Fusarium Head Blight of Wheat: Evaluation of the efficacies of fungicides towards *Fusarium graminearum* 3-ADON and 15-ADON isolates in spring wheat and assess the genetic differences between 3-ADON isolates from Canada and China

By

Chami Chathurangi Amarasinghe

A Thesis

Submitted to the Faculty of Graduate Studies

In partial Fulfillment of the Requirements for the degree of

MASTER OF SCIENCE

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba, Canada

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

Fusarium Head Blight of Wheat: Evaluation of the efficacies of fungicides towards *Fusarium graminearum* 3-ADON and 15-ADON isolates in spring wheat and assess the genetic differences between 3-ADON isolates from Canada and China

BY

Chami Chathurangi Amarasinghe

A thesis submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements for the degree

OF

MASTER OF SCIENCE

ACKNOWLEGMENTS

First of all my sincere gratitude goes to Dr. W. G. D. Fernando, my supervisor, for his great support, guidance, encouragement and advice given to me throughout my Masters studies. He provided a valuable opportunity for me to be a part of his challenging research team. My heartiest gratitude goes to Drs Anita Brule Babel, Lily Tamburic-illinicic, and Jeannie Gilbert my advisory committee members, for the encouragement, help and invaluable guidance given to me throughout my research. They always helped me during my studies and gave me a lot of new ideas to improve my research.

Special thanks to Ms. Paula Parks, for the great support given to me in the field, greenhouse and lab. Paula always helped me and guides me in every aspect.

Also my heartiest gratitude goes to Ms. Mary Meleshko, for the invaluable help given to me for my field trials. I would like to thank Mr. Lyle Frisen, I really appreciate the help and advice given by you, for my data analysis. I would like to thank Nick and Mary, our summer students for helping me in the field. Also I would like to thank the staff in the general office, and green-house for their continuous help.

I would like to convey my heartiest gratitude to my dear friends Ru Li and Dr. Khaled Al Taweel, for the invaluable support given to me.

At last but not least, very special heartfelt thanks goes to my Amma, Thaththa, Nangi and Sajeewa for their invaluable never ending support and encouragement given to me throughout my Masters studies to make it a success.

ABSTRACT

Amarasinghe, Chami Chathurangi. M.Sc., The University of Manitoba, December 2010. Fusarium Head Blight of Wheat: Evaluation of the efficacies of fungicides towards *Fusarium graminearum* 3-ADON and 15-ADON isolates in spring wheat and assess the genetic differences between 3-ADON isolates from Canada and China. Major Professor; Dr. W. G. D. Fernando.

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, is one of the most destructive global diseases of small cereal grains worldwide. Severe epidemic outbreaks of FHB have been reported in North America, South America, Asia and Europe. The most devastating effect of this disease is the deposition of mycotoxins in the grain. Deoxynivalenol (DON) and its analogs 3-ADON, 15-ADON and NIV are the major mycotoxins produced by the fungus. The chemotype, 15-ADON is more prevalent in North America, but recently a chemotypic shift has been observed. The rapid emergence of the 3-ADON chemotype over the traditional 15-ADON chemotype is a major concern in North America. Understanding the behaviour of the 3-ADON chemotype and the reasons for the chemotypic shift is important in several aspects. In this study, the efficacy of fungicides in controlling FHB caused by 3-ADON and 15-ADON chemotypes was evaluated. All fungicides significantly reduced FHB disease symptoms and increased the yield, but some differing results were obtained from a few treatments. The findings of this study eluted to an integrated management approach over the use of a single management strategy to control FHB. This study evaluated the chemotype diversity of *F. graminearum* in corn, in Ontario and revealed that all investigated isolates were 15-ADON. Use of a single primer to differentiate 3-ADON,

15-ADON and NIV chemotypes is more efficient over multiplex polymerase chain reaction (PCR) approach. The reliability of using a single primer based on *TRI13* gene to differentiate 3-ADON and 15-ADON chemotypes of *F. graminearum* was investigated. Interestingly, PCR based on *TRI13* gene revealed genetic differences that exist between the 3-ADON chemotypes of Canada and China. The 3-ADON isolates from Canada had a 61 bp deletion in the *TRI13* gene, but this deletion was absent in 3-ADON isolates from China. Therefore this confirms the presence of genetic differences among 3-ADON chemotypes based on their geographic origin. Another study revealed that the *F. graminearum* 3-ADON isolate can cause more disease over 15-ADON chemotype or from the combined infection of both isolates.

FOREWORD

This thesis is written in manuscript style. A general introduction and review of literature precedes manuscripts that comprised the main part of the thesis. Each manuscript consists of an abstract, introduction, materials and methods, results and discussion. A general discussion and conclusions, a list of references cited and a list of appendices follow the manuscripts.

TABLE OF CONTENTS

CHAPTER 7

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

LIST OF FIGURES

CHAPTER 3

LIST OF APPENDICES

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 General introduction

Fusarium head blight (FHB), also called ear blight or scab, is one of the major fungal diseases of small grain cereals worldwide. This disease can cause severe damage to most important economic cereal grains such as wheat, barley, corn and oats. In North America, *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch) is considered to be the major causative agent of FHB although other *Fusarium* species are also implicated in causing head blight (Bai and Shaner, 1994; Parry et al., 1995; McMullen et al., 1997; Gilbert and Tekauz, 2000).

Epidemics of FHB disease are sporadic worldwide (Fernando et al., 2000). During the period from 1927 to 1980, there were six epidemics of FHB in Canada occurring in Ontario, Quebec and the Maritime provinces in 1980 (Sutton, 1982). In Manitoba, disease severity and incidence of FHB increased from 1984 to 1991 (Wong et al., 1992). In 1993, the most severe outbreak of FHB occurred in south-central Manitoba showing 20-80% disease incidence in infected fields especially in the Red River Valley and adjacent regions in crop districts 7 and 8 (Gilbert et al., 1995). The estimated economic losses to FHB in Canada in the 1990s were US$200 million for Quebec and Ontario and US$300 million for Manitoba (Windels, 2000).

Fusarium head blight of wheat appears as blighted heads they produce shrunken and light-weight kernels. The symptoms of FHB are often characterized by the presence of fungus, which may include pink sporodochia and /or purple black perithecia on glumes and seeds often are shrunken or shrivelled and called “tombstones’and Fusarium damaged kernels (FDK) (Parry et al., 1995). The damage from FHB is multifold; it reduces both

grain quantity and quality. Although the disease reduces the wheat yield, the mycotoxins which may accompany with the disease are often a more serious issue in the wheat industry. This mycotoxin contamination of wheat grain lowers the market grade and presents difficulties in marketing, exporting and processing of grains (McMullen et al., 1997).

The most common mycotoxins associated with *Fusarium*-infected wheat grain are trichothecenes, mainly deoxynivalenol (DON) and its acetylated derivatives, 3-acetyl deoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV) (McCormick, 2003). These trichothecenes can induce acute or chronic toxic effects on humans, animals and plants. Vomiting, feed refusal, bleeding, dizziness, vertigo are the common symptoms associated with trichothecenes (Desjardins, 2006; Yazar and Omurtag, 2008). Trichothecenes are potent phytotoxins produced by many plant pathogenic *Fusarium* species and can cause wilting, chlorosis and necrosis in infected plants (McClean, 1996). Tricothecenes are thought to play a key role in pathogenicity, aggressiveness or in both (Cumagun et al., 2004).

. Host resistance, fungicides, biological control and cultural practices are the common management strategies being used to control FHB. However application of fungicides is still the major control method used in many countries. Several authors have provided conflicting evidence regarding the efficacy of fungicides in controlling FHB. Therefore further research is needed in this area to clarify these conflicting results.

In recent years molecular surveillance on *F. graminearum* chemotypes showed a rapid chemotypic shift from 15-ADON chemotypes to more toxigenic 3-ADON

chemotypes from eastern to western Canada (Guo et al., 2008; Ward et al., 2008). The study done by Ward et al. (2008) showed that both under *in vivo* and *in vitro* conditions 3-ADON chemotypes can produce more trichothecenes than the 15-ADON chemotypes. This rapid shift from the traditional 15-ADON chemotype to 3-ADON chemotype is an increasing concern in the wheat industry in Canada. Therefore, understanding the distribution of chemotypes in Canada, the genetic basis of the chemotypic shift and the impact of this chemotype shift on major control methods such as fungicide applicationis of importance.

In view of this, the major objectives of this study were to examine whether the chemotype shift of *F. graminearum* influences the current disease management strategies such as fungicide application, to investigate the chemotype diversity of *F. graminearum* in corn fields in Ontario and to examine the reliability of using a single primer pair to differentiate 3-ADON and 15-ADON chemotypes of *F. graminearum* in Canada.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1Wheat

2.1.1 Origin of wheat

Wheat (*Triticum* spp. L.) is one of the most important food crops in the world and has been the basic staple food of the major civilizations of Europe, West Asia and North Africa for 8000 years (Curtis, 2002). Wheat is a primary source of energy, protein and dietary fibre in human nutrition and themost important source of human food. It is cultivated on more land area than any other commercial crop with a global production of over 600 million tons produced from about 210 million hectares in many different countries. Approximately 65% of all the wheat grain is used directly as human food, 21% to feed livestock, 8% as seed and the remaining 6% for other uses (Gooding and Davies, 1997; Curtis, 2002; Shewry, 2009).

Wheat is believed to have originated in southwestern Asia. Wheat was first cultivated about 10000 years ago, as a part of the "Neolithic Revolution". Some of the earliest remains of wheat have been found in Syria, Jordan, and Turkey (Curtis, 2002; Shewry, 2009). Primitive relatives of present day wheat have been discovered in the Fertile Crescent, which date back 9,000 years. Other archeological findings show that bread wheat was grown in the Nile Valley around 5,000 B.C. as well as in India, China, and even England at about the same time (Curtis, 2002; Shewry, 2009). Through historical inscriptions it has been affirmed that wheat is the earliest field crop used for human food processing. *Triticum monococcum* (einkorn) and *Triticum dicoccum* (emmer) were the earliest found wheat varieties, grown 12,000 – 17,000 years ago in the Near East (Feldman, 1997; Curtis, 2002; Shewry, 2009; Gustafson et al., 2009).

Wheat is better adapted to harsh environmental conditions than the other crops such as rice and corn. The optimum growing temperature for wheat is about 25 ºC, with minimum and maximum temperatures of 3 ºC to 4 ºC and 30 ºC to 32º C, respectively (Curtis, 2002). The optimum precipitation range for wheat growth lies between 250 mm to 1750 mm. Therefore wheat can be grown within a wide range of locations having diverse environmental conditions. Wheat is most successfully grown in the areas located between the latitudes of 30 º and 60 º N and 27 º and 40 º S (Curtis, 2002).

2.1.2 Classification and Types of Wheat

Wheat is classified under the genus *Triticum*, tribe *Triticinae*, family Gramineae, order Poales, class Liliopsidia, division Magnoliophyta and Kingdom Plantae. There are three types of *Triticum* species based on ploidy level; diploid (14 chromosomes), tetraploid (28 chromosomes) and hexaploid (42 chromosomes) (Gooding and Davies, 1997; Gustafson et al., 2009). Wheat has three different sets of chromosomes; A, B and D. Diploid wheat contains AA, tetraploid and hexaploid wheat contains AABB and AABBDD chromosomes, respectively (Feldman, 1997; Curtis, 2002; Shewry, 2009; Gustafson et al., 2009).

Wheat is mainly divided into two types based on the growing season, spring wheat and winter wheat. This classification is not solely based on the seeding time and harvesting time. Winter wheat usually has higher yield and lower protein concentration than spring wheat (Curtis, 2002). There are about 30 species of wheat, but by far the most important wheat is the common wheat (*T. aestivum* L.syn. *T. Vulgare* Host) or hexaploid

bread wheat. This is the most widely grown type of wheat and includes numerousvarieties indicating diversified agroecological adaptations (Gooding and Davies; 1997). There are a total of 11 types of wheat in the world, 1) hard red spring wheat 2) hard white spring wheat 3) soft red spring wheat 4) soft white spring wheat 5) hard red winter wheat 6) hard white winter wheat 7) soft red winter wheat 8) soft white winter wheat 9) Compactum (*T. compactum*) 10) Spelta (*T. spelta*) and 11) durum wheat (*T. durum*). Common wheat, also known as bread wheat, is commonly grown in the prairies. Durum wheat (AABB), can be grown under hot and dry conditions and used in many pasta products. It is widely grown in Minnesota, North and South Dakota and in Alberta, Canada (Gooding and Davies; 1997; Curtis, 2002).

In Canada, there are eleven main commercial classes of wheat based on the milling properties, dough rheology, bran colour and vernalization requirement; Canada Western Hard Red Spring (CWRS), Canada Western Hard White Spring (CWHWS), Canada Western Amber Durum (CWAD), Canada Prairie Spring Red (CPSR), Canada Western Extra Strong (CWES), Canada Prairie Spring Winter (CPSW), Canada Western Soft White Spring (CWSWS) , Canada Eastern White Winter (CEWW),Canada Western Red Winter (CWRW), Canada Eastern Soft Red Winter (CESRW), Canada Eastern Soft White Winter (CESWW) and Canada Eastern Hard Red Winter (CEHRW) (DePauw and Hunt, 2001; Curtis, 2002).

2.1.3 Importance of wheat

Wheat is important for mankind in multiple ways. With a total of 224 million hectares of crop fields, it is the most widely cultivated crop in the world today. According to the FAO statistics, 690 million metric tonnes of wheat was produced in 2008 (http://faostat.fao.org).

Wheat plays a vital role in the human diets. Wheat has higher protein, fat and fibre content, compared to other grains. It is also rich in vitamins and minerals such as manganese, phosphorus, potassium, zinc, vitamin B6, folate, thiamin, riboflavin and riacin. Therefore, for thousands of years, wheat has been one of the most prominent food sources for human and livestock. Wheat flour is used to make a wide variety of foods such as bread, biscuits, cookies, cakes, breakfast cereal, pasta, noodles, and couscous (McMullen et al., 1997; Pena, 2002). Using fermentation of wheat, beer, other alcoholic beverages, and biofuels are produced.

2.1.4 Growth and Development of Wheat Plant

The growth cycle of wheat consists of five phases; 1). Germination, seedling establishment and leaf production 2). Tillering and spike differentiation 3). Stem and spike growth 4). Spike emergence and flowering 5). Grain filling and maturity (Simmons et al., 1995).

Germination starts when a kernel is sown in the presence of water. The radical and seminal roots initiate from the level of the seed and at the crown of the seedling.

Consequently, the growth of the coleoptile ceases and the first leaf emerges through the tip as soon as it has grown out of the soil (Simmons et al., 1995).

After seedling establishment, the plant starts to produce leaves at a rate of one per 4 or 5 days. Usually there will be a total of 8 or 9 leaves produced. Emergence of the flag leaf is an important stage in the growth of the plant and this helps for timing the application of plant growth regulators (Simmons et al., 1995).

Tillering is one of the most important stages in the growth of the plant; it allows plants to compensate for low plant populations. Lower leaves on the main shoot and the coleoptile are the points of attachment from which tillers are formed. Variety and the growing conditions are the decisive factors influencing the number of tillers formed in a plant (Simmons et al., 1995). Even though all tillers do not produce grain, other than the main shoot, there may be a total of three tillers per plant under usual growing conditions. The tillers which appear fourth to sixth from the main shoot have the higher probability of producing grain (Simmons et al., 1995).

Parallel to the tillering, formation of spikes on the tillers and main shoot can be observed. Parts which will possibly become kernels and floral structures are being formed in this microscopic spike (Simmons et al., 1995). The "boot" stage is just prior to spike emergence, when the flag leaf sheath encloses the growing spike (Simmons et al., 1995). Booting is defined as the stage, when the spike can be felt within the whorl of leaf sheaths, but this is not visible (McMaster, 2009), booting stage ceases when heading of the plant begins.

The ‘heading’ of the plant occurs when stem continues to elongate in such a way that the spikeis pushed out of the flag sheath. Flowering, also known as pollination, initiates in the spike. It starts with the florets in the central spikelets. Flowering can be observed by when anthers are thrust out from the floret. But this depends on the conditions such as weather. When pollination starts, anthers within a floret turn to yellow or gray. Pollination within a single spike takes around four days. The young kernels within a spike vary considerably in size at pollination and maintain this size variation throughout grain filling to maturity (Simmons et al., 1995).

Usually, the growth of the kernel takes around three weeks. The kernel grows in three phases (Simmons et al., 1995). Under the first phase, numerous cells in the endosperm are formed. Endosperm is the main protein and starch storage section of the kernel. Secondly, a couple of weeks after pollination, the kernel starts producing protein and starch. It causes the dry weight of the wheat grain to increase. Unfavourable environmental conditions during the above mentioned growth periods of the kernel might decrease the yield as a result of a low rate of dry matter accumulation. Necessarily, the longer the harmful weather conditions exist during grain filling, the greater the effect on yield (Simmons et al., 1995). Products of photosynthesis that are produced during the grain filling stage contribute 70 to 90% of the final grain yield (Simmons et al., 1995). The flag leaf and the spike also give a significant contribution (about 50%) of the photosynthate to grain filling, but the amount depends on the environmental conditions; therefore maintenance of green and functional upper leaf blades, sheaths and spikes during grain filling results in better grain yields (Simmons et al., 1995).

2.1.5 Wheat production and trade

World wheat production is almost entirely based on just two wheat species; common wheat or bread wheat that accounts for about 95% of the world production and durum wheat that accounts for the remaining 5% of production (Shewry, 2009). As a result of extensive efforts made through national and international breeding programs wheat production per hectare has almost tripled and the area sown to wheat has doubled over the last 50 years (Curtis, 2002).

China ranks first in world wheat production having the largest cultivated land area for wheat (29.4 million hectares). United States, India, Russian Federation, Kazakhstan and Canada are the other top wheat producers in the world having 25, 24.9, 23.6, 12.6 and 11.5 million hectares of wheat cultivated wheat land, respectively (Curtis, 2002).

Wheat is also the world's most widely traded food grain with about 150 million tons or about 18% of world production traded each year. China being the world's largest wheat producer is also the world's largest wheat importer. Russian Federation, Egypt, Japan and Brazil rank among the other top wheat importers in the world. The United States, Canada, France and Australia are the largest wheat exporters in the world (Curtis, 2002).

2.1.6 Wheat in Canada

Canada is the world's sixth-largest wheat producer and one of the largest exporters, producing an average of over 25 million tonnes and exporting about 19 million tonnes annually. Within Canada, wheat is the most important cultivated crop. In Canada,

wheat probably was first grown at Port-Royal in about 1605; the first exports were made in 1654. In 1870, the cultivar Red Fife, developed in Ontario, became very popular because of its good yield and excellent milling and baking qualities. The cultivar, Marquis developed by crossing Hard Red Calcutta and Red Fife made Canada famous for its high quality hard red spring wheat over the world and rapidly increased wheat production during 1909 (DePauw and Hunt, 2001; http://thecanadianencyclopedia, 10.08.2010).

Ninety five percent of all Canadian wheat is produced in the Prairie Provinces of Manitoba, Saskatchewan and Alberta. Although low precipitation restricts yield, the unique climatic conditions within the Prairies provide ideal conditions for development of better wheat with high protein and baking properties. The most common wheat type grown in Prairies is CWRS, accounting for 70% of total production. CWRS wheat has superior milling and baking qualities offered at various guaranteed levels of protein content. Because of the grain quality, CWRS has a high export demand from other countries. CWAD wheat produces a high yield of semolina with excellent pasta-making quality and it accounts for 20% of total wheat production, and approximately 4 million tonnes are produced each year (Curtis, 2002).

The long warm summer days coupled with adequate moisture levels and fertile soil in Manitoba makes it an ideal place to grow wheat (Curtis, 2002). In Manitoba, approximately 5.5 million acres are cultivated with wheat and contributes 17% to the total wheat production in Canada (Curtis, 2002). CWRS wheat is the most commonly grown wheat variety in Manitoba, accounting for 89% of the total production. Manitoba exports wheat to over sixty countries in the world including United States, Iran, Japan and

Mexico and earns around 500 million dollars annually through wheat production (Curtis, 2002).

2.2 *Fusarium*

Fusarium spp. are considered to be one of the most economically important ascomycetous fungi causing disease in most genera of cultivated plants (Parry et al., 1995). It has been reported that at least one *Fusarium*- associated disease is found on many plants (Leslie and Summerell, 2006). Furthermore, *Fusarium* can cause serious diseases in humans, animals as well as mycotoxicoses (Desjardins, 2006; Yazar and Omurtag, 2008). Ear rot in corn, 'Bakane' disease in rice and fusarium head blight in wheat are the common diseases caused by *Fusarium* spp. in cultivated lands (Parry et al., 1995).

2.2.1 Origin and classification of *Fusarium*

Fusarium spp. are believed to belong to an ancient fungal group that appears in the early stages in the evolution of Ascomycetes (Backhouse et al., 2001). *Fusarium* spp. have been found worldwide including the areas from subarctic to above the Arctic Circle. The distinctive taxa *F. nurragi* and *F. aywerte* that are found in native vegetation in Australia may provide information about the past evolution and migratory patterns (Backhouse et al., 2001). *Fusarium* belongs to anamorphic Hypocreaceous Ascomycetes (*Ascomycota: Hypocreales: Hypocreaceae*) in the genera *Gibberella* and *Nectria* (Liddell, 2003). Most of these fungi are saprophytes that can colonize the living host at any stage during the life cycle of the host. Several species of *Fusarium* have been

identified in association with fusarium head blight and they fall into four biologically distinct sections; *Discolor*, *Roseum*, *Gibbosum* and *Sporotrichiella* (Liddell, 2003).

The major pathogen of FHB, *Fusarium graminearum* (teleomorph: *Gibberella zeae*) is classified under the section *Discolor*. The fungi belonging to section *Discolor* are found to produce vivid carmine-red mycelium on high carbon sources, and the classic banana shaped macroconidia. The production of microconidia is not found among the fungi belonging to this section (Booth, 1971; Liddell, 2003). *Gibberella zeae* (anamorph: *Fusarium graminearum*) is divided into to two groups; Group 1 is responsible for causing crown and root diseases in wheat and generally do not produce perithecia in culture. Group 2 which readily produce perithecia in culture is associated with head blight and stalk rot of maize (Burgess et al., 1975; Francis and Burgess, 1977; Liddell, 2003; Bushnell et al., 2003). Group 1 has been re-classified as a distinct species, *Fusarium pseudograminearum* (teleomorph: *Gibberella coronicola* (Aoki and O'Donnell, 1999a, 1999b).

2.2.2 Morphological characteristics and distribution of *Fusarium* spp.

The morphological characteristics that are used to identify *Fusarium* spp. are described in reviews and *Fusarium* identification manuals (Nelson et al., 1994; Leslie and Summerell, 2006). The formation of macroconidia is considered as one of the key morphological features that distinguishes *Fusarium* spp.from other genera.

Macroconidia are identified as multiseptate, moon crest or banana-shaped spores produced inside a sporodochium. Normally there are three shapes of macroconidia i.e. straight or needle like, dorsiventral curvature, and dorsal curvature. The species

determination within the *Fusarium* genus is based on the shapes of the end, apical and basal cells of the macroconidia. The apical cells basically have four shapes i.e blunt, papillate, hooked and tapering whereas basal cells also have four shapes i.e foot-shaped, elongated foot shaped, distinctly notched and barely notched (Leslie and Summerell, 2006). Microconidia are produced from aerial mycelia and not from sporodochia. They are of various shapes, i.e oval, reniform, obovoid, napiform, globose and fusiform (Leslie and Summerell, 2006). Chlamydospores are thick walled spores having lipid substances; they allow the fungus to survive under harsh environmental conditions.

Fusarium spp. are widely distributed across all geographic regions, including soils, plants and air. Some species show cosmopolitan distribution whereas some species are found only in tropical and subtropical regions, or cool to warm temperate regions, while others are not affected by the climatic conditions (Burgess and Summerell, 1992).

2.2.3 *Fusarium graminearum*

Although the main causative agent of FHB, *F. graminearum*, is considered as a single, panmictic species worldwide, results of several studies have proved the existence of phylogenetically distinct species within the *F. graminearum* clade. The phylogenetic analyses based on DNA sequences from portions of eleven nuclear genes at six independent loci including the mating type locus (MAT locus) and Histone H3, totalling 13.6 kb, identified nine biogeographically structured, phylogenetically distinct species within the *F. graminearum* clade (O'Donnell et al., 2004). All of these species appear to have reached an advanced state of genetic isolation because each was reciprocally monophyletic within six individual nuclear gene genealogies and the combined

phylogeny of these genes (O'Donnell et al., 2004). These species include, *Fusarium austroamericanum* (lineage 1), distributed in South America; *F. meridionale* (lineage 2) frequently found in South and Central America, South Africa, New Caledonia, Nepal and Korea ; *F. boothii* (lineage 3) distributed in South Africa, Mesoamerica, Nepal, Guatemala and Korea ; *F. mesoamericanum* (lineage 4), is found in Central America and Pennsylvania; *F. acacia-mearnsii* (lineage 5), is found in Australia and South Africa; *F. graminearum* (lineage 7) found worldwide ; *F. cortaderiae* (lineage 8) and *F. brasilicum* (not given a lineage designation), found in South America and Oceania (O'Donnell et al., 2004; Starkey et al., 2007). This study was further expanded by Starkey at el. (2007) including two more genes, elongation factor-1α (EF-1α) and reductase (RED) totalling 13 genes and found two new *Fusarium* species. The two new *Fusarium* species were *F. vorosii* collected in Hungary and *F. geralachii* in upper Midwest of USA. O'Donnell et al. (2008) identified another new *F. graminearum* clade species from Ethiopia, *F. aethiopicum* (no lineage number). Yli-Mattila et al. (2009) also identified another new species from the Russian Far East and designated as *F. ussurianum*. The B-trichothecene chemotype polymorphism is maintained through multiple speciation events and may have consequences for the fitness and aggressiveness of FHB pathogens on particular hosts or in specific environments. The phylogenetic and evolutionary studies on *F. graminearum* suggest that the combined species and mycotoxin diversity of FHB pathogens is very high (Ward et al., 2008).

2.3 Fusarium head blight

Fusarium head blight (FHB), also called scab, is an economically important fungal disease in many crops including wheat, barley and oats (Parry et al., 1995). The disease Fusarium head blight was first described as wheat scab by W.G. Smith in 1884. In 1920 Atanasoff used the term "Fusarium blight" (Stack, 2003). Today, both scab and head blight is used to describe the disease. Although many species of *Fusarium* contribute to the FHB complex, *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch) is considered to be the major pathogen of FHB in many countries (Parry et al., 1995; McMullen et al., 1997; Gilbert and Tekauz, 2000; Shaner, 2003). *Fusarium culmorum*, *F. avenaceum*, *Microdochium nivale*, *F. moniliforme*, *F. oxysporum* and *F. poae* are the other related species that play a minor role in FHB development (Liddell, 2003; Parry et al., 1995). Contamination of grain cereals with mycotoxins, especially with deoxynivalenol is a major concern in the wheat industry. The contamination of wheat grain with potent mycotoxins alters the milling, baking and pasta making properties of the grains (Kushiro, 2008). *Fusarium graminearum* is commonly found in continental regions in Asia, North and South America and Europe and *F. culmorum* is more prevalent in temperate regions (Parry et al., 1995). The right combination of high humidity, rain and temperature during flowering in the presence of a susceptible host and aggressive *Fusarium* strains leads to FHB outbreaks. There have been epidemics of FHB in the USA, Canada, Asia, Europe and South America (Xu and Chen, 1993; Parry et al., 1995; McMullen et al., 1997).

2.3.1 Symptoms of FHB

Fusarium head blight symptoms are characterised by the appearance of water-soaked brown-coloured spots at the base or the middle of the glume or on the rachis. Eventually this water soaked appearance and discoloration spread further from the point of invasion (Parry et al., 1995). When all conditions are highly favourable for FHB development, a growth of salmon pink to red coloured mycelia can be seen on the base of the spikelet and spreads through the entire head. Ultimately the infected grains become discoloured, shrunken and chalky white in appearance with black perithecia giving the name "scab" (Parry et al., 1995).

2.3.2 FHB Disease cycle

The components that are necessary to develop and establish a disease are described in the disease triangle. The disease triangle is composed of 1) inoculum 2) suitable host 3) favourable environmental conditions (Shaner, 2003). All diseases, including FHB depend on the above components of the disease triangle.

Inoculum

Fusarium-infected crop residues of various plants, such as wheat, corn, barley, soybean and rice on the soil surface serve as the primary sources of inoculum (Shaner, 2003; Parry et al., 1995; Bai and Shaner, 2004). Although macroconidia, chlamydospores and fusarium hyphal fragments can act as a source of inoculum, ascospores are considered to be the major source of inoculum that initiates infection (Bai and Shaner, 2004). FHB is considered as a monocyclic disease; therefore, the level of primary

inoculum that is available to infect the host plant will have a great effect on epidemic development (Sutton, 1982; Bai and Shaner, 1994). Environmental factors such as temperature, moisture and wind have a direct impact on inoculum production and release and dispersal of spores. During warm, moist and windy environmental conditions, the ascospores disperse by air currents and deposit on a susceptible host plant and initiate infection (Shaner, 2003; Fernando et al., 1997). The amount of inoculum is determined by the amount of crop residue and the degree of infection of the crop residue on the soil surface (Dill-Macky and Jones, 2000). Fernando et al. (1997) examined the spore dispersal gradients for ascospores and macroconidia of *G. zeae*. They concluded that airborne ascospores were the primary inoculum sources and primary infection is the main cause of the disease whereas the secondary spread only has a minor role in disease development.

Asymptomatic inflorescences of wild grasses, copious perithecia and ascospores on the soil surface have been examined as minor sources of *Fusarium* inoculum. Fusarium damaged kernels (FDK) do not act as a source of inoculum, sowing FDK does not cause FHB (Gilbert and Fernando, 2004).

The host plant

Fusarium graminearum has a wide range of hosts and can survive on both living and also dead plant tissue (Xu and Chen, 1993). The flowering stage or shortly after the flowering stage of a host plant is considered the most vulnerable stage for *Fusarium* infection (Bai and Shaner, 1994; Shaner, 2003). In wheat, the window for FHB infection is short and occurs from anthesis through early grain development. Because of this short

vulnerable period, FHB infection is restricted to one infection cycle with no secondary infection (Shaner, 2003). The developmental cycle of *Gibberella zeae* is well studied by Guenther and Trail, (2005). During infection, the ascospores germinate and colonize the developing caryopsis, floral bracts and rachis. Hyphal growth on the exterior surfaces allows the fungus to move to stomates and invade the more susceptible sites within the glume or floret. Consequently, the glumes of the infected floret show dark brown necrotic lesions resulting in blighted florets. The fungus invades the other spikelets through the vascular bundles of the rachilla and rachis causing severe infections on the entire spike. Three distinct morphologies have been observed in the colonizing hyphae: thin and uninucleate, wide and dikaryotic and curled into perithecium initials (Guenther and Trail, 2005). Once florets are infected, they fail to produce grains or they will not be well filled (Bushnell et al., 2003). As mentioned previously, FHB is a monocyclic disease therefore the density of host plant per land is less important in disease development (Shaner, 2003).

Environmental factors

Environmental factors such as temperature and moisture affect the inoculum production, dispersal and the duration of the vulnerability of the host plant to the infection. Optimum temperatures for head blight development range between 25 to 32 ºC (Sutton, 1982). Precipitation has a great impact on epidemic development. Because favourable temperatures for FHB development are common during the flowering stage of cereal crops, moisture has a major role in inoculum production, dispersal and initial infection during disease establishment than the temperature (Sutton, 1982). *Fusarium graminearum* inoculum is generally produced under warm weather conditions than in

cool conditions. The optimal temperatures for *F. graminearum* perithecial and ascospore production were 29 and 25-28 ºC respectively. Paulitz et al. (1996) examined the daily pattern of ascospore release by *Giberella zeae* and reported that, the discharge of inoculum is triggered by the combined effect of drop in air temperature and a rise in relative humidity. Although rainfall has a role in ascospore and perithecial formation, it does not trigger the release of ascospores (Paulitz et al., 1996). According to the studies by Sutton (1982) ascospore release occurs between 10-30 ºC and the optimum temperature was 16 ºC. The studies done by Tschanz et al. (1976) reported that low temperatures were favourable for discharge of ascospores and no discharge of ascospores was observed above 26 ºC. Fernando et al. (1997) reported that the dispersal of *F. graminearum* inoculum occurs downwind from inoculated wheat heads and disease incidence was higher in irrigated plots than the non-irrigated plots. Release of ascospores is correlated with the rain fall events and the time of day (Fernando et al., 2000). Environmental factors such as temperature, pH, humidity, aeration and light affects the germination of ascospores. Beyer and Verreet (2005) reported that age of ascospores, relative humidity, temperature and pH were the key factors that determine the germination of ascospores. After release, ascospores germinated within 4 hrs at 20 ºC and a relative humidity of 100% (Beyer and Verreet, 2005). According to the study done by Gilbert et al. (2008) the germination of *G. zeae* ascospores were highest at 90% relative humidity and lowest at 60 %. It is reported that ascospores can germinate successfully even under extreme environmental conditions (Gilbert et al., 2008). Anderson, (1948) reported that the germination of macroconidia occurred within 3 hr at 28-32 ºC or within 6 hr at 20-32 ºC. A period of 92-94% high relative humidity in combination with warm

temperatures during the flowering stage of host plant makes an ideal environment for FHB development and establishment (Sutton, 1982).

