THE UNIVERSITY OF MANITOBA
FACULTY OF GRADUATE STUDIES
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Development of models to predict fusarium head blight disease and deoxynivalenol in wheat, and genetic causes for chemotype diversity and shifting of *Fusarium graminearum* in Manitoba

BY

Xiaowei Guo

This dissertation is submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Science

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Fusarium head blight (FHB) is one of the most important diseases in wheat. It causes wheat yield loss and affects the quality of wheat. Disease prediction models will help wheat producers with making right decision on fungicide application.

This study revealed the important roles of cumulative rainfall during different periods from seeding to wheat anthesis and in the 14 days after anthesis, and average daily temperature from the first week before to the first week after anthesis in fusarium airborne inoculum level, fusarium head blight (FHB) disease and deoxynivalenol (DON) levels. Cropping practices, which were quantified and expressed as cropping practice index (CPI), significantly affected the number of *Fusarium graminearum* / *Gibberella zeae* spores, FHB disease index and DON level in the mature kernels. The numbers of *F. graminearum* spores were strongly correlated with the disease index and toxin levels. Four models (Chapter 2) were developed to predict the number of *F. graminearum* spores on single wheat heads using CPI and weather conditions. Two types of prediction models for FHB disease index and DON level were developed based on cropping practices, actual or predicted spore counts on single wheat heads, and weather conditions. Type I models were developed using actual spore number, and Type II models were developed based on predicted spore number using the predicted model developed in Chapter 2. The average prediction accuracy was 85% for Type I models and 58% for Type II models. Type I models can be used for the purpose of market prediction by the Canadian Wheat Board. Type II models can be used with the spore prediction model by wheat producers.

FHB is important not only because it causes yield loss but also its agents produce mycotoxins. Deoxynivalenol (DON) is one of the most important mycotoxins produced

by fusarium strains. The 3-acetyldeoxynivalenol (3ADON) chemotype of *F. graminearum* produces more DON than the 15 ADON chemotype and is replacing the 15ADON chemotype in Canada. These findings cause increasing concern within the wheat industry and will potentially affect the prediction accuracy of the FHB and DON models. Therefore, it is necessary to determine distribution and shifting of *F. graminearum* chemotypes in Manitoba.

Chapter 4 of this study revealed that the 15ADON chemotype of *F. graminearum* was predominant in Manitoba. The 3ADON chemotype was predominant in the southeast part of Manitoba, which included Sanford, Morris and Horndean; and shared the same percentage with the 15ADON chemotype in Cartier and Portage la Prairie. The variation of chemotype likely resulted from genetic diversity of *F. graminearum* populations in Manitoba, which was associated with sexual recombination, age of populations and tillage system. Wheat seed shipment and long-distance *F. graminearum* spore dispersal likely contributed to the gene flow in this province.

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GENERAL INTRODUCTION

Wheat is one of the most important crops in the world. Fusarium head blight (FHB) disease has become one of most important diseases in wheat worldwide. This destructive disease is caused by the agent *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum* Schwabe) in North America. Since 1993, FHB disease has become a major problem in Canada (Gilbert and Tekauz, 2000). FHB causes wheat yield loss due to shrunken kernels caused by the pathogen, damages wheat quality due to reduction of test weight and loaf volume in the baking process, and threatens safety of human food and animal feed because of mycotoxins produced by the pathogen (Dexter and Nowicki, 2003).

In Canada, economic losses to FHB in the wheat industry were estimated at US$220 million in Central Canada in the 1990s, and at US$300 million in Manitoba from 1993 to 1998 (Windels, 2000). In the United States, wheat and barley losses to this disease were estimated at US$3 billion. The farmers lost more than 500 million bushels of wheat. Epidemics of FHB are favored by high-moisture weather conditions, planting of susceptible wheat cultivars, and increase of zero or minimum tillage, which contribute to buildup of fusarium inoculum (Windels, 2000).

Different disease control methods are being used, although fungicide application combined with cropping practices is still the first choice for wheat producers due to the lack of resistant cultivars. Most wheat producers spray fungicides without considering weather conditions and pathogen inoculum levels, and thus, waste a great deal of money (personal communication with farmers). Disease prediction models and forecasting systems will help wheat producers with timing and making effective decisions for fungicide application.

Current prediction models and forecasting systems were developed based on weather conditions without considering pathogen effect. However, a good prediction model should include weather and pathogen factors, making prediction more reliable in the long term.

Three chemotypes are found in *F. graminearum* species, 3-acetyldeoxynivalenol (3ADON), 15ADON and nivalenol (NIV) (Miller et al., 1999). In North America, the 15ADON chemotype is predominant (Mirocha et al., 1989). However, the 3ADON chemotype is replacing the 15ADON chemotype from eastern to western Canada (Ward et al., 2005). The 3ADON chemotype produces more deoxynivalenol (DON), which causes more animal feed refusal, than the 15 ADON chemotype (Gilbert et al., 2006). This findings cause increasing concern of the wheat industry in Canada. To understand the chemotype distribution in different provinces in Canada and its underlying causes will benefit the management of FHB disease.

The objectives of this study:

1). to develop models to predict airborne fusarium inoculum level on wheat heads

2). to develop models to predict FHB disease and DON accumulation in wheat grain

3). to investigate the chemotype distribution and shifting of *F. graminearum* in Manitoba

CHAPTER 1

1.0 Literature Review

1.1 Wheat

1.1.1 Wheat origin and distribution

Wheat (*Triticum* spp. L.) was cultivated originally in the Fertile Crescent of the Middle East around 12,000 years ago (Gooding and Davies, 1997), and is grown worldwide today. The optimum temperature for wheat growth is about 25°C with minimum and maximum temperatures of 3-4°C and 30-32°C, respectively. Wheat can grow regions with precipitation ranging from 250 to 1750mm (Curtis, 2002). Therefore, most wheat crops are grown between latitudes 30-60°N and 27-40°S.

Wheat is one of the most important sources of human food, and ranks second to rice in total production as a cereal crop (FAOSTAT database of World Agriculture, 2006). Wheat is used to make flour for leavened, flat and steamed breads, cookies, cakes, pasta, and noodles (Cauvain and Stanley, 2003), and for fermentation to make alcohol (Palmer, 2001). Wheat is planted to a limited extent as a forage crop for livestock.

1.1.2 Wheat classification and production

Wheat is placed in the genus *Triticum*, tribe *Triticinae*, family Gramineae, order Poales, class Liliopsida, division Magnoliophyta and kingdom Plantae. *Triticum* species are grouped into three types based on ploidy level, diploid (14 chromosomes), tetraploid (28 chromosomes) and hexaploid (42 chromosomes) wheat (Gooding and Davies, 1997). There are three different sets of chromosomes, A, B and D. Diploid, tetraploid and hexaploid wheats contain AA, AABB and AABBDD chromosomes, respectively. Within a species, wheat can be further classified into winter wheat and spring wheat according to growing season, hard wheat and soft wheat based on protein content of kernels; or, red, white and amber wheat in terms of grain color.

Common wheat or bread wheat (*T. aestivum*), a hexaploid species, is most widely grown worldwide.

There are a total of 11 types of wheat in the world, soft red winter (SRW), soft white winter (SWW), hard red winter (HRW), hard white winter (HRW), soft red spring (SRS), SWS, HRS, HWS, Durum Wheat (*T. durum*), Compactum (*T. compactum*) and Spelta (*T. spelta*) (Curtis, 2002). The former eight types (common wheat) are most commonly grown. There are eight commercial classes of wheat in Western Canada, Canada Western Hard Red Spring (CWRS), Canada Western Hard White Spring (CWHWS), Canada Western Amber Durum (CWAD), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Extra Strong (CWES), Canada Prairie Red Winter (CPRW), and Canada Western Soft White Spring (CWSWS) (Curtis, 2002).

Wheat is one of the most important food crops based on its annual production rate. World wheat production significantly increased from approximately 250 million tonnes in the 1960s to 550 million tonnes in the 1990s (Curtis, 2002). There in cases occurred in the former USSR, Australia, New Zealand, Europe and Asia between 1974 and 1992 (Gooding and Davies, 1997).

China has the largest cultivated area for wheat of all countries (29.4 million hectares), followed by the United States, India, the Russian Federation, Kazakhstan and Canada which have 25.0, 24.9, 23.6 12.6 and 11.5 million hectares, respectively (Curtis, 2002). China produces approximately 100 million tonnes of wheat annually, far ahead of the United States, India and Canada, which average 62, 59 and 25 million tonnes, respectively. China also has the highest wheat yield per unit area, 3.5 tonnes/hectare, while the United States, India and Canada produce 2.5, 2.4 and 2.2 tonnes/hectare.

Although it is the largest producer, China is also the largest wheat importer in the world. Ten million tonnes of wheat were imported annually by China since 1980 (Curtis, 2002). The Russian Federation, Japan, Brazil and Egypt each imported nearly 5 million tonnes annually. Most wheat is consumed by the producing countries, but on average, 20% of total world wheat production is exported. The United States, Canada and Australia are the three largest exporting countries, accounting for 33%, 18% and 12% of world wheat trade. Canada is famous for its high-quality wheat and large range of grain protein content for pocessing.

In Canada, CWRS wheat is predominant and, on average, 20 million tonnes are produced annually, accounting for 70% of total Canadian wheat production (Curtis, 2002). It can be used for different products due to its good gluten strength, including pan and hearth breads, flat breads, steam breads and noodles. It is grown in Manitoba, Saskatchewan and Alberta. CWAD wheat accounts for 20% of total wheat production, and approximately 4 million tonnes are produced each year. It is desirable for the production of high quality pasta due to its high protein content, strong gluten and high yellow pigment content.

In Manitoba, about 89% of the wheat crop is CWRS, 7.3%, winter wheat, about 1.2% CPSR, about 0.9% CWES, and 1.2% CWAD. Only 0.2%was CWSWS and 0.4% was other varieties. Seeded area of wheat is approximately 2.2 million hectares. Wheat production is around 3.5 million tonnes, accounting for 17% of total wheat production in Canada. Total value of the Manitoba wheat crop is around CAD$500 million annually. Manitoba wheat is exported to over sixty countries. In 2001, approximately 42% of the exported wheat was shipped to the United States, Iran, Japan and Mexico (Curtis, 2002).

1.2 *Fusarium*

1.2.1 Classification and morphological characters of *Fusarium* species

Fusarium head blight was first described as wheat scab by W. G. Smith in 1884 (Stack, 2003). Wollenweber and Reinking published their study in *Fusarium* systematics, entitled *Die Fusarien* in 1935 (Liddell, 2003). *Fusarium* species belong to the Genus *Fusarium*, Family Nectriaceae, Order Hypocreales, Subclass Hypocreomycetidae, Class Sordariomycetidae, Subphylum Pezizomycotina, Phylum Ascomycota, and Kingdom Fungi (Kendrick, 2003).

Genus *Fusarium* is divided into 16 sections and four sections of *Fusarium* species are important for fusarium head blight disease (Liddell, 2003). They are *Discolor*, *Roseum*, *Gibbosum*, and *Sporotrichiella* sections. *Fusarium graminearum* (*Gibberella zeae*), *F. culmorum* and *F. crookwellense* are in the section *Discolor*. *Fusarium equiseti*, *F. scirpi* and *F. acuminatum* (*G. acuminata*) are included in the section *Gibbosum*. *Fusarium avenaceum* (*G. avenacea*) is in the section *Roseum*. *Fusarium poae*, *F. tricinctum* and *F. sporotrichioides* belong to the section *Sporotrichiella*.

Fusarium graminearum is the predominant species causing FHB disease in Canada, the United States, China, some counties in Europe and other temperate climates (Desjardins, 2006). In the sexual stage, this fungus produces its fruiting body, called a perithecium, from which ascospores are released under the high moisture condtions. Perithecia usually form in clusters from a small inconspicuous stroma around the nodes or basal region of the infected wheat stem. They are ovoid with a very rough tuberculate wall, and 140-250 μm in diameter (Booth, 1971). Asci are clavate with a short stipe, and 60-85 × 8-11 μm in size. There are 8, or occasionally 4-6, ascospores in each ascus. Ascospores are hyaline or occasionally very light brown,

curved fusoid with round ends, and 19-24 × 3-4 μm in size. They usually have 3 septa. *Fusarium graminearum* macroconidia are formed from single phialides or multibranched conidiophores, which may aggregate into sporodochia. The conidia range from falcate to sickle shape, and have a well marked foot cell. They have 3 to 7 septa depending on the size of the spores: conidia with 3-4 septa and 5-7 septa are 25-40 × 2.5-4 μm and 48-50 × 3-3.5 μm, respectively (Booth, 1971).

Burgess et al. (1975) differentiated two populations of *F. graminearum* in culture, and designated them Group 1 and Group 2. Group 1 of *F. graminearum* caused crown rot of wheat, and Group 2 caused fusarium head blight of wheat. Burgess et al. (1988) described the diagnostic characters of these two groups. The single-spore strains of Group 1 could not form perithecia in culture and were heterothallic, whereas, single-spore strains of Group 2 formed abundant homothallic perithecia. Aoki and O'Donnell (1999) distinguished Group 1 from Group 2 based on colony growth rates, the widest regions of macroconidia, reactions to near-ultraviolet black-light-blue light, according to different septa numbers of macroconidia, and absence of perithecia and differentiated them by analyzing DNA sequences of the β-tubulin gene. The strains in Group 1, *Fusarium pseudograminearum* O'Donnell et T. Aoki (teleomorph: *Gibberella coronicola* O'Donnell et T. Aoki), grows faster on low nutrient culture media, and have a more uniform length of 3-septate conidia formed under the near-ultraviolet light or in complete darkness than Group 2, *Fusarium graminearum* (Schwabe) (teleomorph: *Gibberella zeae* (Schwein.) Petch). The macroconidia of Group 1 strains have the greatest width at their middle septate, while the macroconidia of Group 2 strains have the greatest width at the one-third to two-fifth distance from the apex, between two septa. The analysis of DNA sequences also

revealed a significant differentiation between these two groups (Aoki and O'Donnell, 1999).

O'Donnell et al. (2004) compared DNA sequences of 11 genes at six independent loci from *F. graminearum* (Group 1) isolates collected worldwide, and found that nine distinct species existed within the *F. graminearum* clade. They included *Fusarium austroamericanum* (lineage 1), distributed in South America, *F. meridionale* (lineage 2), distributed in South and Central America, South Africa, Australia, New Caledonia, Nepal, and Korea, *F. boothii* (lineage 3), found in South Africa, Mexico, Guatemala, Nepal, and Korea, *F. mesoamericanum* (lineage 4), from Central America and Pennsylvania, *F. acaciae-mearnsii* (lineage 5), found in Australia and South Africa, *F. asiaticum* (lineage 6), whose range is Asia and South America, *F. graminearum* (lineage 7), cosmopolitan; *F. cortaderiae* (lineage 8), found in South America and Oceania, and *F. brasilicum*, distributed in South America (O'Donnell et al., 2004).

1.2.2 Fusarium head blight disease symptoms and *Gibberella zeae* life cycle

Fusarium head blight (FHB) disease symptoms are very conspicuous before wheat heads mature and turn yellow in the field. A tan discolouration can first be seen at the base or apex of a floret. As the infection progresses, the lesions become purple-brown or brown with a bleached center. Under moist conditions, more water-soaked and darker olive green symptoms may be visible on the floret veins and interveinal areas (Atanasoff, 1920). Floret discolouration spreads up and down from the infection point on the spike, however the premature ripening phenomenon results from the dysfunction of the rachis vascular system. Symptoms are also indicated by chalky and pinkish mycelium on floret surfaces, and masses of macroconidia showing pink coloration on the surfaces of florets and glumes. Fusarium-damaged kernels (FDK)

(tombstone kernels) are shriveled and lightweight, and a dull grayish or pinkish color.If the infection occurs late in kernel development, the infected kernels will be the same size as the healthy ones (Bushnell et al., 2003).

It is important to understand how the fusarium pathogen survives in the natural environment. The life cycle of *Gibberella zeae* is well documented. This pathogen overwinters on wheat stubble as mycelium or perithecial initials (Trail and Common, 2000; Guenther and Trail, 2005). When spring and summer come and temperatures and precipitation increase, the perithecium initial develops and matures. Eight ascospores develop in each ascus within the perithecium. The mature perithecium emerges through the stomatal cavity of wheat stem and spike debris (Guenther and Trail, 2005). When enough moisture is present in the environment, the perithecium absorbs water, turgor pressure is built up in the asci, and mature ascospores are shot out (Kendrick, 2003). The ascospores are carried by wind, land on susceptible host materials and start infections. Wheat is most sensitive to FHB during the period from the beginning of flowering to the soft dough stage (approximately 14 days after the beginning of anthesis) (Sutton, 1982; Pugh et al., 1933; Groth et al., 1999; Wilcoxson et al., 1992). Susceptibilty to the disease drops prior to anthesis (Andersen, 1948). *Gibberella zeae* is homothalic (Goswami and Kistler, 2004). The fungus initiates its infection with its haploid hypae in the tissues of wheat heads, and then spreads throughout the heads and stems through the vascular bundles (Guenther and Trail, 2005). The first dikaryotic phase is initiated in the haploid hyphae behind the colonization front. The secondary haploid hyphae grow out of the first dikaryotic-phase section, and start to colonize the tissues in the stem epidermis. The secondary dikaryotic phase which forms behind the secondary haploid hyphae front will produce perithecium initials. After harvest of wheat in the fall, the fungus overwinters as

perithecium initials in the wheat stem and head debris (Guenther and Trail, 2005). In the next year, it will start another life cycle. *G. zeae* perithecia are produced and ascospores are discharged from spring to summer in Manitoba (Inch, et al, 2005) and Ontario (Paulitz, 1996; Fernando et al., 1997) in Canada.

1.2.3 Infection of wheat by *Fusarium*

1.2.3.1 Infection pathways of *Fusarium* on wheat spikes

Entry through stomates and space between lemma and palea

Kang and Buchenauer (2000) observed that a hypha of *F. culmorum* entered stomatal openings on the innner surface of wheat lemma. Ribichich et al. (2000) reported that the wheat spike infection by *F. graminearum* caused necrosis of chlorenchyma of the glume, lemma, and palea. This indicates that the thin walled and photosynthetically active chlorenchyma provided an entry point for this fungus. Pugh et al. (1933) found that chlorenchyma of infected glumes contained a number of hyphae. Ribichich et al. (2000) reported that the lemma had more chlorenchyma in apical than basal florets. Therefore, the stomates are one of the infection pathways for head blight pathogens.

Lewandowski and Bushnell (2001) studied the development of *Fusarium graminearum* on the floret surface of field-grown barley and found that the fungal mycelia on overlapping areas of the adaxial surfaces of lemma and palea extended into the floret when wheat plants were irrigated by a misting system in the field, and lesions appeared on the same position of the lemma and palea of the floret.

Florets provide an entry point for fungal spores blown by wind or splashed by rain when they open at anthesis. There are no studies reporting this possibility, however, Cherewick and Robinson (1958) reported that *Siteroptes graminum* mites could carry spores of *F. poae* into wheat florets.

Anthers

Pugh et al. (1933) reported that the exserted anthers could be attacked and colonized by *F. graminearum*, and offered a path for the fungus into the florets. Strange and Smith (1971) inoculated wheat spikelets both with partially exserted anthers and with no exserted anthers using macroconidia of *F. graminearum*, and observed that the spikelets with partially exserted anthers had chlorosis 48 hours after inoculation, whereas, the ones without exserted anthers showed no symptoms. They also found a close relationship between infection and presence of the exserted anthers, and posed a hypothesis that there were some secretions produced by the anther that stimulated hyphal growth. Robson et al. (1994) showed that betaine or choline produced by the anthers improved fungal growth. Ribichich et al. (2000) reported that wheat pollen could be colonized by *F. graminearum*, and played an important role in infection of the florets.

Penetration of the adaxial surfaces of lemma and palea

Kang and Buchenauer (2000) observed the penetration of the adaxial surfaces of the lemma and palea by *F. culmorum* using scanning electron microscope, and found that the hyphae on the surface of lemma created infection pegs which completely penetrated epidermal cell walls. They speculated that this penetration was performed through enzymatic degradation.

Wounded florets

Wounds on florets caused by insects, natural factors (hail, wind, and etc.) and artificial factors may provide the fungus with paths to the epidermis. Boshoff et al. (1996) reported that *F. graminearum* and *F. crookwellense* could penetrate the wheat lemma through wounds. Bushnell et al. (2000) investigated the development of *F. graminearum* on and in the detached lemma and palea of barley florets using a

transformed strain of the fungus containing a gene for green fluorescent protein, and found that the intracellular hyphae were present only in cells at four days after inoculation. However, Pugh et al. (1933) showed that the infection of the lemma surface by *Gilbberella zeae* progressed very slowly. This indicates that the entry and development in the lemma of a floret by the fungus needs a "sufficiently damaged wound".

1.2.3.2 Effects of weather conditions on inoculum production, spore dispersal and germination, and infection by *Fusarium graminearum*

Fusarium graminearum production is favored by warm and humid weather conditions. Tschanz et al. (1976) investigated perithecial production on sterilized carnation leaf discs on water agar, and found that the number of perithecia increased exponentially with an increase in temperature from 15 to 23°C, and reached a peak at 28°C. No perithecia were produced at 30 °C. Sutton (1982) showed that the optimal temperature range for *G. zeae* ascospore production was between 25 and 28°C. Macroconidia of *F. graminearum* are most abundantly produced at temperatures between 28 and 32°C (Andersen, 1948; Tschanz et al., 1976), and are not produced when temperatures are lower than 16°C or higher than 36°C (Tschanz et al., 1976).

F. graminearum macroconidia germinate rapidly. Andersen (1948) found that most *F. graminearum* macroconidia germinated within 3hr at 28-32°C, or within 6hr at 20-32°C. Beyer and Verreet (2005) found an interaction of temperature and relative humidity in *G. zeae* ascospore germination. Four hours were required for spore germination at 20°C and a relative humidity of 100%; 10 hours were needed at 14°C for the same relative humidity. The optimal temperature for *F. graminearum* infection is 24-28°C; the fungus cannot grow at temperatures below 4°C and above 36°C (Andersen, 1948; Sutton, 1982).

Sung and Cook (1981) reported that *G. zeae* perithecia were produced at an optimal water potential of –1.5MPa, and inhibited at –4.0MPa, which is consistent with the results in Suttons' study (Sutton, 1982). Dufault et al. (2006) found that moisture levels of –0.45 to –1.30MPa promoted perithecial production, and moisture levels of –2.36 to –4.02MPa limited their production. The interaction of moisture and temperature in perithecial production is significant (Dufault et al. (2006). Perithecial production requires higher moisture levels when temperature is lower, and less moisture when temperature is higher.

The roles of rainfall and moisture in *Fusarium* spore dispersal have been demonstrated (Sutton, 1982; Paulitz, 1996; Fernando et al., 2000; Trail et al., 2002; Inch et al., 2005). *G. zeae* ascospores are forcibly discharged from perithecia when turgor pressure is built up in asci (Trail et al., 2002; Kendrick, 2003). A drop of water can trigger *G. zeae* ascospore release (Gilbert and Tekauz, 2000). Indirect evidence that ascospores are discharged several hours or 1-2 days after rainfall events indicates the effect of rainfall and high relative humidity on the ascospore dispersal (Paulitz, 1996; Fernando et al., 2000; Inch et al., 2005). However, Gilbert and Tekauz (2000) reported that rainfall of >5mm and relative humidity of >80% inhibited ascospore release. Splash dispersal of *F. graminearum* macroconidia were also reported (Sutton 1982; Fernando et al., 2000).

Ultraviolet (UV) light is reported to be necessary for *G. zeae* perithecial formation (Tschanz, et al., 1976; Sutton, 1982). Tschanz showed that UV light of <390 nm was required for perithecial initiation; and UV light of 300-320nm was most effective for perithecial production. Inch and Gilbert (2003a) found that *G. zeae* ascospores were only formed in the perithecia on the soil surface, though Gilbert and Tekauz (2000) successfully developed perithecia in culture without UV light.

1.2.4 *Fusarium* mycotoxins and *Fusarium graminearum* chemotypes

1.2.4.1 Trichothecenes

Major types of trichothecenes

Mycotoxins are toxins produced by fungi that can cause diseases classified as mycotoxicoses when they are ingested (Mycotoxins. Website: http://www.nwhc.usgs.gov/publications/field_manual/chapter_37.pdf). The most commonly reported *Fusarium* mycotoxins are trichothecenes (De Nijs et al., 1996). Among these mycotoxins, trichothecenes are strongly associated with chronic and fatal toxicoses of humans and animals (Desjardins, 2006). Trichothecenes are sesquiterpenoid mycotoxins characterized by a tricyclic ring structure, a double bond at C-9, 10, and an epoxide at C-13. They include two groups differentiated according to the type of substitution at C-8. Type A trichothecenes have hydrogen, hydroxyl, or ester groups at C-8, such as T-2 and HT-2 toxins. Type B trichothecenes have a keto function at C-8, such as DON and nivalenol (NIV).

Trichothecenes are mainly produced by *F. graminearum*, *F. culmorum*, *F. poae*, *F. sporotrichioides*, and *F. equiseti*. Mirocha et al. (1989) reported that *F. graminearum* isolates collected in fields worldwide produced DON and its derivatives 3-acetyl-DON (3-ADON), 15-ADON, and zearalenone. Abramson et al. (1993) found that *F. graminearum* and *F. poae* isolates in culture produced DON, 15ADON, T-2 and HT-2 toxins and NIV. *Fusarium culmorum* isolates produced DON, 15ADON, NIV and T-2 toxin, *F. equiseti* isolates produced DON, 15ADON and T-2 toxin while *F. sporotrichioides* isolates produced DON, 15ADON, NIV, T-2 and HT-2 toxins. *F. graminearum, F. poae* and *F. sporotrichioides* produced a greater amount of trichothecene mycotoxins (Abramson et al., 1993).

The pathway of trichothecene biosynthesis was constructed based on experiments using *F. culmorum*, *F. sporotrichioides* and *F. graminearum* (McCormick, 2003; Desjardins, 2006). The biosnythesis of trichothecenes starts with farnesyl pyrophosphate. Farnesyl pyrophosphate is converted to trichodiene by synthase Tri5. Trichodiene is oxygenated to form 2-hydroxytrichodiene at the C-2 position by Tri4. 2-hydroxytrichodiene forms trichothecenes through a series of isomerizations, cyclizations and esterifications. There are many different derivatives produced during this process, such as 3-ADON, 15-ADON, and 3,15-diacetyl-DON. Several genes were charaterized in the trichothecene biosynthesis for *F. graminearum* and *F. sporotrichioides*, they are *Tri1* (C-8 monooxygenase), *Tri3* (15-O-acetyltransferase), *Tri4* (C-2 monooxygenase), *Tri5* (sesquiterpene cyclase), *Tri6* (transcriptional regulator), *Tri7* (4-O-acetyltransferase), *Tri8* (8-O-acetyltransferase), *Tri9* and *Tri10* (regulatory), *Tri11* (C-15-monooxygenase), and *Tri101* (3-O-acetyltransferase).

The role of DON in fungal infection

DON and NIV are the primary trichothecene metabolites found in *Fusarium*-infected wheat (Thrane, 2001). Most of the studies on the infection mechanisms of *Fusarium* causing FHB disease have addressed the effects of trichothecene mycotoxins on pathogenicity or virulence. Atanassov et al. (1994) demonstrated that DON and their derivatives impacted the virulence of *Fusarium*, however, they were not necessary for the infection and fungal growth in wheat grains. *Fusarium graminearum* and *F. culmorum* are two trichotheceneproducing fungi that are the most pathogenic species causing FHB disease (Wong et al. 1995). Different strains of these two species have different pathogenicities, however, the pathogenicities are positively correlated to the capabilities of the fungi to synthesize toxins (Atanassov et

al. 1994). Kang and Buchenauer (1999) inoculated wheat spikelets using *F. culmorum* and found that most of the DON was present in the vicinity of the invading mycelia at the early stage of infection. It then spread into the surrounding tissues, and passed through the xylem vessels and phloem sieve tubes to the neighboring spikelets. This indicates that DON can facilitate *Fusarium* invasion of host cells. Desjardins et al. (1996) reported that a *F. graminearum* mutant that did not synthesize trichodiene synthase, the first enzyme in the trichothecene synthesis pathway, caused less FHB disease severity than the wild type isolate, but still caused symptoms.

1.2.4.2 *Fusarium graminearum* chemotypes

Three strain-specific chemotypes of *F. graminearum* have been found, 3ADON chemotype that produces DON and 3ADON, 15ADON chemotype producing DON and 15ADON, and NIV chemotype producing NIV and its acetylated derivatives (Miller et al., 1991). These mycotoxns belong to the B-group trichothecenes. Ward et al. (2002) found that the differences of *F. graminearum* chemotype were not well correlated with its lineages based on the DNA analysis of six independent genes of this species, suggesting that each chemotype originated from its own ancestors.

In terms of pathogenicity and DON production of *F. graminearum* chemotypes, there are no significant differences in pathogenicity between 3ADON and 15ADON chemotypes based on an artificial-inoculation experiment in the greenhouse (Gilbert et al, 2006). However, the amount of DON produced by the 3ADON chemotype isolates is significantly greater than the 15ADON chemotype (Ward et al., 2005; Gilbert et al., 2006).

The 15ADON chemotype is predominant in North America, while the 3ADON chemotype is found in some areas in Asia, including China, and Australia and New Zealand (Mirocha et al. 1989). Recent molecular surveillance showed that the

3ADON chemotype was replacing the 15ADON chemotype from eastern to western Canada (Ward et al., 2005).

1.2.5 Effects of trichothecenes on animals and humans

The disease, alimentary toxic aleukia (ATA), caused by T-2 toxin mainly produced by *F. sporotrichioides* and *F. poae*, first broke out in Russia in 1932 (Beardall and Miller, 1994). The patients showed nausea, vomiting, diarrhea, and a reduction in blood cells. In some serious cases, severe skin rashes and necrotic lesions of the gastrointestinal tract were found. Experimental animals showed similar symptoms to humans in later studies (Beardall and Miller, 1994). The disease akakabi-byo first occurred in Japan in 1890. Patients experienced nausea, vomiting, diarrhea, headache, dizziness, and trembling (Beardall and Miller, 1994). Experimental animals showed similar symptoms. This disease is rarely fatal like ATA. In the 1970s, this disease was found to be associated with deoxynivalenol (DON) and nivalenol (NIV), which were isolated from blighted barley and wheat grain. Swine feed refusal was first found in the Central United States in 1928. In 1972, DON was isolated from contaminated maize used for animal feed (Beardall and Miller, 1994).

Studies on the potential effects of trichothecenes on humans were initiated with a large number of experiments on animals (Bennett and Klich, 2003; D'Mello et al., 1999; Feeding Fusarium-Contaminated Grain to Livestock, website: http://www.gov.mb.ca/agriculture/livestock/nutrition/bza00s01.html). DON causes feed refusal, reduction of weight gain of swine when the toxin concentration is 0.3-0.5 ppm in feed, and a reduction in feed intake resulting in severe weight loss when the concentration is greater than 1 ppm (D'Mello et al., 1999). Pigs fed DON-contaminated feed may also vomit (hence name vomitoxin associated with DON).

Poultry are more tolerant of DON than swine (D'Mello et al., 1999). The microorganisms of the rumen play an important role in detoxifying DON by reducing the epoxide ring.

For dairy cattle, DON is associated with reduced feed intake and reproduction (D'Mello et al., 1999). When DON concentration in feed is greater than 0.3 ppm, milk production is reduced. T-2 toxin is associated with feed refusal, reduction in milk production, intestinal hemorrhages and death. The immune system goes down in calves when T-2 toxin-contaminated feed is consumed. A level of 0.1 ppm of T-2 toxin in the total diet is the threshold for growing or lactating dairy animals.

Trichothecenes demonstrate a wide range of toxicity in animals. During the 1970s, trichothecenes were studied as anticancer agents in humans, because tumor cells were believed to require more protein than normal cells, and trichothecenes were considered to be inhibitors of protein synthesis (Desjardins, 2006). Following treatment with diacetoxyscirpenol, the patients experienced nausea, vomiting, diarrhea, fever, skin rash, hair loss and immunosuppression, symptoms similar to those of ATA and akakabi-byo.

1.2.6 Effects of *Fusarium* mycotoxins on wheat quality

Fusarium head blight disease is important because not only does it cause animal diseases and human illness, but also it damages wheat quality in terms of milling and baking processes. Wheat kernels are contaminated with trichothecenes as the result of *Fusarium* infection. The poor quality of the infected grain limits its usage and lowers its marketing value. The most important trichothecene is DON (Scott, 1990). Infection by *Fusarium* changes kernel composition, especially storage proteins and carbohydrates.

In the infection of developing wheat kernels *F. graminearum* destroys starch granules, storage proteins and cell walls, and spreads in the aleurone and pericarp tissues (Bechtel et al., 1985). If infection occurs at early stage of wheat kernel, the hyphae were observed throughout the endosperm (Bechtel et al., 1985). Nightingale et al. (1999) explained the poor mixing and processing characteristics associated with FHB disease by determining whether degradation of wheat storage proteins by proteolytic enzymes associated with FDK, found that proteolytic enzymes were present in FDK infected by both *F. graminearum* and *F. avenaceum*, and concluded that these enzymes hydrolysed endosperm proteins during dough mixing and fermentation, resulting in weaker dough and decreased loaf volume. Furthermore, DON shows an inhibition of protein synthesis (Casale and Hart, 1988), causes a low level of glutenins, which is the major reason of low baking quality.

The effect of *Fusarium* on wheat processing is reflected in the biochemical changes in wheat grains. Boyacioglu and Hettiarachchy (1995) determined the changes in biochemical components of wheat grains infected by *F. graminearum*, and found that reducing sugars and non-starch lipids increased by 24% and 5%, respectively; cellulose and hemicellulose decreased by 17% and 20% in the slightly infected wheat. Protein, total sugars, reducing sugars, non-starch and starch lipids increased by 6%, 26%, 14%, 20% and 8%; total amylose, cellulose and hemicellulose decreased by 11-20%, 43% and 37% in the moderately infected wheat. The infection decreased proportions of albumin and glutenin by 33% and 80%, respectively, in the moderately infected wheat. The reduction of glutenins lowers gluten quality. Nightingale et al. (1999) reported that *Fusarium* decreased the ratio of glutenins to gliadins, and the amount of high molecular weight glutenins was reduced. The study on infected wheat kernels by Bechtel et al. (1985) verified that large amount of starch

granules were degraded by enzymes and loss of storage proteins in the *Fusarium*-infected kernels.

Wheat mill streams range from flours to feeds. When *Fusarium* infected wheat is milled, the mycotoxins produced by the pathogen will remain in different end products in different proportions. Nowicki et al. (1988) investigated the retention of DON in samples of Canada Western Red Spring (CWRS) wheat of the variety Sinton and Canada Western Amber Durum (CWAD) wheat of the variety Coulter during processing and cooking of spaghetti and noodles, and revealed penetration of *F. graminearum* into the kernels. The highest mill fraction mould counts were found in the bran of Sinton and the shorts of Coulter. The amount of DON retained in Sinton flour and Coulter semolina averaged 29% and 52% respectively. DON concentrations were highest in the bran for both varieties. Distribution of DON in wheat mill fractions for flour and semolina milling of *Fusarium*-infected wheat depends on the degree of penetration of the infection into the endosperm (Nowicki, et al. 1988). Scott et al. (1983) showed that DON was distributed throughout the products of the flour milling process of Hard Red Spring wheat, a high concentration of DON was found in the dockage. Cleaning of wheat did not significantly reduce DON levels.

Seitz et al. (1986) evaluated the quality of flours from *Fusarium*-infected Hard Red Winter wheat using a bread-baking method, and found that there were no significant changes in DON levels from flour to bread, and the higher temperature in crust compared to crumb had little effect on DON concentration.

Flour refinement and yield are important factors of flour quality, which are affected by FDK (Tkachuk et al. 1991). Millers require high quality flours in terms of ash content and brightness. Tkachuk et al. (1991) used a specific gravity table to remove tombstone kernels (FDK) from Eastern White Winter Wheats and obtained

higher flour yield, lower ash content, and brighter color. Dexter et al. (1997) investigated the effect of FHB on semolina milling and pasta-making quality of durum wheat, and found that FDK had a negative impact on kernel weight, causing a lower semolina yield. FDK had a low proportion of glutenins, resulting in weaker gluten strength. FDK had a strong negative effect on pasta color.

Abbas et al. (1988) reported that lime water (2% $Ca(OH)_2$) reduced significantly the levels of zearalenone, and DON and destroyed 15ADON in the process of making tortillas from corn. It is unknown whether these reductions can be expected in wheat tortillas. Nowicki et al. (1988) showed that retention of DON in Chinese alkali noodles (42%) was lower than Japanese noodles (52%), suggesting that DON is not stable under alkaline conditions.

1.3 Disease control methods

1.3.1 Cropping practices

There have been studies and surveys on the effects of crop rotation and tillage on FHB disease. McMullen et al. (1997) stated that there was less FHB in fields in which crop rotation was employed in the United States in 1993. FHB disease was significantly reduced by wheat rotation with non-wheat and corn crops in experiments conducted in the University of Minnesota in 1994, North Dakota State University in 1996, and other places in 1929 and 1942, suggesting that crop rotation was an important key to reducting FHB disease. Parry et al. (1995) also concluded that crop rotation played an important role in controlling FHB disease. Rotation of wheat with non-host crops reduces the amount of infested wheat stubble (Sutton, 1982; Teich and Nelson, 1984; Windels and Kommedahl, 1984; Parry et al., 1995; Dill-Macky and Jones, 2000). However, Dill-Macky and Jones (2000), Gilbert and Tekauz (1994) and Miller et al. (1998) found no significant effects of crop rotation on the disease.

Schaafsma et al. (2001) detected no effect of crop rotation on DON level in wheat. Recently, canola stubble was found to be able to harbour *F. graminearum* (unpublished data). Therefore, the effect of crop rotation on FHB disease is unclear.

The importance of controlling FHB disease by tillage is significant (Miller et al., 1998). Moldboard ploughing significantly reduces FHB disease compared to chisel and zero tillage (Dill-Macky and Jones, 2000). In the soil, wheat stubble is decomposed quickly and only a small amount of dry matter stubble is left after 24 months (Parry et al., 1995; Pereyra et al., 2004). Cereal stubble burning in the previous year was found to lower FHB disease levels in the current year (Dill-Macky and Salas, 2002). Although no effect of tillage on FHB disease (Gilbert and Tekauz, 1994) and DON (Schaafsma et al., 2001) were found in some studies, these were due to the possibility that severe disease masked the effect of tillage (Gilbert and Tekauz, 1994). Corn stubble was a more effective inoculum reservoir than wheat stubble (Schaafsma et al., 2001).

1.3.2 Chemical control

Fungicides are a tool often used for disease control. It is believed that fungicides control not only diseases, but also reduce toxins to acceptable levels. There are two major funigicides, protectant (contact) fungicide that protects a plant from infection by a pathogen, and systemic fungicide that permeats plant's sap and inhibits or kills a pathogen from inside of the plant (Crop Protection, 2007). Fungicides are metabolic inhibitors and their modes of action can be classified into four broad groups: 1. Inhibitors of electron transport chain: respiration in mitochondria — azoxystrobin, kresoxim-methyl, pyraclostrobin, trifloxystrobin, 2. Inhibitors of enzymes — maneb, manzate, dithane, and chlorothalonil, 3. Inhibitors of nucleic acid metabolism and protein synthesis — thiophanate-methyl, mefenoxam, iprodione, and vinclozolin, 4.

Inhibitors of sterol synthesis: inhibit demethylation of ergosterol in fungi and therefore inhibit membrane structure and function — propiconazole, myclobutanil, tebuconazole, triflumazole, imazalil and dimethomorph (Crop Protection, 2007). Short lasting fungicides should be applied at the full flowering stage to kill and protect wheat heads from the pathogens, and can be sprayed more than one time according to fungicide longevity, whereas long lasting ones can be used at the early flowering stage. In Canada, Gilbert and Tekauz (1995) examined wheat seed treatments to protect wheat plants from infection by *Fusarium* spp.. BD-Green L, Dithane M-45, NM Drill Box, Vitaflo 280, and Vitavax S significantly improved seed germination and emergence (Gilbert and Tekauz, 1995). Foliar fungicides are widely applied. Propiconazole products (Tilt 125EC and 250EC) were found to be effective against FHB for susceptible wheat cultivars but not for moderately resistant cultivars (Gilbert and Tekauz, 2000). In the United States, Boyacioglu et al. (1992) found that triadimefon (Bayleton 25 WP) and propiconazole (Tilt products) fungicides significantly reduced *F. graminearum* infection and DON levels, and thiabendazole (Mertect) application resulted in the maximum reduction of DON levels. In Hungary, Mesterházy (2003b) revealed a high efficacy of tebuconazole in controlling FHB disease though the application of this fungicide did not produce a significant increase in wheat yield. Kászonyi et al. (2006. CIMMYT) reported control of disease and DON by prothioconazole fungicides. Gilbert and Tekauz (2000) stated that the application of azoxystrobin fungicide could probably result in an increase of DON levels. The fungicides used today appear either to control or have no effect on FHB disease, suggesting that efficacy of fungicides is related to the conditions under which they are applied, including the environment and the resistance of wheat cultivars.

