

Seasonal and daily variation in the airborne concentration of *Gibberella zeae* (Schw.) Petch spores in Manitoba

S. Inch, W.G.D. Fernando, and J. Gilbert

Abstract: The aerial concentration of ascospores and macroconidia of *Gibberella zeae* (anamorph *Fusarium graminearum*) within small inoculated plots was measured over 2 years (1999 and 2000) in Manitoba. In July 1999, a 30 m × 30 m area was inoculated with corn kernels infested with *G. zeae*. Ten Rotorod spore samplers were set up in a line transect, trapping airborne ascospores of *G. zeae* from 1800 to 0200. Ascospore concentration was high 1 to 4 days after rainfall exceeding 5 mm. Daily ascospore concentrations ranged from 0 to 214 m⁻³. Daily macroconidial concentrations ranged from 0 to 42 m⁻³. In 2000, a 3 m × 3 m area was inoculated with corn kernels infested with *G. zeae*, and a Burkard 7-day spore sampler was set up in the centre. Higher numbers of both ascospores and macroconidia were trapped in 2000 than in 1999. The airborne concentration of ascospores started to increase between 1500 and 1700 and coincided with the lowest daily relative-humidity values. Ascospores continued to be trapped until 0400. The highest concentration of ascospores occurred at 2100, with a maximum of 15 233 m⁻³. Fewer ascospores were trapped between 0500 and 1400, ranging from 0 to 167 m⁻³. As in 1999, high ascospore concentrations were detected after rainfall exceeding 5 mm. Compared with the number of ascospores, fewer macroconidia were trapped, with daily concentrations ranging from 0 to 567 m⁻³. An increased understanding of airborne inoculum is important for developing local-risk assessment models and strategies for managing fusarium head blight.

Key words: *Gibberella zeae*, *Fusarium graminearum*, ascospores, macroconidia, wheat.

Résumé : La concentration d'ascospores et de macroconidies de *Gibberella zeae* (anamorphe *Fusarium graminearum*) dans l'air à l'intérieur de petites parcelles inoculées fut mesurée sur une période de 2 ans (1999 et 2000) au Manitoba. En juillet 1999, une zone de 30 m × 30 m fut inoculée avec des grains de maïs infestés de *G. zeae*. Dix échantillonneurs de spores Rotorod furent installés le long d'un transect pour capturer les ascospores aériennes de *G. zeae* entre 1800 et 0200. La concentration en ascospores était élevée de 1 à 4 jours après une pluie de plus de 5 mm. Les concentrations quotidiennes en ascospores variaient de 0 à 214 m⁻³. Les concentrations quotidiennes en macroconidies variaient de 0 à 42 m⁻³. En 2000, une zone de 3 m × 3 m fut inoculée avec des grains de maïs infestés de *G. zeae*, et un échantillonneur de spores Burkard de type 7 jours fut installé au centre. En 2000, un plus grand nombre d'ascospores et de macroconidies y furent capturées qu'en 1999. La concentration en ascospores dans l'air commença à augmenter entre 1500 et 1700 et correspondit aux valeurs quotidiennes d'humidité relative les plus faibles. Des ascospores ont continué à être capturées jusqu'à 0400. La plus forte concentration en ascospores a été trouvée à 2100, avec un maximum de 15 233 m⁻³. Moins d'ascospores furent capturées entre 0500 et 1400, soit de 0 à 167 m⁻³. Tout comme en 1999, des concentrations élevées en ascospores furent trouvées après une pluie de plus de 5 mm. Avec des concentrations quotidiennes allant de 0 à 567 m⁻³, moins de macroconidies que d'ascospores furent capturées. Une meilleure compréhension de l'inoculum aérien est important pour le développement de modèles prévisionnels d'évaluation du risque local et de stratégies pour lutter contre la fusariose de l'épi.

Mots clés : *Gibberella zeae*, *Fusarium graminearum*, ascospores, macroconidies, blé.

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Introduction

In North America, *Gibberella zeae* (Schw.) Petch (anamorph = *Fusarium graminearum* Schwabe) is the principal cause of fusarium head blight (FHB) on small grain cereals (Clear and Patrick 2000; Gilbert et al. 2000; Gilbert and Fernando 2004). Epidemics have been sporadic in the past years and often associated with above-average rainfall during the growing season (Sutton 1982). The disease has been responsible for significant economic losses in yield and quality of grain (Sutton 1982; Parry et al. 1995; McMullen et al. 1997; Gilbert and Tekauz 2000; Gilbert et al. 2001).

