EVALUATION OF FUSARIUM HEAD BLIGHT CAUSAL SPECIES AND DISEASE FORECASTS
FOR THE MARITIME PROVINCES OF CANADA

by

Emily M. Johnstone

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
April 2023

Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is an important consideration in cereal production as the fungus reduces yields and deposits mycotoxins in grain. Characteristics of the *Fusarium* spp. population found in the Maritime provinces of Canada are not well understood thus, an objective of this study was to describe the local population. Wheat and barley samples from 39 sites across the Maritimes were surveyed to determine *Fusarium* spp. and mycotoxins present from 2018 to 2021. Management of FHB may be supported by disease forecasting systems therefore, FHB forecasts were evaluated in comparison to epidemic records and field experiments. *Fusarium graminearum* was the primary causal species of concern and deoxynivalenol was the most abundant mycotoxin detected each year. FHB was most accurately forecasted using 7-day pre-anthesis relative humidity and temperature. This study represents the first multi-year survey of FHB causal species and evaluation of FHB forecasting in the Maritimes.

Keywords: *F. graminearum*, Fusarium head blight (FHB), wheat, barley, mycotoxins, DON, disease forecasting, fungicides

LIST OF ABBREVIATIONS USED

°C	Degrees Celsius
µg	Microgram
µL	Microlitre
15-ADON	15-acetyldeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
AGC	Atlantic Grains Council
ANOVA	Analysis of Variance
ARCCC	Atlantic Recommending Committee for Cereal Crops
Aug	August
BCA	Biological Control Agents
BEA	Beauvericin
BLAST	Basic Local Alignment Search Tool
d	Day(s)
D3G	Deoxynivalenol-3-glucoside
DAA	Days after application
DMI	Demethylation inhibitor
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
ECCC	Environment and Climate Change Canada
ELISA	Enzyme linked immunosorbent assay
ENN	Enniatin
FDK	Fusarium damaged kernel(s)
FHB	Fusarium head blight
FN	False negative
FP	False positive
FUS	Fusarin
g	Gram
GR	Growth rate
h	Hour
ha	Hectare
ITS	Internal transcribed spacer region gene
Jul	July
Jun	June
k	Thousand
kg	Kilogram
Kt	Thousand tonnes
L	Litre
LC-MS/MS	Liquid chromatography with tandem mass spectrometry

m	Metre
MB	Mung bean media
mg	Milligram
min	Minute(s)
mL	Millilitre
mm	Millimetre
MON	Moniliform
MR	Moderately resistant
MS	Moderately susceptible
Mt	Million tonnes
NB	New Brunswick
NCBI	The National Center for Biotechnology Information
ng	Nanogram
NIV	Nivalenol
NS	Nova Scotia
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PE	Prince Edward Island
PM	Powdery mildew
ppm	Parts per million
qPCR	Quantitative Polymerase Chain Reaction
R	Resistant
RAL	Resorcylic acid lactone
RH	Relative humidity
RNA	Ribonucleic acid
RPM	Revolutions per minute
s	Second(s)
S	Susceptible
SB	Septoria blotch
SDHI	Succinate dehydrogenase inhibitor
Sep	September
SNA	Spezieller Nährstoffarmer agar
t	Metric tons
TEF1α	Translation elongation factor 1
TN	True negative
TP	True positive
TRI	Trichothecene biosynthetic gene
VS	Very susceptible
yr	Year
ZEA	Zearalenone

ACKNOWLEDGEMENTS

Chapter 1 - Introduction

1.0   Literature Review

Wheat and barley are important rotational cash crops for the Maritime farm economy of Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PE), Canada, where grain may be sold or remain on farm to feed livestock. Worldwide, 770.88 Mt of wheat were produced in 2021 (FAOSTAT 2023) with Canada contributing 22.30 Mt (Statistics Canada 2022a) to the total at an approximate value of C$ 8.66 billion (Atlantic Grains Council 2022d). The Maritimes produced a total of 68.70 Kt (Statistics Canada 2022a), contributing about C$ 24.39 million in 2021 to the agricultural economy (Atlantic Grains Council 2022d). Of the Maritime provinces, PE produced the most wheat with 49.83 Kt while NB and NS produced 10.87 Kt and 8.01 Kt, respectively, in 2021 (Statistics Canada 2022a).

Barley is grown worldwide, and 145.62 Mt of barley was harvested in 2021 (FAOSTAT 2023) with Canada producing 6.96 Mt (Statistics Canada 2022a) at an approximate value of C$ 2.92 billion in 2020 (Atlantic Grains Council 2022a). Maritime producers harvested 118.99 Kt (Statistics Canada 2022a) of barley worth about C$ 39.26 million in 2021 (Atlantic Grains Council 2022a). PE produced the most barley in the Maritimes with 89.07 Kt while NB and NS produced 25.94 Kt and 3.98 Kt, respectively, in 2021 (Statistics Canada 2022a).

