. He also observed that rainfall events during the evening reduced ascospore release.

However, some moisture may still be required to trigger ascospore release. Although we did not detect leaf wetness until 3 to 4 h after ascospore release began, the RH near the soil surface around the perithecia may have been higher than what was recorded in the mid canopy. Water vapor evaporating from moist soil may have distilled on lower leaves in the canopy in the early evening, before dew formed in the upper canopy (15). Free water may have been present on the infected corn kernels, which would not be detected with the leaf wetness sensor. A second hypothesis concerning ascospore release is that an increase in RH over a 1- to 2-h period during the early evening may increase the turgor pressure of the asci beyond the bursting point. The large central vacuole of the ascus may contain a high osmotic pressure, calculated at 10 to 30 atmospheres (1 to 3 MPa) (10). If the osmotic concentration remained constant, an increase in the water potential of the surrounding air would increase the effective turgor pressure, assuming the ascus is semipermeable to water. Mechanical rupture may also occur from the swelling of the hydrophilic mucilage around the ascospores (9). The hy-

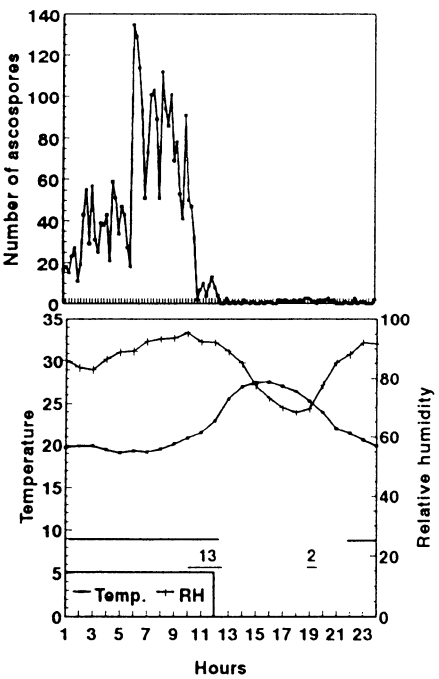

Fig. 3. Number of ascospores sampled, temperature, and relative humidity in an inoculated wheat plot, day 193 (12 July 1993). Solid lines without numbers denote periods of leaf wetness, solid lines with numbers denote periods of rainfall (mm).

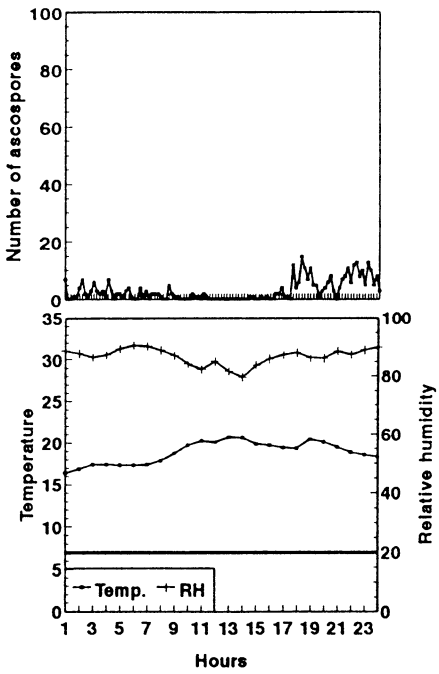

Fig. 4. Number of ascospores sampled, temperature, and relative humidity in an inoculated wheat plot, day 195 (14 July 1993). Solid lines denote periods of leaf wetness.

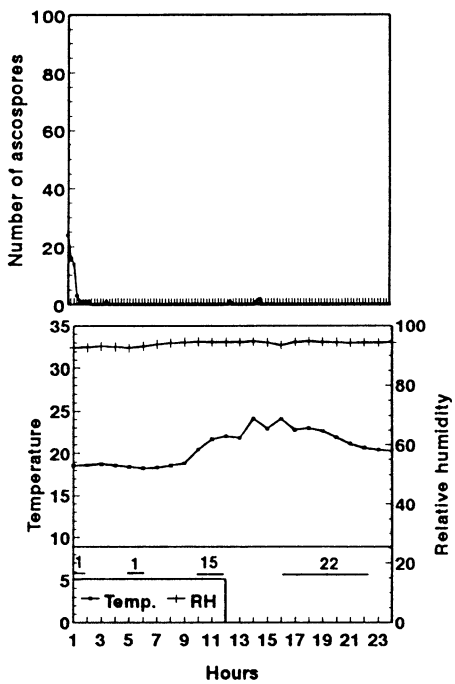

Fig. 5. Number of ascospores sampled, temperature, and relative humidity in an inoculated wheat plot, day 208 (27 July 1993). Solid lines without numbers denote periods of leaf wetness, solid lines with numbers denote periods of rainfall (mm).