2.3.3 Types of resistance against FHB development

Five types of resistance to FHB have been proposed; Type I: resistance to initial infection by the pathogen (Schroeder and Christensen, 1963), Type II: resistance to spread of the pathogen within the head from the point of invasion (Schroeder and Christensen, 1963), Type III: resistance to kernel infection (Mesterházy, 1995) Type IV: tolerance- ability to maintain the yield irrespective of the presence of the disease (Mesterházy, 1995). Type V: resistance to toxins which can be either the ability of host to degrade toxins produced by the pathogen or plant insensitivity to accumulation of toxins within the tissue (Miller et al., 1985; Bai and Shaner, 2004). Type I resistance is common in spring wheat from Brazil such as; Frontana and Type II resistance is exhibited by Chinese resistant varieties such as Sumai 3 and its derivatives (Bai and Shaner, 2004). Type I resistance is assessed by using spray inoculation and Type II by single floret inoculation techniques (Schroeder and Christensen, 1963). Type II resistance is assessed under controlled environmental conditions and found to be more stable than Type I as it is less affected by non-genetic factors (Bai and Shaner, 1994).

2.3.4 Infection of wheat by *Fusarium*

The thick epidermal cell walls in the exterior surfaces of glume, lemma and palea act as barriers for the direct penetration of *Fusarium* spp. (Lewandoski et al., 2006). Therefore, the pathogen must have alternative ways to enter the host tissue. Kang and Buchenauer, (2000a) studied the cytology and ultrastructure of the infection of wheat

spikes by *F. culmorum* using light and electron microscopy. They reported that macroconidia can germinate on wheat glumes within 6-12 hrs after inoculation and produce one to several germ tubes. In a similar study, Pritsch et al. (2000) have reported that macroconidia of *F. graminearum* also germinate 6-12 hrs after infection. Histological studies done by Kang and Buchenauer, (2000a), revealed that, penetration of host tissue occurred through the inner surfaces of lemma, glume and palea, but not from the outer surfaces of lemma, glume and rachis. A clear difference was observed between the hyphal growth on outer surfaces and inner surfaces of lemma, glume and palea. A dense hyphal network was observed on inner surfaces where as no or less hyphal network on outer surfaces (Kang and Buchenauer, 2000a).

The fungus can directly penetrate the host tissue through the wounds. Boshoff et al. (1996) reported that, *F. graminearum* was able to penetrate the wheat lemma through wounds. Anthers also play a key role in pathogen entry. Anthers provide routes for fungus to enter the florets and act as an initial base for floret colonization (Pugh et al., 1933). A positive correlation was found between the rate of partial exsertion of anthers and rate of infection (Strange and Smith, 1971). Kang and Buchenauer, (2000a) and Ribichich et al. (2000) reported that *F. culmorum* and *F. graminearum* can grow on pollen surfaces and both anthers and pollen can accelerate hyphal growth. Another avenue of infection is the abaxial surface at the base of the wheat glume. This has relatively thin epidermal cell walls. It is reported that FHB causing *Fusarium* spp can produce cuticle and wall degrading enzymes. Kang and Buchenauer, (2000b) reported that during infection *F. culmorum* produced cell wall degrading enzymes such as cellulases, xylanases and pectinases. Therefore, these enzymes may serve as weapons when entering the floret..

The spread of the pathogen from spikelet to spikelet occurs through the vascular bundles in the rachilla and rachis (Kang and Buchenauer, 2000a; Kang and Buchenauer 2000c; Ribichich et al., 2000). The fungus colonizes within the vascular bundle and cause major alterations within the vascular tissue, such as, thickening of walls, deposition of wall appositions and occlusions of vessels. As a result of these alterations phloem and phloem parenchyma isblocked and then collapses. Kang and Buchenauer, (2000c) observed the cytological differences during *F. culmorum* infection in resistant and susceptible wheat cultivars using immunogold labelling technique. They reported, although the initial infection process is similarthe pathogen's development is slower in resistant cultivar than the susceptible cultivar.

2.4 FHB control strategies

2.4.1 Cultural control

Cultural control is an environmentally friendly approach that can be used to reduce the risk of FHB epidemics. Numerous studies have been done to evaluate the effect of crop rotation on FHB development. Depending on the previous crop, the severity of FHB can be affected. Rotation of wheat with non-host crops reduces the amount of inoculum in the crop residues (Sutton, 1982; Parry et al., 1995, Dill-Macky and Jones, 2000). Research showed that when wheat was grown following maize, FHB infection increased by 15% compared to only 4% infection when wheat was sown following alfalfa or oats (Pirgozliev et al., 2003). In another study, it has been found that cultural practices such as tillage do not have significant effects on the disease severity and kernel infection (Miller et al., 1998). Miller et al, (1998) examined the effect of tillage on FHB disease

incidence and suggested that the use of FHB resistant cultivars is more important in controlling FHB epidemics than tillage practices. Dill-Macky and Jones, (2000) evaluated the effect of crop rotation and tillage on FHB of wheat and reported that, FHB severity and incidence was less when wheat was grown after soybean than after wheat or corn irrespective of the tillage practice. Dill-Macky and Jones, (2000) also reported that conventional tillage and no till systems contributed to FHB epidemics in the Upper Midwest. Schaafsma et al. (2005) reported that previous crop, field size and tillage affect the FHB index, DON accumulation and, *Fusarium*-damaged kernels in infected fields. Studies done by Schaafsma et al. (2001) also reported that tillage had no effect on DON levels in infected wheat grains. Guo et al. (2010) quantified the effects of cropping practices on *F. graminearum* inoculum levels and developed a cropping practice index (CPI) model to express the relationship. Applications of nitrogen fertilizers, can, however, increase the incidence of FDK in wheat, barley and triticale (Martin et al., 1991). But Teich and Hamilton, (1985) reported that application of nitrogen fertilizers had no significant effect on the FHB disease incidence. According to Yi et al. (2001) application of nitrolime reduced the incidence of FHB by 59% but no significant reduction of DON accumulation was observed. Weed control is another cultural practice that can be adopted to reduce the FHB. Instead weed can act as an alternative source of FHB inoculum, control of weeds can reduce the availability of alternative FHB inoculum (Pirgozliev et al., 2003). Fields with higher weed densities had higher numbers of infected heads than the weed-free fields (Teich and Nelson, 1984).

2.4.2 Biological control

Biological control is an important part of an integrated FHB management system. Biological control is an environmentally friendly, durable method that is compatible with other control strategies (Schisler et al., 2002b). The strategies for biological control of FHB include the control of the pathogen by disrupting the fungal life cycle using non-pathogenic microorganisms. Spikelet infection, colonization, ascospore production and dispersal are considered to be potential points for this biological intervention (Luz et al., 2003). Biological control of FHB mainly includes treatment of crop residue with antagonists to reduce the pathogen inoculum or application of antagonists to wheat heads during anthesis to reduce fungal infection (Schisler et al., 2002b). The biological control of the pathogen may be achieved by aborting, curtailing or delaying the germination of the spores in the infection court of the head (Fernando et al, 2000). Antibiosis, competition, mycoparasitism, induced resistance and inhibition of mycotoxin synthesis are considered to be the major modes of action of biocontrol agents (Schisler et al., 2002a; Luz et al., 2003). Various research groups have examined the use of a wide range of microorganisms against the development of FHB (Stockwell et al., 1997; Luz et al., 2003). The Brazilian isolates of *Bacillus* and *Paenibacillus* are found to be the most effective biocontrol agents that can reduce the FHB disease severity in field by 50-67% (Luz et al., 2003). Schisler et al. (2002) isolated microbial strains from wheat anthers during anthesis and examined the feasibility of using those organisms in biological control of FHB. They could identify four strains that utilize tartaric acid and three that did not utilize tartaric acid as potential biocontrol agents from wheat anthers. These strains reduced the FHB disease severity up to 95% under green-house conditions and 56% under

field conditions (Schisler et al., 2002a). In another study, Schisler et al. (2006) identified 31 choline metabolizing strains from wheat flower tissue; all of them reduced FHB disease severity by 25% in a green house trial where 17 of them reduced the disease severity up to 50% on wheat. Khan and Doohan et al. (2009) reported that, *Pseudomonas fluorescens* strains MKB 158 and MKB 249 significantly reduced both the FHB severity and mycotoxin contamination caused by *F. culmorum* on wheat and barley. Another *Pseudomonas* strain, *Pseudomonas frederikbergensis* also significantly reduced the FHB severity under both green house and field conditions (Khan and Doohan, 2009). Nourozian et al. (2006) reported on use of Bacillus *subtilis* strains 53 and 71, and *Pseudomonas fluorescens* biov1 strain 32 and *Streptomyces* spp. strain 3 as potential biological agents for control of FHB. Also Fernando et al. (2002) examined three bacterial strains of *Bacillus substilis* (Ehrenberg) Cohn strains H-08, S-01, and L-01 and were found to reduce FHB disease severity. Ramarathnam et al. (2007) reported that *Bacillus substilis* strain DFH08 significantly inhibited the radial mycelial growth of *F. graminearum* by 60% compared to the control and reduced the disease severity in a green-house study. Khan et al. (2001) isolated seven novel antagonists from wheat anthers and examined the efficacy of those antagonists against three isolates of *G. zeae* on the wheat cultivar Norm. Xue et al. (2009) found that, strain ACM941 of *Clonostachys rosea* significantly reduced the FHB index, FDK and DON content but less effective than the fungicide tebuconazole. Schisler et al. (2002b) demonstrated the feasibility of using biocontrol agents to control FHB on durum wheat and found that yeast antagonists were more successful in reducing the FHB symptoms than bacterial antagonists on durum wheat. There is a lack of consistency between the performance of biocontol agents under

controlled environmental conditions and natural field conditions. Biocontrol agents that are proven to be effective under controlled conditions do not perform in the same way under field conditions. This is one of the major issues in commercial biocontrol production. Therefore, future research should focus on identifying biocontrol agents and effective application technologies to restrict the FHB colonization and mycotoxin accumulation both under large scale field conditions and glass-house conditions (Luz et al., 2003). As biological control agents are also living organisms, they may require specific or optimum conditions for their functioning (Fernando, 2003). Therefore presence of a favourable environment for a particular biocontrol agent ensures an effective control against *F. graminearum*.

2.4.3 Fungicide control

Chemical control is one of the main parts of an integrated FHB management approach. Fungicides are currently used at both flowering stage and before flowering stage to reduce quantitative yield loss and mycotoxin contamination (Mullenborn et al., 2008). Effective fungicides should be safe products with short pre-harvest interval and have high efficacy in reducing FHB and DON.Traditionally should have optimum application rates and techniques and a reasonable price. To date, many fungicides with different active ingredients are being used to manage FHB.

Little studies have been done regarding control of FHB with fungicides (Mesterházy, 2003b). Several factors such as level of inoculum, cultivar resistance, climatic conditions, crop sensitivity and yield potential affect the success of fungicide application in controlling FHB (Mesterházy, 2003b). Fungicides based on the triazole

chemistry (tebuconazole, metconazole or prothioconazole) are considered to be the most effective among all available registered fungicides (Mesterházy et al., 2003; Edwards et al., 2001; Pirgozliev et al., 2002; Simpson et al., 2001). Triazole based fungicides inhibit the 14α demethylase, an enzyme that is essential for ergosterol biosynthesis (Klix et al., 2007). The efficacy of use of fungicides and the effects on FHB and mycotoxin contamination in the field are often conflicting. In some studies, it has been found that triazole fungicides such as metaconazole, tebuconazole, prothioconazole and tebuconazole were effective, resulting in reductions of head blight severity and mycotoxin contamination by 50-80% and 5-90% respectively (Matthies and Buchenauer, 2000). On the contrary, in another study, application of fungicides has resulted in an increased trichothecene accumulation (Gareis and Ceynowa, 1994; Simpson et al., 2001). Gareis and Ceynowa, (1994) observed that application of the fungicide, Matador to *F. culmorum* infected winter wheat, increased the NIV content in infected seeds. Application of the fungicide, Azoxystrobin also increased the DON content in infected grains (Simpson et al., 2001). Therefore, presence of conflicting evidence in the use of fungicides to control the development of FHB needs to be clarified. Fungicides may affect the severity of FHB symptoms and the amount of DON in harvested grain by either altering the proportion of trichothecene- producing *Fusarium* spp. or altering the rate of DON synthesis (Hasan, 1993; Edwards et al., 2001). Hasan et al. (1993) studied the effect of fungicides on diacetoxyscirpenol and zearalenone produced by *F. graminearum* and observed that fungicides significantly reduced the toxin content and fungal growth. It has been suggested that fungicides are more effective at the early stages of the *Fusarium* infection

process such as during spore germination and growth of the germ tube. An *in vitro* study done by Klix et al. (2007) found triazole based fungicides inhibit ascospore germination.

The two main critical factors in use of fungicides to control FHB are the timing and rate of application. The best time to apply fungicides is the period after the emergence of the head. Because the systemic triazole fungicides do not move from leaves to head from the point of contact, early application can protect only the leaves not the heads (Mesterházy, 2003b). Application of fungicides several weeks before wheat anthesis may be more harmful for non-toxigenic microorganisms and can promote subsequent spread of toxigenic *Fusarium* species in the field (Henriksen and Elen, 2005). Fungicides should be applied from both sides of the plots as partial coverage reduces the control of FHB. The rate of application may vary with the type of fungicides applied and comes with the fungicide label. It has been found that the concentration of fungicides was highest in the glumes and gradually decreases when moving to lemma and the embryo (Mesterházy, 2003b).

It is always recommended that fungicides be used with other management strategies such as tillage, crop rotation and resistant cultivars. The combined effect of severalstrategies would provide a better control with higher yield and less infection. The following table illustrates some of the commonly used fungicides and their mode of action **(Table: 1).**

Table 2.1: Mode of action of commonly used fungicides to control FHB

Fungicide	Mode of action
Tebuconazole	Sterol biosynthesis inhibitor
Metconazole	Sterol biosynthesis inhibitor
Propiconazole	Sterol biosynthesis inhibitor
Asoxystrobin + propiconazole	Respiration and sterol inhibitor
Copper sulphate	Blocking SH group
Fenarimol	Cell membrane synthesis inhibitor
Isoprothiolane	Lipid metabolic inhibitor
Captan	Energy metabolic inhibitor

Source: http://www.agnet.org/library/bc/54012/

2.5 Breeding for FHB resistance

Breeding for commercial wheat cultivars with high levels of FHB resistance with all the other desired agronomic traits is a huge challenge due to the lack of sources combined with high levels of FHB resistance with favourable agronomic traits (Bai and Shaner, 2004). The polygenic nature of FHB resistance, the effect of environment on resistance phenotype and the complex disease evaluation procedures make the breeding process more complicated (Bai and Shaner, 2004).

Wheat resistance to FHB is controlled by multiple genes. Because resistance to FHB is a complex quantitative trait some researchers hypothesised that it is controlled by

many minor genes. But some studies have proven that it is controlled by a few major genes (Snijders, 1994; Buerstmayr et al., 1999; Miedaner, 1997; Bai et al., 2003). Although resistance in some cultivars is determined by additive gene effects, non-additive gene effects such as dominance and epistasis might also have a significant role in FHB resistance (Bai et al., 2000). Spring wheat cultivar, Sumai3 (Funo and Taiwanxiaomai) developed in China has been used in wheat breeding programs worldwide as a resistant parent. Ning 7840 (Abpoba/Anhui11 x Sumai3) developed from Sumai3, is another popular resistant source used in current FHB breeding programs (Bai et al., 2003; Buerstmayr et al.,2002; 2003; Mesterházy, 2003). Ning 7840 has the same resistance level as Sumai3 for FHB with improved yield potential and it shows more resistance to other wheat diseases such as leaf rust, stem rust, stripe rust and powdery mildew (Bai and Shaner, 2004). The resistant parents, Sumai3 and Ning7840, have resistance genes on chromosomes 1B, 2A, 5A, 6D , 7D, 2B, 3B and 6B (Yu, 1982). Wheat cultivar, Wangshuiibai has resistance genes on chromosomes 4A, 5A, 7A, 7B and 4D (Liao and Yu, 1985).

In addition to these Chinese resistant wheat cultivars, other FHB resistant sources such as Brazillian wheat cultivar Frontana and Encruzilhada, United States winter wheat cultivars, Ernie and Freedom, Japanese cultivars Shinchunaga and Nobeokabouzu komugi are being used in FHB breeding programs (Mesterházy, 2003a; Ginkel et al., 1996; Bai and Shaner, 2004). Two cultivars Chokwang from Korea and Fundulea 201R from Romania have sources of resistance different from that of Sumai3 and its derivatives (Bai and Shaner, 2004).

2.6 *Fusarium* mycotoxins

2.6.1 Trichothecenes

Fusarium produces a number of chemically diverse toxins (Desjardins, 2006). Trichothecenes are considered the most common mycotoxins produced by *Fusarium.* Trichothecenes are sesquiterpenoid mycotoxins that have molecular weights typically ranging from 200-500 Da. These are characterized by a tricyclic ring structure containing a double bond at C-9, 10 and an epoxide group at C-13 (Foroud and Eudes, 2009; Desjardins, 2006; Mirocha et al., 2003). Regardless of the size and structural composition, trichothecenes are strongly associated with chronic and fatal toxicosis of humans and animals. They inhibit eukaryotic protein synthesis with specific activity on ribosomal protein L3 within the 60S subunit causing an inhibition of peptidyl transferase activity (Rocha et al., 2005; Desjardins, 2006). *Fusarium graminearum*, *F. culmorum*, *F. poae*, *F. sporotrichioides* are the main trichothecene producers. There are four types of trichothecenes (type A to D) based on the type of substitution at the C-8 position. Type A and B are more common in cereals. Type A trichothecenes, such as T-2 and HT toxins have hydrogen, hydroxyl or an ester group at C-8 whereas Type B trichothecenes, such as DON, nivalenol (NIV) and fusarenon-X (Fus-X) have a keto group at C-8 (Desjardins, 2006).

Trichothecene biosynthesis is a complex process that proceeds from farnesyl phosphate via the trichodiene, followed by a sequence of sesquiterpene cyclization, eight oxygenations and four esterifications (Desjardins, 2006). So far, fifteen genes have been found to involve in the trichothecene biosynthesis pathway. These genes have been

identified within either *F. sporotrichioides, F. graminearum* or in both species (Foroud and Eudes, 2009). The genes involved in the trichothecene biosynthesis pathway were found to be arranged as clusters. Ten pathway genes have been identified within a gene cluster spanning a 23 Kb region of DNA in *F. sporotrichioides* (Hohn et al., 1998). Trichodene synthase (*TRI5*) was the first trichothecene biosynthetic gene to be discoverd. This gene was first isolated as *TOX5* from *F. sporotrichioides* by using specific antibodies (Hohn and Beremand, 1989). *TRI5* gene is located on linkage group 1 of the genetic map of *F. graminearum* and also has been localized on contig 1.159 (Jurgenson et al., 2002). Because *Fusarium* spp. are haploid and the *TRI5* gene occurs as a single copy in the genome, it has been found that, disruption of the *TRI5* gene interrupts the biosynthesis of trichodene and all the other trichothecens. Loss of function mutants of the *TRI5* gene has provided opportunity to examine the involvement of trichothecenes in plant-fusarium interaction (Proctor et al., 1995; Desjardins et al., 1996; McCormick, 2003; Desjardins, 2006). Other genes such as *TRI4*, *TRI1*, *TRI13*, *TRI101*, *TRI8*, *TRI6*, *TRI10*, *TRI11*, *TRI12*, *TRI15*, *TRI7*, *TRI9*, and *TRI14* are also involved in the trichothecene biosynthetic pathway. Except for the genes *TRI1* and *TRI101* gene, all known trichothecene genes are located on the *TRI5* gene cluster (McCormick, 2003; Desjardins, 2006).

The most widespread mycotoxin of fusariotoxin group is deoxynivalenol (DON) (12, 13-epoxy-3α, 7α, 15-trihydroxytrichothec-9-en-8-one) and it is a low molecular weight hapten (MW=296 Da) that has one primary and two secondary hydroxyl groups (Mirocha et al., 2003; Desjardins, 2006). Deoxynivalenol, which is also known as vomitoxin, is primarily produced by *F. graminearium* and *F. culmorum*. Three strain

specific profiles of trichothecene chemotypes have been identified in the B-trichothecene lineage of *Fusarium*: i) nivalenol and acetylated derivatives (NIV), (ii) deoxynivalenol and 3-acetyldeoxynivalenol (3-ADON chemotype), and (iii) deoxynivalenol and 15-acetyldeoxynivalenol (15-ADON chemotype). The chemotypic differences between NIV and DON are based on the single change in hydroxyl group at C-4. The 3-ADON and 15-ADON are two naturally occurring acetylated derivatives of DON (Mirocha et al., 2003). These strain specific chemotype differences are strongly associated with the allelic polymorphisms within the trichothecene biosynthetic gene cluster (O'Donnell et al., 2000; Ward et al., 2002). The genes located at either end of the biosynthetic cluster are found to be highly correlated with chemotype and encode proteins that determine chemotype differences (McCormick et al., 1996). The gene *TRI3*, that encodes a 15-O-acetyl transferase is responsible in producing 15-ADON thus 15-ADON chemotype and *TRI8* gene contributes directly to the 3-ADON chemotype (Kimura et al., 2003). *TRI13* gene, that encodes an oxygenase, is found to be involved in C-4 hydroxylation in NIV biosynthesis pathway and the determination of the NIV chemotype (Lee et al., 2002). Both DON chemotypes are not able to produce NIV due to the presence of a non-functional *TRI13* gene in their gene clusters (Kimura et al., 2003; Lee et al., 2002). The 3-ADON chemotype is more prevalent in China, Europe and Japan, where as 15-ADON chemotype is found most frequently in North America. The chemotype diversity is believed to be depending on the geographical origin of the pathogen and the susceptible host crop (Ward et al., 2002). The level of toxicity of DON, 3-ADON, 15-ADON and NIV are found to be different in different animal and plant cells (Kimura et al., 1998). Goswami and Kistler, (2004) indicated that, the different chemotypes of FHB pathogens

may have different consequences on host plants based on their level of aggressiveness and fitness.

2.6.2 Zearalenones

Zearalenones are one of the most frequently found *Fusarium* mycotoxins in temperate regions of America, Europe and Asia (Yazar and Omurtag., 2008). They are commonly found as contaminants in corn but also are present in other cereals (Krska et al., 2003). Zearalenones are non steroidal estrogenic mycotoxins that are not acutely toxic and have not been associated with any fatal mycotoxicoses in humans or animals. These mycotoxin causes infertility, abortion or other breeding problems especially in swine and sheep (Yazar and Omurtag., 2008). Many *Fusarium* spp. such as *F. graminearum*, *F. culmorum*, *F. equiseti, F. pseudograminearum, F. semitectum* and *F. crookwellense*. have been reported to produce zearalenones. However, the species *F. oxysporum* and *F. solani* do not produce zearalenones (Desjardins, 2006). Contamination of corn with zearalenones causes adverse effects for the corn industry and for animal and human food and feed (Yazar and Omurtag., 2008; D'Mello et al., 1999; Desjardins, 2006). The maximum accepted level of zearalenon in diets is 0.25 ppm (D'Mello et al., 1999; Desjardins, 2006).

2.6.3 Fumonisins

Fumonisins have relatively simpler chemical structures compared to zearalenones and trichothecenes (Desjardins, 2006). To date, twenty-eight fumonisins have been isolated and are classified into four series known as A, B, C and P. FB1, FB2 and FB3 are the main natural contaminants of cereals (Yazar and Omurtag, 2008). Fumonisins are

produced by many *Fusarium* spp., including *F. verticillioides* (formerly *Fusarium moniliforme=Gibberella fujikuroi*), *F. proliferatum*, *F. anthophilum*, *F. nygamai* as well as *Alternaria alternata* f. sp. *lycopersici*. FB2 is a carcinogenic mycotoxin and was first found in *Aspergillus niger*, an industrially important species (Yazar and Omurtag, 2008). Fumonisins can cause leukoencephalamalacia, a fatal brain disease in horses and porcine pulmonary edema (PPE), a fatal disease in pigs. Fumosins are also found to be carcinogenic in experimental rodents (Desjardins, 2006). The International Agency for Research on Cancer (IARC) has identified the cancer risk of fumonisins to humans and classified them under group 2B as carcinogenic substances. It has been found that cereals contaminated with FB1 can cause human esophageal cancer and several cases were reported in Africa, northern Italy and in Iran (Yazar and Omurtag, 2008).

2.7 Effects of *Fusarium* mycotoxins

2.7.1 Effects of trichothecenes on animal and human health

Trichothecenes are commonly found mycotoxins on cereals worldwide (Visconti, 2001). The mode of action of trichothecens includes inhibition of DNA, RNA synthesis, mitochondrial function, effects on cell division and membrane effects. These toxins bind to the peptidyl transferase, which is an integral part of the 60S ribosomal subunit of mammalian ribosome. Toxins are also reported to be interfering with the metabolism of membrane phospholipids and affect the cell membrane (Yazar and Omurtag., 2008; Foroud and Eudes, 2009; Visconti, 2001; McCormick, 2003).

The common symptoms of trichothecenes toxicity in animals include weight loss, decreased feed conversion, feed refusal, vomiting, bloody diarrhea, severe dermatitis,

hemorrhage, decreased egg production, abortion, and death (Yazar and Omurtag., 2008; Foroud and Eudes, 2009).

T–2 toxins can cause inhibition of DNA, RNA and protein synthesis and mitochondrial function. The T–2-contaminated products can result in severe health disorders both in humans and animals and heavy doses can even cause death of animals. The main target of the T-2 toxins is the immune system. The general symptoms of T-2 include nausea, emesis, dizziness, chills, abdominal pain, diarrhea, dermal necrosis, abortion, irreversible damage to the bone marrow and aleukia.

The mode of action of vomitoxin, DON, involves disruption of normal cell structure by inhibiting protein synthesis via binding to the ribosome. It has been reported that DON can activate important cellular kinases in the cell that are involved in signal transduction related to proliferation, differentiation, and apoptosis. DON is less toxic than T-2 and HT –toxins but high doses can cause sudden death of animals. Immunostimulation is one of the main toxic effects of DON at the cellular level. The general symptoms of DON toxicity include abdominal distress, increased salivation, malaise, diarrhea, emesis and anorexia (Yazar and Omurtag, 2008; Desjardins, 2006). Deoxynivalenol can cause similar adverse effects in humans, and several cases have been reported in Asia, where humans suffered with vomiting, nausea, dizziness and headaches, after consumption of cereals contaminated with DON (Yazar and Omurtag, 2008).

2.7.2 Effects of trichothecenes in plants

Trichothecenes can cause adverse effects in plant systems including inhibition of seed germination and growth of seedling shoot and root. These toxins also can cause

wilting, chlorosis and necrosis in a wide variety of whole plants and plant tissues (McClean, 1996; McCormick., 2003; Desjardins, 2006). The phytotoxic effects of trichothecenes are associated with the inhibition of ribosomal protein synthesis, disruption of membrane integrity and alteration in electron transport and leaf chlorophyll content in plants (Desjardins, 2006). Several efforts have been made to develop trichothecene resistance in plants through breeding. The proposed mechanisms include alterations of the ribosomal target sites, inhibition of trichothecene biosynthesis and detoxification of trichothecenes (McCormick., 2003; Desjardins, 2006).

2.7.3 Effects of mycotoxins on wheat quality

The shrivelled and light-weight fusarium damaged kernels (FDK) are difficult to remove from the uninfected kernels, during processing. Hence, mycotoxins within the FDK affect the quality of the end products. Dexter et al. (1997) investigated the level of fusarium damage on semolina milling, gluten strength and pasta making quality of ten durum wheat cultivars in Manitoba, and found that, FDK have a significant effect on the processing quality of durum wheat. Fusarium damage reduced semolina yield and affected color which became duller and red. Because the fungus attacks via the kernel, the fusarium mycotoxins that are concentrated in the mill feeds are mainly derived from the outer layer of the wheat kernel. Young et al. (1984) examined the effects of milling and baking on DON content of eastern Canadian wheat and reported that milling resulted in fractionation of both DON and ergosterol. After milling, a higher content of DON was found in the outer kernel portions and a lower content in the inner flour portions. Levels of mycotoxins were similar in both the best quality flour and low quality flour. It is

difficult to remove DON and other fusarium mycotoxins completely from flour by milling as the fungus is commonly found in the aleurone and pericarp tissues (Dexter and Nowicki, 2003). It is reported that debranning or pre-processing before milling can better reduce the mycotoxin concentration in flour than flour from conventional milling (Dextar and Wood, 1996). Secondary processing like baking, frying and toasting can have various effects on the retention of fusarium mycotoxins in the end products (Scott, 1984). In the presence of high fusarium mycotoxins the wet gluten becomes soft and sticky and reduces the dough development time and stability (Dextar et al., 1996). Heavy fusarium infection can reduce baking performance and the level of reduction depends on the wheat variety. It is reported that these detrimental effects on dough processing is based on two factors; 1) fungal proteolytic activity 2) availability of high ratio of free fatty acids due to the increased fat breakdown in wheat samples after fungal infection (Nightingale et al., 1999).

2.7.4 Trichothecenes as pathogenicity factors

Aggressiveness is the quantity of disease caused by a pathogenic isolate on a susceptible host and this determines the outcome of a particular disease. Pathogenicity is the ability of the pathogen to cause the disease (Gilbert et al., 2001; Cumagun et al., 2004). It is reported that aggressiveness of *G. zeae* is non host specific (Van Euwijik et al., 1995; Gilbert et al., 2001). Numerous studies have been done to examine the role of trichothecene as an aggressiveness factor in FHB but this still remains unclear. Hestbjerg et al. (2002) and Gang et al. (1998) found a positive correlation between aggressiveness and DON production by *G. zeae* and *F. culmorum,* but in other studies no or inconsistent

correlations were observed (Adams and Hart, 1989; Liu et al., 1997; Walker et al., 2001). Research done by Adams and Hart, (1989) found that, DON and 15-ADON did not act as pathogenicity factors on maize or carnation. It has been found that trichothecene-chemotype of isolates and the cumulative impact of multiple trichothecenes produced either by a single isolate or combinations of isolates may increase disease severity (Arseniuk et al., 1999; Mesterházy et al., 1999). In addition, some FHB-resistance sources have been shown to have the ability to detoxify DON, primarily by glycosylation (Boutigny et al., 2008). Acetylation and de-epoxydation are the other two detoxification processes found especially in transgenic plants. These findings suggest that the ability of *Fusarium* species to cause disease is correlated to trichothecene accumulation in the host, and that reduced aggressiveness may be observed by either reduced toxin production by the pathogen or removal/degradation of the toxin by the host (Foroud and Eudes, 2009).

Studies on the *TRI5* gene, the gene encoding the first enzyme in the trichothecene pathway, reveal the role of tricothecens in aggressiveness (Desjardins, 2006). The *tri5* mutants were still pathogenic to wheat, rye and maize but were less aggressive than their wild-type genotypes (Desjardins et al., 1996; Proctor et al., 1997; Langevin et al., 2004). DON acts as an aggressiveness factor in FHB rather than a pathogenicity determinant (Bai and Shaner, 2004). The non- DON producing *Fusarium* strains can only cause initial infection but not further invasion. Therefore, it is suggested that DON is not necessary for initial infection but for the spread of the pathogen within the spike (Bai et al., 2002; Langevin et al., 2004; Desjardins, 2006).

2.8 Chemotype diversity and geographical distribution patterns

The chemotypes appeared to differ in geographical distribution, with both DON and NIV chemotypes reported in several countries of Africa, Asia, and Europe (Miller et al. 1991 ; Jennings et al. 2004) but only the DON chemotypes were reported in North America (Mirocha et al. 1989). In a recent study Starkey et al. (2007) identified six *F. gramineaum* isolates with a NIV or 3-ADON chemotype in Louisiana, USA. In China, two *Fusarium* phylogenetic species appeared to have different geographical distribution patterns. Ninety-five percent of *F. asiaticum* species lineage 6 that produce DON, 3-ADON, 15-ADON and NIV mycotoxins originated from warmer regions, whereas *F. graminearum* species lineage 7, which consists only of 15-ADON chemotypes, was predominantly from cooler regions (Zhang et al., 2007). Ji et al. (2007) also examined the differences in geographical distribution of chemotypes in China and found that 15-ADON chemotypes are predominant in colder wheat growing areas and 3-ADON in warmer areas in Yangtaz River valley. Both *F. graminearum* (teleomorph *Gibberella zeae*) and *F. pseudograminearum* (teleomorph *G. coronicola*) are important pathogens of wheat in Australia. Lineages 2 (*F.meridionale*), 5 (*F. acaciae-mearnsii*), 7 (*F. graminearum*)and 8 (*F. cortaderiae*) were reported in Australia (Akinsanmi et al., 2006).

In Italy, all three chemotypes were found in infected durum wheat fields and the 15-ADON chemotypes were most frequent (87.2%) followed by the 3-ADON (8.1%) and NIV (2.7%) (Prodi et al., 2009). Jennings et al. (2004) investigated the chemotype diversity in wheat crops in Wales and England and found that the 15-ADON chemotype is predominant (95%) and only 5% were 3-ADON. According to the study done in

Poland, 15-ADON chemotypes were predominant among the *F. graminearum* isolates and 3-ADON chemotypes prevailed among *F. culmorum* isolates. The ability of Argentinean *Fusarium* isolates to produce trichothecenes is controversial and the pattern of mycotoxin production is not well defined yet. Faifer et al. (1990) reported that *F. graminearum* isolated from wheat in Argentina produced DON, 15-ADON, 3-ADON and zearalenone (ZEA) while Maria et al. (2006) found only DON and 3-ADON producers. All those studies were carried out in crops heavily affected by FHB.