1.3.3 Biological control

Biological control is an environmentally friendly means to control of pathogens by microbial agents used as part of an integrated disease management system. Fernando et al. (2002) screened three bacterial strains, *Bacillus subtilis* (Ehrenberg) Cohn strains H-08, S-01 and L-01 that were found to reduce FHB disease levels. Jochum and Yuen (2002) reported that *Lysobacter enzymogenes* strain C3 reduced FHB disease in one wheat cultivar through a heat-stable elicitor, which induced resistance of the cultivar. Luz et al. (2003) suggested that the strategies for control of FHB disease should be to isolate microbial strains from wheat anthers secreting the stimulatory nitrogenous compounds choline and betaine. Inch and Gilbert (2003b) investigated effects of *Trichoderma* species on perithecial formation on wheat stubble and revealed a significant inhibition effect by *T. harzianum*. The volatile compounds produced by bacteria (benzothiazole, cyclohexanol, and etc.) completely inhibit germination and formation of reproductive structure of *Sclerotinia sclerotiorum* (Lib.) de Bary, and are being tested against *Fusarium* species (Fernando, 2003). Currently there are a number of biological methods available for controlling FHB disease, including bacteria *Bacillus megaterium*, *Paenibacillus lentimorbus*, yeast *Crytococcus* spp. and *Sporobolomyces roseus* (Luz et al., 2003) Harsh environmental conditions can, however, limit the activities of these biological control agents, including levels of low moisture, high UV radiation and high temperature (Gilbert and Fernando, 2004).

1.3.4 Genetic control

Use of resistant cultivars saves costs of pesticide application and also reduces environmental pollution. Breeding for resistance to FHB in wheat is the most economical and effective means of reducing disease and DON levels. There are five types of resistance mechanisms, Type 1, resistance against initial infection; Type II,

resistance against pathogen spreading within an infected spike; Type III, resistance to kernel infection; Type IV, tolerance against the disease, meaning tolerant wheat cultivars can maintain their yields when the disease is present; Type V, resistance to toxins (Mesterházy, 2003a). Type II resistance is most commonly assessed.

The Suzhou Institute of Agriculture Science in Jiangsu Province in China developed a well-known spring wheat resistant cultivar, Sumai 3 by crossing two moderately susceptible cultivars in 1972 (Bai et al., 2003). Another resistant cultivar Ning 7840 was developed using Sumai 3 by Jiangsu Academy of Agricultural Science in the same province in China. These two cultivars are widely used as the sources of resistance to FHB disease. Other sources of resistance include Brazilian spring wheat cultivar "Frontana" and Japanese spring wheat cultivar "Nobeoka-bouzu" (Rudd et al., 2001); and European winter wheat cultivars Praag 8 and Novokrumka (Gilbert and Tekauz, 2000).

Wheat resistance to FHB disease is controlled by multiple genes (Bai et al., 2003). Three major genes along with several minor genes affect FHB disease resistance in Sumai 3 and Ning 7840 (Bai et al., 2003). Resistance of Frontana is controlled by two major genes with additive interaction of three minor genes (Ginkel et al., 1996). Resistance genes are located on several chromosomes. Sumai 3 has resistance genes on chromosomes 1B, 2A, 5A, 6D and 7D (Yu, 1990). Liao and Yu (1985) showed that resistance genes in the Chinese cultivar Wangshuibai were on chromosomes 4A, 5A, 7A and 4D. Yu (1990) found the resistance genes of Chinese cultivar PHJZM on chromosomes 6D, 7A, 3B, 5B and 6B. Different locations of resistance genes on chromosomes make it difficult to screen for resistant cultivars to FHB disease (Gilbert and Tekauz, 2000).

Canadian spring wheat breeding programs use resistance sources from the cultivars Sumai 3 and Ning 7840 and its derivatives, and winter wheat breeding programs use the resistance source cultivar Frontana (Gilbert and Tekauz, 2000). Durum wheat, which has no D chromosome, is susceptible to FHB disease. Attempts to cross Sumai 3 and durum wheat were not successful (Gilbert and Tekauz, 2000). The Canadian cultivar Neepawa, showing few symptoms under moderate disease pressure, was developed using resistance source from the cultivar Frontana; and the current CWRS cultivars were developed from the cultivar Neepawa, including AC Barrie, AC Cora and Katepwa (Gilbert and Tekauz, 2000). Two moderately resistant varieties, 5602 and Waskada were registered more recently (Seed Manitoba, 2007).

1.3.5 Prediction of fusarium head blight disease

There are some prediction models available for FHB disease and DON accumulation (De Wolf et al., 2003; Hooker et al., 2002; Moschini and Fortugno, 1996).

De Wolf et al. (2003) developed regression models for FHB disease epidemics based on the weather conditions in three different U.S. wheat-production regions (Ohio, North Dakota and Missouri) for a total of 50 location-years. Weather conditions, including the levels and duration of temperatures, relative humidity and rainfall, were collected from two periods, 7 days prior to anthesis and 10 days from anthesis of wheat. The most useful predictor variables were the duration of precipitation, relative humidity greater than 90% and temperatures of 15-30°C 7 days prior to anthesis. The prediction accuracy ranged from 62% to 85%. Hooker et al. (2002) developed multiple regression models for predicting DON concentration in winter wheat in Ontario, Canada. The duration of rainfall of >5mm and temperatures of <10°C were collected 4 to 7 days before heading for early-period prediction. The

duration of rainfall of >3mm and temperatures of >32°C were collected 3 to 6 days after heading for the middle-period prediction and duration of rainfall of >3mm was collected 7 to 10 days after heading for the late-period prediction. The accuracy of prediction was approximately 73%. Moschini and Fortugno (1996) developed two linear regression models for FHB disease incidence based on weather factors, including the number of two day periods with rainfall and relative humidity of >81% in the first day and relative humidity of >78% in the second, and days of temperatures of 9-26°C and relative humidity of >83%, all of which were collected from 50% heading stage to about 30 days after that. The prediction accuracy was more than 83%, however, the model was not feasible for application due to the long prediction period.

Several FHB disease forecasting systems were developed based on the relationship between disease occurrence and environmental conditions, including Manitoba Fusarium Head Blight Risk (website: http://www.gov.mb.ca/agriculture/crops/diseases/index.html), Fusarium head blight in Canada (website: http://www.cgc.ca/Pubs/fusarium/fusarium-e2.htm), U.S. Wheat and Barley Scab Initiative (website: http://www.scabusa.org), USDA Cereal Research Lab (website: http://www.crl.umn.edu/scab/scab.html), and forecasting systems developed by the North Dakota State University (website: http://www.ag.ndsu.nodak.edu/cropdisease/cropdisease.htm), the Ohio State University (website: http://www.oardc.ohio-state.edu/ohiofieldcropdisease), the South Dakota State University (website: http://plantsci.sdstate.edu/smallgrainspath/scab_advisory/index.html), and the University of Minnesota (website: http://mawg.cropdisease.com). These forecasting systems are used through a dialogue window on a computer, in which a user is asked

to enter location and wheat cultivars, and then the risk of the disease occurrence, risk map along with advice is provided.

The models developed so far to predict FHB and DON were mainly based on weather conditions and did not consider cropping practices and pathogen inoculum level in the development of prediction models. The latter two factors are important for disease epidemic, and were taken into account in this study.

CHAPTER 2

2.0 Development of Models to Predict *Fusarium graminearum* Spore Inoculum in Wheat Fields

Abstract

The objectives of this study were to investigate the effects of cropping practices and weather conditions on airborne fusarium inoculum levels on wheat heads, and to develop models to predict *Fusarium graminearum* spore counts on wheat heads. This study was conducted in 58 wheat producers' fields in Manitoba from 2003 to 2006. Two wheat cultivars, Superb, moderately susceptible, and AC Barrie intermediately resistant to fusarium head blight (FHB) disease were grown. Cropping practices were first quantified by assigning different scores to different crops, years, tillage methods, and wheat cultivars in resistance employed in the previous three years, and expressed as cropping practice index (CPI). CPI and cumulative rainfall during the following periods significantly affected the number of *Fusarium graminearum* spores on wheat heads (S): rainfall from seeding to jointing and anthesis, from jointing to anthesis, in the second and third weeks and in the four weeks prior to wheat anthesis, and rainfall in the first and second weeks after anthesis. Average daily temperature from seeding to anthesis and in the first week after anthesis significantly affected spore number. Four models were developed for the number of *F. graminearum* spores using a stepwise procedure. Model 6 would be used for predicting FHB index and DON level and was expressed as, $S = -26.32124 + 0.55326CPI - 5.67071T_{+2} + 6.64223T_{-1+1} + 0.02861RT_{+2}$, where T_{+2} was average daily temperature in the second week after anthesis, T_{-1+1} was average daily temperature from the first week before to the first week after anthesis, RT_{+2} was the interaction between cumulative rainfall and average daily temperature in the second week after anthesis. R^2 value was 0.66 ($P < 0.0001$). Prediction accuracy of Model 6 was 57% using the Jack-Knife full cross validation method. The differences between predicted and actual spore counts on artificial wheat heads (spore traps) were estimated using a field test in 2006, and ranged from 1 to 23.

The *F. graminearum* spore models will be used in the risk estimation of FHB disease epidemic, FHB disease index and in the prediction of deoxynivalenol (DON).

2.1 Introduction

Fusarium head blight (FHB) is one of the most important diseases in wheat. It is caused by *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) and other *Fusarium* species. Epidemics of FHB are sporadic worldwide (Fernando et al., 2000). Since the first description of FHB by Arthur in 1884 in the UK, this disease has been reported in North America and Europe (Andersen, 1948; Leonard and Bushnell, 2003). In Canada, this disease was detected from the 1940s to 1980s in the Maritime Provinces, Quebec, and Ontario (Sutton, 1982).

Fusarium pathogens that infect the wheat head cut off the translocation of photosynthetic metabolites from leaves to heads, resulting in shrunken kernels and reduced yield (Leonard and Bushnell, 2003). The pathogen also produces mycotoxins in grains, damaging wheat quality and causing human illness and animal feed refusal, weight loss, and poor sexual reproduction (Dexter and Nowicki, 2003). Wheat is most sensitive to FHB from the flowering to soft dough stages (Groth et al, 1999; Wilcoxson et al., 1992), and thus fusarium spores discharged during this period threaten wheat crops the most.

Gibberella zeae ascospores are the primary source of inoculum (Inch and Gilbert, 2003a; Sutton, 1982). The seasonal and diurnal patterns of *G. zeae* ascospores have been described in previous studies (Fernando et al., 2000; Inch et al, 2005; Paulitz, 1996). Ascospores are discharged from 1600 to 0900 hours with high moisture from the beginning to middle of July in Ontario (Fernando et al., 2000; Paulitz, 1996). Significant release of ascospores occurs from 1600 to 0400 hours from the beginning of July to the end of August in Manitoba (Inch et al, 2005). However, air-borne

fusarium inoculum and effects of weather conditions are not quantified in these patterns of spore release, though peaks of *G. zeae* ascospore release were observed after rainfall events. The spore traps used in the above studies were Burkard and rotorod from which the data collected could not indicate *F. graminearum* inoculum levels on wheat heads. Furthermore, they are driven by electrical power, which is not feasible for application of a disease forecast. Colony forming units (CFU) of *G. zeae* on wheat spikes were investigated from the flowering to soft dough stages (Francl et al, 1999), and this method can be used to develop accurate and reliable prediction models for the FHB epidemics. However it must be performed every day, making it difficult to be applied in practice. Also, no quantitative relationship was established among CFU, fusarium inoculum levels on wheat stubble and amount of rainfall in the study.

Weather conditions are critical for *G. zeae* inoculum formation and important for spore prediction. *Gibberella zeae* perithecia are formed from 15°C to 28°C with high relative humidity (Tschanz et al., 1976). In the field, perithecia initials form in wheat debris in the form of the second dikaryotic mycelium under favorable conditions after harvest in the fall (Trail, et al., 2005). Perithecia start their dormancy when the winter comes. In the spring and summer with temperature rising and precipitation increasing, perithecia develop and mature. Ascospores are discharged when enough turgor pressure is built up in the asci with moisture. The optimal temperature for ascospore formation is from 25°C to 28°C (Sutton, 1982). Ascospore release often occurs shortly (several hours or even 2-3 days) after rainfall events in the field (Fernando et al., 2000; Inch et al., 2005; Paulitz, 1996).

The prevalence of FHB worldwide has triggered significant efforts in disease management. Crop rotation and tillage have been deployed for several decades to

control disease including FHB. However, their effects on FHB are unclear. Wheat stubble, the major source of fusarium inoculum on the soil surface that is associated with epidemics of FHB disease (Sutton, 1982), can be reduced by crop rotation and tillage (Parry et al., 1995; Teich and Nelson, 1984). Although there have been no reports on the relationship between fusarium-infested wheat stubble and airborne inoculum at the flowering stage, appropriate cropping practices have been shown to reduce airborne inoculum for another host-pathogen system (Guo et al., 2005).

The objectives of this study were, 1). to understand the relationships between the number of *F. graminearum* spores on single wheat heads, rainfall and temperature during different periods of wheat growth, 2). to develop models to predict fusarium inoculum level on single wheat heads, from flowering to soft dough stages, as a function of cropping practices and weather conditions, and 3). to provide a model to predict FHB and DON levels.

2.2 Materials and methods

Wheat fields. The study experiments, consisted of 58 wheat fields, with 14, 18, 19 and 7 wheat fields in 2003, 2004, 2005 and 2006, respectively, in Manitoba, Canada (Fig. 1A and Table 1). In 2003, seven fields were sown to CWRS wheat cultivar Superb, moderately susceptible to FHB , and the other seven fields were sown to CWRS AC Barrie, intermediate in resistance to FHB disease (Seed Manitoba, 2006). In 2004, nine fields were sown to Superb and other nine fields were sown to AC Barrie. In 2005, ten fields were sown to Superb and nine fields were sown to AC Barrie. In 2006, three fields were sown to Superb and four fields were sown to AC Barrie. Prediction model were developed using the data collected from 2003 to 2005, and the models were validated in the field using the data collected from 2006. Because a model should be developed based on at least three-year data, and the data

sets from 2003, 2004 and 2005 were more than 2006, prediction models developed using the data from 2003 to 2005 were more reliable than other combinations.

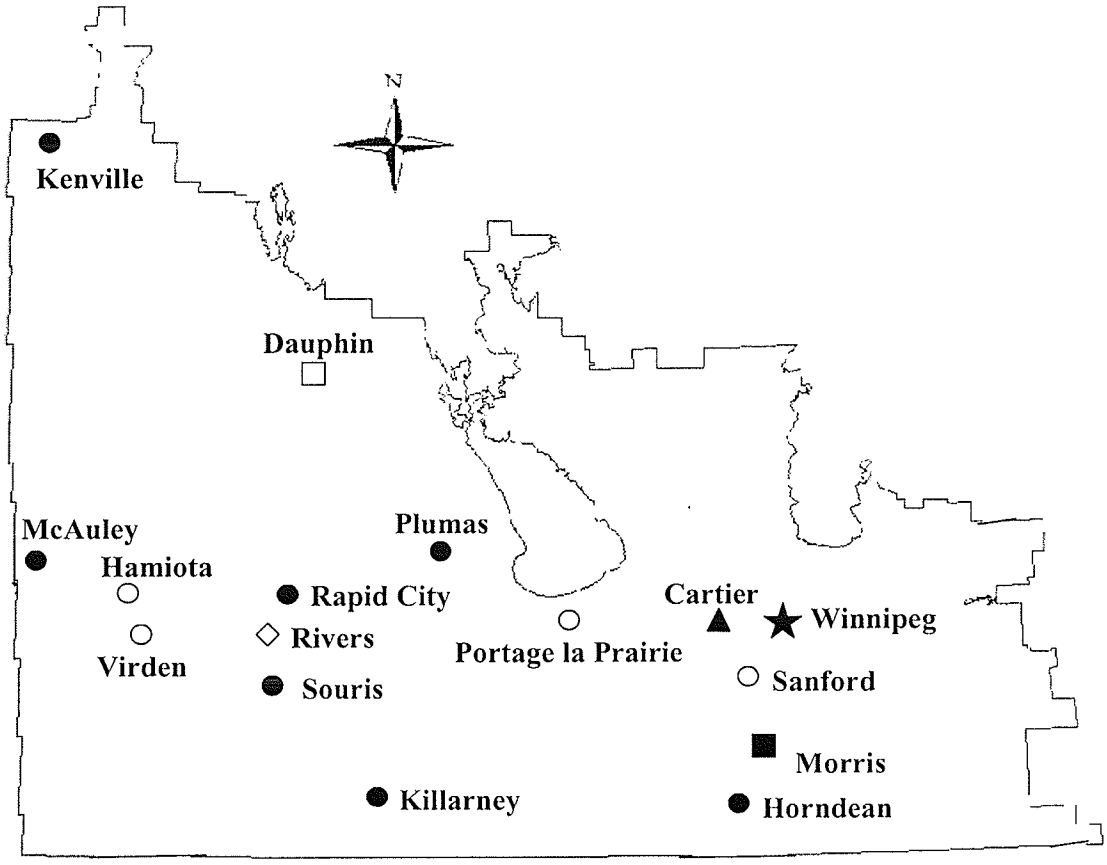


Figure 1A. A map of southern Manitoba. The symbol " ■ " represents the locations in which the experiment was conducted from 2003 to 2006; symbol " ● " represents the locations in which the experiment was conducted from 2003 to 2005; "○ " represents the locations where the experiment was conducted in 2004 and 2005; "△" indicates the locations in 2003, 2004 and 2006; " ▲ " indicates the locations in 2003 and 2005; "◇ " indicates the location in 2003, 2005 and 2006; and " □ " indicates the location in 2004.

Data collection. The information on crop rotation and tillage for each field were collected from the wheat growers. In each field, four sampling sites were lined up approximately 20 m apart. Each sampling site was 1.0m × 0.5m. Wheat stubble was collected from one half of each sampling site (0.5m × 0.5m) 7 days after seeding. Ten 1-m high spore traps were placed 20 cm apart in the other half of each sampling site at wheat anthesis and removed 14 days later (Fig. 1B). The spore trap, consisting of a trap head and a supporting rod, was designed to simulate deposition of spores on wheat heads in the field. The trap head (6mm × 6mm × 90mm) was a square plastic foam rod with four spore-collecting surfaces (6 mm × 90 mm each). Each surface was covered with Melinex tape, which was coated with a thin layer of petroleum jelly that spores would adhere to. After spore sampling, Melinex tape was removed from each face of the spore trap, cut into halves (6mm × 45mm each), and mounted on a glass slide. Total fusarium spores were counted under a compound microscope (400×).

Wheat stubble collected from each sampling site (Fig. 1C) was separated from other crop stubble, and dried at 25°C for 10 days in the drying room. The samples were weighed and 10% of stubble in weight was randomly selected, and cut into 5- to 20-mm pieces. The stubble was surface sterilized using 1% bleach for 1 min, air-dried on sterile filter paper, placed on potato dextrose agar (PDA) medium, incubated in light at 24°C, and observed for colony growth each day. The colonies were marked and numbered, then transferred to a fresh PDA medium to obtain single isolations for identification of *Fusarium* species. Identification was performed according to the standard keys described by Nelson et al. (1983).

Measurements of cumulative rainfall (R) and average daily temperature (T) were made in the following periods: the first week (R_{-1}; T_{-1}), second week (R_{-2}; T_{-2}), third week (R_{-3}; T_{-3}) and fourth week (R_{-4}; T_{-4}) prior to wheat anthesis, and the first week

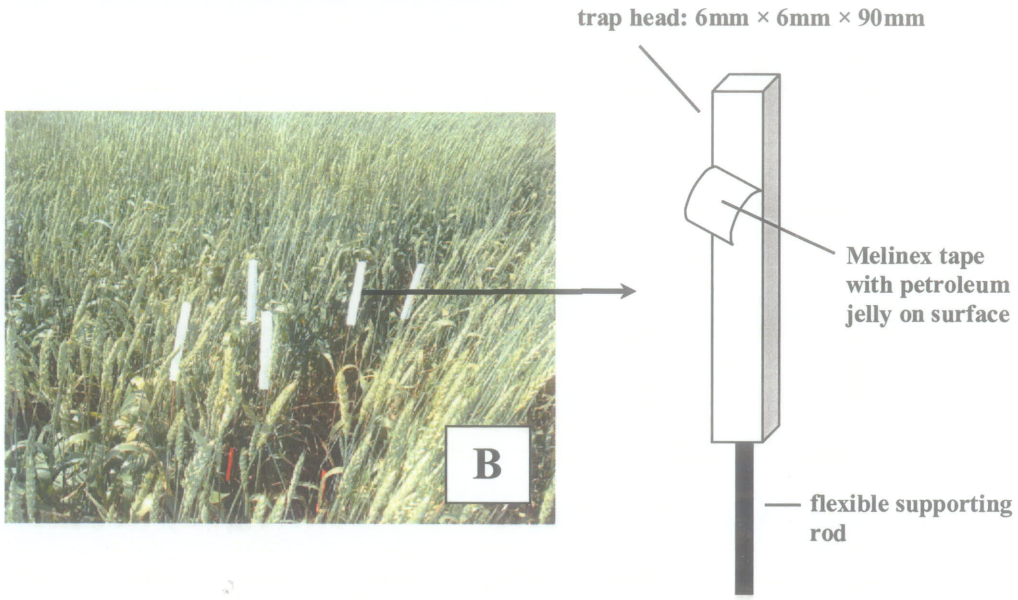


Figure 1B. A picture of spore traps in the field and a spore trap diagram.
Figure 1C. Wheat stubble was sampled from each of four sampling sties in each field. The sampling size was 0.5m × 0.5m.

(R_{+1}; T_{+1}) and second week (R_{+2}; T_{+2}) after wheat anthesis. R and T during different periods with a seven-day interval were computed based on the above individual periods, such as R during the period from the first to fourth week prior to anthesis (R_{-14}), R during the period from the first to second weeks after anthesis (R_{+12}), R during the period from the second week prior to anthesis to the first week after anthesis (R_{-2+1}), and so on. R and T were also measured from seeding to anthesis (R_{sa}; T_{sa}), from seeding to jointing stage (R_{sj}; T_{sj}) , and from jointing to anthesis stage (R_{ja}; T_{ja}).

Cumulative rainfall was measured using a standard rain gauge located next to each field with manual readings taken daily. The rain gauge had an inner cylinder, with 0.1mm increments, capable of collecting and measuring a maximum of 25.4mm of rainfall. The inner cylinder was positioned inside a larger, outer cylinder capable of catching excess rainfall exceeding 25.4mm during high volume rainfall events. The excess rainfall could then be measured using the incremental inner cylinder. Average daily temperatures were interpolated to each field site from neighboring Environment Canada weather stations by use of an inverse distance weighted method (Barnes, 1964).

Data analysis. To analyze the effects of cropping practices on fusarium inoculum level, cropping practices should be quantified. Chongo et al. (2001) reported that canola, field pea, bean and flax could be the hosts of *F. graminearum*. Guo (unpulished data) found that canola stubble had a greater capacity in peritheical production than bean, soybean and pea, and flax stubble had the lowest level in perithecial production. Therefore, cropping practices in the previous three years were assigned with scores to different crops, years, tillage methods, and the wheat cultivars' level of resistance according to capacities of the crops to be infected and

produce *G. zeae* perithecia on the crop stubble. Cropping practices was expressed as cropping practice index (CPI). For crop score, cereal crops and corn were assigned to 4, canola was 3, pea, soybean or bean was 2, and other crops were 1. For year score, three years before, two years before and one year before were assigned scores 1, 2 and 3, respectively. For tillage score, conventional and minimum or zero tillage were assigned 1 and 2 scales, respectively. For cultivar susceptibility to FHB, crops other than cereal crops and corn were assigned 1 scale; wheat resistant, intermediately resistant, moderately susceptible, and susceptible cultivars were assigned 2, 3, 4, and 5 scales. The interaction score of the four factors was expressed as multiplication of the four factors' scores, and designated as cropping practice index (CPI) and was derived based on the following formula:

$$CPI = \sum_{i}^{i=3} (\text{crop score}_i + \text{crop score}_i \times \text{year score}_i \times \text{tillage score}_i \times \text{susceptibility score}_i)$$

where i represented year number; crop score_i × year score_i × tillage score_i × susceptibility score_i represented the interaction score in year i. Therefore, CPI indicated similarity among different cropping practices. The closer the CPI values, the more similar the cropping practices. The number of *F.* graminearum spores on single wheat heads (S) in a field was estimated as total spores of this species trapped by all the spore traps on four collecting faces in this field divided by 40 (spore traps).

The relationships between dependent variable S and each independent variable were examined using the simple linear regression procedure in the SAS statistical program (Version 9.1). Multiple regression models were developed using a stepwise procedure. CPI and the cumulative rainfall and average daily temperature during the different periods were considered in the development of the multiple regression models. In the model that included correlated independent variables, the parameters of

these variables did not accurately indicate the effects of the corresponding variables on S due to the intercorrelations existing among these variables, which may affect the significance of parameter estimates for these variables, but not affect prediction accuracy of a model. The variance inflation factor (VIF) was used for evaluating multicollinearity, and expressed as $1/(1-R_i^2)$, where R_i^2 was the coefficient of determination for regression of the ith independent variable on all the other independent variables (Variance Inflation Factors:http://tce.die.uniroma1.it/personale/blasi/misurels/Dataplot/nist%20Refman/vif.htm). When VIF value was greater than 10, multicollinearity was significant, and the correlated variable should be removed. The number of sampling units should be at least ten times more than the number of predictors in a prediction model (Steel et al., 1997).

Model validation. The model to predict the number of spores was validated using the Jack-Knife full cross-validation method (Walker, 2007). For example, a prediction model M_n was developed using the data collected from n fields. To validate this model, these data were split into two parts, the first part included the data collected from "n-1" fields, and the second part included the data from the remaining field. The data from "n-1" fields were used for fitting a model $M_{(n-1)}$, which was developed using the same variables and considered as the model M_N. The data from the remaining field was for the validation of the model $M_{(n-1)}$. When this was completed for each of n fields, it provided a full cross validation data set, where for each field there was both an independent measured value and a predicted value based on the relationship determined independently from data for all the other fields. A linear regression model was developed and the accuracy of the models to predict S was estimated according to coefficient of determination (R^2) and slope values of this

linear model. When both R^2 and slope values were close to 1, prediction was more accurate.

The prediction models developed based on the data collected from 2003 to 2005 were also validated using the data collected from 2006.

2.3 Results

Effects of year, location, wheat cultivar and two-way interaction. ANOVA showed that year, location and their interaction had significant effects on the number of *F. graminearum* spores on single wheat heads from 2003 to 2005 (Table 2). However, it was not feasible to develop different models according to different year and locations. Therefore, the weather and spore data in the three years were pooled, and significance of year and location effects were not important when a relationship between *F. graminearum* spores and a weather condition during an individual period was significant.

The types of wheat cultivar showed no effect on the number of spores in the three years (Table 2). The number of spores formed and dispersed was affected by the wheat cultivars sown in the previous years, but not by the cultivars sown in the current year.

Relationships between the number of *F. graminearum* spores on artificial single wheat heads (spore traps), fusarium inoculum levels on wheat stubble and cropping practices. A higher CPI value indicated either a shorter crop rotation, less tillage, or more susceptible wheat cultivars were used in the previous three years. CPI significantly affected fusarium inoculum levels on wheat stubble (Fig. 2). The coefficient of determination (R^2) for the linear relationship was 0.69 ($P < 0.05$). With increase of cropping practice index, *F. graminearum* colonies per square meter increased for the three years and two cultivars, though there was great variation for

colonies/m^2 when CPI was approximately 40 and 80. These variations were likely caused by different weather conditions in the previous years.

Fusarium inoculum level on wheat stubble had a significant effect on the number of *F. graminearum* spores on spore traps with R^2 value of 0.46 ($P < 0.05$) (Fig. 3). There was a great variation in spore number at the point of 5000-5500 colonies/m^2, which may have resulted from differences in the weather conditions prior to wheat anthesis.

When the relationship between the number of spores on spore traps and CPI was examined, it was found that the R^2 value (0.40; $P < 0.05$) was lower than the above two relationships (Fig. 4). This may be caused by the variation in the colonies/m^2 and spore number. There was a great variation of the number of spores when CPI was 80, which was likely due to the variation of *F. graminearum* colonies and differences in weather conditions.

Thus, the results indicate that the more cereal crops, zero tillage methods and susceptible wheat cultivars were used in the previous years, the more fusarium inoculum on wheat stubble was accumulated on the soil surface.

Table 1. *Fusarium graminearum* spore counts, CPI, fusarium inoculum levels on wheat stubble and weather conditions in different fields in Manitoba from 2003 to 2006.

Field #	Year	Cultivar	S	CPI	C/m^2	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
1	2003	Superb	70	82	6213	8.4	41.6	52.6	86.0	10.2	21.0	20.1	17.8	18.7	17.7	17.4	18.4
2	2003	Superb	38	82	5894	56.0	96.0	105.0	105.0	0.0	7.0	18.2	17.1	16.8	17.1	18.5	19.4
3	2003	Superb	28	76	4968	0.0	4.0	8.9	8.9	2.2	2.2	17.3	18.5	17.3	17.7	19.7	20.4
4	2003	Superb	0	46	2145	24.6	32.4	61.8	106.6	1.7	5.8	16.1	17.8	18.0	17.3	19.0	18.8
5	2003	Superb	0	42	2145	5.4	5.4	13.4	24.6	4.8	4.8	16.3	17.9	16.8	17.7	19.6	19.9
6	2003	Superb	0	46	1894	5.6	18.2	18.4	62.4	5.8	7.8	18.2	17.4	18.0	16.9	16.8	18.5
7	2003	Superb	0	33	887	8.1	36.5	39.0	49.2	16.3	25.7	20.1	18.0	18.9	17.9	17.3	18.2
8	2004	Superb	84	82	6197	0.0	0.0	6.0	30.8	0.0	102.0	16.4	17.6	18.0	16.6	15.4	13.9
9	2004	Superb	74	82	6264	3.9	36.9	52.0	52.6	26.5	29.3	21.0	19.0	18.0	16.7	17.6	16.9
10	2004	Superb	66	82	5466	16.0	45.0	53.0	66.0	3.0	3.0	19.2	19.3	18.0	17.5	16.4	16.5
11	2004	Superb	70	65	2829	16.0	45.0	63.0	81.0	4.0	15.0	20.9	19.7	17.8	17.1	17.2	16.5
12	2004	Superb	36	68	3015	17.0	40.0	58.6	77.8	0.0	0.0	18.2	19.2	18.1	17.6	16.1	16.0
13	2004	Superb	55	50	2280	10.0	50.5	50.5	53.5	11.6	17.6	20.8	18.2	17.6	16.2	19.5	18.6
14	2004	Superb	33	56	1779	25.8	51.2	64.3	80.8	3.5	3.5	19.4	19.5	17.9	17.4	16.8	16.8
15	2004	Superb	38	34	1278	15.8	22.2	22.2	35.2	14.4	18.2	20.3	17.9	17.2	15.9	18.8	18.3
16	2004	Superb	23	31	966	0.0	20.2	30.0	40.8	2.2	4.8	18.7	17.2	16.9	15.3	17.8	17.5
17	2005	Superb	98	82	5388	48.8	155.0	210.4	212.6	22.0	25.0	23.9	20.5	19.4	19.8	19.7	18.5
18	2005	Superb	86	82	5001	21.4	97.0	100.0	123.1	39.6	39.6	18.8	16.7	18.5	18.0	22.7	20.2
19	2005	Superb	68	82	4778	39.4	116.8	116.8	175.3	38.1	38.1	18.6	16.6	18.2	17.7	22.6	20.0
20	2005	Superb	75	82	3019	64.0	164.5	171.0	180.5	29.5	36.5	21.7	19.7	19.7	19.7	21.8	20.3
21	2005	Superb	52	68	4583	82.6	159.9	209.8	214.2	26.3	28.7	23.5	20.5	18.8	19.3	18.0	17.2
22	2005	Superb	60	42	5460	106.6	173.3	173.3	216.0	10.6	20.8	18.9	16.9	17.9	17.7	21.9	19.7
23	2005	Superb	42	44	4828	9.4	20.2	73.8	91.6	0.0	0.0	16.9	18.4	19.9	19.1	20.0	19.6

Field #	Year	Cultivar	S	CPI	C/m^2	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
24	2005	Superb	28	34	2759	5.0	54.0	72.0	88.0	13.0	13.0	20.1	17.8	17.2	17.2	19.5	17.9
25	2005	Superb	34	23	1045	18.0	35.8	83.4	85.6	3.4	5.2	22.6	20.9	18.9	19.3	17.7	16.8
26	2005	Superb	20	54	2470	72.0	89.0	140.0	245.0	2.5	18.5	19.2	21.9	20.9	19.7	17.1	19.7
27	2006	Superb	11	46	no	10.6	1.2	12.8	10.2	1.8	1.8	19.4	19.7	17.8	19.1	22.2	21.2
28	2006	Superb	22	46	no	3.7	13.6	48.1	9.5	1.0	0.2	19.8	19.3	17.6	18.4	21.6	20.1
29	2006	Superb	14	75	no	1.9	32.2	28.6	8.9	1.0	1.0	19.1	18.8	17.1	18.3	21.0	19.7

S: actual *F. graminearum* spores on wheat heads.
C/m^2: *F. graminearum* colonies on wheat stubble/m^2.

Table 1 (continued). ***Fusarium graminearum*** **spore counts, CPI, fusarium inoculum levels on wheat stubble and weather conditions in different fields in Manitoba from 2003 to 2006.**

Field #	Year	Cultivar	S	CPI	C/m^2	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
30	2003	AC Barrie	68	82	5541	9.0	27.0	45.0	49.0	19.0	34.0	18.9	18.0	18.3	18.5	19.1	19.4
31	2003	AC Barrie	20	82	5541	1.8	3.8	10.7	10.7	2.2	2.2	19.5	17.9	18.0	17.8	20.8	20.7
32	2003	AC Barrie	20	75	4802	23.4	30.2	59.2	81.8	10.0	18.6	17.1	19.2	17.9	17.4	21.4	19.3
33	2003	AC Barrie	0	50	2011	80.2	80.2	80.2	80.2	0.0	23.0	18.7	18.5	19.0	17.9	17.3	18.7
34	2003	AC Barrie	15	48	1979	11.4	14.3	35.8	42.9	9.5	29.5	18.6	18.6	18.1	18.3	19.6	20.3
35	2003	AC Barrie	15	47	1788	11.4	21.6	29.2	38.1	2.5	2.5	18.6	17.8	17.3	17.5	19.4	20.1
36	2003	AC Barrie	0	34	1546	2.8	17.6	17.6	54.6	5.6	7.6	19.3	17.4	18.1	17.1	17.2	19.2
37	2004	AC Barrie	80	119	6749	3.9	36.9	52.0	52.6	26.5	29.3	21.0	19.0	18.0	16.7	17.6	16.9
38	2004	AC Barrie	62	94	5331	32.0	63.6	65.6	70.4	23.2	25.6	20.1	17.3	16.8	15.6	19.9	18.7
39	2004	AC Barrie	60	65	5858	0.2	22.2	22.2	35.2	14.4	21.6	19.8	17.6	17.2	15.8	18.7	18.1
40	2004	AC Barrie	52	76	4156	6.1	29.1	37.1	38.9	0.0	10.0	20.1	18.0	17.6	16.1	19.0	17.9
41	2004	AC Barrie	54	68	4387	48.5	50.5	50.5	59.5	9.0	18.0	21.0	18.1	17.3	16.0	19.6	18.6
42	2004	AC Barrie	42	42	2130	0.0	20.2	30.0	37.8	2.2	9.2	20.1	19.2	17.7	16.4	17.1	16.2
43	2004	AC Barrie	28	59	1763	25.8	51.2	64.3	80.8	3.5	3.5	19.4	19.5	17.9	17.4	16.8	16.8
44	2004	AC Barrie	112	82	4899	104.1	129.5	129.5	172.7	43.2	94.0	17.2	18.0	18.8	18.1	24.8	22.5
45	2004	AC Barrie	88	82	5231	46.2	161.6	163.8	195.6	20.8	42.8	17.9	17.0	18.6	17.9	24.1	21.4
46	2005	AC Barrie	54	82	4769	63.5	111.8	144.8	170.2	0.0	0.0	21.8	18.9	18.2	18.4	19.7	18.2
47	2005	AC Barrie	60	101	5372	21.8	124.6	128.6	132.0	29.8	30.6	20.2	18.3	18.7	18.6	22.3	20.3
48	2005	AC Barrie	48	42	2691	30.5	127.5	127.5	149.5	73.0	80.0	20.1	18.4	19.5	19.0	23.7	21.2
49	2005	AC Barrie	30	87	4846	88.1	159.9	212.4	237.6	26.3	27.1	22.1	19.2	18.6	18.8	19.9	18.4
50	2005	AC Barrie	34	79	4213	104.6	145.7	183.9	183.9	10.2	13.2	22.8	19.5	18.4	18.7	18.7	17.6
51	2005	AC Barrie	26	42	3347	21.8	31.6	105.4	133.4	3.6	5.0	20.0	21.5	19.9	19.2	16.9	18.6

Field #	Year	Cultivar	S	CPI	C/m^2	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
52	2005	AC Barrie	20	29	879	22.9	104.1	104.1	127.0	0.0	12.7	18.6	16.5	18.2	17.8	22.6	20.0
53	2005	AC Barrie	22	42	2262	14.2	72.8	77.8	103.2	24.8	27.8	21.0	18.2	18.1	18.3	20.2	18.8
54	2005	AC Barrie	16	59	2101	3.5	91.5	115.5	242.5	16.0	16.0	19.0	20.9	21.1	20.2	18.5	20.2
55	2006	AC Barrie	8	34	no	1.0	2.5	7.9	12.9	2.6	0.0	19.2	19.9	17.7	19.8	22.0	20.5
56	2006	AC Barrie	10	42	no	10.6	1.2	12.8	10.2	1.8	1.8	19.4	19.7	17.8	19.1	22.2	21.2
57	2006	AC Barrie	17	68	no	3.7	13.6	48.1	9.5	1.0	0.2	19.8	19.3	17.6	18.4	21.6	20.1
58	2006	AC Barrie	9	54	no	1.9	32.2	28.6	8.9	1.0	1.0	19.1	18.8	17.1	18.3	21.0	19.7

S: actual *F. graminearum* spores on wheat heads.
C/m^2: *F. graminearum* colonies on wheat stubble/m^2.

Table 2. ANOVA for year, cultivar and location effects on the number of *Fusarium graminearum* spores on single artificial wheat heads.

Source	Type III SS	Mean square	F value	Pr > F
Year	7195.01296	3597.50648	663.69	0.0001
Location	17449.55568	918.39767	169.43	0.0006
Cultivar	28.54176	28.54176	5.27	0.1055
Year*Location	6649.81630	474.98688	87.63	0.0018

Year*Location: interaction of year and location.

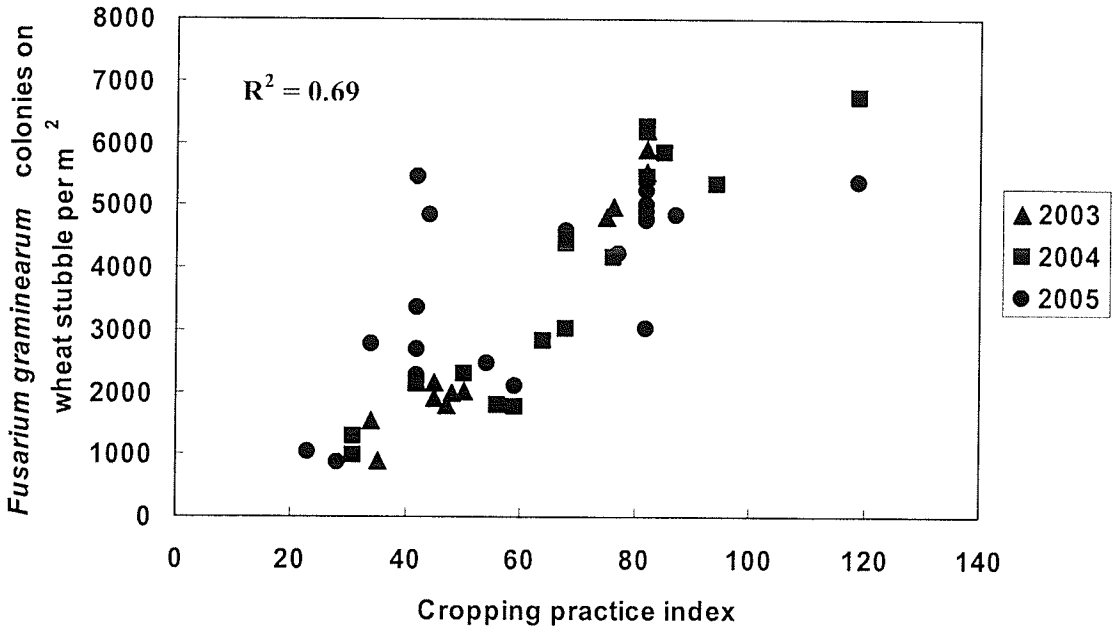


Figure 2. Relationship between the number of *Fusarium graminearum* colonies per m^2 on wheat stubble (colonies/m^2) and cropping practice index (CPI).