Both macroconidia and ascospores are able to infect and colonize wheat heads (Stack 1989). Macroconidia are asexual spores and are produced in sporodochia on infected crop residues and heads. These spores may be splash dispersed to neighboring plants (Sutton 1982). Perithecia of *G. zeae* develop in the spring on overwintered crop residues (Sutton 1982; Paulitz 1996). In response to environmental stimuli such as rainfall and high relative humidity (RH), the ascospores are forcibly discharged from perithecia because of a build-up of turgor pressure (Trail et al. 2002). Although infection can occur at all stages of head maturity, wheat is considered most susceptible at anthesis (Fernando et al. 1997; Hart et al. 1984). Once airborne, ascospores may be transported some distance in wind currents and eventually deposited on spikes of cereals and other grasses. Moisture and ultraviolet (UV) light are thought to play an important role in the development of perithecia (Paulitz 1996; Trail et al. 2002). In eastern Canada, Paulitz (1996) determined that ascospore discharge was correlated with a rise in RH that began between 1600 and 1800 and continued until 0900. Airborne ascospores have been trapped 1–6 days after a rainfall event, with many studies demonstrating a distinct lag phase between rainfall and ascospore release (Markell and Francl 2003; de Luna et al. 2002; Fernando et al. 2000; Paulitz 1996). Daily and seasonal variations in the magnitude of airborne ascospore concentration have been observed (Fernando et al. 2000; Paulitz 1996).

Studies in Quebec and Ontario have investigated spore dispersal and disease spread, using susceptible spring-wheat cultivars (Paulitz 1996; Fernando et al. 1997, 2000), yet no published studies have examined the variation in airborne spore concentration over time in Manitoba. The objective of this study was to investigate the seasonal and daily patterns in the aerial concentrations of ascospores and macroconidia under environmental conditions in Manitoba.

Materials and methods

Inoculum was prepared using autoclaved corn kernels inoculated with five different field isolates of *G. zeae* that produced perithecia in culture and were tested for pathogenicity on wheat. The fungi were grown in Petri dishes containing potato dextrose agar (PDA) (BBL, Cockeysville, Md.), at 20 °C, under continuous fluorescent white light for 5 days. The cultures and agar were removed from the Petri dishes and placed into a blender with 500 mL of distilled water. The mixture was poured onto the sterile corn kernels and mixed thoroughly. The corn kernels were covered and

allowed to incubate at room temperature for 3 weeks. The infested corn kernels were then dried on a greenhouse bench for 1 week and stored at 4 °C until required.

In 1999 and 2000, the study site was established at the Cereal Research Centre experimental research farm at Glenlea, Manitoba. The main crops grown in the area are *Triticum aestivum* L. (wheat), *Avena sativa* L. (oats), *Hordeum vulgare* L. (barley), and *Brassica napus* L. or *Brassica rapa* L. (canola). In previous years, the field had been planted with wheat followed by a rotation with fall rye (*Secale cereale* L.). In 1999, the field was planted with experimental wheat lines and used for FHB disease trials.

In 1999, a 30 m × 30 m area was inoculated on 8 and 14 July with 40 g/m² of corn kernels infested with *G. zeae*. Ten Rotorod samplers (Aerobiology Research Laboratories, Nepean, Ont.) were placed 10 m apart in a line transect running northeast to southwest across the field and inoculated area. The samplers collected airborne spores for the first 15 min of each hour from 1800 to 0200. The clear-polystyrene Rotorod samplers were coated with silicone high-vacuum grease (Dow Corning, Midland, Mich.) to retain impacted spores. The rods were collected daily from 1 July through to 26 August and examined for spores, using a compound microscope (magnification, × 400). Spore concentration was calculated using the following formula:

$$C_s = N_{ts}/RKT$$

where C_s is spore concentration, N_{ts} is the total number of spores, R is the number of rotations per minute (2400), K , is the rotation constant (0.0197), and T is the total sampling time in minutes.