In Canada, according to variable number tandem repeat (VNTR) analysis, Ward et al. (2008) revealed that 27.4% of the isolates were 3-ADON producers and the remaining was typical 15-ADON producers whereas no NIV chemotypes were observed. The 3-ADON producers found in North America are more related to the Italian population than the North American 15-ADON population. The chemotype distribution across Canada showed an interesting longitudinal cline in which the frequency of 3-ADON producers gradually decreased in each province when moving from East to West (Ward et al., 2008). In western Canada, the percentage of 3-ADON is highest in Manitoba, where nearly half of the isolates studied were 3-ADON. Studies by researchers, of FHB pathogen diversity, revealed that 3-ADON producing *F. graminearum* are now widely prevalent in North America and there has been a significant population shift in FHB pathogen composition towards 3-ADON producers (Guo et al., 2008; Ward et al., 2008). Between 1998 and 2004 the frequency of 3-ADON producers in western Canada has increased more than 14-fold. This significant increase in 3-ADON producers suggested that they must have selective advantage over the native 15-ADON chemotypes (Ward et al., 2008).

2.9 Corn

Corn or maize, a major source of food for both humans and animals, is grown in more countries than any other crop. Corn is classified under Division: Magnoliophyta, Class: Liliopsida, Order: Cyperales, Family: Poaceae, Genus: Zea and Species: Zea mays. Today, the United States, China, the European Union, Brazil and Mexico are the world's largest producers of corn. Canada ranked 11 th in world corn production and contributed 1.34% is of the total world corn production in 2008 (http://www.grains.org/corn). In Canada, corn has been a principal crop in south-western Ontario since 1800 as the climatic conditions within that region favours corn production (Daynard et al., 1977).

Corn has a wide variety of uses. Corn is made into cereals, bread and corn syrup. At present corn is used as a major source in ethanol production. Diseases on corn may significantly reduce corn production and would affect those industries associated with corn production. One of the main diseases in corn is the *Fusarium* ear or cob rot. This can be a serious disease of all corn types. Infection of the maize cob with *Fusarium* spp. can lead to yield reductions and mycotoxins produced by the fungus can have serious implications for the end users of the grain. Fusarium ear rot is commonly caused by *F. verticillioides*, *F. proliferatum* and *F. subglutinans*. Gibberella ear rot is caused by *Gibberella zeae* (asexual state *F. graminearum*). *Gibberella zeae* can also cause stalk rot in corn (Watson, 2007). Therefore identification of these fungi and their mycotoxins is critical because it allows the implementation of grain harvest, storage and feeding management options to reduce mycotoxin contamination and maintain a better grain yield.

CHAPTER 3

3.0 DETERMINATION OF EFFICACY OF FUNGICIDES ON *Fusarium graminearum* 3-ADON AND 15-ADON CHEMOTYPES BY VISUAL DISEASE ASSESSMENT AND REAL-TIME POLYMERASE CHAIN REACTION

Abstract

Fusarium head blight (FHB) continues to threaten the economic sustainability of many small grain producers in Manitoba by causing losses in grain yield and quality. To date, only a small number of spring wheat cultivars with a moderate level of FHB resistance are available commercially. Therefore timely application of fungicide is considered to be the most important disease management strategy. Two field trials were carried out in 2009 and 2010 at the University of Manitoba, Winnipeg to assess the efficacies of fungicides in controlling FHB caused by 3-ADON and 15-ADON chemotypes. In the 2009 field experiment, all four fungicide treatments significantly reduced the FHB index, FDK and DON compared to the fungicide unsprayed control in cultivar Glenn (rated moderately resistance) and all four fungicide treatments significantly increased the yield compared to the unsprayed control. In the cultivar Roblin (rated highly susceptible) although all fungicides significantly reduced the FHB index, and increased the yield compared to the unsprayed control, inconsistent results were observed for FDK and DON in a few treatments. In 2010, all fungicide treatments significantly decreased the FHB index and FDK and increased the yield in both cultivars, Glenn and Roblin. Similarly all fungicides reduced the DON contamination in infected grains compared to the unsprayed control. Significant differences were observed between the two chemotypes 3-ADON and 15-ADON for all variables in both years. The two cultivars Glenn and Roblin had significant differences on all the FHB variables examined except for DON. The experiments confirmed that host resistance plays an important role in host-pathogen-fungicide interaction. In 2009, strong correlations were observed between FHB Index-DON, and FDK-DON suggesting that FHB Index and FDK can be used as predictors of

DON. The real-time quantitative PCR assay on selected samples showed that the amount of fungal DNA in fungicide treated samples was less than the unsprayed control, but there were not strong correlations between the amount of fungal DNA and DON content in infected grains. This study suggests that currently grown wheat cultivars, fungicide applications and other protective precautions cannot individually guarantee sufficient protection against the disease but by growing less susceptible spring wheat cultivars plus application of fungicides can control FHB to a greater extent.

3.1 Introduction

Fusarium head blight (FHB) of wheat is a devastating fungal disease that occurs frequently in Canada. The pathogen *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch] is considered to be the most common causal agent of FHB worldwide (Goswami and Kistler, 2004). Fusarium head blight has been reported to be one of the major threats to wheat production in many parts of the world. In Manitoba annual losses of up to $75 million have been reported due to FHB (King, C. 2008). Due to fungal infection the whole or part of the wheat head becomes bleached and the peduncle tissues turn brown or tan and senesce prematurely (Parry et al., 1995). The pathogen further invades the stem tissue causing bleaching of the entire spike (Osborn and Stein, 2007). Fusarium damaged kernels (FDK) appear shrunken having a characteristic bleached colour with decreased seed quality and weight (Osborne and Stein, 2007).

One of the major concerns of FHB is the contamination of the seeds with potent mycotoxins specifically deoxynivalenol (DON). Deoxynivalenol poses a health hazard in food and feed causing neurotoxic and immunotoxic effects in mammals (D'Mello et al., 1999; Desjardins, 2006). Due to the toxicity of DON, the United States Food and Drug Administration has set maximum acceptable DON levels for human consumption in wheat grain. These levels have been set from 0.5 to 2 ppm for wheat grain and 1.0 mg/kg for all finished wheat flour products (Bai and Shaner, 2004; Pirogozliev et al., 2002). Currently, highly resistant wheat cultivars to FHB and DON accumulation are not available. Therefore integrated FHB management strategies are considered to be the most effective approach to control the disease (Bai and Shaner, 2004). Appropriate farming

practices such as, crop rotation, tillage, seed treatment and the use of fungicides or biological agents are being used to control FHB (Parry et al., 1995; Edwards et al., 2001; Matthies and Buchenauer, 2000).

Application of fungicides plays a major role in integrated FHB management. Fungicides are being used at the anthesis to reduce the quantitative yield losses and mycotoxin contamination (Mesterházy, 2003b). In general, fungicides having triazole as the active ingredient (tebuconazole, metconazole and bromuconazole) are considered as the most effective chemical compounds against *Fusarium* spp (Simpsons et al., 2001; Edwards et al., 2001; Pirgozliev et al., 2002; Mesterházy, 2003b). However, the use of fungicides in controlling FHB has been unsatisfactory and variable. Most studies reported inadequate or inconsistent control of FHB (Mesterházy, 2003b). For example, several studies have shown that triazole-based fungicides can reduce FHB incidence and DON accumulation whereas, fungicides containing azoxystrobin as active ingredients can increase the DON accumulation although they partially control the FHB incidence (Simpsons et al., 2001; Edwards et al., 2001; Pirgozliev et al., 2002; Mesterházy et al, 2003; Haidukowski et al., 2005). Therefore success with fungicide application depends on other factors, such as level of resistance of the cultivar, timing and coverage of fungicide application, the rate of application and aggressiveness of the isolate (Mesterházy et al, 2003).

Evaluation of effects of fungicides in controlling FHB relies on various pathogen quantification methods, such as: (i) FHB disease severity and incidence (ii) Fusarium damaged kernels (FDK), (iii) DON content in grain, and (iv) detection of *TRI5* DNA of

the *Fusarium* spp. by quantitative polymerase reaction (qPCR) (Doohan et al., 1999; Homdock et al., 2000; Zhang et al., 2008; Edwards et al., 2001). The first three techniques are species non-specific as they determine pathogen population directly based on the incidence of the FHB pathogens which are a complex of *Fusarium* spp. But the fourth technique which is based on polymerase chain reaction (PCR) provides the basis for a rapid quantification of toxigenic *Fusarium* spp. in cereal samples (Zhang et al., 2008). Therefore, a more accurate quantification of trichothecene- producing *Fusarium* spp. within harvested grain can be assessed using real time quantitative PCR. This method can be used to determine whether fungicides are affecting the proportion of trichothecene-producing *Fusarium* spp. within the FHB complex (Zhang et al., 2008). Also, qPCR could be used to quantify the amount of *F. graminearum* 3-ADON and 15-ADON isolates in grain and correlate their levels with the amount of DON accumulation.

In recent years, FHB has been severe in Canada, especially in the Prairies. In Manitoba, the incidence of FHB has been increasing since 1984 (Wong et al., 1992). Ward et al. (2008) found a chemotypic shift from 15-ADON to the 3-ADON chemotypes. Recent studies done by Guo et al. (2008) reported that 3-ADON chemotypes are more prevalent in the Red River Valley area. This chemotypic change may have an effect on the success of fungicide application in North America. Therefore, the objectives of this study were to 1) evaluate the efficacy of fungicides against 3-ADON and 15-ADON chemotypes of *F. graminearum* 2) determine correlations between FHB visual disease variables and DON content in infected grains and 3) determine the fungal biomass in infected wheat grain using real time quantitative PCR analysis.

3.2 Materials and Methods

3.2.1 Fungal inoculum

The strains of *F. graminearum* ON-06-05 (15-ADON), Q-06-10 (15-ADON), 0N-06-39 (3-ADON), M8-06-02 (3-ADON), M5-06-01 (3-ADON) and Q-06-32 (3-ADON) were used in the 2009 growing season and DF-Fg-144 (15-ADON) was used in the 2010 growing season instead of M8-06-02. All the strains were collected from Fusarium damaged kernels (FDK) of wheat grown by farmers in Western and Eastern Canada in 2006 **(Table: 3.1)**. Spores were obtained by growing the fungal strains in 2-liter flasks containing liquid carboxy methyl cellulose (CMC) medium previously sterilized by autoclaving for 20 min at 121 ºC. Then the medium was aerated through a glass tube. After 7 days of incubation at room temperature (21 ºC to 24 ºC) contents in the flasks were filtered through three layers of cheese cloth to obtain a spore suspension. The concentration of the conidia was measured using a hemacytometer and adjusted to 5×10^4 conidia per millilitre using sterilized distilled water.

Table 3.1. Information on *Fusarium graminearum* isolates used in field trial 2009 and 2010

NRRL	EQ	Wheat Class	Geographic Origin	Year Collected	Luminex Chemotype
44433	M5-06-1	CWRS	NA Canada, Manitoba, Bowsman	2006	3-ADON
44509	M8-06-2	CWRW	NA Canada, Manitoba, Winkler	2006	3-ADON
43919	ON-06-39	CERS	NA Canada, Ontario	2006	3-ADON
45121	Q-06-32	CERS	NA Canada, Quebec	2006	3-ADON
45099	Q-06-10	CERS	NA Canada, Quebec	2006	15-ADON
43885	ON-06-5	CERS	NA Canada, Ontario	2006	15-ADON
	DF-Fg-144		NA Canada, Manitoba	2006	15-ADON

Wheat Classes are as Follows: CWRS= Canada Western Red Spring;

CWRW= Canada Western Red Winter; CERS= Canada Eastern Red Spring

3.2.2 Fungicides

Four commercially available products- Proline® (prothioconazole) , Prosaro® (prothioconazole plus tebuconazole) and Folicur® (tebuconazole) manufactured by Bayer Crop Science Inc. (Canada), and Caramba® (metconazole), manufactured by BASF Chemical Company, (USA) were used in the field experiments **(Table:3.2)**.

Table 3.2 . Active ingredients, product name and application rate of fungicides used in the field trials conducted in 2009 and 2010 field trials

Trademark[a]	**Active ingredient (a.i)**	**Active ingredient concentration (g a.i. l^{-1})**	**Rate of application (ml ha^{-1})**
Proline®	prothioconazole	480	315-420
Prosaro®	prothioconazole + tebuconazole	211+211	480-605
Folicur®	tebuconazole	432	292
Caramba®	metconazole	90	1000

Proline®, Prosaro® and Folicur® manufactured by Bayer Crop Science Inc. (Canada), and Caramba®, manufactured by BASF Chemical Company (USA)

3.2.3 Wheat genotypes

Two spring wheat cultivars (*Triticum aestivum* L) Glenn (moderately resistant to FHB) and Roblin (highly susceptible to FHB) were used (Seed Manitoba, 2008).

3.2.4 Field trials

During the 2009 and 2010 growing seasons, two spring wheat cultivars Glenn and Roblin were grown under normal agronomic practices in the field at the University of Manitoba, Winnipeg, with three replications using a split–split plot design. Four fungicide treatments plus a control without fungicide spraying served as the main plots and two wheat cultivars served as the one of the split plot effects. Within each wheat cultivar block, six isolates of *F. graminearum* and a control (inoculated with distilled water) served as split-split plot effect. The plot size was 3m x 1.5 m and wheat was sown at a rate of 1200 seeds per plot in six rows with 17 cm row spacing. A taller wheat cultivar, Amazon, was used as the buffer to separate between fungicide treatments. Fungicides were applied at 30% anthesis, with a carbon dioxide back-pack sprayer. Each fungicide was applied in 250 ml water per plot, with one-half applied from each side of the plot so that heads could be thoroughly covered. Two days after fungicide application, at 50% anthesis, pathogen inoculum was applied using a CO_2 backpack sprayer calibrated at 30 psi, at a rate of 1-liter inoculum per plot. The uninoculated plots were sprayed with distilled water. A second inoculation of the same rows were performed three days after the first inoculation. Plots were mist-irrigated using an over-head sprinkler system in the evening of, and the morning after each inoculation for 12 hours at an interval of 5 min/hour.

3.2.5 Disease evaluation and yield

Disease incidence and disease severity of each plot were rated 10, 14, 18, 22 and 26 days after first inoculation using a FHB disease scale (Stack and McMullen,1995). Disease severity and disease incidence ratings were taken by examing five random locations within the plot. Disease severity and disease incidence data were used to calculate FHB index for each plot using the following formula:

FHB index = (Disease severity x Disease incidence)/100

After maturity, plots were harvested mechanically using a Wintersteiger Elite combine and placed in separate bags. Seeds were then threshed using a belt thresher and placed in bags and dried for one week at 36 ºC using a forced air system. Grain yields of each plot were determined (kilogram per hectare). A 10 g random sample of each plot was visually assessed to determine the percentage of FDK based on the weight of the kernels. Only those kernels with characteristic white or rose discoloration were considered damaged by *Fusarium* spp.

3.2.6 DON analysis

DON analysis was carried out by taking 10 g of grain samples randomly from two replicates (140 plots) and using Enzyme-linked immunosorbent assay (ELISA) DON identification kits (www.diagnostix.ca)

3.2.7 Determination of the efficacy of fungicides

Efficacy of each fungicide treatment for FHB index, FDK, yield and DON content was calculated separately for the mean of all 3-ADON isolates, 15-ADON isolates inoculated plots and the uninoculated plots using the following formula:

Efficacy percentage (FHB Index) = (FHB Index of unsprayed control- FHB Index of fungicide treated)/ FHB Index of unsprayed control x 100

Efficacy of each fungicide treatment for yield was calculated using the following formula:

Efficacy percentage (yield) = (yield of fungicide treated- yield of unsprayed control)/ yield of unsprayed control x 100

In the above formula unsprayed control referred to the control for fungicide that is, with no fungicide application but inoculated with the respective isolate.

3.2.8 Determination of percent yield reduction

Percent yield reduction was calculated in unsprayed control treatments, for example: percent yield reduction in Glenn after inoculation with 15-ADON was calculated as:

Percent yield reduction = (yield of Noisolates- yield of 15-ADON)/ yield of Noisolates*100)

3.2.9 Extraction of DNA from pure fungal isolates

DNA was extracted from monoconidial fungal cultures of *F. graminearum* isolates ON-06-05 (15-ADON), Q-06-10 (15-ADON), 0N-06-39 (3-ADON), M8-06-02 (3-ADON), M5-06-01 (3-ADON) and Q-06-32 (3-ADON) using the method described by Fernando et al. (2006). Seven day old cultures were taken for DNA extraction and the fungal mycelia were harvested. The harvested mycelia were freeze dried and ground to a fine powder in a 1.5 microcentrifuge tube. Six hundred microliter of TES buffer (100mM Tris, 10mM EDTA and 2% sodium dodecyl sulphate) was added to a 1 g of mycelial powder in the tube. Then, 140 µl of 5M NaCl and 70 µl of 10% CTAB were added vortexed for 5 min, and incubated at 65 ºC for 20 min. To remove proteins, 600 µl of phenol:chloroform:isoamylalcohol (25:24:1) was added and centrifuged at 10,000 rpm for 15 mins. The supernatant was then transferred into a new tube and the above step was repeated. To precipitate DNA, 1000 µl of 100% ethanol and 80 µl of 5M NaCl were added and the solution was centrifuged at 13000 rpm for 5 mins. The DNA pellet was washed with 200 µl of ice-cold 80% ethanol and air dried. It was re-suspended in 200 µl of sterilized distilled water at 65 ºC. To remove RNA, 5 µl of RNase (10 mg/ml) was added to the solution which was incubated at 37 ºC for 20 mins. The DNA was quantified using 1% agarose gel. The DNA concentration was measured using a NanoDrop3300 (ThermoFisher Scientific Inc.).

3.2.10 Extraction of *Fusarium*-DNA from infected grain samples

To extract Fusarium-DNA from infected grain samples 10 g of grains was taken from selected plots and ground. DNA was extracted using the upscaled CTAB-based

method described by Brandfass and Karlovsky, (2008). One gram of ground grian samples were blended in a 50 ml tube with CTAB-buffer (10 ml, 10mM Tris, 20 mM EDTA, 0.02 M CTAB, 0.8 M NaCl, 0.03 M N-laurylsarcosine, 0.13 M sorbitol, 1% (W/V) polyvinylpolypyrolidone, pH set to 8.0 with NaOH). Then, 20 µl of mercaptoethanol and 10 µl of proteinaseK (20 mg/ml stock solution) were added. The mixture was treated in an ultrasonic bath for 5 sec and then incubated at 42ºC for 10 mins following incubation at 65ºC for 10 mins. After incubation, 8 ml of chloroform:isoamylalcohol (24:1) was added, thoroughly emulsified and incubated on ice for 10 min. The mixture was then centrifuged at 5000 g for 10 min. Six hundred µl of supernatant was transferred into a 1.5 ml micro-centrifuge tube containing 194 µl of 30% PEG and 100 µl of 5M NaCl and centrifuged for 15 min at 13000 rpm. Then the resultant pellet was washed with 70% ethanol and air dried. The dried DNA pellet was re-suspended in 200 µl of 1X TE buffer and incubated overnight at 4 ºC. Then 5 µl of RNase (20mg/ml) was added and incubated at 37ºC for 20 mins. DNA was quantified using 1% agarose gel and the NanoDrop3300 (ThermoFisher Scientific Inc.).

3.2.11 Real-time quantitative PCR protocol for quantification

A light cycler MiniOpticon™ Real-Time PCR (Bio-Rad Laboratories, Inc.) system was used for amplification and quantification of the DNA prepared. The gene specific primers *TRI5* forward primer (5' AGCGACTACAGGCTTCCCTC 3') and *TRI5* reverse primer (5' AAACCATCCAGTTCTCCATCTG 3') were used to amplify a 544 bp product from the trichodiene synthase gene (*TRI5*) of trichothecene producing *Fusarium* spp. (Zhang et al., 2008). The real time PCR mixture consisted of 6.5 µl of iQ Syber

Green SuperMix (Bio-Rad Laboratories, Inc.), 0.1 µl of forward *TRI5* primer (10mM) , 0.1 µl of reverse *TRI5* primer (10mM), 2 µl (50ng/ µl) of template DNA and 11.1 µl of sterilized distilled water making a total volume of 20 µl. Fifty samples were run in a MiniOpticon™ Real-Time PCR machine (Bio-Rad Laboratories, Inc.) using the following thermal cycling protocol; initial preheating at 95 º C for 5 min followed by 40 cycles of 30 s at 95 º C, 1min 15 sec at 65 º C , 1min 15 sec at 72 º C. Detection of fluorescent product was carried out after every 3 steps.

3.2.12 Quantification of *Fusarium*-DNA in infected wheat grains

DNA standards were made from pure culutres of M8-06-02 (from the concentrations 500, 100, 10, 1 ng/µl, 100, 1 pg/ µl) and Q-06-10 (from the concentrations 200, 100, 10, 1 ng/µl, 100, 1 pg/ µl) . The amount of fungal DNA in unknown samples was calculated from the standard curve run in parallel reactions in three replicates. The *Fusarium* biomass was assessed in terms of picograms of fungal DNA in a total of 100 ng of wheat-*Fusarium* DNA.

3.2.13 Statistical analysis

Analysis of variance (ANOVA) for FHB index, FDK, yield and DON for each year was performed using the "PROC GLM" procedure of the SAS software (SAS version 9.2, SAS Institute, Inc, Cary, NC, USA). The model statement used in the analysis was "variables = fungicide cultivar isolate fungicide*cultivar fungicide*isolate cultivar*isolate fungicide*cultivar*isolate rep fungicide*rep cultivar*rep fungicide*cultivar*rep". Replicates and the interaction with replicates were considered random and all the other factors were fixed. The error terms for fungicide, cultivar and

fungicide*cultivar, were fungicide*rep, cultivar*rep and fungicide*cultivar*rep respectively. Other sources were tested against the residuals.

In order to see the effect of fungicides on FHB variables, first, all five fungicide treatments (including the unsprayed control) were analysed. Then a separate analysis was performed without the unsprayed control to see the significant differences within the four fungicides. The same pattern of analysis was performed for the isolate component. First, an overall analysis was performed for all isolates. Then individual analyses were carried out for each component within the isolates such as, for all 3-ADON isolates, 15-ADON isolates, 3-ADON and 15-ADON isolates and inoculated (DON) and uninoculated (NoIsolates).The individual analyses were carried out to determine the significant differences within 3-ADON and 15-ADON isolates, between 3-ADON and 15-ADON isolates and between the inoculated and uninoculated samples. Fusarium head blight index was analysed by considering the disease severity and incidence ratings taken at 22 days after the first inoculation. The area under disease progress curve (AUDPC) for FHB index was also calculated and the statistical analysis on AUDPC for FHB index is presented in appendix 1.

The correlations between the response variables for each year were analysed using the “PROC CORR” procedure of the SAS (version 9.2) software package considering raw data in two replicates. The correlation coefficient between the DON and the amount of fusarium DNA in infected grains was also analysed using the “PROC CORR” procedure.

3.3 Results

Results of the two field seasons (2009 and 2010) are presented separately. Results relevant to field season 2009 are summarized in tables 3.3, 3.5A, 3.5B, 3.7A, 3.7B and 2.10A and 2010 are in 3.4, 3.6A, 3.6B, 3.8A, 3.8B and 3.10B

Table 3.3. Significance of mean square values for fusarium head blight index (FHB index), percentage of Fusarium damaged kernels (FDK), yield and concentration of deoxynivalenol (DON) within each source of variation in 2009

Source of variance	df	Mean square values (Type III)				df for DON
		FHB Index(%)	FDK (%)	Yield(kg/ha)	DON (ppm)	
Overall Fungicide	4	3567.83*	1251.96*	5973864.74*	728.59 ns	4
Within Fungicides	3	118. 63ns	304.53ns	135196.87ns	109.20ns	3
Fungicides vs control	1	13915.41*	4094.25*	23489868.32*	2586.79ns	1
Cultivar	1	164626.00*	188268.70*	127878498.40*	7276.04ns	1
Fungicide x cultivar	4	807.35*	1319.20*	2033110.5*	523.87*	4
Isolate	6	7412.69*	1448.90*	10908364.20*	1067.51*	6
3-ADON	3	384.21ns	146.51ns	288432.43*	213.44*	3
15-ADON	1	52.77ns	3.90ns	316.30ns	30.99ns	1
3-ADON vs 15-ADON	1	2940.42*	520.80*	985949.00*	542.31*	1
Inoculated vs Non- inoculated	1	40330.33*	7729.18*	63598622.03*	5191.46*	1
Fungicide x isolate	24	209.30 ns	127.11ns	135891.10*	34.25 ns	24
Cultivar x isolate	6	4193.76*	60.21ns	809492.60*	272.22*	6

Fungicide x cultivar x isolate	24	146.82 ns	144.04ns	131391.00*	77.88*	24
Rep	2	45.26ns	16.09ns	288378.60*	12.70ns	1
Fungicide x rep	8	256.87 ns	101.86 ns	497982.80*	121.18*	4
Cultivar x rep	2	226.24ns	123.46ns	32534ns	474.42*	1
Fungicide*Cultivar*Rep	8	144.18ns	184.39ns	234663.5*	72.34ns	5
Error	120	142.67	94.18	76709.00	29.19	60
Total	209					139

*** significant at P≤ 0.05; ns= not significant.**

Table 3.4. Significance of mean square values for fusarium head blight index (FHB index), percentage of Fusarium damaged kernels (FDK), yield and concentration of deoxynivalenol (DON) within each source of variation in 2010

Source of variance	df	Mean square values (TypeIII)				Df for DON
		FHB Index (%)	FDK (%)	Yield(kg/ha)	DON(ppm)	
Overall fungicide	4	6341.15*	6716.35*	10851062.70*	318.70*	4
Within fungicides	3	231.28ns	783.14*	2854224.47*	61.19*	3
Fungicide vs control	1	24670.75*	24516.00*	34841517.38*	1086.38ns	1
Cultivar	1	146245*	125426.08*	17420803.29*	2.22ns	1
Fungicide x cultivar	4	604.71*	929.38*	506824.13ns	43.80 ns	4
Isolate	6	8059.67*	3541.69*	354366.55*	122.04*	6
3-ADON	2	218.84*	307.10*	205593.00ns	59.90*	2
15-ADON	2	3333.34*	519.64*	18250.00ns	7.11ns	2
3-ADON vs 15-ADON	1	1504.53*	2.26ns	7735.29ns	198.99*	1
Inoculated vs Non-inoculated	1	39749.00*	19594.42*	1670766.08*	398.57*	1
Fungicide x isolate	24	224.90*	170.00*	182465.23ns	4.55ns	24
Cultivar x isolate	6	2822.17*	1220.56*	266009.13ns	5.76ns	6

Fungicide x cultivar x isolate	24	212.10 *	355.44*	92714ns	7.26ns	24
Rep	2	163.85ns	139.43*	4242560.46*	12.37ns	1
Fungicide x rep	8	134.43*	32.81ns	495708.15*	27.03ns	4
Cultivar x rep	2	25.97ns	14.88ns	507371.38*	21.50ns	1
Fungicide x cultivar x rep	8	86.96 ns	26.63ns	629476.24*	10.14ns	5
Error	120	56.22	20.83	126384.80	7.05	59
Total	210					138

*** significant at P≤ 0.05; ns= not significant.**

3.3.1 Visual assessment of FHB

In 2009, fungicides, fungal isolates and cultivars, all had a significant effect on FHB disease index. The two-way interactions fungicide by cultivar and cultivar by isolate were significant but fungicide by isolate interaction was not significant. The three way interaction, fungicide by cultivar by isolate was also not significant **(Table: 3.3)**. All four fungicide treatments were significantly different from the unsprayed control but no significant differences were observed within the four fungicide treatments for FHB index **(Table: 3.3)**. There were no significant differences within the 3-ADON *F. graminearum* isolates and within the 15-ADON isolates. But significant differences were found between 3-ADON and 15-ADON isolates and between the fungal isolates (inoculated)

and the uninoculated (non-inoculated) controls for FHB index (**Table: 3.3**). Within the four fungicides used in the field trial, the lowest mean FHB index was shown in the Proline-Glenn-15-ADON interaction (0.59%) and the highest in Caramba-Roblin-3-ADON interaction (79.5%) **(Tables: 3.5A and 3.5B)**. There was a significant difference between the two cultivars Glenn and Roblin, the FHB index across all fungicides and fungal isolates were higher for Roblin, the susceptible cultivar, than for the more resistant cultivar Glenn **(Tables: 3.5A and 3.5B)**. Fungal isolates were analysed according to their chemotypic origin as 3-ADON and 15-ADON. Significant differences were found between the 3-ADON and 15-ADON fungal isolates but not within the 3-ADON and 15-ADON isolates **(Table: 3.3)**.

In 2010, FHB index results were similar to 2009, fungicide, isolates and cultivars all had significant effects on FHB index **(Table: 3.4)**. The two way interactions fungicide by cultivar, isolate by cultivar and fungicide by isolate were significant and the three way interaction fungicide by isolate by cultivar was also significant **(Table: 3.4)**. Although all four fungicides were significantly different from the unsprayed control, no significant differences were observed within the fungicides. Unlike in 2009, significant differences were found within the 3-ADON and 15-ADON isolates. Also significant differences were found between 3-ADON and 15-ADON isolates **(Table: 3.4)**. In 2010, within the four fungicides, the lowest mean FHB index was given by Prosaro, Glenn, 15-ADON interaction (5.6%) and highest was Folicur, Roblin, 3-ADON interaction (81.7%). The FHB index was higher in the highly susceptible cultivar Roblin than the moderately resistant cultivar Glenn **(Tables: 3.6A and 3.6B)**.

In 2009, FHB index was significantly reduced upto 97% in cultivar Glenn, inoculated with 3-ADON and 15-ADON isolates (up to 97%) by the fungicide treatments as compared with the inoculated unsprayed control. In the susceptible cultivar the percentage of reduction was up to 31% **(Tables: 3.7A and 3.7B)**. In 2010, the percent reduction of FHB index in Glenn was up to 87% and in Roblin up to 35% (**Tables: 3.8A and 3.8B)**. Although the fungicides used in this study had different active ingredients, there were no significant differences within the four fungicides either in 2009 or 2010. In the cultivar Glenn Prosaro, Proline and Caramba tended to be more effective in reducing the FHB index compared to Folicur, but in cultivar, Roblin, those fungicides aced in a similar way confirming the variability of efficacy of fungicides in controlling FHB.

Table 3.5A.The overall mean values for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for cultivar Glenn inoculated with 3-ADON, 15-ADON isolates and control under different fungicide treatments, 2009

Cultivar	Fungicide	Isolate	FHB Index (%)	FDK (%)	Yield (kg/ha)	DON (ppm)
GLENN	**Prosaro**	15-ADON	1.8	19.4	3190	7.7
		3-ADON	1.9	23.9	3146.67	7.2
		Noisolates	0	9.4	4226.67	1.0
	Proline	15-ADON	0.59	14.2	3353.56	8.6
		3-ADON	1.16	16	3348.45	6.8
		Noisolates	0	7.4	3831.78	0.9
	Folicur	15-ADON	2.76	21.4	2868.89	10.2
		3-ADON	6.8	25.9	2734.44	13.4
		Noisolates	0	10.1	4201.33	1.84
	Caramba	15-ADON	1.2	18.4	3451.78	8.5
		3-ADON	3.8	23.1	3257.33	8.8
		Noisolates	0	10.3	4342.22	2.36
	Control	15-ADON	28.11	43.7	1998.44	28.6
		3-ADON	40	50.3	1827.33	30.1
		Noisolates	0	10.1	3776.89	4.1

Table 3.5B.The overall mean values for fusarium head blight index (FHB index), Fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for cultivar Roblin inoculated with 3-ADON, 15-ADON isolates and control under different fungicide treatments, 2009

Cultivar	Fungicide	Isolate	FHB Index (%)	FDK(%)	Yield (kg/ha)	DON (ppm)
ROBLIN	**Prosaro**	15-ADON	65.4	87.11	1152.22	30.1
		3-ADON	79.2	86.3	1047.78	38.5
		Noisolates	0.23	69.2	3358.44	4.05
	Proline	15-ADON	58.3	74.1	1653.33	21.6
		3-ADON	78.7	85.7	1117.78	36.0
		Noisolates	0	79.9	3362.22	4.46
	Folicur	15-ADON	57.6	85.5	1740.89	19.1
		3-ADON	63.2	89.8	1605.78	28.5
		Noisolates	0	78.5	3399.11	4.14
	Caramba	15-ADON	69.5	89.2	1338.89	22.5
		3-ADON	79.5	88.2	1311.78	22.5
		Noisolates	0	46.5	2932.22	6.04
	Control	15-ADON	74.6	83.5	917.78	25.0
		3-ADON	91.9	85.9	702.67	35.3
		Noisolates	0.4	68.8	2909.56	9.0

Table 3.6A. The overall mean values for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK) yield and deoxynivalenol content (DON) for cultivar Glenn inoculated with 3-ADON, 15-ADON isolates and control under different fungicide treatments, 2010

Glenn	Isolate	FHB Index (%)	FDK(%)	Yield (kg/ha)	DON (ppm)
Prosaro	15-ADON	5.6	20.23	3227.65	4.67
	3-ADON	6.3	23.20	3261.48	7.16
	Noisolates	0.9	2.63	3648.89	0.93
Proline	15-ADON	8.81	10.84	3510.86	2.73
	3-ADON	11.61	13.68	3588.88	6.04
	Noisolates	0.7	3.53	3390.37	0.55
Folicur	15-ADON	9.36	17.10	2983.70	7.69
	3-ADON	11.9	17.90	3199.01	8.96
	Noisolates	1.4	23.73	3496.29	1.14
Caramba	15-ADON	6.31	17.90	3315.80	3.74
	3-ADON	8.51	14.48	3245.18	6.67
	Noisolates	1.4	6.23	3410.37	1.52
Control	15-ADON	44.22	48.67	1942.47	10.32
	3-ADON	54.01	51.38	2484.69	11.39
	Noisolates	4.2	16.02	3031.11	1.60

Table 3.6B. The overall mean values for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for cultivar Roblin inoculated with 3-ADON, 15-ADON isolates and control under different fungicide treatments, 2010

Roblin	Isolate	FHB Index (%)	FDK (%)	Yield (kg/ha)	DON (ppm)
Prosaro	15-ADON	72.93	83.03	2540.49	3.53
	3-ADON	63.47	72.90	2201.48	4.27
	Noisolates	8.6	50.00	2988.15	1.51
Proline	15-ADON	58.66	82.86	3278.52	3.69
	3-ADON	80.70	79.75	3271.60	5.32
	Noisolates	15.1	19.50	3094.07	1.34
Folicur	15-ADON	70.9	65.12	2539.75	2.73
	3-ADON	81.7	61.87	2317.28	10.00
	Noisolates	13.4	9.70	2031.85	0.99
Caramba	15-ADON	69.45	65.43	2921.73	2.06
	3-ADON	80.47	72.02	2752.84	4.27
	Noisolates	7.7	12.33	2957.09	1.51
Control	15-ADON	90.78	91.35	1645.18	11.9
	3-ADON	95.56	92.40	1714.81	17.6
	Noisolates	19.4	79.80	1872.59	9.38

3.3.2 Grain yields and FDK

In 2009, grain yield values in unsprayed controls (with fungal inoculation with 3-ADON and 15-ADON isolates) ranged from 702.67 to 1998.4 kg/ha **(Tables: 3.5A and 3.5B)**. A significant yield increase was observed when cultivars were sprayed with the fungicides, but no significant differences were found within the four fungicides **(Table: 3.3)**. Within the four fungicide treatments, under inoculation, the highest mean yield,

3451.78 kg/ha was obtained for the cultivar Glenn inoculated with 15-ADON isolates after Caramba treatment and the lowest, 1047.78 kg/ha for the cultivar Roblin inoculated with 3-ADON isolates after Prosaro treatment **(Tables: 3.5A and 3.5B)**. Cultivars and fungal isolates had a significant effect on yield. The moderately resistant cultivar Glenn had higher yield compared to the highly susceptible cultivar Roblin **(Tables: 3.5A and 3.5B)**. There were significant differences between the inoculated treatments and the uninoculated treatments. Significant differences were found between the 15-ADON and 3-ADON isolates for effect on yield and within 3-ADON isolates but no significant differences were observed for effect on yield within 15-ADON isolates **(Table: 3.3)**.