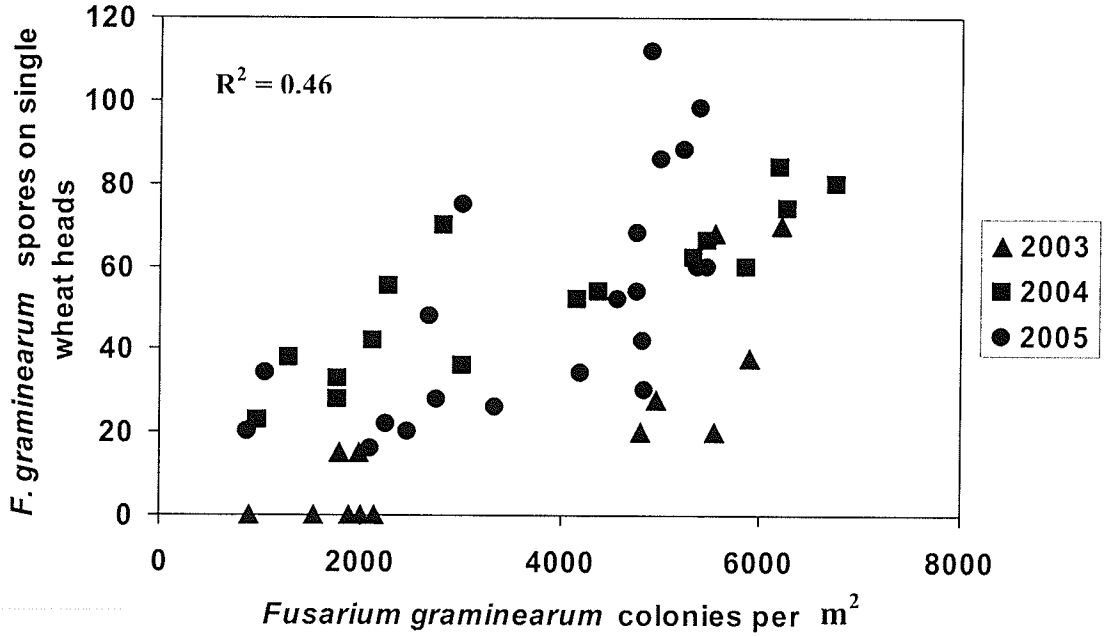


Figure 3. Relationship between *Fusarium graminearum* spores isolated from artificial wheat heads and *F. graminearum* colonies per m^2 on wheat stubble.

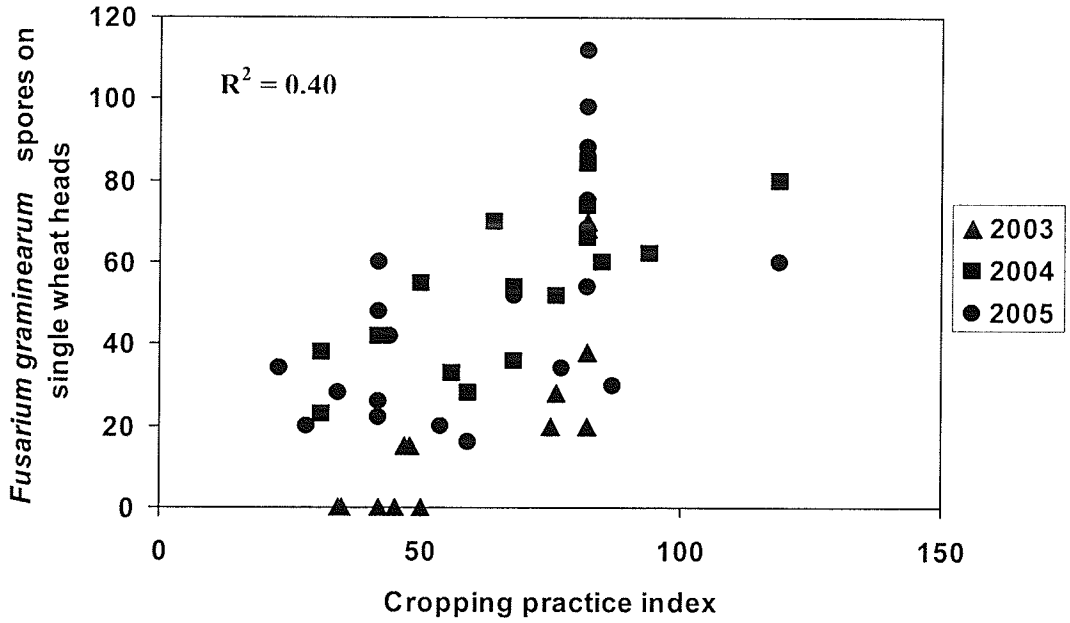


Figure 4. Relationship between *Fusarium graminearum* spores on single artificial wheat heads (S) and cropping practice index (CPI).

Effects of weather conditions during different periods on the number of *F. graminearum* spores on artificial single wheat heads (spore traps). Cumulative rainfall significantly ($P < 0.05$) affected the number of *F. graminearum* spores on spore traps during the following periods: from seeding to jointing and anthesis, from jointing to anthesis, in the four weeks, the third and second weeks prior to wheat anthesis; in the first, second and two weeks after anthesis; from the first week before to the first and second weeks after anthesis (Fig. 5).

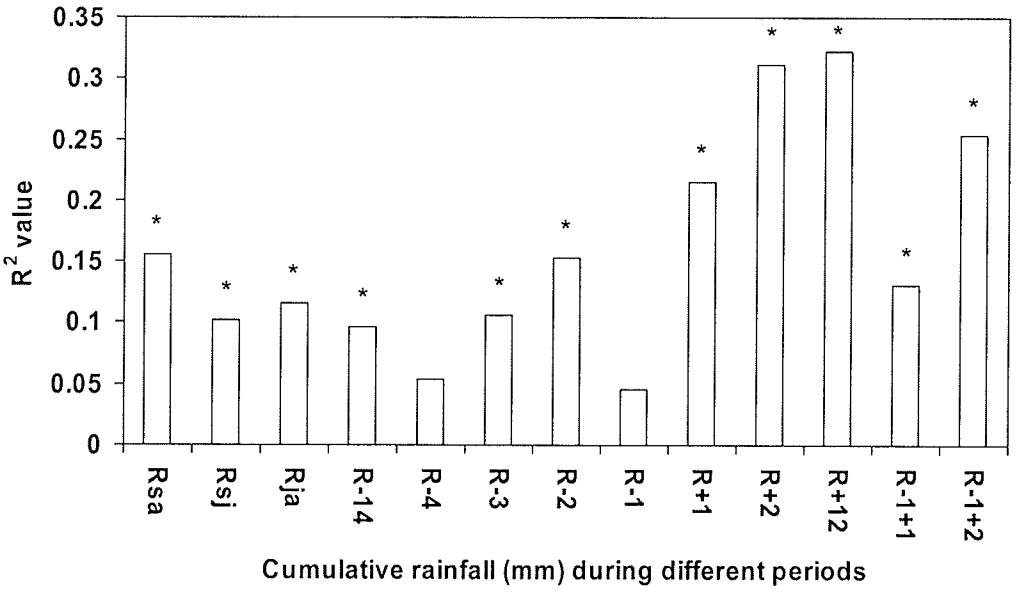


Figure 5. R^2 value for the relationships between *Fusarium graminearum* spores on single artificial wheat heads from anthesis to 14 days after and cumulative rainfall (mm) during different wheat growth periods from 2003 to 2005.
The stars on the top of bars represented significant relationship at 5% level.
R_{sa}: cumulative rainfall from seeding to anthesis.
R_{sj}: cumulative rainfall from seeding to jointing.
R_{ja}: cumulative rainfall from jointing to anthesis.
R_{-14}: cumulative rainfall in the 28 days prior to anthesis.
R_{-4}: cumulative rainfall in the fourth week prior to anthesis.
R_{-3}: cumulative rainfall in the third week prior to anthesis.
R_{-2}: cumulative rainfall in the second week prior to anthesis.
R_{-1}: cumulative rainfall in the first week prior to anthesis.
R_{+1}: cumulative rainfall in the first week after anthesis.
R_{+2}: cumulative rainfall in the second week after anthesis.
R_{+12}: cumulative rainfall in the 14 days after anthesis.
R_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis.
R_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

With increase of cumulative rainfall in the four weeks prior to anthesis, spore number increased, although there was a great variation in spore number in 2005 when cumulative rainfall ranged from 350 to 700 mm (Fig. 6). R^2 value was 0.10 ($P < 0.05$). The variation in this period may have resulted from the variation in the second (R^2=0.15; $P < 0.05$) and third weeks (R^2=0.11; $P < 0.05$) prior to anthesis (Fig. 5, 7

and 8), which were likely caused by effects of cropping practices and weather conditions in other periods. This indicates that cumulative rainfall in the third and second weeks and in turn in the four weeks prior to anthesis plays an important role in spore dispersal.

Cumulative rainfall from jointing to anthesis overlapped with cumulative rainfall in the four weeks prior to anthesis, and significantly affected spore number (Fig. 5). Effect of cumulative rainfall from seeding to anthesis resulted from the effects of rainfall from seeding to jointing and from jointing to anthesis on spore number (Fig. 5). Both periods exhibited significant cumulative rainfall from seeding to jointing and anthesis and promoted spore dispersal.

Cumulative rainfall in the 14 days after anthesis significantly affected the spore number more than rainfall during the other periods (Fig. 5 and 11). With increase of cumulative rainfall during this period, spore number exhibited an increasing trend (R^2=0.33). The greater amount of rainfall in 2005 contributed to the greater number of spores than in either 2003 or 2004 (Fig. 11). The relationship between spore number and rainfall during this period resulted from the relationships between spore number and rainfall in the first and second weeks after anthesis, which had similar trends (Fig. 5, 9 and 10).

Although there were significant relationships between spore number and cumulative rainfall from the first week before to the first week after (R^2=0.13) and to the second week after (R^2=0.25) anthesis, no relationship was found between spore number and cumulative rainfall in the first week before anthesis (Fig. 5, 12 and 13). Therefore, the effects of rainfall during the two former periods were contributed to the effects of the rainfall in the first and second weeks after anthesis on spore counts.

The average daily temperature during the periods from seeding to anthesis, the first week after anthesis, and from the first week before to the first and second weeks after anthesis significantly affected spore number (Fig. 14). The significant effects of temperature during the two later periods on spore numbers were due to the effect of temperature in the first week after anthesis. There was a similar increasing trend of spore number with temperature from the first week before to the first week after anthesis and from the first week before to second week after anthesis (Fig. 15 and 16).

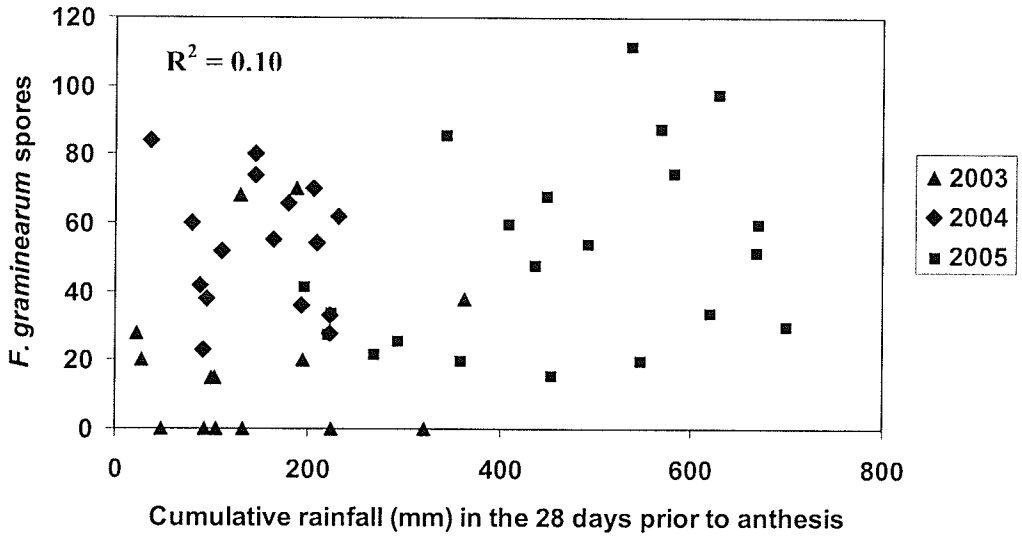


Figure 6. Relationship between cumulative rainfall (mm) in the 28 days prior to anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

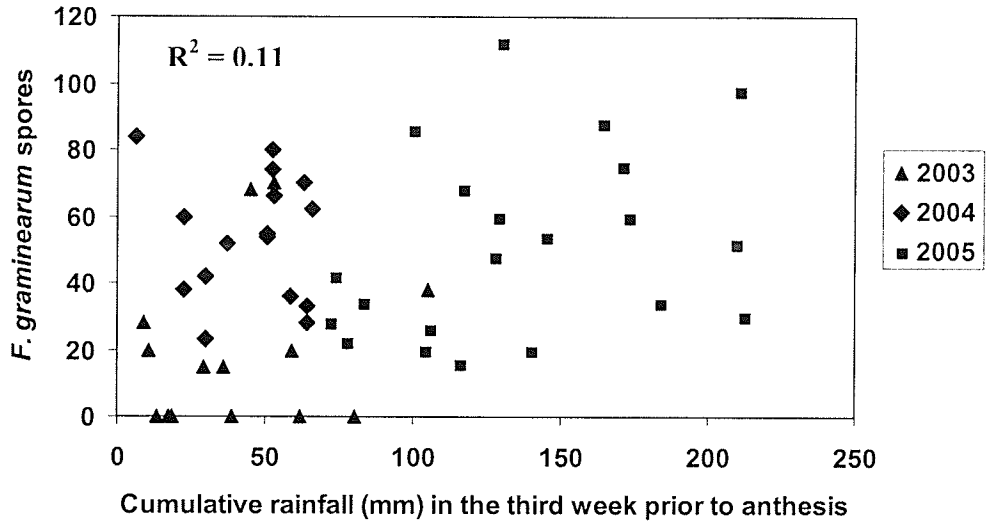


Figure 7. Relationship between cumulative rainfall (mm) in the third week prior to anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

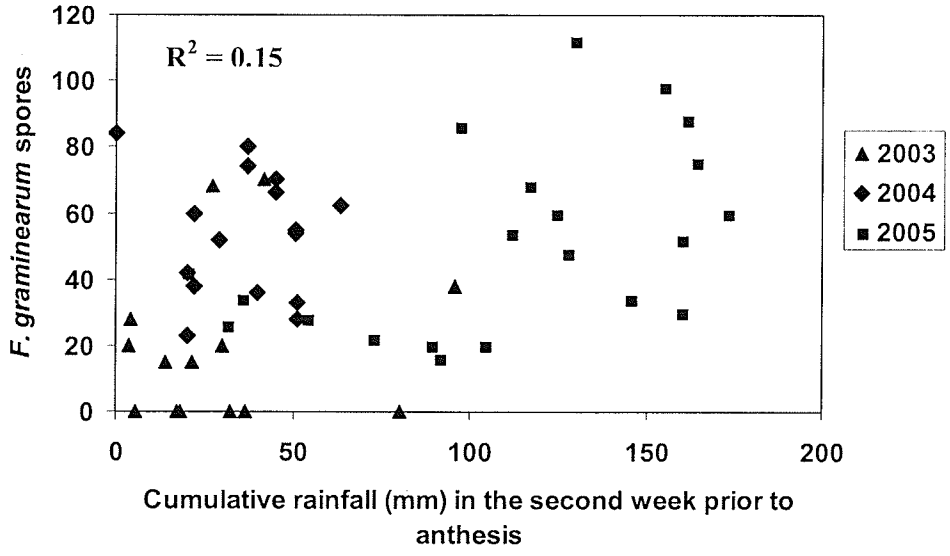


Figure 8. Relationship between cumulative rainfall (mm) in the second week prior to anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

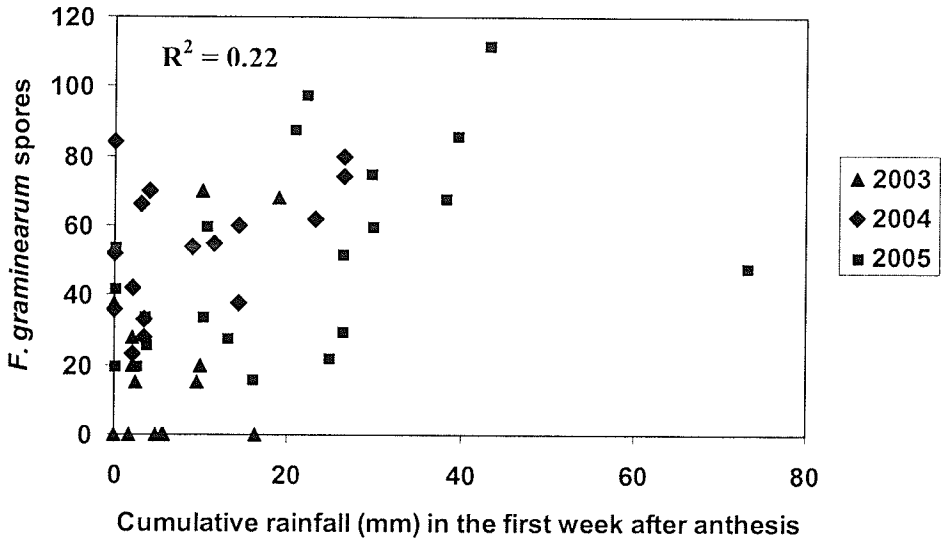


Figure 9. Relationship between cumulative rainfall (mm) in the first week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

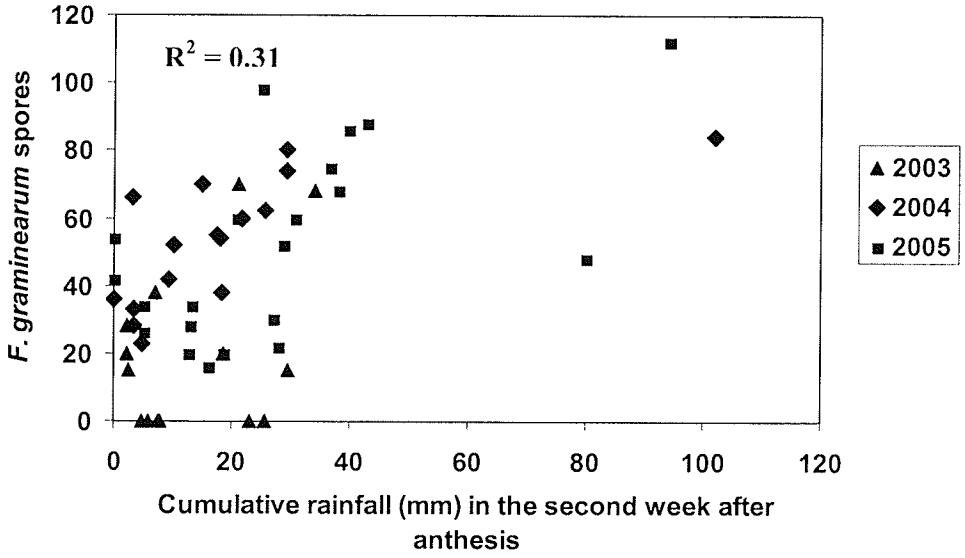


Figure 10. Relationship between cumulative rainfall (mm) in the second week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

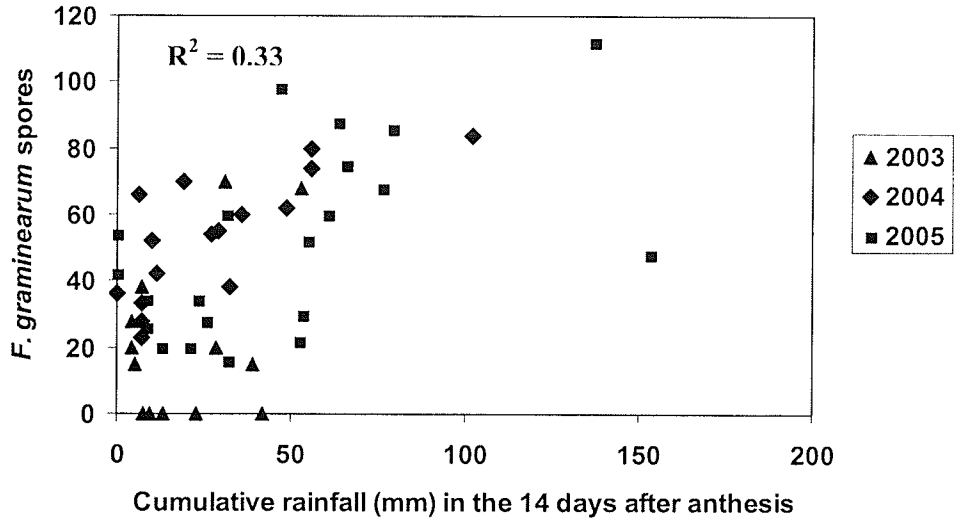


Figure 11. Relationship between cumulative rainfall (mm) in the second week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

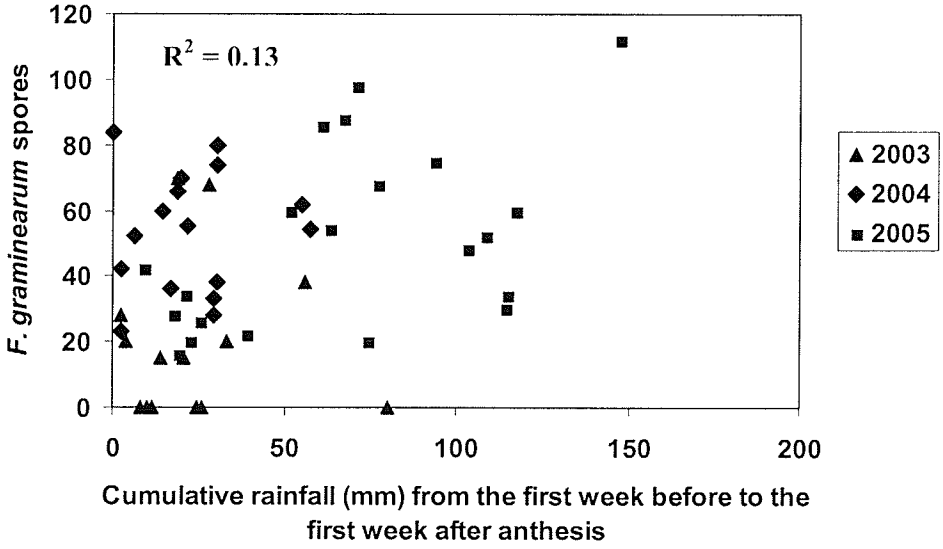


Figure 12. Relationship between cumulative rainfall (mm) from the first week before to the first week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

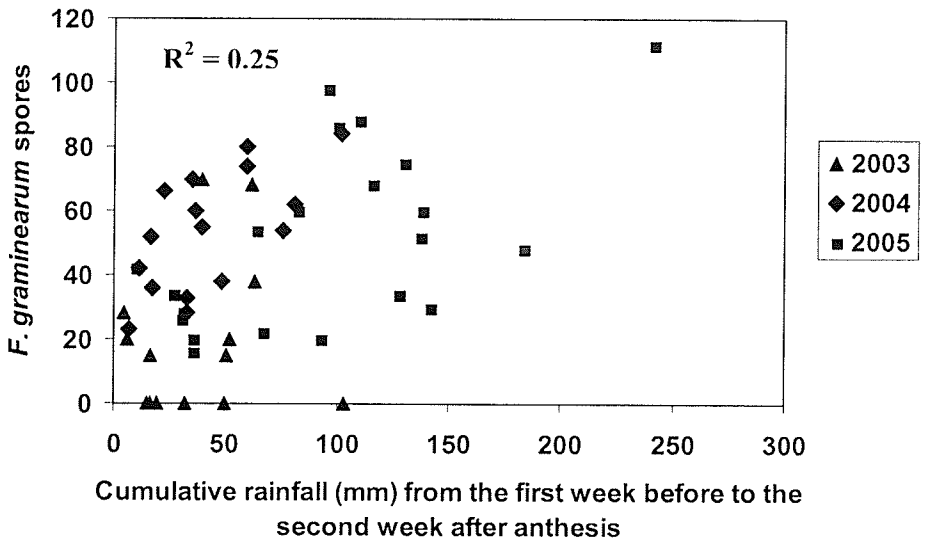


Figure 13. Relationship between cumulative rainfall (mm) from the first week before to the second week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

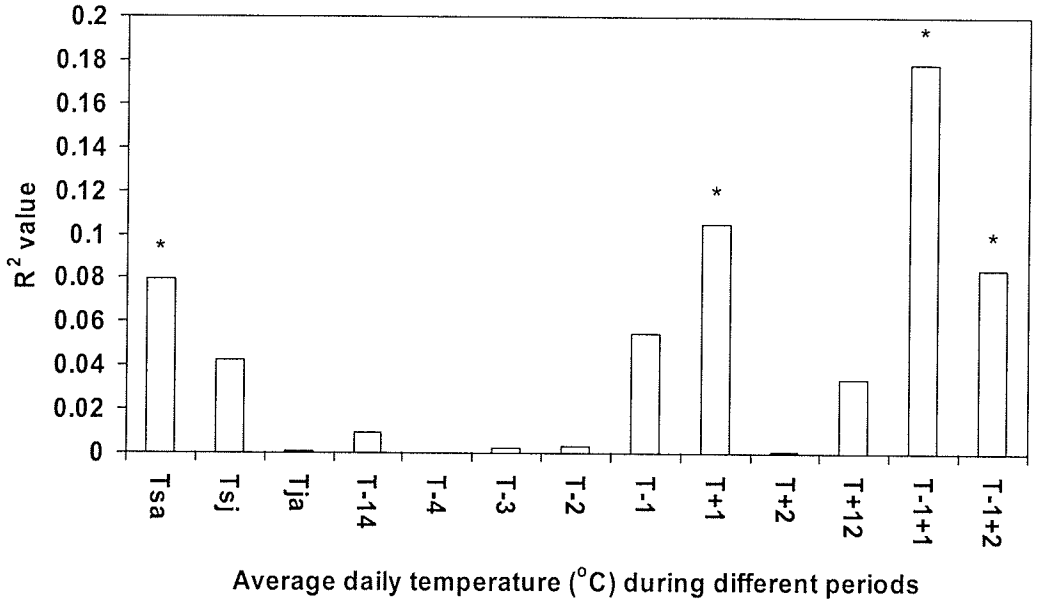


Figure 14. R^2 value for the relationships between *Fusarium graminearum* spores on the artificial wheat heads (spore traps) from anthesis to two weeks after and average daily temperature (°C) during different periods from 2003 to 2005.
The stars on the top of bars represented significant relationship at 5% level.
T_{sa}: cumulative rainfall from seeding to anthesis.
T_{sj}: cumulative rainfall from seeding to jointing.
T_{ja}: cumulative rainfall from jointing to anthesis.
T_{-14}: cumulative rainfall in the 28 days prior to anthesis.
T_{-4}: cumulative rainfall in the fourth week prior to anthesis.
T_{-3}: cumulative rainfall in the third week prior to anthesis.
T_{-2}: cumulative rainfall in the second week prior to anthesis.
T_{-1}: cumulative rainfall in the first week prior to anthesis.
T_{+1}: cumulative rainfall in the first week after anthesis.
T_{+2}: cumulative rainfall in the second week after anthesis.
T_{+12}: cumulative rainfall in the 14 days after anthesis.
T_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis.
T_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

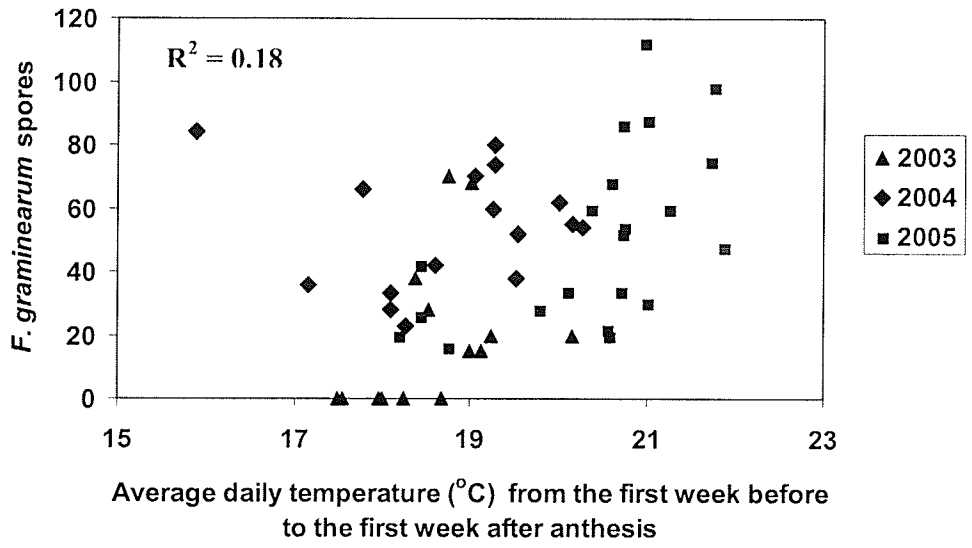


Figure 15. Relationship between average daily temperature (°C) from the first week before to the first week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

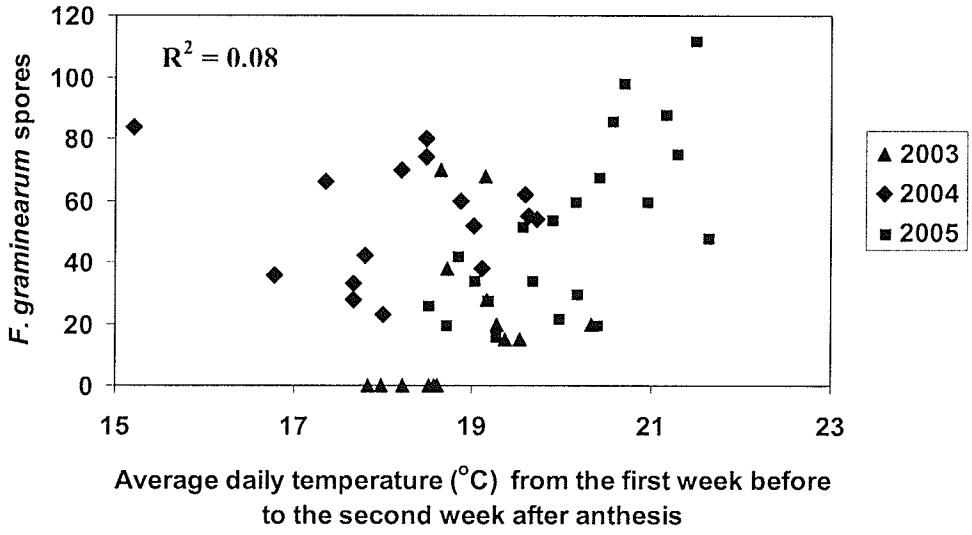


Figure 16. Relationship between average daily temperature (°C) from the first week before to the second week after anthesis and *Fusarium graminearum* spore counts on the artificial wheat heads (spore traps).

Development of models to predict the number of *F. graminearum* spores on single wheat heads. Variables CPI, R_{-14}, R_{-4}, R_{-3}, R_{-2}, R_{-1}, R_{-+1}, R_{+2}, R_{+12}, R_{-1+1}, R_{-1+2}, R_{-2+1}, R_{-2+2}, R_{-3+1}, R_{-3+2}, R_{-4+1}, R_{-4+2}, R_{-1+2}, R_{12}, R_{13}, R_{14}, R_{23}, R_{24} and R_{34}, and T_{-14}, T_{-4}, T_{-3}, T_{-2}, T_{-1}, T_{-+1}, T_{+2}, T_{+12}, T_{-1+1}, T_{-1+2}, T_{-2+1}, T_{-2+2}, T_{-3+1}, T_{-3+2}, T_{-4+1}, T_{-4+2}, T_{-1+2}, T_{-12}, T_{-13}, T_{-14}, T_{-23}, T_{-24} and T_{-34}, and interactions between cumulative rainfall and average daily temperature (RT) during the same periods were selected for development of prediction models using a stepwise procedure. R_{sa}, R_{sj}, R_{ja}, T_{sa}, T_{sj} and T_{ja} were not selected because difference in seeding and jointing dates would lead to prediction errors in the model application. There were in total 12 multiple regression models obtained (Table 3). The independent variables in the Models 1, 2, 3, 6, 9 and 11 were significant according to statistical analysis, and the variables in the other models were not. In Model 9, VIF values for variables R_{+12}, RT_{+2}, RT_{-1}, RT_{-1+2} were greater than 10, multicolinearity of these variables with other variables in the model was significant. In Model 11, VIF values for variables R_{+12} and RT_{-1} were greater than 10. Therefore, Models 5, 7, 8, 9, 10 and 11 were not acceptable. Models 1 was simplest, including one variable CPI however its R^2 value was 0.38. Models 2 and 3 included two and three independent variables, and their R^2 values were 0.56 and 0.60, respectively, therefore they could be used for predicting *F. graminearum* spore counts when model simplicity is interest. Model 6 included four variables with R^2 value of 0.66, it could be used for both predicting spore counts and FHB and DON levels.

Model validation. Models 6 was validated based on the Jack-Knife full cross validation method. The predicted S explained 57.3 % of the variation in the actual data, and its slope was close to 0.62 (Fig. 17). Models 6 was also tested by comparing their predicted data for 2006 with the corresponding actual data collected from the

same year (Table 4). When a predicted data value was zero or minus, it would be considered zero. The difference greater than 10 between predicted and actual spore counts was found in one field sown to Superb and two fields sown to AC Barrie.

Table 3. Prediction models for *F. graminearum* spore counts (S) developed using a stepwise procedure.

Model #	Items	Models: S =					
1	Variable	-6.31540	0.77750CPI				
	$P > F$	0.50	<0.0001				
	R^2	0.40 (<0.0001)					
2	Variable	-8.78498	0.63964CPI	$0.02813RT_{+2}$			
	$P > F$	0.28	<0.0001	0.0001			
	VIF		1.08	1.08			
	R^2	0.56 (<0.0001)					
3	Variable	-69.76193	0.59470CPI	$3.22414T_{-1}$	$0.02998RT_{+2}$		
	$P > F$	0.02	<0.0001	0.036	<0.0001		
	VIF		1.11	1.04	1.10		
	R^2	0.60 (<0.0001)					
4	Variable	-10.11494	0.58933CPI	$2.67112T_{-1}$	$-2.66359T_{+2}$	$0.03319RT_{+2}$	
	$P > F$	0.84	<0.0001	0.086	0.13	<0.0001	
	R^2	0.62 (<0.0001)					
5	Variable	4.88081	0.54855CPI	$-3.88940T_{-1}$	$-8.48014T_{+2}$	$11.7622T_{-1+1}$	$0.02468RT_{+2}$
	$P > F$	0.91	<0.0001	0.17	0.003	0.008	0.001
	R^2	0.67 (<0.0001)					
6	Variable	-26.32124	0.55326CPI	$-5.67071T_{+2}$	$6.64223T_{-1+1}$	$0.02861RT_{+2}$	
	$P > F$	0.52	<0.0001	0.002	0.004	<0.0001	
	VIF		1.13	1.38	1.47	1.26	
	R^2	0.66 (<0.0001)					
7	Variable	-47.84942	0.58183CPI	$-6.79306T_{+2}$	$-0.60907R_{+12}$	$8.88710T_{-1+1}$	$0.07353RT_{-2}$
	$P > F$	0.25	<0.0001	0.001	0.067	0.001	0.005
	R^2	0.68 (<0.0001)					

Table 3 (continued). Prediction models for *F. graminearum* spore counts (S) developed using a stepwise procedure.

Model #	Items	Models: S =								
8	Variable	-82.69094	0.60230CPI	-7.44480T_{+2}	-0.82593R_{+12}	11.5169T_{-1+1}	-0.0082RT_{-1}	0.09063RT_{+2}		
	$P > F$	0.007	<0.0001	0.0002	0.019	0.0002	0.075	0.001		
	R^2	0.71 (<0.0001)								
9	Variable	-7.86177	0.62914CPI	-10.24524T_{+2}	-1.62946R_{+12}	10.2884T_{-1+1}	-0.05725RT_{-1}	0.06743RT_{+2}	0.0522RT_{-1+2}	
	$P > F$	0.89	<0.0001	<0.0001	0.002	0.001	0.016	0.017	0.035	
	R^2	0.74 (<0.0001)								
10	Variable	-33.06384	0.60160CPI	-9.60069T_{+2}	-1.03192R_{+12}	11.0094T_{-1+1}	-0.11573RT_{-1}	-0.0826RT_{+1}	-0.03519RT_{+2}	0.1148RT_{-1+2}
	$P > F$	0.55	<0.0001	<0.0001	0.092	0.0002	0.008	0.100	0.600	0.013
	R^2	0.75 (<0.0001)								
11	Variable	-31.07877	0.61067CPI	-9.81620T_{+2}	-1.23408R_{+12}	11.1148T_{-1+1}	-0.09732RT_{-1}	-0.05875RT_{+1}	0.0948RT_{-1+2}	
	$P > F$	0.57	<0.0001	<0.0001	0.010	0.0002	<0.0001	0.004	0.001	
	R^2	0.75 (<0.0001)								
12	Variable	-8.28232	0.61046CPI	-10.06120T_{+2}	-1.34978R_{+12}	9.91996T_{-1+1}	-0.11294RT_{-1}	0.00681RT_{-3}	-0.06621RT_{+1}	0.1026RT_{-1+2}
	$P > F$	0.88	<0.0001	<0.0001	0.005	0.001	<0.0001	0.087	0.001	<0.0001
	R^2	0.77 (<0.0001)								

R_{-14}: cumulative rainfall in the 28 days prior to anthesis. R_{-4}: cumulative rainfall in the fourth week prior to anthesis. R_{-3}: cumulative rainfall in the third week prior to anthesis. R_{-2}: cumulative rainfall in the second week prior to anthesis. R_{-1}: cumulative rainfall in the first week prior to anthesis. R_{+1}: cumulative rainfall in the first week after anthesis. R_{+2}: cumulative rainfall in the second week after anthesis. R_{+12}: cumulative rainfall in the 14 days after anthesis. R_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis. R_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

T_{-14}: average daily temperature in the 28 days prior to anthesis. T_{-4}: average daily temperature in the fourth week prior to anthesis. T_{-3}: average daily temperature in the third week prior to anthesis. T_{-2}: average daily temperature in the second week prior to anthesis. T_{-1}: average daily temperature in the first week prior to anthesis. T_{+1}: average daily temperature in the first week after anthesis. T_{+2}: average daily temperature in the

second week after anthesis. $T_{+12:}$ average daily temperature in the 14 days after anthesis. $T_{-1+1:}$ average daily temperature from the first week before to the first week after anthesis. $T_{-1+2:}$ average daily temperature from the first week before to the second week after anthesis.
RT: interaction between cumulative rainfall and average daily temperature.
The models in table 3 were selected from all steps of the stepwise procedure until no further improvement in R^2 value was possible.

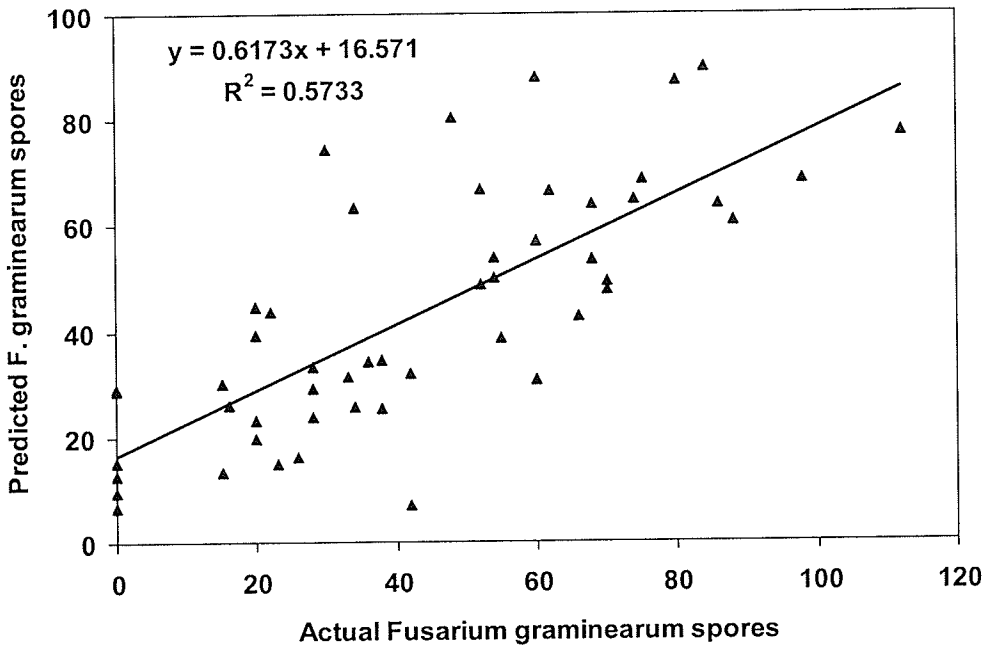


Figure 17. Jack-Knife full cross validation for Model 6.

Table 4. Validation of Model 6 using actual data of *Fusarium graminearum* spores trapped in the seven fields in Manitoba in 2006.

	Field 52	Field 53	Field 54	Field 55	Field 56	Field 57	Field 58
	Superb			AC Barrie			
Model 6	18[a]	23	37	13	16	35	26
Actual data	11	22	14	8	10	17	9

[a] Values in the table represent the predicted number of *Fusarium graminearum* spores on single wheat heads using Models 6 and actual data collected from seven fields in 2006.

2.4 Discussion

To my knowledge, this is the first study to show the relationship between airborne fusarium inoculum levels and weather conditions, and to develop prediction models for the number of *F. graminearum* spores on wheat heads using cropping practices and weather factors. In this study the spore data were collected using the spore traps designed to simulate the way wheat heads trap spores in the field. It has several advantages compared to other commercial spore samplers. The spore counts collected using our spore traps can indicate actual airborne fusarium inoculum levels on wheat heads. Spore data do not need to be collected everyday because the spores on the surface of a spore trap are stable in terms of size and shape for one month under wet or dry weather conditions. The number of spores on the surface of a spore trap, which indicates spore counts on the wheat head, can be transferred to the number of spores per unit volume in the air as other spore samplers (Burkard and rotorod spore samplers). The spore traps in this study are easy to set up in the field and do not need electrical power to run, which facilitated this study.