In 2000, wheat straw residues from the previous year were plowed under, except for a 3 m × 3 m area in the centre of the field. On 2 June, a 100 m × 100 m area was planted with the FHB susceptible wheat 'CDC Teal'. Rows were seeded 8 cm apart. The 3 m × 3 m plot was inoculated, on 12 June and 7 July, with 40 g/m² of corn kernels infested with *G. zeae*. Anthesis in the 'CDC Teal' crop occurred from 6 August through to 16 August.

A Burkard 7-day spore sampler (Burkard Scientific Ltd., Uxbridge, Middlesex, UK) was set up in the centre of the 3 m × 3 m inoculated area to monitor aerial spore concentration of *G. zeae*. The sampler was powered by a 12-V battery charged with a solar panel. The volume of air sampled was 10 L/min with a tape speed of 2 mm/h. Melinex[®] tape and a thin coating of silicone high-vacuum grease was applied to retain impacted spores. The tape was changed at weekly intervals and cut into 48-mm (representing 24 h) sections. The sections were mounted onto glass microscope slides and covered with 51 mm × 22 mm glass cover slips, using Permout[®] (Fisher Scientific, Nepean, Ont.) to fix the tape to the slides. Spore sampling began on 1 July and continued until 28 August. Ascospores and macroconidia were identified as *G. zeae* based on size and septation, using a compound microscope (magnification, × 400). The concentration of spores trapped during 1 h was calculated using the following formula:

$$C_s = (N_{ss} \times 20)/0.6$$

where N_{ss} is the number of spores per 100 μm wide transverse section, 20 is the total number of sections representing

Fig. 1. Daily concentration of *Gibberella zeae*: (A) ascospores (m^{-3}) and (B) macroconidia (m^{-3}) trapped with Rotorod samplers, between 1800 and 0200, during July and August 1999, at Glenlea, Manitoba.