In 2010, grain yield values for unsprayed control plots with fungicide spray (with artificial inoculation of *F. graminearum*) ranged from 1645.18 to 2484.69 kg/ha **(Tables: 3.6A and 3.6B)**. In 2010, similar to 2009, fungicides, cultivars and isolates had a significant effect on yield **(Table: 3.4)**. All fungicides significantly increased yield compared to the control. In 2010, there were significant differences within the fungicides. Two fungicides Proline and Caramba were significantly different from Prosaro and Folicur (least significant difference (LSD)= 130.52, α=0.05). In both cultivars Glenn and Roblin, under artificial inoculation, the highest yield was observed in the plots sprayed with the fungicide Proline (In cultivar Glenn 3588.88 kg/ha and cultivar Roblin 3278.52 kg/ha). In 2010, the moderately resistant cultivar Glenn also gave higher yield compared to the highly susceptible cultivar Roblin **(Tables: 3.6A and 3.6B)**.

In 2009, the inoculation of cultivar Glenn with 15-ADON and 3-ADON isolates significantly reduced the yield by 47% and 51% and in cultivar Roblin by 68% and 75%

respectively **(Tables: 3.5A and 3.5B)**. In 2010, the percent reduction of yield after inoculation with 15-ADON and 3-ADON isolates were, 35% and 18% for cultivar Glenn and 12% and 8% for cultivar Roblin respectively **(Tables: 3.6A and 3.6B)**. Application of fungicides eliminated the suppressive effect of the *Fusarium* infection and improved the yields by 43-72% in cultivar Glenn inoculated with 15-ADON isolates and 49-83% with 3-ADON isolates in 2009. In cultivar Roblin the percent increase of yield after fungicide application was 25-89% inoculated with 15-ADON isolates and 49-128% inoculated with 3-ADON isolates **(Tables: 3.7A and 3.7B)** . In 2010, the efficacy percentages of fungicides for yield were 35-45% for 15-ADON isolates and 22-30% for 3-ADON isolates in cultivar Glenn. For Roblin the values were 35-50% for 15-ADON and 22-48% for 3-ADON isolates **(Tables: 3.8A and 3.8B)**.

In 2009, the four fungicides were significantly different from the unsprayed control for FDK, but there were no significant differences within the fungicides. Some fungicide treatments (Prosaro, Folicur and Caramba) increased the FDK compared to the unsprayed control on the highly susceptible cultivar Roblin, while other treatments reduced the FDK compared to the unsprayed control **(Tables: 3.5A and 3.5B)**. There also were significant differences between the two cultivars and fungal isolates for FDK. Significant differences were observed between the 3-ADON and 15-ADON isolates, but no significant differences were observed within the 3-ADON or 15-ADON isolates **(Table: 3.3)**.

In 2010, fungicide, cultivar and isolates all had a significant effect on FDK. All fungicides were significantly different from the unsprayed control and there were

significant differences within the four fungicides. All fungicides reduced FDK compared to the unsprayed control. In contrast to 2009 significant differences were found within the 3-ADON and 15-ADON isolates. But no significant differences were found between 3-ADON and 15-ADON isolates **(Table: 3.4)**.

In 2009, in the moderately resistant cultivar Glenn, fungicide application significantly reduced the FDK percentage by 48-68%, but in the susceptible cultivar Roblin, except for Proline other fungicides increased the FDK percentage compared to the unsprayed control **(Tables:3.7A and 3.7B)**. In 2010, the percent reductions of FDK in Glenn were 54-78% and in Roblin 9-33% for both isolates **(Tables: 3.8A and 3.8B)**.

3.3.3 DON contamination of grains

In 2009, no significant differences were observed for DON between treatments with fungicides and fungicide treatments were not significantly different from the unsprayed control. Also no significant differences were observed between the two cultivars Glenn and Roblin **(Table: 3.3)**. Fungal isolates had a significant effect on the DON contamination. There were significant differences within the 3-ADON isolates and between the 3-ADON and 15-ADON fungal isolates but no significant differences were found within the 15-ADON isolates. DON contamination was significantly higher on the susceptible cultivar, Roblin than the moderately resistant cultivar, Glenn **(Tables: 3.5A and 3.5B)**. In 2009, application of Prosaro and Proline increased the DON content in infected grains compared to the control in the highly susceptible cultivar Roblin **(Table: 3.5B)**. In 2010, the DON results were different from 2009. In 2010, significant differences were found within the four fungicides. There were no significant differnces

found between the two cultivars Glenn and Roblin. There were significant differences between the 3-ADON and 15-ADON isolates and within 3-ADON isolates but not within 15-ADON isolates **(Table: 3.4)**. In 2010, the fungicide Folicur was significantly different from other fungicides (LSD= 2.816, α=0.05). In both years, according to the pooled mean values for 3-ADON and 15-ADON isolates, 3-ADON isolates had higher DON contamination compared to the 15-ADON isolates, in cultivars Glenn and Roblin

In 2009, the percent reduction of DON content in Glenn ranged between 64-73% for heads inoculated with 15-ADON and 55-77% with 3-ADON, but in Roblin the range was 10-23% and 19-36% for 15-ADON and 3-ADON respectively **(Tables: 3.7A and 3.7B)**. In cultivar Roblin, the application of Prosaro (prothioconazole + tebuconazole) had increased the level of DON content for both chemotypes and application of Proline for 3-ADON isolates. In 2010, the percent reduction of DON content by fungicides was 25-73% for 15-ADON isolates and 21-47% for 3-ADON isolates in Glenn and in Roblin, 69-82% for 15-ADON and 43-75% for 3-ADON isolates **(Tables: 3.8A and 3.8B)**.

Table 3.7A. The efficacy percentage of fungicide treatments for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for the 3-ADON, 15-ADON isolates in cultivar Glenn, 2009

Glenn	Isolate	FHB Index	FDK	Yield	DON
Prosaro	15-ADON	93.60	55.61	59.62	73.08
	3-ADON	95.25	52.49	72.20	76.08
Proline	15-ADON	97.90	67.51	67.81	69.93
	3-ADON	97.10	68.19	83.24	77.41
Folicur	15-ADON	90.18	51.03	43.56	64.34
	3-ADON	83.00	48.51	49.64	55.48
Caramba	15-ADON	95.73	57.89	72.72	70.28
	3-ADON	90.50	54.08	78.26	70.76

Table 3.7B. The efficacy percentage of fungicide treatments for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for the 3-ADON, 15-ADON isolates in cultivar Roblin, 2009

Roblin	Isolate	FHB Index	FDK	Yield	DON
Prosaro	15-ADON	12.33	-4.32	25.54	-20.40
	3-ADON	13.82	-0.47	49.11	-9.07
Proline	15-ADON	21.85	11.26	80.14	13.60
	3-ADON	14.36	0.23	59.08	-1.98
Folicur	15-ADON	22.79	-2.40	89.68	23.60
	3-ADON	31.23	-4.54	128.53	19.26
Caramba	15-ADON	6.84	-6.83	45.88	10.00
	3-ADON	13.49	-2.68	86.69	36.26

Table 3.8A. The efficacy percentage of fungicide treatments for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for the 3-ADON, 15-ADON isolates in cultivar Glenn, 2010

Glenn	Isolate	FHB Index	FDK	Yield	DON
Prosaro	15-ADON	87.16	58.43	39.82	54.47
	3-ADON	87.16	54.84	23.82	37.13
Proline	15-ADON	80.08	77.72	44.67	73.54
	3-ADON	78.50	73.37	30.77	46.97
Folicur	15-ADON	78.82	64.86	34.90	25.48
	3-ADON	77.84	65.16	22.33	21.33
Caramba	15-ADON	85.73	63.63	41.42	63.57
	3-ADON	84.24	71.03	23.43	41.43

Table 3.8B. The efficacy percentage of fungicide treatments for fusarium head blight index (FHB index), fusarium damaged kernel percentage (FDK), yield and deoxynivalenol content (DON) for the 3-ADON, 15-ADON isolates in cultivar Roblin, 2010

Roblin	Isolate	FHB Index	FDK	Yield	DON
Prosaro	15-ADON	19.66	9.10	35.24	70.33
	3-ADON	33.57	21.10	22.11	73.06
Proline	15-ADON	35.37	9.29	49.82	68.99
	3-ADON	15.55	13.69	47.58	69.77
Folicur	15-ADON	21.90	28.71	35.22	77.05
	3-ADON	14.50	33.04	26.00	43.18
Caramba	15-ADON	23.49	28.37	43.69	82.68
	3-ADON	15.78	22.05	37.71	75.73

3.3.4 *Fusarium* DNA in infected wheat grains

Selected plots were subjected to qPCR analysis to evaluate the amount of fungal biomass in infected wheat grains. Field plots infected with M8-06-02 were selected to examine the effect of 3-ADON isolates and Q-06-10 to examine the effect of 15-ADON isolates in year 2009. According to the results obtained from real time standard curves, except for several treatments (Prosaro and Proline in Glenn) grains infected with 3-ADON isolates had higher amounts of fungal DNA than those infected with 15-ADON

isolates **(Table:3.9)**. The amount of *Fusarium* DNA was higher in the samples extracted from the highly susceptible cultivar Roblin than the moderately resistant cultivar Glenn.

Table 3.9. *Fusarium* DNA in 100 ng of Wheat-Fusarium DNA and deoxynivalenol content (DON) in selected field samples, M8-06-02 (3-ADON), Q-06-10 (15-ADON), 2009

Glenn	**M8-06-02 (3-ADON)**		**Q-06-10 (15-ADON)**		**NoIsolates**	
	DNA (pg/100ng)	**DON (ppm)**	**DNA (pg/100ng)**	**DON (ppm)**	**DNA (pg/100ng)**	**DON (ppm)**
Prosaro	0.18	6.56	6.47E-05	5.77	3.25E-05	0.69
Folicur	7.25	15.95	0.167	9.22	1.12E-11	1.50
Caramba	12.76	14.42	7.23	8.2	4.45E-06	1.17
Proline	0.017	6.26	0.42	3.44	8.99E-07	0.82
Control	12104.26	41.91	925.58	33.89	9.75E-05	3.02
Roblin	**M8-06-02 (3-ADON)**		**Q-06-10 (15-ADON)**		**NoIsolates**	
	DNA (pg/100ng)	**DON (ppm)**	**DNA (pg/100ng)**	**DON (ppm)**	**DNA (pg/100ng)**	**DON (ppm)**
Prosaro	158.9	53.29	0.017	33.0	0.001	4.64
Folicur	27.28	38.77	15.83	20.84	0.0004	4.24
Caramba	160.24	30.47	147.99	23.21	0.006	4.91
Proline	215.48	43.16	0.017	5.63	0.00012	4.70
Control	812.4	33.39	44558.28	23.9	8.23E-05	8.39

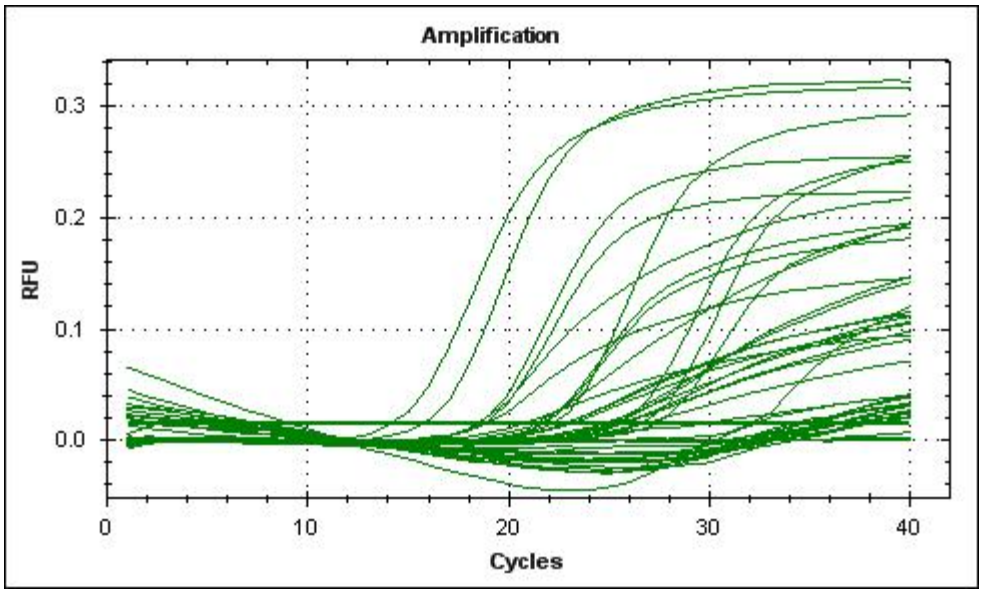


Figure 3.1: Amplification curves of the standards and the unknown samples. (RFU- relative fluorescence units)

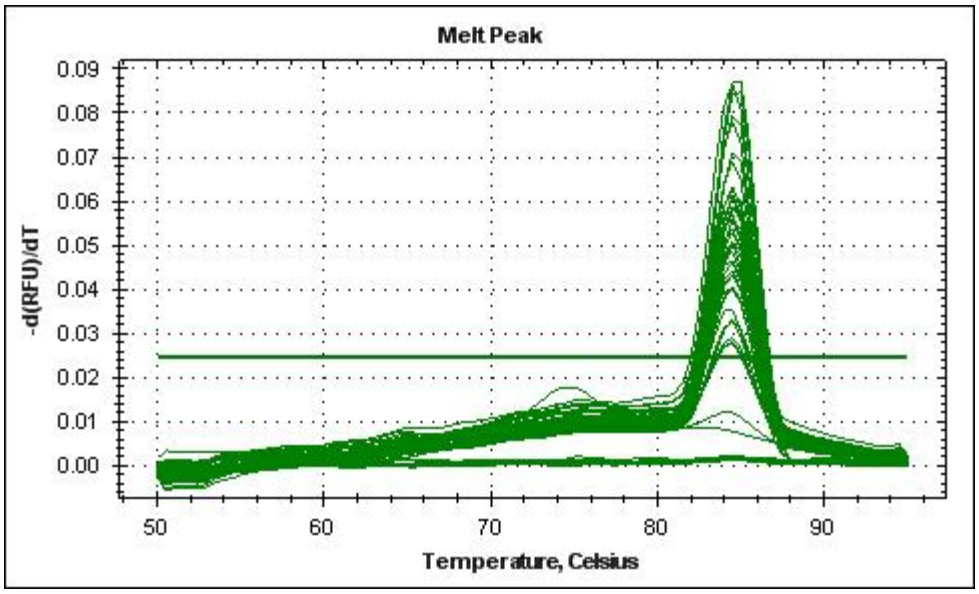


Figure 3.2: Melt curve of the standards and the unknown samples. The melting temperature of the primer is 84º C

3.3.5 Correlation between the FHB response variables

In the 2009 field trial, FHB index, yield, FDK and DON content were highly correlated with each other when correlation analysis was performed for raw data (**Table: 3.10A)** . But in 2010, lowcorrelation was observed between the FHB index-DON and FDK-DON **(Table: 3.10B)**. In 2009, low correlation was observed between the amounts of *Fusarium* DNA in infected grains and the DON content in the analysed samples (for both cultivars) (r= 0.188). But when the correlation analysis was carried out separately for each cultivar, in cultivar Glenn a strong positive correlation was observed between the DON content and amount of *Fusarium* DNA in infected grains (r= 0.75908), but in highly susceptible cultivar Roblin the Pearson correlation coefficient was 0.03738, so no correlation was observed between the two variables in Roblin.

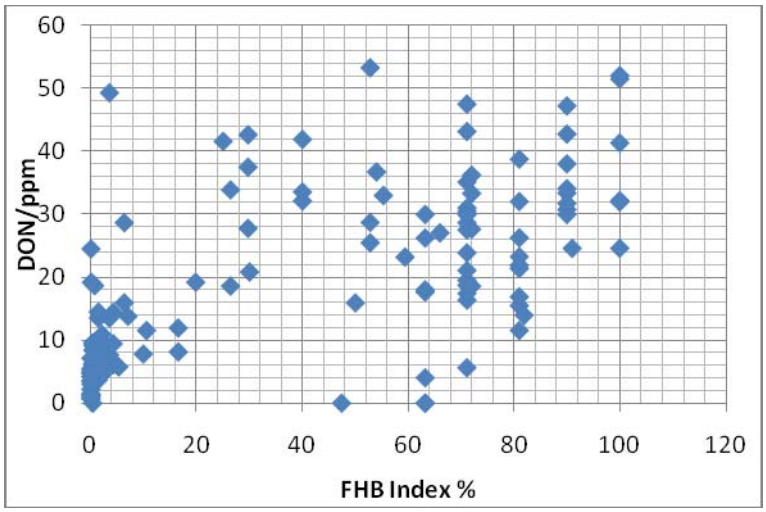
Table 3.10A. Pearson correlation coefficients between fusarium head blight index (FHB index), yield, fusarium damaged kernel percentage (FDK) and deoxynivalenol content (DON), 2009

	FHB Index	FDK	Yield	DON
FHB Index	1.000	0.8119	-0.88611	0.70829
n=140		<.0001	<.0001	<.0001
F DK	0.8119	1.000	-0.80302	0.62559
n=140	<.0001		<.0001	<.0001
Yield	-0.88611	-0.80302	1.000	-0.7683
n=140	<.0001	<.0001		<.0001
DON	0.70829	0.62559	-0.7683	1.000
n=136	<.0001	<.0001	<.0001	

Table 3.10B. Pearson correlation coefficients between fusarium head blight index (FHB index), yield, fusarium damaged kernel percentage (FDK) and deoxynivalenol content (DON), 2010

	FHB Index	FDK	Yield	DON
FHB Index	1.000	0.86053	-0.55188	0.36772
n=140		<.0001	<.0001	<.0001
FDK	0.86053	1.000	-0.60494	0.33993
n=140	<.0001		0.0001	<.0001
Yield	-0.55188	-0.60494	1.000	-0.43213
n=140	<.0001	0.0001		
DON	0.36772	0.33993	-0.43213	1.000
n=139	<.0001	<.0001	<.0001	

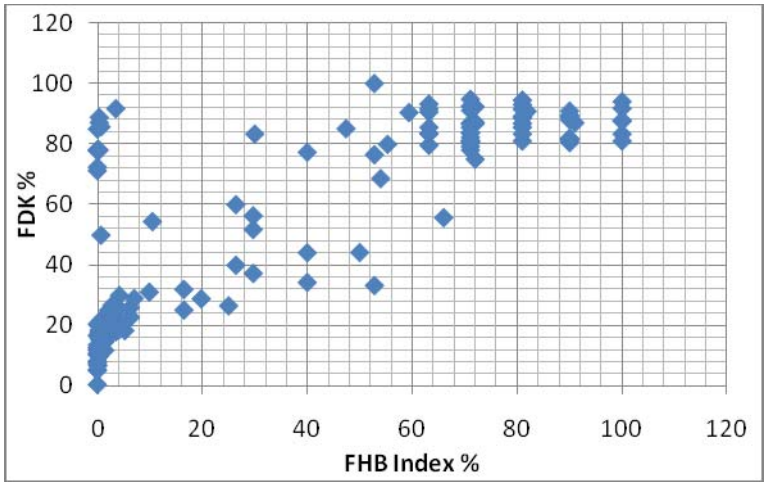
A



R= 0.70829

Figure 3.3A: Scatter plot between deoxynivalenol content (DON) and fusarium head blight index (FHB index), 2009

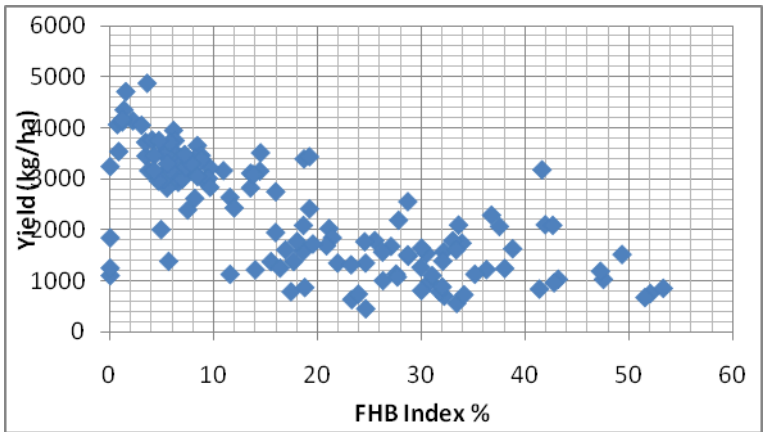
B



R= 0.8119

Figure 3.3B: Scatter plot between fusarium damaged kernel percentage (FDK) and fusarium head blight index (FHB index), 2009

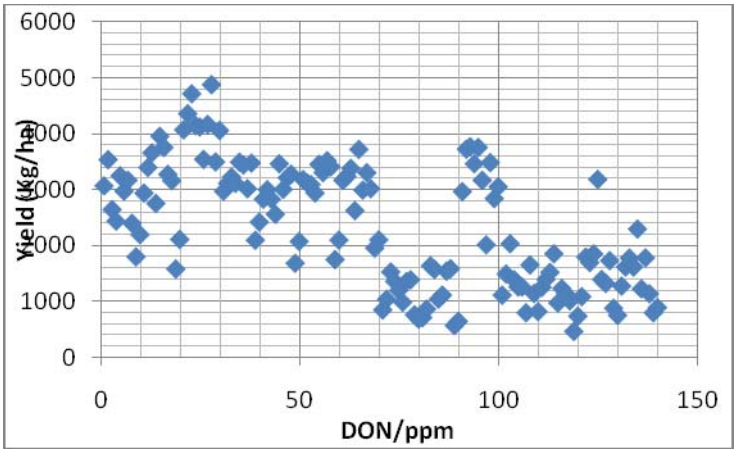
C



R= -0.8861

Figure 3.3C: Scatter plot between yield and fusarium head blight index (FHB index), 2009

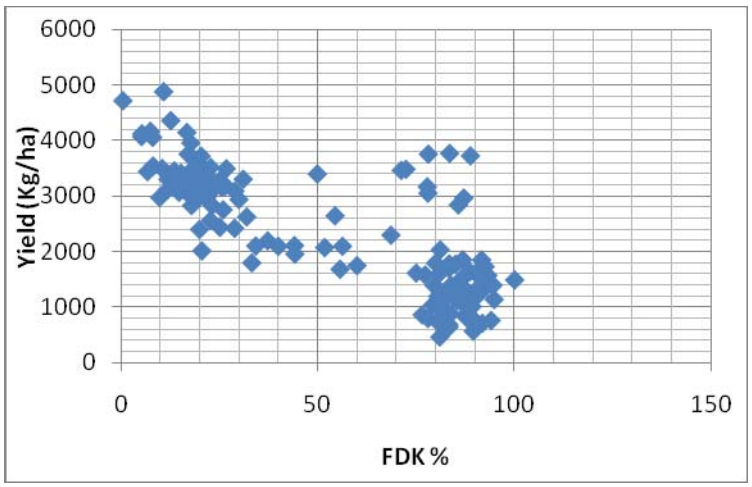
D



R= -0.7683

Figure 3.3D: Scatter plot between yield and deoxynivalenol content (DON), 2009

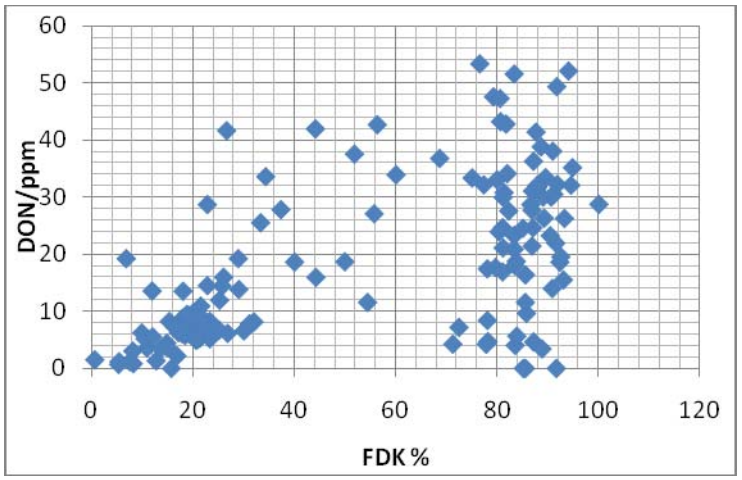
E



R= -0.8030

Figure 3.3E: Scatter plot between yield and fusarium damaged kernel percentage (FDK), 2009

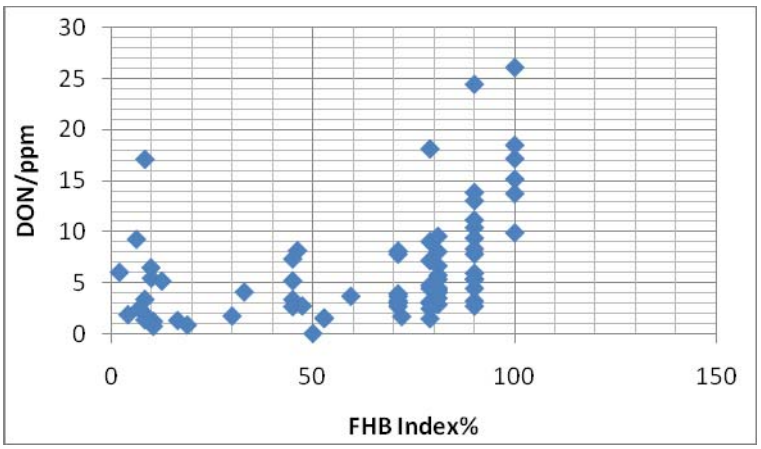
F



R= 0.6255

Figure 3.3F: Scatter plot between deoxynivalenol content (DON) and fusarium damaged kernel percentage (FDK), 2009

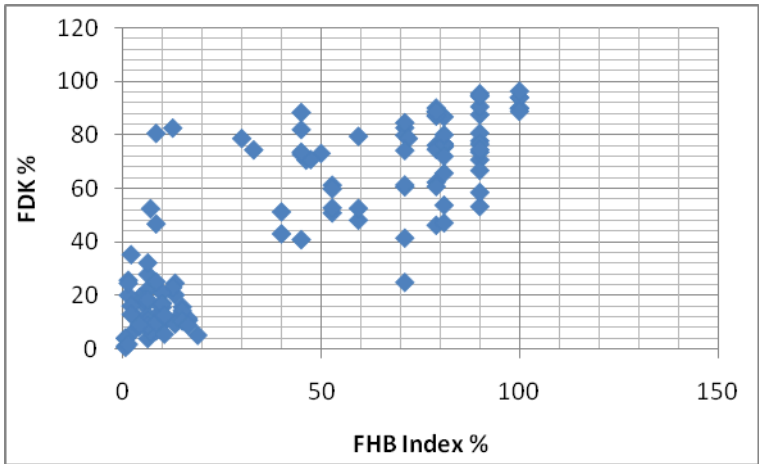
A



R= 0.3677

Figure 3.4A: Scatter plot between deoxynivalenol content (DON) and fusarium head blight index (FHB index), 2010

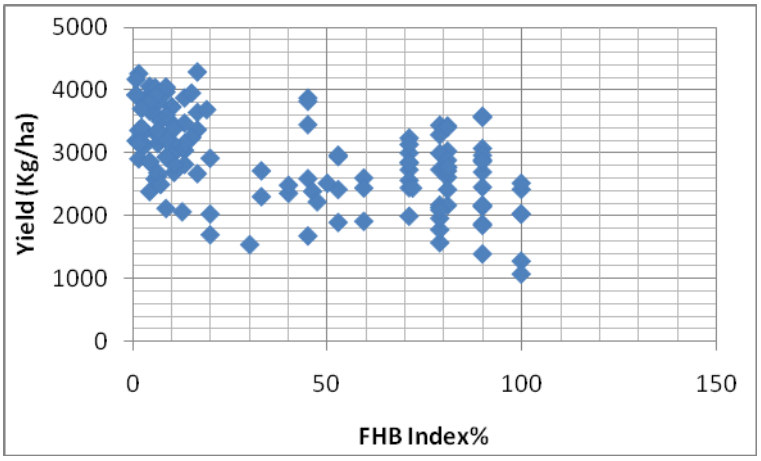
B



R= 0.8605

Figure 3.4B: Scatter plot between fusarium damaged kernel percentage (FDK) and fusarium head blight index (FHB index), 2010

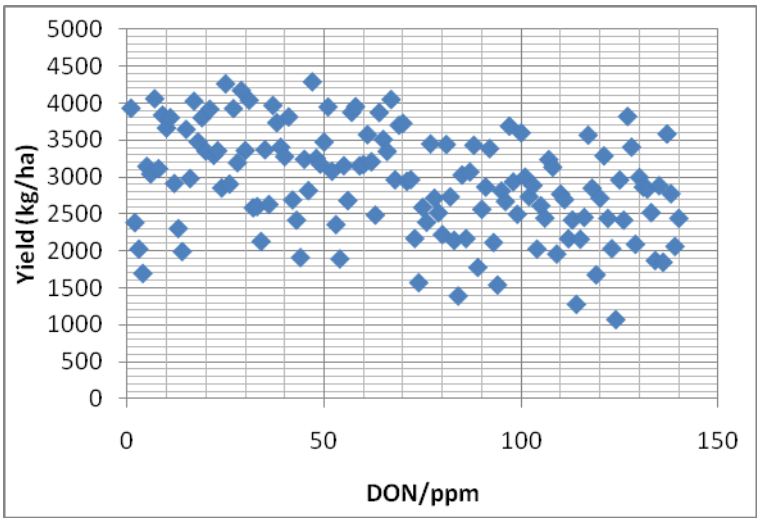
C



R= -0.55188

Figure 3.4C: Scatter plot between yield and fusarium head blight index (FHB index), 2010

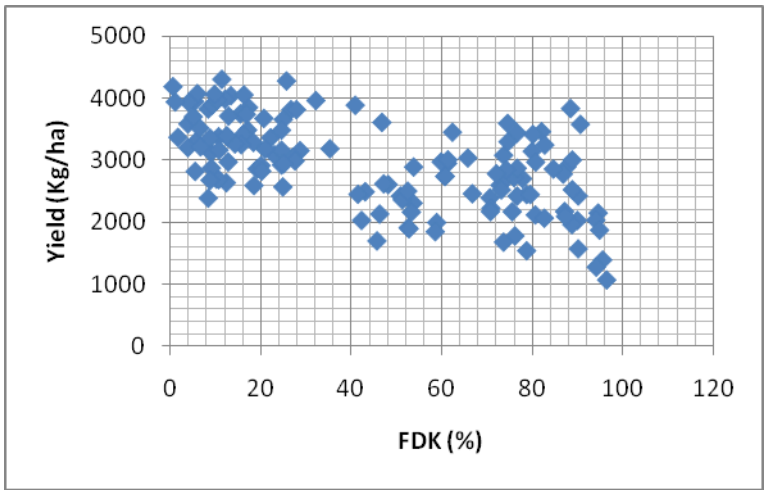
D



R= 0.4321

Figure 3.4D: Scatter plot between yield and deoxynivalenol content (DON), 2010

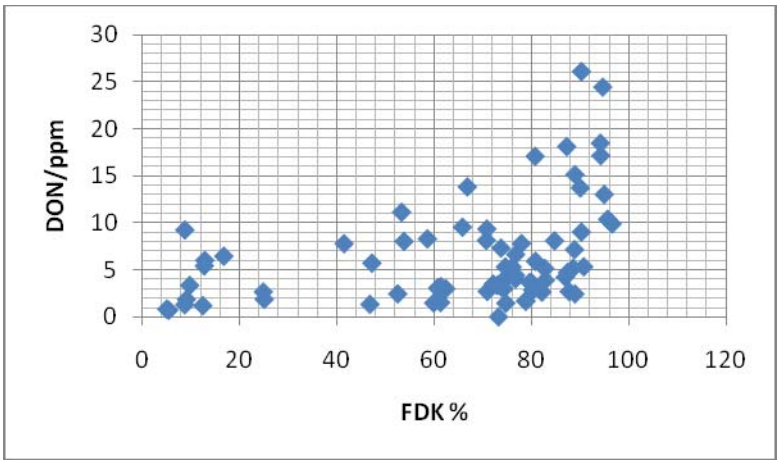
E



R= -0.6049

Figure 3.4E: Scatter plot between yield and fusarium damaged kernel percentage (FDK), 2010

F



R= 0.3399

Figure 3.4F: Scatter plot between deoxynivalenol content (DON) and fusarium damaged kernel percentage (FDK), 2010

3.4 Discussion

This was the first field study done in Canada to examine the efficacy of fungicides against the 3-ADON and 15-ADON isolates of *F. graminearum*. Both in 2009 and 2010 the environmental conditions in Winnipeg, Manitoba, Canada were particularly favourable to the development of FHB in wheat. Although Milus and Parsons, (1994) reported that, fungicide treatments do not have an effect on FHB incidence, DON levels, yield and FDK in the presence of artificial inoculum, in our experiments, most of the fungicide treatments significantly reduced FHB index, FDK and DON content and increased the yield compared to the unsprayed inoculated controls. These findings are in agreement with other reports, confirming the effectiveness of triazole fungicides in controlling FHB both under green house and field conditions (Matthies and Buchenauer., 2000; Homdork et al., 2000; Pirgozliev et al., 2002; Mennitti et al., 2003). The variability of efficacy of fungicides in controlling the FHB has been also reported by other authors too. Paul et al. (2007) reported that, the efficacy of tebuconazole was variable, even though a susceptible cultivar was used, and the fungicide applied at a single growth stage with the same active ingredient rate per area.

