

AN EPIDEMIOLOGICAL STUDY
OF FUSARIUM HEAD BLIGHT (FHB)
OF WHEAT CAUSED BY
GIBBERELLA ZEAE

BY

SHARON ANNE INCH

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the degree of

MASTER OF SCIENCE

Department of Botany
University of Manitoba
Winnipeg, Manitoba

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Abstract

In Manitoba, *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) is the predominant causal agent of fusarium head blight (FHB) on small grain cereals. Since 1993, the disease has resulted in significant economic losses. Certain aspects of the epidemiology of the disease under Manitoba conditions are unknown including the survival of *G. zeae* on crop residues, alternative host plants, and the effects of environmental variables on timing of spore release. The objectives of the project were to: 1) investigate the survival of *G. zeae* on fusarium damaged kernels (FDK). 2) determine the spatial and temporal aspects of spore release. 3) examine the host range of *Fusarium graminearum*. Fusarium damaged kernels were either left on the soil surface or buried at 5 and 10 cm at Glenlea, Morden, Portage la Prairie, and Winnipeg, and monitored for up to 24 months. *Gibberella zeae* was successfully re-isolated from FDK under all conditions with isolation frequency values ranging from 85-99% after 24 months. Perithecia developed on FDK from all locations and treatments, but ascospores developed only on FDK located at the soil surface. A similar experiment was conducted under controlled conditions to test survival of the fungus on kernels left at the surface or buried at 5 cm in sterile and non-sterile soil at -10°C, 2°C, and 20°C. General survival rate ranged from 92-100%. The percentage of kernels from which *G. zeae* developed was reduced to 83% and 76% when kernels were incubated at 20°C, in sterile and non-sterile soil, respectively. Kernels incubated at 20°C and 2°C developed perithecia, but ascospores developed only on FDK at 20°C. As survival of *G. zeae* on seed was not diminished after 24 months, rotations of at least two years are necessary to avoid infection of new crops by seed-borne *G. zeae*. In July and August 1999, ten rotorod

spore samplers were set up in a line transect, trapping air-borne ascospores from 1800-0200 h. Ascospore discharge occurred from 1 to 4 days after rainfall exceeding 5 mm. Daily ascospore concentrations ranged from 0-214 ascospores/m³ and were trapped from the air the day after a rainfall event (>5 mm). Daily macroconidia concentrations ranged from 0-42 macroconidia/m³. In 2000, an area of 3 x 3 meters was inoculated with *G. zeae* infested corn kernels and a Burkard 7-day spore sampler was set up in the centre. Ascospore release began at 1700 h, which coincided with the lowest daily RH values, and continued as RH increased, until 0400 h. Peak ascospore releases occurred at 2100 h, with a maximum concentration of 5064 ascospores/m³. Few ascospores were trapped between 0500 and 1500 h, ranging from 0-167 ascospores/m³. Ascospore release occurred 2 to 5 days after rainfall exceeding 5 mm. Daily ascospore concentration ranged from 0-15,233 ascospores/m³. Few macroconidia compared to ascospores were trapped from the air. Daily concentrations ranged from 0-567 macroconidia/m³. Grasses were collected from Glenlea, Tolstoi Tall-Grass Prairie Preserve, and Living Prairie Museum, in June, July, and August 1999 and 2000. *Fusarium graminearum* was isolated from 9% of the grass inflorescences examined (149/1766). Other *Fusarium* species isolated included, *F. sporotrichioides* (2.3%), *F. equiseti* (1.2%), *F. oxysporum* (1.1%), *F. avenaceum* (0.9%), *F. culmorum* (0.8%), and *F. poae* (0.7%). *Fusarium graminearum* occurred on 11 grass species, seven of which are reported for the first time. No visible symptoms of *Fusarium* infection were observed on any of the plants collected. This suggests that grass hosts are symptomless carriers of *F. graminearum* as well as other species of *Fusarium*. Wild grass species may help bridge the gap between susceptible host species and provide a mechanism for survival.

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Introduction and Literature Review

Introduction

In Manitoba, *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) is the predominant causal agent of fusarium head blight (FHB), accounting for over 96% of the *Fusarium* species isolated from wheat kernels collected during annual disease surveys (Gilbert *et al.* 2001). Several other species of *Fusarium* cause FHB including *F. acuminatum* Ellis and Everhart, *F. avenaceum* (Fr.) Sacc., *F. crookwellense* Burgess, Nelson, Toussoun, *F. culmorum* (W.G. Smith) Sacc., *F. equiseti* (Corda) Sacc., *F. poae* (Peck) Wollenw., and *F. sporotrichioides* (Sherb.). Of these, *F. graminearum*, *F. culmorum*, and *F. avenaceum* develop significant visual head blight symptoms (Miller 1994). The predominant *Fusarium* species within a particular region has been found to vary from year-to-year depending on prevailing temperatures and moisture (Magan and Lacey 1984). *Fusarium graminearum* is generally dominant in warmer climates, such as North America and China (Wang and Miller 1988), whereas *F. culmorum* tends to be the dominant species in several European countries with cooler climates (Snijders and Perkowski 1990). Pathogenicity will also influence the predominance of a particular species. The most pathogenic and commonly found *Fusarium* species are *F. graminearum* and *F. culmorum*. *Fusarium avenaceum* is isolated from wheat in relatively low frequencies, in part due to its low pathogenicity (Visconti *et al.* 1989). The other *Fusarium* species, including *F. acuminatum*, *F. equiseti*, *F. poae*, and *F. sporotrichioides* have low pathogenicity and vary in isolation frequency depending on climate and region (Abramson *et al.* 1987, Clear and Patrick 1990, Duthie *et al.* 1986). *Fusarium crookwellense* is rarely isolated in Canada and its importance has

not been examined (Abramson *et al.* 1987), although it has been reported as the primary cause of FHB in irrigated areas of South Africa (Scott *et al.* 1988).

Symptoms of Fusarium Head Blight

In general, only *F. graminearum*, *F. culmorum*, and *F. avenaceum* produce significant head blight symptoms on wheat (Miller 1994). These symptoms include premature bleaching of one or more spikelets of the head and the infected spikelets generally become sterile. Pink/orange sporodochia, or spore masses, may be seen around the edges of glumes on infected florets, and the rachis and peduncle can darken, turning a chocolate-brown color. Fusarium damaged kernels (FDK) are shriveled, chalky white in appearance, and are lighter in weight than normal healthy kernels. The degree to which the kernels are infected depends on what stage in the wheat life cycle they were infected. Early season infections result in kernels that are small and almost entirely colonized by the fungus. When kernels are infected late in the season, they may resemble healthy kernels. Symptoms of FHB caused by *F. poae* are distinct from other *Fusarium* species. On blighted heads the lesions appear with a bleached centre and a dark brown margin around the edge (Polley *et al.* 1991).

Historical Perspective

Fusarium head blight is not a new disease and epidemics have occurred sporadically over the years. It was first reported in the late 1800's in England by Smith (1884), who attributed the disease to *Fusisporium culmorum*. In 1917, FHB caused by *Gibberella zeae*, formerly *G. saubinetii*, was reported on many of the small grain cereal

crops in the eastern United States (Atanasoff 1920). From 1927 to 1980, periodic epidemics have been reported in eastern Canada during periods of above average rainfall (Sutton 1982). In Manitoba, *F. graminearum* was isolated from just 0.9% of cereal seed samples in the mid decades of the 1900's, and was not considered the predominant *Fusarium* species (Gordon 1944). The three common species of *Fusarium* that were isolated from seed samples were *F. avenaceum* (20.1 %), *F. poae* (16.4%), and *F. equiseti* (7.8%) (Gordon 1944). It has been suggested that since the 1940's, *F. graminearum* has been spreading northwest from the U.S into southern Manitoba and now reaches into parts of eastern Saskatchewan and Alberta (Clear and Patrick 2000). In 1984, two FHB-contaminated wheat samples from southern Manitoba were identified. High levels of deoxynivalenol (DON) were found in the hard red spring wheat cultivar, Sinton, and in the amber durum wheat cv. Coulter, at levels of 12.6 and 9.6 ppm, respectively. Plentiful rainfall in the area of Letellier, near the U.S. border, during anthesis and corn/wheat rotations appeared to have favoured the development of the disease (Clear and Abramson 1986). Studies have shown that continuous crops of wheat or wheat/corn rotations can potentially increase the amount of inoculum and lead to epidemics of FHB (Snyder and Nash 1968; Teich and Nelson 1984). Since 1984, the incidence of FHB caused by *F. graminearum* has steadily increased (Tekauz *et al.* 1986). In 1992 in southern Manitoba, FHB was found in 36% of the wheat fields sampled and *F. graminearum* was the principal causal species (Gilbert *et al.* 1993). In 1993, during the most severe outbreak on record, infected heads were found in 96% of the fields examined and *F. graminearum* accounted for 88% of the *Fusarium* isolations. Losses from the

epidemic were estimated at \$75 million dollars and annual losses have been estimated at approximately \$50 million (Gilbert *et al.* 1994; Gilbert and Tekauz 2000).

Epidemiology

Spore Production

Two states of the pathogen exist; the sexual state, *G. zeae*, and the asexual state, *F. graminearum*. Macroconidia are 45 x 3.5 µm in size (3-7 septate) and are produced by asexual reproduction in sporodochia on crop debris (Booth 1971). The optimal temperature range for macroconidia production is 28-32°C and decreases sharply below 16°C or above 36°C (Tschanz *et al.* 1976). Ascospores (3 septate) are 22 x 3.5 µm in size and develop in purplish black perithecia (sexual fruiting bodies) on over-wintered wheat and corn residues (Sutton 1982). Under controlled conditions several hours of UV light in the range of 300-320 nm is required for initiation of the sexual state (Tschanz *et al.* 1976). Optimal temperatures for the production of perithecia range from 15 to 28.5°C (Tschanz *et al.* 1976), and for ascospore production, from 25 to 28°C (Sutton 1982). Tschanz *et al.* (1975) reported that dehydration of the perithecia and substrate triggered ascospore release. Temperature did not influence the timing of release (Tschanz *et al.* 1975).

Under natural field conditions in eastern Canada, perithecia form on over-wintered crop residues in late spring and early summer (Paulitz 1996). Ascospores are forcibly discharged and dispersed by wind and may be deposited on the heads of grasses and small grain cereal crops which are most susceptible at anthesis (Fernando *et al.* 1997). The presence of choline and betaine (fungal growth stimulants), present in anthers,

was found to stimulate hyphal extension but not ascospore germination (Strange and Smith 1978). Both ascospores and macroconidia can infect at any time during head maturity (Hart *et al.* 1984) and are equally effective in the development of FHB, despite their difference in size (Stack 1989). Macroconidia are generally splash-dispersed (Sutton 1982). In eastern Canada, Fernando *et al.* (2000) trapped few macroconidia from the air relative to the number of ascospores. Macroconidia that arise from sporodochia may be an important source of inoculum causing infection on heads of secondary tillers later in the growing season. However, the relative importance of macroconidia vs. ascospores in the development of the disease is unclear (Francl *et al.* 1999).

Under field conditions, in eastern Canada, ascospore discharge was correlated with a drop in air temperature and a rise in relative humidity (RH). Ascospore release occurred at temperatures of 11 to 30°C and RH values of 60 to 95% (Paulitz 1996). Ascospore release from 0800 to 1600 h was low, with a concentration of less than 133 ascospores/m³. Peak ascospore release occurred between 1600 and 2400 h with the highest ascospore concentration of 4333 ascospores/m³ at 2300 h. Ascospore release events occurred 1 to 4 days after a significant amount of rainfall. Release was inhibited by periods of rain greater than 5 mm, continuous RH of more than 80%, or high RH with intermittent rainfall during the day (Paulitz 1996). Other studies have shown similar patterns of ascospore release. Ascospore release occurred in the late afternoon and continued into the evening (Ayers *et al.* 1975; Fernando *et al.* 2000). Several ascospore release events were also observed in July and August (Paulitz 1996; Fernando *et al.* 2000). This would suggest that a heterogenous population of perithecia with varying levels of maturity exist at any given time. Ascospore discharge is in part, related to the

number of mature ascospores available at the time when optimal conditions for discharge occur (Tschanz *et al.* 1975).

Spore Dispersal

Wind plays an important role in the dispersal of ascospores. Fernando *et al.* (1997) found wind-driven gradients in plots inoculated with ascospores. The infection gradient upwind was much steeper than the gradient produced downwind with gradient slopes ranging from -10 to -43 m⁻¹. Seed infection declined up to 90% within 5-22 m from the inoculum source. In plots inoculated with macroconidia a symmetrical pattern away from the focal centre was observed, in which the gradient was much steeper than that produced by ascospores; slopes ranged from -0.48 to -0.79 m⁻¹. The authors concluded that wind plays a lesser role in the dispersal of macroconidia (Fernando *et al.* 1997). Based on the steep gradients formed from artificially inoculated wheat plots, it was concluded that FHB is a monocyclic disease, as secondary inoculum (macroconidia) would have flattened out the gradient (Fernando *et al.* 1997).

Insect damage may provide another point of entry and allow for the colonization of *F. graminearum*. The orange wheat blossom midge may be a vector responsible for the transmission and spread of FHB. Sporadic outbreaks of wheat midge have been recorded in Manitoba and Saskatchewan, corresponding to FHB epidemics (Lamb *et al.* 1999). Studies have shown that wheat spikes exposed to wheat midge, inoculated with *F. graminearum*, had a higher incidence (29%) of disease than plants exposed to the control midge (0%) (Mongrain *et al.* 1999). However, no direct correlation has been observed between wheat midge and the spread of FHB, under field conditions. In Saskatchewan,

wheat midge is a serious problem, but not only in areas where FHB occurs (Lamb *et al.* 1999). Further investigation is required to determine the role of the wheat midge in the epidemiology of FHB.

Survival of *Gibberella zeae* on Crop Residues

Soil type has been found to influence survival of *G. zeae*. Under field conditions in Australia, the recovery of *G. zeae* was significantly greater from straw residues collected from soils located at Blue Nobby (104 weeks) than from those buried at Tremain (42 weeks) or at the North-West Wheat Research Institute (NWWRI) (96 weeks) (Burgess and Griffin 1968). However, the soil type and climatic conditions at the three locations were not defined in the study. When naturally infected wheat debris overwintered in the field, the recovery of *G. zeae* decreased from 40 % to 24 % (Warren and Kommedahl 1973). The survival of *G. zeae* is longer on tissues that resist breakdown. Recovery of *G. zeae* after 52 weeks was greater on nodal than internodal tissues, 94 and 0%, respectively (Burgess and Griffin 1968). However, rapid internodal tissue breakdown and the difficulty in recovering the tissues may have influenced the decline in survival. In Minnesota, *Gibberella zeae* was isolated from 84% of rotted corn stalks in November. In the following spring, recoveries were reduced to 61% from over-wintered cornstalks and to 31% in the fall on 1-year old basal stalk pieces (Windels and Kommedahl 1984). In eastern Canada, *G. zeae* was isolated after 3 years from all types of corn and wheat residues located above the soil and from corn kernels, wheat stems, and wheat spikelets from the soil surface. The duration of survival of *G. zeae* was only 1 year

on buried residues and 2 years on wheat kernels and corn stems located on the soil surface (Konga and Sutton 1988).

Increased soil moisture (more frequent wetting) has been found to reduce the recovery of *G. zeae*. After 28 weeks, the recovery of *G. zeae* decreased when moisture was adjusted every three weeks to a set moisture level (pF 2.5), compared to 72 weeks when soil moisture was adjusted only every 6 weeks (Burgess and Griffin 1968).

Survival of *G. zeae* in Soil

Macroconidia of *F. graminearum* will form chlamydospores, or thick-walled survival spores, when placed in soil. (Nyvall 1970). However, *F. graminearum* is not found in high frequencies in the soil and is generally associated only with plant residues (Sutton 1982). The predominant species of *Fusarium* found in soil samples from cereal plots in Manitoba in 1953 were *Fusarium oxysporum* Schlecht. Emend. Snyder & Hans (51.91 %) and *F. equiseti* (24.95 %) (Gordon 1954). No isolates of *F. graminearum* were obtained in that study. There are no recent studies that examine the *Fusarium* species composition in Manitoba soils. In Colorado, the most commonly isolated *Fusarium* species from the root zone of grassland plants were *F. roseum* 'Culmorum' (71 to 40%), *F. solani* (Mart.) Appel & Wollenw. Emend. Snyder & Hans. (15-31%), and *F. oxysporum* (8-20%) (Kreutzer 1972). In plots where corn was grown annually for a decade, *F. oxysporum* (62%), *F. solani* (21%), and *F. roseum* (8%) were the predominant *Fusarium* species isolated from corn roots, plant residues, the rhizosphere and soil (Warren and Kommedahl 1973). A second study (Windels and Kommedahl 1974) yielded similar results with *Fusarium oxysporum*, *F. solani*, and *F. roseum* being the three predominant

species isolated from prairie and cornfield soils, as well as roots of grasses, forbs and corn. *Fusarium graminearum* was found in approximately 30% of the soils sampled study. In eastern Australia, *F. graminearum* was isolated from small pieces of organic debris in 4 out of 5 soils. However, it was only isolated from 2 of 5 soils using the soil dilution technique (Wearing and Burgess 1978). This suggests that the sampling technique can influence results. In grassland soils in North Dakota, the recovery of *G. zeae* was low, with a relative density of 0.8%. *Fusarium oxysporum* and *F. equiseti* tended to be the dominant *Fusarium* species isolated, at a relative density of 46.1 % and 22.2 %, respectively (McMullen and Stack 1983).

Alternative Hosts for Survival

Alternative hosts species may bridge the gap between seasons, crops, locations, and unfavorable environmental conditions leading to a continuous source of inoculum which may contribute to disease outbreaks when susceptible hosts and proper conditions are met (Dinoor 1974). It is important that plant pathogens have mechanisms to ensure their survival when the host plant is absent. An extensive body of literature exists which examines alternative hosts of plant viruses and the associated dispersal vectors. Much less literature exists on alternative hosts for fungal pathogens.

Fusarium graminearum is pathogenic to many small grain cereal crops in the Gramineae family. There is little information on the role of alternative hosts for *F. graminearum*. Earlier studies indicate that *F. culmorum* was the dominant species from cereal crops in the Red River Valley (Gordon 1959). In recent years, isolation of *F. graminearum* from cereal crops has increased. This may have been due to the differences

in the aggressiveness of the two species, as well as a shift in the timing of perithecial and ascospore development. Gordon (1959) identified several *Fusarium* species from a range of plant species from several provinces in Canada, but *F. graminearum* was not one of them. Conners (1942) indicated that *F. graminearum* was rare in Manitoba, although it was reported on several grass species in Minnesota, and North Dakota, South Dakota. These species included *Agropyron cristatum* (L.) Gaertn., *A. subsecundum* (Lk.) Hitchc., *Agrostis* sp., *Bromus tectorum* L., *Cenchrus echinatus* L., *Cortaderia selloana* (Schult.) Aschers. and Graebn., *Echinochloa crusgalli* (L.) Beauv., *Hordeum distichon* L., *Lolium perenne* L., *Poa annua* L., *P. pratensis* L., and *Setaria lutescense* (Weigel) (Sprague 1950). *Fusarium graminearum* also has been reported on broad-leaved species including *Matricaria* spp. and *Viola arvensis* Murr. (Jenkinson and Parry 1994). In many cases these plant hosts showed no visible symptoms of the disease. Symptomless hosts are generally not included on host range lists because they are difficult to identify and require careful examination. However, it is important to identify such symptomless hosts in order to gain a better understanding of the pathogen. In most published studies *F. oxysporum* was the dominant *Fusarium* species found on weeds (non-planted plant species). Helbig and Carroll (1984), examined roots and stems of weed species from soybean fields. From the 21 species collected, 16 were found to be asymptomatic carriers of *Fusarium oxysporum* (74.5%). Other *Fusarium* species isolated at low frequencies included *F. solani*, *F. graminearum*, *F. acuminatum*, *F. moniliforme* Sheldon, *F. tricinctum* (Corda) Sacc., *F. semitectum* Beck. & Rav., *F. equiseti*, and *F. avenaceum*. Katan (1971) isolated *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synd. & Hans., the cause of tomato wilt, from roots of weeds belonging to the genera *Oryzopsis*, *Digitaria*, *Amaranthus*, and

Malva, none of which showed any symptoms. When the roots of the plants from these genera were inoculated with *Fusarium oxysporum* under controlled conditions, they generally showed no symptoms and colonization of the roots was low, ranging from 11 to 41%. However, when tomatoes were inoculated with isolates obtained from weed species, colonization was 100%. MacDonald and Leach (1976), isolated *Fusarium oxysporum* Schlecht. f. sp. *betae* (Stewart) Synd. & Hans., the cause of stalk blight in sugarbeet (*Beta vulgaris* L.), from *Chenopodium album* L., *Brassica nigra* (L.) Koch, and *Anethum graveolens* L. All three species were symptomless carriers of the pathogen. *Amaranthus retroflexus* L. was the only species identified that exhibited *Fusarium* wilt symptoms. Cultures obtained from diseased plants were morphologically identical to isolates from diseased sugarbeets. Haware and Nene (1982) found four weed species, *Cardiospermum halicacabum* L., *Convolvulus arvensis* L., *Cyperus rotundus* L., and *Tribulus terrestris* L., which harbored the pathogen responsible for chickpea wilt, *F. oxysporum* Schlecht. f. sp. *ciceri* (Padwick) Synd & Hans. None showed symptoms of the disease. When isolates obtained from these genera were inoculated on to chickpea plants, they were found to be non-pathogenic. This may have been due to the race specificity of the pathogen.