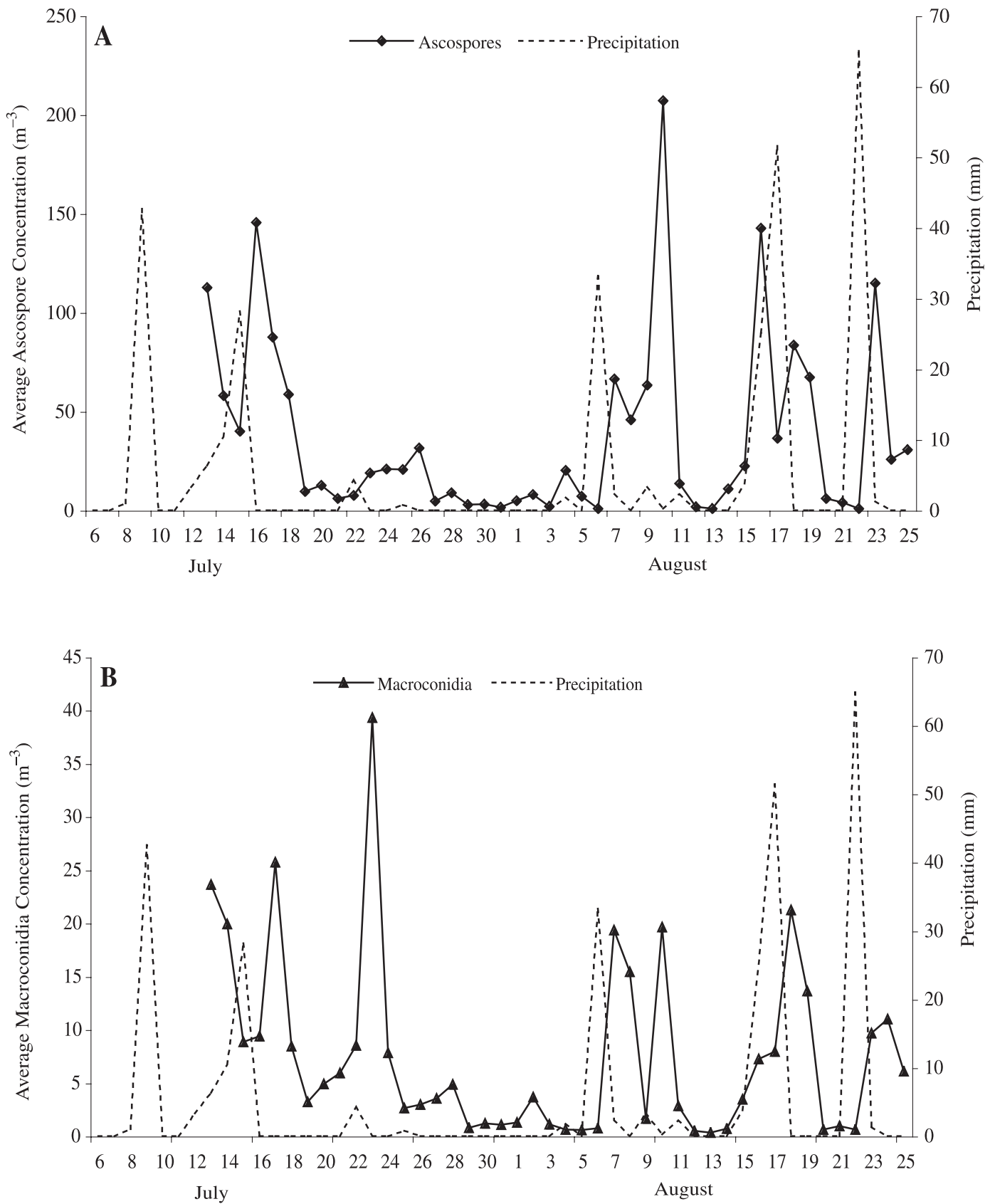
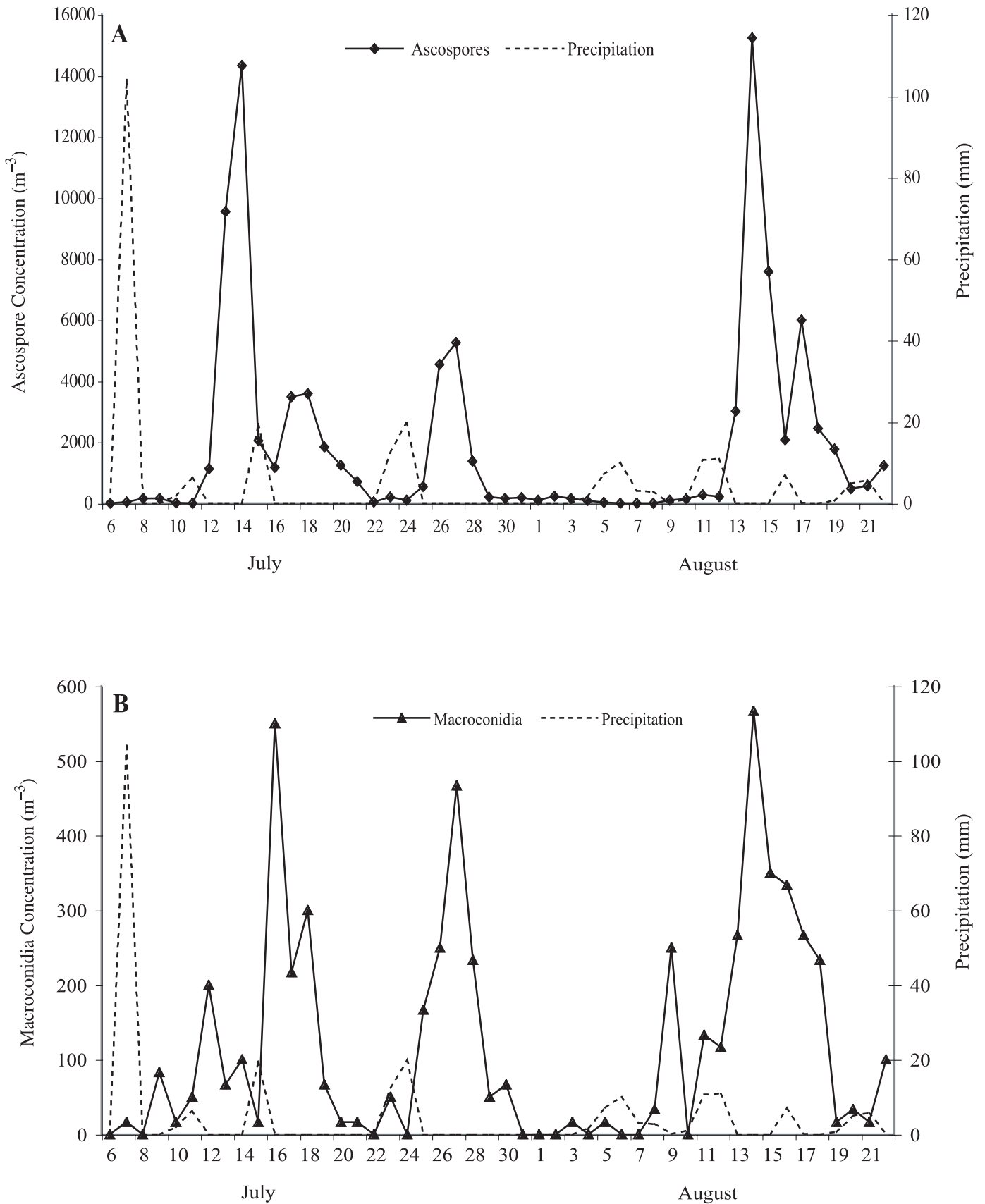