Cereal producers in Canada are faced with many challenges including plant disease. Fusarium head blight (FHB) is a serious fungal disease of cereal crops world-wide, causing yield and quality losses. Among the *Fusarium* species that cause FHB, the most concerning is *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schweinitz) Petch.) as this species produces the mycotoxin deoxynivalenol (DON) during infection. Ingestion of DON-

contaminated grain by humans and livestock results in serious adverse health effects (Pestka and Smolinski 2005). The causal species of FHB and associated mycotoxins are relatively unknown in the Maritimes as surveillance has only identified occurrence of the disease.

Infection of grain by *F. graminearum* and other FHB causal species is dependent on the presence of disease inoculum and warm, humid weather coinciding with wheat anthesis or barley head emergence (McMullen et al. 2012). Management of this disease requires an integrated approach. Current control practices are primarily in the form of crop rotation, residue management, and preventative fungicide applications. Uncertainty can surround the economic suitability of fungicides and could be eased with the use of disease forecasts that predict risk of FHB epidemics or DON accumulation (Wegulo et al. 2015). Both economic and environmental benefits could arise from improved fungicide decision making that results in reduced use of fungicides. FHB forecasting, however, has yet to be developed for the Maritime provinces. This research aims to identify and characterize the primary causal *Fusarium* species of FHB and evaluate FHB forecasting models in the Maritime provinces.

1.1   Wheat Production

Both spring and winter bread wheat (*Triticum aestivum* L.) types are produced in the Maritime provinces. Spring wheat is sown in spring and harvested the same year while winter wheat is sown in late summer or early fall and harvested the following summer. Winter cultivars establish before winter and resume growth the following spring. This is due to a vernalization requirement preventing the transition from vegetative to reproductive growth unless a cold period is satisfied (Yan et al. 2004).

Wheat is a major source of nutrients in human diets and is an important ingredient in many food products including pastries, bread, and pasta (Shewry and Hey 2015). Low protein

cultivars may be used as a brewing grain in the production of wheat beers (Faltermaier et al. 2014). The grain ration of livestock may also include wheat or its milling by-products (OMAFRA 2021).

1.2   Barley Production

For many regions of the world, barley (*Hordeum vulgare* L.) is an important food crop while North America's primary use of barley is for animal feed and brewing purposes (Newman and Newman 2006). However, there is increasing interest in barley for food stuffs in North America as diets trend to include more whole grains (Ullrich 2011). In the diet of ruminant animals, barley is readily degradable and provides a more rapid starch fermentation than corn. Rumen microbial assimilation is also improved with the synchronous release of energy and nitrogen from barley (Nikkhah 2012). Given the fermentability of barley, it is the most sought-after grain for malting. Barley malt is used in the production of beer and some whiskies imparting flavor, colour, mouthfeel, and foam stability to the finished products (Mallett 2014).

1.3   Fusarium Biology

Fusarium is a genus of filamentous fungi in the family *Nectriaceae*, phylum *Ascomycota*. Many *Fusarium* species are economically important plant-pathogenic fungi attacking roots, stems, seedlings, and floral structures on a wide range of hosts (Summerell 2019) as causal agents of diseases such as wilts, blights, rots, and cankers (Ma et al. 2013). *Fusarium* species are saprophytes that exist primarily as haploid hyphae with distinct asexual and sexual reproduction stages (Ma et al. 2013). Sexual spores are produced within a sac-like ascus that is enclosed in a fruiting body, known as a perithecium, that upon maturity, develops a pore through which haploid ascospores are forcibly discharged. Asexual haploid spores include microconidia and macroconidia, and some *Fusarium* species produce chlamydospores (Leslie and Summerell

2006). Microconidia are borne on conidiophores while larger macroconidia are borne in a dense cluster of conidiophores called sporodochia (Ma et al. 2013). Morphological characteristics of conidia size, shape, and presence are important in species identification. Microconidia may be reniform, oval, pear, turnip, spherical, or fusiform in shape and are not produced by all *Fusarium* species (Leslie and Summerell 2006). Macroconidia are identified by their multi-septate and banana-shaped form with specific shaping of apical and foot cells (Leslie and Summerell 2006).

1.3.1 Fusarium Head Blight (FHB)

FHB is a serious disease of not just bread wheat and barley, but durum wheat (*Triticum durum* Desf.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), triticale (*X Triticosecale* Wittmack), and rice (*Oryza* L.) (Parry et al. 1995). *Fusarium graminearum* also infects maize (*Zea mays* L.) causing ear rot disease (Sutton 1982). Producers impacted by FHB experience yield losses and reduced grain quality due to Fusarium damaged kernels (FDK), low test weights, and reduced germination capacity. The fungi destroy cell walls, storage proteins and starch (Bechtel et al. 1985). The most important concern associated with FHB, however, is the contamination of grain with mycotoxins, including DON, which at certain concentrations is unsafe for consumption (Charmley and Trenholm 2017). These quality factors negatively impact the grade and economic value of the grain.