Control of Fusarium Head Blight:

Recommendations for the control of FHB include burying crop residues to help accelerate decomposition and potentially eliminate this source of inoculum (Sutton 1982). Gilbert and Tekauz (1994) showed there was no apparent effect of tillage practice on disease development in Manitoba when disease pressure was high in the sever

epidemic of 1993. However, the effects of various tillage practices were difficult to demonstrate, due to high background levels of inoculum. Rotations away from wheat, after a susceptible host such as corn or small grain cereals, with a non-host crop, may reduce disease incidence (Sutton 1982; Teich and Hamilton 1985; Parry *et al.* 1995). Crop densities may affect the incidence of FHB. High stand densities increase the humidity in the canopy and may lead to a higher incidence of disease (Hatmenu 1972). The amount and type of nitrogen fertilizer used also may influence the development of disease. Teich (1987) found that the use of urea fertilizer on wheat tended to result in lower incidence of disease compared to wheat fertilized with ammonium nitrate. In commercial fields, weed control may also reduce disease incidence. Fields with an excess of weeds present were shown to have higher incidence of FHB than fields free of weeds (Teich and Nelson 1984). Weeds may potentially increase the amount of inoculum available and may increase the humidity and shading of crop plants, which can lead to higher incidence of disease (Hanson *et al.* 1950).

Chemical control is an effective management option for reducing disease incidence and DON accumulation in FHB-affected crops. Seed treatment fungicides have been found to be effective in preventing seedling blight, and result in increasing seed germination (Gilbert and Tekauz 1995). Foliar fungicides, such as propiconazole and triadimefon, reduced disease severity and DON accumulation. However, control can vary depending on the cultivar (Boyacioglu *et al.* 1992; Tekauz *et al.* 1998; Tekauz and Gilbert 1998).

Genetic resistance to FHB is the most promising method for control. However, at this time no registered resistant cultivars are available. Resistant cultivars may soon be

registered, but until then, alternative management strategies are essential to reduce the impact of the disease (Gilbert and Tekauz 2000).

Chapter 1

Survival of *Gibberella zeae* on Fusarium Damaged Kernels

1.1 Introduction

Fusarium head blight (FHB) is an important disease of wheat and other small grain cereals. The principal pathogen associated with FHB in Manitoba is *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) (Gilbert *et al.* 1999). *Gibberella zeae* over-winters on infested crop residues and gives rise to perithecia (sexual fruiting bodies) in the spring (Sutton 1982). Above average rainfall in spring favors the development of perithecia in which ascospores mature later in the growing season. In eastern Canada, ascospores are regarded as the primary source of inoculum; they are forcibly discharged from perithecia, may be carried by the wind, and deposited on heads of susceptible crops at or around the time of anthesis (Fernando *et al.* 1997). Wheat heads are most susceptible to infection at anthesis and compounds produced by the anthers, choline and betaine, have been found to promote hyphal growth (Strange *et al.* 1978). However, heads can become infected at any point during flowering (Hart *et al.* 1984). Macroconidia are produced on infected heads and crop residues, they are splash-dispersed by rain and may be important for the secondary infection of tillers later in the growing season (Fernando *et al.* 1997).

Several practices have been suggested to control FHB and to reduce inoculum levels. These include: crop rotation of wheat and other susceptible small grain cereals with non-host crops such as canola or alfalfa (Dickson 1956; Sutton 1982; Teich and Hamilton 1985; Parry *et al.* 1995), and plowing under of crop to eliminate inoculum by accelerating decomposition and blocking ascospore dispersal. However, buried crop

residues can support *G. zeae* as a saprophyte (Sutton 1982). Fungal viability was found to be greater in tissues that decomposed slowly. Survival of *G. zeae* on nodal and internodal tissues was 94% and 0%, respectively, after being buried for 52 weeks (Burgess and Griffin 1968). However, rapid internodal tissue breakdown and the difficulty in recovering the tissues may have influenced the decline in survival. In Minnesota, initial recovery of *G. zeae*, from pieces of naturally infested wheat straw was 41% and decreased to 24 % over the winter (Warren and Kommedahl 1973). In a second study, Windels and Kommedahl (1984) isolated *Gibberella zeae* from 84% of rotted corn stalks in November. In the following spring, recoveries were reduced to 61% from overwintered cornstalks and to 31% in the fall on 1-year old basal stalk pieces. In eastern Canada, *G. zeae* was isolated after 3 years from all types of corn and wheat residues located above the soil and from corn kernels, wheat stems, and wheat spikelets from the soil surface (Konga and Sutton 1988). With the increase in zero tillage practices, large amounts of crop residues remain on the surface of the soil. Macroconidia were produced mainly in the first year, while perithecia developed on residues on or above the soil surface for up to three years, (Konga and Sutton 1988). Soil type and environmental conditions also play an important role in determining rates of decomposition and duration of survival. After 2 years under field conditions, recovery of *G. zeae* from wheat stubble buried in three locations in Australia ranged from 42 to 104 weeks (Burgess and Griffin 1968).

A practical recommendation to reduce the potential for downgrading of grain at delivery to the elevator, is to blow as many of the light weight fusarium damaged kernels

(FDK) out the back of the combine during harvest. However, the ultimate fate and duration of survival of this seed-borne *G. zeae* is unknown (Gilbert and Tekauz 2000).

1.2 Objectives

The objectives of the study were to:

- 1) Determine the duration of survival of *G. zeae* in FDK when these are left on the soil surface or buried at 5 and 10 cm in the field.
- 2) Examine the effects of sterile verses non-sterile soil, and temperature, on the survival of the pathogen in FDK under controlled conditions.

1.3 Materials and Methods

1.3.1 Seed Source

Samples of FHB-infected grain of the hard red spring wheat cultivars AC Domain and Roblin were used. To determine species present on the infected samples, 100 FDK were surface sterilized in a 0.3% solution of NaOCl (1:40) for 1 minute. The sterile kernels were placed in a laminar flow cabinet and allowed to dry for 3 hours and then plated on potato dextrose agar (PDA) (BBL Cockeysville, MD) amended with the antibiotic, streptomycin (0.1%). Plates were incubated at 20°C under continuous cool white light for 5 days. *Fusarium* species were identified based on colony and spore morphology using dissecting and compound microscopes at magnifications of 50X and 400X, respectively.

1.3.2 Field Study

Fusarium damaged kernels were placed in nylon mesh (1 mm) bags (8 x 8 cm). In October 1998, bags were left on the soil surface or buried at 5 or 10 cm at the Cereal Research Centre (CRC) experimental research farm, MB., in a 4 replicate randomized complete block design (1 bag, 50 FDK per replicate). Bags were sampled at 6-month intervals for 24 months. In October 1999, additional bags were placed on the soil surface or buried at 5 or 10 cm at the CRC in Winnipeg, Morden Research Centre and the Manitoba Crop Diversification Centre (MCDC) site at Portage la Prairie, Manitoba. Bags were sampled and examined for *G. zeae* and perithecial development on a monthly basis from May through September 2000.

1.3.3 Soil Analysis

Soil samples were collected from the four study locations, in September 2000. The surface debris was brushed aside using a small trowel and approximately 20 grams of soil removed from the top 15 cm of soil. This was repeated five times at each of the sample sites. The sub-samples were combined into a single paper bag and mixed thoroughly by shaking. The samples were placed in a drying oven at 37.5 °C for 1 week. The dried samples were ground using a mortar and pestle then passed through a 2 mm sanding sieve to remove any large particles. To determine sand, silt, and clay content of the soil, 50 g of soil was placed into a beaker and 100 ml of dispersing solution (50 g water softener and 1 L distilled water) was added. The solution was mixed by hand for 5 minutes and left to soak overnight. The mixture was poured into a 1000 ml graduated cylinder and distilled water was added to bring final volume up to 1000 ml. The cylinder was covered with a rubber stopper and inverted several times. After 40 seconds a hydrometer was placed into the solution and the first reading was recorded. The solution was mixed again and a second hydrometer reading was recorded after 6 hours (Foth *et al.* 1974). Percent sand, silt, and clay, were calculated with the following formulae:

$$\text{Grams of silt and clay (WS)} = \frac{V_t (D - D_w)}{(1 - (D_w / D_s))} \quad (1.1)$$

Where: D = hydrometer reading at 40 seconds

D_w = density of water = 1.0 g cm⁻³

D_s = density of soil = 2.65 g cm⁻³

V_t = total volume (ml)

To determine sand content:

$$\text{Sand (g)} = \text{Soil used (g)} - \text{Silt and clay (g)} \quad (1.2)$$

To determine clay content:

After 6 hours, only clay remains in suspension. Therefore, the hydrometer reading after 6 hours was used to calculate grams of clay (formula 1.1)

To determine silt content:

$$\text{Silt (g)} = \text{Silt and clay (g)} - \text{clay (g)} \quad (1.3)$$

$$\text{Percent sand, silt, and clay} = \frac{\text{grams of (sand, silt, or clay)}}{\text{Weight of soil used}} \times 100 \quad (1.4)$$

1.3.4 Decomposition of FDK

After harvested kernels were surface sterilized and air dried for 3 hours and their mass was determined. The natural logarithm of the percent mass remaining was calculated with the following formula:

$$\text{Ln (\%mass remaining)} = \text{Ln (original mass-final mass/ original mass)} \times 100 \quad (1.5)$$

$$\text{Ln (\% mass remaining)} = -kt \quad (1.6)$$

Where:

k = rate of decomposition (the slope of the linear regression model)

t = time

1.3.5 Effects of Soil and Temperature

Soil mixture consisted of a 3:1 mix of Glenlea soil and Turface (Profile Products LLC, Buffalo Grove, IL.). Half the soil was sterilized for 2 hours at 14 to 18 atm. *Fusarium* damaged kernels selected from severely infected samples of Roblin and AC Domain wheat were placed into nylon mesh (1 mm) bags (8 x 8 cm), 25 FDK/bag. The bags were left on the soil surface or buried at 5 cm in the sterile or non-sterile soil. The trays (30 x 15 x 10 cm) and mesh bags were incubated at -10°C, 2°C, or 20°C. The trays incubated at 20°C, were watered with distilled water every second week and the trays at 2°C and -10°C were covered with plastic to prevent the soil from drying out. Bags were sampled using a pre-determined randomization pattern at 6-month intervals for 24 months. A three-way analysis of variance (ANOVA) was performed on the 20°C treatment using SAS version 8 (SAS Institute Inc., Cary, NC). Data were transformed using the arcsine function of percent viability to normalize the variance.

1.3.6 *Fusarium* Isolation

At each sampling time the retrieved FDK were rinsed with distilled water to remove soil, and surface sterilized in a 0.3% solution of NaOCl (1:40) for 1 minute. The kernels were then placed in a laminar flow cabinet and allowed to dry for 3 hours. Kernels from each treatment were weighed and examined for perithecial and ascospore development (see page 20) and then plated on to PDA amended with streptomycin. The plates were incubated at 20°C under continuous cool white light for 5 days. *Fusarium* species were identified based on colony and spore morphology using dissecting and compound microscopes, at magnifications of 50X and 400X, respectively.

1.3.7 Perithecial Development

After the kernels were harvested and surface sterilized, they were examined for perithecial development under a compound microscope at a magnification of 50X. When perithecia were observed, five kernels from each treatment were further examined for ascospore development by removing perithecia from kernels and examining them under a compound microscope at a magnification of 400X. The kernels were rated using the following scale developed for this project to classify maturity levels of perithecia:

<u>Rating</u>	<u>Perithecia and ascospore development</u>
0	No perithecia, no ascospores
1	<25% perithecial coverage, no ascospores
2	25-50% perithecial coverage, no ascospores
3	>50-100% perithecial coverage, no ascospores
4	50% perithecial coverage, ascospores still in asci
5	50% perithecial coverage, free ascospores, some in asci
6	<25% perithecial coverage, free ascospores
7	25-50% perithecial coverage, free ascospores
8	>50-100% perithecial coverage, free ascospores

1.4 Results

1.4.1 Seed Source

The initial presence of *G. zeae* on AC Domain and Roblin wheat was 100% and 97%, respectively. Low levels of other species were also isolated, including *Fusarium culmorum*, *Epicoccum nigrum* Link., and *Alternaria* spp. Ness.

1.4.2 Field Study

At Glenlea (1998 test), after 24 months there was very little decrease in the survival of *G. zeae* on FDK on the soil surface or buried at 5 and 10 cm. Survival ranged from 85-99% (Table 1.1). At 24 months FDK left on the soil surface were difficult to retrieve due to the decomposition of the nylon bags. The remaining seeds were still intact, but few (20/200) were recovered.

At Morden, Portage la Prairie, and Winnipeg (1999 test), both FDK retrieved and fungal survival were high after 11 months, 100% retrieval, and survival ranged from 97-100% (Table 1.2). The soil composition of Morden was a sandy loam, at Portage it was a silty loam, and at Winnipeg a silty clay loam (Table 1.3). Soil composition and rate of decomposition did not influence fungal survival over the period tested. The kernels buried at 5 and 10 cm from Morden and Winnipeg decomposed at similar rates, whereas the kernels buried at Portage had a slightly higher rate of decomposition. The FDK left on the soil surface at Portage, Morden, and Winnipeg decomposed at a slower rate than those buried at 5 and 10 cm. After 331 days, the biomass of the FDK located on the surface, decreased by approximately 50% (Figure 1.1).

Table 1.1: Survival of *Gibberella zeae* on fusarium damaged kernels (FDK) at the soil surface (0 cm) or buried at 5 and 10 cm, at Glenlea, MB.

Time of sampling*	FDK Depth (cm)	# FDK recovered [†]	# kernels with <i>G. zeae</i>	FDK producing <i>G. zeae</i> (%)
April 1999	0	198	194	99
	5	201	195	97
	10	200	193	97
Oct. 1999	0	190	187	98
	5	195	195	100
	10	181	181	100
April 2000	0	202	201	100
	5	181	180	100
	10	175	175	100
Oct. 2000	0	20	17	85
	5	157	153	98
	10	139	137	99

* Trial initiated Oct. 1998

[†] Out of 200 FDK (Sum of 4 replicates, 50 kernels/replicates)

Table 1.2: Survival of *Gibberella zeae* on fusarium damaged kernels (FDK) at the soil surface (0 cm) or buried at 5 and 10 cm, at Morden, Portage la Prairie, and Winnipeg, MB.

Location	FDK Depth (cm)	Viability (%) [†]				
		2000				
		May	June	July	August	September
Morden	0	94*	98	98	100	97
	5	99	99	100	100	97
	10	97	98	100	100	100
Portage	0	91	99	100	99	98
	5	96	100	100	99	100
	10	94	99	100	98	100
Winnipeg	0	96	97	98	100	100
	5	91	100	100	100	98
	10	96	100	100	100	100

*Based on average values of 4 replicates of 50 FDK

[†] % FDK producing *G. zeae*

Table 1.3: Soil type and percent sand, silt, and clay, from study sites in Morden, Portage la Prairie, and Winnipeg, sampled in September 2000.

Location	Sand	Silt	Clay	Soil Type
Morden	55.03	32.12	12.85	Sandy Loam
Portage	3.64	77.09	19.27	Silty Loam
Winnipeg	3.64	64.24	32.12	Silty Clay Loam

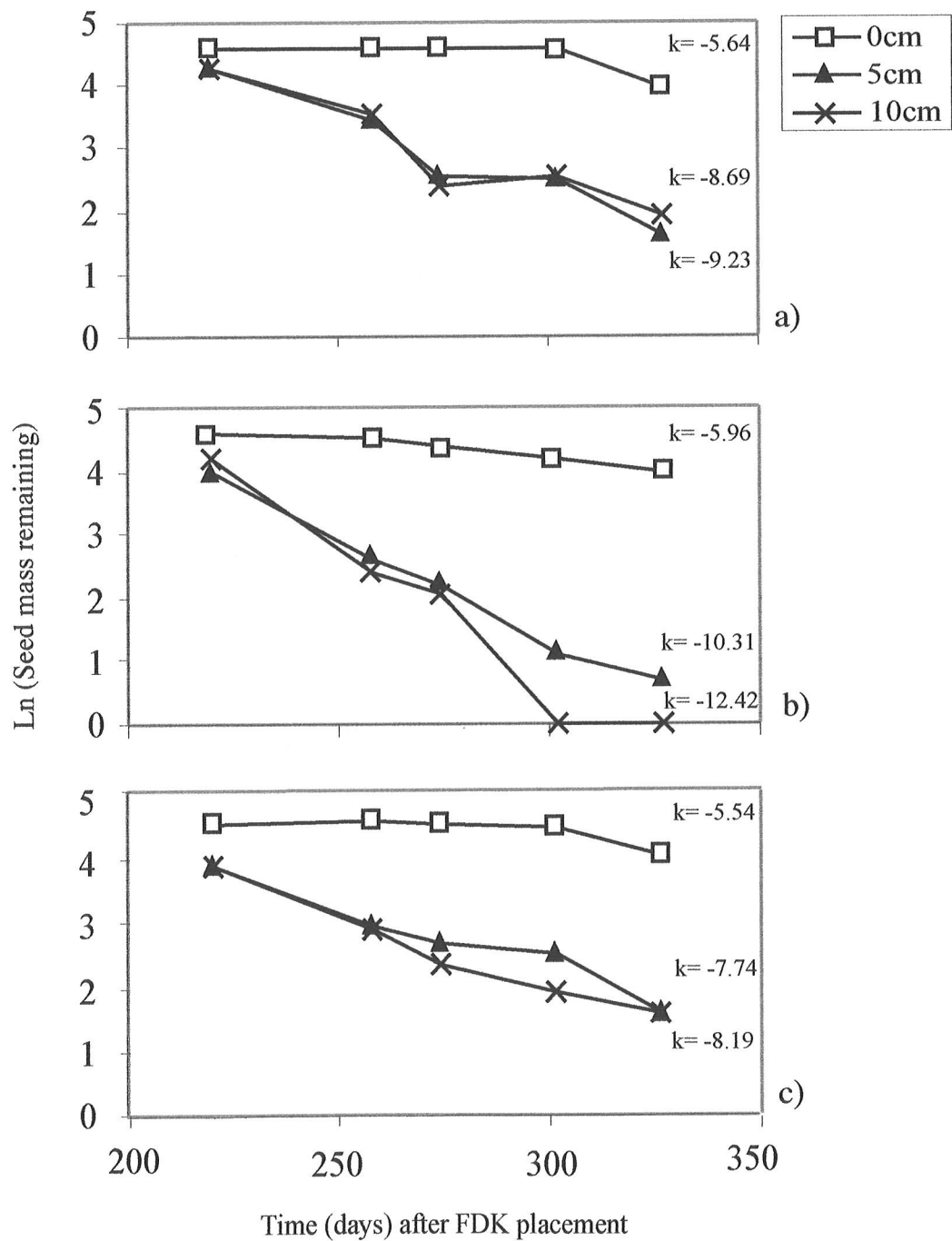


Figure 1.1: Decomposition rates (k) and seed mass remaining of fusarium damaged kernels (FDK) after 11 months at the soil surface (0 cm) or buried at 5 and 10 cm from a) Winnipeg, b) Portage la Prairie, and c) Morden, MB.