Fig. 2. Daily concentration of *Gibberella zeae*: (A) ascospores (m^{-3}) and (B) macroconidia (m^{-3}) trapped with a Burkard 7-day spore sampler during July and August 2000 at Glenlea, Manitoba.



1 h, and 0.6 represents the air sampling volume per hour in cubic metres.

Environmental data in both 1999 and 2000 were recorded every half hour with a CR10X datalogger (Campbell Scientific, Logan, Utah). Temperature and RH were monitored with a sensor (model HMP35A) installed under a RM Young radiation shield (model 41004-HMP). Wind speed and direction were monitored with a Campbell Scientific wind set (model 034A-L). Rainfall was measured with a Texas Electronics rain tipping bucket (model TE525M). No environmental data were recorded from 4 to 10 August in 2000 because of a malfunction of the datalogger. Precipitation data for the missing dates were obtained from rainfall data collected at the experimental research farm at Glenlea.

To monitor perithecial and ascospore development, straw residue and corn inoculum were collected from the inoculated area on a weekly basis. Samples were rinsed with distilled water, surface disinfected with a 0.3% NaOCl solution for 1 min, and then examined for the development of perithecia and ascospores. Maturity levels of the perithecia were determined by squashing perithecia under a glass cover slip. For confirmation and identification of *G. zeae*, 2-cm sections of surface-sterilized straw residue were plated in Petri dishes containing PDA amended with streptomycin (39 g of PDA, 0.1 g of streptomycin, and 1 L of distilled water). Inoculated plates were incubated at 20 °C under continuous fluorescent white light for 5 days. Isolates of *G. zeae* were identified based on colony and spore morphology, using a standard identification key (Nelson et al. 1983).

Results

In 1999, ascospores were first observed on 13 July. High concentrations of ascospores, equal to or greater than 113 m⁻³ were trapped 1 to 4 days after 5 mm or more of rainfall on 15 July and 10, 17, and 24 August. Ascospore concentration declined during periods of rain as seen on 6 and 17 August (Fig. 1A). Macroconidia were generally trapped from the air within a day after a rainfall event (>5 mm). Daily macroconidia concentrations ranged from 0 to 42 m⁻³ (Fig. 1B). There were no significant differences in the number of ascospores trapped on the 10 Rotorod spore samplers (data not shown). Any concentration gradient was probably masked because of high levels of background inoculum.

In 2000, mature perithecia were first detected on 5 July on overwintered infected straw residue. Ascospores were first trapped on 13 July, approximately 7 days after perithecia were observed on substrates in the field. A second smaller peak was observed on 27 and 29 July, approximately 3 weeks after the corn kernels were laid out in the field. Several peaks in ascospore concentrations occurred over the 2-month period, with two major events on 14 July and 14 August, during which ascospore concentrations of 14 333 and 15 233 m⁻³, respectively, were observed. A lag phase of 3 to 7 days between rainfall events exceeding 5 mm and increases in ascospore concentrations were apparent (Fig. 2A). On days that ascospores were trapped, a daily pattern was observed. Increases in ascospore concentrations began at approximately 1500, which corresponded

to a drop in RH and a rise in air temperature. Higher numbers of spores were trapped as RH increased, peaking at 2100, and continued to be trapped until 0400. Few ascospores were trapped between 0500 and 1400, with maximum hourly ascospore concentration ranging from 0 to 167 m⁻³ (Fig. 3).