Symptoms of FHB in wheat first appear as pre-mature senescence of individual spikelets of the seed head and may continue to fully bleach the seed head. Infected heads can be identified by the presence of pink-orange aggregations of sporodochia and fluffy white mycelium on the spikelets and glumes if warm, humid conditions persist (Schmale and Bergstrom 2003). Perithecia may develop on the spikelets with a spherical shape that are dark

blue to black. (Canadian Grain Commission 2019). Infected seeds have a shrunken, wrinkled, scabbed appearance and may have pink discolouration (Canadian Grain Commission 2020). Symptoms of FHB on barley heads are subtle in comparison to wheat (Canadian Grain Commission 2019). Individual spikelets scattered throughout the head become discoloured with an orange to brown appearance (Tekauz et al. 2000; Canadian Grain Commission 2019). Harvested barley grain may appear less shrunken and scabbed than wheat (Tekauz et al. 2000). The visual symptoms of the seed head differ between wheat and barley due to differences in host resistance (Section 1.4).

1.3.2 Causal Species of FHB

Many *Fusarium* species are implicated in the development of FHB. These species include *F. graminearum*, *Fusarium culmorum* (W.G. Smith.) Sacc., *Fusarium avenaceum* (Fr.) Sacc. as well as *Fusarium sporotrichioides* Sherb., and *Fusarium poae* (Peck) Wollenw, which are less pathogenic (Wong et al. 1995; Bottalico and Perrone 2002). Other causal agents of FHB include *Fusarium equiseti* (Corda) Sacc and *Fusarium cerealis* (syn. *Fusarium crookwellense* L.W. Burgess, P.E. Nelson & Toussoun).

F. graminearum is widely known as the primary causal species of FHB and is also believed to be the primary causal agent in the Maritimes (Martin 2004). Annually, The Canadian Phytopathological Society publishes disease surveys indicating occurrence, severity and losses associated with important plant diseases affecting agriculture in Canada, including FHB (Canadian Phytopathological Society n.d.). Before 2019, these surveys provided only information on the presence of FHB in the Maritimes, not specific causal species. However, Canada-wide studies on FHB have included work on *F. graminearum* isolates collected from the Maritime region. Disease surveillance from PEI in 2019 and 2020 indicated the presence of

F. graminearum, *F. sporotrichioides*, *F. poae*, and *F. avenaceum* (Foster and Matters 2020;

Johnstone et al. 2021)

1.3.3 FHB Disease Cycle

Development of FHB is dependent on the production and presence of inoculum and suitable

environmental conditions to infect and develop within a susceptible host. Inoculum includes

hyphal fragments, ascospores, and conidia (Bai and Shaner 2004) found in the soil and

colonized crop residue (Sutton 1982) or ascospores deposited from distant sources (Keller et al.

2014). Ascospores are the principal inoculum of *F. graminearum* however, conidia may act as

an effective source of primary inoculum via splash dispersal (Stack 2000). Secondary infections

may occur if warm, moist conditions persist (OMAFRA 2017). In the asexual phase, *F.*

graminearum produces only macroconidia (Leslie and Summerell 2006).

Environmental conditions within a specific air temperature and relative humidity range

support development of inoculum and infection. Manstretta and Rossi (2016) found that

perithecia of *F. graminearum* developed at temperatures ≥ 5˚C and ≤ 30˚C with relative

humidity ≥ 75.5%. Maturity of perithecia and ascospore production occurred at temperatures ≥

20˚C and ≤ 25˚C with relative humidity ≥ 85%. FHB develops at temperatures of 15-35˚C with

persistent moisture (Sutton 1982). Not all FHB causal species are observed in one region since

individual species are adapted to different microclimates (Xu et al. 2008). *Fusarium*

graminearum infection is associated with warm, humid conditions, where a drier climate is

associated with *F. poae* infection. Cool, humid climates promote infection by *F. avenaceum* and

F. culmorum (Xu et al. 2008).

Once inoculum is present in a suitable environment, it is essential for infection and

development of FHB that the host plant is at a susceptible growth stage. Wheat is susceptible to

Fusarium infection from the time of anthesis, when anthers extrude from the floret, until the soft dough stage (McMullen et al. 2012). Spores germinate on the host where mycelia develop and infiltrate the host tissues. Seeds do not develop when the fungi infect at anthesis as the floret is colonized and killed. A seed can still develop, but is shriveled and chalky, if the floret is infected after anthesis (Schmale and Bergstrom 2003).

The most susceptible growth stage of barley is different from that of wheat. Barley undergoes anthesis while the seed head is still enveloped by the flag leaf, at boot stage and is most susceptible to *Fusarium* infection when the seed head emerges (McMullen et al. 2012). One study suggested that rainfall may funnel spores down the flag leaf to the exposed anthers within, thus extending the period of susceptibility (Schöneberg et al. 2018). The fungus infiltrates the developing barley seed through the crevice opening between the lemma and the palea (Lewandowski et al. 2006).

1.3.4   Fusarium Mycotoxins

Fusarium spp. are mycotoxigenic fungi that produce toxins as secondary metabolites of growth, which are not considered essential for development and reproduction. Causal species of FHB produce various mycotoxins with more than one toxin associated with most species. Fusarium mycotoxins include beauvericin (BEA), enniatins (ENN), fusarins (FUS), moniliform (MON), zearalenone (ZEA), and trichothecenes (Table 1.1). Trichothecenes include toxins such as DON, nivalenol (NIV), T-2 and HT-2 (Birr et al. 2020), NX-2 (Varga et al. 2015) and diacetoxyscirpenol (DAS) (Munkvold et al. 2021). Mycotoxins produced by *F. graminearum* are of particular importance since this species is considered the primary causal agent of FHB. Due to the severe health effects associated with chronic mycotoxin exposure, regulations and

guidelines have been set by many countries to safely limit the concentration of mycotoxins in

food and feed products.