1.4.3 Effects of Soil Type and Temperature

Under controlled conditions, temperature and depth of burial did not influence survival of *G. zeae* on kernels sampled from 2°C and -10°C. Survival ranged from 98% to 100% after 24 months. The survival of *G. zeae* decreased when incubated at 20°C. Fewer FDK at 5 cm depth yielded *G. zeae*, 83 and 76%, in sterile and non-sterile soil respectively; whereas survival of *G. zeae* was 92% and 96%, on sterile and non-sterile soil, respectively. (Table 1.4a). The ANOVA results indicated that at 20°C, sampling time (date) was significant at $P < 0.0001$; soil (sterile and non-sterile) and burial depth were significant at the $P = 0.01$ level (Table 1.4b).

Other fungal species isolated from 1-24% of the kernels, under field and controlled conditions, included *F. equiseti*, *F. culmorum*, *Epicoccum nigrum*, *Alternaria* spp., and *Penicillium* spp. Link.

1.4.4 Perithecial Development

Perithecial production was observed on kernels from all locations and treatments. Under field conditions, at Glenlea the perithecia were first noted on FDK both at the soil surface or buried in April 1999, after 6 months exposure. However, few ascospores were detected (Table 1.5). The kernels buried at Winnipeg, Morden, and Portage la Prairie in October 1999 did not develop perithecia until June 2000 after 9 months exposure. Kernels at all burial depths developed perithecia, but ascospores developed only on the kernels left at the soil surface (Table 1.6). Under controlled conditions, kernels exposed to 20°C and 2°C developed perithecia, but only those incubated at 20°C formed ascospores. A few perithecia formed on kernels buried at -10°C (Table 1.7).

Table 1.4a: Survival of *Gibberella zeae* on fusarium damaged kernels (FDK) incubated at 20°C, 2°C, and -10°C

Temperature (°C)	Position of FDK	Soil type	Viability (%) [†]			
			May 1999	Nov. 1999	May 2000	Nov. 2000
20	Surface	Sterile	100*	96	94	92
		Non-sterile	99.5	99	95	96
	Buried	Sterile	100	87	85	83
		Non-sterile	91.9	87	80	76
2	Surface	Sterile	99.5	100	99	100
		Non-sterile	100	100	100	100
	Buried	Sterile	100	100	100	100
		Non-sterile	99.5	100	99	100
-10	Surface	Sterile	99.5	100	100	99
		Non-sterile	100	100	100	100
	Buried	Sterile	99.5	98	98	100
		Non-sterile	99.5	98	100	98

* Based on average values of 4 replicates of 25 FDK

[†] % FDK producing *G. zeae*

Table 1.4b: Analysis of variance (ANOVA) of the percent survival of *Gibberella zeae* on fusarium damaged kernels at the surface or buried in sterile or non-sterile soil at 20°C.

Source	Type III SS	DF	Mean Square	F Value	P > F
Soil	0.0515	1	0.0515	10	0.0025
Position	0.0428	1	0.0428	8.32	0.0056
Position*Soil	0.0414	1	0.0414	8.04	0.0064
Date	0.6391	1	0.6391	124	<0.0001
Date*Soil	0.037	1	0.037	7.18	0.0097
Date*Position	0.0151	1	0.0151	2.93	0.092
Date*Position*Soil	0.0063	1	0.0063	1.22	0.2736

Table 1.5: Perithecial production on fusarium damaged kernels (FDK) at the soil surface (0 cm) or buried at 5 and 10 cm, at Glenlea, MB.

FDK Depth* (cm)	Perithecial Production [†]			
	April 1999	Oct. 1999	April 2000	Oct. 2000
0	1	3	3	2
5	2	2	1	1
10	2	2	1	1

* Test initiated Oct. 1998

[†] See page 19 for rating scale for perithecial development

Table 1.6: Perithecial and ascospore production on fusarium damaged kernels (FDK) at soil surface (0 cm) or buried at 5 and 10 cm, at Morden, Portage, and Winnipeg, MB.

Location*	FDK Depth (cm)	Perithecial Production [†]				
		May 2000	June 2000	July 2000	Aug. 2000	Sept. 2000
Morden	0	0	7	8	8	8
	5	0	1	1	1	1
	10	0	0	1	1	1
Portage	0	0	7	8	8	8
	5	0	2	2	1	1
	10	0	2	2	1	1
Winnipeg	0	0	6	8	8	8
	5	0	1	2	1	1
	10	0	0	0	1	1

* Test initiated Oct. 1999

[†] See page 19 for rating scale for perithecial and ascospore development

Table 1.7: Perithecial and ascospore production on fusarium damaged kernels (FDK) incubated at 20°C, 2°C, and -10°C.

Temperature (°C)	Position of FDK	Soil type	Perithecial Production [†]			
			May 1999	Nov. 1999	May 2000	Nov. 2000
20	Surface	Sterile	7	8	8	8
		Non-sterile	7	8	8	8
	Buried	Sterile	8	8	8	8
		Non-sterile	8	8	8	8
2	Surface	Sterile	2	2	2	2
		Non-sterile	2	2	2	2
	Buried	Sterile	3	3	3	3
		Non-sterile	3	3	3	3
-10	Surface	Sterile	0	0	0	0
		Non-sterile	0	0	0	0
	Buried	Sterile	1	1	1	1
		Non-sterile	1	1	1	1

[†] See page 19 for rating scale for perithecial and ascospore development

1.5 Discussion

In Manitoba, seed-borne *G. zeae* was found to survive for at least 24 months on infected FDK left on the soil surface or buried. In Ontario, Konga and Sutton (1988) found that *G. zeae* survived 1-2 years longer in residues placed on or above the soil compared to those buried at 10 cm. This inconsistency may be partly explained by differences in the climates and growing seasons in Manitoba and Ontario. Frequent wetting of the soil has been found to decrease the survival of *G. zeae* (Burgess and Griffin 1968). This study examined the duration of survival of *G. zeae* on FDK. Other studies have emphasized survival of *G. zeae* on wheat straw and corn residues. In Minnesota, initial recovery of *G. zeae*, from pieces of naturally infested wheat straw was 41% and decreased to 24% over the winter (Warren and Kommedahl 1973). In a second study, Windels and Kommedahl (1984) isolated *Gibberella zeae* from 84% of rotted corn stalks in November. In the following spring, recoveries were reduced to 61% from overwintered cornstalks and to 31% in the fall on 1-year old basal stalk pieces. The rate in which tissues decompose influence the survival of *G. zeae*. Recovery of *G. zeae* after 52 weeks was greater on nodal than internodal tissues, 94% and 0%, respectively (Burgess and Griffin 1968).

Small pieces of buried FDK fragments may increase disease levels in susceptible crop plants when planted in contaminated soils. Chongo *et al.* (2001) found that in cereal and non-cereal crops that are planted in soils infected with *F. graminearum*, the seedling emergence is reduced and root and crown rot severity increased. If infected kernel fragments are brought to the surface through tillage practices, these may colonize other

surface debris from which perithecia and ascospores may develop to infect the current crop.

Survival of *G. zeae* was highest on surface residues, which also had a slower decomposition rate. Soil type did not appear to influence the short-term survival of *G. zeae* on FDK located at Portage, Morden, and Winnipeg. Soil type did, however, influence the decomposition rate of FDK, which would in turn influence the long-term survival of *G. zeae*. Burgess and Griffin (1968) also found soil type influenced survival of *G. zeae*. Under field conditions in Australia, the recovery of *G. zeae* was significantly greater from straw residues collected from soils located at Blue Nobby (104 weeks) than from those buried at Tremain (42 weeks) or at the North-West Wheat Research Institute (NWWRI) (96 weeks) location. The soil type and climatic conditions at the three locations were not defined in the study. The survival of *G. zeae* is longer on tissues which breakdown slowly (Burgess and Griffin 1968). Once a residue decomposes, the fungus will need to colonize a new substrate. Dill-Macky *et al.* (1998) observed that the reduction in percent colonization of *G. zeae* of wheat residues corresponded to the decomposition of residues. *Gibberella zeae* is a poor competitor with other soil microflora and requires a suitable substrate within the soil to survive (Nyvall 1972). Over the long-term, the rate of decomposition will help determine duration of survival. The longer the infested residue persists the greater the duration of survival.

Soil-microorganisms also will influence the duration of survival. If an organism is a better competitor of the substrate it may out-compete *G. zeae*. After 24 months, survival decreased to 83% and 76%, in FDK that were buried at 20°C in sterile and non-sterile soil, respectively, presumably because of the presence of microorganisms on the non-

sterile soil. The duration of survival at 20°C decreased from 100% to 76% after 24 month compared with the other treatments (-10°C and 2°C) in which survival ranged from 100% to 98%, respectively. This would suggest that temperature influences the duration of survival of *G. zeae*. Gilbert *et al.* (1997) found that *Fusarium* infected grain stored at 20°C had a higher germination rate than seeds stored at -10°C, 2.5°C, and 10°C, which was attributed to a decrease in the viability of *G. zeae* on grain stored at the higher temperature. Cold temperature storage is recommended to control insect pests, mites and fungal growth, but at low temperatures, the survival of *G. zeae* is also enhanced. Seedling survival in the spring may be compromised due to the presence of viable *G. zeae* after cold temperature storage.

In the field, perithecia did not form until the following spring indicating that they are not over-wintering survival structures. Similar observations were made by Trail *et al.* (1997). Perithecia of *G. zeae* were not seen on over-wintered crop debris until temperatures reached 15°C in the spring. At Glenlea the kernels were examined for perithecia and ascospore development in April and October. Abundant perithecia were observed on the FDK located on the surface in April. However, no ascospores were detected as they develop later in the season in June and are discharged in July and August (Sutton 1982). In 2000, perithecia did not form until June on the FDK left on the soil surface at Portage, Morden, and Winnipeg. The delay in perithecial production in 2000 may have been caused by high moisture in spring. Early studies indicated that the fungus may not tolerate wet conditions (Dickson 1923).

The survival structures found in this study on buried FDK included macroconidia, chlamydospores, and hyphal fragments. *Gibberella zeae* can survive in soil as

chlamydospores for up to 500 days in non-autoclaved soils, but will only develop under certain conditions (Nyvall 1970). Under field conditions ascospores developed only on FDK left on the soil surface. As ascospores are wind dispersed (Sutton 1982), the formation of ascospores in the soil would have no advantage to the pathogen.

1.6 Conclusions and Recommendations

As survival of *G. zeae* on seed was not diminished after 24 months and was not influenced by burial depth, rotations of at least two years are necessary to avoid infection of new crops by seed-borne *G. zeae*. Perithecia and ascospore development occurred mainly on FDK located on the surface of the soil. This suggests kernels left on the surface are an inoculum source and may contribute to disease outbreaks. On the buried FDK, perithecial and ascospore production was low, but fungal survival was not compromised. Placement of FDK influenced the rate of decomposition. Kernels that were buried decomposed faster than the kernels left on the surface. This will indirectly affect the duration of survival of *G. zeae*. Once residues decompose duration of fungal survival in the soil may be reduced.

Chapter 2

Temporal and Spatial Relationship Between Spore Dispersal and Environmental Variables

2.1 Introduction

Gibberella zeae (anamorph = *Fusarium graminearum*) is the principal cause of fusarium head blight (FHB) on small grain cereals in North America. Epidemics have been sporadic over the years and are associated with above average rainfall during the growing season (Sutton 1982). The disease has been responsible for significant economic losses in yield and quality. (Sutton 1982; Gilbert *et al.* 1994; Parry *et al.* 1995; Gilbert and Tekauz 2000).

The sexual state the pathogen produces 3-septate ascospores, which are forcibly discharged from perithecia. Perithecia and ascospores develop in the spring on overwintered crop residues (Sutton 1982; Paulitz 1996). Wheat is most susceptible to infection at anthesis, although infection can occur at all stages of head maturity (Fernando *et al.* 1997; Hart *et al.* 1984). After significant rainfall, the wind-borne, ascospores are discharged, taken up by wind currents and may eventually be deposited on spikes of cereals and other grasses. In eastern Canada, Paulitz (1996) determined that spore release began at 1700-1800 h, 3-4 hours before leaf wetness was detected. Spore release was highly correlated with an increase in relative humidity (RH) and a decrease in temperature. Fernando *et al.* (2000) observed four spore release events over a 20-day

period, in which the highest density of spores was trapped in the evening. Moisture is thought to play an important role in the development of perithecia (Paulitz 1996).

In the asexual state, macroconidia (3-7 septate) are produced in sporodochia on infected crop residues and on infected heads. Macroconidia generated on infected heads may be an important source of inoculum for the infection of secondary tillers. These spores are generally splash-dispersed for short distances on to neighboring plants (Sutton 1982). Interception of macroconidia by the crop canopy also prevents the splash-dispersed spores from being carried over a long distance.

Previous studies in Quebec and Ontario, have investigated spore dispersal and disease spread using susceptible spring wheat cultivars (Paulitz 1996, Fernando *et al.* 1997, 2000). However, in Manitoba, the timing of spore release and the role of *G. zeae* ascospores verses macroconidia in the development of FHB have not been examined.

2.2 Objectives

The objectives of the study were to:

- 1) Investigate the seasonal and daily patterns of ascospore and macroconidial dispersal in southern Manitoba.
- 2) Determine the relationship between environmental variables and spore discharge.
- 3) Examine the spatial relationship between a point source of inoculum and disease spread.

2.3 Materials and Methods

2.3.1 Fungal Inoculum

Cultures of *G. zeae* isolates, CRC N1, CRC N2, CRC N3, CRC N14, and CRC IT, were grown on PDA under cool white light for 7 days at room temperature. The five cultures were placed into a blender, mixed thoroughly with distilled water and poured on to autoclaved corn kernels. The dry corn kernels (8 kg) were soaked in water at room temperature for 16 hours, drained, covered, and placed into a soil sterilizer for 2 hours at 14 to 18 atm. The corn was cooled for 24 hours before inoculating. The inoculated corn was mixed thoroughly after inoculation, covered, and allowed to incubate at room temperature for 3 weeks. Inoculated corn was dried for 1 week and stored at 4°C until required.

2.3.2 Study Site

In 1999 and 2000, the study site was established at the CRC experimental research farm, at Glenlea MB. which is located 12 km south of Winnipeg on Highway 75. The main crops grown in the area are *Triticum aestivum* L. (wheat), *Avena sativa* L. (oats), *Hordeum vulgare* L. (barley) and *Brassica napus / 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 various cultivars of wheat and used for FHB disease trials.

2.3.3 Inoculation of Field

In 1999, an area measuring 30 x 30 m, was inoculated on July 8 and 14 (Julian days 189 and 195) with 40g/m² of corn kernels infested with *G. zeae*. In 2000, wheat straw residues from the previous year were plowed under except for an area of 3 x 3 m. On June 2 (Julian day 154), an area of 100 x 100 m was planted with the FHB susceptible wheat cultivar, CDC Teal. Rows were seeded 8 cm apart. The 3x3 m plot was inoculated, on June 12 and July 7 (Julian days 166 and 189), with 40g/m² of corn kernels infested with *G. zeae*. Anthesis in the CDC Teal crop occurred approximately from August 6 through to August 16 (Julian days 219 to 229).

2.3.4 Perithecial Development on Straw Residues and Corn Kernels

To monitor perithecial and ascospore development, straw residue and corn inoculum were collected from the inoculated area on a weekly basis. Residues were rinsed with distilled water and surface sterilized with 0.3 % NaOCl solution. Residues were then examined for the development of perithecia and ascospores. For confirmation and identification, 2 cm sections were plated on to PDA amended with streptomycin (39 g PDA, 0.1 g streptomycin and 1 L distilled water). Inoculated plates were incubated at 20°C under continuous cool 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).

2.3.5 Spore Sampling and Environmental Monitoring

In 1999, ten rotorod samplers (Aerobiology Research Laboratories, Nepean, Ontario) (Figure 2.1a) were placed 10 m apart in a line transect running northeast to southwest across the field. The motors were powered by a 12-volt battery, and samplers rotated at 2400 RPMs, collecting air-borne spores, for the first 15 minutes of each hour from 1800 h to 0200 h. The clear polystyrene rotorods were coated with silicone high vacuum grease (Dow Corning, Midland, Mich.) to retain impacted spores. The rods were collected daily from July 1 through to August 31 and examined for spores using a compound microscope (400x). Spore concentration was calculated using the following formula (2.1):

$$\text{Spore concentration} = \frac{\text{Total particles}}{(\text{RPM})(K)(\text{Min})}$$

Where: RPM = 2400

 K = 0.0197 (conversion factor)

 Min = total sampling time

In 2000, a Burkard 7-day spore sampler (Burkard Scientific Ltd. Uxbridge, Middlesex, U.K.) (Figure 2.1b) was set up in the centre of the inoculated area to monitor spore dispersal. The sampler was powered by a 12-volt battery charged with a solar panel. The volume of air sampled was 10 liters of air per minute 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 (24h) sections. The sections were mounted onto glass microscope slides and covered with 51 x 22 mm glass cover slips using permount (Fisher Scientific, Nepean, Ontario) to fix

the tape to the slides. Spore sampling began on July 1 and continued until August 28.

Ascospores and macroconidia were identified based on size and septation using a compound microscope (400X). The concentration of spores trapped during one hour was calculated using the following formula (2.2):

$$\text{Concentration} = \frac{(\# \text{ spores on a } 100 \text{ microns wide transverse} \times 20)}{0.6\text{m}^3} \times \frac{1.0}{0.6\text{m}^3}$$

Environmental data in both 1999 and 2000, were recorded every half hour with a Campbell CR10X datalogger (Campbell Scientific, Logan, Utah). Temperature and relative humidity 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) (Figure 2.2).

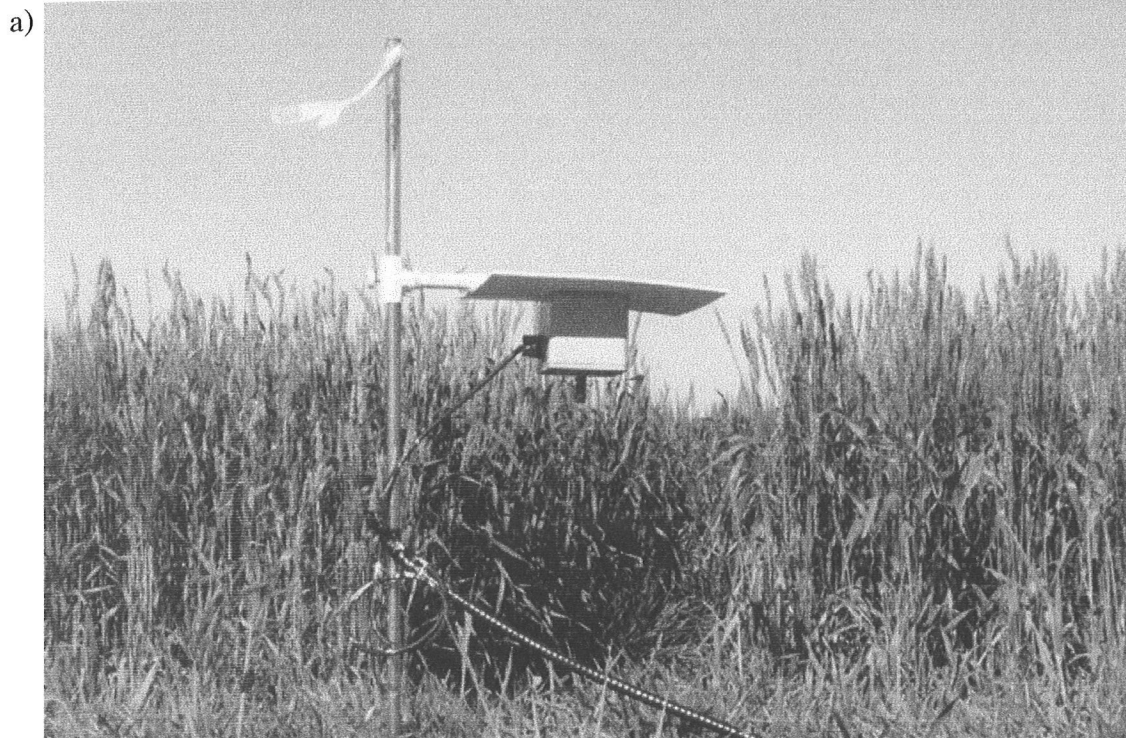


Figure 2.1: a) Rotorod spore samplers (Aerobiology Research Laboratories, Nepan, Ontario) placed at Glenlea, MB. in 1999.

b) Burkard 7-day spore sampler (Burkard Scientific Ltd. Uxbridge, Middlesex, U.K.) with solar panel in centre of field at Glenlea, MB. in 2000.