The FHB index results from this study were similar to those reported from Homdork et al. (2000). The systemic fungicides that were used to control FHB also controled the other foliar diseases therefore the yield increase was not solely dependent on the control of FHB but also possibly due to the control of other foliar diseases. Zhang et al. (2010) examined the effect of fungicides on active oxygen species and the

antioxidant system during leaf senescence and found, fungicides can delay the senescence of wheat resulting in increase in grain yield.

Although the 2009 FDK results were in contrast with the results obtained by Mesterhazy et al. (2003) and Homdork et al. (2000), the FDK results of 2010 were in agreement with their findings. The discrepancy between the FHB index values and the FDK values in uninoculated control treatments were due to the lag in the disease development especially in 2009, in 2009 due to the cooler weather conditions the rate of disease development was less, the FHB index values were taken 22 days after the first inoculation but the FDK values were calculated after the heads were harvested.

The increase in DON content, after application of fungicides also noted in other studies (Milus and Parsons, 1994; Matthies and Buchenauer, 2000; Simpson et al., 2001; Pirgozliev et al., 2002; Menniti et al., 2003). A combined *in vivo/in vitro* approach showed that sub-lethal prothioconazole concentrations act as a trigger for DON biosynthesis and further concluded that DON biosynthesis resulted from the interaction of fungal genomics and external environmental triggers (Audenaert et al., 2010). In the same study they monitored the accumulation of hydrogen peroxide (H_2O_2) that was produced as a response to stress. They observed that sub-lethal triazole application resulted in an increased H_2O_2 accumulation compared to the control and this stress-induced H_2O_2 accumulation acted as a trigger to induce DON concentration (Audenaert et al., 2010). In an *in vitro* study, D'Mello et al. (1998), observed acceleration in 3-ADON production, in a fungicide insensitive strain of *F. culmorum* upon exposure to that particular fungicide. Based on several *in vitro* studies, D'Mello et al. (2001), hypothesized that the acquisition of fungicide resistance by *Fusarium* phytopathogens might impair fungicide control of

mycotoxin contamination. It is suggested that the development of fungicide insensitivity and resistance by *Fusarium* phytopathogens may contribute to an increase in mycotoxin production (D'Mello et al., 1998). To date, insufficient evidence has been presented to explain the role of fungicides in increasing DON in infected grains; therefore further research is needed in this area. Mesterhazy et al. (2003) and Homdork et al. (2002) all reported that the application of fungicides having tebuconazole reduced DON content in infected grains and Pirgozliev et al. (2002) and Dardis and Walsh et al. (2000) reported that metconazole based fungicides reduce both FHB and DON content greatly in infected grain and are very effective against both *F. graminearum* and *F. culmorum* (Pirgozliev et al., 2002). Paul et al. (2007) reported that tebuconazole was more effective in reducing the disease severity than DON content.

In this study, wheat cultivars were a significant source of variability, except for DON (2009 and 2010) and Yield (2010). All the variables had a significant fungicide-cultivar interaction. Therefore this finding confirms that the level of resistance in a cultivar plays a key role in fungicide-cultivar-isolate interaction.

Another source of variability is the aggressiveness of the pathogen and the level of inoculation. It is believed that 3-ADON isolates produce more toxins and have higher fecundity than 15-ADON isolates (Ward et al., 2008). Significant differences for FHB index, FDK, yield and DON concentration were found between the inoculated vs uninoculated treatments in both years. Also, significant differences were found between the 3-ADON and 15-ADON isolates for FHB index, FDK, yield and DON concentration except for FDK and yield in 2010. Only for some variables significant differences were found within the 3-ADON or 15-ADON isolates. Except for yield (2009), FHB index and

FDK (2010) the fungicide-isolate interactions were insignificant for other variables. Therefore the fungicides used in this study can be used to control FHB caused either by 15-ADON or 3-ADON *F. graminearum* isolates.

Other sources of variability in the fungicide field trials are the coverage, timing and rate of fungicide application. Because wheat has a short window for *Fusarium* infection, the timing of fungicide application is a critical factor that determines the success in controlling the FHB. Application of fungicides at early growth stage (GS), between GS 32 and GS 50 failed to significantly reduce FHB. However, the application of fungicides between GS 59 and 70 closer to the time of natural infection by *Fusarium* spp. reduced both the severity of FHB and mycotoxin concentration significantly in harvested grain (Siranidou and Buchenauer 2001; Pirgozliev et al., 2008).

Kang et al. (2001) confirmed that the effects of tebuconazole fungicides are similar to those of other ergosterol-biosynthesis-inhibiting (EBI) fungicides. In the presence of tebuconazole fungicides, *F. culmorum* shows various morphological and cytological changes such as, hyphal swelling, excessive branching, increased vacuolation, irregular thickening of hyphal cell walls and necrosis (Kang et al., 2001). It is expected that similar changes occur in *F. graminearum*. Although a good fungicide should have a high efficacy against the target fungus, it should be less toxic to the other animals. The relative toxicities (LD_{50}) of prothioconazole, metconazole and tebuconazole are 0.10, 1.00 and 0.35 respectively (Klix et al., 2007). In the same study, they examined the relative efficacies (EC_{50}) of prothioconazole, metconazole and tebuconazole that inhibited the germination of 50% of the spores. The relative efficacies (EC_{50}) were 1.00, 0.27 and 0.l5

for prothioconazole, metconazole and tebuconazole respectively (Klix et al., 2007). In an *in vitro* study, it was reported that prothioconazole fungicides can effectively inhibit the *F. graminearum* conidial germination at field dosage but azoxystrobin fungicides didn't inhibit the conidial germination (Audenaert et al., 2010).

Uniform coverage of a fungicide on wheat heads is one of the critical factors that have a significant effect on the final result. Hooker and Scaafsma, (2004) examined that different nozzle types gave highly different coverage of heads. Most of the traditional spraying technologies have been developed to cover the leaves, therefore are not efficient at covering the vertically standing heads. In fungicide field trials, wheat heads should be sprayed from both sides to ensure the uniform coverage of heads. In our study, a good correlation between FHB visual ratings and DON content was observed in 2009. But in 2010 although the correlations were significant, poor correlations were observed, especially the correlations between FHB index-DON and FDK-DON. Several authors have reported a good correlation between FHB visual ratings and DON content in infected grains (Homdork et al., 2000; Bai et al., 2001; Mesterhazy et al., 2003; Haidukowski et al., 2005). However other researchers have observed that DON content is either moderately or not correlated with the FHB visual ratings (Arseniuk et al., 1999; Edwards et al., 2001; Shaner and Buechley, 2004). Schaafsma et al. (2004) indicated that the correlation coefficients between the FDK and DON content can vary from r=0.28 to 0.99 with a mean of 0.73. Because the association between FHB intensity and DON accumulation is not fully understood, Paul et al. (2005) performed a meta analysis using results from 163 published and unpublished data, including 60 fungicide studies and 103 genotype studies to determine the relationship between measures of FHB intensity and

DON accumulation. According to the analysis they found that the estimated correlation coefficients (r) ranged between -0.58 to 0.99.Of all the examined FHB intensity parameters FDK had the highest association with DON (r= 0.73) (Paul et al., 2005). The differences of the correlations among the FHB visual disease ratings and DON content in the two field seasons may be due to the weather conditions during the two field seasons **(Table: 3.11)**. The temperature and the moisture at the flowering stage could have affected the disease levels. Hope et al. (2005) studied the environmental profiles for growth and DON production by *F. graminearum* on wheat grains. The growth of *F. graminearum* was optimum at 0.99a_w (water activity) at 25 ºC and 0.98 a_w at 15 ºC, and the optimum DON was produced at 0.99 a_w at 15ºC and 0.98 a_w at 25 ºC respectively (Hope et al., 2005).

Table 3.11: Average temperatures, relative humidity and precipitation during June, July, August and September in 2009 and 2010 field seasons at the "Point" field station, Department of Plant Science, University of Manitoba

	June		**July**		**August**		**September**	
	2009	2010	2009	2010	2009	2010	2009	2010
Temperature °C	16.2	17.0	17.5	20.8	17.7	19.5	17.8	11.9
Relative Humidity	62.4	71.6	67.1	66.3	73.9	70.3	70.0	69.9
Precipitation mm	2.9	2.5	3.5	3.6	2.9	4.5	1.4	3.0

Source: The point weather station, Department of Plant Science, University of Manitoba

In 2010, the DON levels in infected grains were low compared to 2009. Mesterházy, (2002) also reported the climatic effects on DON production. The same isolate may have highly significant effectson DON production during different years. A significant close correlation has been found between the DON production and amount of precipitation (r=0.642) (Mesterházy, 2002). In this study also, significant differences were observed between the two years, including the year*isolate interactions therefore the results of two years were reported separately (data not shown). The experimental errors that occur during threshing also would have an effect on the DON content in infected grains. Due to the fan speed, the threshing machine may blow away heavily infected light weight seeds which would have otherwise contributed to higher DON content.

To establish a correlation between *Fusarium* DNA and DON content, Fusarium – DNA content calculated from the standard curve was plotted against the respective DON concentrations in all analysed samples. A poor correlation was observed between the *Fusarium* DNA and the DON content in the infected grains in the cultivar Roblin. But a good correlation was observed between DON content and the *Fusarium* DNA in the moderately resistant cultivar Glenn. Studies done by Schnerr et al. (2002), Edwards et al. (2001) and Zhang et al. (2008) found good correlation between the DON content and the *Fusarium* DNA. The difference in this study might be the small sample size that was been taken for DNA extraction and which was not sufficient enough to represent the effects of the whole plot. Therefore from this study it revealed that the amount of DON produced by *Fusarium* might vary varied depending on the amount of *Fusarium* colonization on that particular grain.

The results of this study revealed that fungicides can be used to manage FHB efficiently. In this study, similar to other studies, conflicting evidence were observed specifically for the effect of fungicides on DON and FDK, where some fungicides increased the DON and FDK compared to the unsprayed control. Significant differences were observed for the two *F. graminearum* chemotypes 3-ADON and 15-ADON for FHB index, FDK, yield and DON. From this study, it was clearly confirmed that the level of resistance in the wheat cultivar influences the efficacy of fungicides. Therefore growing moderately resistant wheat cultivars plus application of fungicides would provide better protection against FHB.

CHAPTER 4

4.0 MULTIPLEX PCR ASSAY FOR THE IDENTIFICATION OF CHEMOTYPE DIVERSITY IN *Fusarium graminerum* ISOLATES COLLECTED FROM CORN FIELDS IN ONTARIO

Abstract

The objective of this study was to investigate the frequency of 15-ADON and 3-ADON chemotypes of *Fusarium graminearum* isolates collected from corn fields in Ontario. *Fusarium graminearum* can cause severe economic damage to important crops such as wheat, barley, oat, rye and corn. *Gibberella zeae* is the most common corn ear mould found in Ontario, Canada. The contamination of maize with fungal mycotoxins reduces the quality of the grain. Recent research showed that the higher trichothecene producer, 3-ADON is replacing the 15-ADON chemotype populations from east to west in Canada. Sixty one isolates collected from four different corn fields in Ontario were transferred to a *Fusarium*-specific nutrition poor agar (SNA) medium. DNA was extracted from pure cultures and *F. graminearam* chemotypes were identified using the multiplex PCR primers 3CON, 3Na, 3D15A and 3D3A. All the isolates were confirmedas *F. graminearum* using primers specific for *F. graminearum* and the multiplex PCR assay revealed that all investigated *F. graminearum* isolates were of the 15-ADON chemotype and none of the 3-ADON chemotype.

4.1 Introduction

The genus *Fusarium* consists of a large, complex group of fungi and contains numerous species that are associated with serious plant diseases. Several species of *Fusarium* have been associated with diseases of corn at all growth stages. These *Fusarium* species can cause stalk rot, leaf spot, ear and kernel rot and seedling blight of corn; they can be either soil borne or seed borne pathogens (Leslie et al., 1990; Ocamb and Komedahl, 1994; Dodd and White, 1999; Broders et al., 2007). *Fusarium* spp. can cause two distinct diseases on ears of corn, 1) Gibberella ear rot or red ear rot, 2) Fusarium ear rot or pink ear rot. Although these two diseases are distinct, they possess several overlapping epidemiological characteristics. Therefore the accurate identification of the symptoms is important (Munkvold, 2003). Gibberella ear rot caused by the fungus *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch) is the most common disease of corn grown in Ontario and can cause substantial yield and quality losses (Tamburic-llincic and Schaafsma, 2008). Gibberella ear rot is characterized by a reddish/white fungal growth that usually begins at the tip of the ear and spreads over the entire ear (Munkvold, 2003). The pathogen produces mycotoxins named deoxynivalenol (DON), its acetylated derivatives 3-ADON and 15-ADON, nivalenol (NIV) and zearalenone (ZEN). These toxins contaminate grain causing health problems in many monogastric animals specially swine. The vomitoxin, DON induces emesis in swine and other animals, characterised by vomiting, feed refusal and decreased weight gain. In addition, the mycotoxin zearalenone which has estrogenic properties can cause infertility and birth defects in ruminants (D'Mello et al., 1999; Ali et al., 2005; Desjardins, 2006).

The primary infection pathway of *F. graminearum* on corn ear is through the silk channel, although the infection can also occur through wounds created by insects or birds. Corn is more vulnerable to fusarium infection at the early stages of silk emergence and disease development is highly dependent on the environmental conditions. Cool weather preceded by warm, humid conditions makes an ideal environment for Gibberella ear rot epidemics (Sutton, 1982; Reid and Hamilton, 1996; Reid et al. 1996; Munkvold, 2003; Ali et al., 2005).

Resistance to ear rot in corn is a quantitative trait governed by one or several genes influenced by environmental factors. Two types of resistance to Gibberella ear rot have been found; silk resistance and kernel resistance (Ried et al. 1992; Chungu et al. 1996). Silk resistance is the mechanism of preventing the fungus from growing rapidly down the silk to the kernels. Kernel resistance is the ability to prevent the spread of the fungus from kernel to kernel (Reid et al. 1992; Chungu et al. 1996).

The distribution of chemotype diversity across Canada has been analysed by Ward et al. (2008) using DNA sequence analysis, multilocus genotyping assays and variable number tandem repeat analysis (VNTR). It has been found that 3-ADON chemotypes are more common in eastern Canada than in the Western provinces and 3-ADON producers are quickly displacing the 15-ADON producers in Western Canada. The 3-ADON chemotypes have higher fecundity and can accumulate more trichothecenes than the 15-ADON producers (Ward et al., 2008). This chemotypic shift could have a negative effect on food safety and economics of corn production in Canada. The studies

on *F. graminearum* chemotype diversity on different host crops are essential to understand the behaviour of these chemotypes under different host genetic backgrounds.

Studies on chemotype diversity of *F. graminearum* in corn have received relatively less attention than in wheat. Therefore, the objectives of this study were to (i) confirm the morphologically identified *F. graminearum* using species specific PCR (ii) investigate the chemotype diversity in *F. graminearum* isolates collected from corn ears in different corn field locations in Ontario.

4.2 Materials and methods

4.2.1 Isolate collection and DNA extraction

Sixty one *F. graminearum* isolates were collected from corn fields in Kerwood, Croton, Tilbury and G. Pointae, Ontario (sent by Dr. L. Tamburic-llinicic, University of Guelph) **(Table:4.1)**. To obtain single spore cultures, isolates were transferred on to a *Fusarium*- specific nutrition-poor agar (SNA) medium, and incubated for 7 days for sporulation under fluorescent light at room temperature. After incubation, the developed *F. gramineraum* sporodochia on the SNA medium were washed 3-4 times using 50 µl of sterilized distilled water, and the washings were added in to 100 µl sterilized water and mixed well. Ten microliter of the spore suspension was spread and plated on water agar (WA) medium. Plates were incubated at room temperature under fluorescent light for 6 hr, and a single germinating macroconidium was transferred on to a potato dextrose agar (PDA) (Fisher Scientific, NJ, USA) medium, under the mid power of light microscope (Fisher Scientific, NJ, USA). The PDA plates were then incubated at room temperature

for 10 days. After 10 days mycelium was harvested, freeze dried for 10 hrs, and stored at -80 ºC.

DNA was extracted from the freeze-dried mycelium using the method described by Fernando et al. (2006). The freeze-dried fungal mycelium was first crushed using sterilized tooth picks and then ground in 600 µl of Tris-EDTA-SDS (TES) buffer (100mM Tris; 10mM EDTA; 2% SDS) in a 1.5-ml microcentrifuge tube. One hundred and forty microliters of 5M NaCl and 70 µl of 10% CTAB were added in the tube and vortexed thoroughly for 2 min and incubated for 20 min at 65 ºC. After incubation, 600 µl of phenol: chloroform: isoamylalcohol (PCI) (Fisher Scientific, NJ, USA) (v/v 25:24:1) was added, and the mixture was centrifuged at 10000 rpm for 15 min. The supernatant was then transferred into a new microcentrifuge tube, and the latter step was repeated 2-3 times. To precipitate DNA, 80 µl of 5M NaCl and 1000 µl 100% ethanol were added and the solution was centrifuged at 13000 rpm for 5min. The resultant DNA pellet was washed with 200 µl of ice-cold 80% ethanol and dried at room temperature for about 20mins. The dried pellet was re-suspended in 400µl of sterilized water (65 ºC) and kept at 4 ºC to allow the DNA to dissolve completely. To remove RNA, 5 µl of RNase was added to 50µlof DNA and incubated at 37 ºC for 20 min. Then DNA was quantified both using the GeneQuant spectrophotometer (Biochrom Ltd, Cambridge, England) at 260nm and 280 nm and on 1% agarose gel. DNA was diluted using sterilized distilled water for a final concentration of 10 ng/ µl.

4.2.2 Identification of *F. graminearum* and chemotypes

Identification of the isolates to be *F. graminearum* or not was confirmed using the specific PCR marker described by Demeke et al. (2005). Two *F. graminearum*- specific primers, Fg16F (5'-CTCCGGATATGTTGCGTCAA-3') and Fg16R (5'-GGTAGGTATCCGACATGGCAA-3'), producing a fragment of 450bp were used in the PCR. The PCR was performed in a 25µl volume, containing 20ng of template DNA, 1.5mM $MgCl_2$, 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 (Invitrogen, Carlsbad, CA, USA). Once identity was confirmed, *F. graminearum* chemotypes were identified using the multiplex PCR primers described by Ward et al. (2002). The four primers, 3CON (5`-TGGCAAAGACTGGTTCAC-3`), 3D15A (5`-CGCATTGGCTAACACATG-3`), 3D3A (5`- CGCATTGGCTAACACATG-3`) and 3NA (5`-GTGCACAGAATATACGAAGC -3`) were used in the PCR mixture. The primers 3D3A/3CON produced a 243 bp fragment for 3-ADON chemotype, 3D15A/3CON produced a 610 bp fragment for 15-ADON chemotype and 3NA/3CON produced a 840 bp fragment for NIV chemotype. The PCR reaction was performed in a 15µl volume, containing 20ng of template DNA, 2.0mM $MgCl_2$, 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.

4.3 Results and Discussion

This was the first study done to investigate the chemotype diversity of *F. graminearum* collected from corn fields in Ontario. The species-specific primer Fg16 confirmed that all the investigated isolates were *F. graminearum* **(Fig: 4.1)**. These results are in agreement with the studies done by Tamburic-llincic and Schaafsma, (2008). They reported that, *F. graminearum* was the most frequently recovered fungus from pink discoloration on stalks, close to harvest time in infected corn fields of south-western Ontario. The multiplex PCR assay revealed that all the *F. graminearum* isolates examined were of the 15-ADON chemotype and none were of the 3-ADON or NIV chemotypes **(Fig: 4.2)**. Ward et al. (2008) reported the distribution of trichothecene chemotype frequencies across Canada based on a multilocus genotyping assay. In Canada, the distribution of trichothecene chemotypes shows a distinct longitudinal cline. The frequency of 3-ADON producers was significantly higher in the eastern provinces than in western provinces (Ward et al., 2008). Among all provinces, Prince Edward Island, Quebec and Manitoba have higherfrequencies of 3-ADON chemotypes than Saskatchewan or Alberta. In Manitoba a chemotypic shift from thr more prevalent 15-ADON producers to 3-ADON producers have been reported by Guo et al. (2008) and Ward et al. (2008). Although no significant differences were found between the pathogenicity of 3-ADON and 15-ADON on wheat cultivars Roblin and 5602 HR, it has been found that 3-ADON chemotypes produce higher levels of trichothecenes than 15-ADON chemotypes (Ward et al., 2008). The reasons for this chemotypic shift are not yet clear. It is hypothesised that 3-ADON isolates were introduced into eastern Canada and then have subsequently spread across Canada and into the Midwest of the United States

(Ward et al., 2008). Although no 3-ADON *F. graminearum* isolates were found from this study, Gilbert et al. (2001) reported the presence of 3-ADON producing *F. graminearum* isolates from corn in Ontario. In 1999, the worst epidemic of *Fusarium* since 1986, was experienced by the corn producers in 5 counties in Ontario with 5-10 ppm DON contamination in grain (Schaafsma, 1999). The most recent *Fusarium* epidemic in corn was occurred in 2006 (Tamburic-Ilincic et al., 2010).

In this study no 3-ADON isolates were found among the *F. graminearum* isolates collected from corn fields, therefore this confirms that 15-ADON chemotypes are still prevalent in Ontario at least on maize and there is no rapid chemotypic shift from 15-ADON to 3-ADON chemotypes in Ontario as observed in Manitoba. These findings are in agreement with the study done by Tamburic-llincic et al. (2006), in which they observed that *F. graminearum* isolates collected from winter wheat in Ontario were mainly of the 15-ADON chemotypes.

Crop residue is the primary source of inoculum for FHB epidemics (Parry et al., 1995). The common crop rotation practice in Ontario is corn-soybean-wheat. These crop rotation practices may enhance the survival of *F. graminearum* both on corn and soybean debris (Leslie et al., 1990; Baird et al., 1997). Several severe FHB epidemics were reported from different regions in USA when wheat was introduced to fields that followed after corn (Stack, 2003). Teich and Hamilton, (1985) and Dill-Mackey and Jones, (2000) reported that wheat followed by corn increases the incidence of head blight and DON accumulation. Therefore chemotype diversity in corn also has a direct impact on the wheat industry in Ontario. Corn residue facilitates the survival and sporulation of

Giberella zeae (Khonga and Sutton, 1988). Therefore the findings from this study are important for providing suitable recommendations to manage FHB epidemics.

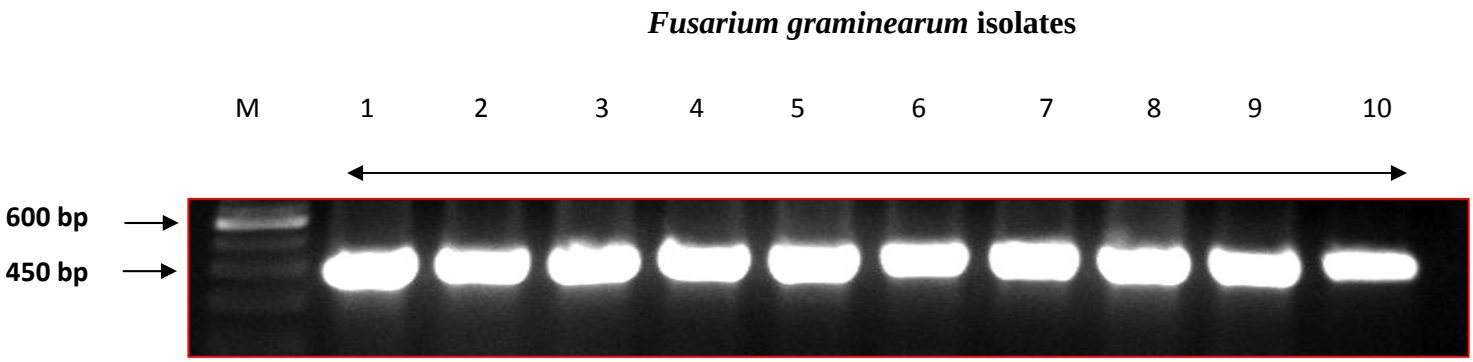


Figure 4.1: *Fusarium graminearum* DNA amplified using the species-specific PCR. The 450 bp fragments represent the *F. graminearum* species on 2% agarose gel.

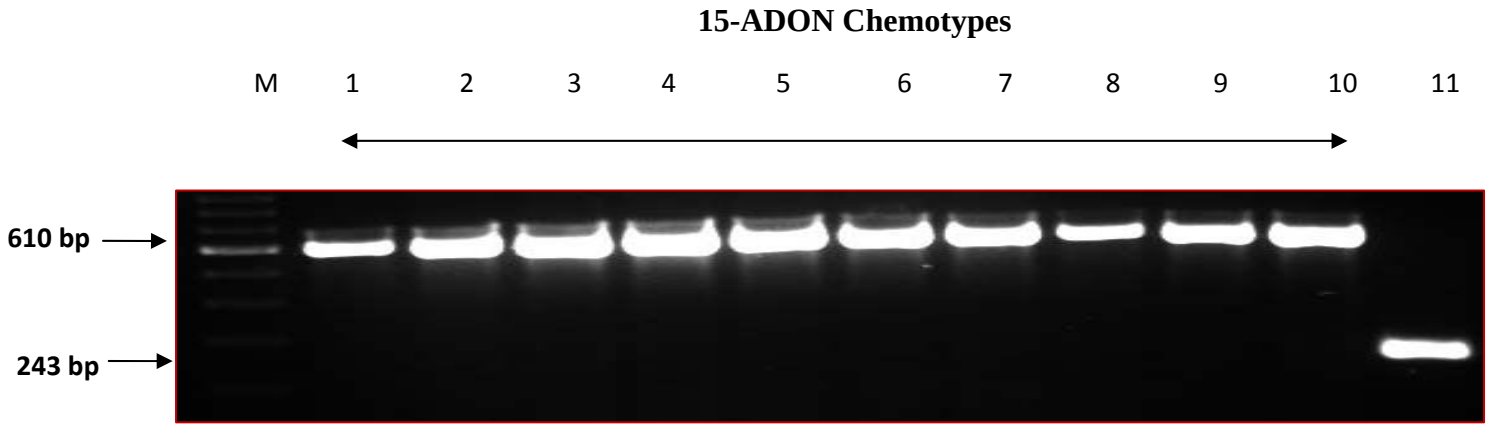


Figure 4.2: DNA of chemotype isolates amplified using multiplex PCR. The 610 fragments represented 15-ADON chemotypes on 2% agarose gel.

1-V-1562**, 2**- V-2109**, 3**- V-1611-1**, 4**- V-1706-1**, 5**- V-2626-1d**, 6**- V-1809-2**, 7**- V-1538**, 8**- V-2000-1**, 9**- V- 2093-3**, 10**- V-1500, **11**- (3-ADON control)

Table 4.1. Mycotoxin chemotypes of *Fusarium graminearum* from corn fields in Ontario, determined by multiplex PCR

***F. graminearum* Isolate**	**Crop**	**Location**	**Luminex Chemotype**
V-1562	corn	Croton	15-ADON
V-2142	corn	Kerwood	15-ADON
V-1706- 4	corn	Croton	15-ADON
V-1500	corn	Croton	15-ADON
V-2109	corn	Kerwood	15-ADON
V-1599-4	corn	Croton	15-ADON
V-2109	corn	Kerwood	15-ADON
V-2202-4	corn	Kerwood	15-ADON
V-1562-2	corn	Croton	15-ADON
V-1980-1	corn	Kerwood	15-ADON
V-2093-1	corn	Kerwood	15-ADON
V-1538-3	corn	Croton	15-ADON
V-2069-5	corn	Kerwood	15-ADON
V-2025-1	corn	Kerwood	15-ADON
V-1536-5	corn	Croton	15-ADON
V-1462-2	corn	Croton	15-ADON
V-2023-5	corn	Kerwood	15-ADON
V-2074-2	corn	Kerwood	15-ADON
V-1443-5	corn	Croton	15-ADON
V-2085-2	corn	Kerwood	15-ADON
V-4596-5	corn		15-ADON
V-2093-3	corn	Kerwood	15-ADON
V-1988-3	corn	Kerwood	15-ADON
V-1611-1	corn	Croton	15-ADON
V-2626-2Q	corn	Tilbury	15-ADON
V-1611-2	corn	Croton	15-ADON
V- 2061-1	corn	Kerwood	15-ADON
V-1969-4	corn	Kerwood	15-ADON
V-2194-1	corn	Kerwood	15-ADON
V-2626-2b	corn	Tilbury	15-ADON
V-1443-3	corn	Croton	15-ADON
V-1443-2	corn	Croton	15-ADON
V-2000-1	corn	Kerwood	15-ADON
V-1581-3	corn	Croton	15-ADON
V-2007-2	corn	Kerwood	15-ADON
V-2023-1	corn	Kerwood	15-ADON

***F. graminearum* Isolate**	**Crop**	**Location**	**Luminex Chemotype**
V-1809-1	corn	G. Pointe	15-ADON
V-2007-1	corn	Kerwood	15-ADON
V-1473-4	corn	Croton	15-ADON
V-2626-5Q	corn	Tilbury	15-ADON
V-1835-4	corn	G. Pointe	15-ADON
V-2694-2Q	corn	Tilbury	15-ADON
V-2023-1	corn	Kerwood	15-ADON
V-2626-1d	corn	Tilbury	15-ADON
V-2626-1b	corn	Tilbury	15-ADON
V-1769-2l	corn	G. Pointe	15-ADON
V-1858-4Q	corn	G. Pointe	15-ADON
V-1903-1	corn	G. Pointe	15-ADON
V-1840-1b	corn	G. Pointe	15-ADON
V-2626-1Q	corn	Tilbury	15-ADON
V-1706-1	corn	Croton	15-ADON
V-1428-1	corn	Croton	15-ADON
V-1902-4b	corn	G. Pointe	15-ADON
V-1902-4c	corn	G. Pointe	15-ADON
V-2129-2Q	corn	Kerwood	15-ADON
V-2129-1l	corn	Kerwood	15-ADON
V-2129-1Q	corn	Kerwood	15-ADON
V-1538	corn	Croton	15-ADON
V-2129-2b	corn	Kerwood	15-ADON
V-1809-2	corn	G. Pointe	15-ADON

CHAPTER 5

5.0 EVIDENCE FOR DIFFERENCES IN *TRI13* GENE SEQUENCES BETWEEN 3-ACETYLDEOXYNIVALENOL PRODUCING *Fusarium graminearum* CHEMOTYPES FROM CANADA AND CHINA

Abstract

Fusarium head blight is a fungal disease that infects wheat, barley and corn. *Fusarium graminearum* is the predominant causal agent of this disease in Manitoba. The fungus produces mycotoxins that leave grains unmarketable. One of these toxins is deoxynivalenol (DON) and its acetylated derivatives, 3A-DON and 15A-DON. Positive-negative PCR assays based on the genes involved in the trichothecene biosynthesis pathway are useful in assessing the risk of trichothecene contamination of grains and are important in epidemiological studies. A single PCR detection method based on the structural gene sequence of *TRI13* gene have been developed to predict the 3-ADON, 15-ADON and NIV chemotypes in China. The chemotypic differences are based on the deletions within the *TRI13* gene. The objective of this study was to assess the reliability of using this single primer based on the *TRI13* gene to differentiate the *Fusarium graminearum* chemotypes in Canada. In this study, we found that this single PCR detection method based on the deletions in the *TRI13* gene cannot be used to differentiate the 3-ADON and 15-ADON chemotypes in the Canadian *F. graminearum* isolates; further sequence analysis of the PCR products confirmed that both Canadian 3-ADON and 15-ADON chemotypes have the 61 bp deletion in the *TRI13* gene. This 61 bp deletion was absent in the Chinese 3-ADON isolates. Therefore these findings revealed that there are genetic differences between the examined 3-ADON *F. graminearum* isolates so far examined from Canada and China. To our knowledge, this is the first report of a difference in the *TRI13* gene sequence of 3-ADON isolates from different regions in the world, namely of Chinese origin and Canadian 3-ADON isolates. These genetic

changes might be due to the geographical distribution of *F. graminearum* isolates and further research is needed to investigate the reasons for these genetic changes.