This study quantified cropping practices including crop rotation, tillage and resistance of wheat cultivars. Wheat rotation with clover, alfalfa, potato and flax; alfalfa and oat, or soybean reduced FHB disease in some studies (Parry et al., 1995). However, other studies have shown that wheat rotation with corn and soybean (Miller et al., 1998), or other types of crop rotations (Gilbert and Tekauz, 1994) did not. Recently, canola stubble was found to be able to harbour *F. graminearum* (Fernando and Gilbert, unpublished data), and whether other crop stubble was able to do so is unclear. There have been no studies done to look at the relationship between crop rotation and tillage and airborne fusarium inoculum; however the effects of cropping practices on wheat stubble and pathogen survival have been well documented.

Rotation of wheat with non-host crops reduces the amount of infested wheat stubble and FHB (Sutton, 1982; Teich and Nelson, 1984; Windels and Kommedahl, 1984; Parry et al., 1995). The period of rotation should be more than two years due to the fact that *Fusarium* pathogens can survive on stubble and wheat kernels for more than two years on the soil surface (Inch and Gilbert, 2003a; Pereyra et al., 2004). Reduction of FHB by using tillage is significant. In the soil, wheat stubble is decomposed quickly and only a small amount of dry matter stubble is left after 24 months (Parry et al., 1995; Pereyra et al., 2004). Although no direct evidence has been found that differences in resistance of wheat cultivars grown in the previous years affect FHB disease in the following year, the pathways of infection in wheat heads and spread in stems, and perithecial formation in stubble have been studied and reported (Guenther and Trail, 2005; Trail et al., 2005). The pathogen can spread throughout the stem tissue following penetration of the wheat head (Trail et al., 2005). Between harvest and winter, the pathogen produces perithecial initial. In the spring, the perithecia mature and ascospores develop and are released at maturity. Therefore, the more resistant the wheat cultivars grown, the less perithecia should be produced in the stubble of the wheat crops grown in the current year. Therefore, the cropping practices including crop rotation, tillage and resistance of previous crop species to the disease was considered and quantified by assigning different scores to components of cropping practices. This study showed that a lower frequency of wheat crops grown, more tillage deployed and more resistant cultivars used in the previous years all resulted in a lower level of fusarium inoculum on wheat heads. Thus, CPI may be a good predictor for airborne fusarium inoculum level on wheat heads.

This study revealed an important influence of cumulative rainfall in the second and third weeks prior to wheat anthesis on spores dispersed in the 14 days after

anthesis, which was consistent with previous studies showing that sufficient moisture was important for perithecial formation (Atanasoff, 1920; Dufault et al., 2006). Perithecial development from initial to maturity usually takes three to four weeks (Atanasoff, 1920; Trail and Common, 2000; Guenther and Trail, 2005), and two weeks when favorable weather conditions occur (Atanasoff, 1920; Leonard and Bushnell, 2003). This study found that the rainfall in the first and fourth weeks prior to anthesis had no significant effects on spore counts. The rainfall in the first week prior to anthesis likely made perithecia mature earlier, which triggered earlier ascospore release than wheat anthesis; whereas, the rainfall in the second week prior to anthesis could result in later ascospore discharge than 14 days after anthesis.

This study also found that cumulative rainfall from seeding to jointing and anthesis significantly affected spore number. However, based on the R^2 value, the effect of rainfall from seeding to jointing was less important than the effect of rainfall from jointing to anthesis, for example rainfall in the second and three weeks before anthesis (Fig. 5). The rainfall from seeding to jointing could possibly affected development of the perithecia but the perithecia developed slowly due to low temperatures in the early spring, thus the relationship between the rainfall from seeding to jointing was not significant.

Cumulative rainfall in the 14 days after anthesis played an important role in spore dispersal in our study. When sufficient moisture (rainfall and relative humidity) is present in the environment, mature perithecia absorb water, subsequently turgor pressure is built up in the asci, and then mature ascospores are discharged (Kendrick, 2003).

The effect of temperature on *G. zeae* perithecial formation and ascospore production has been well documented (Dufault et al., 2006; Sutton, 1982; Tschanz et

al., 1976; Atanasoff, 1920). Perithecial formation occurs from 16°C to 31°C (Tschanz et al., 1976). With increase of temperature, the number of perithecia increases. Ascospores are produced from 13°C to 33°C, and 28°C is the optimal temperature (Sutton, 1982). This study found a significant relationship between spore number and temperature from seeding to anthesis. However, no significant relationship between spore number and temperature during all the individual periods from seeding to anthesis, which was likely caused by the variation of cumulative rainfall during the same periods. This indicates that cumulative rainfall prior to anthesis is more important in spore dispersal than temperature.

Average daily temperature in the first week after anthesis significantly affected spore numbers in this study. Tschanz et al. (1976) reported that *G. zeae* ascospores were discharged in temperatures less than 11.5°C and greater than 22.5°C, and the optimal temperature was 16.5°C. The average daily temperature in the first week after anthesis ranged from 15°C to 25°C in our study, which could improve spore release. Cumulative rainfall in this week showed a similar effect on spore counts and did not mask the temperature effect. However, comparison of rainfall and temperature effects on spore counts needs further analysis. The significant effect of temperature in this week resulted in the effect of temperature from the first week before to the first week and two weeks after anthesis.

There were four models developed in this study. Models 1, 2 and 3 which were simpler and included CPI, or CPI and one to two weather variables, can be selected for the interests of model simplicity and variable effects in the model on the number of spores. Model 6, which included CPI and three wether variables, can be selected for the higher prediction accuracy and the effect of CPI and an overall effect of weather conditions on spore number. Although the four models were close to each

other in prediction accuracy using the field test, Model 6 would likely be more accurate in the long term because the field validation was based on only one-year data.

Model 6 included weather variables collected from the period after the initiation of anthesis, therefore the cumulative rainfall and average daily temperature in this model should be forecasted using weather stations close to individual fields. A wrong weather forecast would affect the prediction accuracy for spore numbers. The prediction accuracy of the number of *F. graminearum* spores on wheat heads is likely to occur in the near future.

This study took into account cropping practices and weather conditions for prediction of the number of *F. graminearum* spores on wheat heads. Another factor that probably affects prediction accuracy is long-distance spore dispersal of *F. graminearum* from one place to another, driven by wind. *Gibberella zeae* ascospores could spread 5-20m away from the artificial inoculum source center (Fernando et al., 1997); however, another study showed that the pathogen's living ascospores were trapped in the planetary boundary layer (Schmale III et al., 2002). In our study, R^2 value for the relationship between spore number and CPI was approximately 40%, thus the effect of the long-distance spore dispersal could partially contribute to 60% of the variation. It is difficult to take this factor into account in the development of prediction models. However, periodical correction and calibration of spore data using the spore traps designed in this study can improve prediction accuracy and solve the above problem in the future.

This study showed that cumulative rainfall in the first and second weeks after anthesis significantly affected spore dispersal in the 14 days after anthesis, in which wheat is most susceptible to FHB (Pugh et al., 1933; Wilcoxson et al., 1992; Groth et al., 1999). There are different types of protectant and systemic fungicides available

for FHB (Mesterházy, 2003), however no studies suggested that fungicides should be sprayed more than once for FHB. Therefore the longevity of all labeled fungicides for FHB should be greater than 10-14 days. However, if there is an exemption, the fungicides whose longevity is shorter than 10-14 days should be sprayed two times, one at the 0-30% flowering stage and the other around the 14th day after anthesis.

The models developed in our study can be used for the prediction of *F. graminearum* spores on wheat heads, and Model 6 can be used for the risk estimation of FHB epidemic of wheat and deoxynivalenol (DON) accumulation in wheat kernels. This study only considered *F. graminearum* species, thus, if other *Fusarium* species, which are predominant in different regions, and have similar biological characters and responses to weather conditions, these models developed in our study can be tentatively used in these regions. They should be corrected by validating them using actual data, which willsave time, money and energy.

2.5 Conclusions

This study successfully quantified cropping practices by assigning different scores to different host crops, rotation years, tillage methods and resistance of wheat cultivars. CPI was significantly related to *F. graminearum* spore counts on wheat heads. Cereal crops, zero tillage or minmum tillage and susceptible wheat cultivars used in the previous years would result in increase of FHB and DON levels in the current year. CPI was a good predictor for FHB index and DON concentration at maturity.

Cumulative rainfall in the second and third weeks prior to anthesis significantly improved perithecial development and formation, and in turn spores discharged at anthesis. The rainfall in the second week after anthesis had a greater effect than the rainfall in the first week in spore dispersal at anthesis. Effect of cumulative rainfall

had a greater effect than and could mask the average daily temperature during the same periods.

Three significant regression models were obtained for *F. graminearum* spore counts. Model 6 including CPI and three weather variables had the prediction accuracy of 57%. This model would be used for predicting FHB index and DON levels.

CHAPTER 3

3.0 Development of Models to Predict Fusarium Head Blight Disease and Deoxynivalenol Levels in Wheat

Abstract

The objectives of this study were to investigate effects of airborne fusarium inoculum on wheat heads, cropping practices and weather conditions on fusarium head blight (FHB) disease and deoxynivalenol (DON) level and to develop models to predict FHB index and DON level. This study was conducted in 14 fields in 2003, 17 fields in 2004, and 19 fields in 2005 in Manitoba, Canada. Seven fields in 2006 were used for validation of the models. I selected two spring wheat cultivars; Superb (moderately susceptible to FHB disease) and AC Barrie (intermediate in resistance to FHB disease) sown by farmers in the fields each year. *Fusarium graminearum* spore counts on wheat heads and cropping practice index (CPI) significantly affected FHB and DON levels for both cultivars. It was observed the number of cereal crops, zero or minimum tillage and susceptible wheat cultivars were used in preceding years, the higher the level of FHB and DON level. Cumulative rainfall in the following periods significantly affected fusarium head blight (FHB) disease index and deoxynivalenol (DON) accumulation for both cultivars: from seeding and jointing to anthesis, in the second week prior to and after anthesis. Superb responded more to cumulative rainfall than AC Barrie in FHB. Cumulative rainfall was more correlated to FHB and DON level than average daily temperature for both cultivars. Two types of models were developed for FHB index and DON level. Type I models with actual spore counts had prediction accuracy of 85%, and Type II models had prediction accuracy of 58%. These models will help wheat producers to reduce FHB infection through management practices and assist the Canadian Wheat Board to reduce the risk of DON contamination in grain shipments.

3.1 Introduction

Fusarium head blight (FHB) disease of wheat is caused by *Gibberella zeae* (Schwein.) Petch (anamorph = *Fusarium graminearum* Schwabe) and other *Fusarium* species. FHB reduces wheat yield and quality by causing shrunken and light kernels, fusarium-damaged kernels (FDK) and mycotoxins (Parry et al., 1995). Severe FHB lowers test weight and reduces milling and baking quality for bread wheat (Dexter and Nowicki, 2003). Deoxynivalenol (DON), one of the most important trichothecenes produced by *F. graminearum*, causes animal feed refusal and illness in humans (Dexter and Nowicki, 2003).

Epidemics of FHB are sporadic worldwide (Fernando et al., 2000). Since the first description of FHB disease by Arthur in 1884, this disease had been reported in North America and Europe (Leonard and Bushnell, 2003). In Canada, this disease was detected from the 1940s to 1980s in the Maritime Provinces, Quebec, and Ontario (Sutton, 1982). From 1984 to 1991, the disease emerged and increased in Manitoba (Wong et al., 1995). The most severe epidemic occurred in south-central Manitoba in 1993, and disease incidence was as high as 70% in some fields (Gilbert et al., 1995). Manitoba continues to be a hotbed for fusarium inoculum and infection. Economic losses to FHB in Canada in the 1990s were estimated at US$200 million in Quebec and Ontario and at US$300 million in Manitoba from 1993 to 1998 (Windels, 2000). Wheat and barley losses in the United States in the 1990s were estimated at US$3 billion (Windels, 2000).

The increasing occurrence of FHB has been favored by increasing wet and warm weather, a lack of resistant wheat cultivars, and a buildup of fusarium inoculum in the fields (Leonard and Bushnell, 2003). Therefore, intense efforts have been exerted in improving agronomic practices, especially lengthening intervals between host crops,

changing sequences of crops and applying fungicides. Various types of fungicides in tests and applications were reported to have good efficacy (Mesterházy, 2003). However, crop growers usually make decisions on fungicide application based on their own experiences. Available and reliable disease-forecasting systems can warn growers of the risks of the disease and consequence mycotoxin accumulation. Given the precise timing of necessary fungicide application, growers may take actions to reduce the disease and toxin accumulation in wheat grain. Meteorologically-based regression models were developed for prediction of FHB disease incidence, severity and index in Argentina, in which were included several predictors, the number of two-day periods with precipitation of more than 0.2mm, relative humidity of >81% and 78% for day 1 and day 2, respectively, and temperatures of 9-26°C (Moschini and Fortugno, 1996). The prediction accuracy ranged from 86% to 88%. Growth stage-based logistic regression model was built up for assessing the risk of FHB epidemics using the information of duration of rainfall and temperatures of 15-30°C from 7 days before anthesis to 10 days after the start of wheat anthesis, and the prediction accuracy ranged from 62% to 85% (De Wolf et al., 2003). Another model was developed for predicting DON levels in wheat grain using days of >5-mm rainfall with temperature of >10°C 4 to 7 days prior to heading stage, days of >3-mm rainfall with temperature of <32°C 3 to 6 days after heading stage, and days of >3-mm rainfall 7-10 days after heading stage (Hooker et al., 2002). The prediction accuracy was 73%. Other FHB-forecasting systems based on weather conditions favorable for disease and epidemic development are available (The North Dakota State University, 2005; The Ohio State University, 2005). The development of more accurate prediction models for FHB disease and DON level needs to take into account factors such as fusarium inoculum and cropping practices.

Cropping practices, as a disease management strategy has been deployed for several decades. However, its effects on certain diseases including fusarium head blight are unclear. Wheat stubble, the major source of fusarium inoculum on the soil surface can be reduced by crop rotation and tillage (Parry et al., 1995; Sutton, 1982; Teich and Nelson, 1984), which in turn may reduce FHB (Dill-Macky and Jones, 2000; Schaafsma et al., 2001). Although there have been no reports on the relationship between fusarium-infested wheat stubble and airborne inoculum at the flowering stage when wheat is most sensitive to fusarium infection (Andersen, 1948; Fernando et al., 1997; Pugh et al., 1933), appropriate cropping practices have been shown to reduce airborne inoculum for blackleg disease in canola (Guo et al., 2005).

Production of fusarium inoculum, infection by the pathogen, and effects of weather conditions are well understood (Atanasoff, 1920; Andersen, 1948; Tschanz, 1976; Sutton, 1982; Fernando et al., 2000). However, the relationship between airborne fusarium inoculum and mycotoxins in wheat grain is unclear, though a study has shown that FHB severity, percentage of fusarium damaged kernels (FDK) and DON level were correlated (Dill-Macky and Jones, 2000).

The objectives of this study were, 1). to understand the relationships between FHB and DON accumulation in wheat grain at maturity and cropping practices, weather conditions and inoculum level on wheat heads, and 2). to develop prediction models for FHB index and DON levels for wheat cultivars that differ in disease resistance using cropping practices, average daily temperature, cumulative rainfall, and the number of *F. graminearum* spores on single artificial wheat heads (spore traps).

3.2 Materials and methods

Experimental plots and measurements. The experiment was conducted in the same fields as the experiment in Chapter 2 (Fig 1A in Chapter 2, and Table 1). CWRS wheat cultivar Superb, moderately susceptible to FHB disease (Seed Manitoba, 2006), and CWRS AC Barrie, intermediate in resistance to FHB disease (Seed Manitoba, 2006) were each sown in seven fields in 2003. Superb was sown in nine fields and AC Barrie was sown in eight fields in 2004. Superb was sown in ten fields and AC Barrie was sown in twelve fields in 2005. In 2006 three fields were sown to Superb and four fields were sown to AC Barrie. Prediction model were developed using the data collected from 2003 to 2005, and the models were validated in the field using the data collected from 2006. A prediction model should be developed based on at least three-year data, and the data sets from 2003, 2004 and 2005 were more than 2006, prediction models developed using the data from 2003 to 2005 were more reliable than other combinations. In each field, four sampling sites, close to the places where the spore traps were set in the experiment in Chapter 2, were lined up approximately 20 m apart for data collection. Each sampling site was 1.0 m × 0.5 m. Wheat heads were collected from one half of each sampling site (0.5 m × 0.5 m) 21 days after anthesis. Mature wheat heads were collected from the other half of the sampling site. The method of assessing cropping practice index (CPI) for each field was presented in Chapter 2.

The wheat heads sampled from each field were used for the estimation of FHB disease index (Fig. 1). The wheat heads collected at maturity were threshed using a stationary thresher (Model: 3876; Redmond Company, Inc. Mich., USA). Fifty grams of the threshed kernels were sent to the Canadian Grain Commission and analyzed for

DON. DON (ppm) was determined through a procedure of sample extraction, cleanup and derivatization using gas chromatography-mass spectrometry (GC-MS) method.

Cumulative rainfall and average daily temperature were measured during the same periods and using the same methods as described in Chapter 2.

Figure 1. Wheat heads were sampled from each of four sampling sties in each field 21 days after anthesis. The sampling size was 0.5m × 0.5m.

Data analysis and model development. The number of *F. graminearum* spores on single wheat heads (S) was estimated as total spores of this species trapped on four collecting faces of a spore trap. FHB disease index (%), using a 0 to 100% scale, was calculated as percentage of infected spikes multiplied by percentage of infected spikelets and divided by 100 (Gilbert et al., 1995). Analysis of variance (ANOVA) (SAS, version 9.1) was used to test effects of year, location, cultivar and interaction of year and location on FHB disease index and DON levels. A simple linear (one independent variable) regression procedure was used to examine effect significance of the individual factors on the disease index and toxin levels. The multiple regression models were developed using a stepwise procedure. Two types of models were developed for FHB index and DON levels. Type I models were developed using actual spore number, and Type II models were developed based on predicted spore number using Model 6 in Chapter 2. In the models including independent variables that were correlated with each other, the interactions of the variables could mask the significance of their parameters in the models. The variance inflation factor (VIF) method (Chapter 2) was used for testing the effect significance of each correlated variable. The VIF value greater than 10 indicated that multicollinearity was significant and the model would not be acceptable.

Model validation. The prediction models (full models) for FHB index and DON were tested using the Jack-Knife full-cross validation method (Good and Hardin, 2003) as used in Chapter 2. A new prediction model was developed using the data of all the fields except for one, and then this model was considered as the full model and tested using the data of the remaining field, and one validation data set of predicted vs. actual values was obtained. The same procedure was run for all the other data including the data used previously each time, so that the same number of validation

data sets as the total number of the fields was obtained. A linear regression model was developed using the predicted vs. actual data sets using a regression analysis procedure. The R^2 and slope values indicated the predicting accuracy.

The prediction models developed based on the data collected from 2003 to 2005 were also tested by comparing the predicted values of FHB index and DON level with the data collected from 2006. The actual FHB index (%) and DON concentration (ppm) were averaged according to wheat cultivars in 2006. Type I models for the disease and toxin for Superb were tested using the corresponding actual averaged values based on three fields for the same cultivar, and Type I models for AC Barrie were tested using the actual averaged values based on four fields. The same method was applied for testing Type II models.

3.3 Results

Effects of year, location, wheat cultivar and interaction of year and location on FHB index and DON levels. ANOVA showed that year effect was significant ($P < 0.05$) for FHB index and DON levels, indicating the disease and toxin distribution among the three years were significantly different (Table 2A and 2B). This could be caused by other factors, including cropping practices, the number of *F. graminearum* spores and weather conditions. In Chapter 2, spore counts were significantly affected by year, which likely resulted in the difference in FHB index and DON levels among the three years. Differences in cropping practices and weather conditions among years could also cause differences in disease and toxin levels. Location and its interaction with year significantly affected DON levels, which could result from the differences in cropping practices and weather conditions (Table 2B). ANOVA also exhibited no significant effect of wheat cultivar on either FHB index or DON level (Table 2A and 2B).

Although the effect of year was significant, and the effect of cultivar was not it was possible to examine the relationships between individual factors and the FHB index and toxin levels based on the pooled three-year data for cultivar, because the models developed based on three individual years would not be feasible.

Table 1. FHB index (%) and DON levels (ppm) in wheat kernels at maturity in different fields in Manitoba from 2003 to 2006.

Field #	Year	Cultivar	FHB index (%)	DON (ppm)	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
1	2003	Superb	6	1.6	8.4	41.6	52.6	86.0	10.2	21.0	20.1	17.8	18.7	17.7	17.4	18.4
2	2003	Superb	2	0.2	56.0	96.0	105.0	105.0	0.0	7.0	18.2	17.1	16.8	17.1	18.5	19.4
3	2003	Superb	0	0.3	0.0	4.0	8.9	8.9	2.2	2.2	17.3	18.5	17.3	17.7	19.7	20.4
4	2003	Superb	0	0	24.6	32.4	61.8	106.6	1.7	5.8	16.1	17.8	18.0	17.3	19.0	18.8
5	2003	Superb	0	0	5.4	5.4	13.4	24.6	4.8	4.8	16.3	17.9	16.8	17.7	19.6	19.9
6	2003	Superb	0	0	5.6	18.2	18.4	62.4	5.8	7.8	18.2	17.4	18.0	16.9	16.8	18.5
7	2003	Superb	0	0	8.1	36.5	39.0	49.2	16.3	25.7	20.1	18.0	18.9	17.9	17.3	18.2
8	2004	Superb	7.5	2.1	0.0	0.0	6.0	30.8	0.0	102.0	16.4	17.6	18.0	16.6	15.4	13.9
9	2004	Superb	5.5	1.1	3.9	36.9	52.0	52.6	26.5	29.3	21.0	19.0	18.0	16.7	17.6	16.9
10	2004	Superb	3.1	1.1	16.0	45.0	53.0	66.0	3.0	3.0	19.2	19.3	18.0	17.5	16.4	16.5
11	2004	Superb	2.6	1.2	16.0	45.0	63.0	81.0	4.0	15.0	20.9	19.7	17.8	17.1	17.2	16.5
12	2004	Superb	2	0.4	17.0	40.0	58.6	77.8	0.0	0.0	18.2	19.2	18.1	17.6	16.1	16.0
13	2004	Superb	1.6	0.6	10.0	50.5	50.5	53.5	11.6	17.6	20.8	18.2	17.6	16.2	19.5	18.6
14	2004	Superb	0.4	0.4	25.8	51.2	64.3	80.8	3.5	3.5	19.4	19.5	17.9	17.4	16.8	16.8
15	2004	Superb	0.5	0.2	15.8	22.2	22.2	35.2	14.4	18.2	20.3	17.9	17.2	15.9	18.8	18.3
16	2004	Superb	0.6	0.2	0.0	20.2	30.0	40.8	2.2	4.8	18.7	17.2	16.9	15.3	17.8	17.5
17	2005	Superb	9.2	2.7	48.8	155.0	210.4	212.6	22.0	25.0	23.9	20.5	19.4	19.8	19.7	18.5
18	2005	Superb	9.1	2.3	21.4	97.0	100.0	123.1	39.6	39.6	18.8	16.7	18.5	18.0	22.7	20.2
19	2005	Superb	8.8	2.1	39.4	116.8	116.8	175.3	38.1	38.1	18.6	16.6	18.2	17.7	22.6	20.0
20	2005	Superb	6.1	1.8	64.0	164.5	171.0	180.5	29.5	36.5	21.7	19.7	19.7	19.7	21.8	20.3
21	2005	Superb	5.9	1.4	82.6	159.9	209.8	214.2	26.3	28.7	23.5	20.5	18.8	19.3	18.0	17.2
22	2005	Superb	4.3	1.5	106.6	173.3	173.3	216.0	10.6	20.8	18.9	16.9	17.9	17.7	21.9	19.7
23	2005	Superb	2.3	1.3	9.4	20.2	73.8	91.6	0.0	0.0	16.9	18.4	19.9	19.1	20.0	19.6
24	2005	Superb	1.1	0.9	5.0	54.0	72.0	88.0	13.0	13.0	20.1	17.8	17.2	17.2	19.5	17.9

Field #	Year	Cultivar	FHB index (%)	DON (ppm)	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
25	2005	Superb	1.1	0.9	18.0	35.8	83.4	85.6	3.4	5.2	22.6	20.9	18.9	19.3	17.7	16.8
26	2005	Superb	0.5	0.2	72.0	89.0	140.0	245.0	2.5	18.5	19.2	21.9	20.9	19.7	17.1	19.7
27	2006	Superb	0.1	0.2	10.6	1.2	12.8	10.2	1.8	1.8	19.4	19.7	17.8	19.1	22.2	21.2
28	2006	Superb	0.2	0.2	3.7	13.6	48.1	9.5	1.0	0.2	19.8	19.3	17.6	18.4	21.6	20.1
29	2006	Superb	0.1	0.3	1.9	32.2	28.6	8.9	1.0	1.0	19.1	18.8	17.1	18.3	21.0	19.7

Table 1 (continued). FHB index (%) and DON levels (ppm) in wheat kernels at maturity in different fields in Manitoba from 2003 to 2006.

Field #	Year	Cultivar	FHB index (%)	DON (ppm)	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
30	2003	AC Barrie	3	1.2	9.0	27.0	45.0	49.0	19.0	34.0	18.9	18.0	18.3	18.5	19.1	19.4
31	2003	AC Barrie	0	0.1	1.8	3.8	10.7	10.7	2.2	2.2	19.5	17.9	18.0	17.8	20.8	20.7
32	2003	AC Barrie	0	0.1	23.4	30.2	59.2	81.8	10.0	18.6	17.1	19.2	17.9	17.4	21.4	19.3
33	2003	AC Barrie	0	0.0	80.2	80.2	80.2	80.2	0.0	23.0	18.7	18.5	19.0	17.9	17.3	18.7
34	2003	AC Barrie	0	0.1	11.4	14.3	35.8	42.9	9.5	29.5	18.6	18.6	18.1	18.3	19.6	20.3
35	2003	AC Barrie	0	0.1	11.4	21.6	29.2	38.1	2.5	2.5	18.6	17.8	17.3	17.5	19.4	20.1
36	2003	AC Barrie	0	0.0	2.8	17.6	17.6	54.6	5.6	7.6	19.3	17.4	18.1	17.1	17.2	19.2
37	2004	AC Barrie	6.7	1.7	3.9	36.9	52.0	52.6	26.5	29.3	21.0	19.0	18.0	16.7	17.6	16.9
38	2004	AC Barrie	3.9	1.1	32.0	63.6	65.6	70.4	23.2	25.6	20.1	17.3	16.8	15.6	19.9	18.7
39	2004	AC Barrie	2.6	0.9	0.2	22.2	22.2	35.2	14.4	21.6	19.8	17.6	17.2	15.8	18.7	18.1
40	2004	AC Barrie	2.1	0.6	6.1	29.1	37.1	38.9	0.0	10.0	20.1	18.0	17.6	16.1	19.0	17.9
41	2004	AC Barrie	1.4	0.4	48.5	50.5	50.5	59.5	9.0	18.0	21.0	18.1	17.3	16.0	19.6	18.6
42	2004	AC Barrie	1	0.4	0.0	20.2	30.0	37.8	2.2	9.2	20.1	19.2	17.7	16.4	17.1	16.2
43	2004	AC Barrie	0.2	0.1	25.8	51.2	64.3	80.8	3.5	3.5	19.4	19.5	17.9	17.4	16.8	16.8
44	2004	AC Barrie	8	2.7	104.1	129.5	129.5	172.7	43.2	94.0	17.2	18.0	18.8	18.1	24.8	22.5
45	2004	AC Barrie	7.5	2.3	46.2	161.6	163.8	195.6	20.8	42.8	17.9	17.0	18.6	17.9	24.1	21.4
46	2005	AC Barrie	5.6	1.4	63.5	111.8	144.8	170.2	0.0	0.0	21.8	18.9	18.2	18.4	19.7	18.2
47	2005	AC Barrie	4.1	1.6	21.8	124.6	128.6	132.0	29.8	30.6	20.2	18.3	18.7	18.6	22.3	20.3
48	2005	AC Barrie	1.3	1.3	30.5	127.5	127.5	149.5	73.0	80.0	20.1	18.4	19.5	19.0	23.7	21.2
49	2005	AC Barrie	1.1	1.1	88.1	159.9	212.4	237.6	26.3	27.1	22.1	19.2	18.6	18.8	19.9	18.4
50	2005	AC Barrie	0.8	1.0	104.6	145.7	183.9	183.9	10.2	13.2	22.8	19.5	18.4	18.7	18.7	17.6
51	2005	AC Barrie	0.7	0.9	21.8	31.6	105.4	133.4	3.6	5.0	20.0	21.5	19.9	19.2	16.9	18.6

Field #	Year	Cultivar	FHB index (%)	DON (ppm)	R_{-1}	R_{-2}	R_{-3}	R_{-4}	R_{+1}	R_{+2}	T_{-1}	T_{-2}	T_{-3}	T_{-4}	T_{+1}	T_{+2}
52	2005	AC Barrie	0.5	0.6	22.9	104.1	104.1	127.0	0.0	12.7	18.6	16.5	18.2	17.8	22.6	20.0
53	2005	AC Barrie	0.5	0.7	14.2	72.8	77.8	103.2	24.8	27.8	21.0	18.2	18.1	18.3	20.2	18.8
54	2005	AC Barrie	0.2	0.1	3.5	91.5	115.5	242.5	16.0	16.0	19.0	20.9	21.1	20.2	18.5	20.2
55	2006	AC Barrie	0	0.1	1.0	2.5	7.9	12.9	2.6	0.0	19.2	19.9	17.7	19.8	22.0	20.5
56	2006	AC Barrie	0.1	0.2	10.6	1.2	12.8	10.2	1.8	1.8	19.4	19.7	17.8	19.1	22.2	21.2
57	2006	AC Barrie	0.2	0.3	3.7	13.6	48.1	9.5	1.0	0.2	19.8	19.3	17.6	18.4	21.6	20.1
58	2006	AC Barrie	0.1	0.1	1.9	32.2	28.6	8.9	1.0	1.0	19.1	18.8	17.1	18.3	21.0	19.7

R_{-4}: cumulative rainfall in the fourth week prior to anthesis. R_{-3}: cumulative rainfall in the third week prior to anthesis. R_{-2}: cumulative rainfall in the second week prior to anthesis. R_{-1}: cumulative rainfall in the first week prior to anthesis. R_{+1}: cumulative rainfall in the first week after anthesis. R_{+2}: cumulative rainfall in the second week after anthesis.

T_{-4}: average daily temperature in the fourth week prior to anthesis. T_{-3}: average daily temperature in the third week prior to anthesis. T_{-2}: average daily temperature in the second week prior to anthesis. T_{-1}: average daily temperature in the first week prior to anthesis. T_{+1}: average daily temperature in the first week after anthesis. T_{+2}: average daily temperature in the second week after anthesis.

Table 2A. ANOVA for year, cultivar and location effects on fusarium head blight (FHB) index (%).

Source	Type III SS	Mean square	F value	Pr > F
Year	58.8703671	29.4351835	26.33	0.0125
Location	168.7964008	8.8840211	7.95	0.0563
Cultivar	0.5340919	0.5340919	0.48	0.5391
Year*Location	60.9376206	4.3526872	3.89	0.1448

Table 2B. ANOVA for year, cultivar and location effects on deoxynivalenol (DON) (ppm) in wheat grain.

Source	Type III SS	Mean square	F value	Pr > F
Year	8.20715071	4.10357536	601.86	0.0001
Location	12.34776044	0.64988213	95.32	0.0015
Cultivar	0.00767045	0.00767045	1.13	0.3667
Year*Location	3.51905580	0.25136113	36.87	0.0063

Fusarium inoculum level on single artificial wheat heads (spore traps) vs. FHB. The number of *F. graminearum* spores on single artificial wheat heads significantly ($P < 0.05$) affected FHB index for both cultivars (Fig. 2A). With increase in spore number from 0 to 97 for Superb, and from 0 to 110 for AC Barrie, FHB index increased from 0 to 9% and from 0 to 8%, respectively. Coefficient of determination (R^2) of the linear regressions was 0.77 and 0.83 for Superb and AC Barrie, respectively. Predicted spore number using Model 6 (Chapter 2) was significantly correlated to FHB index as well (Fig. 2B). R^2 values for Superb and AC Barrie were 0.75 and 0.45, respectively. Therefore, both actual and predicted spore numbers could be considered as predictors of FHB disease index.

Cropping practices vs. FHB. Cropping practice index had a significant ($P < 0.05$) effect on FHB index (Fig. 3). R^2 values of the linear regressions were 0.50 and 0.42 for Superb and AC Barrie, respectively. However, there was great variation in FHB index for both cultivars when CPI was approximately 80. This was likely caused by the great variation of spore number at the same CPI point (Fig. 4 in Chapter 2). At the same level of CPI, Superb showed a higher disease level than AC Barrie.

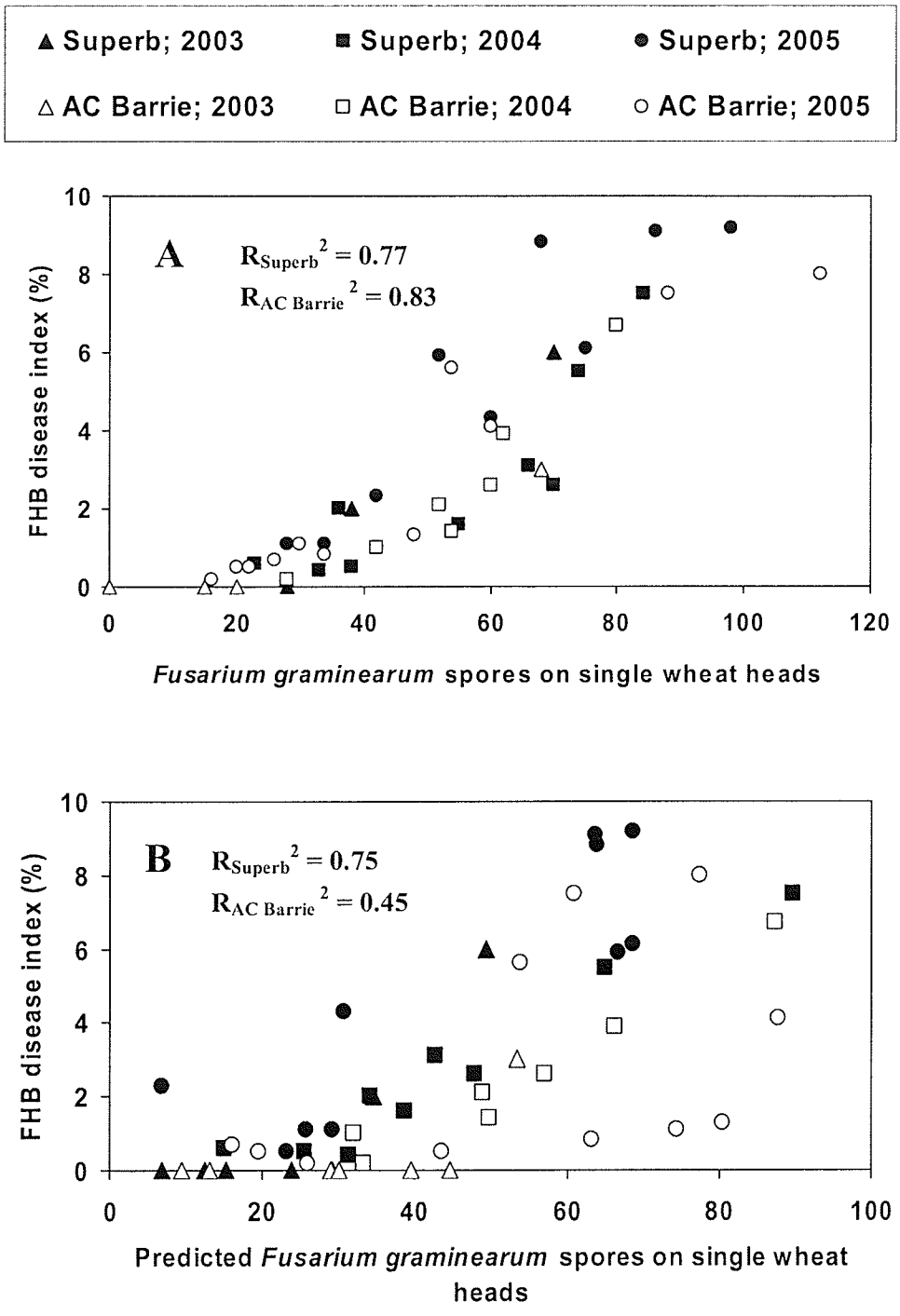


Figure 2. Relationships between fusarium head blight (FHB) disease index (%) and the actual (A) and predicted (B) number of *Fusarium graminearum* spores on artificial wheat heads (spore traps).

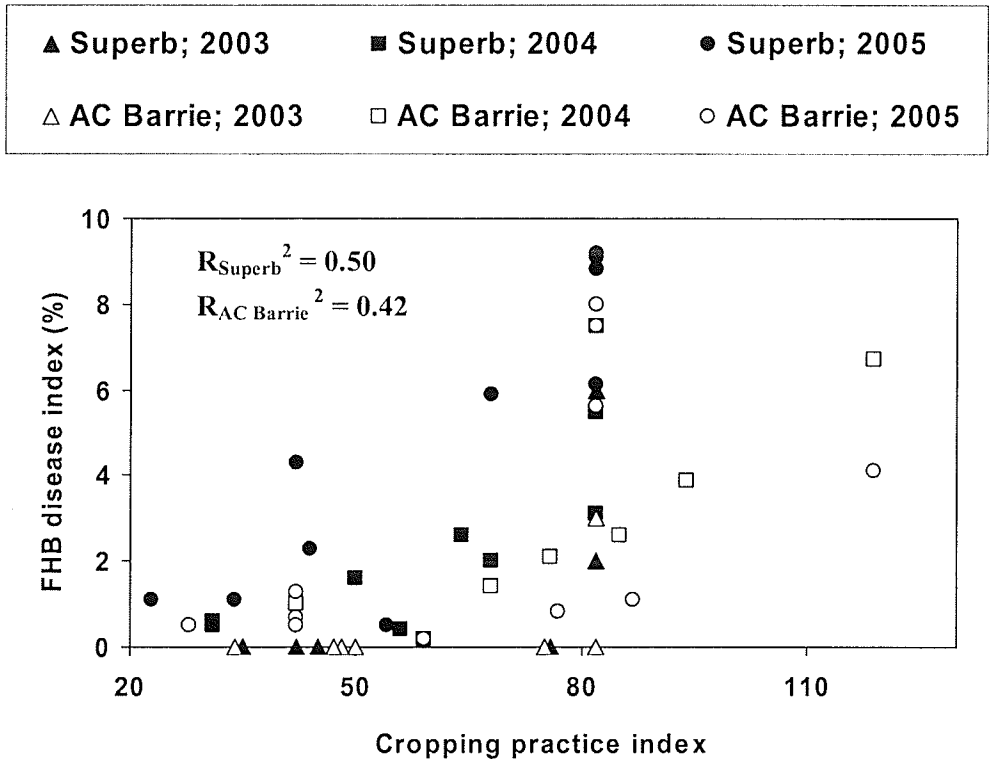


Figure 3. Relationship between fusarium head blight (FHB) disease index (%) and cropping practice index.

Cumulative rainfall vs. FHB. Cumulative rainfall in the second, third and fourth weeks before anthesis, from jointing to anthesis of wheat, and cumulative rainfall in the first and second weeks after anthesis significantly affected FHB index for Superb (Fig. 4). This resulted in the significant effects of cumulative rainfall in the four weeks prior to anthesis, from seeding to anthesis, and from the first week before to the first and second weeks after anthesis on the disease index for the same cultivar (Fig. 4). The R^2 values for the significant relationships ranged from 0.20 to 0.63 for Superb. For AC Barrie, the relationships were significant for the periods of the second week

prior to anthesis, from jointing to anthesis, and from seeding to anthesis, and in the second week and two weeks after anthesis, and from the first week before to the first week and second weeks after anthesis (Fig. 4). The R^2 values for the significant relationships ranged from 0.15 to 0.25. This indicates that cumulative rainfall prior to wheat anthesis is more important for the FHB for Superb than AC Barrie.

For Superb, cumulative rainfall in the second week prior to anthesis was more important than in the first, third and fourth weeks prior ro anthesis (Fig. 4 and 5B-5D). The plots for the relationships between the disease index and cumulative rainfall in the third (Fig. 5C) and fourth (Fig. 5B) weeks prior to anthesis scattered more than in the second week (Fig. 5D). R^2 value for the latter relationship (0.33) was higher than the two former relationships.

The cumulative rainfall in the second week contributed most to the significant effect of rainfall in the 28 days prior to anthesis on FHB index for Superb (Fig. 4 and 5A). Cumulative rainfall from seeding to anthesis and from jointing to anthesis significantly affected disease index (Fig. 4). The latter period resulted from the significant effect of rainfall in the 28 days prior to anthesis, because most of the period from jointing to anthesis and the 28-day period prior to anthesis was overlapped. The significant effect of cumulative rainfall from seeding to anthesis originated from the effect of rainfall from jointing to anthesis (Fig. 4).