Figure 2.2: Campbell Scientific weather station and CR10X datalogger (Campbell Scientific, Logan, Utah) used in 1999 and 2000 at Glenlea, MB.

2.3.6 Spatial Analysis

In 2000, plots of CDC Teal (1x1 m), 5 to 25 m apart, were rated 21 days post-anthesis for disease incidence and severity. Incidence is the frequency of the disease, or the percentage of heads with some infection. Severity is the average percentage of disease on infected heads. From these values the FHB index was calculated with the formula (2.3) (Snijders 1990):

$$\text{FHB index} = \frac{\text{Incidence (\%)} \times \text{Severity (\%)}}{100}$$

The mean and the unbiased estimate of variance were calculated using the program Excel (Microsoft Office 1998) for incidence, severity and FHB index. A variance-to-mean ratio was determined for each of the measurements and was tested by a chi-square statistic (2.4) (Bliss 1958);

$$\chi^2 = ns^2 / x$$

Where: n = degrees of freedom (sample size minus 1)

s^2 = unbiased estimate of variance

x = sample mean

A variance-to-mean ratio not significantly different from unity (=1) indicates an even or random distribution. A variance-to-mean ratio greater than unity (>1) may indicate a clustering or aggregation of diseased plants (Campbell and Pennypacker 1980).

A variogram was calculated to obtain an estimate of the distance traveled before the data points become uncorrelated, using the following formula (2.5) (Carr 1995):

$$\gamma(h) = \frac{1}{2N} \sum_{i=1}^N [Z(x_i) - Z(x_i + h)]^2$$

Where: $\gamma(h)$ = similarity measure
 h = lag distance
 N = number of pairs found
 $Z(x_i)$ = value of the variable of interest (incidence, severity, and FHB index) at x_i

A semivariogram graph (Fig. 2.3) was constructed with the x-axis representing the lag distance (h), and the y-axis being the semivariance $\gamma(h)$. A line was fitted through the data points to determine the spatial correlation from the formation of the sill (flat region of the curve, in which there is no further correlation between sample points).

'Kriging' uses the semivariogram to determine an optimal set of weights for the estimate of any values from unsampled locations by calculating the weighted moving averages to estimate the unknown values. The following formula can be used for a spherical model (2.6) (Carr 1995):

$$\gamma(h) = C_0 + C[1.5(h/A_0) - 0.5(h/A_0)^3] \quad \text{for } h \leq A_0$$

$$\gamma(h) = C_0 + C \quad \text{for } h > A_0$$

Where: h = the lag distance interval
 C_0 = nugget variance (the value of the semivariogram at a zero lag distance)
 C = structural variance
 A_0 = range

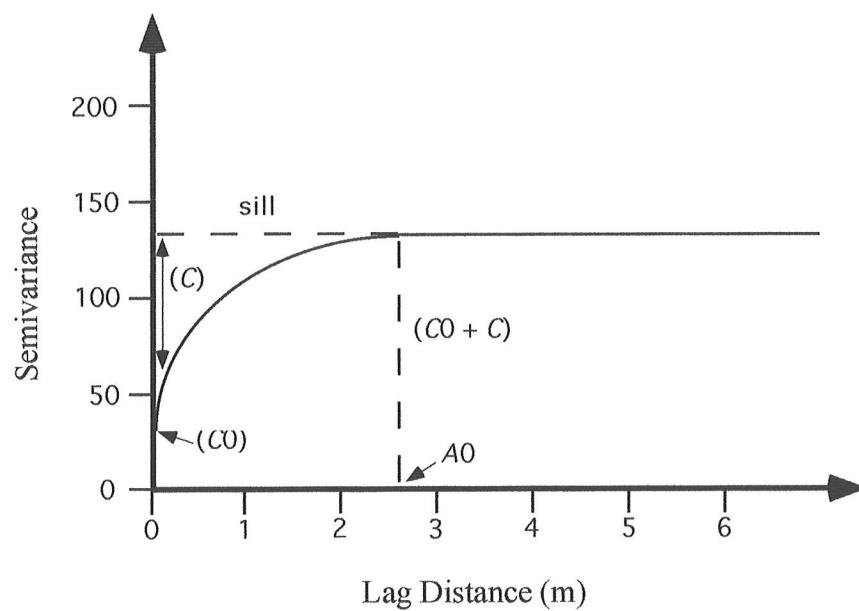


Figure 2.3: Characteristics of a semivariogram showing the nugget variance (C_0), structural variance (C), range of influence (A_0) and the sill.

2.4 Results

2.4.1 Spore Dispersal

In 1999, ascospore release was first observed July 13 (Julian day 194). Four spore release events, greater than 113 spores/m^3 , occurred in July and August. Ascospore discharge occurred between 1 to 4 days after 5 mm or more of. Ascospores were washed from the air by rain on August 6 and 17 (Julian days 218 & 229) (Fig. 2.4). Macroconidia were generally trapped from the air within a day after a rainfall event ($>5 \text{ mm}$). Daily macroconidia concentrations ranged from $0\text{-}42 \text{ conidia/m}^3$ (Fig. 2.5).

In 2000, mature perithecia with ascospores were first detected on July 5 (Julian day 187) on over-wintered infected straw residues. Ascospores were first collected from the air on July 13 (Julian day 195), approximately 4 weeks after infested corn kernels were placed in the field. Ascospore release did not directly correspond with rainfall events, occurring 2 to 5 days after rainfall exceeding 5 mm. Two major ascospore release events occurred over the 2-month period with peak ascospore concentrations of 5067 and 5400 ascospores/ m^3 on July 14 and August 14 (Julian days 196 and 227), respectively. Daily concentration ranged from $0\text{-}15,233 \text{ ascospores/m}^3$ (Fig. 2.6). Spore release began at approximately 1700 h and continued until 0400 h peaking at 2100 h. Few ascospores were trapped from 0500 to 1500 h, with maximum hourly ascospore concentration of 167 ascospores/ m^3 during these hours (Fig. 2.7).

Macroconidia were first detected on July 9 (Julian day 191) following a rainfall event. Several release events were observed over the 2-month period. Daily concentrations ranged from $0\text{-}567 \text{ macroconidia/m}^3$ (Fig. 2.8). No daily pattern of

macroconidial dispersal was observed, and release was highly variable over a 24-hour period (Fig 2.9).

No environmental data were recorded from August 4 to 10 (days 217-223) due to an error in the datalogger program. Precipitation data for days 217-223 were obtained from rainfall data collected at the experimental research farm at Glenlea, MB.

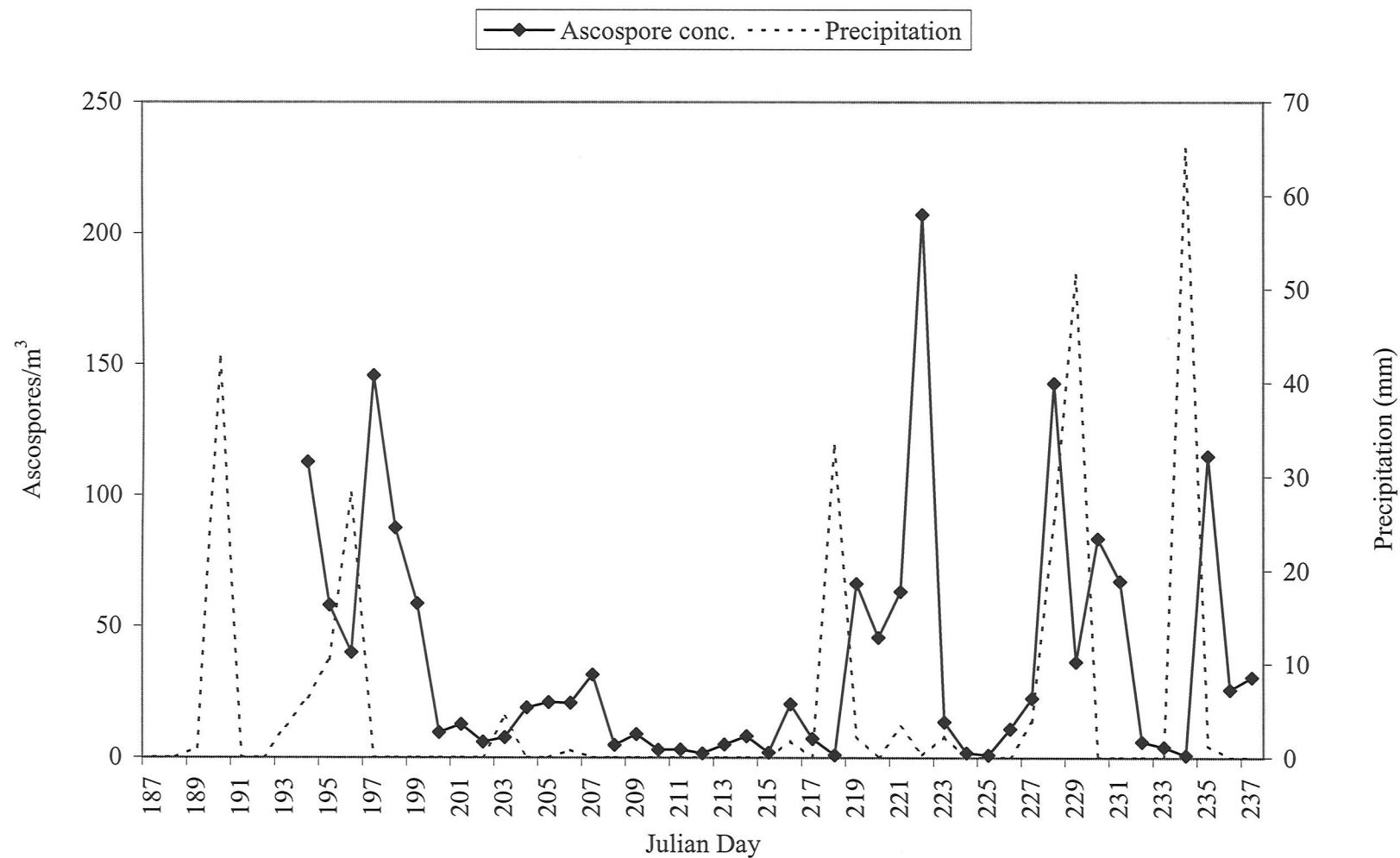


Figure 2.4: Concentration of *Gibberella zeae* ascospores (ascospores/m³) trapped with rotorod spore samplers, between 1800 and 0200 h during July and August 1999, at Glenlea, MB.

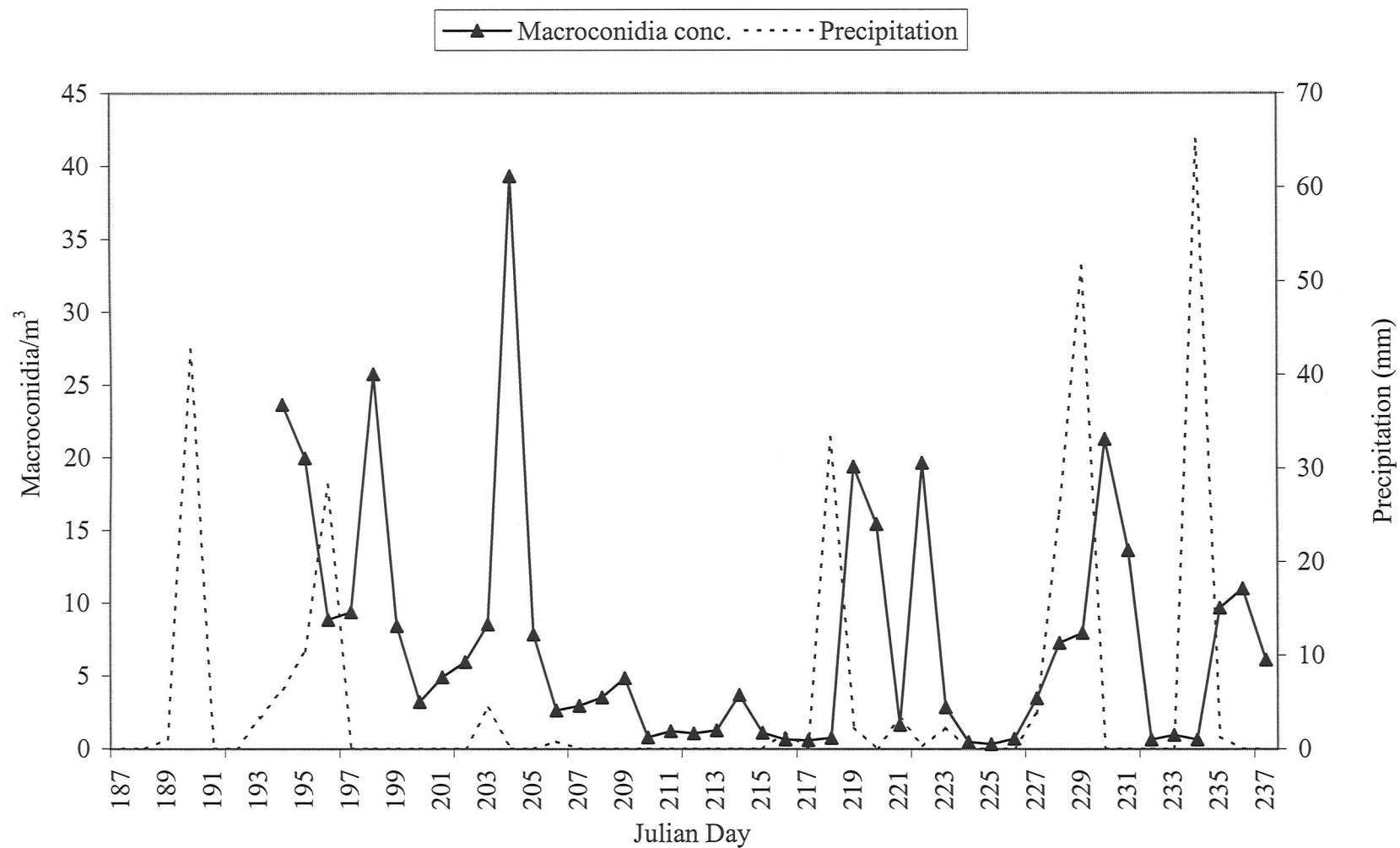


Figure 2.5: Concentration of *Fusarium graminearum* macroconidia (macroconidia/m³) trapped with rotorod spore samplers, between 1800 and 0200 h during July and August 1999, at Glenlea, MB.

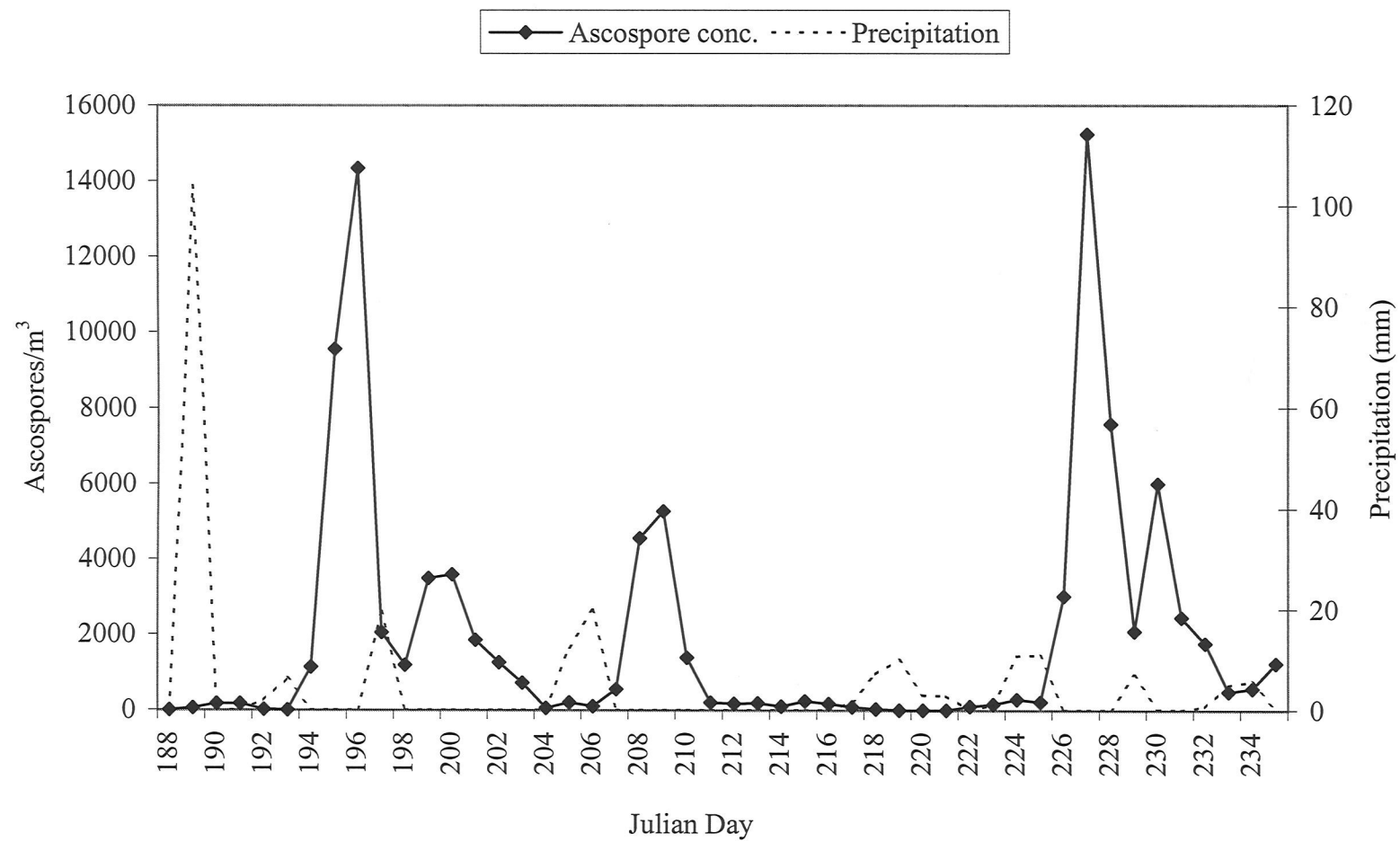


Figure 2.6: Concentration of *Gibberella zeae* ascospores trapped with Burkard 7-day spore sampler during July and August 2000, at Glenlea, MB.