In 2000, macroconidia were first detected on 9 July, following a rainfall event. Several dispersal events were observed over the 2-month period. Daily macroconidia concentrations ranged from 0 to 567 m⁻³ (Fig. 2B). Concentrations of macroconidia were highly variable over the 24-h period, and no daily pattern was observed (data not shown).

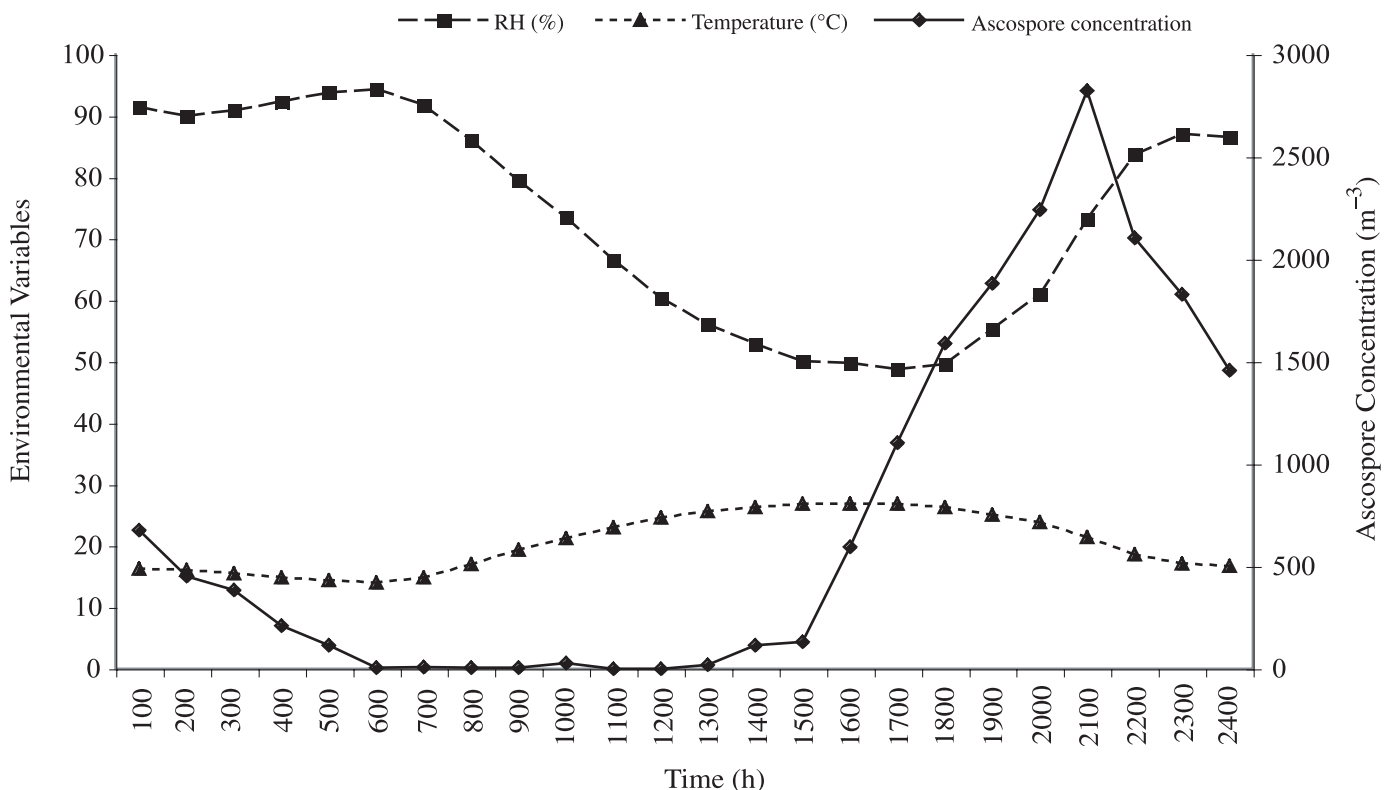
Discussion

This study provides the first detailed account of airborne concentrations of ascospores and macroconidia in Manitoba. A diurnal pattern of ascospore concentration was observed. In eastern Canada, ascospore release was correlated with a rise in RH early in the evening (Fernando et al. 2000; Paulitz 1996; Ayers et al. 1975). It has been postulated that a decrease in RH may cause the perithecia to dehydrate (Tschanz et al. 1975), resulting in the mechanical rupturing of the ascus wall. Ascospores are forcibly discharged through an ostiole at the apex of the perithecium (Trail and Common 2000). As the RH increases, the osmotic pressure rises in the perithecium, leading to the forcible discharge of the ascospores (Trail et al. 2002).