Table 1.1 *Fusarium* species and associated mycotoxins (Munkvold et al. 2021).

Fusarium spp.	Mycotoxins Produced
F. graminearum	DON, NIV, ENN, FUS, ZEA, NX-2*
F. sporotrichioides	T-2, HT-2, DAS BEA, ENN, FUS, MON
F. avenaceum	BEA, ENs, FUS, MON
F. poae	NIV, T-2$^{\wedge}$, HT-2$^{\wedge}$, DAS, BEA, ENN, FUS
F. culmorum	DON, NIV, ENN, FUS, MON, ZEA
F. cerealis[+]	DON, NIV, BEA, ENN, ZEA
F. equiseti	NIV, T-2, DAS, BEA, MON, ZEA

*Varga et al., 2015 ; ^ Thrane et al., 2004 ; + Palacios et al., 2021.
DON = deoxynivalenol, NIV = nivalenol, ENN = enniatins, FUS = fusarins, ZEA = zearalenone, DAS = diacetoxyscirpenol, BEA = beauvericin, MON = moniliform.

Among the mycotoxins produced by FHB causal species, trichothecenes are the most

frequently reported and studied, as they are most associated with FHB and have significant

economic impact on cereal production. Trichothecenes are sesquiterpenoids, which share a

common tricyclic structure with a 9, 10 double bond and 12, 13 epoxide group and are

differentiated by the functional groups attached to this structure (Desjardins and Proctor 2007;

Villafana et al. 2019). Type A trichothecenes have a hydroxyl group, an ester, or no substituent

at C-8 while Type B have a ketone at this position as well as a hydroxyl group located at C-7.

(McCormick et al. 2011). Type A trichothecenes include T-2 and HT-2 toxins as well as

recently discovered NX-2 (Varga et al. 2015). Type B includes NIV as well as DON and its

acetylated derivatives, 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-

ADON). DON is the primary mycotoxin produced by *F. graminearum*.

Toxicity of trichothecenes is experienced in both animals and plants. Synthesis of

proteins, DNA, and RNA are inhibited, and cell membrane and mitochondrial function are

altered in the presence of trichothecenes (Rocha et al. 2005). Ingestion of contaminated grain results in different symptoms in humans and livestock species. Most common symptoms of trichothecene toxicity include intestinal irritation inducing vomiting and diarrhea leading to feed refusal (Pestka 2007). Swine are the most susceptible to the toxic effects of DON compared to poultry and ruminant animal livestock (CAST 2003; Pestka 2007). Cellular function is similarly affected in plants resulting in chlorosis, necrosis and slowed growth (Rocha et al. 2005). Plants are capable of detoxifying DON by attaching a glucoside to the molecule resulting in deoxynivalenol-3-glucoside (D3G), however, when consumed by mammals the glucose is removed, resulting in the toxic DON molecule (Berthiller et al. 2005).

Trichothecenes are not the only mycotoxin produced by *F. graminearum* that can negatively impact mammalian health. Producers should be aware of *F. graminearum*'s ability to produce ZEA, which is a resorcylic acid lactone (RAL). RALs are nonsteroidal estrogenic compounds (Kuiper-Goodman et al. 1987). ZEA, therefore may be better classified as a mycoestrogen as it is not acutely toxic but results in reproductive issues in humans and livestock (Bennett and Klich 2003). Although the economic impact of ZEA is minor in comparison to DON, it may be an important consideration in livestock breeding operations (Bridges et al. 2010).

Regulations and guidelines have been set by many countries to safely limit the concentration of mycotoxins in food and feed products. Guidelines for maximum chemical contaminants including DON in food and feed have been issued in Canada and are presented in Table 1.2. The United States has also published advisory levels for DON while the European Union has set regulatory limits for DON contamination as low as 0.2 ppm for processed cereal-based foods (European Union 2006). Mycotoxins can be identified and quantified and through

analytical chemistry methods such as LC-MSMS (liquid chromatography with tandem mass spectrometry) or ELISA (enzyme linked immunosorbent assay) (CAST 2003).

Table 1.2 Guidelines for maximum DON contamination in Canadian food and feed products (Charmley and Trenholm 2017; Health Canada 2020).