pothesis that increasing RH triggers ascospore release is contrary to Tschanz et al. (24), who claimed that ascospore release was triggered by perithecial dehydration. However, their data showed initiation of ascospore release always occurred during humidification (100% RH), but peak ascospore release could also occur during periods when the humidity returned to 70% but dew was still present.

Light may also play a role in triggering ascospore release. Near-UV light was required for the production of perithecia of *G. zeae* (20), and the perithecia of *Sordaria* showed a positive phototropism and light-stimulated ascospore release (7,8,10). Some perithecial/pseudothecial pathogens are stimulated by light and release their spores during the day (12). However, release of *G. zeae* ascospores in the field occurred 4 to 5 h before sunset. Even under the wheat canopy, they would still receive some light at the time of release. A more logical hypothesis is that there may be a lag between the stimulus and response. The transition from dark to light in the morning may trigger events leading to ascospore release that evening. The relationship between rising RH and ascospore release may be coincidental.

Another possibility is that a daily rhythm may have developed, resulting in an endogenous response independent of external stimuli. *Daldinia concentrica* has a nocturnal rhythm of ascospore release that continued for a time after the illumination stimulus ceased (8,11). This would explain attenuated releases still occurring even on days with a lack of low-high RH stimulus (Fig. 4).

Ascospores often occurred in clusters on the tape. This may indicate that ascospores are held together by a gelatinous matrix, which may increase their mass and range (8,10). Sanderson (20) observed that all eight ascospores of *Calonectria nivalis* were released simultaneously, and 3 to 4 ascospores were often joined together on the glass slide from the spore sampler. The periodicity of ascospore release over the season suggests that there is a heterogeneous population of perithecia on the kernels, with different levels of maturity. Once the perithecia have released their ascospores, they cannot form new ones, but younger perithecia may mature a few days later. In both years, spores were sampled over a 3-week period, suggesting a range of perithecial maturities.