A seasonal pattern of ascospore concentration was observed in 1999 and 2000. High numbers of ascospores were detected in July and August. Corn kernels were laid out twice during the summer, and overwintered straw residue was left on the surface of the soil, which ensured an abundant inoculum source and may have produced a heterogeneous population of perithecia of different ages. Once the ascospores are discharged, the perithecia do not produce new ones (Paulitz 1996). This may be responsible for the peaks in ascospore concentrations throughout the sampling time.

High ascospore concentrations were observed 1 to 7 days after rainfall events of more than 5 mm, suggesting that rainfall may be important for perithecial development and for ascospore discharge. Moisture appears to be important for perithecial production (Sung and Cook 1981). In Manitoba, moisture tends to be a limiting factor. During dry-weather conditions, ascospore release did not occur. This was evident during a 10-day period at the end of July and the beginning of August 2000. Ascospore concentrations increased 7 days after a rainfall event. Perithecia are able to survive extended dry periods and resume activity after rewetting of the substrate occurs (Fernando et al. 2000). Paulitz (1996) found that moisture was not a limiting factor, as rain and (or) high RH occurred every 3 to 5 days and perithecia remained hydrated throughout the study. The relationship between rainfall and ascospore dispersal may be coincidental. It has been demonstrated under laboratory conditions that after an initial stimulus such as light or moisture is removed or changed, ascospore discharge occurs in a rhythmic pattern (Tschanz et al. 1975). However, in the field, moisture appears to play an important role in

Fig. 3. Relationship between relative humidity (RH), temperature, and average ascospore concentration of *Gibberella zeae* (m^{-3}) trapped at hourly intervals with a Burkard 7-day spore sampler on days with ascospore release $> 1000 \text{ m}^{-3}$ at Glenlea, Manitoba, in 2000.



perithecial development and ascospore release (Ayers et al. 1975; Paulitz 1996; Fernando et al. 2000; Trail et al. 2002).

Relatively low concentrations of macroconidia were detected compared with ascospore concentrations. Fernando et al. (2000) also trapped low numbers of macroconidia from the air. Macroconidia are considered to be splash dispersed and thus are transported only over short distances in rain droplets (Paul et al. 2004; Sutton 1982). The highest concentrations of macroconidia were trapped during the early morning and late afternoon. A possible explanation for this is that water droplets containing trapped macroconidia dry from wind and sun exposure, and the spores are then taken up and transported by wind currents. One advantage to macroconidial dispersal after a rainfall event is that the spores will not be washed off the plants as they would if deposited before rainfall events (Madden et al. 1996).

One advantage of the Burkard spore sampler is the ability to accurately monitor spore concentration over a 24-h and weekly time period. The Rotorod spore sampler monitors spore concentrations over a specified time period (i.e., from 1800 to 0200). One disadvantage of both the Burkard 7-day spore sampler and the Rotorod spore sampler is that the spores trapped do not remain viable on the adhesive surface. Therefore, the *Fusarium* species cannot be cultured to get a more accurate identification. In this study, *G. zeae* was identified based on the size and shape of the ascospores and macroconidia. Spore collection is different for the two spore samplers. The Rotorod spore sampler collects spores by direct impact. The Burkard spore sampler draws in airborne spores by suction, which allows a greater volume of air to be sampled. This may explain the great disparities between

the numbers of spores that were trapped in 2000 (using the Burkard sampler) as compared with 1999 (using the Rotorod samplers), even though levels of FHB in Manitoba during 1999 and 2000 were similar. The average FHB index, a measure of incidence and severity, in 1999 and 2000 were 4.3% and 7.9%, respectively, (Gilbert et al. 2000, 2001).

Parameters for FHB development will vary according to regions. Under Manitoba conditions, the highest numbers of ascospores were trapped primarily between 1500 and 0400 and were triggered by rainfall and changes in RH. With a greater understanding of the relationship between inoculum development and the influence of environmental parameters on the aerial spore concentration appropriate disease forecasting models can be developed, leading to more effective and efficient control methods of FHB.

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