Cumulative rainfall in the 14 days after anthesis significantly affected FHB index for Superb (Fig. 5G), which was due to the effects of rainfall in the first (Fig. 5E) and second (Fig. 5F) weeks after anthesis. With increasing cumulative rainfall in the 14 days after anthesis from 0-10mm to 80-100mm, FHB index increased from 0% to 9% (Fig. 5G). The effects of rainfall from the first week before anthesis to the first week

and second weeks after anthesis were also due to the significant effect of rainfall in the first (Fig. 5E) and second (Fig. 5F) weeks after anthesis.

For AC Barrie, the significant effect of cumulative rainfall in the second week prior to anthesis contributed most to the effects of rainfall from jointing to anthesis and from seeding to anthesis (Fig. 4). As cumulative rainfall increased from 0 to 160mm, FHB index increased from 0% to 8% (Fig. 5D).

With increasing cumulative rainfall in the second week after anthesis from 0 to 90mm, the disease index increased from 0% to 8% (Fig. 5F). This relationship contributed to the significant effect rainfall in the 14 days after anthesis (Fig. 4 and 5G). Although cumulative rainfall in the first week before and the first week after anthesis had no effect on the disease index, total rainfall from the two weeks and rainfall from the first week before to the second week after anthesis significantly affected FHB index (Fig. 4, 5H and 5I).

FHB index was higher in Superb than AC Barrie when cumulative rainfall during the different periods was at the same or similar levels (Fig. 5A-5I). For Superb, cumulative rainfall during different periods affected FHB index by influencing the number of *F. graminearum* spores during the same periods, because there was a similar pattern of cumulative rainfall effect on the number of spores to the effect on FHB index (Fig. 5 in Chapter 2, and Fig. 4 in this Chapter).

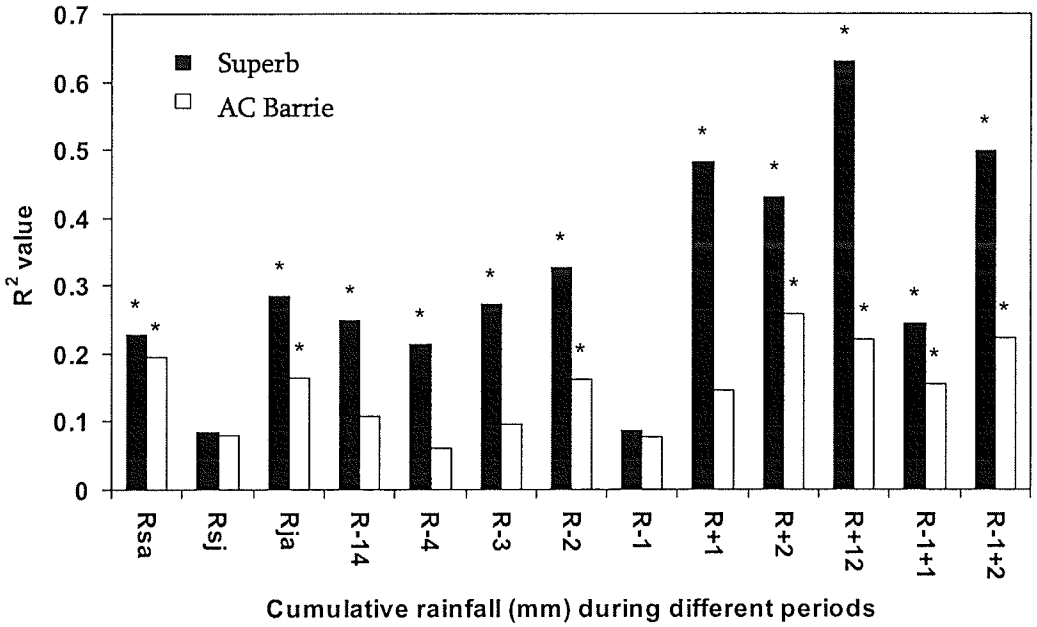


Figure 4. R^2 values for the relationships between fusarium head blight (FHB) index (%) and cumulative rainfall (mm) from seeding to 14 days after anthesis.
The stars on the top of bars represented significant relationship at 5% level.
R_{sa}: cumulative rainfall from seeding to anthesis. R_{sj}: cumulative rainfall from seeding to jointing. R_{ja}: cumulative rainfall from jointing to anthesis. R_{-14}: cumulative rainfall in the 28 days prior to anthesis. R_{-4}: cumulative rainfall in the fourth week prior to anthesis. R_{-3}: cumulative rainfall in the third week prior to anthesis. R_{-2}: cumulative rainfall in the second week prior to anthesis. R_{-1}: cumulative rainfall in the first week prior to anthesis. R_{+1}: cumulative rainfall in the first week after anthesis. R_{+2}: cumulative rainfall in the second week after anthesis. R_{+12}: cumulative rainfall in the 14 days after anthesis. R_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis. R_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

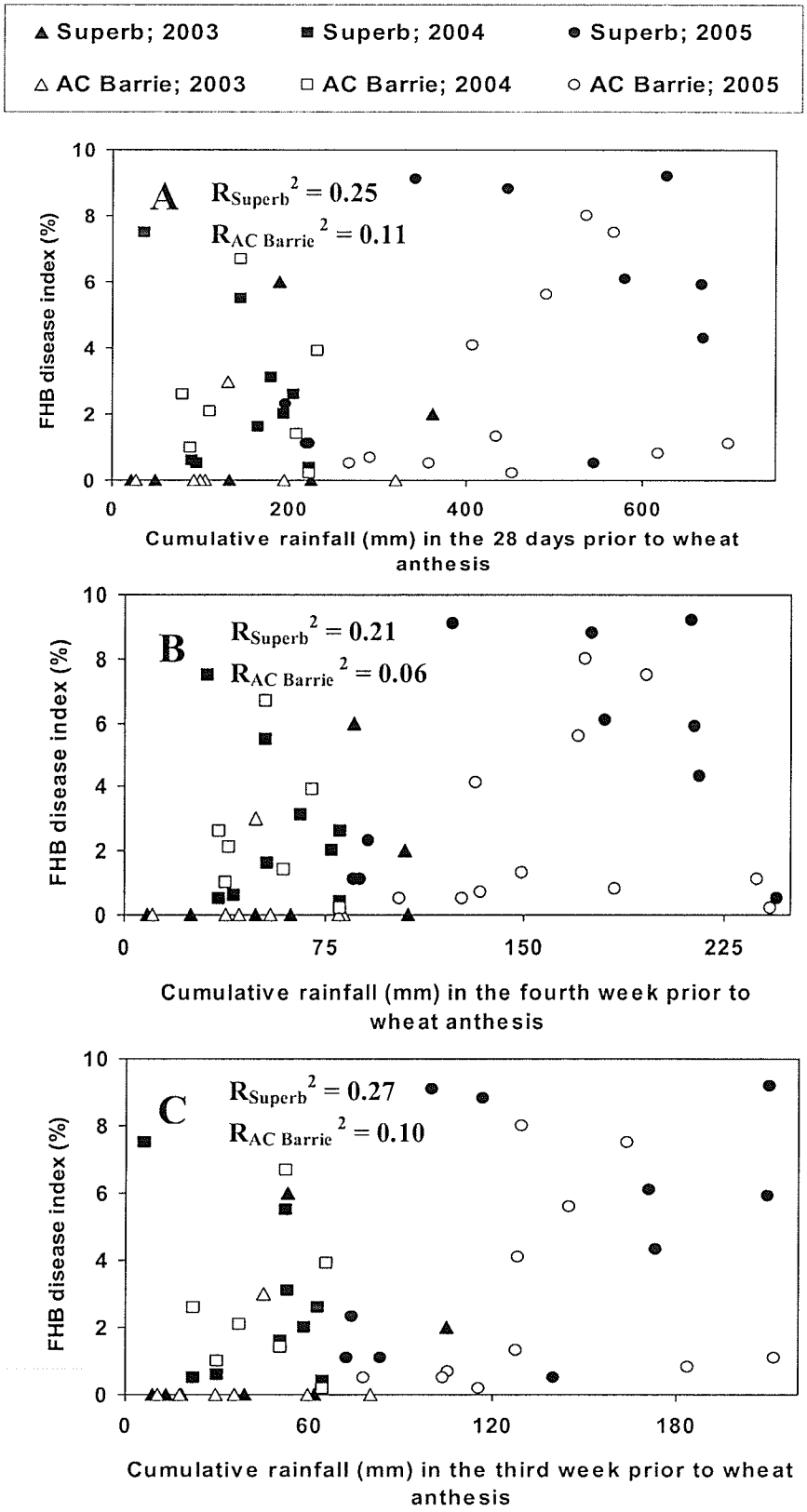


Figure 5 (A-C). Relationship between fusarium head blight (FHB) index (%) and cumulative rainfall (mm) during the different periods.

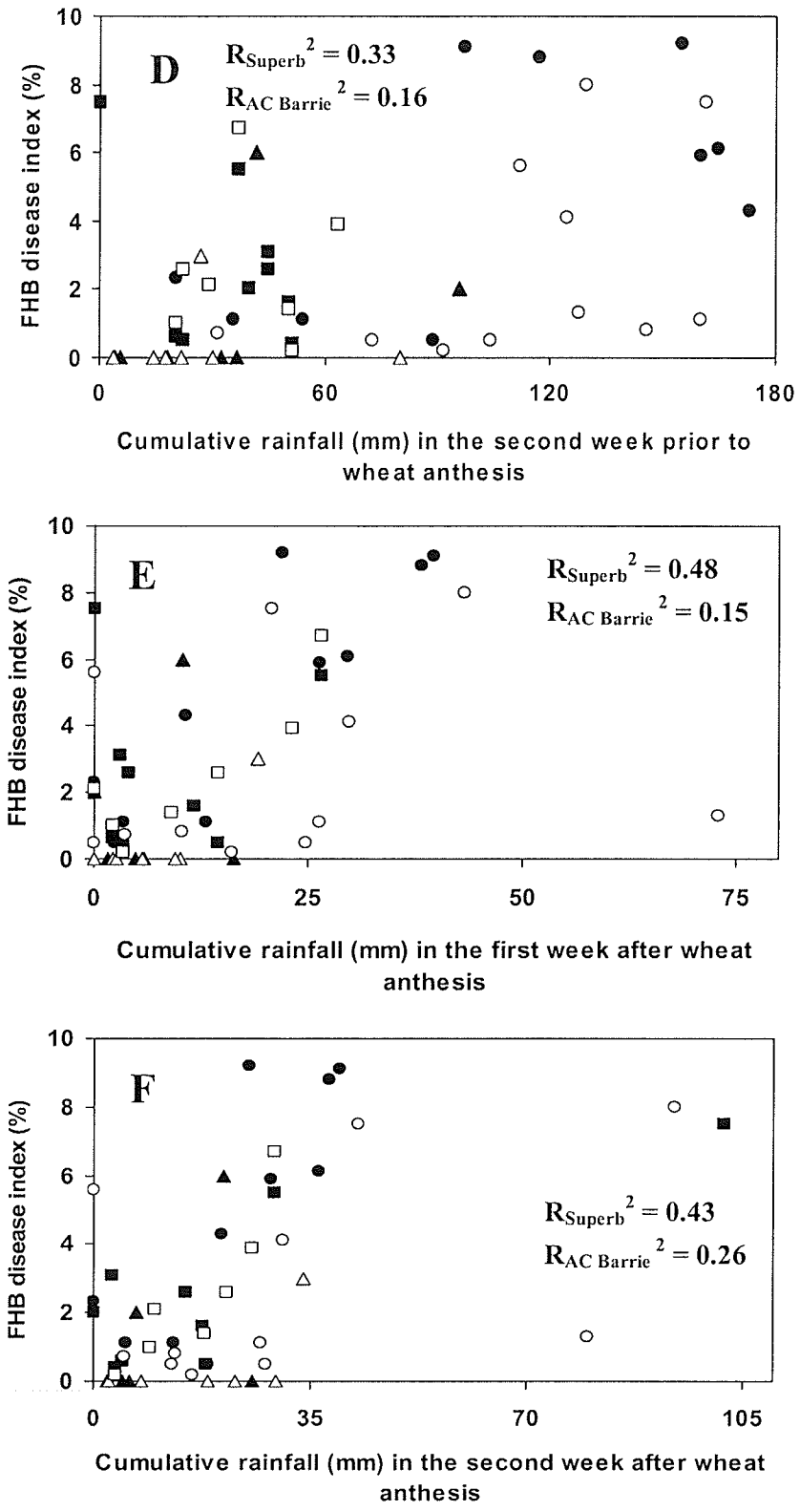


Figure 5 (D-F). Relationship between fusarium head blight (FHB) index (%) and cumulative rainfall (mm) during the different periods.

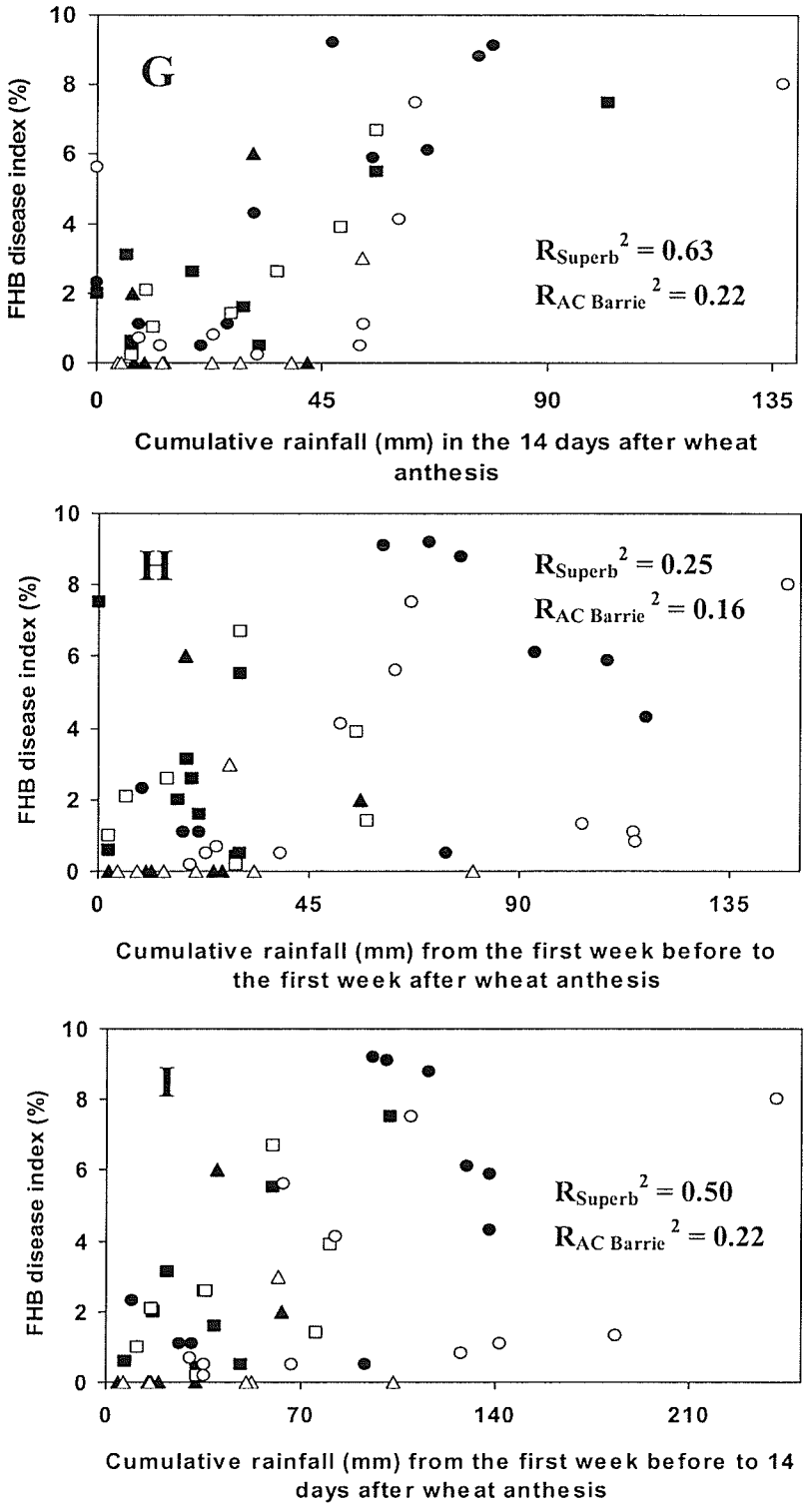


Figure 5 (G-I). Relationship between fusarium head blight (FHB) index (%) and cumulative rainfall (mm) during the different periods.

Average daily temperature vs. FHB. Effect of average daily temperature on FHB disease index was not as strong as cumulative rainfall (Fig. 6). For Superb, average daily temperature from the first week before to the first and second weeks after anthesis significantly affected FHB index (Fig. 6). R^2 values ranged from 0.13 to 0.23. For AC Barrie, average daily temperature from seeding to anthesis, in the first week after anthesis, and from the first week before to the first and second weeks after anthesis significantly affected FHB index (Fig. 6). R^2 value ranged from 0.15 to 0.20.

With increasing average daily temperature from the first week before to the first week after anthesis from 18 to 22°C for Superb and from 17 to 21°C for AC Barrie, FHB index increased from 0% to 9% and 8%, respectively (Fig. 7A). A similar increasing trend was apparent in the relationship between the disease index and average daily temperature from the first week before to the second week after anthesis (Fig. 7B). There was a higher level of FHB index in Superb than AC Barrie at the same or similar levels of temperature (Fig. 7A and 7B).

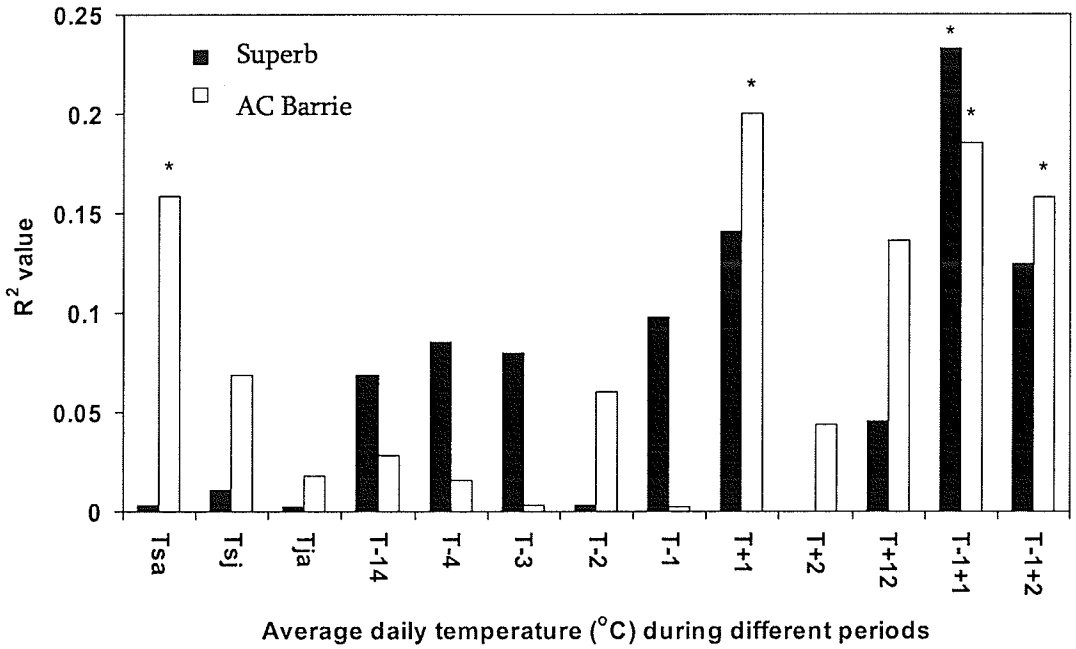


Figure 6. R^2 values for the relationship between fusarium head blight (FHB) index (%) and average daily temperature (°C) from seeding to 14 days after anthesis.
The stars on the top of bars represented significant relationship at 5% level. T_{sa}: cumulative rainfall from seeding to anthesis. T_{sj}: cumulative rainfall from seeding to jointing. T_{ja}: cumulative rainfall from jointing to anthesis. T_{-14}: cumulative rainfall in the 28 days prior to anthesis. T_{-4}: cumulative rainfall in the fourth week prior to anthesis. T_{-3}: cumulative rainfall in the third week prior to anthesis. T_{-2}: cumulative rainfall in the second week prior to anthesis. T_{-1}: cumulative rainfall in the first week prior to anthesis. T_{+1}: cumulative rainfall in the first week after anthesis. T_{+2}: cumulative rainfall in the second week after anthesis. T_{+12}: cumulative rainfall in the 14 days after anthesis. T_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis. T_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

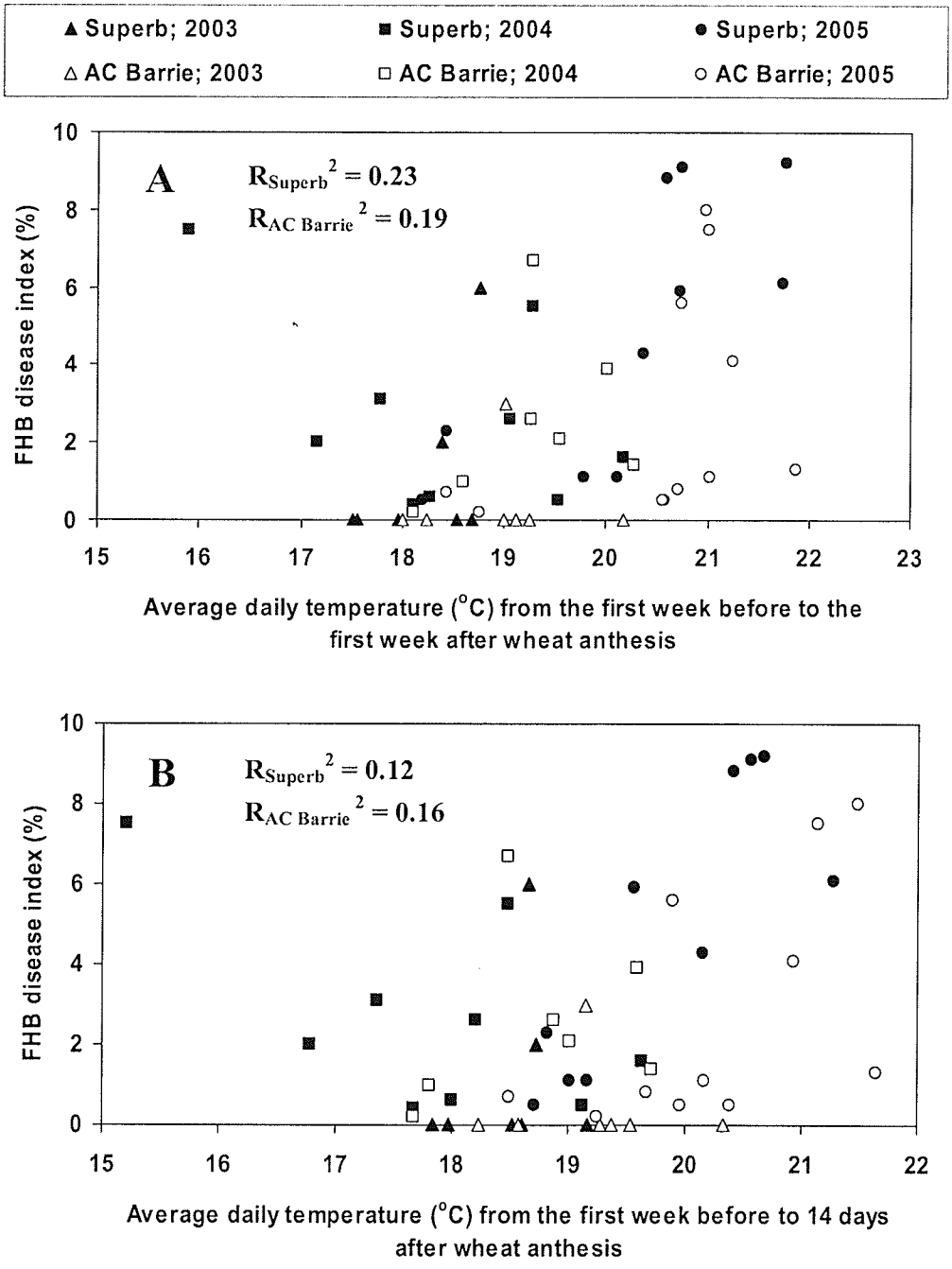


Figure 7 (A and B). Relationships between fusarium head blight (FHB) disease index (%) and average daily temperature (°C) during the different periods.

The number of *F. graminearum* spores and cropping practices vs. DON accumulation. Both the actual and predicted numbers of *F. graminearum* spore counts on wheat heads significantly affected DON levels for cultivars Superb and AC Barrie (Fig. 8). R^2 values for the linear relationship between actual spore counts and DON levels were 0.81 and 0.78 for Superb and AC Barrie, respectively (Fig. 8A), and R^2 values for the relationship between predicted spore counts and DON levels were 0.63 and 0.57 for Superb and AC Barrie (Fig. 8B). This indicates that both actual and predicted *F. graminearum* spore counts on wheat heads was a good predictor for DON levels.

There was significant relationship between CPI and DON levels for both cultivars (Fig. 9). This indicates that the more cereal crops are grown, more zero or minimum tillage and resistant wheat cultivars are used in the previous years, the higher level of DON concentration in the kernels at maturity, and CPI was a good factor for predicting DON levels. There was a great variation of DON levels around the point of CPI 80 (Fig. 9), and the similar variation was found in the relationship between CPI and FHB index (Fig. 3). This was likely caused by the difference in weather conditions between locations in the previous years and the difference in *G. zeae* perithecial formation in the current year.

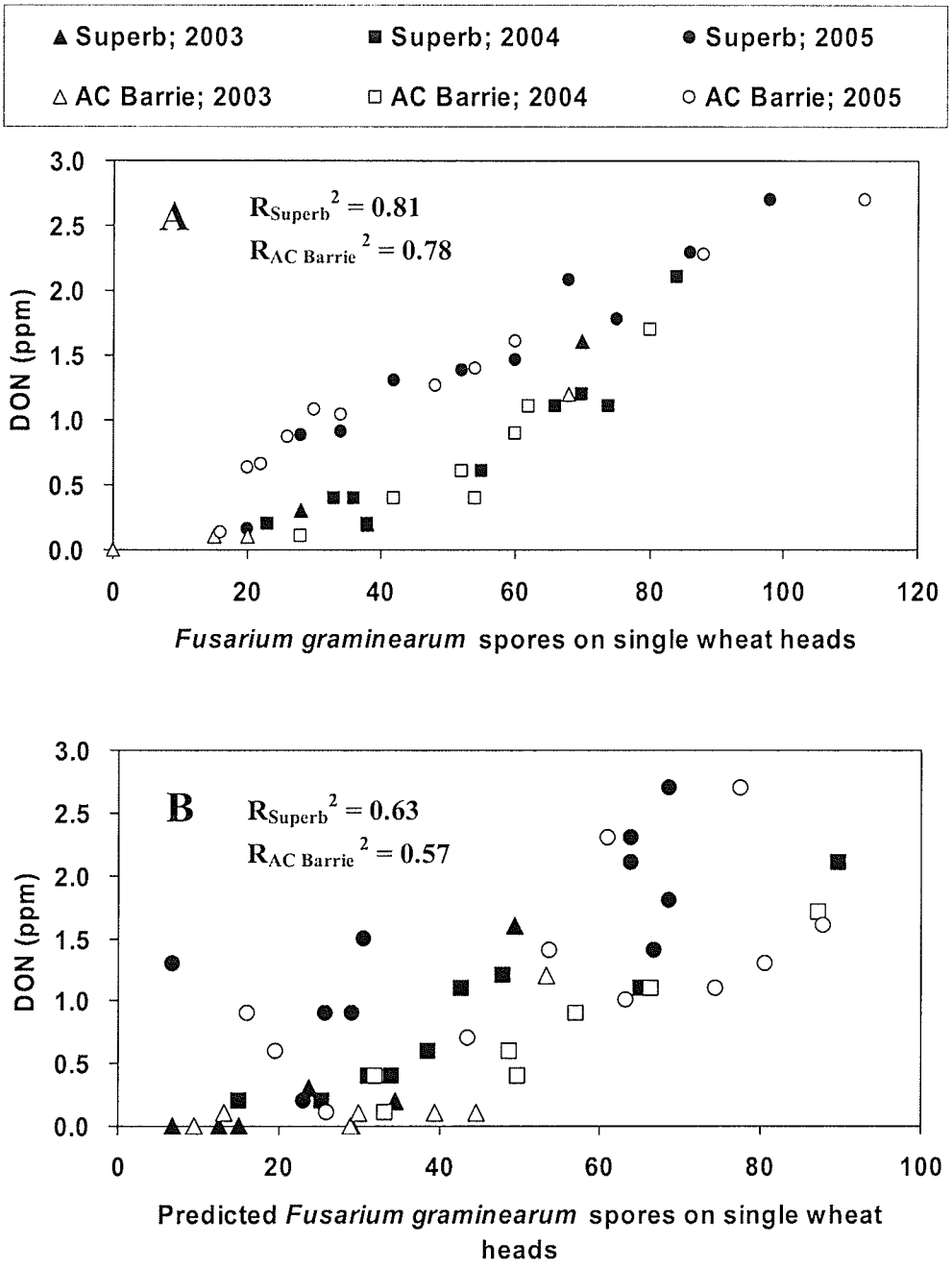


Figure 8. Relationships between deoxynivalenol (DON) (ppm) in grains and the actual (A) and predicted (B) number of *Fusarium graminearum* spores on artificial wheat heads (spore traps).

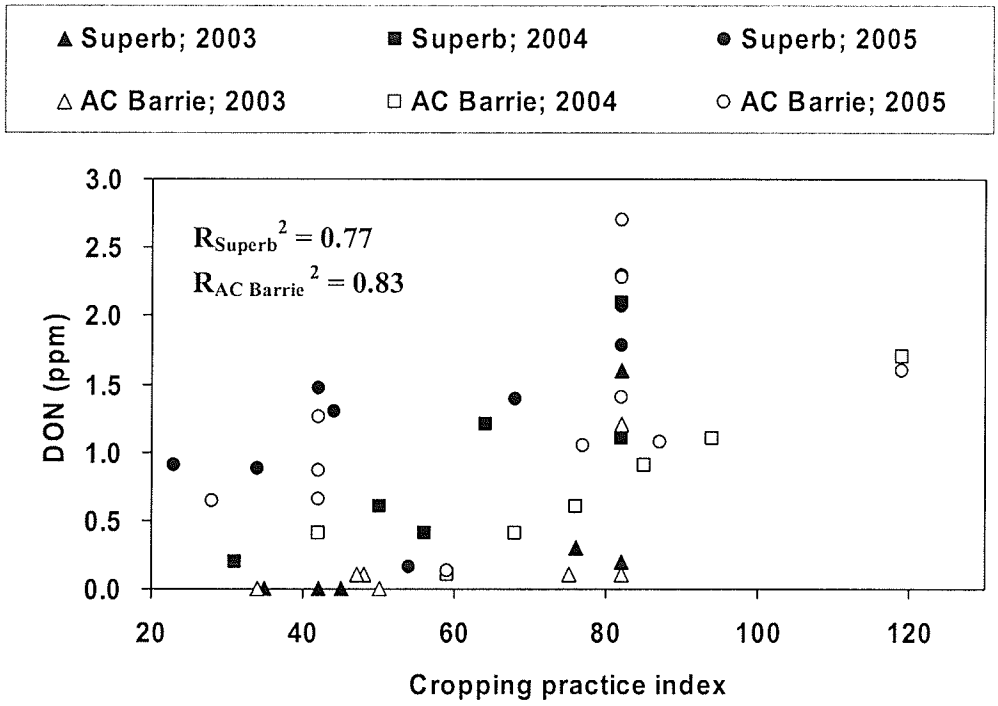


Figure 9. Relationships between deoxynivalenol (DON) (ppm) in wheat kernels and cropping practice index.

Cumulative rainfall and average daily temperature vs. DON accumulation. Cumulative rainfall in the second, third and fourth weeks prior to anthesis, in the first and second weeks after anthesis, and from jointing to anthesis significantly improved DON levels for both cultivars Superb and AC Barrie (Fig. 10). The effects of cumulative rainfall during these periods contributed to the effects of rainfall during other periods (Fig. 10). This was different from the relationships between cumulative rainfall and FHB index, in which Superb was more sensitive to the rainfall than AC Barrie (Fig. 4). R^2 values for AC Barrie for the rainfall was greater than Superb during the following periods: the first, second and third weeks and four weeks prior to

anthesis, from jointing and seeding to anthesis, the second week after anthesis, and from the first week before to the first and second weeks after anthesis. This indicates that AC Barrie is more sensitive to rainfall in DON accumulation than Superb, and there was a better relationship between rainfall and DON accumulation for AC Barrie than for Superb. However, it does not indicate that more DON was accumulated in AC Barrie than Superb.

With increase of cumulative rainfall in the second, third and fourth weeks prior to anthesis, DON level significantly increased for both cultivars (Fig. 10, 11B, 11C and 11D). The effects of rainfall during the above periods contributed to the effects of rainfall in the four weeks prior to anthesis. Significant effect of rainfall from jointing to anthesis resulted in the effect of rainfall from seeding to anthesis on DON levels, because rainfall from seeding to jointing had no effect on DON levels (Fig. 10). This indicates that cumulative rainfall before anthesis plays an important role in the perithecial development and formation, and in turn the spores discharged at anthesis and DON accumulation at maturity.

Cumulative rainfall in the first and second weeks after anthesis significantly affected DON levels for both cultivars (Fig. 10). This indicates that rainfall during the two periods improved spore dispersal and infection on wheat heads, which resulted in increase of DON levels. The effects of rainfall during the same periods contributed to the effects of rainfall in the two weeks after anthesis and from the first week before to the first and second weeks after anthesis on DON levels (Fig. 10). R^2 values for the cumulative rainfall in the two weeks after anthesis were greater than the values for the rainfall in the four week prior to anthesis for both cultivars (Fig. 10), because rainfall before anthesis improved peritheical formation but not spores discharged at anthesis,

and the rainfall in the two weeks after anthesis improved not only spore dispersal but also infection.

Effects of cumulative rainfall on DON levels prior to anthesis in 2003 and 2004 were different from in 2005 (Fig. 11A, 11B, 11C and 11D). There was a higher level of rainfall in 2005 than in 2003 and 2004. For example, when rainfall in the second week prior to anthesis increased from approximately 5 to 35mm, DON concentration increased from 0 to 1.7ppm in 2003 and 2004, and when rainfall increased from 70 to 155mm, DON concentration increased from 0.6 to 2.7ppm (Fig. 11D). However, it is feasible to pool all the data from 2003 to 2005 to analyze the relationship between rainfall and DON levels and to develop prediction models.

There were fewer periods for average daily temperature significantly affecting DON levels than cumulative rainfall for both cultivars (Fig. 12). Average daily temperature in the first week after anthesis and from the first week before to the first week after anthesis affected DON levels for both cultivars. This indicates that rainfall effect was greater than temperature effect and could mask temperature effect on DON levels. Year effect on the relationship between temperature and DON levels was also found (Fig. 13A and 13B). Higher temperatures in 2005 most likely caused the rapid increase of DON levels than in 2003 and 2004.

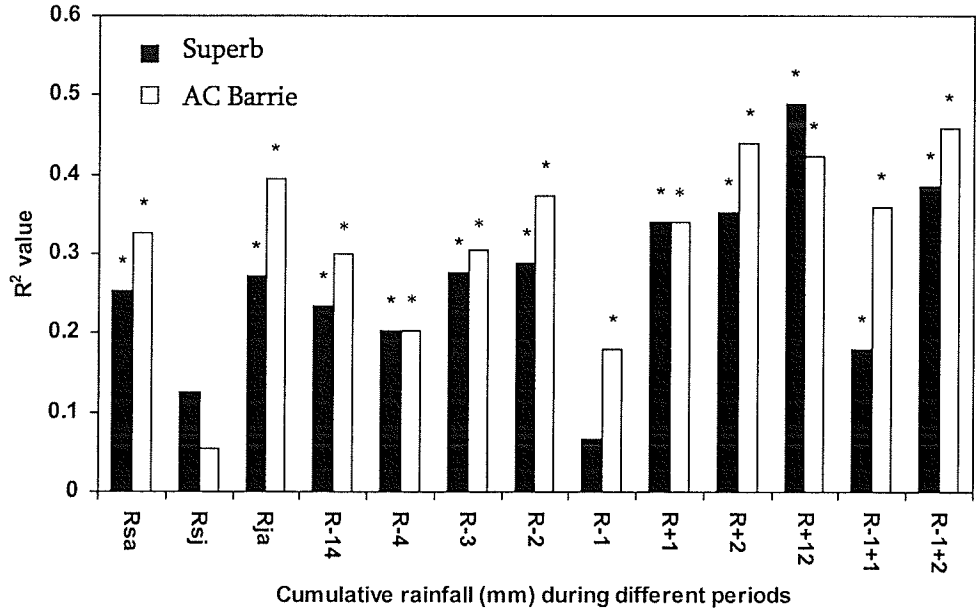


Figure 10. R^2 values for the relationships between deoxynivalenol (DON) (ppm) and cumulative rainfall (mm) from seeding to 14 days after anthesis.
The stars on the top of bars represented significant relationship at 5% level.
R_{sa}: cumulative rainfall from seeding to anthesis. R_{sj}: cumulative rainfall from seeding to jointing. R_{ja}: cumulative rainfall from jointing to anthesis. R_{-14}: cumulative rainfall in the 28 days prior to anthesis. R_{-4}: cumulative rainfall in the fourth week prior to anthesis. R_{-3}: cumulative rainfall in the third week prior to anthesis. R_{-2}: cumulative rainfall in the second week prior to anthesis. R_{-1}: cumulative rainfall in the first week prior to anthesis. R_{+1}: cumulative rainfall in the first week after anthesis. R_{+2}: cumulative rainfall in the second week after anthesis. R_{+12}: cumulative rainfall in the 14 days after anthesis. R_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis. R_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

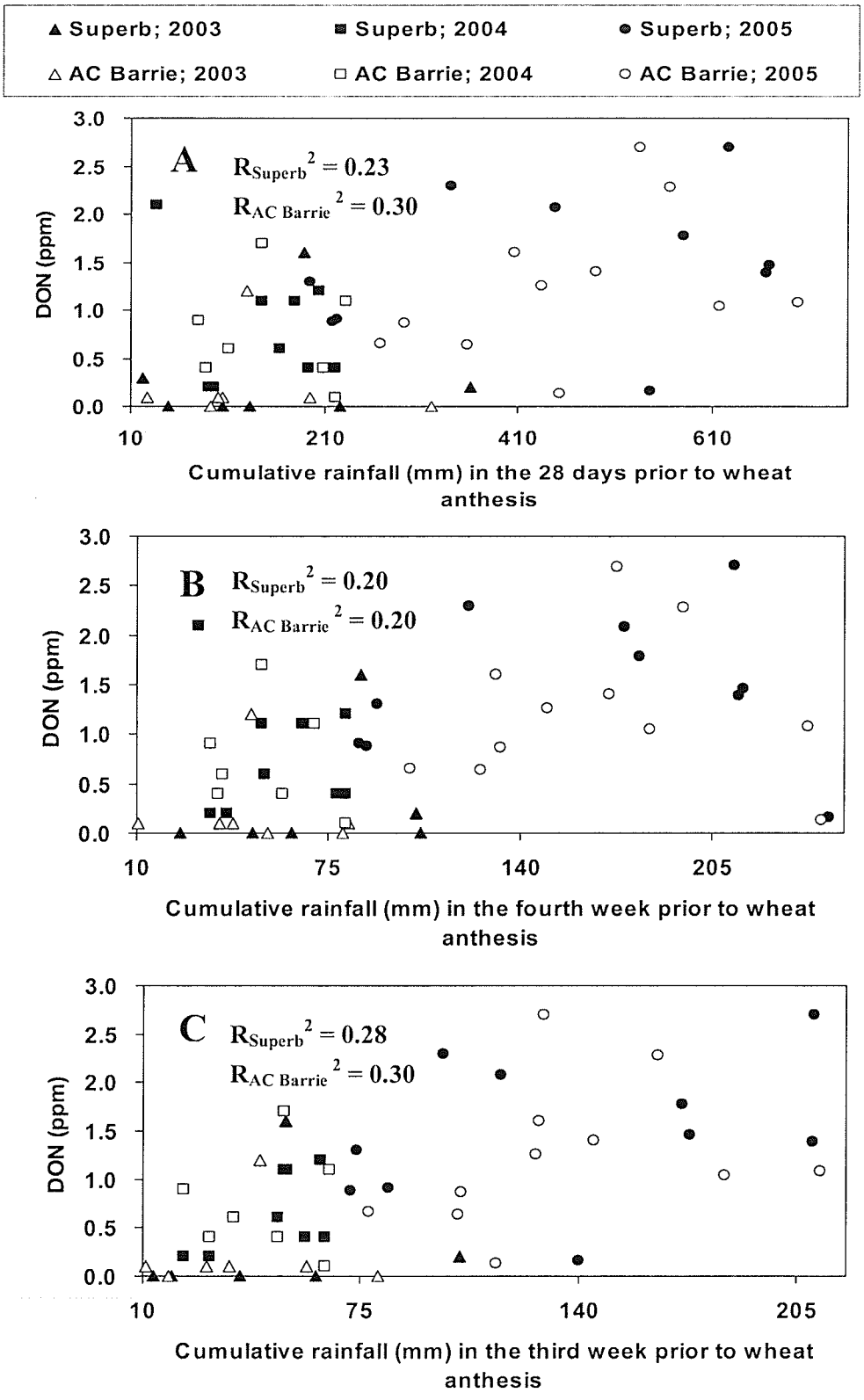


Figure 11 (A-C). Relationship between deoxynivalenol (DON) (ppm) and cumulative rainfall (mm) during the different periods.