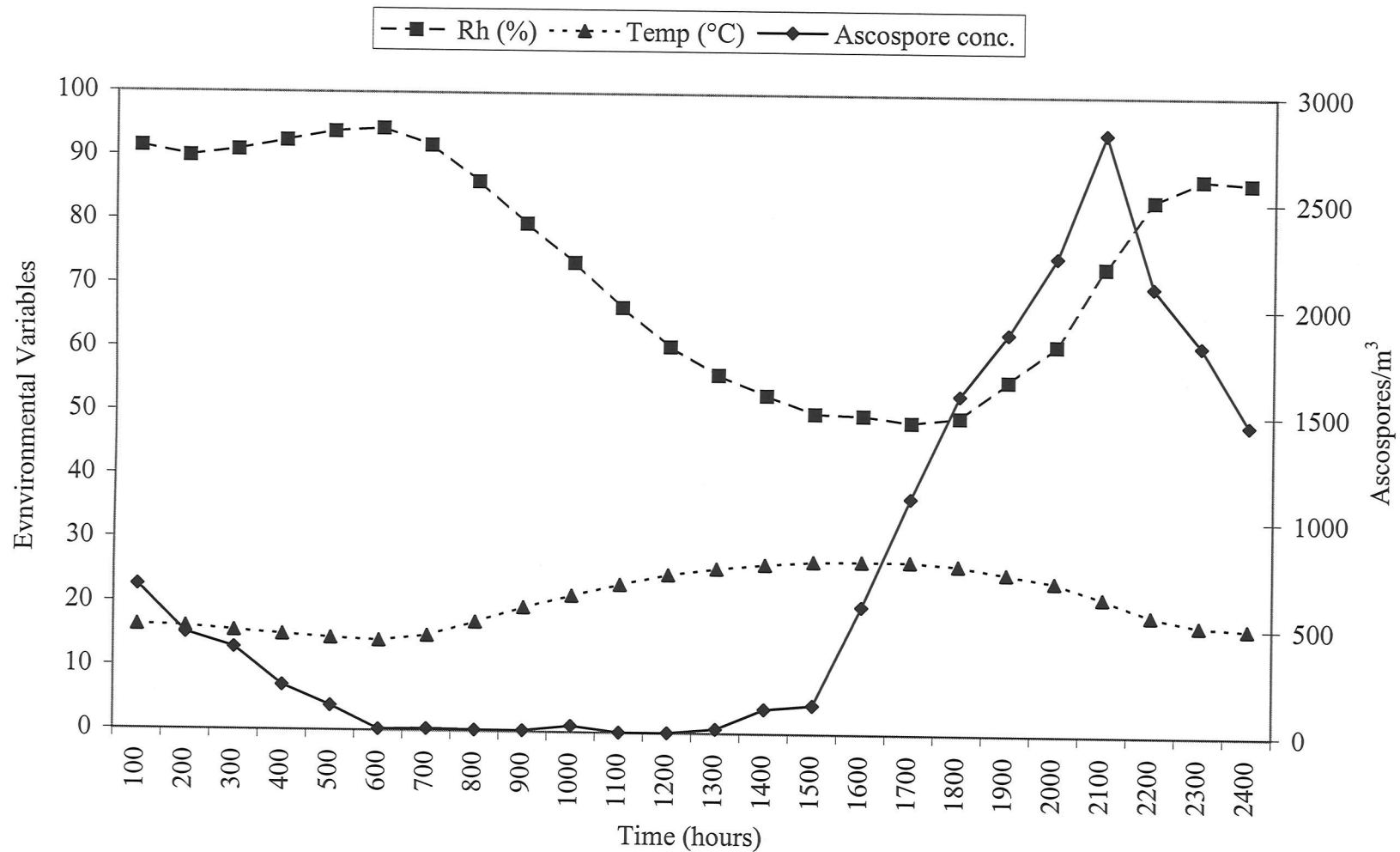


Figure 2.7 Average ascospore concentration of *Gibberella zeae*, trapped at hourly intervals with the Burkard 7-day spore sampler on days in with spore release of >1000 ascospores/ m^3 at Glenlea, MB. in 2000.

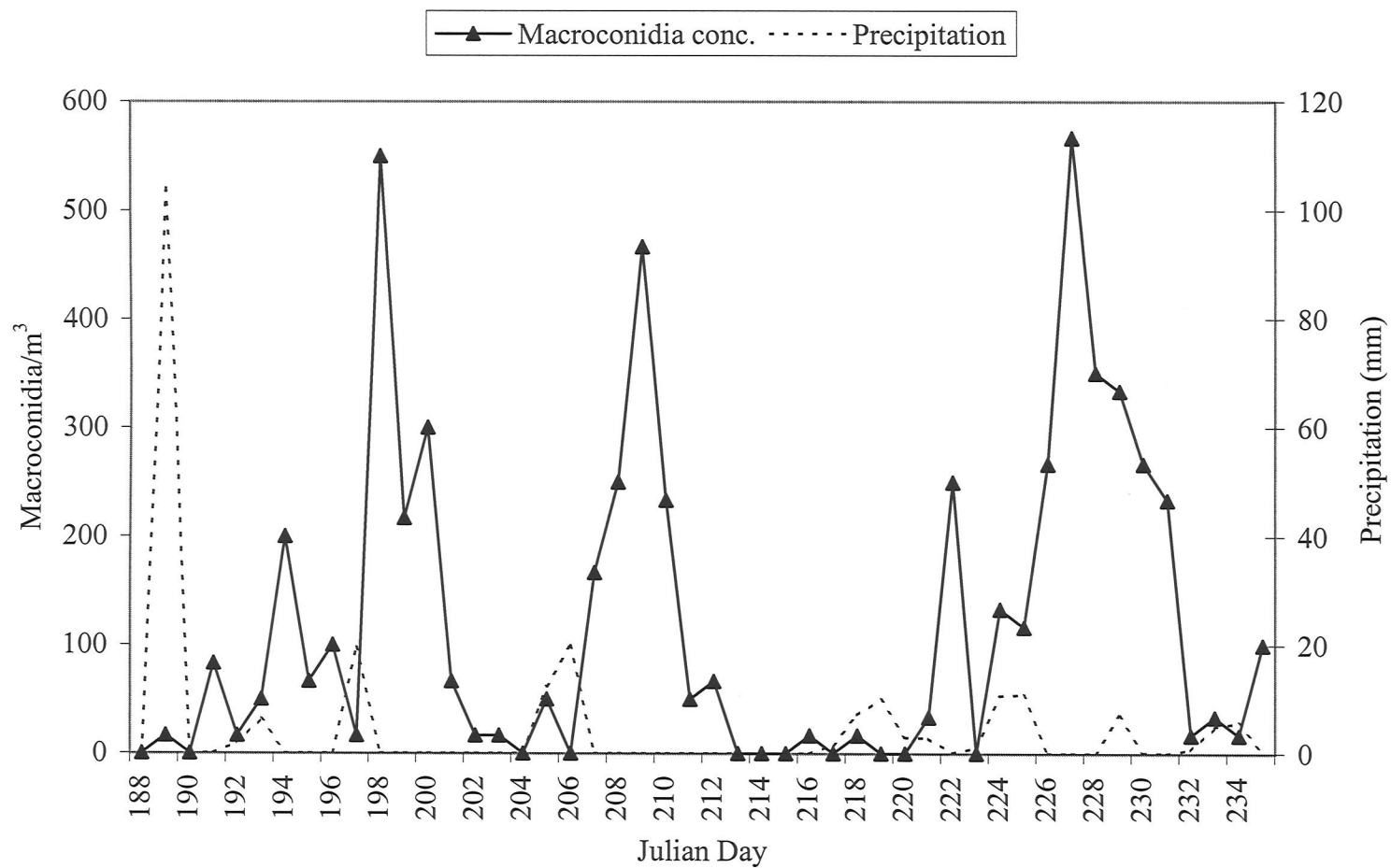


Figure 2.8: Concentration of *Fusarium graminearum* macroconidia trapped with Burkard 7-day spore sampler during July and August 2000, at Glenlea, MB.

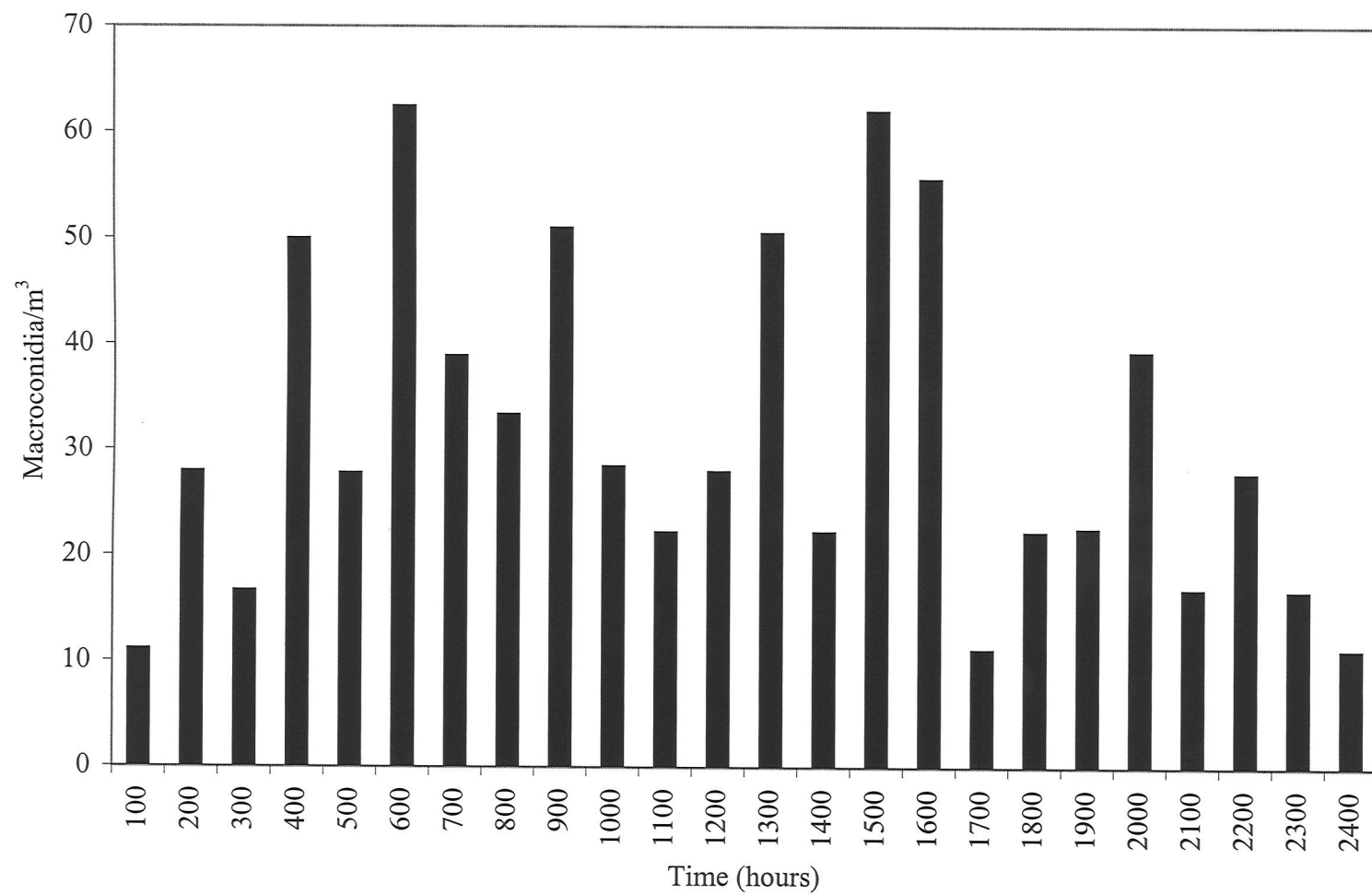


Figure 2.9: Average macroconidia concentrations of *Fusarium graminearum* trapped at hourly intervals with a Burkard 7-day spore sampler on days with spore release of >20 macroconidia/m³, at Glenlea, MB in 2000.

2.4.2 Spatial Relationship

The chi-square analysis indicated that the mean-to-variance ratios for incidence, severity and FHB index were significantly greater than one (Table 2.1). The disease incidence was greater on the east side, whereas severity was greater on the southwest corner of the field in 2000 (Fig. 2.10). Wind direction was highly variable, shifting within a matter of hours (Appendix A1). The semivariogram indicated that there was no spatial autocorrelation between points after a distance of 5 meters by the lack of formation of the sill (flat region of the curve) (Fig. 2.11). Due to the lack of spatial autocorrelation, the 'kriging' weights were not calculated.

Table 2.1: Variance-to-mean ratio analysis for incidence, severity, and FHB index on wheat in field plots at Glenlea in 2000.

Rating	variance:mean	Chi-square*	Probability of exceeding chi- square value
incidence	8.68	781.50	<0.001
severity	6.62	595.67	<0.001
FHB index	4.28	385.33	<0.001

*90 degrees of freedom

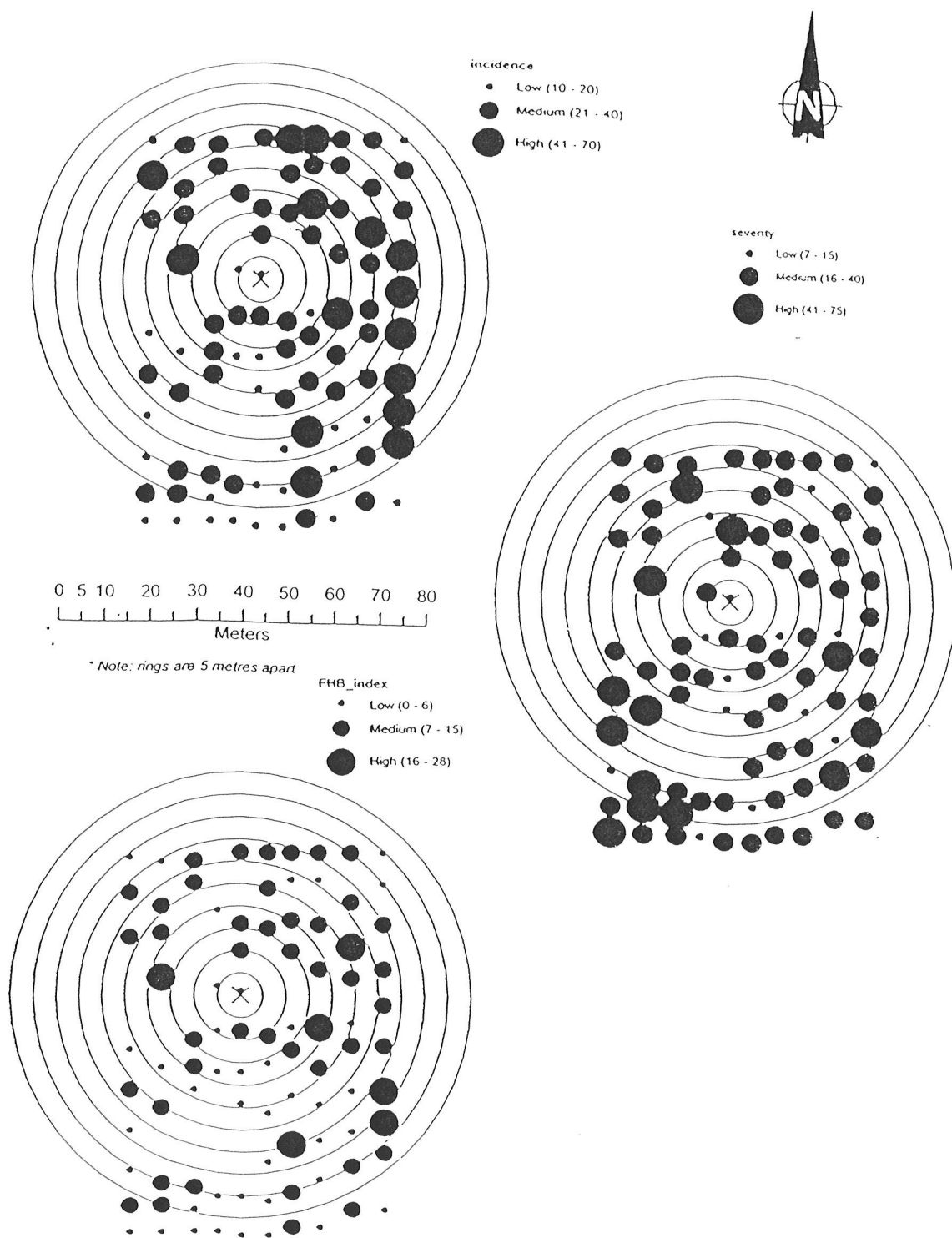


Figure 2.10: Graduated maps of incidence, severity, and fusarium head blight (FHB) index of wheat in plots at Glenlea, MB. in 2000.

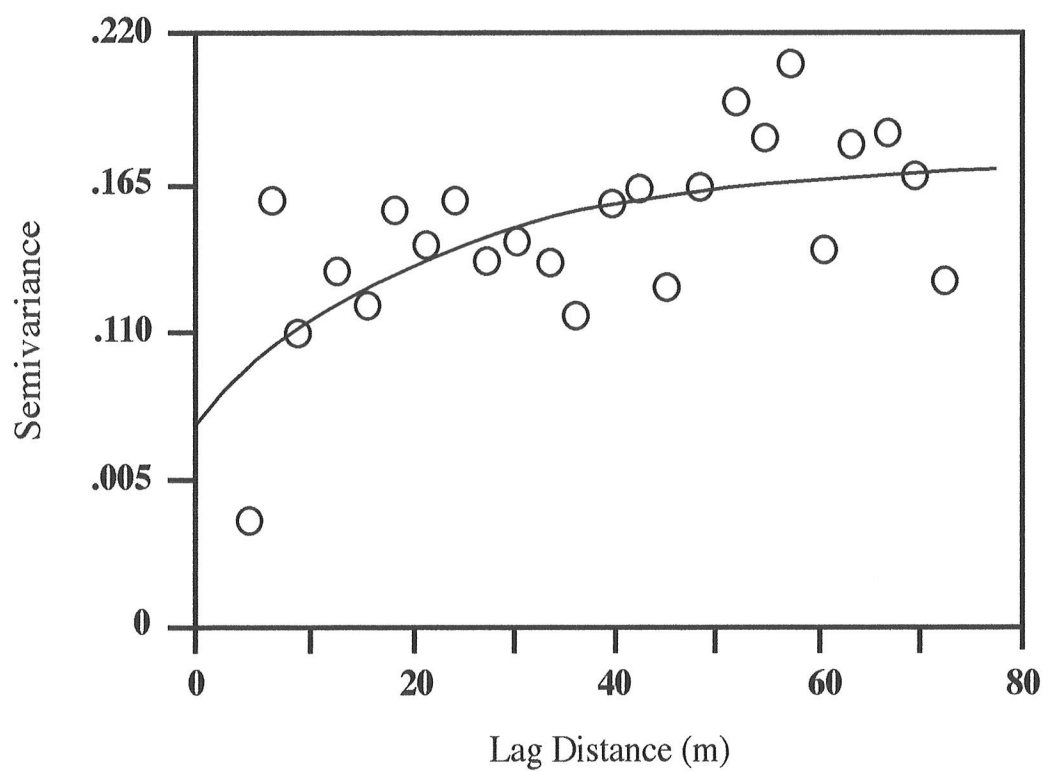


Figure 2.11: Semivariogram for FHB disease index on wheat in plots at Glenlea, MB. in 2000.

2.5 Discussion

A seasonal pattern of ascospore release was observed in 1999 and 2000. High levels of inoculum, primarily ascospores, were detected in the middle of July and at the end of August, but several ascospore release events were detected throughout the season. Once the ascospores are discharged the perithecia do not produce new ones (Paulitz 1996). This suggests that a heterogeneous population of perithecia existed with different times of maturity. However, corn kernels were laid out throughout the summer, which allowed for more than one population of perithecia. Other crop residues, at various stages, would also have produced perithecia. In this study perithecia developed on the straw residues, by July 5, followed by the first ascospore release two weeks later. In both years of the study, ascospore release was found to correspond with the time of anthesis for many of the spring wheat cultivars grown in Manitoba. Time of anthesis is dependent on crop seeding date, and on temperature and precipitation.

A diurnal pattern of ascospore release was also observed in this study. Ascospore release generally began at 1700 h and peaked at approximately 2100 h. This agrees with reports of previous studies in which a similar pattern of release was observed. In eastern Canada, ascospore release began at 1600 h with the highest numbers trapped in the evening between 1800 and 2100 h (Fernando *et al.* 2000; Paulitz 1996; Ayers *et al.* 1975). In this study, RH had decreased to approximately 40 % at 1700 h, this was followed by a rapid increase in RH. Relative humidity decreased as temperature increased during the day and RH increased as the temperature decreased early in the evening. A decrease in RH may cause the perithecia to dehydrate (Tschanz *et al.* 1975), which, in turn, may cause mechanical rupturing of the ascus wall. Ascospores are usually

discharged forcibly through a small tear or slit at the apex of the perithecium (Trail and Common 2000). As the RH begins to increase, the osmotic pressure may also rise in the perithecium causing an increase in the number of the ascospores to be discharged.