DON (ppm)	Product
1	Feed for swine, young calves, lactating dairy cattle Wheat for baby foods
2	Wheat for human consumption Non-staple foods
5	Feed for cattle and poultry

1.3.5 Population Genetics

F. graminearum can be assigned population membership by the mycotoxin genotype of an individual isolate. Trichothecene producers are identified by their acetylated DON forms, 3-ADON, or 15-ADON, NIV or NX-2. There are 15 *TRI* genes encoding enzymes in the trichothecene biosynthetic pathway of which loss or disruption specific alleles result in the end trichothecene produced (Alexander et al. 2009). Genotypes predicting the trichothecene produced by *F. graminearum* can be determined by PCR assays targeting specific genes (Villafana et al. 2019). The literature often refers to the trichothecene genotype as the chemotype, the chemical phenotype of the isolate. However, chemical analysis of the *F. graminearum* isolate should be performed to confirm the chemotype as it may contradict the genotype (Desjardins 2008).

The 3-ADON genotype has been reported as the most prevalent trichothecene genotype in the Maritimes and is increasing in the western provinces of Canada where the 15-ADON genotype is most prevalent (Ward et al. 2008; Kelly et al. 2015). It was determined by Ward *et al.* (2008) that the 3-ADON populations of *F. graminearum* have greater reproductive ability

which has been suggested as a reason for the displacement of the 15-ADON genotype in North America. It should be noted that DON has been identified as a virulence factor, contributing to the pathogen's ability to spread disease within the host. Deoxynivalenol-nonproducing *Fusarium* strains in which the trichodiene synthase gene, *TRI5*, was disrupted, caused initial infection in wheat but were unable to spread and cause severe disease within the host (Proctor et al. 1995; Eudes et al. 2001; Bai et al. 2002). Additionally, Kelly and Ward (2018) identified North American populations of *F. graminearum* with fitness traits involved with fungicide resistance and virulence. While fungicide resistance has not been reported in Canada, there has been a resistant isolate identified in New York, USA (Spolti et al. 2014).

Beyond cereal crops, *F. graminearum* has a wide host range including soybean (*Glycine max* (L.) Merr.), potato (*Solanum tuberosum* L.) and grass and non-grass weeds in and outside of the field (Broders et al. 2007; Estrada Jr et al. 2010; Mourelos et al. 2014). These alternative hosts may provide a reservoir for pathogen diversity and inoculum (Fulcher et al. 2019). The adaptive ability of FHB causal species underscores the importance of monitoring for future population shifts in virulence and fungicide resistance.

1.4 Management of FHB

Agronomic management practices, including cultural, chemical, and biological controls, provide practical in-field strategies for producers to minimize yield and quality losses associated with FHB (Wegulo et al. 2015). The goal of an FHB management program is to prevent DON accumulation greater than 1 ppm and to minimize grain damage. Seasonal risk of FHB varies by region and is dependent on *Fusarium* spp. inoculum and a favourable environment; therefore, the effectiveness of each strategy depends on these conditions as well. The most effective

management program will integrate multiple strategies to reduce infection and mycotoxin contamination (McMullen et al. 2008; Blandino et al. 2012).

Cultural control methods include selecting the most resistant cultivars, crop rotation, and tillage to reduce infection and sources of pathogen inoculum. Choosing the least susceptible cultivars with different maturities or staggering planting between fields are basic management practices (McMullen et al. 2012; Friskop et al. 2018). Data on the susceptibility to FHB and other diseases is provided in cultivar descriptions. Currently, only moderately resistant (MR) cultivars are registered in the Maritimes such as Island barley (Choo et al. 2003) and 25R40 winter wheat (CFIA 2010). FHB resistance is a quantitative trait, slowing breeding efforts towards resistant cultivars (Bai et al. 2018). Five types of host resistance have been described: Type I, resistance to initial infection; Type II, resistance to spread of infection; Type III, resistance to kernel infection; Type IV, tolerance; Type V, resistance to accumulation of toxins (Mesterházy 1995). A source of type II resistance in wheat comes from Sumai3 and its descendants (Bai and Shaner 2004). There is no known, single source of resistance in barley although it has natural type II resistance. Greater resistance to FHB in barley has been derived from 6-row Chevron, and 2-row cultivars Zhedar 1 and 2 and Harrington (Rudd et al. 2001).

Some wheat and barley cultivars have phenotypes that are beneficial to avoiding *Fusarium* infection. Taller cultivars with seed heads further from the soil surface may escape inoculum dispersal but are associated with lower yields and lodging (Choo et al. 2004). Cultivars with improved lodging resistance should also be considered as adverse weather events can topple plants resulting in seed heads close to the soil where humidity and inoculum levels are increased (Choo et al. 2004).

Crop rotation is perhaps the most important agronomic practice because wheat and barley, following another host crop of *F. graminearum*, such as corn, provides ample inoculum for infection. Host crop residues support overwintering as *F. graminearum* survives saprotrophically on dead plant tissue (Leplat et al. 2013). Incidence and severity of FHB in wheat has been found to increase when wheat follows corn in rotation (Dill-Macky and Jones 2000; Schaafsma et al. 2005; Tillmann et al. 2017). Dill-Macky and Jones (2000) found DON accumulation in wheat preceded by soybeans was reduced by 25% and 49% compared to wheat preceded by wheat or corn, respectively. Few studies observe rotational effects on FHB in barley; however, a Saskatchewan study found an increase of *F. graminearum* in barley seeds after pulse and oilseed crops compared to previous cereal crops (Fernandez et al. 2007).