5.1 Introduction

Fusarium head blight (FHB) is an economically important disease worldwide. Apart from causing significant yield losses the fungus can produce trichothecene mycotoxins. Harvested grain, contaminated with trichothecene mycotoxins can cause both acute and chronic illness in livestock and humans. It has been reported that 25% of the world crop is affected by mycotoxins (Charmley et al., 1995). In plants, these mycotoxins have been shown to act as virulence factors during pathogenesis (Proctor et al., 1995). There are mainly two classes of trichothecenes; class A and class B. Class B contains a keto group at the C-8 position of the trichothecene ring (Miller et al., 1991). Different *Fusarium* spp such as*, F. graminearum*, *F. culmorum* and *F. cerealis* can produce different types of class B trichothecenes, such as deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives, 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) and 4-acetylnivalenol (4-ANIV). Based on type B tricothecene production, three different chemotypes of *F. graminearum* have been identified. The 3-ADON chemotype produces both DON and 3-ADON; 15-ADON chemotype produces both DON and 15-ADON; and the NIV chemotype produces NIV and 4-ANIV (Miller et al., 1991). The differences among chemotypes are governed by the different genes involved in the trichothecene biosynthesis pathway. Trichothecene biosynthesis is a complex process, mediated by at least 10 genes that are involved in series of oxygenation, isomerization and esterification steps (Desjardins, 2006). The

trichothecene gene cluster includes trichodiene synthase (*TRI5*), P450 oxygenase (*TRI4* and *TRI11*), acetyltransferases (*TRI3* and *TRI7*), transcription factors (*TRI6* and *TRI10*), a toxin efflux pump (*TRI12*), and two unidentified hypothetical proteins (*TRI8* and *TRI9*). The acetyltransferase gene *TRI101*, unlinked to the *TRI* cluster, is also found to be involved in the biosynthesis pathway. Two genes *TRI13* and *TRI14* have been identified from *F. sporotrichioides* (Lee et al., 2002; Desjardins, 2006). Among the *TRI* cluster genes *TRI13* is found to be the determinant for the DON-NIV switching in *Fusarium* and *TRI7* is involved in further modification of NIV to its acetylated derivative 4-ANIV (Lee et al., 2002). NIV chemotypes have functional copies of *TRI13* and *TRI7* genes whereas both genes are non-functional in DON chemotypes (Lee et al., 2002).

Various PCR-based assays have been developed to examine the trichothecene mycotoxin genotypes of *F. graminearum*. These assays are based on the amplification of a part of a gene that encodes for key enzymes involved in the trichothecene biosynthesis pathway (Lee et al., 2001; Ward et al., 2002; Jennings et al., 2004; Quarta et al., 2006). These PCR based assays provide a rapid and cost-effective way of identifying the different *F. graminearum* chemotypes (Ward et al., 2002).

The genotypic differences among the *F. graminearum* isolates collected from different populations in diverse geographic regions have been extensively studied (O'Donnell et al., 2002). To date, 13 phylogenetically distinct species have been identified within the *F. graminearum* clade based on DNA sequences from 13 genes (O'Donnell et al., 2004; Starkey et al., 2007; O'Donnell et al., 2008; Yli-Mattila et al., 2009). These lineages are morphologically cryptic. *Fusarium graminearum* (lineage 7) is

the predominant species found in North America (Goswami and Kistler, 2005). In China, *F. asiaticum* (lineage 6) and *F. graminearum* (lineage 7) are the most prevalent FHB causing species (Zhang et al., 2007). O'Donnell et al. (2000) and Ward et al. (2002) reported that the evolution of genes involved in the B-tricothecene biosynthesis does not correlate with the *F. graminearum* clade phylogeny. It is believed that chemotype polymorphism is trans-specific and has been maintained through multiple speciation events by balancing selection (Ward et al., 2002). High levels of genetic variations have been found in the virulence associated genes within the *F. graminearum* clade (Ward et al., 2002). The different lineages of *F. graminearum* have different geographic distributions; also differ in the type of mycotoxins that they produced and they may differ in their ability to cause disease on particular crops (Cumagun et al., 2004). Environmental factors within a certain geographical area may influence the prevalence of a particular chemotype. Zhang et al. (2007) investigated the mycotoxin chemotype frequency of the *F. graminearum* strains isolated from wheat in FHB epidemic regions of China. The chemotypes investigated appeared to have different geographical distribution patterns and these were associated with annual average temperatures in the region (Zhang et al., 2007). Qu et al. (2008b) also investigated the geographical distribution of *F. graminearum* and *F. asiaticum* isolates from all regions in China and demonstrated that *F. graminearum* isolates were more prevalent in the cooler regions whereas *F. asiaticum* predominated in the warmer regions.

Ward et al. (2002) discussed the impact of environment on pathogen fitness. It has been reported that the chemotypes of *F. graminearum* from China and North America were different (Miller et al., 1991; Bai and Shaner, 1996).The results from phylogenetic

studies are important to prevent the unintentional introduction of species or genetically different strains from one genetically unique population to another population especially with international trade (Brasier et al., 1999). The introduction of new *Fusarium* species to the existing population may affect the current management strategies and breeding programs.

Therefore studies on the genetic diversity of *F. graminearum* chemotypes found in different geographical regions are important to prevent the future FHB epidemics. Wang et al. (2008) developed a generic PCR detection method based on the *TRI13* gene to identify 3-ADON, 15-ADON and NIV chemotypes using a single primer pair. The objective of this study is to examine the applicability and reliability of this generic PCR detection method based on *TRI13* gene to identify the chemotypes of *F. graminearum* isolates from Canada.

5.2 Materials and Methods

5.2.1 Fungal isolation and DNA extraction

Fusarium graminearum isolates collected from different regions of Canada (120 isolates) and China (24 isolates) were used **(Table: 5.1)**. Single spore cultures of *F. graminearum* isolates growing from fusarium damaged kernels (FDK) were used for the DNA extraction. All isolates were grown on potato dextrose agar (PDA) plates for 7 days and genomic DNA was extracted from the freeze dried aerial mycelium using a CTAB based protocol described by Fernando et al. (2006).

5.2.2 PCR assays for trichothecene mycotoxin genotypes

Two PCR assays were carried out. In the first PCR, the fungal DNA was amplified using the single primer set, developed by Wang et al. (2008). The single primer set used for amplification was as follows: TRI13P1 (5`-CTCSACCGCATCGAAGAASTCTC-3`) and TRI13P2 (5`-GAASGTCGCARGACCTTGTTTC-3`), these primers generate a 859 bp fragment from NIV producing strains, a 644 bp fragment from 3-ADON producers and a 583 bp fragment from 15-ADON producers, respectively (Wang et al., 2008).

In the second PCR, the same DNA was amplified by the multiplex primer set developed by Ward et al. (2002). The primers for multiplex PCR were as follows: 3CON (5`-TGGCAAAGACTGGTTCAC-3`), 3D15A (5`-CGCATTGGCTAACACATG-3`), 3D3A (5`-CGCATTGGCTAACACATG-3`) and 3NA (5`-GTGCACAGAATATACGAAGC-3`) and they produce a 243 bp fragment for 3-ADON chemotypes, a 610 bp fragment for 15-ADON chemotypes and a 840 bp fragment for NIV chemotypes (Ward et al., 2002).

5.2.3 PCR conditions

Both PCR assays were conducted using 50 ng of fungal DNA in a total volume of 25 µl containing 1.25 mM $MgCl_2$, 1 U Taq polymerase (Invitrogen, Carlsbad, CA, USA), 2.5 mM dNTPs and 10 mM from each primer. The PCR amplification of TRI13P1 and TRI13P2 primers consisted of an initial step at 94 ºC for 4 min, followed by 35 cycles of 94 ºC for 1 min, 58 ºC for 40 sec, 72 ºC for 40 sec, then a final extension at 72 ºC for 6 min. The PCR amplification of multiplex primers consisted of an initial step at 94 ºC for 5

min, followed by 45 cycles of 94 ºC for 30 sec, 52 ºC for 30 sec, 72 ºC for 1 min , then a final extension of 72 ºC for 8 min. Resulting PCR products were separated by 1% gel electrophoresis, stained with ethidium bromide (EtBr) at a final concentration of 0.2 µg/ml and visualized under UV light.

5.2.4 Confirmation of PCR products using sequencing

A representative sample of the 120 Canadian isolates and 24 of the Chinese isolates were used for sequencing. Selected PCR products (4 Canadian 15-ADON isolates, 4 Canadian 3-ADON isolates, 5 Chinese 15-ADON isolates, 5 Chinese 3-ADON isolates and 4 Chinese NIV isolates,) were sequenced (Macrogene Corp, USA) and the sequences were subjected to multiple alignment using ClustalX (1.8) software to examine the expected deletions within the sequences.

5.3 Results

One hundred and twenty (120) *F. graminearum* isolates from different provinces in Canada, 24 *F. graminearum* isolates from China were selected to examine the reliability of the TRI13P1 and TRI13P2 primers for the identification of 3-ADON and 15-ADON chemotypes in Canadian *F. graminearum* isolates. Our PCR assay with TRI13P1/P2 primers revealed that this single primer pair cannot be used to differentiate the two chemotypes within the Canadian isolates, although they could identify the 3-ADON, 15-ADON and NIV isolates within Chinese isolates. The TRI13P1 and TRI13P2 amplified a 583 bp fragment for all analysed Canadian isolates regardless of whether they are 3-ADON or 15-ADON producers **(Fig: 5.1B)**. In this study all the above isolates were tested using multiplex PCR assay as well and it could differentiate the chemotypes

within both Canadian and Chinese isolates**.** The multiplex primers amplified a 610 bp fragment for 15-ADON chemotypes, a 243 bp fragment for 3-ADON chemotypes **(Fig: 5.1A)**. The sequence analysis of the amplified fragments of the TRI13P1 and TRI13P2 primers of the selected 3-ADON, 15-ADON and NIV chemotypes from Canada, and China clearly showed the difference in *TRI13* gene. Compared with the NIV-producers, the 3-ADON chemotypes from Canada had both the 61 bp deletion and the 36 bp deletion whereas the 3-ADON chemotypes from China had only the 36 bp deletion; the 15-ADON chemotypes from both Canada and China had 36 bp and 61 bp deletions **(Fig: 5.3)**.

5.4 Discussion

The *TRI13* gene sequences of the examined Canadian 3-ADON isolates are different from that of examined Chinese 3-ADON isolates. This is the first report of a difference in the *TRI13* gene sequence of 3-ADON isolates from any region in the world. Our study investigated the differences of isolates Chinese origin and Canadian 3-ADON isolates.

Determination of the *F. graminearum* chemotypes using multiple primers makes the evaluation process tedious, time consuming and also they may generate false negative results (Li et al., 2005; Wang et al., 2008). Therefore use of a single primer that could differentiate the two chemotypes would accelerate the identification of chemotypes.

The *TRI13* gene is functional only in NIV producers and encodes 3-acetyltrichothecene C-4 hydroxylase, the enzyme that catalyses the C-4 oxygenation of calonectrin. The disruption of the gene resulted in the loss of NIV production and accumulation of DON (Brown et al., 2002; Lee et al., 2002). The *TRI13* gene consists of a

unique intron of 62 bp. The TRI13P1/P2 primers were designed to identify the three chemotypes based on the deletions within the *TRI13* gene **(Fig: 5.2A).** The primers derived from *TRI13* gene have been used to separate the NIV producers and DON producers. This identification was based on the largest deletion of 178 bp fragment present only in DON producers but not in NIV producers (Brown et al., 2001; Lee et al., 2002; Chandler et al., 2003; Quarta et al., 2006). The molecular differentiation between 3-ADON and 15-ADON chemotypes was based on the remaining two smaller deletions, 61 bp and 36 bp within the coding region. The 15-ADON chemotypes have both 61 bp and 36 bp deletions and amplified a product of 583 bp with TRI13P1/TRI13P2 primers; 3-ADON chemotypes have only the 36 bp deletion and amplified a product of 644 bp. In this study, all the Canadian isolates examined (both 3-ADON and 15-ADON) amplified a product of 583 bp, revealing that Canadian 3-ADON chemotypes have the 61 bp deletion in the *TRI13* gene which is not present in the Chinese 3-ADON chemotypes. Sequencing results clearly confirmed the 61 bp deletion in the *TRI13* gene within the Canadian 3-ADON isolates. Lee et al. (2002) reported that several deletions, substitutions and insertions were found in putative *TRI13* genes in DON producing strains collected from Korea, Nepal and the United states. Further, the nuclear alignment of the putative *TRI13* fragments indicated that these characteristics were highly conserved among the DON-producing strains from different geographical regions.

The multiplex PCR assay was based on the primers derived from trichothecene 15-O-acetyltransferase (*TRI3)* gene (Ward et al., 2002). McCormick et al. (1996) isolated *TRI3* gene from *F. sporotrichioides*. The *tri3* mutants of *F. sporotrichioides* were able to acetylate the trichothecene C-3 hydroxyl group but not the C-15 hydroxyl group. These

findings indicated that, acetylation of C-15 hydroxyl is mediated by the *TRI3* gene. Multiplex PCR assay based on the *TRI3* gene differentiated the 3-ADON and 15-ADON chemotypes in the Canadian isolates that could not be differentiated using the TRI13P1/TRI13P2 primers **(Fig: 5.1A)**. This confirmed that the *TRI13* gene sequences of the Canadian 3-ADON isolates examined are different from that of the Chinese 3-ADON isolates examined. To our knowledge, this is the first report of a difference in the *TRI13* gene sequence of 3-ADON isolates between different regions of the world, and particularly between Chinese and Canadian 3-ADON isolates.

It has been reported that variations in trichothecene production may be caused by alleleic polymorphisms in the tricothecene biosynthesis gene cluster, as a result of selection pressure from the environment (O'Donnell et al., 2000; Ward et al., 2002). Several authors have examined the relationship between chemotype diversity and geographical distribution but have not reported a difference in the *TRI13* gene sequences between regional 3A-DON isolates. Both DON and NIV chemotypes have been reported in Africa, Asia and Europe whereas in North America only DON chemotypes were reported (Mirocha et al., 1989; Miller et al., 1991; Jennings et al., 2004). However, in a recent study Starkey et al. (2007) identified six *F. graminearum* isolates with a NIV or 3-ADON chemotype in Louisiana, USA. Also Gale et al. (2010) reported that NIV type populations of *F. graminearum* and *F. asisticum* were prevalent on wheat in Southern Louisiana. They reported a presence of high proportion of NIV type *F. graminearum* among isolates (79%) collected from small grain growing regions of Louisiana and also NIV type isolates were identified in collections from Arkansas, North Carolina and Missouri. Studies done on phylogenetics of *Fusarium* spp. showed that significant levels

of sexual recombination took place within populations of the *F. graminearum* clade (Bowden and Leslie, 1999; O'Donnell et al., 2000). Members of all lineages are cross fertile with strains belonging to lineage 7 and with strains in other lineages. Lineage 7 is considered to be a universally cross-fertile lineage (Bowden et al., 2006). The identification of a hybrid strain between two *F. graminearum* clade species (O'Donnell et al., 2000) and reports by Bowden and Leslie (1999) on the laboratory out-crossing amongst the lineages provides evidence for the possibility of developing novel lineages in appropriate geographical locations. The genetic diversity that was found among the 3-ADON chemotypes from Canada and China, based on the *TRI13* gene may be due to sexual recombination occurring within the population. Further investigation is needed to confirm the above findings using more *F. graminearum* isolates representing different geographical regions from Canada, China and elsewhere where FHB is a problem.

The genetic diversity of the species within the *F. graminearum* clade from different geographical regions in the world may affect the current disease management, quarantine regulations and breeding strategies (O'Donnell et al., 2004). Therefore studies on the genetic diversity of the *F. graminearum* clade species are important in preventing future outbreaks of FHB epidemics. The findings from this study provide a foundation for further investigations to understand the genetic diversity of *F. graminearum* chemotypes from different geographical regions.

Table 5.1. Mycotoxin chemotypes of *F. graminearum* isolates determined by multiplex PCR

Fg code #	Location	Wheat Class	Chemotype
12-8	ALTONA, MB	CWRW	3ADON
12-9	ALTONA, MB	CWRW	15ADON
12-10	ALTONA, MB	CWRW	3ADON
12-11	ALTONA, MB	CWRW	15ADON
47-1	ST JEAN, MB	CWRW	3ADON
47-2	ST JEAN, MB	CWRW	15ADON
47-3	ST JEAN, MB	CWRW	3ADON
47-4	ST JEAN, MB	CWRW	15ADON
47-5	ST JEAN, MB	CWRW	15ADON
47-6	ST JEAN, MB	CWRW	3ADON
47-7	ST JEAN, MB	CWRW	15ADON
47-8	ST JEAN, MB	CWRW	3ADON
82-1	DELORAINE, MB	CWRW	15ADON
82-3	DELORAINE, MB	CWRW	3ADON
82-4	DELORAINE, MB	CWRW	3ADON
82-6	DELORAINE, MB	CWRW	3ADON
82-7	DELORAINE, MB	CWRW	15ADON
82-8	DELORAINE, MB	CWRW	3ADON
44-1	OAKVILLE, MB	CWRW	3ADON
44-2	OAKVILLE, MB	CWRW	3ADON
44-3	OAKVILLE, MB	CWRW	3ADON
44-4	OAKVILLE, MB	CWRW	15ADON
44-6	OAKVILLE, MB	CWRW	3ADON
11-1	ALTONA, MB	CWRW	15ADON
11-2	ALTONA, MB	CWRW	3ADON
11-3	ALTONA, MB	CWRW	3ADON
11-4	ALTONA, MB	CWRW	3ADON
11-5	ALTONA, MB	CWRW	3ADON
11-6	ALTONA, MB	CWRW	3ADON
11-7	ALTONA, MB	CWRW	15ADON
1-1	KANE, MB	CWRW	15ADON
1-2	KANE, MB	CWRW	3ADON
1-2	KANE, MB	CWRW	3ADON

Fg code #	Location	Wheat Class	Chemotype
1-3	KANE, MB	CWRW	3ADON
1-4	KANE, MB	CWRW	3ADON
1-5	KANE, MB	CWRW	3ADON
1-6	KANE, MB	CWRW	15ADON
1-7	KANE, MB	CWRW	15ADON
55-1	RED RIVER VALLEY, MB	CWRW	15ADON
55-2	RED RIVER VALLEY, MB	CWRW	3ADON
55-3	RED RIVER VALLEY, MB	CWRW	3ADON
55-4	RED RIVER VALLEY, MB	CWRW	3ADON
55-5	RED RIVER VALLEY, MB	CWRW	3ADON
84-1	SOURIS, MB	CWRW	3ADON
84-2	SOURIS, MB	CWRW	15ADON
84-3	SOURIS, MB	CWRW	15ADON
84-5	SOURIS, MB	CWRW	3ADON
84-6	SOURIS, MB	CWRW	15ADON
84-7	SOURIS, MB	CWRW	15ADON
40-1	NEWTON, MB	CWRW	15ADON
40-2	NEWTON, MB	CWRW	15ADON
40-3	NEWTON, MB	CWRW	3ADON
40-5	NEWTON, MB	CWRW	3ADON
40-6	NEWTON, MB	CWRW	3ADON
3-2	CARMAN, MB	CWRW	15ADON
3-3	CARMAN, MB	CWRW	15ADON
3-4	CARMAN, MB	CWRW	3ADON
3-5	CARMAN, MB	CWRW	3ADON
M8-06-2	WINKLER, MB	CWRW	3ADON
M5-06-1	BOWSMAN, MB	CWRS	3ADON
139-8	RHEIN, SK	CWRW	3ADON
139-9	RHEIN, SK	CWRW	15ADON
139-10	RHEIN, SK	CWRW	15ADON
142-2	YORKTON, SK	CWRW	15ADON
142-7	YORKTON, SK	CWRW	3ADON
142-10	YORKTON, SK	CWRW	15ADON
142-11	YORKTON, SK	CWRW	3ADON
142-11 (FDK)	YORKTON, SK	CWRW	15ADON
142-12	YORKTON, SK	CWRW	3ADON

Fg code #	Location	Wheat Class	Chemotype
142-13	YORKTON, SK	CWRW	3ADON
S1A-06-3	ALANEDA, SK	CWRS	3ADON
S1A-06-4	CARIEVALE, SK	CWRS	15ADON
S3AN-06-1	EYEBROW, SK	CWRS	15ADON
S3BS-06-1	BRACKEN, SK	CWRS	3ADON
S8A-06-1	BROOKSBY, SK	CWRS	3ADON
NB-06-17	NB	CERS	3ADON
NB-06-18	NB	CERS	15ADON
NS-06-2	NS	CERS	3ADON
ON-06-05	GREY #1, ON	CERS	15ADON
ON-06-17	OXFORD #1, ON	CERS	15ADON
ON-06-39	WELLIGNTON#1, ON	CERS	3ADON
A6-06-1	WESTLOCK, AB	CWRS	3ADON
PEI-06-33	PEI	CERS	3ADON
PEI-06-34	PEI	CERS	15ADON
Q-06-10	QC	CERS	15ADON
Q-06-32	QC	CERS	3ADON
Q-06-23	QC	CERS	3ADON
DF-Fg-2	MB	Not known	3ADON
DF-Fg-3	MB	Not known	3ADON
DF-Fg-5	MB	Not known	3ADON
DF-Fg-7	MB	Not known	15ADON
DF-Fg-8	MB	Not known	3ADON
DF-Fg-9	MB	Not known	3ADON
DF-Fg-10	MB	Not known	3ADON
DF-Fg-11	MB	Not known	3ADON
DF-Fg-13	MB	Not known	3ADON
DF-Fg-14	MB	Not known	3ADON
DF-Fg-16	MB	Not known	3ADON
DF-Fg-17	MB	Not known	3ADON
DF-Fg-73	MB	Not known	3ADON
DF-Fg-77	MB	Not known	3ADON
DF-Fg-80	MB	Not known	15ADON
DF-Fg-81	MB	Not known	15ADON
DF-Fg-82	MB	Not known	15ADON
DF-Fg-83	MB	Not known	3ADON
DF-Fg-86	MB	Not known	3ADON
DF-Fg-88	MB	Not known	15ADON

Fg code #	Location	Wheat Class	Chemotype
DF-Fg-89	MB	Not known	15ADON
DF-Fg-93	MB	Not known	15ADON
DF-Fg-95	MB	Not known	3ADON
DF-Fg-96	MB	Not known	3ADON
DF-Fg-125	MB	Not known	3ADON
DF-Fg-126	MB	Not known	15ADON
DF-Fg-131	MB	Not known	15ADON
DF-Fg-134	MB	Not known	15ADON
DF-Fg-135	MB	Not known	15ADON
DF-Fg-142	MB	Not known	15ADON
DF-Fg-144	MB	Not known	15ADON
DF-Fg-184	MB	Not known	3ADON

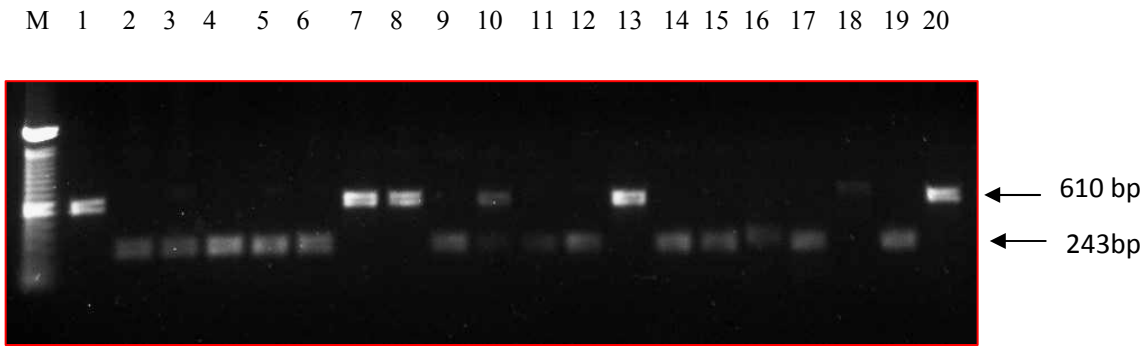
Note:

Alberta (AB), Manitoba (MB), New Brunswick (NB), Nova Scotia (NS), Prince Edward Islands (PEI), Quebec (QC) and Saskatchewan (SK)

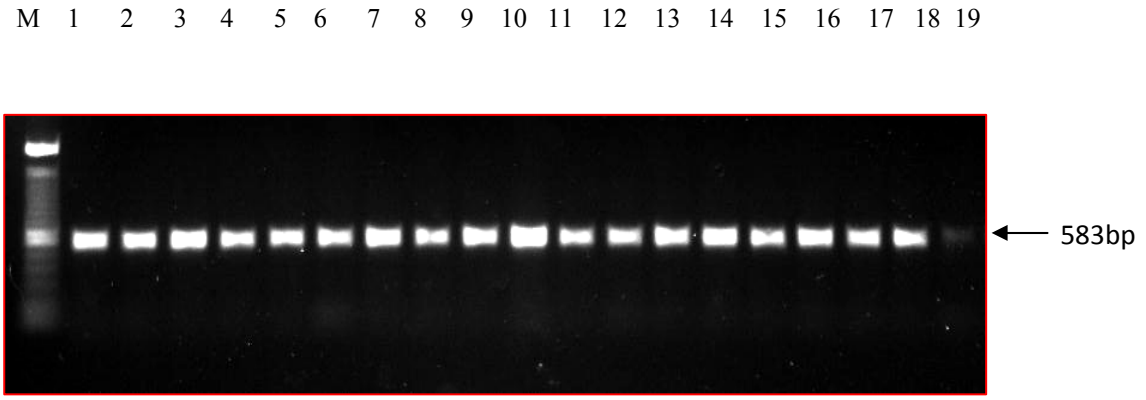
Wheat Classes are as Follows: CWRS= Canada Western Red Spring; CWRW= Canada Western Red Winter; CERS= Canada Eastern Red Spring

A

***Fusarium graminearum* isolates (Canada)**



B



C

***Fusarium graminearum* isolates (China)**

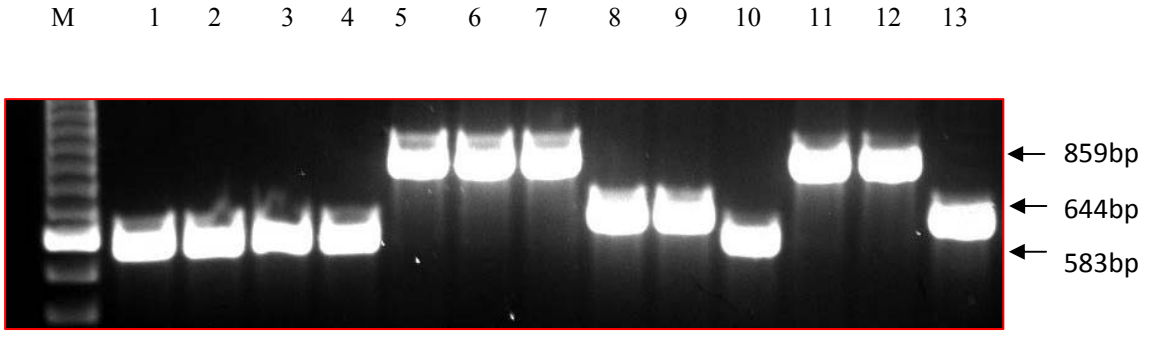


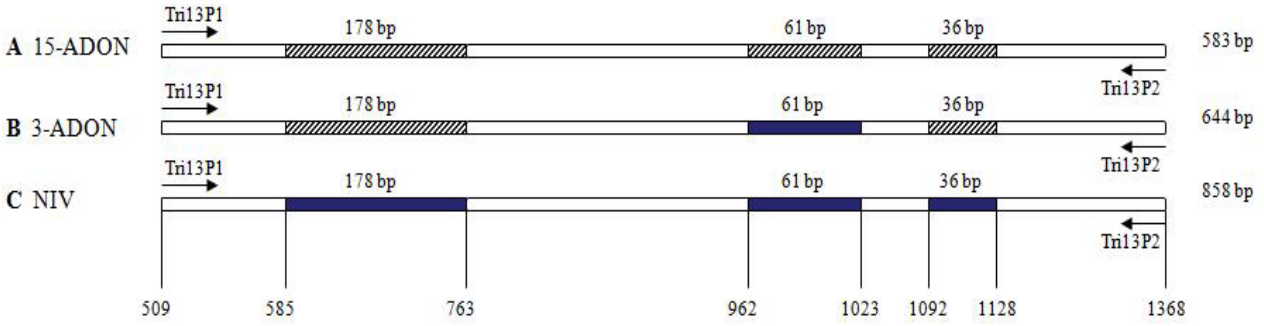
Figure 5.1A: DNA of Canadian chemotype isolates amplified using multiplex PCR (Ward et al., 2002). The 610 bp fragments represented 15-ADON chemotypes and 243 bp fragments represented 3-ADON chemotype**.** Lanes 1, 7, 8, 10, 13, 18 and 20: 15-ADON chemotypes (PEI-06-34, ON-06-05, ON-06-17, Q-06-10, DF-Fg-144, 55-1,3-3); Lanes 2-6, 9, 11, 12, 14-17 and 19: 3-ADON chemotypes (M8-06-02, M5-06-01, SIA-06-03, PEI-06-33, ON-06-39, Q-06-32, DF-Fg-2, DF-Fg-3, DF-Fg-95, DF-Fg-96, DF-Fg-184, S8A-06-01, 1-3).

5.1B: DNA of Canadian chemotype isolates amplified using TRI13P1/ Tri13P2 primers (Wang et al., 2008). The 583 bp fragments represented both 3-ADON and 15-ADON chemotypes. Lanes 1,7,8, 10, 13 and 18: 15-ADON chemotypes (PEI-06-34, ON-06-05, ON-06-17, Q-06-10, DF-Fg-144, 55-1); Lanes 2-6,9, 11,12,14-17 and 19: 3-ADON chemotypes (M8-06-02, M5-06-01, SIA-06-03, PEI-06-33, ON-06-39, Q-06-32, DF-Fg-2, DF-Fg-3, DF-Fg-95, DF-Fg-96, DF-Fg-184, S8A-06-01, 1-3).

5.1C: DNA of Chinese chemotype isolates amplified using TRI13P1/ TRI13P2 primers (Wang et al., 2008). The 583 bp fragments represented 15-ADON chemotypes, 644 bp represented 3-ADON chemotypes and 858 bp fragments represented NIV chemotypes. Lanes 1-4 and10: 15-ADON chemotypes (Fg-0819, 1960, 0963, 0938, 0952); Lanes 8-9 and 13: 3-ADON chemotypes (Fg-0919, 0926, 0970); Lanes 5-7, 11 and 12: NIV chemotypes (Fg-0921, 0905, 0973, 0970, 0903).

(M=Marker)

A



B

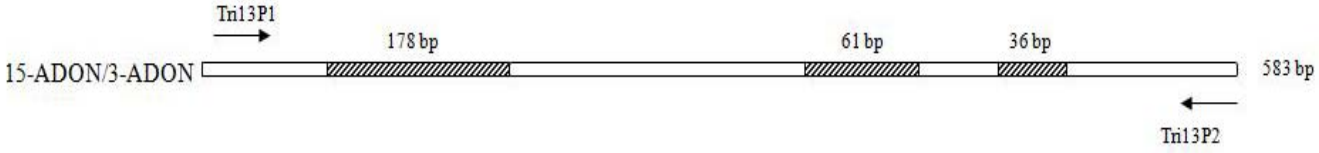


Figure 5.2A: Diagrammatic representations of *TRI13* genes of 15-ADON, 3-ADON and NIV chemotypes in Chinese isolates of *Fusarium graminearum* (Boxes with vertical lines represent the deletions; boxes with solid fill represent the respective insertions and boxes with no fill represent the remaining parts of the gene).

5.2B: Diagrammatic representations of *TRI13* genes of 15-ADON and 3-ADON chemotypes in Canadian isolates of *Fusarium graminearum*.


178 bp deletion
61 bp deletion
36 bp deletion

Figure 5.3: Sequence alignments of *TRI13* gene amplified from Canadian *Fusarium graminearum* 3-ADON, 15-ADON chemotypes and Chinese 3-ADON, 15-ADON and NIV chemotypes.

3-ADON chemotypes, Canada (1-M8-06-02, 2- ON-06-39, 3- Q-06-32, 7-55-3), 15-ADON chemotypes, Canada (4-ON-06-17, 5- DF-Fg-144, 6- Q-06-10, 8- 55-1), 3-ADON chemotypes, China (11-Fg-0919, 12-Fg-0926, 15-Fg-0970), 15-ADON chemotypes, China (9- Fg-0819, 10-Fg-1960, 14-Fg-0963), NIV chemotypes, China (13-Fg-0921, 16-Fg-0905)

CHAPTER 6

6.0 EVALUATION OF THE AGGRESSIVENESS OF *Fusarium graminearum* ISOLATES INOCULATED UNDER GREENHOUSE CONDITIONS ON SPRING WHEAT

Abstract

This study evaluated the aggressiveness of selected *F. graminearum* 3-ADON, 15-ADON and a combined inoculation of 3-ADON and 15-ADON isolates on spring wheat with different levels of resistance to fusarium head blight. The aggressiveness of the *Fusarium graminearum* isolates was assessed based on FHB disease severity and DON accumulation after single floret inoculation under green-house conditions. Results from this study revealed that FHB disease severity was higher in wheat plants inoculated with 3-ADON isolates than in plants inoculated with the 15-ADON or the combined inoculation. Also the cultivar Roblin, which is highly susceptible to FHB showed significantly higher disease severity than the moderately resistant cultivars Glenn and Waskada.