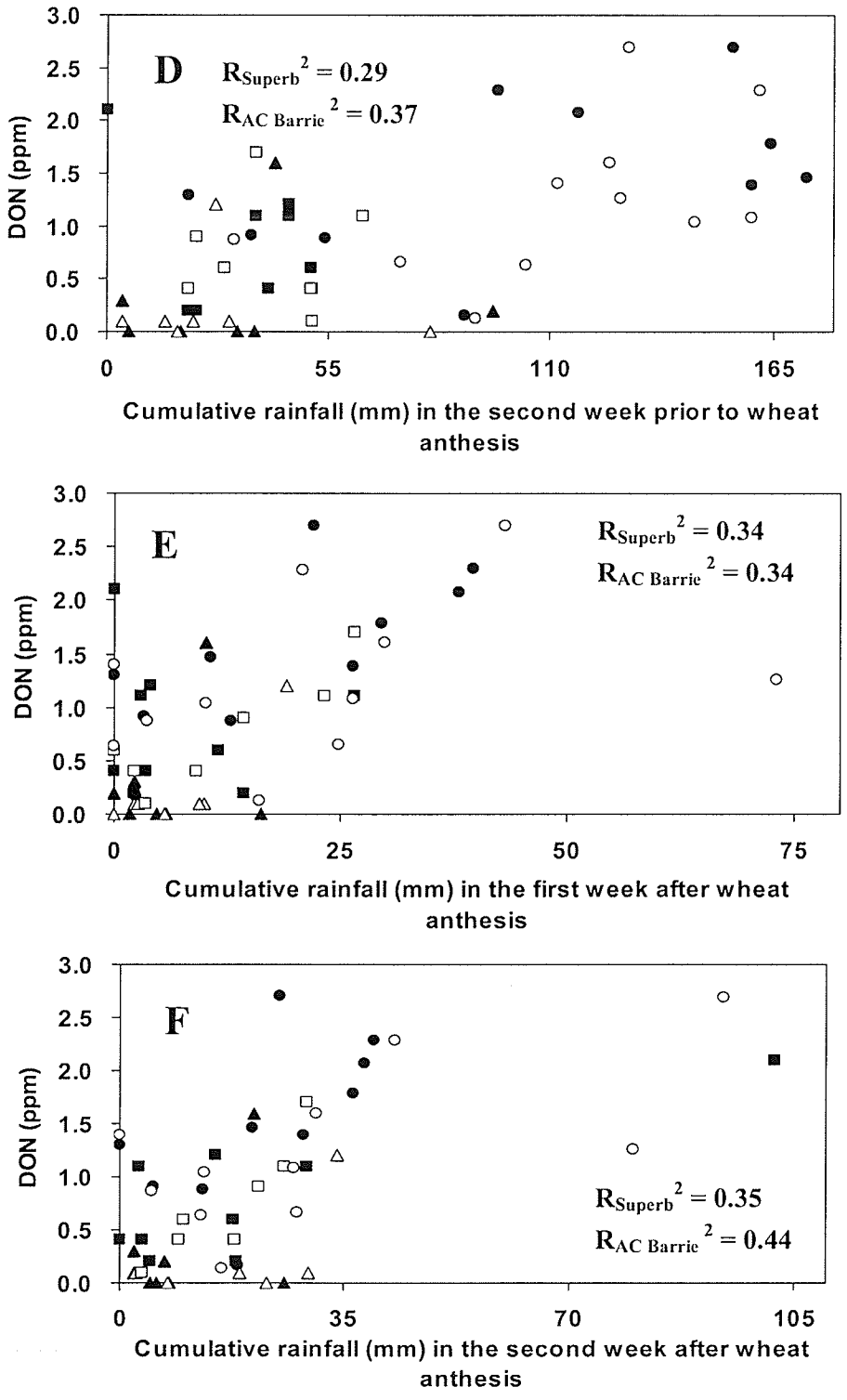


Figure 11 (D-F). Relationship between deoxynivalenol (DON) (ppm) and cumulative rainfall (mm) during the different periods.

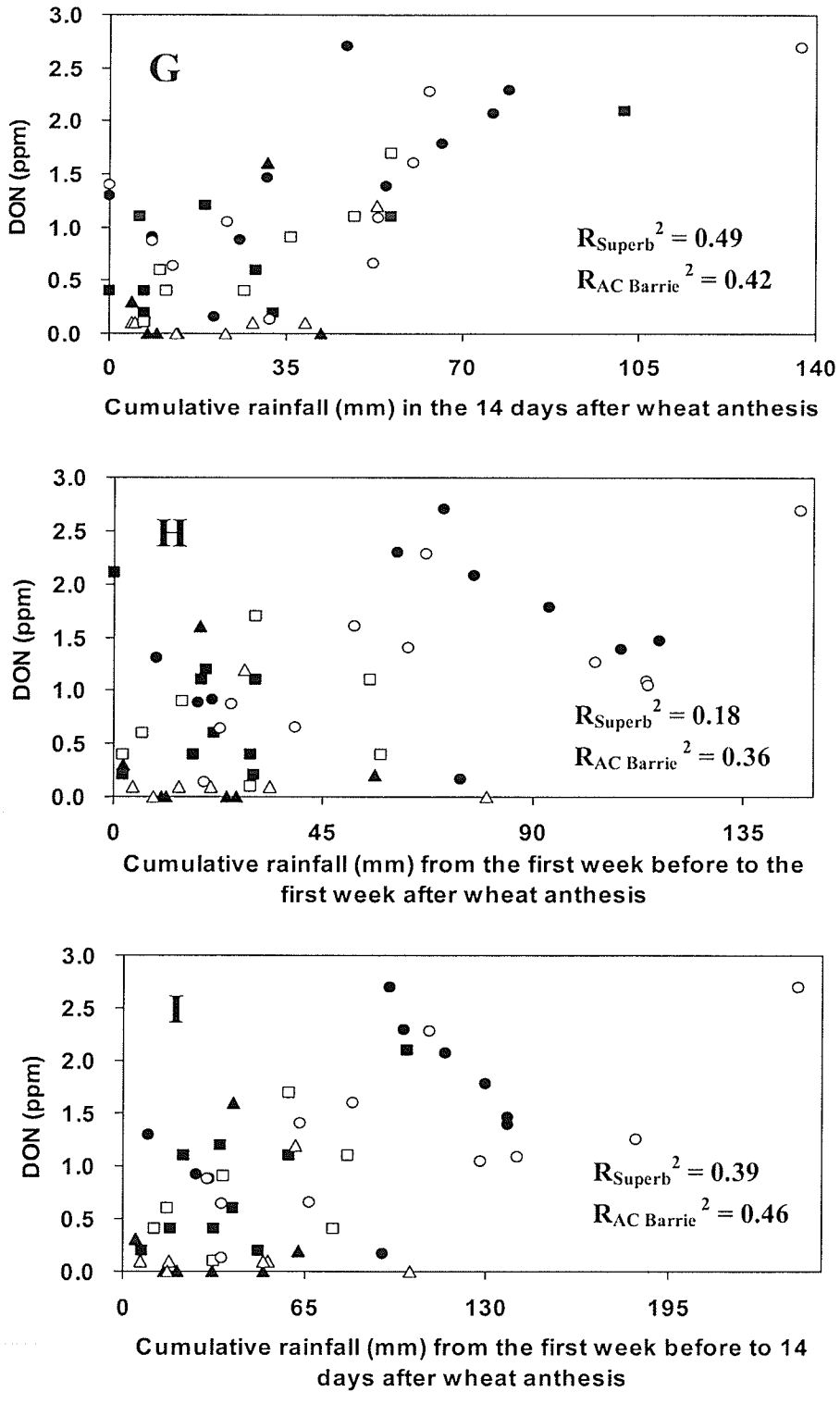


Figure 11 (G-I). Relationship between deoxynivalenol (DON) (ppm) and cumulative rainfall (mm) during the different periods.

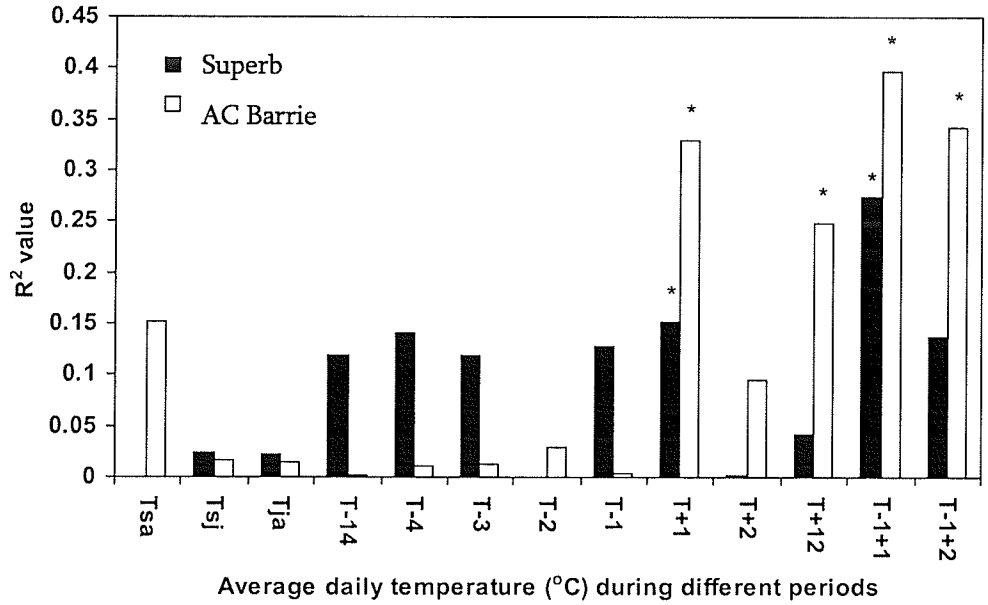


Figure 12. R^2 values for the relationships between deoxynivalenol (DON) (ppm) and average daily temperature (°C) from seeding to 14 days after anthesis.
The stars on the top of bars represented significant relationship at 5% level. T_{sa}: cumulative rainfall from seeding to anthesis. T_{sj}: cumulative rainfall from seeding to jointing. T_{ja}: cumulative rainfall from jointing to anthesis. T_{-14}: cumulative rainfall in the 28 days prior to anthesis. T_{-4}: cumulative rainfall in the fourth week prior to anthesis. T_{-3}: cumulative rainfall in the third week prior to anthesis. T_{-2}: cumulative rainfall in the second week prior to anthesis. T_{-1}: cumulative rainfall in the first week prior to anthesis. T_{+1}: cumulative rainfall in the first week after anthesis. T_{+2}: cumulative rainfall in the second week after anthesis. T_{+12}: cumulative rainfall in the 14 days after anthesis. T_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis. T_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

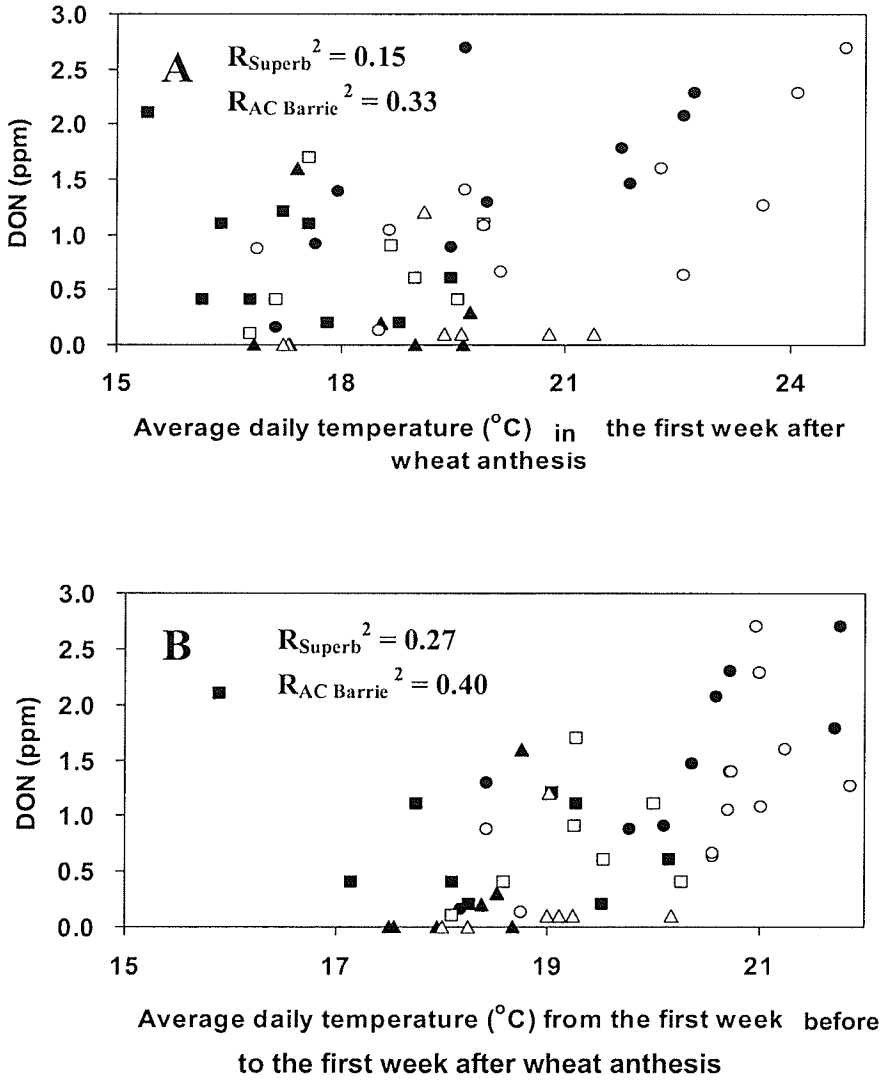


Figure 13 (A and B). Relationship between deoxynivalenol (DON) (ppm) and average daily temperature (°C) during the different periods.

Prediction models for FHB index and DON accumulation. Two types of prediction models were developed for each of FHB index and DON level for each wheat cultivar using a stepwise procedure, and in turn twenty Type I models for both disease index and toxin concentration and both cultivars with actual spore number collected from the fields, and twenty-four Type II models with using predicted spore number were obtained (Table 3). Variables actual *F. graminearum* spore counts, CPI, R_{-14}, R_{-4}, R_{-3}, R_{-2}, R_{-1}, R_{+1}, R_{+2}, R_{+12}, R_{-1+1}, R_{-1+2}, R_{-2+1}, R_{-2+2}, R_{-3+1}, R_{-3+2}, R_{-4+1}, R_{-4+2}, R_{-1+2}, R_{-12}, R_{-13}, R_{-14}, R_{-23}, R_{-24} and R_{-34}, and T_{-14}, T_{-4}, T_{-3}, T_{-2}, T_{-1}, T_{-+1}, T_{+2}, T_{+12}, T_{-1+1}, T_{-1+2}, T_{-2+1}, T_{-2+2}, T_{-3+1}, T_{-3+2}, T_{-4+1}, T_{-4+2}, T_{-1+2}, T_{-12}, T_{-13}, T_{-14}, T_{-23}, T_{-24} and T_{-34}, and interactions between cumulative rainfall and average daily temperature (RT) during the same periods were selected for the development of Type I models, and predicted *F. graminearum* spore counts, CPI, and same weather factors and their interactions were used for the development of Type II models. The weather conditions from seeding to jointing, from jointing to anthesis and from seeding to anthesis were not considered in the model development, because seeding and jointing dates varied with different years, which would most likely cause prediction errors in the model application.

There were ten Type I regression models for FHB index for Superb (Table 3A). Independent variables in Models 1, 2, 4, 7, 8, 9 and 10 significantly affected the disease index, and the ten models were statistically acceptable. However, Models 4, 7, 8, 9 and 10 contained 4, 5, 6, 7 and 8 independent variables, which would affect reliability of these models in application, because the ratio of sampling units to predictors in a prediction model should be greater than 10:1 (Steel et al., 1997), and there were 25 samples for the model development for each cultivar in this study, therefore Models 1 and 2 were acceptable in application. Model 2 contained two variables S_a (actual spore counts) and RT_{+12}, VIF value for multicollinearity of the

two variables was 1.43, and it had higher R^2 value (0.88) than Model 1, thus Model 2 was selected for predicting FHB index. There was one Type I model with one variable S_a (Model 11) obtained for FHB index for AC Barrie, R^2 value was 0.83 (Table 3B).

Five Type I regression models for DON level for Superb were obtained, including Models 12, 13, 14, 15 and 16 (Table 3C). All the variables in the models were significant; however, Models 14, 15 and 16 contained more than two variables, therefore, they were not acceptable. Model 13 contained two variables S_a and T_{-4}, VIF value for these was 1.0, and the R^2 value (0.86) was greater than Model 12. Thus, Model 3 was used for DON prediction for Superb. Four Type I regression models were obtained for AC Barrie, including Models 17, 18, 19 and 20 (Table 3D). However, Models 19 and 20 contained more than two variables and some variables did not significantly affect DON level. Model 18 was acceptable because it had a greater R^2 value (0.91) than Model 17, included two variables with VIF value of 1.04.

There were two Type II regression models obtained for FHB index for Superb including Models 21 and 22 and two for AC Barrie including Models 23 and 24 (Table 3E and 3F). Model 22 including variables S_p (predicted spores) and T_{+1} with R^2 value of 0.85 and Model 23 including variable S_p with R^2 value of 0.45 were acceptable. There were nine and eleven Type II regression models obtained for DON level for Superb and AC Barrie, respectively (Table 3G and 3H). Model 26 for Superb including variables S_p and RT_{-3} with R^2 value of 0.74 and Model 35 for AC Barrie including variables S_p and RT_{-2} with R^3 value of 0.65 were acceptable.

Table 3A. Development of models with actual *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict FHB index (%) for cultivar Superb using a stepwise procedure.

Model #	Items	Models: FHB index =						
1	Variable	-1.25525	$0.09579S_a$					
	$P > F$	0.039	<0.0001					
	R^2	0.77 (<0.0001)						
2	Variable	-1.37864	$0.06565S_a$	$0.00265RT_{+12}$				
	$P > F$	0.003	<0.0001	0.0001				
	VIF		1.43	1.43				
	R^2	0.88 (<0.0001)						
3	Variable	-7.42108	$0.06385S_a$	$0.34781T_{-4}$	$0.00259RT_{+12}$			
	$P > F$	0.029	<0.0001	0.068	<0.0001			
	R^2	0.90 (<0.0001)						
4	Variable	-4.77044	$0.06988S_a$	$-0.52143T_{-2}$	$0.74768T_{-4}$	$0.00200RT_{+12}$		
	$P > F$	0.122	<0.0001	0.013	0.003	0.001		
	R^2	0.93 (<0.0001)						
5	Variable	-0.03886	$0.06914S_a$	$-0.80036T_{-2}$	$1.06705T_{-4}$	$-0.28235T_{+12}$	$0.00206RT_{+12}$	
	$P > F$	0.992	<0.0001	0.002	0.001	0.064	0.003	
	R^2	0.94 (<0.0001)						
6	Variable	4.37482	$0.06901S_a$	$-0.92425T_{-2}$	$1.16532T_{-4}$	$-0.49253T_{+12}$	$0.00300RT_{+1}$	$0.00092RT_{+12}$
	$P > F$	0.303	<0.0001	0.001	0.0002	0.010	0.067	0.226
	R^2	0.95 (<0.0001)						
7	Variable	6.73235	$0.07272S_a$	$-1.03680T_{-2}$	$1.25598T_{-4}$	$-0.59365T_{+12}$	$0.00453RT_{+1}$	
	$P > F$	0.086	<0.0001	<0.0001	<0.0001	0.001	0.001	
	R^2	0.94 (<0.0001)						

Table 3A (continued). Development of models with actual *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict FHB index (%) for cultivar Superb using a stepwise procedure.

Model #	Items	Models: FHB index =								
8	Variable	12.50598	$0.07041S_a$	$-1.10919T_{-2}$	$1.03913T_{-4}$	$-0.65599T_{+12}$	$0.00040RT_{-4}$	$0.00422RT_{+1}$		
	$P > F$	0.010	<0.0001	<0.0001	0.0004	0.0002	0.045	0.0001		
	R^2	0.96 (<0.0001)								
9	Variable	16.76634	$0.07472S_a$	$-0.61614T_{-2}$	$1.27317T_{-4}$	$-0.46001T_{+12}$	$-1.17576T_{-4+1}$	$0.00048RT_{-4}$	$0.00543RT_{+1}$	
	$P > F$	0.001	<0.0001	0.046	<0.0001	0.010	0.043	0.014	<0.0001	
	R^2	0.96 (<0.0001)								
10	Variable	26.12881	$0.07479S_a$	$-0.87826T_{-2}$	$0.99087T_{-4}$	$-0.09112R_{-4}$	$-0.49623T_{+12}$	$-1.10165T_{-4+1}$	$0.00540RT_{-4}$	$0.00516RT_{+1}$
	$P > F$	0.0003	<0.0001	0.007	0.001	0.039	0.003	0.038	0.026	<0.0001
	R^2	0.97 (<0.0001)								

Table 3B. Development of models with actual *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict FHB index (%) for cultivar AC Barrie using a stepwise procedure.

Model #	Items	Models: FHB index =	
11	Variable	-1.31754	$0.08201S_a$
	$P > F$	0.002	<0.0001
	R^2	0.83 (<0.0001)	

Table 3C. Development of models with actual *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict DON levels (ppm) for cultivar Superb using a stepwise procedure.

Model #	Items	Models: DON =					
12	Variable	-0.20793	$0.02539S_a$				
	$P > F$	0.136	<0.0001				
	R^2	0.81 (<0.0001)					
13	Variable	-3.00743	$0.02428S_a$	$0.16108T_{-4}$			
	$P > F$	0.004	<0.0001	0.007			
	VIF		1.0	1.0			
	R^2	0.86 (<0.0001)					
14	Variable	-2.00818	$0.02405S_a$	$-0.18528T_{-2}$	$0.29929T_{-4}$		
	$P > F$	0.016	<0.0001	0.001	<0.0001		
	R^2	0.92 (<0.0001)					
15	Variable	-0.60969	$0.02278S_a$	$-0.26309T_{-2}$	$0.40161T_{-4}$	$-0.09361T_{+2}$	
	$P > F$	0.492	<0.0001	<0.0001	<0.0001	0.017	
	R^2	0.94 (<0.0001)					
16	Variable	-1.21045	$0.02016S_a$	$-0.21229T_{-2}$	$0.37351T_{-4}$	$-0.29170T_{+2}$	$0.21087T_{+12}$
	$P > F$	0.162	<0.0001	0.001	<0.0001	0.004	0.030
	R^2	0.95 (<0.0001)					

Table 3D. Development of models with actual *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict DON levels (ppm) for cultivar AC Barrie using a stepwise procedure.

Model #	Items	Models: DON =				
17	Variable	-0.13404	$0.02325S_a$			
	$P > F$	0.298	<0.0001			
	R^2	0.78 (<0.0001)				
18	Variable	-0.44836	$0.02125S_a$	$0.00025481RT_{-3}$		
	$P > F$	0.0002	<0.0001	<0.0001		
	VIF		1.04	1.04		
	R^2	0.91 (<0.0001)				
19	Variable	-0.45349	$0.02120S_a$	$0.00071RT_{-3}$	$-0.0001286RT_{-14}$	
	$P > F$	0.0001	<0.0001	0.026	0.135	
	R^2	0.92 (<0.0001)				
20	Variable	-0.39792	$0.01875S_a$	$0.0007853RT_{-3}$	$-0.00023RT_{-14}$	$0.000107RT_{-3+2}$
	$P > F$	0.492	<0.0001	<0.0001	<0.0001	0.017
	R^2	0.94 (<0.0001)				

Table 3E. Development of models with predicted *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict FHB index (%) for cultivar Superb using a stepwise procedure.

Model #	Items	Models: FHB index =		
21	Variable	−1. 67029	0. 12259S_p	
	P > F	0.015	<0.0001	
	R^2	0.75 (<0.0001)		
22	Variable	-10.87376	0.11940S_p	0.50003T_{+1}
	P > F	0.0001	<0.0001	0.001
	VIF		1.00	1.00
	R^2	0.85 (<0.0001)		

Table 3F. Development of models with predicted *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict FHB index (%) for cultivar AC Barrie using a stepwise procedure.

Model #	Items	Models: FHB index =		
23	Variable	-1.41905	0.07241S_p	
	P > F	0.125	0.0003	
	R^2	0.45 (0.0003)		
24	Variable	11.83580	0.07748S_p	-0.70629T_{-12}
	P > F	0.106	<0.0001	0.070
	R^2	0.85 (<0.0001)		

Table 3G. Development of models with predicted *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict DON levels (ppm) for cultivar Superb using a stepwise procedure.

Model #	Items	Models: DON =					
25	Variable	-0.17896	$0.02891S_p$				
	P > F	0.385	<0.0001				
	R^2	0.63 (<0.0001)					
26	Variable	-2.74096	$0.02802S_p$	$0.02802T_{+1}$			
	P > F	0.003	<0.0001	0.004			
	VIF		1.11	1.11			
	R^2	0.74 (<0.0001)					
27	Variable	-5.33585	$0.02672S_p$	$0.12815T_{+1}$	$0.15885T_{-34}$		
	P > F	0.002	<0.0001	0.005	0.059		
	R^2	0.78 (<0.0001)					
28	Variable	-4.90544	$0.01921S_p$	$0.29291T_{+1}$	$-0.25865T_{+2}$	$0.24294T_{-34}$	
	P > F	0.002	0.0002	0.0004	0.012	0.005	
	R^2	0.84 (<0.0001)					
29	Variable	4.37482	$0.00885S_p$	0.01034CPI	$0.36342T_{+1}$	$-0.37305T_{+2}$	$0.27397T_{-34}$
	P > F	0.001	0.246	0.110	0.0002	0.004	0.002
	R^2	0.86 (<0.0001)					
30	Variable	-4.89762	0.01649CPI	$0.43067T_{+1}$	$-0.47955T_{+2}$	$0.31090T_{-34}$	
	P > F	0.001	<0.0001	<0.0001	<0.0001	0.0002	
	R^2	0.85 (<0.0001)					
31	Variable	-3.92382	0.01598CPI	$0.39636T_{+1}$	$-0.49159T_{+2}$	$-0.38392T_{-24}$	$0.69435T_{-34}$
	P > F	0.011	0.0001	<0.0001	<0.0001	0.134	0.013
	R^2	0.87 (<0.0001)					

Table 3G (continued). Development of models with predicted *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict DON levels (ppm) for cultivar Superb using a stepwise procedure.

Model #	Items	Models: DON =							
32	Variable	-4.14931	0.01610CPI	$0.34336T_{+1}$	$-0.44389T_{+2}$	$0.25266T_{-13}$	$-0.92133T_{-24}$	$0.99276T_{-34}$	
	$P > F$	0.005	<0.0001	<0.0001	<0.0001	0.046	0.014	0.002	
	R^2	0.89 (<0.0001)							
33	Variable	-5.61373	0.01876CPI	$0.40070T_{+1}$	$-0.46223T_{+2}$	$-0.01670R_{+1}$	$0.42406T_{-13}$	$-1.26579T_{-24}$	$1.20441T_{-34}$
	$P > F$	0.001	<0.0001	<0.0001	<0.0001	0.100	0.011	0.004	0.001
	R^2	0.91 (<0.0001)							

Table 3H. Development of models with predicted *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict DON levels (ppm) for cultivar AC Barrie using a stepwise procedure.

Model #	Items	Models: DON =							
34	Variable	-0.32079	$0.02382S_p$						
	$P > F$	0.177	<0.0001						
	R^2	0.57 (<0.0001)							
35	Variable	-0.25900	$0.01742S_p$	$0.00052528RT_{+2}$					
	$P > F$	0.238	0.002	0.032					
	VIF		1.49	1.49					
	R^2	0.65 (<0.0001)							
36	Variable	-0.38053	$0.02310S_p$	$-0.02029R_{+12}$	$0.00187RT_{+2}$				
	$P > F$	0.086	0.0004	0.066	0.018				
	R^2	0.70 (<0.0001)							
37	Variable	-0.76836	$0.02636S_p$	$-0.02788R_{+12}$	$0.01048R_{34}$	$0.00233RT_{+2}$	$-0.00030469RT_{-14}$		
	$P > F$	0.003	<0.0001	0.010	0.025	0.003	0.044		
	R^2	0.79 (<0.0001)							
38	Variable	-0.75408	$0.02087S_p$	$-0.02389R_{+12}$	$0.02433R_{34}$	$-0.00078214RT_{-4}$	$0.00218RT_{+2}$	$-0.00048091RT_{-14}$	
	$P > F$	0.002	0.001	0.017	0.004	0.042	0.003	0.005	
	R^2	0.84 (<0.0001)							
39	Variable	-4.18846	$0.01816S_p$	$0.17837T_{-1}$	$-0.03557R_{+12}$	$0.02945R_{34}$	$-0.000817RT_{-4}$	$0.00337RT_{+2}$	$-0.000657RT_{-14}$
	$P > F$	0.025	0.003	0.059	0.003	0.0001	0.024	0.001	0.001
	R^2	0.87 (<0.0001)							
40	Variable	-6.79115	$-0.0162S_p$	0.01994CPI	$0.29728T_{-1}$	$-0.02224R_{+12}$	$0.035R_{34}$	$-0.0012RT_{-4}$	$0.003RT_{+2}$	$-0.0007\ RT_{-14}$
	$P > F$	0.002	0.299	0.029	0.005	0.052	0.0001	0.002	0.004	0.003
	R^2	0.90 (<0.0001)							

Table 3H (continued). Development of models with predicted *Fusarium graminearum* spore counts on artificial wheat heads (spore traps) to predict DON levels (ppm) for cultivar AC Barrie using a stepwise procedure.

Model #	Items	Models: DON =							
41	Variable	-5.59123	0.01143CPI	0.23998T_{-1}	-0.02894R_{+12}	0.03190R_{34}	-0.00101RT_{-4}	0.00334RT_{+2}	-0.0006651RT_{-14}
	$P > F$	0.002	0.0004	0.006	0.004	0.0001	0.002	0.0003	0.0003
	R^2	0.90 (<0.0001)							
42	Variable	-7.19757	0.01126CPI	0.32555T_{-1}	-0.04056R_{+12}	0.02706R_{34}	0.0176R_{3+1}	0.000185RT_{-4}	0.00356RT_{+2}	-0.0015RT_{-14}
	$P > F$	0.0004	0.0002	0.002	0.001	0.001	0.071	0.785	<0.0001	0.005
	R^2	0.92 (<0.0001)							
43	Variable	-6.97737	0.01122CPI	0.31378T_{-1}	-0.03901R_{+12}	0.02820R_{34}	0.01527R_{3+1}	0.00353RT_{+2}	-0.00141RT_{-14}	
	$P > F$	<0.0001	0.0002	0.0003	0.0001	<0.0001	0.0003	<0.0001	<0.0001	
	R^2	0.92 (<0.0001)							
44	Variable	-3.85445	0.01179CPI	0.37051T_{-1}	-0.03916R_{+12}	0.03087R_{34}	0.01622R_{3+1}	-0.23209T_{-2+1}	0.00366RT_{+2}	-0.0015RT_{-14}
	$P > F$	0.109	<0.0001	0.0001	<0.0001	<0.0001	0.0001	0.113	<0.0001	<0.0001
	R^2	0.93 (<0.0001)							

R_{-14}: cumulative rainfall in the 28 days prior to anthesis. R_{-4}: cumulative rainfall in the fourth week prior to anthesis. R_{-3}: cumulative rainfall in the third week prior to anthesis. R_{-2}: cumulative rainfall in the second week prior to anthesis. R_{-1}: cumulative rainfall in the first week prior to anthesis. R_{+1}: cumulative rainfall in the first week after anthesis. R_{+2}: cumulative rainfall in the second week after anthesis. R_{+12}: cumulative rainfall in the 14 days after anthesis. R_{-1+1}: cumulative rainfall from the first week before to the first week after anthesis. R_{-1+2}: cumulative rainfall from the first week before to the second week after anthesis.

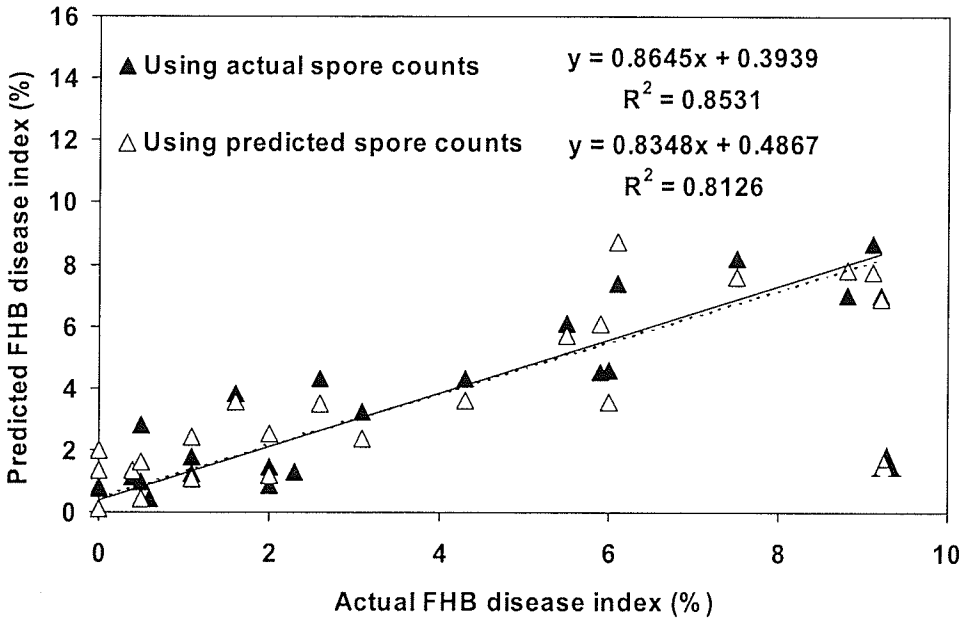
T_{-14}: average daily temperature in the 28 days prior to anthesis. T_{-4}: average daily temperature in the fourth week prior to anthesis. T_{-3}: average daily temperature in the third week prior to anthesis. T_{-2}: average daily temperature in the second week prior to anthesis. T_{-1}: average daily temperature in the first week prior to anthesis. T_{+1}: average daily temperature in the first week after anthesis. T_{+2}: average daily temperature in the second week after anthesis. T_{+12}: average daily temperature in the 14 days after anthesis. T_{-1+1}: average daily temperature from the first week before to the first week after anthesis. T_{-1+2}: average daily temperature from the first week before to the second week after anthesis.

RT: interaction between cumulative rainfall and average daily temperature.

The models in table 3 were selected from all steps of the stepwise procedure until no further improvement in R^2 value was possible.

Model validation. Simple linear regression models were developed using the predicted data sets and corresponding actual data to test the predicting accuracy. R^2 values for Type I FHB models for both cultivars ranged from 0.81 to 0.85, and slope values ranged from 0.79 to 0.86 (Fig. 14 A and 14B). Type II FHB model for AC Barrie had the least R^2 and slope values (Fig. 14B). Higher R^2 (0.89) and slope (0.90) values were found for Type I DON model for AC Barrie than other DON models (Fig. 14C and 14D). The R^2 values for Type I and Type II DON models for Superb and Type II DON model for AC Barrie ranged from 0.49 to 0.83, and the slope values ranged from 0.55 to 0.84.

The field test for two types of models using the actual values from 2006 showed that both Type I and Type II models were close to each other in prediction accuracy for FHB index (Table 4). However, Model 22 had the prediction accuracy of 81% in the full-cross validation, but had a great prediction error in the field test (Fig. 14A). Model 26 had the prediction accuracy of 68% in the full-cross validation, but also had a great prediction error in the field test (Fig. 14C).



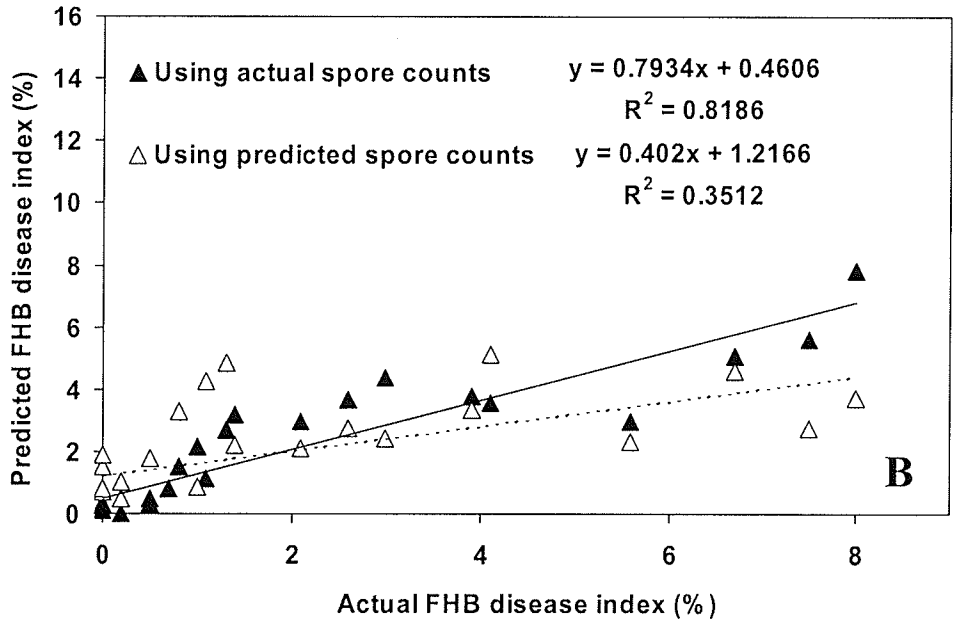


Figure 14A and 14B. Jack-Knife full-cross validation for FHB disease index models for Superb (A) and AC Barrie (B).

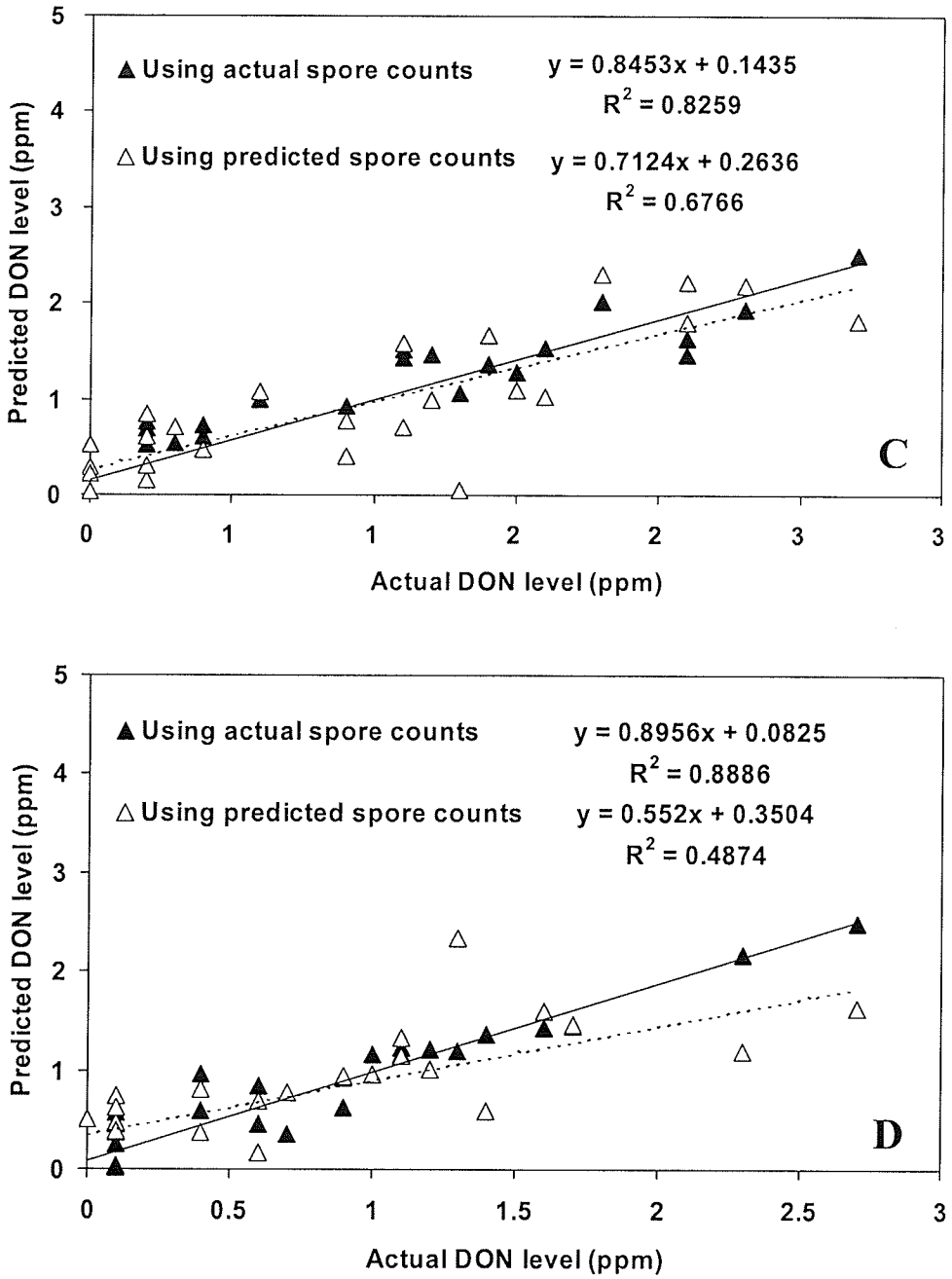


Figure 14C and 14D. Jack-Knife full-cross validation for DON models for Superb (C) and AC Barrie (D).