Development of perithecia and dispersal of primary inoculum may be impeded by tillage as colonized crop and weed residues are buried (Pereyra and Dill-Macky 2008). *Fusarium graminearum* can survive for at least two years on infected wheat and corn residues on or above the soil surface (Khonga and Sutton 1988; Pereyra et al. 2004). Khonga and Sutton (1988) found no perithecia or macroconidia were produced on buried residues while Pereyra *et al*. (2004) found buried residues decayed faster with increased microbial activity than residues on the soil surface. Dill-Macky and Jones (2000) found that chisel plough and no-till systems resulted in greater disease incidence and severity of FHB in comparison to moldboard ploughed fields however, crop rotation away from cereals was found more effective than tillage practice to reduce FHB. Similarly, Schaafsma *et al*. (2005) found FHB index and DON in winter wheat was higher when following a corn crop with minimum or no-till systems. With adoption of soil conservation practices, incidence of FHB has increased since the early 1990s (Aboukhaddour et al. 2020), which further emphasizes the importance of crop rotation.

Chemical control options for FHB are limited and only provide suppression when environmental conditions are forecasted to be optimal for disease development at anthesis and head emergence. Triazoles are a group of fungicides known as demethylation inhibitors (DMI) belonging to FRAC resistance group 3 (FRAC 2022), which prevent sterol production essential for the development of the fungus after spore germination (Mueller 2006). Fungicide treatment can significantly increase yield and reduce FHB and DON. The effectiveness, however, is dependent on weather and application timing as well as disease intensity and cultivar resistance (Paul et al. 2010).

Prosaro® XTR (Bayer CropScience Inc.) is a triazole product available in Canada. This fungicide is formulated with prothioconazole and tebuconazole for suppression of FHB and control of certain leaf diseases. In wheat, the application of Prosaro® XTR should occur between 75% of main stem emergence, and until 50% of main stem heads have reached anthesis. In barley, the application should take place when 70-100% of heads have completely emerged on main stems until 3 days after full head emergence (Bayer CropScience Inc.).

A recent addition to the Canadian marketplace is Miravis® Ace (Syngenta Canada Inc.), registered for suppression of FHB and control of certain leaf diseases of wheat and barley. Miravis® Ace is formulated with propiconazole and includes a FRAC resistance Group 7 active ingredient, pydiflumetofen, belonging to the chemical group N-methoxy-(phenylethyl)-pyrazole carboxamides (FRAC 2022). Group 7 products function as succinate dehydrogenase inhibitors (SDHI), blocking the enzyme from its involvement in fungal cell respiration. Miravis® Ace can be applied to wheat when at least 75% of main stem heads have fully emerged to when 50% of the main stem heads have reached anthesis. In barley, Miravis® Ace may be applied when 70%

of the main stem heads have emerged until 3 d after full head emergence (Syngenta Canada Inc. 2021).

Suppression of FHB may alternatively be achieved with organic amendments or biological control agents (BCAs). BCAs are naturally occurring organisms that are antagonists of pests and are non-phytotoxic. These organisms work by way of competition, antibiosis, mycoparasitism, induction of host resistance, or plant growth promotion (Legrand et al. 2017; Köhl et al. 2019). Chitosan, an extract of crustacean shells has been evaluated as a potential organic control for *F. graminearum*. Preventative applications of chitosan on wheat heads in a greenhouse study significantly reduced FHB severity compared to untreated heads (Deshaies et al. 2022). Chitosan is also noted to induce plant defense mechanisms (El Hadrami et al. 2010). Numerous microorganisms including *Bacillus* spp., *Cryptococcus* spp., *Trichoderma* spp., and *Clonostachys rosea f. rosea* ((Link) Schroers; synonym: *Gliocladium roseum*) have been studied for control of FHB (Schisler et al. 2002; Gilbert and Fernando 2004; Xue et al. 2009). BCAs may be applied like fungicides at head emergence and anthesis to supress fungal growth, as well as to the senescing plant and residues to prevent perithecia development (Gilbert and Fernando 2004; Legrand et al. 2017). Only the bacterial agent, *Bacillus subtilis* var. *amyloliquefaciens* Strain FZB24 is available commercially and registered for FHB on wheat under the product name Taegro® 2 (Novozymes BioAg Limited 2021).

C. rosea is a necrotrophic fungus in the family *Bionectriaceae*, phylum *Ascomycota*, which functions as a mycoparasite and biocontrol agent (Sun et al. 2020). *C. rosea* first secretes cell wall degrading enzymes then parasitizes the host fungus (Chatterton and Punja 2009). *C. rosea* ACM941, isolated from field pea has been evaluated for its efficacy to control *F. graminearum* and work has begun to commercialize production and register the organism for

FHB management (Xue et al. 2009; Xue et al. 2014). Applications of *C. rosea* have resulted in significant reductions of infected spikelets, FDK, FHB index and DON. *C. rosea* however, was less effective than triazoles and DON was not reduced below 1 ppm in an epidemic (Xue et al. 2009; Xue et al. 2014). Future use of BCAs would be best suited in an integrated management plan on organic farms and in combination with conventional practices. However, their effectiveness as living organisms often relies on similar environmental conditions to those of the pathogen (Gilbert and Fernando 2004).