Tschanz *et al.* (1975) suggested that a decrease in the atmospheric moisture would cause the substrate and perithecia to dry out, which would subsequently cause ascospore discharge. In eastern Canada, Paulitz (1996) found that moisture was not a limiting factor, as rain and/or high RH occurred every 3 to 5 days. Perithecia were hydrated and did not dry out throughout the release period. In Manitoba, moisture tends to be more of a limiting factor. The relationship between ascospore discharge and RH may be coincidental. It has been suggested that ascospore discharge will occur in a rhythmic pattern after an initial stimulus such as light or RH are removed or changed (Tschanz *et al.* 1975).

Ascospore discharge occurred 2 to 5 days after > 5 mm of precipitation. In this study, ascospore release was inhibited on days in which intermittent rainfall was recorded. Intermittent rainfall and continuous $RH > 80\%$ has been found to inhibit ascospore release (Paulitz 1996). This pattern suggests that moisture is required for perithecial development and ascospore maturation, but is not responsible for the actual release event. Perithecial production was found to be greatest at a water potential of -15 bars, few perithecia developed below -50 bars (Sung and Cook 1981). During dry weather conditions, ascospore release did not occur. Perithecia can survive extended dry periods and resume activity after re-wetting of the substrate occurs (Fernando *et al.* 2000). This was evident at the end of July and the beginning of August 2000; ascospore release was inhibited when there was no rainfall for 10 days.

Low concentrations of macroconidia were detected when air was sampled. This was expected because macroconidia are not normally air-borne and are unlikely to be sampled with the equipment used. In addition, interception by the crop canopy may have prevented larger numbers of macroconidia from being trapped by the spore sampler. Macroconidia are primarily splash-dispersed and thus are transported only over short distances in rain droplets (Sutton 1982). High amounts of kinetic energy would be required to release macroconidia into the air (Fernando *et al.* 2000). The highest concentrations of macroconidia were trapped during the early morning and late afternoon. A possible explanation for this is that as water droplets containing trapped macroconidia dry from wind and sun, the spores are 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 (Madden *et al.* 1996).

One disadvantage to both the Burkard 7-day spore trap and the rotorod spore sampler is the spores trapped do not remain viable on the adhesive surface. Therefore, the *Fusarium* species cannot be accurately identified. In this study, *G. zeae* and *F. graminearum* were 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 air and spores by suction, which would allow a greater volume of air to be sampled. This may partly explain why a greater number of spores were trapped in 2000 as compared to 1999. Environmental conditions vary from year to year which also would influence inoculum levels.

The spatial autocorrelation between points was approximately 5 meters. Disease development was unevenly distributed (clustered) in the field as indicated by the Chi-square value for the variance-to-mean ratio. Disease developed locally in several areas of the field. This may have been caused by local areas of inoculum, such as pockets of crop debris. The pattern of distribution may vary depending on cultural practices (Campbell and Pennypacker 1980). No disease gradients from the disease focus were observed. Fernando *et al.* (1997) found steeper gradients downwind from the inoculum source, which extended 4 to 22 m away from the centre focus. There are two possible reasons why no gradients were observed in this study: 1) High levels of background inoculum may have prevented the formation of a distinct disease gradient. Background levels of *G. zeae* were not determined due to time constraints. In 2000, *F. graminearum* was the predominant species, accounting for over 96% of the isolations from the fields surveyed in Manitoba, the average FHB index was 7.9% and ranged from 3.5% to 47% (Gilbert *et al.* 2001). At Glenlea, levels of FHB caused by *F. graminearum* are high due to the high disease pressure caused by artificial inoculation of *F. graminearum*. To determine background levels in this study, the use of a morphologically distinct strain, for example, one with yellow colony color, as used by Fernando *et al.* (1997), could have been implemented to differentiate the background strains. Amplified fragment length polymorphisms (AFLP) or random amplified polymorphic DNA (RAPD) could have been used to detect background levels of inoculum (Ouellet and Seifert 1993). However, the isolates used in this study were the same as those used in the FHB disease nursery adjacent to the study field, and were used to inoculate wheat in previous years. High levels of disease were observed along the edges of the field plots, which suggests that

some infection was initiated by outside sources of inoculum; 2) Shifting wind direction and interference by the crop canopy also could have modified the direction of spore dispersal.

2.6 Conclusions

Ascospore release occurred primarily between 1700 and 0400 h and was triggered by environmental factors such as rainfall and relative humidity. The rate at which perithecia mature determines when ascospores are released. Generally, perithecia develop on residues in 7-8 days after an initial stimulus, such as rainfall, followed by ascospore development in approximately 7 days. Ascospore release coincided with the time of anthesis for spring wheat cultivars in Manitoba.

Fewer macroconidia were trapped, compared to the number of ascospores. The highest numbers were found in the air following a rainfall event. This would suggest that macroconidia are primarily splash-dispersed, but further investigation on how the macroconidia become air-borne (likely from water droplets) is needed.

Chapter 3

Host range of *Fusarium graminearum*

3.1 Introduction

Alternative hosts plants harbor a wide variety of plant pathogens including many viruses, bacteria, and fungi (Duffus 1971; Karunakaran 1980; Stone 1962; Hepperley *et al.* 1980; Cerkaskas *et al.* 1983). Alternative hosts may increase inoculum levels and play an important role in the survival of the pathogen by bridging the gap between susceptible crops or unfavorable environmental conditions (Dinoor 1974). *Fusarium graminearum* is a disease of grasses. It is the principal *Fusarium* species that causes fusarium head blight (FHB) in North America. While other species of *Fusarium* can cause FHB they do not cause significant economic losses. Over the last 100 years the predominant species causing FHB has shifted. Connors (1940) reported that *F. graminearum* was rare in Manitoba. In the 1950's Gordon (1959) identified several *Fusarium* species from a range of plants from several provinces in Canada, but *F. graminearum* was not one of these. *Fusarium culmorum* was more frequently isolated from a wide variety of crops and grasses (Gordon 1959). Sprague (1950) reported in Minnesota, North Dakota, and South Dakota, *F. graminearum* attacked the heads of small grain cereals and many graminaceous forage crops. It has also been reported on two broad-leaved species, *Matricaria spp.* and *Viola arvensis* (Jenkinson and Parry 1994). The present outbreak of FHB in Manitoba may have its beginnings in 1984 when two contaminated wheat samples with high levels of DON were identified (Clear and Abramson 1986). Since that time, the incidence of FHB caused by *F. graminearum* has steadily increased. In 1993, Manitoba experienced the most severe outbreak to date;

estimated losses were \$75 million dollars (Gilbert *et al* 1994). Subsequent losses have been approximately \$50 million dollars annually (Gilbert and Tekauz 2000).

Grasses are commonly found along the roadsides and ditches adjacent to fields. If these grasses harbor *F. graminearum* this could potentially increase disease pressure. Fields where weeds (non-planted species) were not controlled had a higher incidence of FHB (Teich and Nelson 1984). Several plant species have been identified as symptomless carriers of *F. oxysporum* (Katan 1971; MacDonald and Leach 1976; Haware and Nene 1982; Helbig and Carroll 1984). Very little recent literature exists which examines the host range of *Fusarium graminearum* on alternative host species in Manitoba.

3.2 Objectives

The objective of the study was to identify possible grass species as alternative hosts for *F. graminearum* in southern Manitoba.

3.3 Materials and Methods

3.3.1 Field study

Grasses were collected at three southern Manitoba locations: CRC experimental research farm at Glenlea, Tolstoi Tall-Grass Prairie Preserve, and Living Prairie Museum in Winnipeg. Each site was visited on a monthly basis, in June, July, and August, 1999 and 2000. At each site 16 plants per species were collected from fields and adjacent areas. Samples were placed in plastic bags and transported back to the lab where they were stored in a plant press. The grasses were identified to the species level using Budd's Flora of the Canadian Prairie Provinces (1987). A total of 1766 plants representing 34 species (Appendix A2) was collected from the three locations and examined for presence of *Fusarium* species.

3.3.2 Site Locations

The Tolstoi Tall-Grass Prairie preserve is located 100 km south of Winnipeg on Highway 59 and 2.4 km east of Tolstoi on Highway 209. Areas adjacent to the preserve are pasturelands for beef cattle. The principal crops grown in the area are, alfalfa and alfalfa with mixed grasses.

Experimental research farm at Glenlea, MB. is located 12 km south of Winnipeg on Highway 75. The main crops grown in the area are wheat, oats, barley and canola. The field station belongs to the Cereal Research Centre and is involved in extensive FHB trials on small grain cereals. *Fusarium* head blight levels in this area are very high.

Living Prairie Museum is located in Winnipeg at 2795 Ness Avenue. The adjacent areas are residential homes and business.

3.3.3 Isolation of *Fusarium* species

For each of the plants, ten florets from a single head were surface sterilized in a 50:50 solution of 95% ethanol and water for 30 seconds and rinsed in distilled water. The florets were placed on sterile filter paper and allowed to dry for 30 minutes at room temperature. When dry, florets were plated onto PDA amended with 0.1 g streptomycin. The plates were incubated under cool white light at 20°C for 1 week. *Fusarium* species were identified based on colony and conidial morphology using dissecting and compound microscopes and a standard identification key (Nelson et al 1983). When necessary *Fusarium* isolates were sub-cultured onto synthetic nutrient agar (SNA) (1.0 g KH_2PO_4 , 1.0 g KNO_3 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g Glucose, 0.2 g Sucrose, 20 g Agar and 1 L distilled water), to aid in identification. These plates were also incubated at 20°C for 1 week.

3.3.4 Incidence and Isolation Frequency

Each grass inflorescence was rated for incidence and severity of *Fusarium* species. Incidence was the frequency of *Fusarium* species isolated from heads. Isolation frequency was the percentage of florets harboring the pathogen on grass inflorescences.

3.4 Results:

No visual symptoms of the disease were observed on any of the grass species collected. Some grass species had other wheat pathogens present such as, *Claviceps purpurea* (ergot), *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph, *Stagonospora nodorum* (Berk.) Castellani and E.G. Germano) (stagonospora nodorum blotch), *Mycosphaerella graminicola* (Fuckel.) J. Schröt. in Cohn. (anamorph, *Septoria tritici* Roberge in Desma.) (septoria tritici blotch), *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph, *Drechslera tritici-repentis* (Died.) Shoemaker) (tan spot), and *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. Ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker) (spot blotch). *Fusarium graminearum* was isolated from 9% of the grass plants examined (149/1766). Other *Fusarium* species isolated included, *F. sporotrichioides* (2.3%), *F. equiseti* (1.2%), *F. oxysporum* (1.1%), *F. avenaceum* (0.9%), *F. culmorum* (0.8%), and *F. poae* (0.7%) (Fig. 3.1). *Fusarium graminearum* occurred on 11 grass species, seven of which are a first report. These include *Agropyron trachycaulum* (Link) Malte (slender wheat grass), *Agropyron repens* (L.) Beauv. (quack grass), *Agrostis stolonifera* L. (red top), *Bromus ciliatus* L. (fringed brome), *Bromus inermis* Leyss. (smooth brome), *Calamagrostis canadensis* (Michx.) Beauv. (canary reed grass), *Echinochloa crusgalli* (L.) Beauv. (barnyard grass), *Hierochloe odorata* (L.) Beauv. (sweet grass), *Phleum pratense* L. (timothy), *Poa pratensis* L. (kentucky blue grass), and *Spartina pectinata* Link (prairie cord grass).

At Glenlea, the highest incidence of the pathogen was on *Agropyron trachycaulum*, *Bromus inermis*, and *Calamagrostis canadensis*. Other grass species hosting *F. graminearum* were *Agropyron repens*, *Agrostis stolonifera*, *Echinochloa*

crusgalli (Figure 3.2). These grass species are commonly found adjacent to fields.

Tolstoi, had a higher incidence of *Fusarium* species, as well as a greater overall diversity of plant species (Appendix A2). *Fusarium graminearum* was isolated from *Agropyron trachycaulum*, *Agropyron repens*, *Agrostis stolonifera*, *Bromus inermis*, *Bromus ciliatus*, *Calamagrostis canadensis*, *Hierochloe odorata*, *Phleum pratense*, *Poa pratensis*, and *Spartina pectinata* (Figure 3.3). At the Living Prairie Museum, three grass species, *Bromus inermis*, *Calamagrostis canadensis*, and *Dactylis glomerata* harbored one *Fusarium* sp., *F. sporotrichioides* (Figure 3.4).

Fusarium graminearum was the dominant *Fusarium* species isolated from Glenlea and Tolstoi, comprising, 62 and 59 %, respectively. Incidence of the other species of *Fusarium* ranged from 3 to 13 % (Table 3.1). Grass species with a high incidence of *Fusarium graminearum*, did not necessarily have a high isolation frequency value. Grass species had either: a) high incidence and a low isolation frequency, b) low incidence and high isolation frequency, or c) similar levels of incidence and isolation frequency (Figures 3.5 and 3.6).

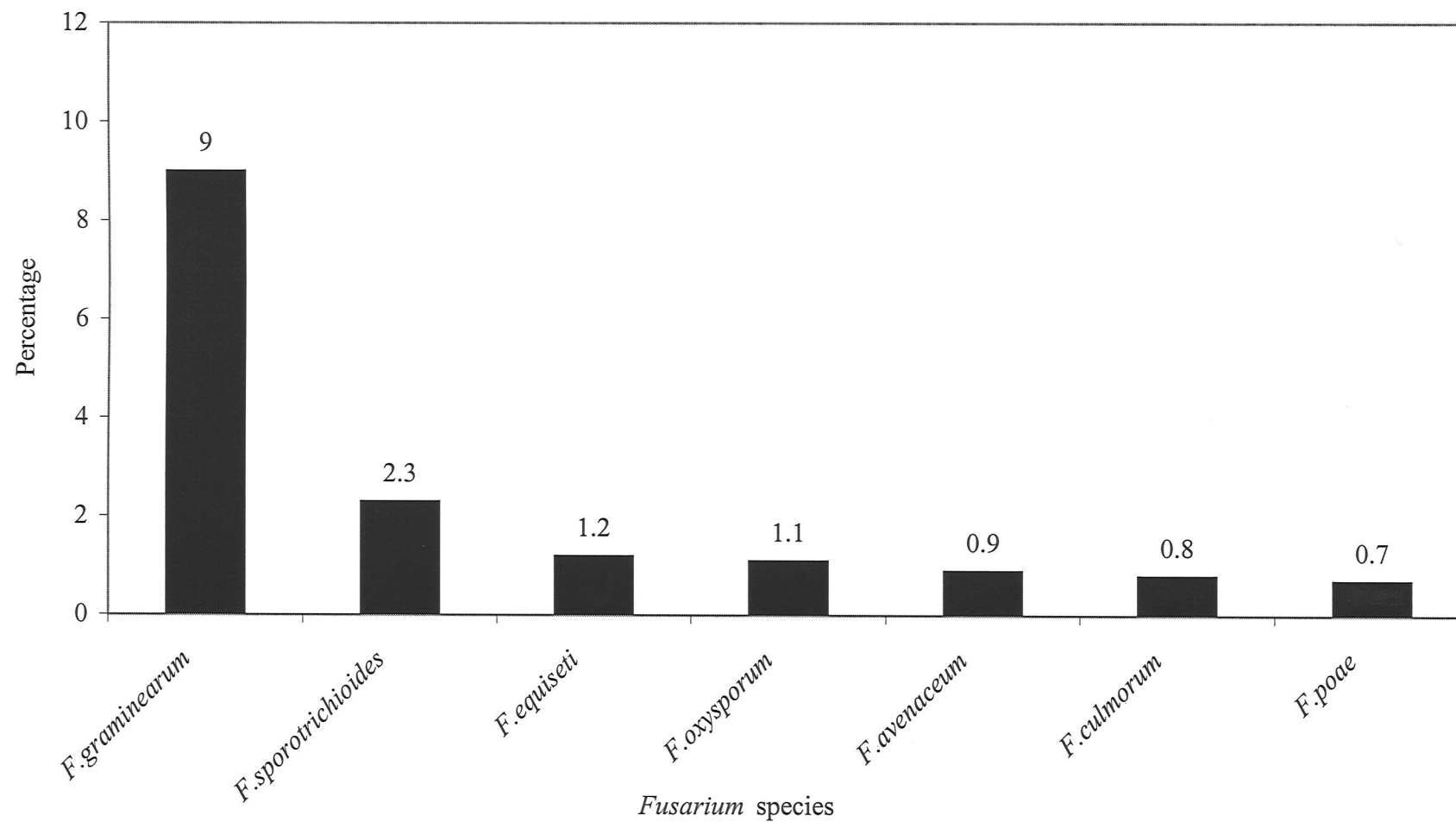


Figure 3.1: Percent of total grass inflorescences (n= 1766) harbouring *Fusarium* species from Glenlea, Tolstoi, and Living Priarie Museum (combined) in 1999 and 2000.

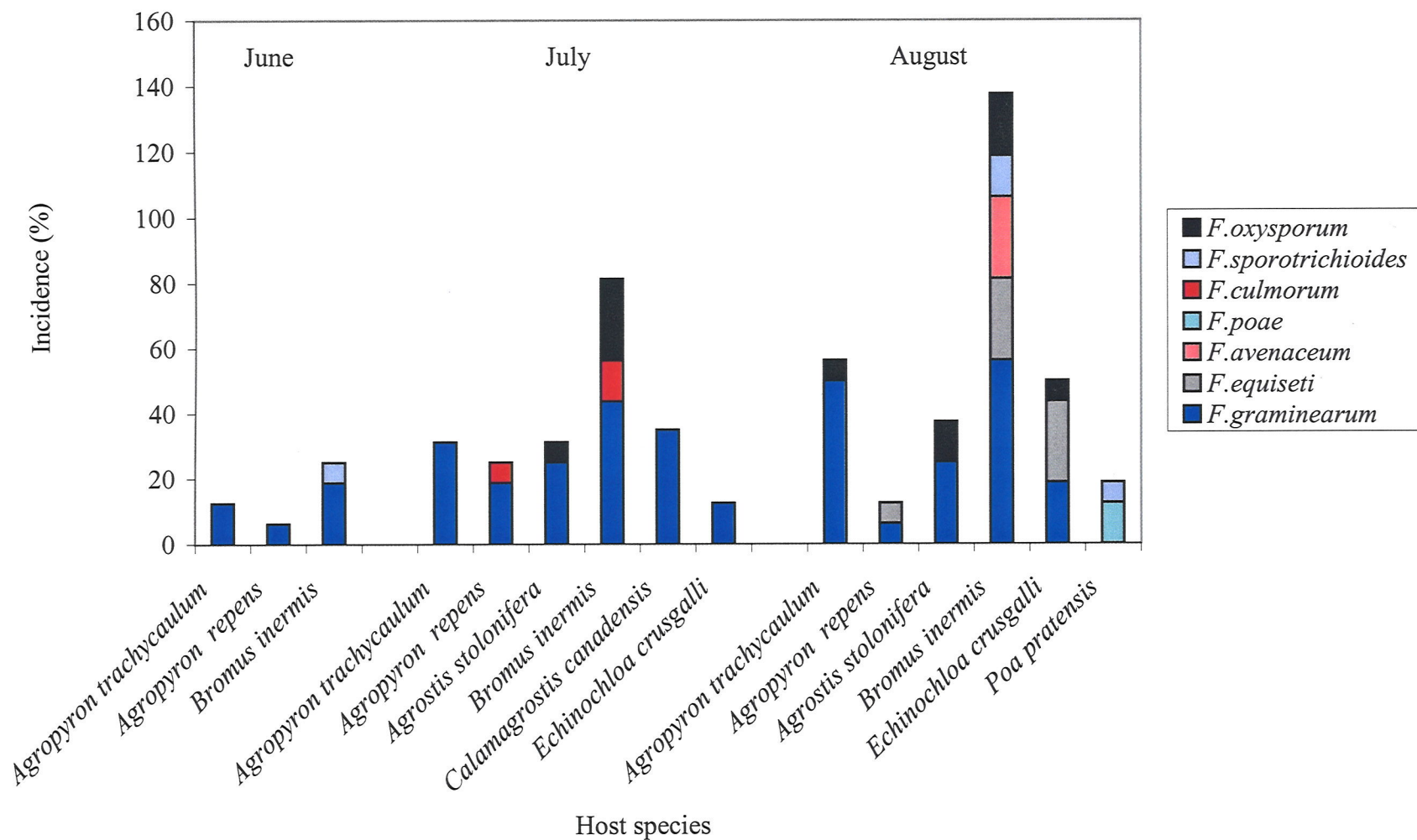


Figure 3.2: Incidence of *Fusarium* species isolated from grass inflorescences (n=16) from Glenlea, MB. in 1999 and 2000.