Table 4. Validation of fusarium head blight (FHB) index and deoxynivalenol (DON) level models by comparing the predicted and actual data.

Model number	2	11	22	23	13	18	26	35
Predicted FHB index (%)	0	0	3.02	0.21	-	-	-	-
Predicted DON level (ppm)	-	-	-	-	0.37	0	0.99	0.14
[a] Average actual value	0.15	0.11	0.15	0.11	0.21	0.16	0.21	0.16

[a] Actual FHB index and DON levels for the three Superb fields and four AC Barrie fields in 2006 were averaged respectively, and used for model testing.

3.4 Discussion

This study first revealed that *Fusarium graminearum/G. zeae* spores on single wheat heads, cropping practices in the previous years, and cumulative rainfall during different periods prior to and after wheat anthesis were critical for FHB and DON accumulation in wheat grains. To my knowledge, this is the first time disease prediction models have taken into account fusarium inoculum and cropping practices which are important for disease epidemics. Two types and total eight prediction models for the disease and toxin levels were obtained in this study, which can be used for different purposes. For Type II models, the number of *F. graminearum* spores on single wheat heads was predicted using Model 6 developed in Chapter 2.

Availability of *F. graminearum* spores for infection. The number of *F. graminearum* spores on single wheat heads is strongly related to FHB disease index

and DON concentration. The errors of the spore prediction models affect the prediction accuracy for the disease and toxin. However, the number of *F. graminearum* spores on a single what head is still a good predictor. The germination rate of spores on wheat heads could be another important factor. Most or all the spores are germinated under conditions of moisture but not in dry areas. The number of available spores for infection was not considered in this study. Deposition of the spores on a wheat head is also important for successful infection. There are different entry paths on a wheat head, through which the pathogen can invade, including stomata, glume's epidermis, openings of florets, and the wounds (Leonard and Bushnell, 2003). The spores, which land in the space between a glume and palea or lemma, have more chance for germination due to the higher moisture compared to spores landing in other positions. The total number of *F. graminearum* spores cannot indicate the above situation, which will partially cause FHB disease and DON prediction errors.

Cropping practices vs. FHB index and DON accumulation. It was not clearly explained how crop rotation affected FHB by previous studies (Gilbert and Tekauz, 1994; Miller et al., 1998). One study reported that the disease was reduced when wheat was rotated with clover, alfalfa, potato and flax, alfalfa and oat, or soybean (Parry et al., 1995). Another study showed that wheat rotation with corn and soybean had no significant effect on the disease (Miller et al., 1998). Different crops and crop sequences in the rotation are likely associated with disease level. A recent study found that canola stubble could harbor *F. graminearum* (Fernando and Gilbert, unpublished data). It is unknown if other crop stubble has the same characteristics. Nevertheless, reduction of FHB by using conventional tillage has been confirmed (Dill-Macky and Jones, 2000). There is no direct evidence that resistance level of wheat cultivars

grown in the previous years affects the fusarium inoculum level on wheat stubble in the current year, though the studies of systemic pathways of infection from wheat heads to stems indirectly proved this hypothesis (Guenther and Trail, 2005). This study showed that cropping practices significantly affected disease and DON accumulation in wheat grain, the more wheat and canola crops were grown in the previous year, the more disease and toxin were found.

Susceptible period of wheat to FHB. The susceptible period of a crop to a disease is a key factor for the development of a disease prediction model. It has been generally accepted that susceptibility of wheat to FHB disease is high from anthesis to about 14 days after (Pugh et al., 1933; Wilcoxson et al., 1992; Groth et al., 1999), and is low prior to the flowering stage (Pugh et al., 1933; Andersen, 1948; Schroeder and Christensen, 1963), because in the most cases the pathogen invades florets through anthers, temporary opening of floret at dehiscence, and expended crevices between the palea and lemma when the caryopsis enlarges (Leonard and Bushnell, 2003). This is consistent with our study, which revealed a high correlation between FHB index and DON level and cumulative rainfall in the 14 days after anthesis.

Rainfall and temperature vs. FHB index and DON accumulation. Cumulative rainfall in the second, third and fourth weeks, and in the 28 days prior to wheat anthesis for Superb, and in the second week before anthesis for AC Barrie, and rainfall from seeding and jointing to anthesis for both cultivars were important for FHB index and DON levels (Fig. 4 and 10). Effect of rainfall on the FHB and DON level can be explained by its effect on perithecial development and maturation, discharge of ascospores from perithecia. There is no direct evidence of how rainfall affects perithecial formation under field conditions. However, perithecial formation requires high water potential (Sung and Cook, 1981; Dufault et al., 2006). Several

studies showed that ascospore discharge occurred several days after rainfall events (Fernando et al., 1997; Inch et al., 2005), which might indicate perithecial development and maturation were associated with rainfall. Previous studies reported that perithecia could be formed within 2-4 weeks under favorable conditions (Atanasoff, 1920; Leonard and Bushnell, 2003). The above studies support the results of the effect of rainfall during different periods in the 28 days before anthesis on disease and toxin levels. However, they were not sufficient to explain the effect of rainfall on spore dispersal from seeding to jointing (Fig. 5 in Chapter 2) and the effect of rainfall on FHB index and DON levels from seeding to anthesis (Fig. 4 and 10 in this Chapter). Therefore, there are two possibilities. First, *G. zeae* perithecia develop when precipitation is high in early spring and mature a month before anthesis due to low temperatures in spring (Tschanz et al., 1976); when temperature rises and rainfall increases in the summer, perithecia develop more rapidly before and mature around anthesis. Secondly, perithecia can be formed and mature in the spring and summer with favorable moisture and temperature (Dufault et al., 2006; Tschanz et al., 1976) and ascospores are discharged throughout the two periods. However, there are no studies reporting that *G. zeae* ascospores are dispersed in the early spring. Based on these two possibilities, cumulative rainfall in different periods in the 28 days prior to anthesis is most important for the spore dispersal in the 14 days after anthesis, in which wheat is most susceptible to the FHB (Pugh et al., 1933; Wilcoxson et al., 1992; Groth et al., 1999).

Cumulative rainfall in the first and second weeks after anthesis and in the 14 days after anthesis for Superb and rainfall in the second week and 14 days after anthesis for AC Barrie were critical for FHB and DON levels (Fig. 4 and 10). Rainfall during the above periods not only increases spore discharge, but also improves fungal

germination, and infection on wheat heads. Rainfall in the second week after anthesis was important for both cultivars according to FHB index and toxin levels (Fig. 4 and 10). In the first week, spores start to land on the wheat heads and germinate, and they are easily washed off by rain; whereas, in the second week, most spores will have already germinated though new spores still land on the spikes. Thus, rainfall in the first week could have a two-sided effect on the spore number on the wheat heads.

Effects of temperature on perithecial formation have been well documented. Perithecia are not formed when the temperature is lower than 9°C (Tschanz et al., 1976). Perithecial production increases when temperature rises from 16 to 28°C, and decreases when temperature is greater than 31°C (Tschanz et al., 1976). However, our study did not show the same effect of temperature before anthesis on disease and toxin levels as rainfall.

Average daily temperature from the first week before to the first week after anthesis was important for disease and toxin levels in this study (Fig. 6 and 12). This temperature could play an important role in perithecial maturation before anthesis, and spore germination and infection after anthesis. Andersen (1948) found that most *F. graminearum* macroconidia germinated at 28-32°C in 3hr, or at 20-32°C in 6hr. The optimal temperature for *F. graminearum* infection is 24-28°C; the fungus cannot grow at temperatures below 4°C and above 36°C (Andersen, 1948; Sutton, 1982). Average daily temperature from the first week before to the first week after anthesis ranged from 17 to 22°C.

Model comparison. There are three major prediction models developed for FHB epidemics of wheat by De Wolf et al. in the United States (De Wolf et al., 2003), DON content by Hooker et al. (2002) in Canada, and FHB disease incidence by Moschini and Fortugno in Argentina (Moschini and Fortugno, 1996). De Wolf et al.

(2003) developed the models which excluded the correlated variables, then used the models developed using two-way and three-way interactions of the same variables in the previous models to select the best models. Their models did not include the interaction terms or correlated variables, and in turn had lower R^2 values. Type I models in this study included no correlated variables, however the strong relationships of actual *F. graminearum* spore counts with FHB index and DON level resulted in the higher R^2 values for all Type I models. Type II models in this study included both correlated and uncorrelated variables, however the R^2 values were lower than Type I models due to the prediction errors of the spore model. The correlated variables in the models were acceptable after the test of multicollinearity, however, the parameters of the correlated variables in the models could not accurately indicate the effects of their corresponding variables. Hooker's models (Hooker et al., 2002) included the variable days of rainfall and its squared term to increase R^2 values of the models but in turn this increases the bias of parameter estimation in the models. Moschini and Fortugno's models (Moschini and Fortugno, 1996) also included correlated variables.

De Wolf et al. (2003) used the periods of 7 days prior to the flowering stage and 10 days after the beginning of the flowering stage for disease prediction. The 7-day period is too short for perithecia to develop from initial to mature stage, and thus most perithecia will not be considered as a potential source for ascospore dispersal after flowering. The model presented here used weather conditions during different periods in the 28 days prior to and 14 days after anthesis. Hooker et al. (2002) used the 4-7 days before the heading stage and 3-6 days after the heading stage (approximately 13 days before anthesis), and 7-10 days after the heading stage (approximately 3-5 days after anthesis) as the predicting periods for their models. The importance of rainfall in the second week after anthesis for FHB index and toxin levels was not reflected in

their models. Models 2 (Type I), 22, 23, 26 and 35 (Type II) in this study predict FHB index and DON using weather conditions in the 14 days after anthesis, but weather conditions in these models would be predicted from weather stations. Moschini and Fortugno (Moschini and Fortugno, 1996) used the period from 8 days prior to heading stage to 26-32 days after, which was too long for early disease management.

De Wolf et al. (2003) and Moschini and Fortugno (1996) used weather factors, total rainfall, relative humidity, average temperature and their durations. Hooker et al. (2002) used duration of rainfall and temperature. These models employed cumulative rainfall, because the days of rainfall were not as good as cumulative rainfall in our study (data not shown in this study). Relative humidity was measured by weather stations using an inverse distance weighted method (Barnes, 1964), rather than by individual crop producers. Whereas rainfall is easily measured by crop producers, it is difficult to be measured or predicted for individual fields by weather stations.

Model application. In this study, two types of models were developed. Type I models with actual spore number cannot be used together with the spore prediction models developed in this study, because Type I models were not developed based on the spore number predicted using Model 6 in Chapter 2, and in turn did not consider interactions between the variables in Type I models and spore models. However, Type I models can be used in other regions, or can be used with spores measured or predicted using different systems or models from Model 6 in Chapter 2. For example, they would be useful for the Canadian Wheat Board for grain market prediction if the spore determining methods (spore traps) are economical and easy to operate. Type II models should be used with the spore prediction models developed in Chapter 2; however, actual spore number, and spore number predicted using other models rather

than Model 6 in Chapter 2 should not be used for Type II models, which otherwise, will result in a greater prediction error compared to using Model 6.

Type II prediction models for FHB index and DON levels for AC Barrie were not accurate as the other models. This was likely caused by two factors. One was that spore prediction model (Model 6 in Chapter 2) was not accurate enough, which caused low prediction accuracy for the disease index and DON level. The other factor was that the relationships between weather conditions and FHB index and DON levels for AC Barrie were not as good as Superb. This problem can be solved using spore traps to correct and improve prediction accuracy for spore counts and using actual FHB index and DON concentration to correct and predicted data in the future.

The models in this study were developed using the variables that could be measured 28 days before and 2-3 days after anthesis when fungicides are applied, and the variables that could be predicted for the following 11-12 days. The models using the prior-to-anthesis variables can provide crop producers with an early prediction of FHB and DON level. To use the models with the variables collected from 14 days after anthesis, weather conditions need to be forecast using weather stations close to the fields. Currently, weather can be forecast for 5 days (personal communication, Dr. Paul Bullock). Therefore, at 30% flowering stage (around 2 days after the beginning of the flowering stage), a longer-range forecast would have to be available to fill in the remaining 12-day period.

This study showed that the model estimation using the Jack-Knife full cross validation and comparison of predicted and actual data was not completely consistent because the comparison test was performed based on only a single year of data, and the full-cross validation method had approximately 25 validation data sets for two

cultivars. With more years for model testing, R^2 value and slope for linear regression testing it is expected the model will have a higher prediction accuracy.

FHB index and DON accumulation models were developed for two wheat cultivars that were moderately susceptible and intermediate resistance to FHB. The variables in the models were the same for both cultivars. Alternatively, different cultivars are different in the parameters of regression models. Therefore, new models having the same variables for cultivars with different levels of resistance can be developed using the following method: FHB and DON levels collected from the new cultivars are compared with the two cultivars used in our study, and ratios of FHB index or DON concentration for the new cultivars and the two cultivars used in this study can be calculated and used for parameter transformation for each variable in new prediction models.

In all, prediction models developed in this study will provide crop producers with a risk assessment for FHB and DON level so that they can decide whether the cost of applying fungicide for control of FHB is economical. On application, the models should be further tested and corrected using actual data, and are ecpected to become more accurate in the future.

3.5 Conclusions

Airborne fusarium inoculum on wheat heads significantly affected FHB and DON levels. It was a good predictor for FHB index and DON level. FHB and DON levels were related to cropping practices in the previous years and weather conditions. Cropping practices were quantified by assigning different scores to the different factors of cropping practices according to the potential capacities of different crops to produce inoculum. CPI significantly affected FHB index and DON levels for both cultivars Superb and AC Barrie. The more cereal crops, zero or minimum tillage and

susceptible wheat cultivars were used in the previous years, the higher levels of FHB and DON.

Cumulative rainfall in the second week prior to and after anthesis significantly affected FHB and DON level for both cultivars. Superb was more sensitive to rainfall in FHB than AC Barrie. Rainfall had a greater effect on FHB and DON level than temperature effect. Effects of rainfall and temperature before and after anthesis were due to their effects on *G. zeae* perithecial formation, spore dispersal and infection, respectively.

Two types of models were developed to predict FHB and DON level. Type I models with actual *F. graminearum* spore counts were more accurate than Type II models, and can be used for wheat market prediction by the Canadian Wheat Board based in Winnipeg. Type II models with predicted spore counts can be used for fungicide application by wheat producers. The average prediction accuracy of Type I and Type II models were 85% and 58%, respectively.

CHAPTER 4

4.0 Genetic Causes for Chemotype Diversity and Potential Chemotype Shifting of *Fusarium graminearum* in Wheat Fields in Manitoba

Abstract

The objectives of this study were to investigate chemotype diversity, distribution and potential chemotype shifting of *Fusarium graminearum* in Manitoba. This study was conducted in 15 locations, consisting of 39 farmers' fields sown to wheat cultivars Superb and AC Barrie, in Manitoba from 2004 to 2005. The 15 locations were further grouped into 7 regions. Percentages of 3ADON and 15ADON chemotypes of *Fusarium graminearum* (*Gibberella zeae*) ranged from 0 % to 95.7 %, and 4.3 % to 100 %, respectively. The 3ADON chemotype was distributed in the south part of Manitoba. The 3ADON chemotype was predominant in Region 1 including Sanford, Morris and Horndean; the two chemotypes were equally abundant in Region 2, including Cartier and Portage la Prairie; and 15ADON was predominant in Regions 4 and 5, including Killarney, Souris, McAuley and Virden. There was no 3ADON chemotype found in the two northern regions Kenville and Dauphin. Significant gene flow was found in the sub grouped populations from locations Sanford, Portage la Prairie, Hamiota, Plumas, Rapid City and Virden; the populations from Cartier, Rivers, Killarney and Souris; and the populations from Morris, Kenville and Dauphin. Significant gene flow was also observed in the populations from Regions 1 to 5. Gene flow occurred between the populations close to or far from each other. No genetic migration was found between Regions 1 to 5 and Regions 6 and 7. There was a great variation of percentage of 3ADON chemotype within the sub grouped population from different locations and regions, which could result from a high level of genetic diversity of *F. graminearum* populations. It is suggested that sexual recombination, population age and cropping system could be associated with genetic and chemotypic diversities of *F. graminearum* populations. Wheat seed shipment and long-distance spore dispersal of *F. graminearum/G. zeae* likely

contributed to the genetic migration between locations and regions; and potentially caused chemotype shifting in Manitoba.

4.1 Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe) Petch (teleomorph = *Gibberella zeae* Schwein.) and other *Fusarium* species, is one of the most destructive diseases in wheat worldwide. Since it was first reported in 1884 (Leonard and Bushnell, 2003), FHB has spread quickly over cereal production areas, including North America, Europe, and East Asia (Leonard and Bushnell, 2003). FHB causes yield loss of up to 50 % in wheat (McMullen et al., 1997; Windels, 2000), damages protein quality, reduces kernel test weight and color, threatens safety of human food and causes animal disease (Dexter and Nowicki, 2003), all of which cause difficulties for wheat marketing, exporting and processing.

Molecular techniques allowed identification of *F. graminearum* from other species (Demeke et al., 2005) and genetic information of the species populations (Carter et al., 2002; Gale et al., 2002; Cumagun et al., 2004; Fernando et al., 2006). Aoki and O'Donnell (1999) grouped *F. graminearum* into two separate species, Group 1 (*F. pseudograminearum*) and Group 2 (*F. graminearum*). O'Donnell et al. (2004) proposed nine geographically structured lineages of *F. graminearum* based on the DNA sequences of eleven unclustered genes from the isolates collected worldwide. Genetic structures of *F. graminearum* have been characterized in different places in Europe (Cumagun et al., 2004; Gagkaeva and Yli-Mattila, 2004), Asia (Gale et al., 2002; Gagkaeva and Yli-Mattila, 2004), America (Walker et al., 2001; Zeller et al., 2003), and Canada (Fernando et al., 2006; Dusabenyagasani et al., 1999); and different regions worldwide (Miedaner et al., 2001; Carter et al., 2002).

Fusarium graminearum produces trichothecene mycotoxins, one of the most important is deoxynivalenol (DON), known as vomitoxin. DON inhibits protein biosynthesis in eukaryotic organisms (Desjardins, 2006), causes feed refusal, diarrhea, emesis, alimentary haemorrhaging and contact dermatitis in animals (Desjardins, 2006). Human illness aleukia and Akakabi, and syndromes, nausea, vomiting, anorexia and convulsion are associated with the fungus *F. graminearum*.

Three chemotypes, strain-specific profiles of trichothecene metabolites, were found in *F. graminearum* (Desjardins, 2006). They produce nivalenol (NIV) that is DON's C-4 oxygenated derivative, 15ADON the acetyl ester derivative of DON at 15-position oxygen, and 3ADON the acetyl ester derivative of DON at 3-position oxygen, respectively (Desjardins, 2006). The biosynthesis pathway of trichothecene toxin has been well characterized in *F. graminearum* (Desjardins, 2006). The 15ADON chemotype is predominant in North America, and 3ADON chemotype is predominant in some areas in Asia, including China, and Australia and New Zealand (Mirocha et al., 1989).

A higher frequency of 3ADON chemotype isolates was found in North Dakota and Minnesota in recent years than before (Gale et al., 2005). Recent molecular surveillance showed that the 3ADON chemotype was replacing 15ADON chemotype Canada from east to west (Ward et al., 2005). However, the relationship between the chemotype changing and genetic diversity and migration of *F. graminearum* populations and genetic evidence whether there was really a chemotype shifting in the above regions has not been studied. Recent studies showed that a 3ADON chemotype isolate produced more DON than a 15 ADON chemotype isolate (Ward et al., 2005; Gilbert et al., 2006). These findings cause increasing concern to the cereal industries.

Therefore the objectives of this study were, 1). to investigate variations of chemotype of *F. graminearum* and potential chemotype shifting of *F. graminearum* in Manitoba, and 2). to understand the genetic causes for the above by analyzing genetic diversity and gene flow in the populations of *F. graminearum* isolates in Manitoba.

4.2 Materials and methods

Isolate collection and DNA extraction. In total, 291 *F. graminearum* isolates were collected from wheat heads in 17 farmers' fields in 2004 and 22 fields in 2005 in Manitoba. In 2004, nine fields were sown to wheat cultivar Superb, moderately susceptible to FHB disease, and eight fields were sown to AC Barrie, intermediate in resistance to FHB disease (Seed Manitoba, 2006). In 2005, 10 fields were sown to Superb and 11 fields were sown to AC Barrie. The position of each farmer's home was considered as one location, and there were in total 15 locations from 2004 to 2005 (Fig. 1 and Table 1). The collected wheat heads were surface sterilized using 1% bleach for 1min, washed using sterilized distilled water (sd water) for 1min, and then dried under a flow hood. The spikelets were taken off the wheat heads, and put on a potato dextrose agar (PDA) (Difco Laboratories, MD) medium, incubated under fluorescent light at room temperature for 7 days. *F. graminearum* colonies were initially identified according to Nelson et al. (1983), and transferred to a *Fusarium*-specific nutrition-poor agar (SNA), and incubated for 7 days for sporulation under the same conditions as the above. *F. graminearum* sporodochia in the SNA medium were washed 3-4 times using a drop of 50 μl sd water, and the washings of sd water with macroconidia was spread over the surface of a water agar (WA) medium. The macroconidia on the WA were incubated under the same conditions for 6hr, and a single macroconidium was transferred to a PDA medium, and incubated for 10 days. The mycelium was harvested and lyophilized for 10hr, and stored at –80 °C until use.

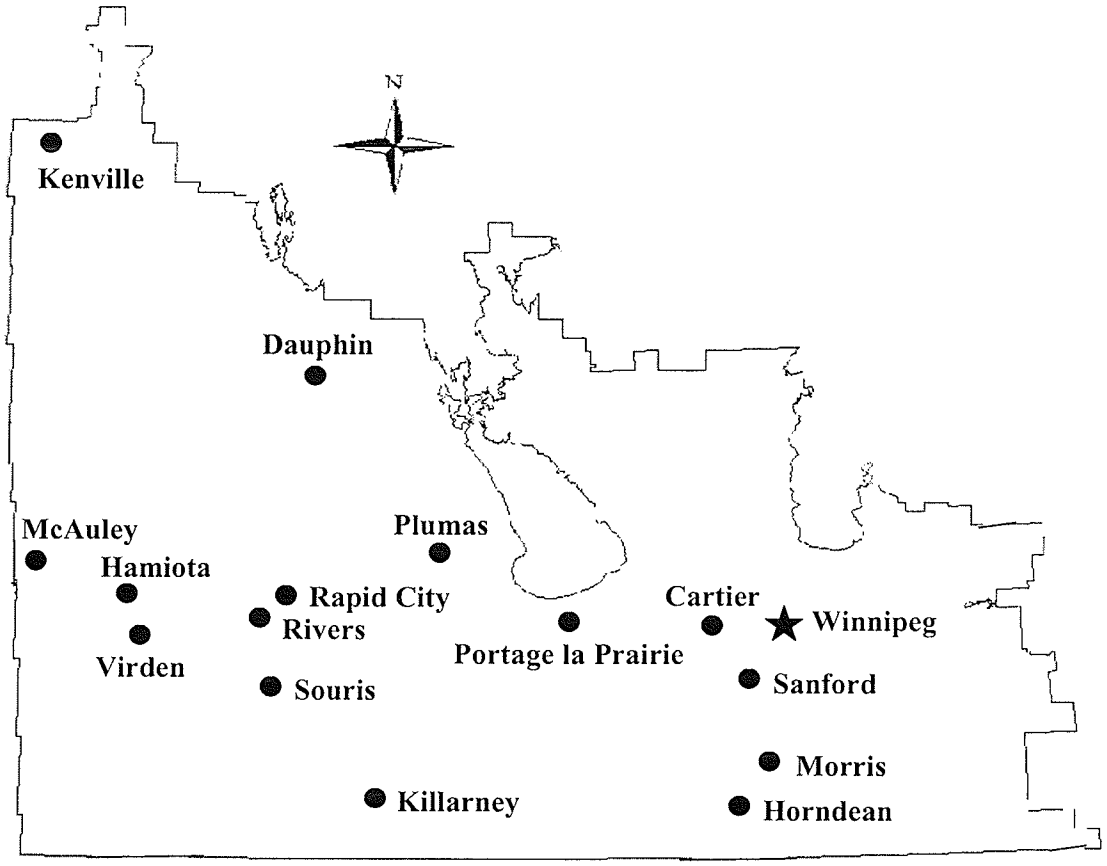


Figure 1. A map of south part of Manitoba, Canada. Symbol "★" represents Winnipeg, Manitoba. Symbol "●" indicates the locations of the fields sampled from 2004 to 2005.

DNA extraction was performed using the method described by Fernando et al. (2006). The lyophilized fungal mycelium was ground in 600μl of TES buffer (100mM Tris; 10mM EDTA; 2% SDS) in a 1.5-ml microcentrifuge tube. One hundred and forty microlitres of 5M NaCl and 70μl of 10% CTAB were added in the tube and vortexed. The mixture was incubated at 65°C for 20min. Six hundred microlitres of a mixture of chloroform and isoamyl alcohol (v/v: 24 : 1) was added, and then centrifuged at 10000rpm for 15min. The supernatant was transferred into a new tube. The latter step was repeated. Eighty microlitres of 5M NaCl and 1000μl of 100% ethanol were added to precipitate the DNA, and the solution was centrifuged at 13000rpm for 5min. The DNA pellet was washed using two hundred microlitres of cold 80% ethanol. After it dried, the pellet was suspended in 100μl of warm sd water (37 °C). The DNA was qualified using 1% agarose gel, and diluted to 10ng/μl.

Identification of *Fusarium graminearum* and chemotype. Identification of *F. graminearum* was performed using the specific PCR marker described by Demeke et al. (2005). Two primers were used in the PCR, Fg16F (5'-CTCCGGATATGTTGCGTCAA-3') and Fg16R (5'-GGTAGGTATCCGACATGGCAA-3'), which produced a fragment of 450bp (Fig. 2A). The PCR reaction was performed in a 25-μl volume, containing 20ng template DNA, 1.5mM $MgCl_2$, 50mM KCl, 10mM Tris HCl (pH 8.0), 0.2mM of each dNTPs, 0.4mM of each primers, and 0.75 units of *Taq* DNA polymerase.

Fusarium graminearum chemotype was identified using the multiplex PCR marker developed by Ward et al. (2002). The three primers used in the PCR were, 3CON (5'-TGGCAAAGACTGGTTCAC-3'), 3D15A (5'-ACTGACCCAAGCTGCCATC-3'), and 3D3A (5'-CGCATTGGCTAACACATG-3'). They produced a 610-bp fragment for the 15ADON chemotype, and a 243-bp

fragment for the 3ADON chemotype (Fig. 2B). The PCR reaction was performed in a 15-μl volume, containing 20ng template DNA, 2.0mM $MgCl_2$, 50mM KCl, 10mM Tris HCl (pH 8.0), 0.2mM of each dNTPs, 0.4mM of each primer, and 0.75 units of *Taq* DNA polymerase.

Amplification of polymorphisms. The sequence-related amplified polymorphism (SRAP) technique (Li and Quiro, 2001) was used for the analyses of genetic diversity and migration of *F. graminearum*. Four SRAP primer pairs were screened out of 36 primer pairs based on efficiency of the primers producing polymorphic bands. They were, ODD9 (5'-AGTTCCTCAGACGCTACC-3') and ODD15 (5'-GCGAGGATGCTACTGGTT-3'), DC1 (5'-TAAACAATGGCTACTCAAG-3') and RP1 (5'-CATTGTGGATGGCATCTGA-3'), ODD30 (5'-GCGATCACAGAAGGAAGGT-3') and EM1(5'-GACTGCGTACGAATTCAAT-3'), and ODD30 and DC1. SRAP PCR reaction was performed in a 15-μl volume, containing 20ng template DNA, 10 mM Tris-HCl (pH 8.0), 50mM KCl, 1.5mM $MgCl_2$, 0.1mM each of dNTP, 0.4mM each of two primers, and 0.75 units of *Taq* polymerase (Invitrogen Life Technologies, ON, Canada). The PCR products were separated by electrophoresis using 5% polyacrylamide DNA sequence gel with 7.5M urea for denaturing DNA. Gel fixing, staining and developing were followed to visualize the DNA bands using the silver-staining kit (Promega Corp., Madison, WI) (Fig. 2C).

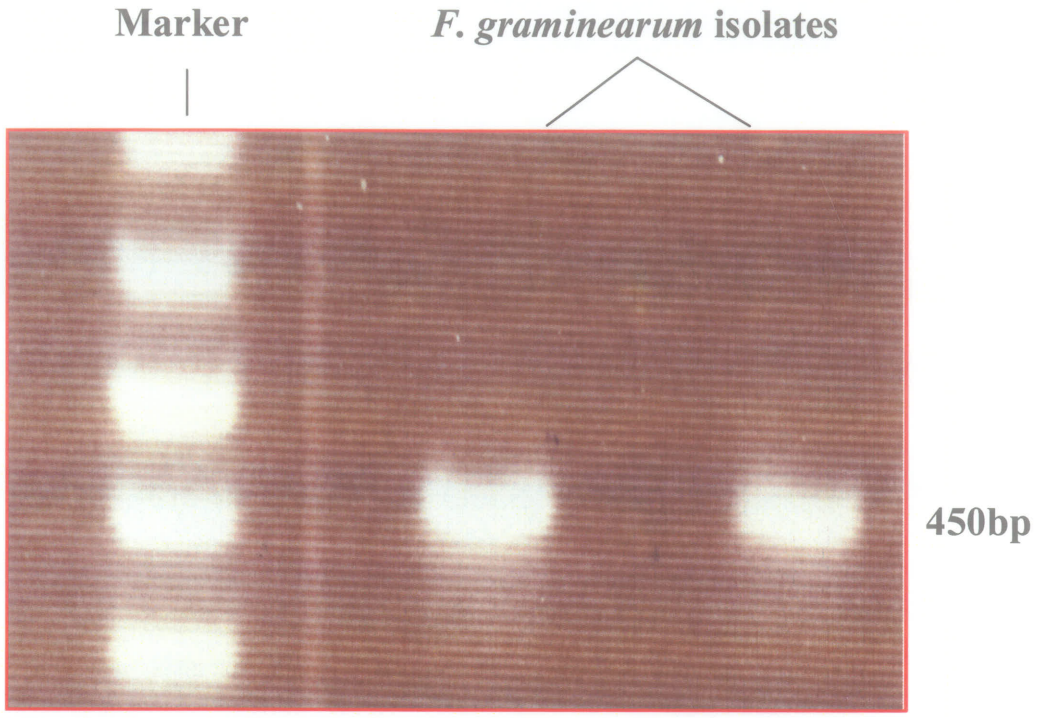


Figure 2A. *Fusarium graminearum* DNA fragments amplified using *Fusarium graminearum* specific PCR (Demeke et al., 2005). The 450-bp fragment represented *F. graminearum* species on the 2% agarose gel.

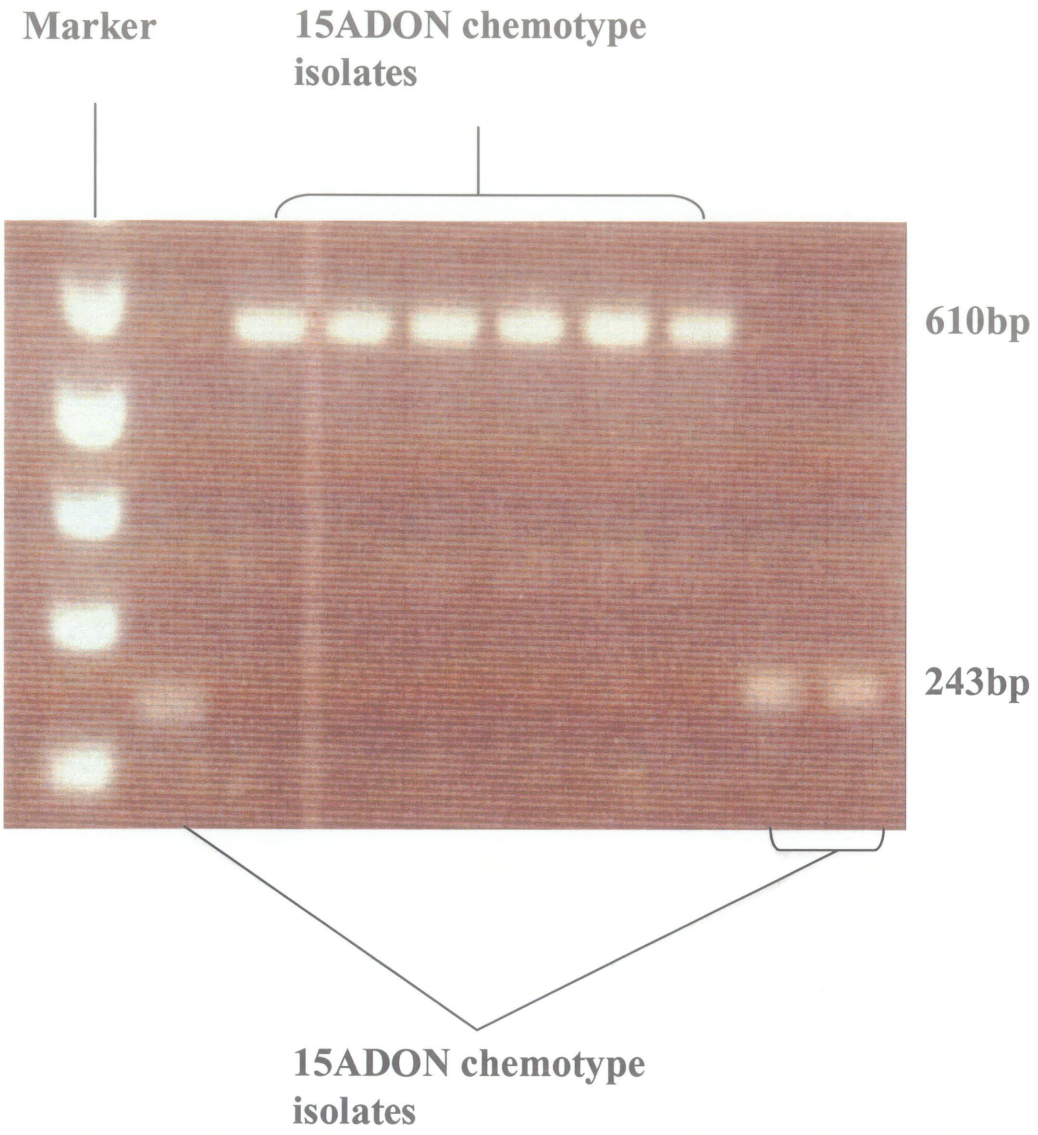


Figure 2B. DNA fragments of *Fusarium graminearum* chemotypes amplified using the multiplex PCR (Ward et al., 2002). The 610-bp fragments represented the 15ADON chemotype, and the 243-bp fragments represented the 3ADON chemotype on the 2% agarose gel.

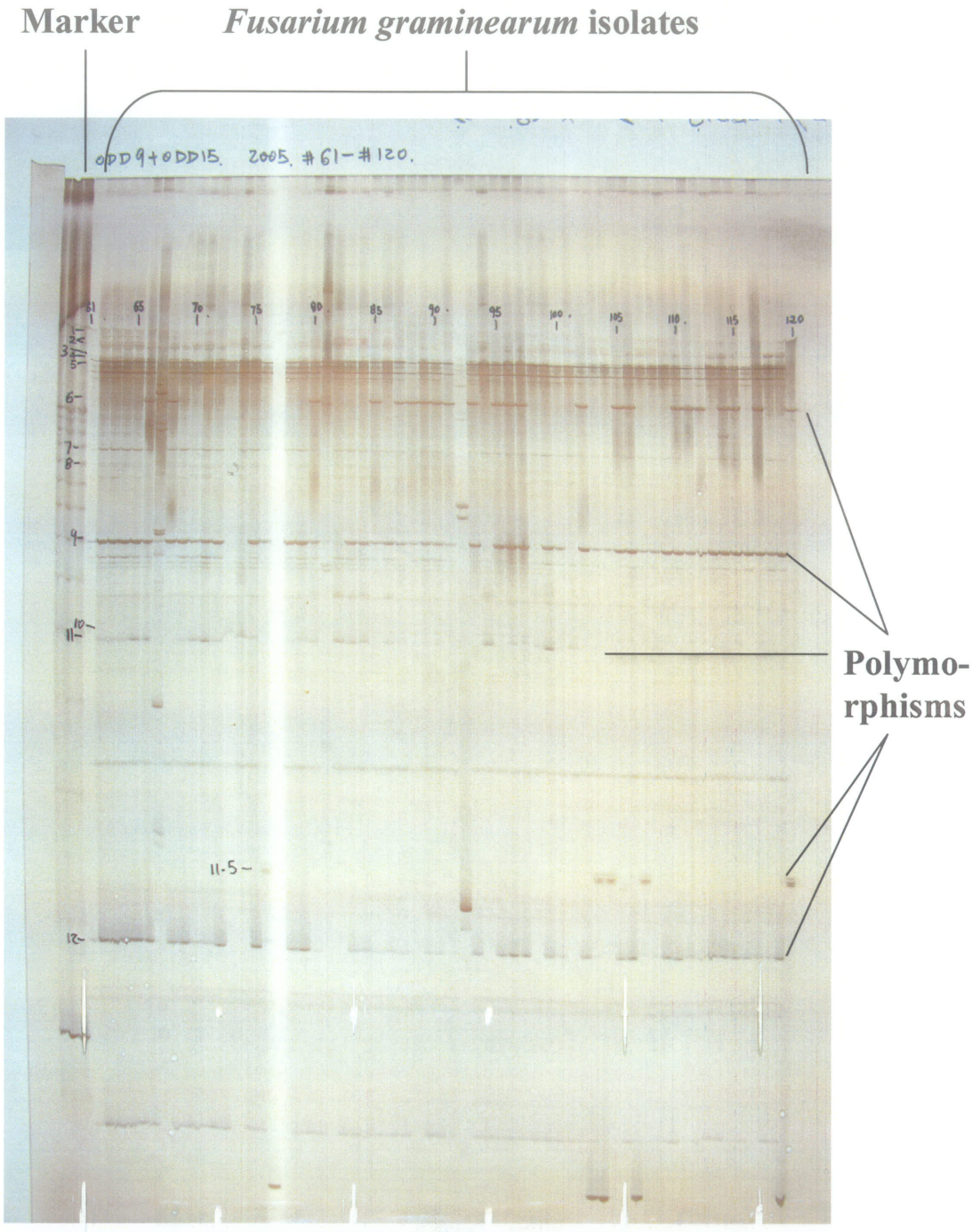


Figure 2C. Polymorphisms of *Fusarium graminearum* DNA on a 5% polyacrylamide gel, which were amplified using sequence-related amplified polymorphism (SRAP) PCR.

Data analysis. Percentage of a chemotype (%) was assessed as the number of the isolates with this chemotype divided by the total number of *F. graminearum* isolates from a location and region, and multiplied by 100. A *F. graminearum* population was defined as all the isolates collected from one location, region, wheat cultivar, or chemotype. Polymorphic DNA bands were estimated as two-allele loci with the presence of fragment assigned a 1 and the absence of fragment assigned a 0. Therefore, a binary matrix including the number of *F. graminearum* isolates and DNA polymorphisms was developed. The software POPGENE (version 1.32; Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, Canada) was used for genetic data analysis. Genetic diversity of the populations from different locations, regions, wheat cultivars and chemotypes (H) was estimated using the formula, $H = 1 - \Sigma P_i^2$, where P_i was frequency of allele i at the locus (Nei, 1973). Heterozygosity and percent polymorphic loci were assessed for all the types of populations. Genotypic diversity was estimated using Shannon's information index (s) (Shannon and Weaver, 1949). An exact test was used for differentiation of different types of populations. Gene flow (genetic migration) was calculated using the formula, $N_m = (1 - F_{st})/(4 \times F_{st})$ (Slatkin, 1987), where N was population size, m was immigration rate of gene flow, N_m was average number of migrants among the populations. F_{st} was variance in allele frequencies between the populations (Slatkin, 1987). The cluster phenograms for different locations and regions were constructed using the unweighted pair-group method with arithmetic average (UPGMA) from Nei's genetic distance (Nei, 1973).

4.3 Results

Chemotypes of *F. graminearum*. Based on the data collected from the 15 locations from 2004 to 2005, there was great variation for percent chemotype between

the locations and regions. Isolates with 15ADON chemotype were predominant in all locations except for Morris and Horndean, in which isolates with 3ADON chemotype accounted for 95.7% and 60.0%, respectively (Table 1). The 3ADON chemotype was predominant in Region 1, the southeast part of Manitoba, including Sanford, Morris and Horndean, was similar in percentage with 15ADON chemotype in Region 2, and accounted for approximately 10-25% in Region 3, Region 4 and Region 5, and was 0% in Regions 6 and 7 in Manitoba. Overall, 3ADON and 15ADON chemotypes accounted for 33.7% and 66.3%, respectively, in Manitoba from 2004 to 2005 (Table 1).