Each of the above methods have demonstrated the ability to reduce FHB and DON contamination, however they cannot stand alone. Integrated management of using multiple strategies is most effective. Blandino *et al*. (2012) reported a 97% reduction of DON in winter wheat when a field was ploughed and planted to a MR cultivar with a fungicide application at heading compared to an untreated susceptible cultivar in a no-till situation. Greater reductions of FHB and DON under favourable conditions were found when an MR cultivar was treated with triazole fungicides than a treated susceptible cultivar (McMullen et al. 2008; Wegulo et al. 2011; Amarasinghe et al. 2013). Similar effects of *C. rosea* were also described with greater FHB control in MR cultivars (Xue et al. 2014). Studies specific to FHB management in barley are limited and often present inconsistent results however, the same on-farm mitigation strategies apply (Tekauz et al. 2000; Horsley et al. 2006; Choo 2009).

Cereal producers in the Maritimes should consider an integrated management plan to mitigate impacts of FHB, especially those with a field history of FHB, and previous cereal crop production. Although the economic cost and sporadic nature of FHB make the decision to apply a fungicide uncertain, a well-timed fungicide application may reduce FDK and DON contamination, improving grain quality and yields over an untreated crop (Mueller 2006). This

decision-making process may be enhanced by the development of a disease forecasting system which delivers advanced warnings of elevated disease pressure with appropriate environmental conditions.

1.4.1 Disease Forecasting

Disease forecast modeling provides information on environmental conditions and periods of crop susceptibility that will be suitable to the epidemic development of disease (van Maanen and Xu 2003). There are many disease forecasting tools used around the world to predict epidemics in important crops including FHB in wheat production. FHB models are diverse in their objectives, development, and prediction criteria. When used in an integrated management plan for FHB, disease forecasts function as a decision support system for fungicide application.

Forecasting models may be empirical or mechanistic, with the former being easier to understand and implement. Empirical methods include descriptive models that use environmental data and statistical models to elucidate the relationship between environmental conditions and disease development to predict occurrence and severity of disease epidemics (van Maanen and Xu 2003; Prandini et al. 2009). Mechanistic models rely on theory rather than observation, making their development more difficult to understand and apply, however, this allows for improved modeling of genotype and environment interactions (Prandini et al. 2009).

FHB forecasting models for wheat have been developed and or implemented for use in cereal growing regions of the United States (De Wolf et al. 2003; Molineros 2007; Bondalapati et al. 2012; Shah et al. 2013; USWBSI 2022), Canada (Hooker et al. 2002; Alberta Climate Information Service 2022; SaskWheat Development Commission 2022), Argentina (Moschini and Fortungo 1996; Moschini et al. 2001), Switzerland (Musa et al. 2007), Belgium (Landschoot et al. 2013), and Italy (Rossi et al. 2003). Molineros (2007) summarized the

development and use of the models listed above. The objective of these models is to predict epidemic risk, FHB index, FHB severity or DON accumulation as the response variable. Independent variables may include time frames of daily or hourly temperature (°C), rainfall (mm), and/or relative humidity (% RH) surrounding head emergence or anthesis of wheat or barley. Until 2005, information on crop residue management or cultivar susceptibility were not included in model calculations but are now incorporated in American forecasts by the US Wheat and Barley Scab Initiative (USWBSI) (Molineros 2007).

FHB forecasting is not without limitations. Many models predict FHB based on forecasted weather conditions; therefore, the accuracy of FHB models rely on the accuracy of weather forecasts (De Wolf et al. 2003). Variability in environment, pathogen population and management also complicate modeling for large scale, regional use (Molineros 2007). If models are to be used outside of their region of origin, they must first be calibrated before implementation (Prandini et al. 2009) as causal species of FHB may have different adaptations to the local environment (Xu et al. 2008). To make the most of model predictions, crop growth stage must be accurately recorded as this determines fungicide timing. Evaluating growth stage in large fields can be difficult due to the heterogeneous nature of field conditions resulting in varied crop stage throughout (Wegulo et al. 2015).

Several established FHB forecasting models have been evaluated under weather conditions of Quebec to support future implementation (Giroux et al. 2016). The study compared forecasted FHB epidemics to independent crop damage indicators at multiple locations over two years to determine the most accurate model using the published decision thresholds as well as adjusted thresholds. It was determined that empirical models with thresholds adjusted for the regional environment performed best in Quebec (Giroux et al. 2016).

FHB forecasting models have not been established in the Maritimes but may benefit cereal producers. This advanced warning system guides fungicide application decisions with timely recommendations and could therefore, prevent unnecessary applications and production costs (Wegulo et al. 2015). Reduced fungicide use could also prevent non-target species exposure and development of fungicide resistance (Hosack and Miller 2017). A disease warning system would also provide time for grain handlers and food processors to obtain necessary infrastructure to test for mycotoxins and manage damaged grain (De Wolf et al. 2003).