6.1 Introduction

Fusarium graminearum and other *Fusarium* spp. cause head blight of small grain cereals affecting yield and quality as well as contaminating the grain with potent mycotoxins. *Fusarium graminearum* is the primary fusarium head blight (FHB) pathogen in Canada (Gilbert et al., 1995). Pathogenicity is defined as the ability of an organism to cause disease while aggressiveness refers to the amount of disease caused by pathogenic isolates on a susceptible host (Gilbert et al., 2001; Cumugan et al., 2004). Pathogenicity and aggressiveness of an isolate are the key characteristics that are being considered in developing FHB resistant wheat cultivars (Mesterházy, 2003a). Although many *Fusarium* spp. can cause FHB, studies on pathogenicity revealed that only *F. graminearum* and *F. culmorum* were highly pathogenic, while other *Fusarium* spp. were intermediate or weakly pathogenic (Xue et al., 2004; Wong et al., 1995). Still no clear reasons have been identified as to the cause of pathogenicity and aggressiveness in *Fusarium* spp. The ability to produce deoxynivalenol is considered to be one of the pathogenicity factors in *Fusarium* spp. (Proctor et al., 1995; Mirocha et al., 1997). A significant correlation has been observed between the DON content and aggressiveness of the isolates on wheat (Goswami and Kistler, 2005).

Level of resistance to FHB is different among wheat lines. The resistance of wheat to FHB is a complex quantitative trait controlled by many genes (Mesterházy, 2002; Bai and Shaner, 2004). Five types of resistance have been identified in wheat against the *Fusarium* infection: 1) Type I -resistance to initial infection 2) Type II- resistance to spread of the disease from the point of infection 3) Type III- resistance to kernel infection 4) Type IV-tolerance- 5) Type V- resistance to accumulation of DON- (Schroeder and

Christensen., 1963; Mesterházy, 1995; Mesterházy, 2002; Bai and Shaner, 2004). Most of the commercially available wheat cultivars are either moderately resistant or susceptible to FHB. The pathogenicity of the species and aggressiveness of the isolates are the key factors that should be considered in breeding for FHB resistant cultivars (Mesterházy, 2003a). The resistant wheat cultivars should have a complex resistance to different *Fusarium* spp.

The presence of chemotaxonomically different groups of *F. graminearum* was first proposed by Ichinoe et al. (1983). The distribution of chemotypes shows regional differences. According to a study done in Manitoba, it has been found that 3-ADON chemotypes were more prevalent in the Red River Valley and moving westwards confirming the rapid chemotypic shift from 15-ADON producers to 3-ADON producers (Guo et al., 2008). Although no significant differences were found among the aggressiveness of two chemotypes; phenotypic analysis of two chemotypes showed that 3-ADON chemotypes were capable of producing more trichothecenes than 15-ADON chemotypes and the rate of fecundity is higher in 3-ADON chemotypes (Ward et al., 2008).

The objectives of this study were to determine 1) the aggressiveness of ON-06-39 (3-ADON), Q-06-10 (15-ADON) *F. graminearum* isolates and a combined inoculation of the two isolates by evaluating the FHB disease severity and DON content and 2) whether the spring wheat cultivars with different resistance levels perform similarly to 3-ADON and 15-ADON chemotypes of *F. graminearum.*

6.2 Materials and methods

6.2.1 Genotypes

Spring wheat cultivars with different levels of FHB resistance, Glenn (FHB moderately resistant), Waskada (FHB moderately resistant) and Roblin (FHB highly susceptible) were used (Seed Manitoba, 2008).

6.2.2 Isolates

Two different chemotypes of *F. graminearum* ON-06-39 (3-ADON) from Ontarioand Q-06-10 (15-ADON) from Quebec were used in this study.

6.2.3 Greenhouse experiment

Three spring wheat cultivars Glenn, Waskada and Roblin were grown in the greenhouse, Department of Plant Science, University of Manitoba, in 2010. A total of 4 treatments including *F. graminearum* Q-06-10 (15-ADON), ON-06-39 (3-ADON) and a combination of ON-06-39 and Q-06-10 (1: 1) and an untreated control were examined. There were five replicates per each treatment and a completely randomized block design. Each replicate had a pot containing two plants and at anthesis two to three heads per pot were artificially inoculated. Inoculation was achieved by point inoculation. Each spike was inoculated by injecting a 10 µl spore suspension (5 $x10^4$ per/ml) between the lemma and palea of the primary and secondary florets positioned at the inoculation point. The spikelets that positioned at two third of the way from the base of the spike were considered as the point of inoculation. Two spikelets were inoculated that arranged opposite to each other. The un-inoculated heads were point inoculated with sterilized

distilled water. After inoculation, pots were transferred to a humidity chamber at 100% relative humidity for 24 hrs to provide conditions for FHB development. After 24 hrs pots were transferred back to the greenhouse bench.

6.2.4 Disease evaluation

Disease severity (DS-disease spread within the spike) was assessed 7, 14 and 21 days after inoculation (dai) using a FHB visual scale by Stack and McMullen, (1995). The DS ratings taken at 21 dai were used for data analysis.

6.2.5 DON analysis

After maturity, plants were hand harvested and threshed. The amount of seeds obtained for each replicate was not sufficient for DON analysis; therefore the seeds from five replicates were pooled and sent for DON analysis. DON analysis was done using ELISA method at University of Guelph (www.diagnostix.ca).

6.2.6 Statistical analysis

Analysis of variance (ANOVA) for DS was performed using the "PROC GLM" procedure for the SAS software package (SAS Institute Inc., Version 9.2). The model statement used in the analysis was DS= cultivar isolate cultivar*isolate rep cultivar* rep Replicates (rep) and interactions with rep considered as random factors whereas all the other factors were considered fixed. Cultivar was tested against the cultivar*rep interaction. The correlation between DS and DON content was analysed using the PROC CORR procedure in SAS (version 9.2) software package.

6.3 Results and Discussion

In this study the aggressiveness of ON-06-39 (3-ADON), Q-06-10 (15-ADON) and a combination of the two were examined on different genetic backgrounds. Three wheat cultivars used in this study had different levels of resistance to FHB. According to the statistical analysis there were significant differences among cultivars, isolates and the two way interaction cultivar x isolate **(Table: 6.1)**. In this study significant differences were found between the two moderately resistant cultivars Glenn and Waskada, Bai and Shaner, (1996) also observed large variations in disease severity among isolates on cultivars with moderate degree of resistant to FHB.

In this study, significant differences were observed among the four different isolate treatments. The heads treated with 3-ADON showed higher FHB DS mean value in the cultivars Glenn and Roblin and the combined treatment gave higher FHB DS in the cultivar Waskada. These results were in contrast with the study done by Gilbert et al. (2010), in their study no significant differences in aggressiveness were observed among isolates based either on geographic origin or mycotoxin type. Ward et al. (2008) showed that 3-ADON isolates produced more trichothecenes *in vitro* than 15-ADON isolates. Gilbert et al. (2010) also confirmed that 3-ADON chemotypes produce more DON than 15-ADON chemotypes. DON is considered as one of the major pathogenicity factors in *Fusarium* spp. and several studies have revealed a good positive correlation between FHB visual disease symptoms and the DON content in infected grains. Bai et al. (2001) reported the possibility of using visual disease assessment of the disease to predict the mycotoxin content in infected grains and suggested that disease severity can be used as a good predicator of DON content. Therefore the higher DS would reflect the accumulation

of higher amounts of trichothecenes in infected grains. In this study, the DS indicated that 3-ADON isolates can cause more disease on spring wheat than 15-ADON isolates and the combination of 3-ADON and 15-ADON isolates except for the wheat cultivar Waskada, where the combined treatment showed more disease than the other two treatments. But when considering the DON content in infected grains, in cultivars Waskada and Roblin 15-ADON isolate, Q-06-10 showed higher DON content than the 3-ADON isolate, ON-06-39 but in the cultivar Glenn 3-ADON isolate showed higher DON content in infected grains. The un-inoculated plants also had trace levels of DON (in Waskada and Glenn), although they didn't show any disease symptoms. In this study we couldn't get reliable results for DON content as only one sample from five replicates were pooled and analysed for DON content. However, a significant positive correlation was observed between the DS and DON content in grains. The Pearson correlation coefficient between DS and DON content was 0.7979 (p= 0.0019). For further confirmation of the results this study should be replicated and supported with DON results.

Table 6.1. Analysis of variance for disease severity (DS)

Source	DF	Sums of square	Mean square	F Value	Pr>F
Cultivar	2	14180.03	7054.01	48.35	<.0001
Isolate	3	14800.31	4933.43	48.22	<.0001
Cultivar*Isolate	6	7426.23	1237.70	12.01	<.0001
Rep	4	196.06	49.01	0.48	0.7508
Cultivar*Rep	8	1167.13	145.89	1.43	0.2194
Residual	36	3683.20	102.31		

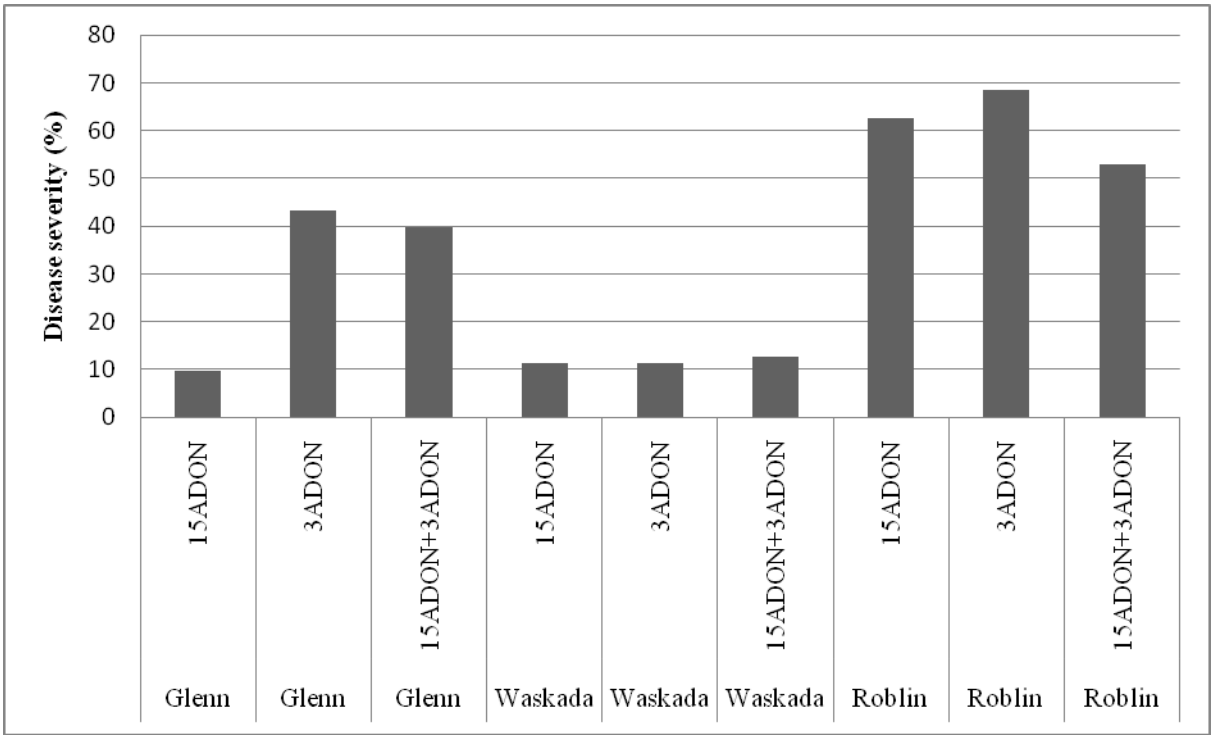


Figure 6.1: Effects of point inoculations of *Fusarium graminearum* isolates, Q-06-10 (3-ADON), ON-06-39 (15-ADON) and a combination of Q-06-10and ON-06-39 on disease severity of wheat cultivars Glenn, Waskada and Roblin.

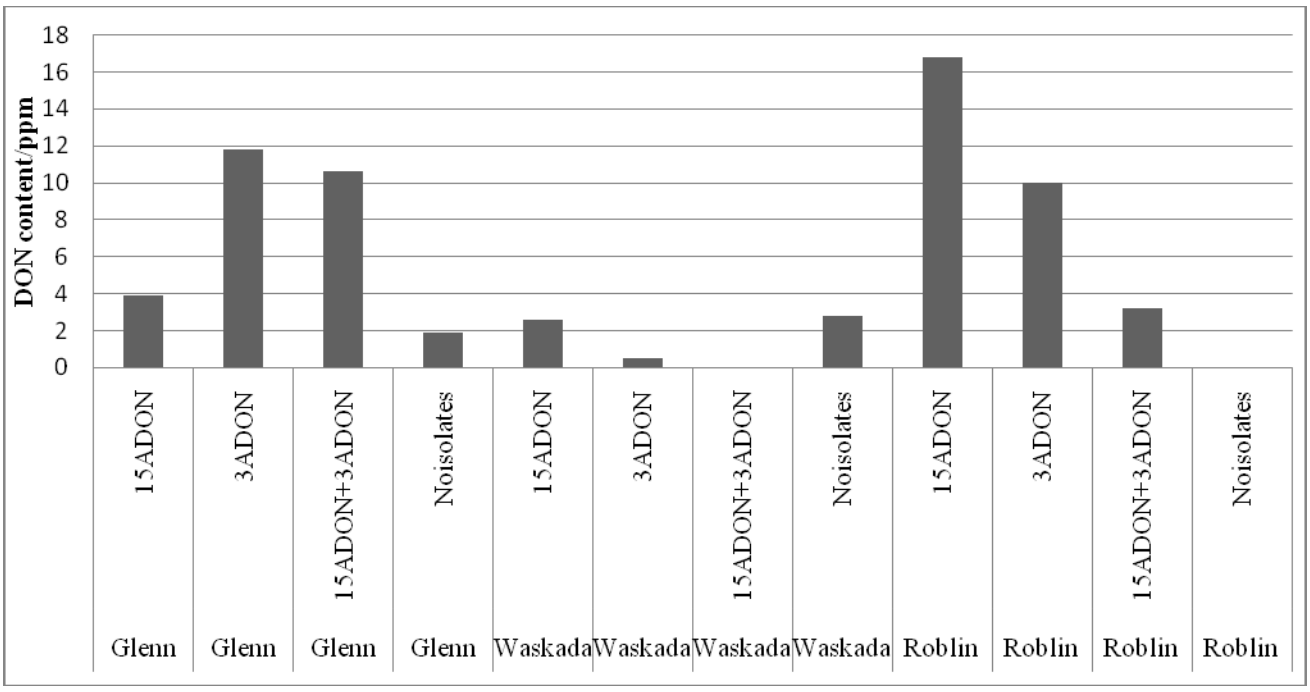


Figure 6.2: Effects of point inoculations of *Fusarium graminearum* isolates, Q-06-10 (3-ADON), ON-06-39 (15-ADON) and a combination of Q-06-10and ON-06-39 on deoxynivalenol content (DON) of wheat cultivars Glenn, Waskada and Roblin.

CHAPTER 7

7.0 GENERAL DISCUSSION AND CONCLUSIONS

7.1 General discussion and conclusions

Application of fungicides is one of the main control strategies that are in use to combat FHB. The studies on efficacy of use of fungicides in controlling FHB often resulted in inconsistent results, suggesting that study or environmental specific factors would influence the relative activity of fungicides. The efficacy of commonly used fungicides having different active ingredients (prothioconazole, tebuconazole and metconazole) was evaluated. In general, all fungicides used in this study significantly reduced the FHB index, FDK and DON content and increased the yield compared to the unsprayed controls except in some treatments. In a few treatments application of fungicides increased the DON concentration and percent FDK. These unexpected results have also been reported in other studies, especially the higher DON levels in infected grain after fungicide application although they reduced the FHB disease severity (Milus and Parsons, 1994; Matthies and Buchenauer, 2000; Simpson et al., 2001; Pirgozliev et al., 2002; Menniti et al., 2003). Tanaka et al. (1988) reported that, the production of mycotoxins by fungi is greatly influenced by environmental factors such as temperature and relative humidity. Temperature and humidity did not directly influence the mycotoxin production, but rather as a function of the influence of those parameters on fungal growth (Doohan et al., 2003). Also the mycotoxin production starts in the field and it continues during the storage of grain (Doohan et al., 2003). Therefore although the disease severity is less, infected grain can have higher DON content.

This study also demonstrated, that efficacy of fungicides is influenced by the level of resistance in wheat cultivars. Efficacy of fungicides was higher when applied to

moderately resistant cultivar Glenn than when applied to the highly susceptible cultivar Roblin. As highly susceptible cultivars could not be effectively protected by fungicides, those cultivars should be withdrawn and replaced by moderately resistant cultivars in commercial wheat production (Mesterházy, 2003).

In this study significant differences were found for FHB index, FDK, yield and DON concentration between the 3-ADON and 15-ADON chemotypes of *F. graminearum*. Considering the mean values for FHB index, FDK, yield and DON, except for several treatments, in both years, 3-ADON chemotypes showed higher FHB index, FDK and DON content compared to 15-ADON chemotypes. This confirms that 3-ADON can cause more disease on spring wheat than 15-ADON chemotypes.

The efficacy of fungicides in controlling FHB mainly relies on the level of resistance in wheat cultivar and the aggressiveness of the isolate. Application of fungicides could not completely control the development of FHB, but the combined effect of fungicides plus growing moderately resistant wheat cultivars would result in better management of FHB.

The species specific PCR assay on *F. graminearum* isolates collected from corn fields in Ontario confirmed that *F. graminearum* is the most common *Fusarium* spp. found in infected corn. Wheat and corn are the two important crops grown in Canada; therefore the diseases on these crops have a direct impact on Canadian agriculture and economics. Multiplex PCR assay on *F. graminearum* isolates showed that all investigated isolates were 15-ADON chemotypes. Therefore findings from this study are important

and add important information to identify the chemotype cline in Canada and the most prevalent *F. graminearum* chemotypes in Ontario.

Evaluation of chemotypes of *F. graminearum* is done by PCR assays based on the genes in the DON biosynthesis pathway. The multiplex PCR assay is based on the *TRI3* gene (Ward et al., 2002). Use of multiple primers makes the evaluation process tedious, time-consuming and also they may generate false negative results (Li et al., 2005; Wang et al., 2008). Therefore use of a single primer that could differentiate the two chemotypes would accelerate the identification of chemotypes. The study in chapter 4 evaluated the reliability of using a single primer based on *TRI13* gene, to differentiate the two chemotypes found in Canadian *F. graminearum* clade. Interestingly, PCR results revealed that although the *TRI13* primer could differentiate the 3-ADON and 15-ADON chemotypes in Chinese isolates (Wang et al., 2008), they cannot be used to differentiate the two chemotypes in Canadian isolates. The sequencing results showed that Canadian 3-ADON chemotypes have a 61 bp deletion in the *TRI13* gene sequence which is absent in the 3-ADON chemotypes of Chinese isolates. Findings of this study concluded that, the examined Chinese 3-ADON chemotypes examined are genetically different from Canadian 3-ADON chemotypes. Therefore this confirms a presence of genetic diversity among different chemotypes of *F. graminearum* with respect to their geographical distribution. To our knowledge, this is the first report of a genetic difference identified within the 3-ADON isolates between two geographic regions in the world. As future work, this study can be further expanded, to examine the genetic diversity that exists among the different chemotypes of *F. graminearum* including more isolates representing different geographical regions. Still no clear reasons are found for the rapid chemotypic

shift that has taken place in North America. Investigation of genetic diversity among the chemotypes from different geographical regions may provide a basis to unravel the mystery behind this rapid chemotypic shift.

The studies in chapter 5 demonstrated the aggressiveness of selected *F. graminearum* isolates causing FHB in highly suscepyible and moderately resistant wheat cultivars. *Fusarium graminearum* ON-06-39 isolate (3-ADON) can cause higher FHB disease severity than Q-06-10 isolate (15-ADON) and the combined application of ON-06-39 and Q-06-10.

In summary, the studies described in this thesis attempted to provide an understanding of the performance of the two *F. graminearum* chemotypes 3-ADON and 15-ADON, in several aspects. Rapid emergence of the novel 3-ADON chemotype over the traditional 15-ADON chemotype in the prairies including other regions in North America may affect several economically important industries such as wheat, barley, corn and the hog industry. Therefore understanding the behaviour of these chemotypes is important in breeding new resistant varieties, manipulating new management strategies and setting new regulations in international trade.

LITERATURE CITED

Adams, G. C., and Hart, L. P. 1989. The role of deoxynivalenol and 15-acetyldeoxynivalenol in pathogenesis by *Gibberella zeae*, as elucidated through protoplast fusions between toxigenic and nontoxigenic strains. Phytopathology. 79: 404-408.

Akinsanmi, O. A., Backhouse, D., Simpfendorfer, S. and Chakraborty, S. 2006. Genetic diversity of Australian *Fusarium graminearum* and *F. pseudograminearum.* Plant Pathology. 55: 494-504.

Ali, M. L., Taylor, J. H., Jie, L., Sun, G., William, M., Kasha, K. J., Reid, L. M. and Pauls, K. P. 2005. Molecular mapping of QTLs for resistance to *Gibberella* ear rot, in corn, caused by *Fusarium graminearum*. Genome. 48: 521-533.

Anderson, A. L. 1948. The development of *Gibberella zeae* head blight of wheat. Phytopathology. 38: 595-611.

Aoki, T. and O'Donnell, K. 1999a. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the group1 population of *F. graminearum*. Mycologia. 91: 597-609.

Aoki, T. and O'Donnell, K. 1999b. Morphological characterization of Gibberella cornicola sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*. Mycoscience. 40: 443-453.

Arseniuk, E., Foremska, E., Goral, T., Chelkowski, J. 1999. Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye. J. Phytopathol. 147: 577-590.

Audenaert, K., Callewaert, E., Höfte, M.,Saeger, S. D., and Haesaert, G. 2010. Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. BMC Microbiology. 10: 112.

Backhouse, D., Burgess, L. W., and Summerell, B. A. 2001. Biogeography of *Fusarium*. *In Fusarium*, Summerell, B. A., Leslie, J. F., Backhouse, D., Bryden, W. L, and Burgess L. W. Eds. The American Phytopathological Society. St. Paul, Minnesota. USA. 2001. Chapter 9. pp. 122-138.

Bai, G. H., Desjardins, A. E., and Plattner, R. D. 2002. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection but does not cause disease spread in wheat spikes. Mycopathologia. 153: 91-98.

Bai, G.H., and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78: 760-766.

Bai, G.H., and Shaner, G. 1996. Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis. 80: 975-979.

Bai, G.H., and Shaner, G. 2004. Management and resistance in wheat and barley to fusarium head blight. Annu. Rev. Phytopathol. 42: 135-161.

Bai, G.H., Chen, L.F. and Shaner, G.E. 2003. Breeding for resistance to fusarium head blight of wheat in China. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, Chapter 11, pp. 296-317.

Bai, G.H., Plattner, R., Desjardins, A., Kolb, F. 2001. Resistance to fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed. 120: 1-6.

Bai, G.H., Shaner, G.E., and Ohm, H.W. 2000. Inheritance of resistance to *Fusarium graminearum* in wheat. Theor. Appl. Genet. 100: 1-8.

Baird, R. E., Mullinix, B. G., Peery, A. B., and Lang, M. L. 1997. Diversity and longevity of the soybean debris mycobiota in a no-tillage system. Plant Dis. 81: 530-534.

Beyer, M., and Verreet, J.-A. 2005. Germination of *Gibberella zeae* ascospores as affected by age of spores after discharge and environmental factors. Eur. J. Plant Pathol. 111: 381-389.

Booth, C. 1971. *The genus Fusarium.* Commonwealth Mycological Institute, Kew, Surrey, England.

Boshoff, W. H. P., Pretorius, Z. A., Swart, W. J., and Jacobs, A. S. 1996. A comparison of scab development in wheat infected by *Fusarium graminearum* and *Fusarium crookwellense*. (Abstr.) Phytopathology. 86: (Suppl.) S58.

Boutigny, A.L., Richard, F.F. and Barreau, C. 2008. Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. Eur. J. Plant Pathol. 121: 411-423.

Bowden, R. L, Leslie, J. F, 1999. Sexual recombination in *Gibberella zeae*. Phytopathology. 89: 182-188.

Bowden, R. L., Leslie, J. F., Lee., J. and Lee., Y. W. 2006. Cross fertility of lineages in *Fusarium graminearum* (*Gibberella zeae*). *In* The Global Fusarium Initiative for International Collaboration: a strategic planning workshop. Eds by, T. Ban, J. M. Lewis, and E. E. Phipps , CIMMYT, El Batan, Mexico. pp. 54-60.

Brasier, C. M., Cooke, D. E. L., and Duncan, J. M. 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. Proc. Natl. acad. Sci. 96: 5878-5883.

Brandfass, C. and Karlovsky, P. 2008. Upscaled CTAB-Based DNA extraction and real-time PCR assays for *Fusarium culmorum* and *F. graminearum* DNA in plant material with reduced error. Int. J. Mol. Sci. 9: 2306-2321.

Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91: 1155-1160.

Brown, D. W., McCormick, S. P., Alexander, N. J., Proctor, R. H., and Desjardins, A. E. 2002. Inactivation of a cytochrome P-450 is a determinant of trichothecene diversity in *Fusarium* species. Fungal Genet. Biol. 36: 224-233.

Brown, D.W., McCormick, S.P., Alexander, N.J., Proctor, R.H., Desjardins, A. E. 2001. A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genet. Biol. 32: 121-133.

Buestmayr, H., Lemans, M., Fedak, G., and Ruckenbauer, P. 1999. Back-cross reciprocal analysis of Fusarium head blight resistance in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 98: 76-85.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., and Ruckenbauer, P. 2002. Molecular mapping of QTL for fusarium head blight resistance in spring wheat. I.Resistance to fungal spread (type II resistance). Theor. Appl. Genet. 104: 84-91.

Buerstmayr, H., Steiner, B., Hartl, L., Griesser, M., Angerer, N., Lengauer, D., Miedaner, T., Schneider, B., Lemmen, M. 2003.Molecular mapping of QTL for fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor. Appl Genet. 107: 503-8.

Burgess, L. W., and Summerell, B. A. 1992. Mycogeography of fusarium: Survey of *Fusarium* spp. in subtropical and semi-arid grassland soils from Queensland, Australia. Mycol. Res. 96: 780-784.

Burgess, L. W., Wearing, A. H. and Toussoun, T. A. 1975. Surveys of the Fusaria associated with crown rot of wheat in Eastern Australia. Australian Journal of Agricultural Research. 26**:** 791-799.

Bushnell, W. R., Hazen, B. H., and Pritsch, C. 2003. Histology and Physiology of Fusarium Head Blight. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, 2003, Chapter 3, pp.44-83.

Carter, J. P., Rezanoor, H. N, Holden, D., Desjardins, A. E, Plattner, R. D and Nicholson P . 2002. Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. Eur. J. Plant Pathol. 108: 573-583.

Chandler, E.A., Simpson, D.R., Thomsett, M.A. and Nicholson, P. 2003. Development of PCR assays to *Tri7* and *Tri13* trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. Physiol Mol Plant Pathol. 62: 355-367.

Charmley, L. L, Trenholm, H. L, Prelusky, D. A and Rosenberg A. 1995. Economic losses and decontamination. Nat Toxins. 3: 199-203.

Chungu, C., Mather, D.E., Reid, L.M., and Hamilton, R.I. 1996. Inheritance of kernel resistance to *Fusarium graminearum* in maize. J. Heredity. 87: 382-385.

Cumagun, C. J. R., Bowden, R. L., Jurgenson, J. E., Leslie, J. F., and Miedaner, T. 2004. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. Phytopathology. 94: 520-526.

Curtis, B. C. 2002. Wheat in the world. *In* Bread Wheat Improvement and Production. Eds by B. C. Curtis, S. Rajaram and H. G. Macpherson. ISBN 92-5-104809-6. Food and Agriculture Organization of the United Nations, Rome.

D'Mello, J. P. F., Macdonald, A. M. C., and Dijksma W. T. P. 1998. 3-acetyl deoxynivalenol and esterase production in a fungicide-insensitive strain of *Fusarium culmorum*. Mycotoxin Research. 14: 9-18.

D'Mello, J. R. F., Macdonald, A. M. C. and Rinna, R. 2001. Effects of azoxystrobin on mycotoxin production in a carbendazim-resistant strain of *Fusarium sporotrichioides*. Phytoparasitica. 29: 431-440.

D'Mello, J.P.F. Placinta, C. M., and Macdonald, A. M. C. 1999. Fusarium mycotoxins: a review of global implifications for animal health, welfare and productivity. Ani. Feed Sci. Technol. 80: 183-205.

Dardis, J. And Walsh E. J. 2000. Studies of the effectiveness of metconazole in controlling Fusarium Head Blight caused by *Fusarium culmorum* in spring wheat (*Triticum aestivum L.*). Cereal Research Communications. 28: 443-448.

Daynard, T. B., Tollenaar, M., and Edmeades, G. O. 1977. Ontario research on maize physiology. Annals of Applied Biology. 87: 245-250.

Demeke, T., Clear, R. M., Patrick, S. K. and Gaba, D. 2005. Species-specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. Int. J. Food Microbiology. 103: 271-284.

DePauw, R. and Hunt, T. 2001. Canadian wheat pool. *In* The world wheat book, A history of wheat breeding. Eds by A. P. Bonjean and W. J. Angus. Lavoisier Publishing, France. Chapter 19. pp. 479-519.

Desjardins, A. E. 2006. Fusarium mycotoxins-chemistry, genetics, and biology. The American Phytopathological Society Press.

Desjardins, A. E., Proctor, R. H., Bai, G. H., McCormick, S. P., Shaner, G., Buechley, G., and Hohn, T. M. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol Plant-Microbe Interact. 9: 775-781.

Dexter, J. E. and Wood, P. J. 1996. Recent applications of debranning of wheat prior to milling. Trends Food Sci. Tech. 7: 35-41.

Dexter, J. E., and Nowicki, T. W.2003. Safety Assurance and Quality Assurance Issues Associated with Fusarium Head Blight in Wheat. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, Chapter 16, pp. 420-460.

Dexter, J. E., Clear, R. M., and Peterson, K. R. 1996. Fusarium head blight: Effect on the milling and baking of some Canadian wheat. Cereal Chem. 73: 695-701.

Dexter, J. E., Marchylo, B. A., Clear, R. M., and Clarke, J. M. 1997. Effect of fusarium head blight on semolina and pasta making quality of durum wheat. Cereal Chem. 74: 519-525.

Dill-Macky, R. and Jones, R. K. 2000. The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. Plant Dis. 84: 71-76.

Dodd, J. L., and White, D. G. 1999. Seed rot, seedling blight, and damping-off. *In* Compendium of Corn Diseases. D. G. White, eds. American Phytopathological Society, St. Paul, MN.

Doohan, F. M., Weston, G., Rezanoor, H. N., Parry, D.W. and Nicholson. P. 1999. Development and use of reverse transcription PCR assay to study expression of *Tri5* by *Fusarium* species *in vitro* and *in planta*. Appl. Environ. Microbiol. 65: 3850-3854.

Doohan, F.M, Brennan, J. and Cooke, B. M. 2003. Influence of climatic factors on Fusarium species pathogenic to cereals. Eur. J. Plant Pathol. 109:755-768.

Edwards, S. G., Pirgozliev, S. R., Hare, M.C. and Jenkinson, P. 2001. Quantification of trichothecene- producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against fusarium head blight of winter wheat. Applied and Environmental microbiol. 67: 1575-1580.

Faifer, G., De Miguel, M. and Godoy, N., 1990. Patterns of mycotoxin production by *Fusarium graminearum* isolated from Argentine wheat. Mycopathologia. 109: 165-170.

Feldman, M. 1997. Origin of cultivated wheat. *In* The world wheat book, A history of wheat breeding. Eds by A. P. Bonjean and W. J. Angus. Lavoisier Publishing, France. Chapter 1. pp. 4-56.

Fernando, W. G. D. 2003. Biological control methods to manage fusarium head blight disease of wheat: is it short or long term solution to the problem? *In* Proceedings of 3rd Canadian Workshop on Fusarium Head Blight. Delta Winnipeg, Winnipeg, Manitoba. December 9-12, 2003. pp: 112.

Fernando, W. G. D., Chen, Y., and Parks, P. 2002. Effect of three *Bacillus sp.* from wheat on FHB reduction. In 2002 National Fusarium head Blight Forum Proceedings. Holiday Inn Cincinnati-Airport, Erlanger KY. December 7-9. 2002. pp. 112.

Fernando, W. G. D., Paulitz, T. C., Seaman W.L., and Seifert, K. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species samples over wheat fields. Can. J. Bot. 78: 497-505.

Fernando, W. G. D., Zhang, J. K., Dusabenyagasani, M., Guo, X. M., Ahmed, H., and McCallum, B. 2006. Genetic diversity *of Gibberella zea* isolates from Manitoba. Plant Dis. 90: 1337-1342.

Fernando, W.G.D., Paulitz, T.C., Seaman, W.L., Dutilleul, P. and Miller, J. D. 1997. Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology. 87: 414-421.

Foroud, N. and Eudes, F. 2009**.** Trichothecenes in Cereal Grains. Int. J. Mol. Sci. 10: 147-173.

Francis, R. G. and Burgess, L. W. 1977. Characteristics of two populations of *Fusarium roseum* 'Graminearum' in Eastern Australia. Transactions of the British Mycological Society. 68: 421-427.