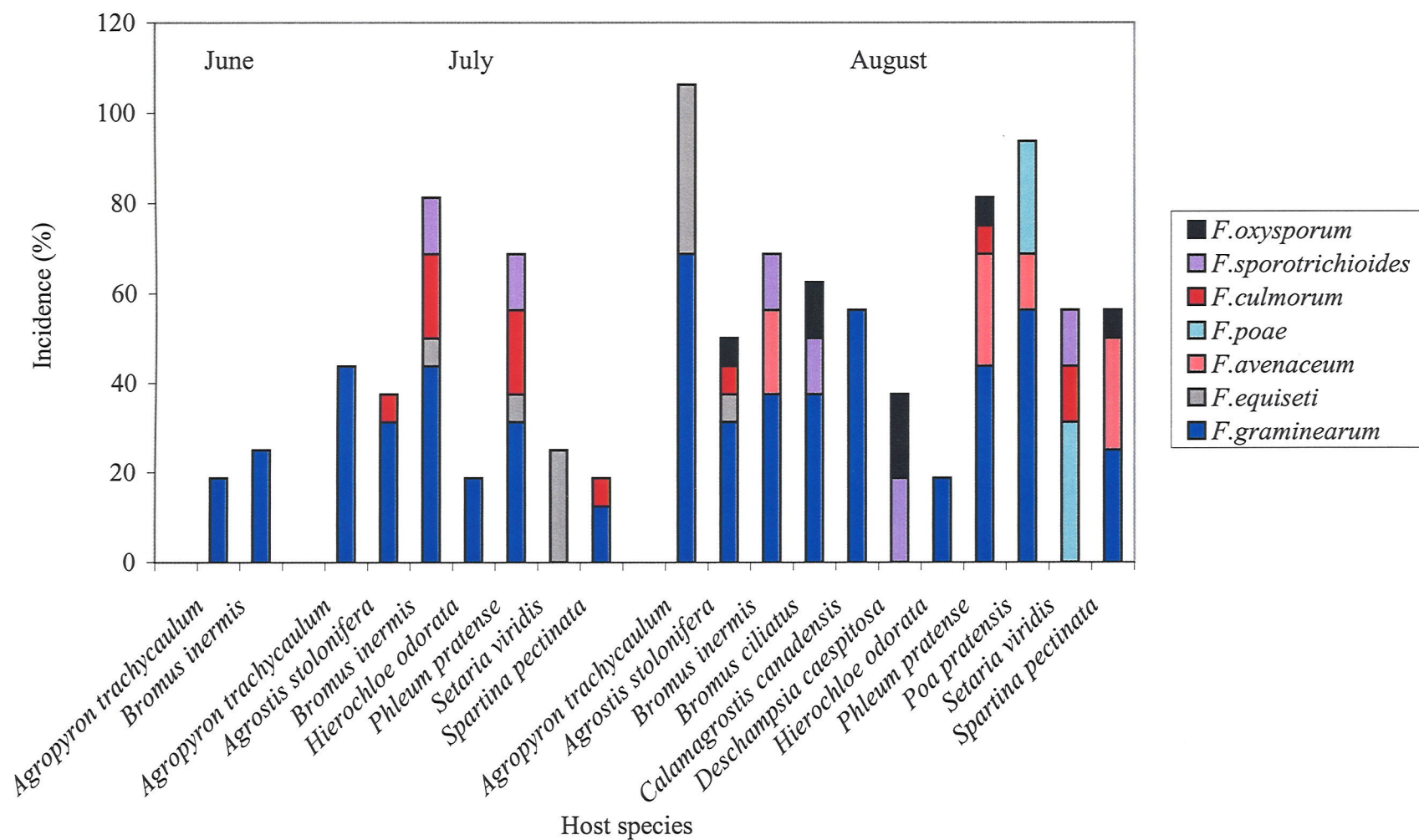


Figure 3.3: Incidence of *Fusarium* species isolated from grass inflorescences (n=16) sampled from Tolstoi Tall-Grass Prairie Preserve, MB. in 1999 and 2000.

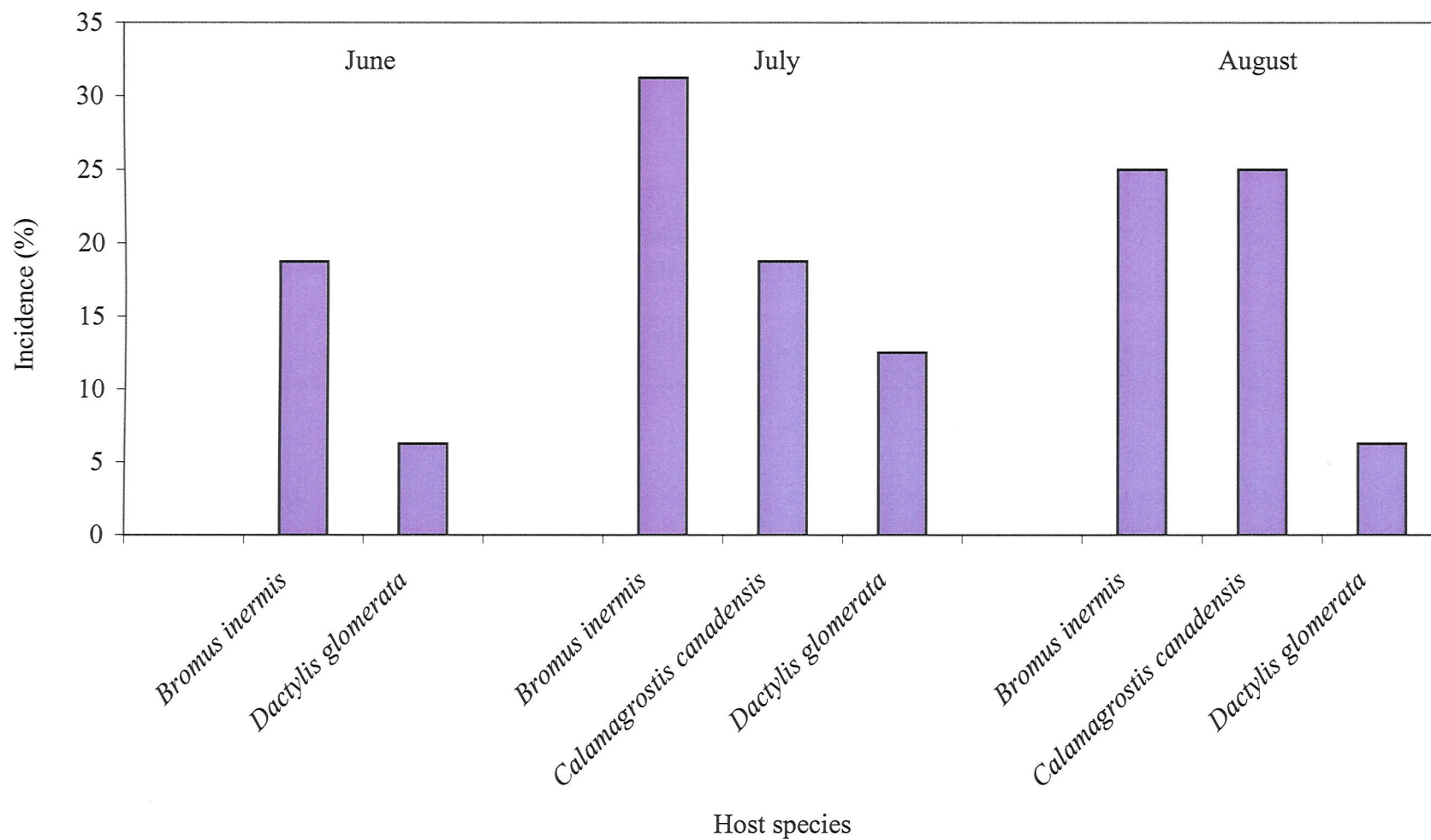


Figure 3.4: Incidence of *Fusarium sporotrichioides* isolated from grass inflorescences (n=16) sampled from the Living Prairie Museum, Winnipeg, MB. in 1999 and 2000.

Table 3.1: Incidence and percent recovery of *Fusarium* species from grass inflorescences sampled from Glenlea, Tolstoi, and Living Prairie Museum in 1999 and 2000.

Species	<u>Glenlea</u>		<u>Tolstoi</u>		<u>Living Prairie Museum</u>	
	Incidence	Percent	Incidence	Percent	Incidence	Percent
<i>F.graminearum</i>	58	62	96	59	0	0
<i>F.oxysporum</i>	12	13	8	5	0	0
<i>F.equiseti</i>	9	10	13	8	0	0
<i>F.avenaceum</i>	4	4	13	8	0	0
<i>F.sporotrichioides</i>	4	4	13	8	23	100
<i>F. poae</i>	4	4	9	5	0	0
<i>F.culmorum</i>	3	3	12	7	0	0
total	91	100	152	100	23	100

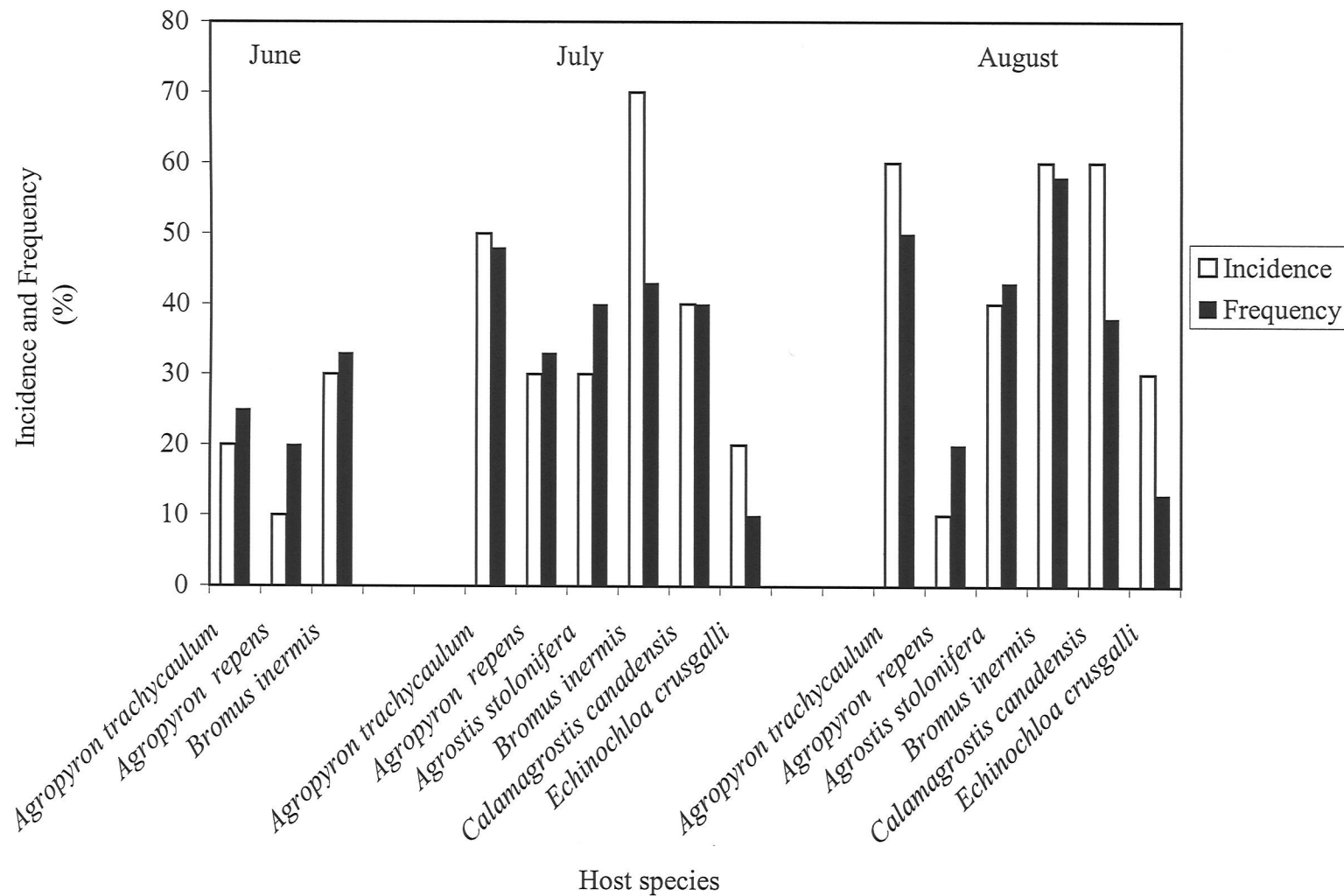


Figure 3.5: Incidence and isolation frequency of *Fusarium graminearum* on grass inflorescences (n=10) collected from Glenlea, MB. in 2000.

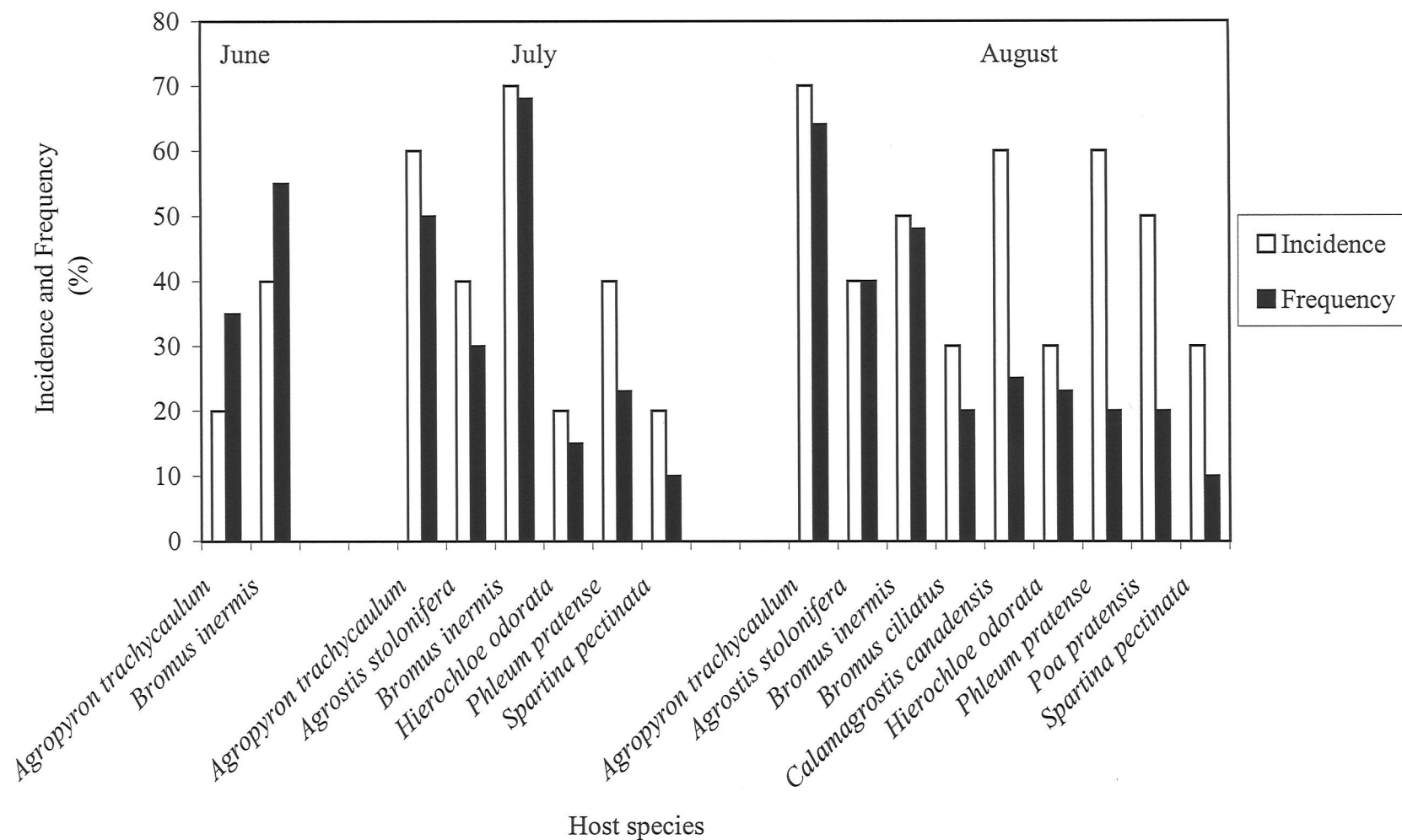


Figure 3.6: Incidence and isolation frequency of *Fusarium graminearum* on grass inflorescences (n=10) collected from Tolstoi, MB. in 2000.

3.5 Discussion:

Inflorescences of all the plants examined were surface sterilized with ethanol to remove superficial organisms, therefore the *Fusarium* species isolated were assumed to be from infected tissues. However, all of the plants examined were asymptomatic, suggesting that the *Fusarium* species were endophytic. The endophytic infection of winter wheat by *F. culmorum* and *F. graminearum* has been suggested by Sieber *et al.* (1988).

Isolation of *F. graminearum* from wild grass species from Tolstoi and Glenlea suggests such species play a role in the survival of the pathogen. In an area like Tolstoi, where levels of FHB are low, the isolation frequency of *F. graminearum* was equal to Glenlea, where levels of FHB are high. A small amount of residues of wild grasses were examined throughout the summer, but no perithecia were observed, suggesting that wild grass species may not provide primary ascospore inoculum for the development of FHB on crops. While this would limit the pathogen's ability to spread long distances, grasses may act as a reservoir to ensure the survival of the pathogen between susceptible crops. Infection by *F. graminearum* may develop on residues when the grass dies back, however, this would account for a minor amount of inoculum, compared to that produced by susceptible crop residues.

Many of the grass species harboring the pathogen are common forage crops such as timothy, kentucky blue grass, smooth brome, slender wheat grass, and rye grass. The levels of DON were not determined in this study. Cattle can consume moderate levels of DON (5 ppm) without negative side effects (Gilbert and Tekauz 2000). However, it is

possible that the frequency of the pathogen could be found at high enough levels to cause possible problems in grazing livestock.

The pathogenicity of the *Fusarium* isolates obtained from the wild grasses was not examined. If the form of *F. graminearum* present on wild grasses were nonpathogenic to wheat and other cereal crops, this would suggest that they do not play a role in survival. Therefore the epidemiological significance of *F. graminearum* isolates obtained from grass species can only be speculated at this time. More work is required to understand the role of alternative hosts in the epidemiology of fusarium head blight.

3.6 Conclusions:

Wild grass species harbor many species of *Fusarium* including *F. graminearum*, the predominant species causing FHB in cereal crops in Manitoba. The failure to find *Fusarium* species on certain grasses does not exclude the possibility that they harbor them. Grass hosts appear to be symptomless carriers of *F. graminearum* as well as other species of *Fusarium*. Wild grass species may help bridge the gap between hosts and increase the inoculum potential. Further study is required to determine why some grass species appear to harbor *F. graminearum* while others do not, whether DON is produced and whether the isolates of *F. graminearum* present on grass species are pathogenic to cereal crops.

Summary and General Conclusions

Current provincial recommendations instruct producers at harvest to blow the light weight FDK out the back of the combine to reduce chances of downgrading at the elevator due to FHB. The ultimate fate of this seed-borne *G. zea* is unknown. However, it was found that *G. zea* survives on fusarium damaged kernels (FDK), either buried or left on the surface, for at least 2 years under field conditions. Buried kernels did not produce mature perithecia. However, if brought to the surface at seeding, they may potentially colonize other surface debris. Perithecia and ascospores were produced on kernels located on the soil surface, which may contribute to disease outbreaks. This has important implications for disease management. Rotations exceeding at least two years are necessary to avoid infection of new crops by seed-borne *G. zea*.

Soil composition did not influence survival of *G. zea*, but it did influence the decomposition rate of the kernels. The kernels buried at 5 and 10 cm from Morden (sandy loam) and Winnipeg (silty clay loam) decomposed at similar rates, whereas the kernels buried at Portage (silty loam) had a slightly higher rate of decomposition. All FDK left on the soil surface decomposed at a slower rate than those buried at 5 and 10 cm.