SRAP variation. Fifteen populations of *F. graminearum* developed based on 291 isolates with four pairs of SRAP primers showed different levels of genetic diversity (Table 2). The percentage of polymorphic loci ranged from 11.65% observed in the population from Dauphin to 91.26% from Portage la Prairie. The number of genotypes in a population showed that the population from Dauphin had the lowest percentage of genotype (66.7%), and the population from Rapid City had the highest percentage (92.3%), in which almost every isolate was a unique genotype (Table 2). Shannon's information index (*s*) showed that higher levels of genotypic diversity existed in the populations from Morris (0.4085), Portage la Prairie (0.4038), Sanford (0.3432) and Hamiota (0.3092), than in populations from the other locations (Table 2). The population from Dauphin had the lowest *s* value. Heterozygosity of different populations ranged from 0.0936 (Dauphin) to 0.2726 (Morris), which was consistent with Shannon's information index (Table 2).

Genetic and genotypic diversity of *F. graminearum* populations were measured from both wheat cultivars Superb and AC Barrie, and chemotypes 3ADON and 15ADON strains (Table 3). High percentage of polymorphic loci were observed in all

the populations, ranged from 95.15% (Superb) to 97.09% (AC Barrie) for the cultivars, and from 96.15% (15ADON) to 97.09% (3ADON) for the chemotypes (Table 3). High levels of heterozygosity were found in the populations from cultivars Superb (0.2385) and AC Barrie (0.2533), and in the populations from 15ADON (0.2264) and 3ADON (0.2827) chemotypes (Tables 3). Shannon's information index and ratio genotype and population size also showed a similar trend (Table 3).

Table 1. Number of 15ADON and 3ADON chemotype isolates of *Fusarium graminearum* in different locations in Manitoba from 2004 to 2005.

Region[a]	Location[b]	Total *Fg*[c]	15ADON (%)[d]	3ADON (%)[e]
1	SFD	19	73.7	26.3
	MRS	23	4.3	95.7
	HND	10	40.0	60.0
	Subtotal	52	36.5	63.5
2	CTR	11	72.7	27.3
	PLP	52	51.9	48.1
	Subtotal	63	55.6	44.4
3	HMT	16	68.8	31.2
	RVS	28	89.3	10.7
	PMS	26	65.4	34.6
	RC	13	76.9	23.1
	Subtotal	83	75.9	24.1
4	KLN	43	72.1	27.9
	SRS	12	83.3	16.7
	Subtotal	55	74.5	25.5
5	MAL	11	90.9	9.1
	VD	19	89.5	10.5
	Subtotal	30	90.0	10.0
6	DPN	3	100.0	0.0
	Subtotal	3	100.0	0.0
7	KNL	5	100.0	0.0
	Subtotal	5	100.0	0.0
Total/Average		291	66.3	33.7

[a] Seven regions consisting of 15 locations in Manitoba.
[b] Fifteen locations consisting of 39 farmer's fields sown to two wheat cultivars, Superb and AC Barrie in Manitoba from 2004 to 2005. SFD = Sanford, MRS = Morris, HND = Horndean, CTR = Cartier, PLP = Portage la Prairie, HMT = Hamiota, RVS = Rivers, PMS = Plumas, KLN = Killarney, SRS = Souris, MAL = McAuley, VD = Virden, DPN = Dauphin, and KNL = Kenville.
[c] *Fg* = *Fusarium graminearum*.
[d] 15ADON (%) = percentage of *Fusarium graminearum* isolates with 15ADON chemotype over the total *F. graminearum* isolates.
[e] 3ADON (%) = percentage of *Fusarium graminearum* isolates with 3ADON chemotype over the total *F. graminearum* isolates.

Table 2. Genetic diversity of *Fusarium graminearum* among different locations in Manitoba.

Population	Location[a]	SRAP data[b]				
		n	*g*	*s*	*r* (%)	*H*
1	SFD	19	16	0.3432	67.96	0.2270
2	MRS	23	19	0.4085	46.60	0.2726
3	HND	10	8	0.2693	36.89	0.1761
4	CTR	11	9	0.2757	40.78	0.1774
5	PLP	52	38	0.4038	91.26	0.2584
6	HMT	16	13	0.3092	62.14	0.2012
7	RVS	28	21	0.2067	39.81	0.1287
8	PMS	26	20	0.2980	66.99	0.1910
9	RC	13	12	0.2127	39.81	0.1418
10	KLN	43	30	0.3432	60.19	0.2131
11	SRS	12	9	0.2842	50.49	0.1727
12	MAL	11	9	0.1516	35.92	0.0937
13	VD	19	15	0.2527	56.31	0.1606
14	DPN	3	2	0.1340	11.65	0.0936
15	KNL	5	4	0.2991	31.07	0.1965

[a] Locations, SFD = Sanford, MRS = Morris, HND = Horndean, CTR = Cartier, PLP = Portage la Prairie, HMT = Hamiota, RVS = Rivers, PMS = Plumas, RC = Rapid City, KLN = Killarney, SRS = Souris, MAL = McAuley, VD = Virden, DPN = Dauphin, and KNL = Kenville.

[b] *n* = population size, *g* = number of genotypes in populations, *s* = Shannon's information index, *r* = percentage of polymorphic loci (99 % criterion), and *H* = average unbiased proportion heterozygosity.

Table 3. Genetic diversity of wheat cultivar and *Fusarium graminearum* chemotype populations in Manitoba.

Population[a]	SRAP data[b]				
	n	*g*	*s*	*r* (%)	*H*
Superb	126	89	0.3813	95.15	0.2385
AC Barrie	165	118	0.3993	97.09	0.2533
15ADON chemotype	193	134	0.3634	96.12	0.2264
3ADON chemotype	98	73	0.4379	97.09	0.2827

[a] Populations were defined as groups of isolates from different wheat cultivars, Superb and AC Barrie; and different chemotypes, 15ADON chemotype and 3ADON chemotype.

[b] *n* = population size, *g* = number of genotypes in populations, *s* = Shannon's information index, *r* = percentage of polymorphic loci (99 % criterion), and *H* = average unbiased proportion heterozygosity.

Population structure. Population structure was developed using genetic distance and identity of populations. Nei's genetic identity index showed that populations from Portage la Prairie and Hamiota (0.9554); Rapid City and Virden (0.9571); Cartier and Rivers (0.9803); and Morris and Kenville (0.9100) were closer to each other than to others (Table 4), which was consistent with genetic distance between the same populations. UPGMA cluster analysis using Nei's genetic distance between populations showed that the distances varied from 0.0228 (Rivers vs. Souris) to 1.3388 (Rivers vs. Dauphin) (Table 4). Fifteen populations were clustered into three subgroups, including the populations from Sanford, Portage la Prairie, Hamiota,

Plumas, Rapid City, Virden and McAuley; the populations from Horndean, Cartier, Rivers, Killarney and Souris; and the populations from the other locations (Table 4 and Fig. 3). The genetic distance between populations within these individual subgroups ranged from 0.0438 to 0.1127, 0.0199 to 0.0495, and 0.0943 to 0.3088, respectively (Table 4).

The analysis of possibilities of each population pairwise comparison revealed genetic variations among different locations. The populations from Sanford, Portage la Prairie, Hamiota and Plumas were not significantly differentiated from each other, and a great gene flow was found among them, ranging from 2.4434 between populations from Sanford and Plumas to 18.9608 between Portage la Prairie and Hamiota (Table 5). There were no significant differences between populations from Rapid City and Virden, from Cartier, Rivers and Killarney, and from Morris, Kenville and Dauphin (Table 5). An extensive gene flow was also observed among these locations. The other population pairs were significantly differentiated from each other (Table 5). It was observed that gene flow existed among some populations that were significantly differentiated from each other (Table 5). For example, there was gene flow (N_m>1) among the locations Sanford, Portage la Prairie, Hamiota, Plumas, Rapid City and Virden, however some of populations were differentiated from each other, such as the populations from Sanford and Virden (Table 5). Gene flow was also found among the populations from Cartier, Rivers, Killarney and Souris and populations from Morris, Kenville and Dauphin (Table 5). This was consistent with the results of genetic distance and identity. The above results suggest that gene flow can possibly occur between the populations that are close to or far from each other, and the populations that are significantly different.

Table 4. Pairwise comparison of the genetic identity and distance among different geographic populations of *Fusarium graminearum* in Manitoba.

Population[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	****	0.6707	0.7315	0.7608	0.9402	0.9377	0.7559	0.9274	0.8983	0.7609	0.7494	0.8934	0.8835	0.5709	0.7079
2	0.3994	****	0.3178	0.3215	0.6901	0.6906	0.3147	0.6657	0.6497	0.3386	0.3166	0.5847	0.6651	0.8633	0.9100
3	0.3127	1.1462	****	0.9309	0.7986	0.7435	0.9602	0.7347	0.7827	0.9727	0.9623	0.7666	0.7837	0.2927	0.2888
4	0.2734	1.1349	0.0716	****	0.7942	0.7642	0.9803	0.7738	0.8014	0.9676	0.9517	0.7968	0.7580	0.2488	0.3103
5	0.0617	0.3709	0.2249	0.2304	****	0.9554	0.7948	0.9503	0.9490	0.8098	0.7906	0.8994	0.9480	0.6182	0.6811
6	0.0643	0.3702	0.2964	0.2690	0.0456	****	0.7629	0.9378	0.9019	0.7687	0.7618	0.8903	0.9183	0.6017	0.6845
7	0.2799	1.1560	0.0406	0.0199	0.2296	0.2706	****	0.7445	0.7987	0.9789	0.9775	0.7943	0.7672	0.2622	0.2986
8	0.0754	0.4069	0.3083	0.2564	0.0509	0.0642	0.2951	****	0.8940	0.7677	0.7362	0.8825	0.8859	0.5491	0.7118
9	0.1072	0.4312	0.2451	0.2214	0.0524	0.1032	0.2248	0.1121	****	0.7949	0.7849	0.8774	0.9571	0.6531	0.6068
10	0.2733	1.0829	0.0276	0.0330	0.2109	0.2630	0.0213	0.2643	0.2295	****	0.9702	0.7846	0.7838	0.2926	0.3216
11	0.2884	1.1503	0.0385	0.0495	0.2350	0.2721	0.0228	0.3063	0.2422	0.0302	****	0.7758	0.7727	0.2713	0.2913
12	0.1127	0.5367	0.2658	0.2272	0.1060	0.1162	0.2303	0.1250	0.1308	0.2426	0.2538	****	0.8776	0.4985	0.5829
13	0.1238	0.4078	0.2438	0.2770	0.0535	0.0853	0.2650	0.1212	0.0438	0.2436	0.2578	0.1306	****	0.6401	0.6107
14	0.5605	0.1470	1.2287	1.3912	0.4809	0.5080	1.3388	0.5995	0.4261	1.2289	1.3046	0.6961	0.4461	****	0.7343
15	0.3454	0.0943	1.2421	1.1704	0.3840	0.3790	1.2086	0.3400	0.4995	1.1344	1.2335	0.5397	0.4931	0.3088	****

[a] Populations 1 = Sanford, 2 = Morris, 3 = Horndean, 4 = Cartier, 5 = Portage la prairie, 6 = Hamiota, 7 = Rivers, 8 = Plumas, 9 = Rapid City, 10 = Killarney, 11 = Souris, 12 = McAuley, 13 = Virden, 14 = Dauphin, 15 = Kenville. Nei's genetic identity based on 103 sequence-related amplified polymorphism loci is above the diagonal, and genetic distance coefficients are below the diagonal.

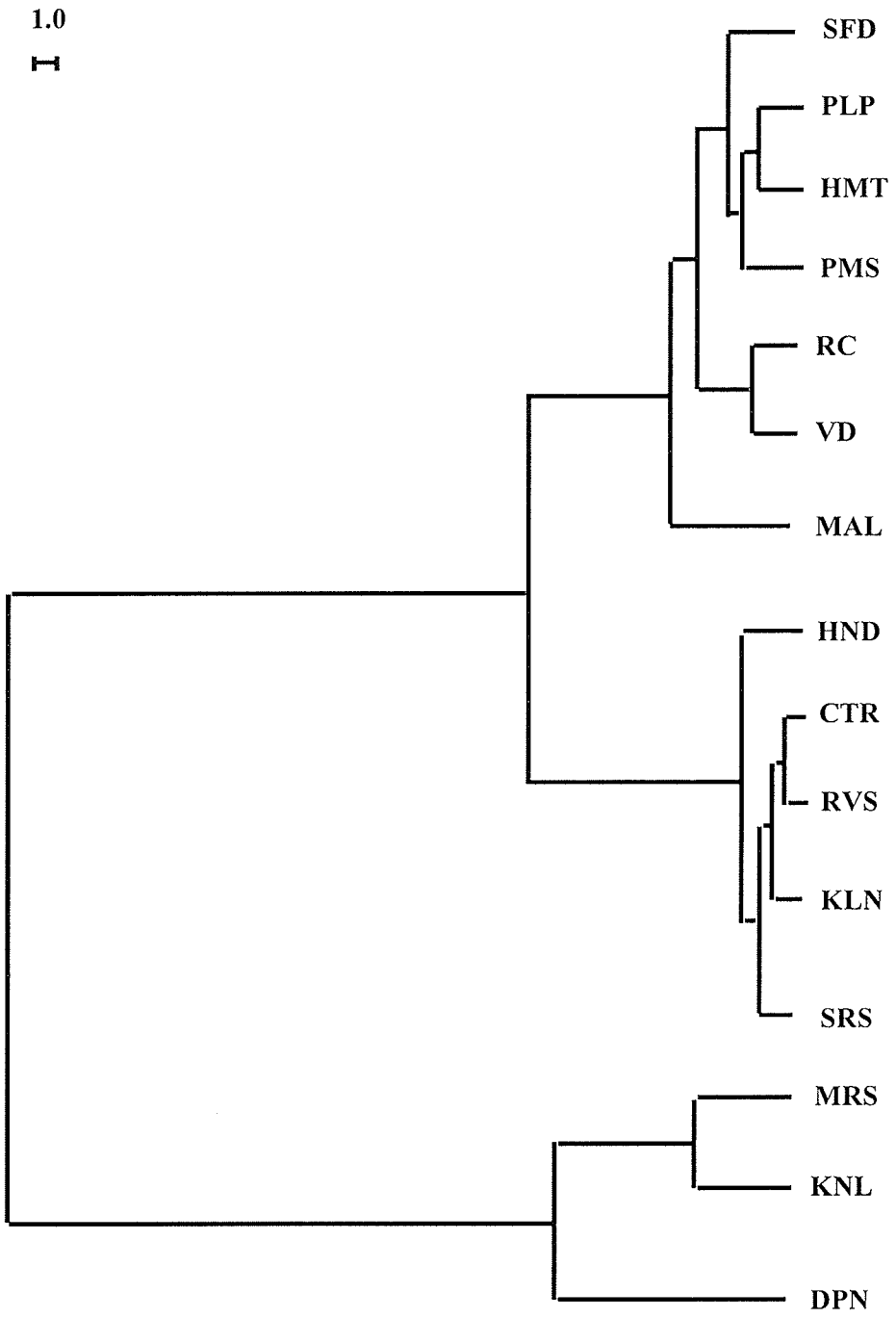


Figure 3. Cluster phenogram of Nei's genetic distance between *Fusarium graminearum* populations from 15 locations in Manitoba. SFD = Sanford, MRS = Morris, HND = Horndean, CTR = Cartier, PLP = Portage la Prairie, HMT = Hamiota,

RVS = Rivers, PMS = Plumas, RC = Rapid City, KLN = Killarney, SRS = Souris, MAL = McAuley, VD = Virden, DPN = Dauphin, and KNL = Kenville.

Table 5. Pairwise comparison of gene flow and probability of population differentiation among different populations of *Fusarium graminearum* from different locations in Manitoba.

Population[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	****	0.1546	1.2388	0.6267	7.3327	4.0053	0.5720	2.4434	2.0137	1.1995	0.9006	0.7276	2.8982	0.9697	0.4689
2	0.0455	****	0.6191	0.3788	1.3570	1.1614	0.3617	0.8986	1.0820	0.6805	0.5081	0.4214	1.2092	1.0619	12.4258
3	<0.0001	<0.0001	****	0.5373	1.1861	0.9461	0.7062	0.7954	1.4651	1.5091	1.4363	0.8012	0.6584	0.6835	0.4834
4	<0.0001	<0.0001	<0.0001	****	1.1024	1.7582	16.8342	1.7163	1.8412	1.7154	1.0560	2.5140	0.3617	0.1624	0.4217
5	0.0727	<0.0001	<0.0001	<0.0001	****	18.9608	1.0678	6.2791	1.7117	2.9218	1.2138	1.0850	2.5565	0.8753	0.9236
6	0.0573	<0.0001	<0.0001	<0.0001	0.5546	****	1.1235	3.5684	2.4536	2.3199	1.0391	1.1510	1.6402	0.4113	0.8725
7	<0.0001	<0.0001	<0.0001	0.2909	<0.0001	<0.0001	****	1.2727	2.1194	3.9229	1.4108	1.8616	0.3859	0.1994	0.3996
8	0.0546	<0.0001	<0.0001	<0.0001	0.0573	0.8273	<0.0001	****	4.1013	0.7107	1.0496	1.2576	1.4072	0.3538	0.9097
9	0.0182	<0.0001	0.0091	0.0182	0.0182	0.0291	0.0182	0.0346	****	1.0032	1.7241	1.5271	11.3205	0.4426	1.3240
10	<0.0001	<0.0001	<0.0001	0.0701	<0.0001	0.0182	0.1123	<0.0001	0.0436	****	1.6272	1.6847	0.7722	0.4406	0.9382
11	<0.0001	<0.0001	0.0091	<0.0001	<0.0001	<0.0001	0.0091	<0.0001	0.0091	<0.0001	****	1.1709	0.5716	0.2498	0.4533
12	<0.0001	<0.0001	<0.0001	0.0364	<0.0001	<0.0001	0.0302	<0.0001	0.0273	<0.0001	0.0182	****	0.4822	0.2220	0.4822
13	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2654	<0.0001	<0.0001	<0.0001	****	0.3350	1.3095
14	0.0091	0.0664	0.0364	0.0182	0.0091	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0091	****	4.4318
15	<0.0001	0.1273	<0.0001	<0.0001	0.0182	<0.0001	<0.0001	0.0455	0.0273	0.0091	<0.0001	<0.0001	0.0364	0.0882	****

[a] Populations 1 = Sanford, 2 = Morris, 3 = Horndean, 4 = Cartier, 5 = Portage la prairie, 6 = Hamiota, 7 = Rivers, 8 = Plumas, 9 = Rapid City, 10 = Killarney, 11 = Souris, 12 = McAuley, 13 = Virden, 14 = Dauphin, 15 = Kenville. Estimates of the number of migrants (*Nm*) between populations are above the diagonal, and probability of each pairwise comparison using the exact test is below the diagonal.

Population structure was also developed for the populations from wheat cultivars and chemotypes (Table 6). Genetic distance and identity for the populations from cultivars were 0.0089 and 0.9911, respectively; and 0.0246 and 0.9757 for the populations of chemotypes (Table 5). This indicates that there are close relationships within the populations from wheat cultivars and chemotypes. The analyses of population pairwise comparison and genetic migration revealed a gene flow within the populations of two wheat cultivars and the ones of two chemotypes, which were not significantly differentiated from each other (Table 7).

Seven geographic regions grouped from the 15 locations were analyzed for population structure (Table 8). The 7 regions were clustered into two subgroups, the population from Regions 1, 2, 3, 4 and 5 located in the southern part of Manitoba, and the population from Regions 6 and 7 in the northern part (Fig. 4). Genetic distance and identity between the populations from Regions 2 and 3 were 0.0147 and 0.9854, respectively, which were closer to each other than to others (Table 8). Genetic distance between the populations from Regions 1 and 2 was greater than the distance between the populations from Regions 1 and 3, which was greater than Regions 1 and 5, and 1 and 4; the distance between the populations from Regions 2 and 3 was greater than Regions 2 and 5, and 2 and 4; and the distance between the populations from Regions 3 and 5 was greater than Regions 3 and 4 (Table 8).

There were no significant differences between the populations from Regions 1, 2 and 3; and the populations from Regions 6 and 7 (Table 9). There was a gene flow among the populations from Regions 1 to 5, and the greatest N_m value was 8.7851 between Regions 2 and 3 (Table 9). A gene flow ($N_m = 4.4318$) was observed between Regions 6 and 7 (Table 9). However, significant difference was found between the populations from Regions 1 and 4 (Table 9).

Table 6. Pairwise comparison of the genetic identity and distance[a] of two wheat cultivar populations and two chemotype populations of *Fusarium graminearum* in Manitoba.

Population[b]	Superb	AC Barrie	15ADON chemotype	3ADON chemotype
Superb	****	0.9911	-	-
AC Barrie	0.0089	****		
15ADON chemotype	-	-	****	0.9757
3ADON chemotype	-	-	0.0246	****

[a] Nei's genetic identity based on 103 sequence-related amplified polymorphism loci is above the diagonal, and genetic distance coefficients are below the diagonal.
[b] Populations were defined as groups of isolates from different wheat cultivars, Superb and AC Barrie; and different chemotypes, 15ADON chemotype and 3ADON chemotype.

Table 7. Pairwise comparison of gene flow and probability of population differentiation[a] among different populations of *Fusarium graminearum* in Manitoba.

Population[b]	Superb	AC Barrie	15ADON chemotype	3ADON chemotype
Superb	****	11.1760	-	-
AC Barrie	0.3243	****	-	-
15ADON chemotype	-	-	****	5.6021
3ADON chemotype	-	-	0.1997	****

[a] Estimates of the number of migrants (*Nm*) between populations are above the diagonal, and probability of each pairwise comparison using the exact test are below the diagonal.

[b] Populations were defined as groups of isolates from different wheat cultivars, Superb and AC Barrie; and different chemotypes, 15ADON chemotype and 3ADON chemotype.

Table 8. Pairwise comparison of the genetic identity and distance[a] among different geographic regions of *Fusarium graminearum* in Manitoba.

Population[b]	1	2	3	4	5	6	7
1	****	0.9816	0.9695	0.8105	0.9533	0.6106	0.6832
2	0.0186	****	0.9854	0.8157	0.9570	0.6137	0.6789
3	0.0310	0.0147	****	0.8125	0.9554	0.5916	0.6830
4	0.2101	0.2037	0.2077	****	0.8053	0.2892	0.3163
5	0.0478	0.0439	0.0456	0.2166	****	0.6247	0.6145
6	0.4933	0.4882	0.5249	1.2406	0.4704	****	0.7343
7	0.3809	0.3873	0.3813	1.1512	0.4869	0.3088	****

[a] Nei's genetic identity based on 103 sequence-related amplified polymorphism loci is above the diagonal, and genetic distance coefficients are below the diagonal.
[b] Population 1 include Sanford, Morris and Horndean, population 2 include Cartier and Portage la prairie, population 3 include Hamiota, Rivers, Plumas and Rapid City, population 4 include Killarney and Souris, population 5 include McAuley and Virden, population 6 include only Dauphin, and population 7 include only Kenville.

Table 9. Pairwise comparison of gene flow and probability of population differentiation[a] among different populations of *Fusarium graminearum* from different regions in Manitoba.

Population[b]	1	2	3	4	5	6	7
1	****	4.6800	1.4489	1.4764	2.5819	0.6777	0.9629
2	0.3240	****	8.7851	4.0330	4.4697	0.7194	0.8047
3	0.2211	0.6091	****	4.6480	3.0408	0.3709	0.7079
4	< 0.0001	0.0543	0.3000	****	2.0325	0.4381	0.8804
5	0.0364	0.5455	0.0555	0.1000	****	0.4398	1.0973
6	< 0.0001	< 0.0001	< 0.0001	0.0427	0.0273	****	4.4318
7	< 0.0001	< 0.0001	0.0364	0.0273	< 0.0001	0.1746	****

[a] Estimates of the number of migrants (*Nm*) between populations are above the diagonal, and probability of each pairwise comparison using the exact test is below the diagonal.

[b] Population 1 include Sanford, Morris and Horndean, population 2 include Cartier and Portage la prairie, population 3 include Hamiota, Rivers, Plumas and Rapid City, population 4 include Killarney and Souris, population 5 include McAuley and Virden, population 6 include only Dauphin, and population 7 include only Kenville.

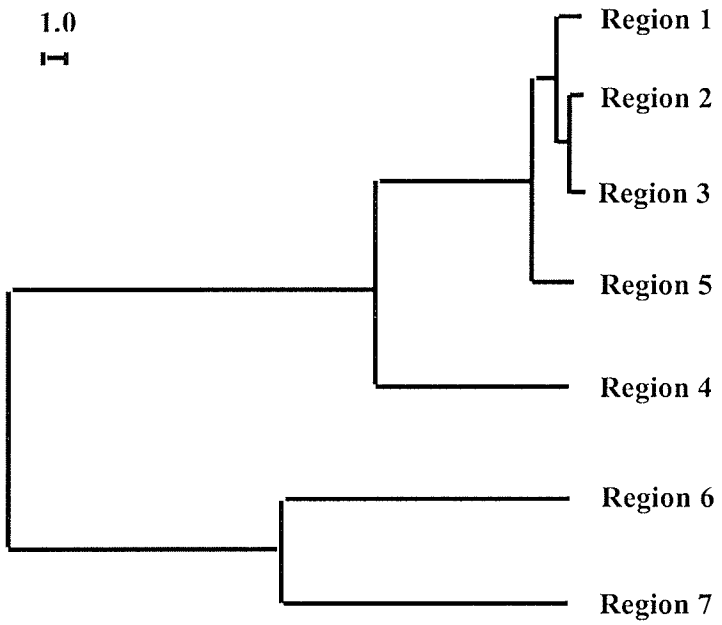


Figure 4. Cluster phenogram of Nei's genetic distance between the populations from 7 regions, consisting of 15 locations in Manitoba. Region 1 included locations Sanford, Morris and Horndean; Region 2 included Cartier and Portage la Prairie; Region 3 included Hamiota, Rivers, Plumas and Rapid City; Region 4 included Killarney and Souris; Region 5 included McAuley and Virden; Regions 6 and 7 included Dauphin and Kenville, respectively.

4.4 Discussion

This was the first study undertaken in Manitoba with reasonally large population (291 isolates) and over two years. This study revealed a large variation in percentage of chemotypes among the *F. graminearum* populations from different locations and regions. Although the 15ADON chemotype is predominant in Manitoba, recently, 3ADON chemotype emerged and showed an increasing trend in southern Manitoba (This study; Ward et al., unpublished data). This study showed that 3ADON chemotype was distributed in the south part of this province, and predominant in the region including Sanford, Morris and Horndean. There are different reasons for variation in percentage of chemotypes including artificial introduction of chemotypes into a new place with seeds or stubble. However this study suggests potential genetic possibility for this variation. Because it is difficult to find the identical farmers' fields between different years to trace chemotype changes, this study did the genetic analysis for the *F. graminearum* populations from different locations and regions to reveal potential chemotype shifting.

The variation of percentage of chemotypes in different locations and regions is associated with the degree of genetic diversity of *F. graminearum* populations from the corresponding locations and regions (Table 2), different chemotypes and cultivars (Table 3) as well. Sexual reproduction of *G. zeae* is one of the most important factors causing genetic diversity. *G. zeae* is a homothallic fungus, but it can be out-crossed both in culture (Bowden and Leslie, 1992) and in nature (Miedaner and Schilling, 1996; Dusabenyagasani et al., 1999). Dusabenyagasani et al., (1999) reported there was a high frequency of *G. zeae* sexual recombination in Ontario and Quebec in Canada. This study revealed great genetic diversities of *F. graminearum* populations from some locations (Table 2). In Sanford, of 19 *F. graminearum* isolates, 16 isolates

were unique genotypes, the genetic diversity index (*H*) was 0.2270, and polymorphic loci accounted for 67.96%. In Morris, 19 different genotypes were found in a total of 23 isolates; genetic diversity index and percentage of polymorphic loci were 0.2726 and 46.6%. The isolates from Portage la Prairie exhibited the highest percentage of polymorphic loci. This suggests that high frequencies of sexual recombination of *G. zeae* existed in these three locations, in which isolates with 3ADON chemotype were predominant. Sexual recombination within *F. graminearum* populations likely leads to the large variation of chemotypes among different locations and regions, though progeny segregation of chemotype has not been reported. In Germany, Cumagun et al. (2004) observed a large genetic variation of *G. zeae* derived from a cross between two DON-producing parents, significant segregation of DON production and pathogenicity was found in the progenies. However the parents and progenies were not identified for chemotype.

The age of a population from a certain location is likely another important factor for genetic diversity. The longer the history of the population, the more chance the *G. zeae* isolates have for sexual recombination or mutation, and the greater is the genetic diversity in this population. Thus, the populations from locations Sanford, Morris and Portage la Prairie could be older than other locations.

Tillage system affects genetic diversity of the population as well. Zero tillage or minimum tillage leaves a greater amount of wheat stubble on the soil surface than conventional tillage, which could be a large reservoir of *G. zeae* perithecia because perithecial formation needs light (Inch and Gilbert, 2003). Therefore, zero tillage or minimum tillage likely causes a greater genetic diversity than conventional tillage. With the introduction of the zero and minimum tillage practices in the prairies in the

past decade and a half, the chances for the genetic diversity of *G. zeae* populations to increase would have been greater.

Genetic identity and migration of *F. graminearum* populations within the sub grouped locations and regions, and the populations within the chemotypes and wheat cultivars revealed potential chemotype shifting. However, it was found that some populations that were not geographiccally close to each other, including Portage la Prairie and Hamiota; Cartier and Rivers; and Morris and Kenville or Dauphin, were similar to each other (Table 5 and 6, and Fig. 3). Wheat seed shipment was likely one reason for the genetic migration between these locations. A study revealed a capability of *G. zeae* ascospores for long distance dispersal through the planetary boundary layer of the atmosphere by day and night (Maldonado-Ramirez et al., 2005), and a great genetic diversity and gene flow of *G. zeae* populations existed spatially and temporally. Therefore, this is another possibility for the genetic migration between populations that are far from each other.

Generally, a high level of gene flow results in a low level of genetic diversity among populations. In this study, great variation of chemotypes was found within the subgroups of locations and regions, in which significant gene flows exists. Nevertheless, this study found that the greatest difference in percentage of 3ADON chemotype was 21.8% between locations Sanford, Portage la Prairie, Hamiota and Plumas; was 17.9% between locations Cartier, Rivers, Killarney and Souris; and was 95.7% between locations Morris, Killarney and Dauphin (Table 1 and Fig. 3). A similar situation was found within the subgroups of regions (Table 1 and Fig. 4). This suggests that there were likely greater genetic diversities and variations of chemotypes in the earlier years.

This study showed genetic distance and migration for *F. graminearum* populations on a large scale by grouping the 15 locations into 7 regions, and revealed the association of genetic factors with a potential chemotype shifting in Manitoba. There was a significant gene flow between the populations in the southern part of Manitoba including regions 1 to 5, and the populations of regions 1, 2, 3 and 5 were closer to each other, in which all the isolates with the 3ADON chemotype existed, suggesting that there was likely a potential for chemotype shifting in the southern part. Whereas, the populations from the two northern locations of this province, Kenville and Dauphin, were not differentiated form each other, in which only isolates with 15ADON chemotype were detected. Although there was a significant gene flow between the population from Morris, in which the 3ADON chemotype was predominant, and the population from Kenville, in which no 3ADON chemotype was found. If isolates with the 3ADON chemotype appear in one of the locations of Kenville or Dauphin, the other one would likely be contaminated by the 3ADON chemotype with the effect of the gene flow in the future.

The genetic migration between the *F. graminearum* populations contributed to the similarity among the populations from regions 1 to 5. The significant gene flow among the populations from locations Sanford, Portage la Prairie, Hamiota, Plumas and Rapid City could contribute to the closeness of the populations from Regions 1 to 3. The similarity between Regions 2 and 5 could be due to the gene flow between the populations from Cartier and McAuley. The same reasons can be found for the similarities between the other regions. The differentiation between the populations from the locations Kenville and Dauphin and the other locations contributed to the differentiation between the populations from Regions 1 to 5 and Regions 6 and 7 (Table 6 and Fig. 3). The genetic diversity and migration of populations on a smaller

scale of locations can be used for prediction of the same genetic characters on a larger scale of regions. The gene flow among the *F. graminearum* populations from different locations and regions caused potential chemotype shifting among the same places. The chemotype shifting among larger areas will likely be forecast incorparating the genetic information available in the smaller areas in the future.

In total, the variations of percentage of chemotypes could result from genetic diversity of *F. graminearum* populations in Manitoba, which could be associated with sexual recombination, age of populations and tillage system. The significant gene flow in southern Manitoba likely caused potential chemotype shifting; however, the direction of shift is difficult to measure. Wheat seed shipment and long-distance *F. graminearum* spore dispersal between different places likely contributed to gene flow in this province.

4.5 Conclusions

Fusarium graminearum's 3ADON chemotype was distributed in the southern part of Manitoba, which included Sanford, Morris and Horndean. It shared the same percentage with the 15ADON chemotype in the area including Cartier and Portage la Prairie, but was not detected in the northern part of Manitoba. High chemotype diversity among different locations and regions was associated with high genetic diversity, which may have been caused by *G. zeae* sexual recombination. High gene flow existed in the southern part of Manitoba and may have caused chemotype shifting, which was likely caused by seed shipment and long-distance spore dispersal.

CHAPTER 5

5.0 General Discussion, Conclusions and Contribution to Knowledge

5.1 General discussion and conclusions

The studies in Chapters 2 and 3 showed the effects of cropping practices on *F. graminearum* spores on wheat heads, FHB disease and DON accumulation, indicating that cropping practices, including crop rotation and tillage, and use of resistant wheat cultivars in the previous years, significantly affect the production of *F. graminearum* airborne inoculum. Fernando et al. (1997) found that most of *G. zeae* ascospores were trapped within 5-20 m of the inoculum source. Paulitz et al. (1999) developed a two-dimensional model for describing short-distance dispersal of *G. zeaea* ascospores. However, Schmale III et al. (2005) found that *G. zeae* ascospores could be discharged approximately 4.6 mm from the mature perithecia in still air, and carried into the atmosphere by wind. Maldonado-Ramirez et al. (2005) trapped over 10,000 visible *G. zeae* ascospores in the planetary boundary layer using a remote airplane. The above studies suggest that a portion of the *G. zeae* ascospores released from perithecia can spread in the same location, and the rest of the spores can be carried by air currents at the soil surface into the atmosphere, and possibly higher into the boundary layer, and thus move to distant locations. These spores could become a new inoculum source in a new area. Results of this study indicate that cropping practices played a more important role in the *F. graminearum* airborne inoculum level than spore long-distance dispersal.

This study demonstarted strong relationships of FHB disease index and DON level with the actual number of *F. graminearum*/*G. zeae* spores from anthesis to 14 days after. Therefore, correcting the predicted number of spores using actual data will make prediction of disease and toxin more accurate, which are concerns of wheat producers (Gilbert and Fernando, 2004). In this study, the prediction accuracy of the models for the FHB disease index and DON level averaged 73.3%. To achieve greater

prediction accuracy for the disease and DON level, collecting spores in the field using a spore trap is a promising method for the future, which will not only improve the prediction accuracy for the number of spores, FHB disease and DON levels, but also solve the problem of the effect of long-distance spore dispersal on the prediction of the above three profiles.

This study developed a prediction model (Model 6) for the number of *F. graminearum*/*G. zeae* spores on wheat heads using the data collected from one week before to two weeks after anthesis. Some factors used in the spore number prediction models were the same as the ones in the disease and toxin prediction models. This seems to mask the actual effect of the spores on the disease and toxin. Alternatively, the disease and toxin prediction models exclude the actual effect of spore numbers, which was predicted using CPI and weather conditions. Furthermore, FHB disease index and DON level have to be predicted by predicting *F. graminearum* spore numbers first, which would reduce prediction accuracy for the disease and toxin levels. However, the importance of fusarium spore numbers cannot be ignored and should be included in the disease and toxin prediction models, even if it will have to be predicted in the short term. In the long term the disease and toxin models including predicted spore numbers will become more accurate, because fusarium spore numbers is the most important factor for the disease and toxin prediction, and the problems can be solved by increasing prediction accuracy for *F. graminearum* spores, which can be achieved by using spore traps or field scouting. The number of the measured spores can be used for the correction of the number of the predicted spores. This can be performed by local agricultural institutes through cooperation or by wheat producers when a cheap and easily operated spore trap is available. By doing this in the future,

the predicted number of spores will be closer to the actual number of spores. The prediction accuracy of FHB disease and DON level will be significantly improved.

These prediction models for the FHB disease and DON level were developed based on the weather conditions in Manitoba, knowledge of *F. graminearum* species, and moderately susceptible and intermediately resistant wheat cultivars; therefore they can be used for wheat cultivars with a similar level of resistance in the wheat-growing areas that have similar climatic conditions and the same predominant *Fusarium* species. However, these models should be used with caution for the following factors: first, the most important factor is the cultivar, as discussed in the Chapter 3; secondly, different *Fusarium* species and how different the local predominant species are from *F. graminearum* in inoculum production; thirdly, the effect of CPI on the disease or toxin, which could be changed under different climatic conditions. Annual precipitation and temperature could affect the availability of fusarium inoculum on crop stubble, especially perithecial formation. The potential for modification of the models developed in our study to fit different regions needs further study.

The prediction models for FHB disease index and DON level in this study did not consider the effect of *F. graminearum* chemotype, which could potentially affect prediction results, especially DON level, due to the difference between the 3ADON and 15ADON chemotypes in DON production (Ward et al., 2005; Gilbert et al., 2006). Therefore, quantification of DON production produced by the two chemotypes, chemotype distribution in the predicted areas, and prediction of chemotype shifting are needed. The study of the first requirement has been undertaken by Dr. Dilantha Fernando's research group. An annual disease and toxin survey for chemotype distribution has been carried out in Manitoba by Dr. Jeannie Gilbert's team. This

study investigated the chemotype distribution in Manitoba from 2004 to 2005, and revealed genetic reasons for potential chemotype shifting by analyzing the genetic diversity and migration of *F. graminearum* populations. The results showed that significant gene flow existed between the populations from different locations, which were close or far from each other, which likely causes chemotype shifting. The gene flow between the closer locations could be caused by short-distance spore dispersal (Fernando et al., 1997), and the genetic migration between the farther locations and regions could result from seed shipment and/or long-distance spore dispersal (Maldonado-Ramirez et al., 2005). A high level of genetic diversity within the locations strongly suggest the possibility of sexual recombination, which has been found in the other locations (Cumagun et al., 2004; Dusabenyagasani et al., 1999), though *G. zeae* is homothalic. Thus, to predict chemotype shifting, the following aspects should be known: the previous-year inoculum level and percentage of *F. graminearum* chemotypes in a field or location, which needs a cooperative survey; long-term quantified cropping practice information, CPI used in this study could be considered, including crop rotation, tillage and cultivar resistance; possibility of sexual recombination in nature, which could be associated with the age of cropping practices used in the same location or region; forecast of long-distance spore dispersal based on weather conditions, especially wind speed and direction. Therefore, a reliable prediction model of *F. graminearum* chemotype shifting is a longway off.

The study of *F. graminearum* chemotype shifting and population genetic analysis not only improves the prediction of chemotype shifting in the future, but also meets the requirement of disease management. For example, in an area where the 3ADON chemotype is predominant, the wheat seeds should be tested and treated using fungicides before shipped to another area; more- than-3-year crop rotation,

cultivation after wheat harvesting and seeding, and resistant cultivars to the disease are in this field or location; whereas in the area where no 3ADON chemotype has not found, disease prediction should be emphasized.

In summary, this study developed four prediction models for *F. graminearum*/*G. zeae* spores on single wheat heads using cropping practices and weather conditions, and two types of prediction models for FHB disease index and DON level based on cropping practices, actual or predicted *F. graminearum* spores on wheat heads, and weather conditions. The average prediction accuracy for the disease and toxin level was 73.3%. The prediction models using the actual spore number (Type I models) can be used for wheat quality prediction by the Canadian Wheat Board in the future. The prediction models using the predicted spore number (Type II models) can be used with spore number prediction model (Model 6) for wheat producers.

The 15ADON chemotype of *F. graminearum* is predominant in Manitoba. However, the 3ADON chemotype is distributed across the southern part of Manitoba, and predominates in Sanford, Morris and Horndean; and it is present in equal proportions with the 15ADON chemotype in Cartier and Portage la Prairie. There was great variation in percentage of chemotypes in different locations and regions. Genetic diversity and gene flow of the *F. graminearum* populations likely caused the diversity of chemotype, and genetic migration contributed to the potential chemotype shifting. Sexual recombination within the populations and long-distance *F. graminearum*/*G. zeae* spore dispersal and seed shipment between locations and regions could play important roles in genetic diversity and gene flow of the populations.

5.2 Contribution to knowledge

This study for the first time took into account cropping practices and airborne fusarium inoculum level in the development of FHB and DON prediction models.

This study successfully quantified cropping practices by assigning a different score to different crops, tillage methods, years and wheat cultivars in resistance in the previous years. CPI significantly indicated cropping practices for FHB and DON level in wheat and can be used for other studies of FHB epidemics.

This study designed a new spore trap to simulate deposition of spores on wheat heads in the field. The number of *F. graminearum* spores on the artificial wheat heads indicated airborne fusarium inoculum level and showed a strong relationship with FHB and DON level. It is the first time this study considered *F. graminearum* spore counts in the model development, which makes FHB and DON prediction more reliable than the other models.

This study also for the first time reported the *F. graminearum* chemotype distribution and potential chemotype shifting in Manitoba. This information can be integrated into development of disease management strategies in the future.

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