Further studies under controlled environmental conditions are needed to deter-

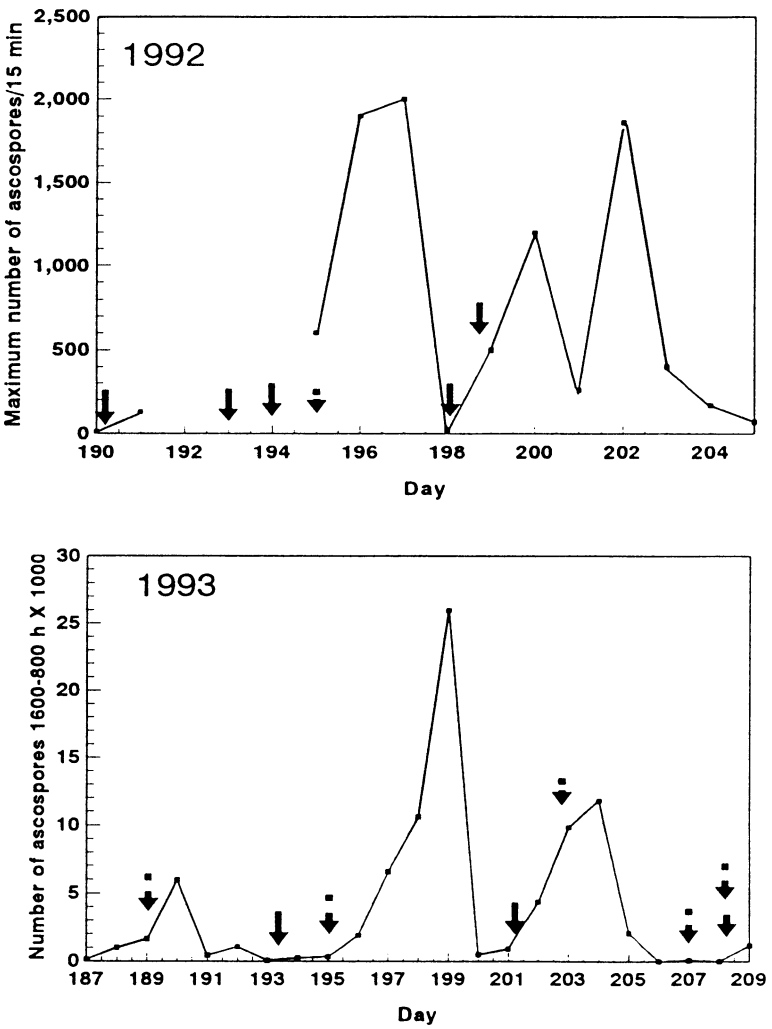

Fig. 6. Number of ascospores released over wheat plot in 1992 and 1993. Solid arrows indicate days with rainfall >5 mm, broken arrows indicate days with relative humidity >80% during entire day.

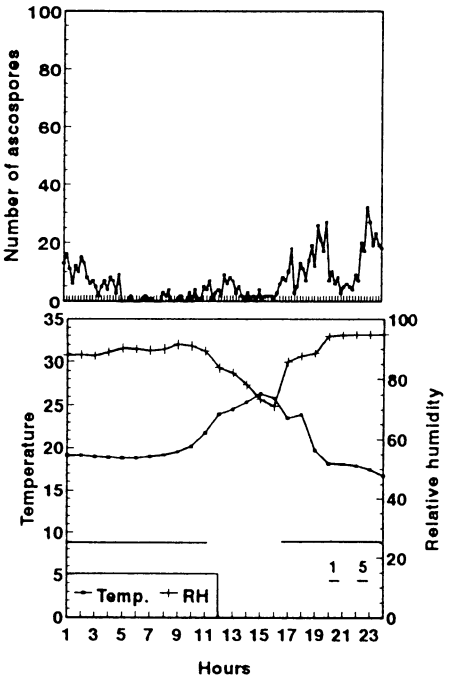

Fig. 7. Number of ascospores sampled, temperature, and relative humidity in an inoculated wheat plot, day 201 (20 July 1993). Solid lines without numbers denote periods of leaf wetness, solid lines with numbers denote periods of rainfall (mm).

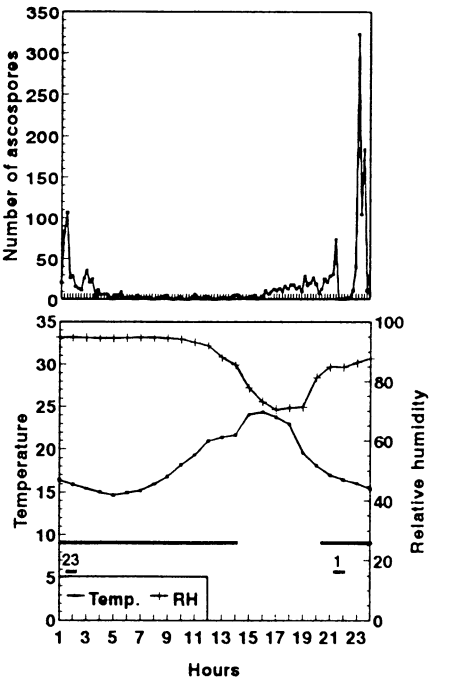

Fig. 8. Number of ascospores sampled, temperature, and relative humidity in an inoculated wheat plot, day 202 (21 July 1993). Solid lines without numbers denote periods of leaf wetness, solid lines with numbers denote periods of rainfall (mm).

mine exactly what triggers ascospore release. In addition, more detailed studies are needed in the field and laboratory to determine environmental conditions (temperature and water potential) necessary for perithecial and ascospore formation and maturity. Greater knowledge of the development of primary inoculum of Fusarium head scab may lead to more effective cultural methods of disease control.

ACKNOWLEDGMENTS

Thanks to Z. Kabir and V. Abraham for help with spore counts and compilation of environmental data; and to W. D. Fernando and W. L. Seaman for reviewing the manuscript. This research was partially supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

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