Under controlled conditions, the survival of *G. zea* on kernels incubated at 20°C decreased significantly after 2 years. Survival of *G. zea* ranged from 76-96%. Time, placement, and soil type (sterile or non-sterile) influenced the duration of survival at 20°C. General survival rate of the kernels incubated at -10°C and 2°C, ranged from 98-100%. This suggests that the survival of *G. zea* decreases at high temperatures and is not affected at low temperatures.

Both seasonal and daily patterns of ascospore release were observed in this study. A seasonal pattern of ascospore release was observed in both years of the study with inoculum, primarily ascospores, first being detected in the middle of July and continuing until the end of August. Several ascospore release events were detected throughout the season. This suggests that a heterogeneous population of perithecia exist with different times of maturity. In both years, peak ascospore release (mid-July and early August) corresponded to the period in which wheat was in anthesis. Anthesis of spring wheat occurs in mid-July to the beginning of August, depending on when the crop was seeded, temperature and rainfall. In 2000, with the use of a volumetric 7-day spore trap, ascospores were found to have a diurnal pattern of dispersal. Ascospore release began at 1700 h, which coincided with the lowest recorded RH value of 40%, and continued as RH increased, until 0400 h. Peak ascospore releases occurred at 2100 h, with a maximum concentration of 5064 ascospores/m³. Very few ascospores were trapped between 0500 and 1500 h, ranging from 0-167 ascospores/m³. Relative humidity decreased as temperature increased during the day and RH increased as the temperature decreased early in the evening. The decrease in RH may have caused the perithecia and substrate to dry out, causing mechanical rupturing of the ascus wall and allowing ascospores to be discharged. Ascospore release occurred 2 to 5 days after a rainfall event (>5 mm). This suggests that rainfall does not trigger ascospore release, but is necessary for maturation of perithecia and ascospores.

Macroconidia were released throughout the day. Daily macroconidia concentrations ranged from 0-567 macroconidia/m³. As macroconidia are primarily splash-dispersed, they are not likely to be sampled routinely from the air. However,

significant kinetic energy may cause the macroconidia to become air-borne. If the droplets in which the conidia are suspended dry from wind and sun it is possible they maybe taken up by the wind. Further investigation on how the macroconidia become air-borne (likely from water droplets) is needed.

The role of grasses in the epidemiology of FHB can only be speculated from the results of this study. *Fusarium graminearum* was isolated from 9% of the grass plants examined. Other *Fusarium* species isolated at low frequencies included *F. sporotrichioides* (2.3%), *F. equiseti* (1.2%), *F. oxysporum* (1.1%), *F. avenaceum* (0.9%), *F. culmorum* (0.8%), and *F. poae* (0.7%). *Fusarium graminearum* occurred on 11 grass species, seven of which are a first report. Wild grass species may help bridge the gap between hosts and increase the inoculum potential. Further study is required to determine why some grass species appear to harbor *F. graminearum* while others do not, whether DON is produced and whether *F. graminearum* present on grass species is pathogenic to cereal crops.

Based on the results of the three projects, a disease life cycle for *G. zeae* was constructed for Manitoba (Fig. 1). In early spring (April – June) perithecia develop on wheat residues which give rise to mature ascospores in July and August. This corresponds with the time of anthesis for many of the spring wheat cultivars grown in Manitoba (mid July early August-depending on seeding time). The ascospores are deposited on the flowering heads and can give rise to blighted heads (August). Macroconidia are produced on the infected heads, which may infect secondary tillers. When wheat and other cereal crops are harvested, the lighter weight, diseased kernels are blown out the back of the combine on to the field. Other residues may also be left on the

surface or tilled under, depending on the cultural practices of the individual producer. The pathogen then over-winters on these residues, as chlamydospores, mycelium, or macroconidia. The following spring the fungus resumes growth and the cycle begins again, pending on the availability of a susceptible host, which may include wild grasses. Proper environmental conditions are required for the pathogen to successfully complete its life cycle. Substantial rain is required to induce perithecial formation and give rise to mature ascospores. During dry periods, ascospore release is inhibited and will only resume when perithecia are re-wetted. Further investigation into the factors (soil and debris moisture) necessary for perithecial and ascospore development on crop residues is required. Also further investigation on the effects of the crop canopy, soil temperature, and wind velocity on ascospore and macroconidial dispersal. A greater understanding of the survival of the pathogen, and the development and spread of inoculum, may lead to more effective means of disease control.

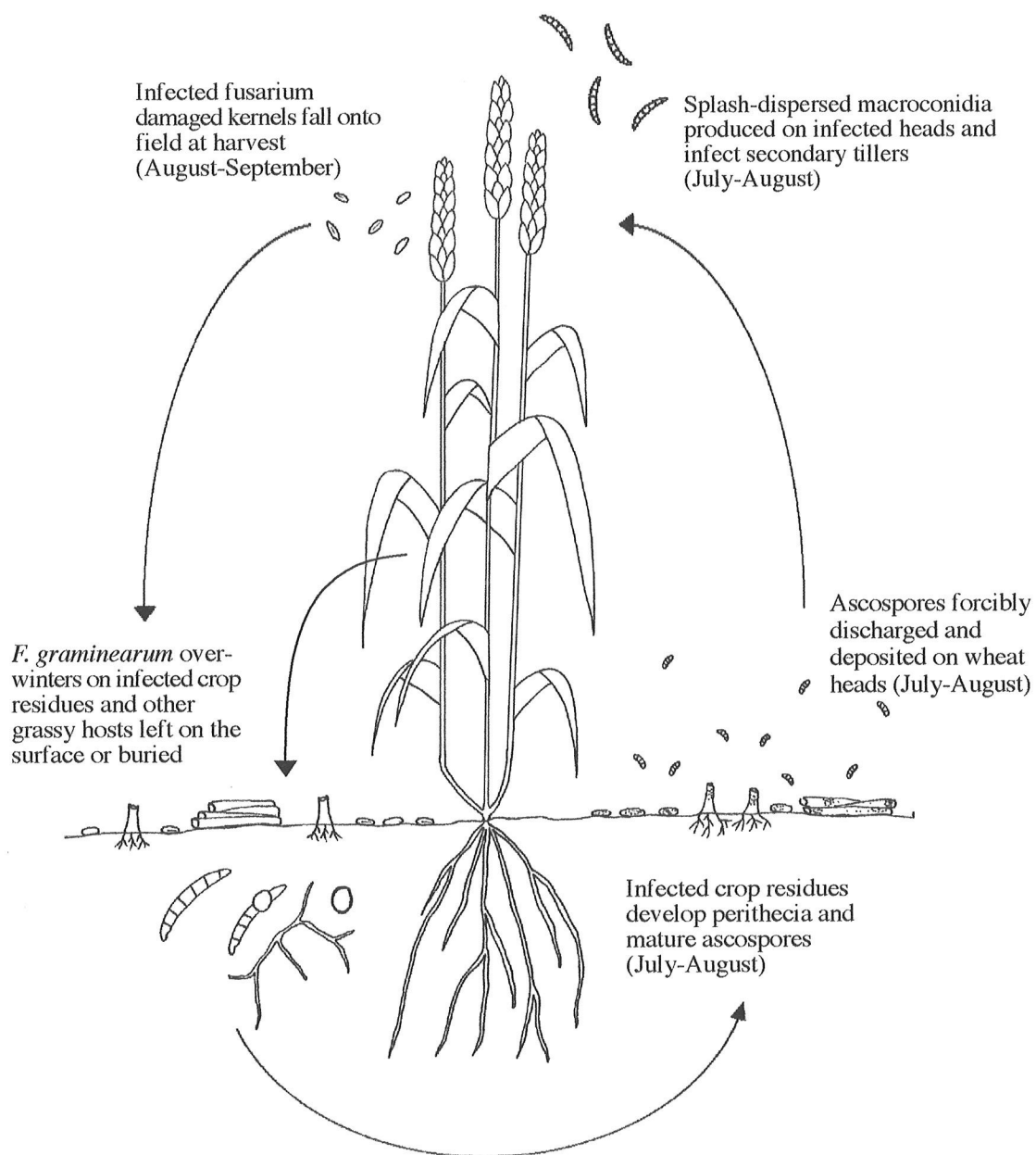


Figure 1: Disease cycle of *Gibberella zeae* and *Fusarium graminearum* (anamorph) on spring wheat in Manitoba.

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APPENDIX

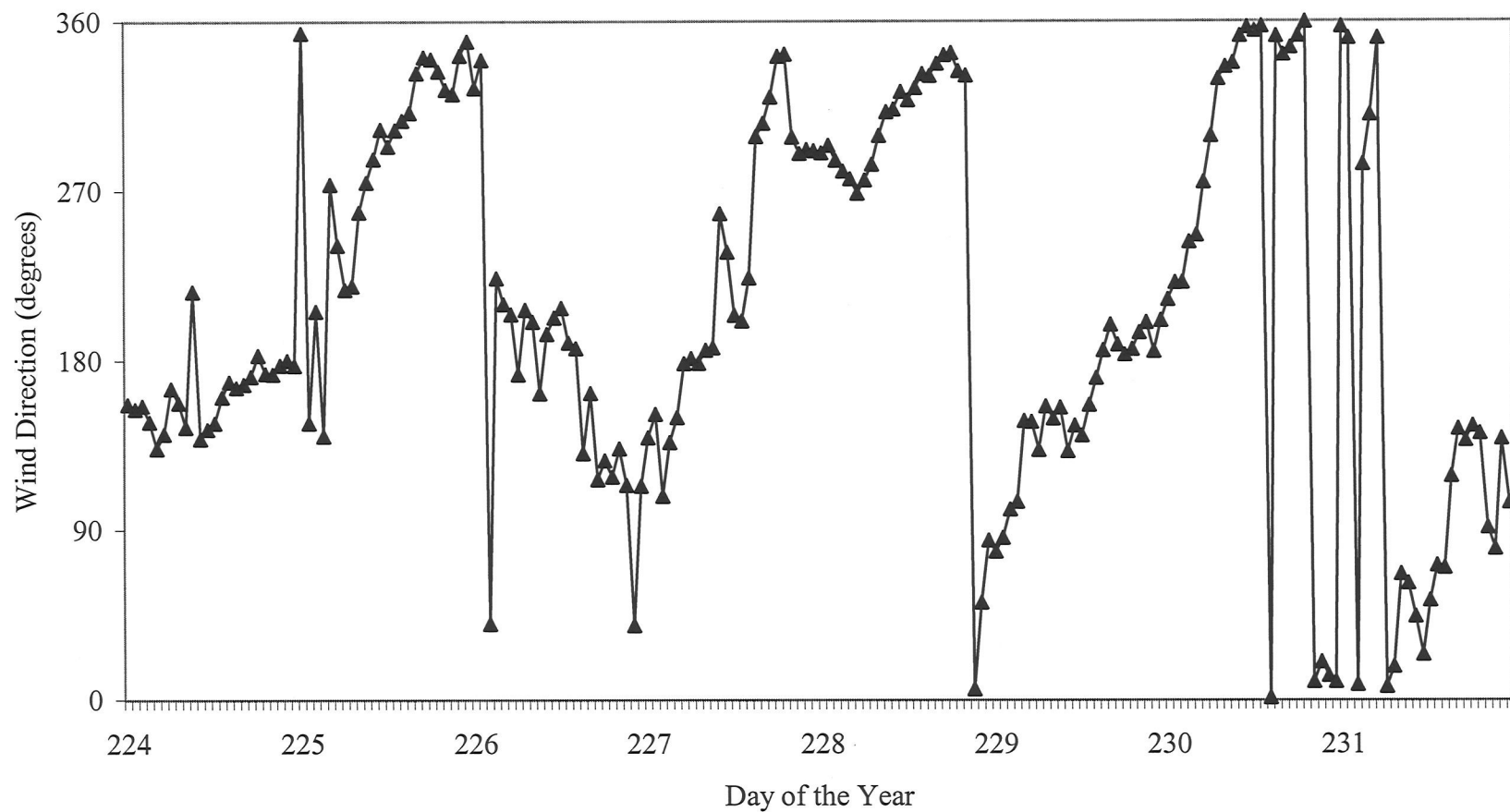


Figure A1: Wind direction (0 & 360 = North, 90 = East, 180 = South) for days for days 224 to 231, the period for ascospore release and time of anthesis at Glenlea, MB., 2000

Appendix A2: Grass collection from Glenlea, Tolstoi Tall-Grass Prairie Preserve,
and Living Prairie Museum for 1999 and 2000.

Glenlea

Sample date	Latin name	Common name
8-Jun-99	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Koeleria gracilis</i> Pers.	June grass
	<i>Lolium multiflorum</i> Lam.	Italian rye grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
8-Jul-99	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Lolium multiflorum</i> Lam.	Italian rye grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
29-Aug-99	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Lolium multiflorum</i> Lam.	Italian rye grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
30-Jun-00	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agropyron cristatum</i> (L.) Gaertn.	Crested wheat grass
	<i>Agrostis stolonifera</i> L.	Red top

	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Lolium multiflorum</i> Lam.	Italian rye grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail
26-Jul-00	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agropyron cristatum</i> (L.) Gaertn.	Crested wheat grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Beckmannia syzigachne</i> (Steud.) Fern.	Slough grass
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Lolium multiflorum</i> Lam.	Italian rye grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail
24-Aug-00	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agropyron cristatum</i> (L.) Gaertn.	Crested wheat grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Lolium multiflorum</i> Lam.	Italian rye grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail

Tolstoi

Sample date	Latin name	Common name
16-Jun-99	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Deschampsia caespitosa</i> (L.) Beauv.	Tufted hair grass
	<i>Hierochloe odorata</i> (L.) Beauv.	Sweet grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Phleum pratense</i> L.	Timothy
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
23-Jul-99	<i>Agropyron subsecundum</i> (Link) Hitchc.	Awed wheat grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Beckmannia syzigachne</i> (Steud.) Fern.	Slough grass
	<i>Bromus ciliatus</i> L.	Fringed brome
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Deschampsia caespitosa</i> (L.) Beauv.	Tufted hair grass
	<i>Hierochloe odorata</i> (L.) Beauv.	Sweet grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Panicum capillare</i> L.	Witch grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Phragmites communis</i> Trin.	Cane grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail
	<i>Sorghastrum nutans</i> (L.) Nash	Indian grass
	<i>Spartina pectinata</i> Link	Prairie cord grass
	<i>Sporobolus heterolepis</i> A. Gray	Prairie dropseed
30-Aug-99	<i>Agropyron subsecundum</i> (Link) Hitchc.	Awed wheat grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Andropogon scoparius</i> Michx.	Little bluestem
	<i>Beckmannia syzigachne</i> (Steud.) Fern.	Slough grass
	<i>Bromus ciliatus</i> L.	Fringed brome
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass

	<i>Deschampsia caespitosa</i> (L.) Beauv.	Tufted hair grass
	<i>Hierochloe odorata</i> (L.) Beauv.	Sweet grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Muhlenbergia cuspidata</i> (Torr.) Rydb.	Prairie muhly
	<i>Muhlenbergia racemosa</i> (Michx.) BSP.	Marsh muhly
	<i>Panicum capillare</i> L.	Witch grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Phragmites communis</i> Trin.	Cane grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Puccinellia nuttalliana</i> (Schultes) Hitchc.	Nuttall's alkali
	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail
	<i>Sorghastrum nutans</i> (L.) Nash	Indian grass
	<i>Spartina pectinata</i> Link	Prairie cord grass
	<i>Sporobolus heterolepis</i> A. Gray	Prairie dropseed
28-Jun-00	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Deschampsia caespitosa</i> (L.) Beauv.	Tufted hair grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Koeleria gracilis</i> Pers.	June grass
	<i>Glyceria grandis</i> S. Wats.	Tall manna grass
	<i>Panicum capillare</i> L.	Witch grass
	<i>Phalaris arundinacea</i> L.	Reed canary grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Spartina pectinata</i> Link	Prairie cord grass
	<i>Stipa spartea</i> Trin.	Porcupine grass
24-Jul-00	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agropyron subsecundum</i> (Link) Hitchc.	Awned wheat grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Bromus ciliatus</i> L.	Fringed brome
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Deschampsia caespitosa</i> (L.) Beauv.	Tufted hair grass
	<i>Glyceria grandis</i> S. Wats.	Tall manna grass
	<i>Hierochloe odorata</i> (L.) Beauv.	Sweet grass

	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Muhlenbergia racemosa</i> (Michx.) BSP.	Marsh muhly
	<i>Phleum pratense</i> L.	Timothy
	<i>Phragmites communis</i> Trin.	Cane grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Setaria viridis</i> (L.) Beauv.	Green foxtail
	<i>Sorghastrum nutans</i> (L.) Nash	Indian grass
	<i>Spartina pectinata</i> Link	Prairie cord grass
20-Aug-00	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agropyron subsecundum</i> (Link) Hitchc.	Awne d wheat grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Agrostis stolonifera</i> L.	Red top
	<i>Andropogon gerardii</i> Vitman	Big bluestem
	<i>Andropogon scoparius</i> Michx.	Little bluestem
	<i>Bromus ciliatus</i> L.	Fringed brome
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Deschampsia caespitosa</i> (L.) Beauv.	Tufted hair grass
	<i>Echinochloa crusgalli</i> (L.) Beauv.	Barnyard grass
	<i>Hierochloa odorata</i> (L.) Beauv.	Sweet grass
	<i>Hordeum jubatum</i> L.	Wild barley
	<i>Muhlenbergia racemosa</i> (Michx.) BSP.	Marsh muhly
	<i>Panicum capillare</i> L.	Witch grass
	<i>Phleum pratense</i> L.	Timothy
	<i>Phragmites communis</i> Trin.	Cane grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Sorghastrum nutans</i> (L.) Nash	Indian grass
	<i>Spartina pectinata</i> Link	Prairie cord grass

Living Prairie Museum

Sample date	Latin name	Common name
17-Jun-99	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Dactylis glomerata</i> L.	Orchard grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
30-Jul-99	<i>Stipa spartea</i> Trin.	Porcupine grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Bouteloua cartipendula</i> (Michx.) Torr.	Side-oat grama
	<i>Bromus inermis</i> Leyss.	Smooth brome
30-Aug-99	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Panicum vigatum</i> L.	Switch grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Stipa spartea</i> Trin.	Porcupine grass
30-Jun-00	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Bouteloua cartipendula</i> (Michx.) Torr.	Side-oat grama
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
26-Jul-00	<i>Panicum vigatum</i> L.	Switch grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Stipa spartea</i> Trin.	Porcupine grass
	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
30-Jun-00	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Dactylis glomerata</i> L.	Orchard grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Stipa spartea</i> Trin.	Porcupine grass
26-Jul-00	<i>Agropyron repens</i> (L.) Beauv.	Quack grass
	<i>Agropyron subsecundum</i> (Link) Hitchc.	Awned wheat grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Andropogon scoparius</i> Michx.	Little bluestem
26-Jul-00	<i>Bouteloua cartipendula</i> (Michx.) Torr.	Side-oat grama
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass

	<i>Hierochloe odorata</i> (L.) Beauv.	Sweet grass
	<i>Panicum vigatum</i> L.	Switch grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Stipa spartea</i> Trin.	Porcupine grass
24-Aug-00	<i>Agropyron subsecundum</i> (Link) Hitchc.	Awned wheat grass
	<i>Agropyron trachycaulum</i> (Link) Malte	Slender wheat grass
	<i>Andropogon gerdii</i> Vitman	Big bluestem
	<i>Andropogon scoparius</i> Michx.	Little bluestem
	<i>Bouteloua cartipendula</i> (Michx.) Torr.	Side-oat grama
	<i>Bromus inermis</i> Leyss.	Smooth brome
	<i>Calamagrostis canadensis</i> (Michx.) Beauv.	Marsh reed grass
	<i>Dactylis glomerata</i> L.	Orchard grass
	<i>Hierochloe odorata</i> (L.) Beauv.	Sweet grass
	<i>Panicum vigatum</i> L.	Switch grass
	<i>Poa annua</i> L.	Annual blue grass
	<i>Poa pratensis</i> L.	Kentucky blue grass
	<i>Sorghastrum nutans</i> (L.) Nash	Indian grass
	<i>Stipa spartea</i> Trin.	Porcupine grass