Gang. G., T. Miedaner, U. Schuhmacher, M. Schollenberger and Geiger, H. H. 1998. Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness towards winter rye. Phytopathology. 88: 879-884.

Gale, L.R, Harrison, S. A, Ward, T.J, O'Donnell, K, Milus, E.A, Gale, S.W and Kistler, H.C. 2010. Nivalenol type populations of *Fusarium graminearum* and *F. asisticum* are prevalent on wheat in Southern Louisiana. *Phytopathology*. doi: 10.1094/PHYTO-03-10-0067.

Gareis, M., and Ceynowa, J. 1994. Influence of the fungicide Matador (tebuconazole-triadimenol) on mycotoxin production by *Fusarium culmorum*. Zeitschrift fur Lebensmittel-Untersuchung und –Forschung. 198: 244-8.

Gilbert, J., Clear, R. M., Ward, T. J. , Gaba, D. , Tekauz, A. , Turkington, T. K. , Woods, S. M. ,Nowicki, T. and O'Donnell, K. 2010. Relative aggressiveness and production of 3- or 15-acetyl deoxynivalenol and deoxynivalenol by *Fusarium graminearum* in spring wheat. Can. J. Plant Pathol. 32: 146-152.

Gilbert, J. and Fernando, W.G. D. 2004. Epidemiology and biological control of *Gibberella zeae* / *Fusarium graminearum*. Can. J. Plant Pathol. 26: 464-472.

Gilbert, J. and Tekauz, A. 2000. Review: Recent developments in research on fusarium head blight of wheat in Canada. Can. J. Plant Pathol. 22: 1-8.

Gilbert, J., Abramson, D., McCallum, B., Clear, R., 2001. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. Mycopathologia. 153: 209-215.

Gilbert, J., Tekauz, A., Kaethler, R., Mueller, E. and Kromer, U. 1995. Occurrence of *Fusarium* head blight in Manitoba in 1994. Canadian Plant Disease Survey. 74: 77-78.

Gilbert, J., Woods, S. M., and Kromer, U. 2008. Germination of ascospores of *Gibberella zeae* after exposure to various levels of relative humidity and temperature. Phytopathology. 98: 504-508.

Ginkel, M. V., Schaar, W. V. D., Zhuping, Y., and Rajaram, S. 1996. Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis. 80: 863-867.

Gooding, M. J., and Davies, W. P. 1997. Wheat production and utilization. Advance Typesetting Oxford, the University of Cambridge. pp: 335.

Goswami, R. S. & Kistler, H. C. 2005. Pathogenicity and *in planta* mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology. 95: 1397-1404.

Goswami, R. S. and Kistler, H. C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Molecular Plant Pathology. 5: 515-525.

Guenther, J. C. and Trail, F. 2005. The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia. 97: 229-237.

Guo, X. W., Fernando, W. G. D., and Seow-Brock, H. Y. 2008. Population structure, chemotype diversity, and potential chemotype shifting of *Fusarium graminearum* in wheat fields of Manitoba. Plant Dis. 92: 756-762.

Guo, X. W., Fernando, W. G. D., Bullock, P. and Sapirstein, H. 2010. Quantifying cropping practices in relation to inoculum levels of *Fusarium graminearum* on crop stubble. Plant Pathology. 59: 1107-1113.

Gustafson, P., Raskina, O., Ma, X., Nevo, E. 2009. Wheat Evolution, Domestication and Improvement. *In* Wheat Science and Trade. Eds. Carver, B. F. Wiley-Blackwell. Ames. Iowa. USA. Chapter 1. pp. 5-23.

Haidukowski, M., Pascale, M., Perrone, G., Pancaldi, D., Campagna, C. and A. Visconti, A. 2005. Effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. J. Sci. Food Agric. 85: 191-198.

Hasan, H. A. H. 1993. Fungicide inhibition of of aflatoxins, diacetoxyscirpenol and zearalenone production. Folia Microbiol. 38: 295-298.

Henriksen, B., and Elen, O.2005. Natural *Fusarium* grain infection level in wheat, barley and oat after early application of fungicides and herbicides. J. Phytopathol. 153: 214-220.

Hestbjerg, H., Felding, G. and Elmholt, S. 2002. *Fusarium culmorum* infection of barley seedlings: correlation between aggressiveness and deoxynivalenol content. Phytopathology. 150: 308-312.

Hohn, T. M and Beremand, P. D. 1989. Isolation and nucleotide sequence of a sesquiterpene cyclase gene from the trichothecene-producing fungus *Fusarium sporotrichiodies.* Gene. 79: 131-138.

Hohn, T. M., McCormick, S. P., Alexander, N. J., Desjardins, A. E. and Procter, R. H. 1998. Function and biosynthesis of trichothecenes produced by *Fusarium* species. *In* Molecular genetics of host-specific toxins in plant disease. Eds by K. Kohomoto and O. C. Yoder. Kluwer Academic Publishers. Netherlands. pp: 17-24.

Homdork, S., Fehrmann, H. and Beck, R 2000. Effects of field application of tebuconazole on yield, yield components and the mycotoxin content of Fusarium-infected wheat grain. J.Phytopathol. 148: 1-6.

Hooker, D.C. and Schaafsma, A.F. 2004. Effective application of fungicides on wheat heads: what's the best? (Fungicide application systems for controlling Fusarium head blight). *In* Proceedings of 2nd International Symposium on Fusarium head blight. Orlando, FL., pp. 330.

Hope, R., Alfred, D., and Magan, N. 2005. Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett. Appl. Microbiol. 40: 295-300.

Ichinoe, M., H. Kurata, Y. Sigiura and Y. Ueno. 1983. Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. 46: 1364-1369.

Jennings, P., Coates, M.E., Turner, J.A., Chandler, E.A. and Nicholson, P. 2004. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum* isolates from England and Wales by PCR assay. Plant Pathology. 53: 643-652.

Ji, L., Cao, K., Hu, T. and Wang, S. 2007. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum* isolates from China by PCR assay. J. Phytopathol. 155: 505-512.

Jurgenson, J.E., Bowden, R. L., Zeller, K.A., and Leslie, J.F., Alexander, N.J., and Plattner, R. D. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics. 160: 1451-1460.

Kang, Z. and Buchenauer, H. 2000a. Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. Mycol. Res. 104: 1083-1093.

Kang, Z. and Buchenauer, H. 2000b. Ultrastructural and cytochemical studies on cellulose, xylan, and pectin degradation in wheat spikes infected by *Fusarium culmorum*. J. Phytopathol. 148: 263-275.

Kang, Z. and Buchenauer, H. 2000c. Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. Physiology and Molecular Plant Pathology. 57: 255-268.

Kang, Z., Huang, L., Krieg, U., Mauler-Machnik, A. and Buchenauer, H. 2001. Effects of tebuconazole on morphology, structure, cell wall components and trichothecene production of *Fusarium culmorum in vitro*. Pest Management Science. 57: 491–500.

Khan, M. R. and Doohan, F. M. 2009. Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain. Biological control. 48: 42-47.

Khan, N. I., Schisler, D. A., Boehm, M. J., Slininger, P. J., 2001. Selection and evaluation of microorganisms for biocontrol of Fusarium Head Blight of wheat incited by *Gibberella zea*. Plant Dis. 85: 1253-1258.

Khonga, E. B., and Sutton, J. C. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Can. J. Plant Pathol. 10: 232-239.

Kimura, M., Kaneko, I., Komiyama, M., Takatsuki, A., Koshino, H., Yoneyama, K. and Yamaguchi, I. 1998**.** Trichothecene 3-O-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. Cloning and characterization of *Tri101*. J. Biol. Chem**.** 273: 1654-1661.

Kimura, M., Tokai, T., O'Donnell, K., Ward, T.J., Fujimura, M., Hamamoto, H., Shibata, T., Yamaguchi, I. 2003. The trichothecene biosynthesis gene cluster of *Fusarium graminearum* F15 contains a limited number of essential pathway genes and expressed non-essential genes. FEB Lett. 539: 105-110.

King, C. 2008. Could fusarium head blight become an even more serious problem?. Disease watch. Top Crop Manager (West) magazine. Annex Publishing & Printing Inc.

Klix, B. M., Verreet, J. A, and Beyer, M. 2007. Comparison of the declining triazole sensitivity of *Gibberella zeae* and increased sensitivity achieved by advances in triazole fungicide development. Crop protection. 26: 683-690.

Krska, R., Pettersson, H., Josephs, R.D., Lemmens, M., MacDonald, S. and Welzig, E. 2003. Zearalenone in maize: stability testing and matrix characterization of a certified reference material. Food Addit. Contam. Part A. 20: 1141-1152

Kushiro, M. 2008. Effects of milling and cooking process on the deoxynivalenol content in wheat. Intl J. of Mol. Sci. 9: 2127-2145.

Langevin, F., Eudes, F., and Comeau, A. 2004. Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium Head Blight development in six cereal species. Eur. J. Plant Pathol. 110: 735-746.

Lee, T., Han,Y. K., Kim, K. H., Yun, S. H, and Lee, Y. W. 2002. *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl. Environ. Microbiol. 68: 2148-2154.

Leonard, K.J. and Bushnell, W. R. 2003. Fusarium Head Blight of Wheat and Barley. The American Phytopathological Society, St.Paul, MN, APS Press. 510-512.

Leslie, J. F. and Summerell, B. 2006. The Fusarium Laboratory Manual. Blackwell publishing professions.

Leslie, J. F., Pearson, C. A. S., Nelson, P. E., and Toussoun, T. A. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. Phytopathology. 80: 343-350.

Lewandowski, S. M., Bushnell, W. R., and Evans, C. K. 2006. Distribution of mycelial colonies and lesions in field-grown barley inoculated with *Fusarium graminearum*. Phytopathology. 96: 567-581.

Li, H. P, Wu, A. B, Zhao, C. S, Scholten, O, Löffler, H., Liao, Y. C. 2005. Development of a generic PCR detection of deoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum*. FEMS Microbiol. Lett. 243: 505-511.

Liao, Y.C and Yu, Y. J. 1985. Genetic analysis of head scab resistance in local wheat cultivar Wangshuibai. J. Huazhong Agricultural University. 4: 6-14.

Liddell, C. M. 2003. Systematics of *Fusarium* species and allies associated with Fusarium head blight. *In* Fusarium Head Blight of Wheat and Barley edited by K. J. Leonard and W. R. Bushnell. pp: 35-83.

Liu, W. Z., Langseth, W., Skinnes, H., Elen, O. N., and Sundheim, L. 1997. Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. Eur. J. Plant Pathol. 103: 589-595.

Luz, W. C. da, Stockwell, C. A., and Bergstorm, G. C. 2003. Biological control of *Fusarium graminearum*. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, 2003, Chapter 14, pp. 381-394.

Maria, L. R., Maria, M. R., Maria, C. F., and Sofia, C. 2006. Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. Eur. J. Plant Pathol. 115: 139-148.

Martin, R.A., MacLeod, J.A. and Caldwell, C. 1991. Influences of production inputs on incidence of infection by *Fusarium* species on cereal seed. Plant Dis. 75: 784-788.

Matthies, A. and Buchenauer, H. 2000. Effect of tebuconazole (Folicur) and prochloraz (Sportak) treatments on *Fusarium* head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with *Fusarium culmorum*. Journal of Plant Disease and Protection. 107: 33-52.

McClean, M. 1996. The phytotoxicity of *Fusarium* metabolites: An update since 1989. Mycopathologia 133: 163-179.

McCormick, S. P., Hohn, T. M., and Desjardins, A. E. 1996. Isolation and characterization of Tri3, a gene encoding 15-O-acetyltransferase from *Fusarium sporotrichioides*. Appl. Environ. Microbiol. 62: 353-359.

McCormick, S.P. 2003. The role of DON in pathogenicity. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, Chapter 7, pp. 165-183.

McMaster, G. S. 2009. Development of the wheat plant. *In* Wheat Science and Trade. Eds. Carver, B. F. Wiley-Blackwell. Ames. Iowa. USA. Chapter 2. pp. 31-50.

McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81: 1340-1348.

Menniti, A. M., Pancaldi, D., Maccaferri, M. and Casalini L. 2003. Effect of fungicides on Fusarium Head Blight and deoxynivalenol content in durum wheat grain. Eur. J. Plant Pathol. 109: 109-115.

Mesterhazy Á. 1995. Types and components of resistance to *Fusarium* head blight of wheat. Plant Breed. 114: 377-86.

Mesterházy A. 2003b. Control of *Fusarium* head blight of wheat by fungicides. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, 2003, Chapter 13, pp.363-80.

Mesterházy, Á. 2003a. Breeding wheat for fusarium head blight resistance in Europe. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, 2003, Chapter 13, pp. 363-381.

Mesterházy, Á. 2002. Role of deoxynivalenol in aggressiveness of Fusarium graminearum and *F. culmorum* and in resistance to fusarium head blight. Eur. J. Plant Pathol. 108: 675-684.

Mesterházy, Á., Bartók, T., and Lamper, C. 2003. Influence of wheat cultivar, species of *Fusarium,* and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight. Plant Dis. 87: 1107-1115.

Mesterházy, Á., Bartók, T., Mirocha, C.G., Komoroczy, R. 1999. Nature of wheat resistance to fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed. 118: 97-110.

Miedaner, T. 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breed. 116: 201-220.

Miller, J. D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Quellet, T., Seaman, W. L, Seifer, K. A, Turkington, K. and Voldeng, H. 1998. Effect of tillage practice on Fusarium head blight of wheat. Can. J. Plant Pathol. 20: 95-103.

Miller, J.D., Greenhalgh, R., Wang, Y.Z. and Lu, M., 1991. Trichothecene chemotypes of three Fusarium species. Mycologia. 83: 121-130.

Miller, J.D., Young, J.C., and Sampson D.R. 1985. Deoxynivalenol and *Fusarium* head blight resistance in spring cereals. J. Phytopathol. 113: 359-67.

Milus, E. A., and Parsons, C. E. 1994. Evaluation of foliar fungicides for controlling Fusarium head blight of wheat. Plant Dis. 78: 697-699.

Mirocha, C. J. Abbas, H. K., Windels, C. E. and Xie, W. 1989. Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. Applied and Environmental Microbiology 55: 1315-1316.

Mirocha, C.J., Xie, W. and Filho, E.R. Chemistry and detection of *Fusarium* mycotoxins. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, 2003, Chapter 6, pp. 144-164.

Mullenborn, C., Steiner, U., Ludwig, M., Oerke, E. C. 2008. Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. Eur. J. Plant. Pathol. 120: 157-66.

Munkvold, G. P. 2003. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur. J. Plant Pathol. 109: 705-713.

Nelson, P. E., Dignani, M.C., and Anaissie, E. J. 1994. Taxonomy, biology and clinical aspects of Fusarium species. Clin. Microbiol. Rev. 7: 479-504.

Nightingale, M. J., Marchylo, B. A., Clear, R. M., Dexter, J. E., and Preston, K. R. 1999. Influence of Fusarium head blight on wheat storage proteins. Cereal Chem. 76: 150-158.

Nourozian, J., Etebarian H. R., and Khodakaramian, G.2006. Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria. Songklankarin J. Sci. Technol. 28(Suppl. 1): 29-28.

O'Donnell, K., Kistler, H. C., Tacke, B. K. and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of

Fusarium graminearum, the fungus causing wheat scab. Proc. Natl. Acad. Sci. USA 97: 7905-7910.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genetics and Biology. 41: 600-623.

O'Donnell, K., Ward, T. J, Aberra, D, Kistler, H. C., Aoki, T., Orwig, N.,Kimura M, Bjørnstad A°, Klemsdal, S. S. 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genetics and Biology. 45: 1514-1522.

Ocamb, C. M., and Komedahl, T. 1994.Rhizosphere competence of *Fusarium* species colonizing corn roots. Phytopathology. 84: 166-172.

Osborne, L. E. and Stein, J. M. 2007. Epidemiology of *Fusarium* head blight on small-grain cereals. International Journal of Food Microbiology. 119**:** 103-108.

Parry, D.W., Jenkinson, P., and McLeod, L. 1995. Fusarium ear blight (scab) in small grain cereals- a review. Plant Pathol. 44: 207-238.

Paul, P. A., Lipps, P. E., Hershman, D. E., and Madden, L. V. 2005. Relationship between visual estimates of fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grains: A meta-analysis. Phytopathology. 95: 1225-1236.

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., and Madden, L. V. 2007. A quantitative review of tebuconazole effect on Fusarium head blight and deoxynivalenol content in wheat. Phytopathology. 97: 211-220.

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

Pena, R. J. 2002. Wheat for bread and other foods. In *Bread Wheat Improvement and Production* edited by B. C. Curtis, S. Rajaram and H. G. Macpherson. ISBN 92-5-104809-6. Food and Agriculture Organization of the United Nations, Rome.

Pirgozliev, S.R., Edwards, S.G., Hare, M.C., and Jenkinson, P. 2002. Effect of dose rate of azoxystrobin and metconazole on the development of fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain. Eur. J. Plant Pathol. 108: 469-478.

Pirgozliev, S.R., Edwards, S.G., Hare, M.C., and Jenkinson, P. 2003. Strategies for the control of *Fusarium* head blight in cereals. Eur. J. Plant Pathol. 109: 731-742.

Pirgozliev, S. R ., Edwards, S. G., Hare, M. C., and Jenkinson, P. 2008. Effect of timing of fungicide application on the development of Fusarium Head Blight and the accumulation of deoxynivalenol (DON) in winter wheat grain. Cereal Research Communications. 36: 289-299.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A. and Vance C.P. 2000. Fungal development and induction of defence response genes during early infection of wheat spikes by *Fusarium graminearum*. Molecular Plant–Microbe Interactions. 12: 159-169.

Proctor, R. H, Hohn, T. M and McCormick, S. P. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Molecular Plant–Microbe Interactions. 8: 593-601.

Proctor, R. H, Hohn, T. M and McCormick, S. P. 1997. Restoration of wild-type virulence of *Tri5* disruption mutants of *Giberella zeae via* gene reservation and mutant complementation. Microbiology. 143: 2583-2591.

Prodi, A., Tonti, S, Nipoti, P, Pancaldi, D. and Pisi, A. 2009. Identification of deoxynivalenol and nivalenol producing chemotypes of *Fusarium graminearum* from durum aheat in restricted Area of northern Italy. Journal of Plant Pathology. 91: 727-731.

Pugh, G.W., Johann, H. and Dickson, J.G. 1933. Factors affecting infection of wheat heads by Gibberella saubinetti. J.Agr. Res. 46: 771-797.

Qu, B., Li H.P., Zhang, J. B., Huang, T., Carter, J., Liao, Y. C., Nicholson, P. 2008a. Comparison of genetic diversity and pathogenicity of fusarium head blight pathogens from China and Europe by SSCP and seedling assays on wheat. Plant Pathology. 57: 642-651.

Qu, B., Li, H. P., Zhang, J.B., Xu, Y.B., Huang, T., Wu, A. B, Zhao, C. S, Carter, J., Nicholson, P., Liao, Y. C., 2008b. Geographic distribution and genetic diversity of *Fusarium graminearum* and *F. asiaticum* on wheat spikes throughout China. Plant Pathology. 57: 15-24.

Quarta, A., Mita, G., Haidukowski, M., Santino, A.,Mule,` G. and Visconti, A. 2006 . Multiplex PCR assay for the identification of nivalenol, 3-and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. FEMS Microbiol Lett. 259: 7-13.

Ramarathnam, R., Shen, B., Chen, Y., Fernando, W. G. D., Xuewen, G., and de Kievit, T. 2007. Molecular and biochemical detection of fengycin and bacillomycin D- producing *Bacillus* spp., antagonistic to fungal pathogens of canola and wheat. Can. J. Microbiol. 53: 901-911.

Reid, L.M. and Hamilton, R.I. 1996. Screening maize for resistance to *Gibberella* ear rot. Agriculture and Agri-Food Canada. Technical Bulletin. 1996-5E.

Reid, L.M., Mather, D.E. and Hamilton, R.I. 1996. Distribution of deoxynivalenol in *Fusarium graminearum*-infected maize ears. Phytopathology. 86: 110-114.

Reid, L.M., Mather, D.E., Hamilton, R.I., and Bolton, A.T. 1992. Diallel analysis of resistance in maize to *Fusarium graminaerum* infection via the silk. Can. J. Plant Sci. 72: 915–923.

Ribichich, K.F., Lopez, S.E., and Vegetti, A.C. 2000. Histopathological spikelet changes produced by *Fusarium graminearum* in susceptible and resistant wheat cultivars. Plant Dis. 84: 794-802.

Rocha, O., Ansari, K., Doohan, F.M. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: a review. Food Addit. Contam. 22: 369-378.

Schaafsma, A. W., Savard, M. E., Clear, R., and Dexter, J. 2004. Methods and issues regarding detection of deoxynivalenol, *Fusarium*-damaged kernels, and *Fusarium* spp. in commercial grain in Canada. Can. J. Plant Pathol. 26: 443-452.

Schaafsma, A. W., Tamburic-llincic, L., and Hooker, D. C. 2005. Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of *Gibberella zeae/ Fusarium graminearum*, fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. Can. J. Plant Pathol. 27: 217-224.

Schaafsma, A. W., Tamburic-llincic, L., Miller, J. D. And Hooker, D. C. 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. Can. J. Plant Pathol. 23: 279-285.

Schaafsma, A.W. 1999. 1996 epidemic in winter wheat-aftermath. *In* Proceedings of the 1999 Canadian Workshop on Fusarium Head Blight. Eds by R. Clear. Canada Grain Commission, Winnipeg, Man. pp. 31-32.

Schisler, D.A., Khan, N. I., and Boehm, M. J. 2002a. Biological control of fusarium head blight of wheat and deoxynivalenol levels in grain via use of microbial antagonists. In Mycotoxins and Food safety, Eds: DeVries, J. W., Trucksess, M. W., Jackson, L. S. Kluwar Academic/Plenum Publishers, New York, pp. 53-69.

Schisler, D.A., Khan, N. I., Boehm, M. J and Slininger, P. J., 2002b. Green-house and field evaluation of biological control of Fusarium Head Blight on durum wheat. Plant Dis. 86: 1350-1356.

Schisler, D.A., Khan, N. I., Boehm, M. J, Lipps, P. E., Slininger, P. J., and Zhang, S. 2006. Selection and evaluation of the potential of choline-metabolizing microbial strains to reduce fusarium head blight. Biological Control. 39: 497-506.

Schnerr, H., Vogel, R. F., and Niessen, L. 2002. Correlation between DNA of trichothecene -producing *Fusarium* species and deoxynivalenol concentrations in wheat samples. Letters in Applied Microbiology. 35: 121-125.

Schroeder, H. W. and Christensen, J. J. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology. 53: 831–38.

Scott, P. M., Kanhere, S. R. , Dexter, J. E. , Brennan, P. W. and Trenholm, H. L. 1984. Distribution of the trichothecene mycotoxin deoxynivalenol (vomitoxin) during the milling of naturally contaminated hard red spring wheat and its fate in baked products, Food Additives & Contaminants: Part A, 1: 4: 313-323.

Seed Manitoba. 2008. Published by the Manitoba Co-operator, 1666, Dublin Ave. Winnipeg, MB. R3H 0H1.

Shaner, G. 2003. Epidemiology of fusarium head blight of small cereals in North America. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, 2003, Chapter 4, pp. 84-119.

Shaner, G., and Buechley, G. 2003. Relation between head blight severity and DON in natural epidemics of FHB. *In* Proceedings of the National Fusarium Head Blight Forum, Bloomington, MN 13–15 Dec 2003. Mich State Univ, East Lansing, MI. pp. 178.

Shewry, P. R. Wheat. 2009. Darwin Review. Journal of Experimental Botany. 60: 1537-1553.

Simmons, S. R., Oelke, E. A., and Anderson, P.M. 1995. Growth and Development Guide for Spring Wheat. The University of Minnesota. WWW-F0-02547. pp. 14.

Simpson, D. R, Weston, G. E, Turner, J. A, Jennings, P and Nicholson, P. 2001 Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur. J. Plant Pathol. 107: 421-431.

Siranidou, E., Buchenauer, H. 2001. Chemical control of Fusarium head blight on wheat. Journal of Plant Disease Protection. 108 : 231-243.

Snijders, C. H. A. 1994. Breeding for resistance to Fusarium head blight in wheat and maize. *In* Mycotoxins in grain: compounds other than aflatoxin. Eds by J. D. Miller and H. L. Trenholm. Eagan Press. St. Paul. Minn. pp. 37-58.

Stack, R. and McMullen, M. 1995. A visual scale to estimate severity of Fusarium head blight of wheat. (http://www.ext.nodak.edu/extpub/plantsci/smgrains/pp1095w.htm).

Stack, R.W. 2003. History of fusarium head blight with emphasis on North America. *In* Fusarium Head Blight of Wheat and Barley, Leonard, K.J., Bushnell, W.R., Eds., The American Phytopathological Society: St. Paul, Minnesota, USA, Chapter 1, pp. 1-34.

Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J. and O'Donnell, K., 2007. Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet. Biol. 44: 1191-1204.

Stockwell, C.A., Luz, W. C. da, and Bergstrom, G.C. 1997. Biocontrol of wheat scab with microbial antagonists. Phytopathology. 87: S94(Abstr).

Strange, R. N. and Smith, H. 1971. A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. Physiol. Plant Pathol. 1: 141-150.

Sutton, J.C.1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol. 4: 195-209.

Tamburic-llincic, L., Limay-Rios. V and Schaafsma, A. W. 2010. Fusarium requirements for wheat registration in Ontario,Canada,http://top-congress.hu/2010/fusarium/index1.htm

Tamburic-llincic, L. and Schaafsma, A. W. 2001. Fusarium head blight survey of winter wheat in 2000 in Ontario. Can.Plant Dis. Surv. 81: 96.

Tamburic-llincic, L. and Schaafsma, A. W. 2008. The prevalence of *Fusarium* spp. colonizing corn seed stalk in southwestern Ontario, Canada. Can. J. Plant Sci. 89: 103-106.

Tamburic-llincic, L., Schaafsma, A. W, Clear, R. M,Ward, T. J and O'Donnell. K. L. 2006. Chemotype of *Fusarium graminearum* isolates from winter wheat collected in Ontario in 2004. Can. J. Plant Pathol. 28: 340. (Abstr).

Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U.S., Sugiura, Y. and Ueno, Y., 1988. Worldwide contamination of cereals by the Fusarium mycotoxins nivalenol, deoxynivalenol, and zearalenone. 1. Survey of 19 countries. J. Agric. Food. Chem. 36: 979-983.

Teich, A. H and Nelson, K. 1984. Survey of Fusarium head blight and possible effects of cultural practices in wheat fields in Lambton County in 1983. Canadian Plant Disease Survey. 6: 11-13.

Teich, A. H. and Hamilton, J. R. 1985. Effect of cultural practices, soil phosphorus, potassium and pH on the incidence of Fusarium head blight and deoxynivalenol levels in wheat. Applied and Environmental Microbiology. 49: 1429-1431.

Tschanz, A. T., Horst, R. K., and Nelson, P. E. 1976. The Effect of environment on sexual reproduction of *Gibberella zeae* . Mycologia. 68: 327-340.

Van Eeuwijk, F. A., Mesterhazy, A., Kling, C. I., Ruckenbauer, P., Saur, L., Bürstmayr, H., Lemmens, M., Keizer, L. C. P., Maurin, N., and Snijders, C. H. A. 1995. Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theor. Appl. Genet. 90: 221-228.

Visconti, A. 2001. Problems associated with *Fusarium* mycotoxins in cereals. Bulletin of the Institute for Comprehensive Agricultural Sciences, Kinki University. 9: 39-55.

Walker, S. L., Leath, S., Hagler, W. M., Jr., and Murphy, J. P. 2001. Variation among isolates of *Fusarium graminearum* associated with Fusarium head blight in North Carolina. Plant Dis. 85: 404-410.

Wang, J., Li, H., Qu, B., Zhang, J., Huang, T, Chen, F. and Liao, Y. 2008. Development of a Generic PCR Detection of 3-Acetyldeoxynivalenol, 15-Acetyldeoxynivalenol-and Nivalenol-Chemotypes of *Fusarium graminearum* Clade. Int. J. Mol Sci. 9: 2495-2504.

Ward, T. J., Clear R. M., Rooney, A. P.,O'Donnell, K., Gaba, D.,Patrick, S., Starkey, D., Gilbert, J., Geiser, D and Nowicki, T. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genetics and Biology. 25: 473-484.

Ward, T., Bielawski, J.P., Kistler, C.H., Sullivan, E. and O'Donnell, K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. Proc Natl Acad Sci USA. 99: 9278-9283.

Watson, A. Fusarium cob rot of corn. 2007. Primefact.243. ISSN 1832-6668 www.dpi.nsw.gov.au/primefacts.

Windels, C. E. 2000. Economic and social impacts of fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology. 90: 17-22.

Wong, L. S. L., Abramson, D., Tekauz, A., Leslie, D., and McKenzie, R. I. H. 1995. Pathogenicity and mycotoxin production of Fusarium species causing head blight in wheat cultivars varying in resistance. Can. J. Plant Sci. 75: 261-267.

Wong, L. S. L., Tekauz, A., Leslie, D., Abramson, D., and McKenzie, R. I. H. 1992. Prevalence, distribution, and importance of *Fusarium* head blight in wheat in Manitoba. Can. J. Plant Pathol. 14: 233-238.

Xu, Y.G., and Chen, L.F. 1993. Wheat Scab: Theory and Practice on Control. Jiangsu Sci.-Tech. Publ. House, Nanjing, China.

Xue, A. G., Armstrong, K. C., Voldeng, H. D., and Babcock, C. 2004. Comparative aggressiveness of isolates of *Fusarium* spp. Causing head blight on wheat in Canada. Can. J. Plant Pathol. 26: 81-88.

Xue, A. G., Voldeng, H. D., Savard, M. E., Fedak, G., Tian, X and Hsiang. T. 2009. Biological control of fusarium head blight of wheat with *Clonostachys rosea* strain ACM941. Can. J. Plant Pathol. 31: 169-179.

Yazar, S. and Omurtag, G. 2008. Fumonisins, trichothecenes and zearalenone in cereals. Int. J. Mol. Sci. 9: 2062-2090.

Yi, C., Kaul, H.P., Kubler, E., Schwadorf, K. and Aufhammer, W. 2001. Head blight (*Fusarium graminearum*) and deoxynivalenol concentration in winter wheat as affected

by pre-crop, soil tillage and nitrogen fertilisation. Journal of Plant Diseases and Protection 108: 217-230.

Yli-Mattila, T., Gagkaeva, T., Ward, T. J., Aoki, T., Kistler, H. C and O'Donnell, K. 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia. 101**:** 841-852.

Young, J. C., Fulcher, R. G., Hayhoe, J. H., Scott, P. M., and Dexter, J. E. 1984. Effect of wheat and milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. J. Agr. Food Chem. 32: 659-664.

Yu, Y.J. 1982. Monosomic analysis of scab resistance and yield components in the wheat cultivar Sumai 3. Cereal Res.Commun. 10: 185-189.

Zhang, J. B., Li, H. P., Dang, F. J., Qu, B., Xu, Y. B., Zhao, C. S., and Liao, Y. C.2007. Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycological research. 111: 967-975.

Zhang, Y. J., Fan, P.S., Zhang, X.,. Chen, C. J. and Zhou, M. G. 2008. Quantification of *Fusarium graminearum* in harvested grain by real-time polymerase chain reaction to assess efficacies of fungicides on Fusarium Head Blight, deoxynivalenol contamination, and yield of winter wheat. Phytopathology. 99: 95-100.

Zhang, Y. J., Fan, P.S., Zhang, X., Chen, C. J. and Zhou, M. G. 2010. Effects of fungicides JS399-19, azoxystrobin, tebuconazole, and carbendazim on the physiological and biochemical indices and grain yield of winter wheat. Pestic. Biochem. Physiol. 98: 151-157.

http://faostat.fao.org

http://thecanadianencyclopedia

http://www.agnet.org/library/bc/54012/

http://www.grains.org/corn

APPENDICES

Appendix 1: Significance of mean square values for area under the disease progress curve (AUDPC) for fusarium head blight index (FHB index) within each source of variation in 2009 and 2010

Source of variance	df for 2009	Mean square values for AUDPC for FHB Index		df for 2010
		2009	2010	
Overall Fungicide	4	126223.0*	160553.0*	4
Within Fungicides	3	14250.3*	9293.12ns	3
Fungicides vs control	1	462141.3*	613801.0*	1
Cultivar	1	3280015.1*	3834719.4*	1
Fungicide x cultivar	4	20475.4ns	4721.25ns	4
Isolate	6	146443.1ns	180666.3*	6
3-ADON	3	20860.8*	5448.38ns	2
15-ADON	1	0.6805ns	53778.6*	2
3-ADON vs 15-ADON	1	85719.5*	6534.55ns	1
Inoculated vs Non-inoculated	1	750895.5*	956547.2*	1
Fungicide x isolate	24	4853.3*	4967.0*	24

Cultivar x isolate	6	102932.6*	79669.6*	6
Fungicide x cultivar x isolate	24	1636.0ns	4356.5*	24
Rep	2	440.1ns	7953.6ns	2
Fungicide x rep	8	9785.0*	2136.9ns	8
Cultivar x rep	2	160.0ns	157.7ns	2
Fungicide*Cultivar*Rep	8	5949.8*	1939.57ns	8
Error	120	1322.4	1642.6	120
Total	209			209

Appendix 2: Experimental spring wheat plots at the "Point", University of Manitoba, 2010

Appendix 3: Wheat field showing fusarium head blight (FHB) symptoms

Appendix 4: Misting of the field using sprinkler system

Appendix 5: Preparation of *Fusarium graminearum* inoculum in carboxy methyl cellulose (CMC) medium