1.5   Research Objectives and Hypotheses

The literature review has identified gaps in the research pertaining to the causal species of FHB in the Maritime provinces and the suitability of FHB forecasting models in the region. Limited disease surveillance of FHB in the region has not confirmed that *F. graminearum* is the primary causal species of FHB. It is important to characterize the Maritime populations to support the use of FHB forecasting as fungicides may not adequately suppress virulent or resistant isolates of *F. graminearum*. FHB forecasting models have been developed with environmental variables that support the development of *F. graminearum* and DON which may not be the primary species or mycotoxin produced in the Maritimes. Increased surveillance identifying the most abundant species and associated mycotoxins would further support regional management practices. Additionally, improved fungicide application decision making could curtail fungicide use, reducing production costs. Mycotoxin contamination and yield losses could also be minimized with improved management of FHB.

The information presented has led to the following research objectives:

1. Conduct surveillance in wheat and barley to determine species composition of Maritime populations of *Fusarium* spp. causing FHB.

2. Determine mycotoxin genotypes, virulence, and fungicide susceptibility of *F. graminearum* found in the Maritimes.

3. Evaluate the suitability of published FHB forecasting models for use in the Maritimes.

4. Evaluate the efficacy of fungicide application when guided by forecasting models.

Experiments and data analysis will be conducted to meet these objectives and test the

following hypotheses:

- The species composition of FHB causal population will vary among provinces. The primary causal species will vary annually with respect to environmental conditions before host anthesis and heading.

- Both 3-ADON and 15-ADON chemotypes of *F. graminearum* will be present in the Maritimes with 3-ADON being more abundant.

- Virulence and fungicide sensitivity will be variable across the region.

- FHB forecasting models developed in similar Maritime climate conditions or using pre-anthesis weather variables will have superior performance.

- Fungicide application will be economically viable only with elevated risk of disease.

This research contributes to the project "Forecasting and managing Fusarium head blight in Atlantic Canada" which is made possible by Agriculture and Agri-Food Canada (AAFC) in partnership with the Atlantic Grains Council (AGC) through the Canadian Agricultural Partnership (CAP) AgriScience Project ASP-008 Activity 3 (CAP ASP-008-Activity #3)

Chapter 2 - Characterizing Fusarium Head Blight Causal Species in the Maritime Provinces of Canada

2.0 Abstract

Fusarium head blight (FHB) is caused by many toxigenic *Fusarium* spp. and is a major concern for wheat and barley producers in the Maritime provinces of Canada. Severe symptoms associated with consumption of deoxynivalenol (DON), and its derivatives produced by *F. graminearum* make this the species of greatest impact. Although yield and quality are reduced by the fungus, the population dynamics of FHB and *F. graminearum* are not well understood in the Maritimes. The objective of this research is to determine the primary causal species of FHB and associated mycotoxins in the Maritimes and characterize *F. graminearum*. Grain samples were collected from sites across Nova Scotia (NS), New Brunswick (NB) and Prince Edward Island (PE) from 2018-2021. *Fusarium* spp. and mycotoxin genotypes were identified using PCR assays. Mycotoxins were quantified by LC-MS/MS and an ELISA lateral flow device. Fungicide sensitivity of *F. graminearum* was also evaluated. In 2018 and 2021, *F. graminearum* was the primary causal species and is the primary cause for concern in the region. DON was the most abundant mycotoxin detected in all years of the study. The *TRI*3 3-ADON genotype was found to be dominant at 71.43% over 15-ADON at 28.57%. Fungicide sensitivity of *F. graminearum* was variable and significant differences were found among year, province, host crop and, *TRI3* genotype. The results of this study serve as the first multi-year report on FHB in the Maritimes, providing a baseline study for future research.

Keywords: *F. graminearum*, Fusarium head blight (FHB), wheat, barley, mycotoxins, *TRI3* genotype

2.1 Introduction

Fusarium head blight (FHB) is a globally important disease of cereal crops. The disease is most often incited by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schweinitz) Petch.) among many other causative species including *F. sporotrichioides* Sherb., *F. avenaceum* (Fr.) Sacc., and *F. poae* (Peck). Substantial economic and grain quality losses can occur when FHB epidemics develop (Dahl and Wilson 2018). Wheat and barley are important crops in the Maritime provinces of Canada where producers have incurred losses due to this devastating disease.

The most important grain quality considerations are mycotoxins. Mycotoxins are secondary metabolites of fungal growth, imparted in the grain by *Fusarium* species. The β-trichothecene, deoxynivalenol (DON) is most commonly associated with Fusarium head blight caused by *F. graminearum* (Schmale and Bergstrom 2003). Human and animal health is negatively impacted by the consumption of these mycotoxins therefore, regulatory restrictions and guidelines have been developed by food inspection agencies globally to limit the concentration of mycotoxins in food and feed stuffs.

The population of FHB causal species is dynamic and varies in species dominance by region and climate (Karlsson et al. 2021). The population *F. graminearum* also has diverse genetics and phenotypes. The population of *F. graminearum* is often defined by the trichothecene genotype, 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), and most recently NX-2, determined by molecular genetic tests targeting the trichothecene (*TRI*) gene cluster (Ward et al. 2002; Liang et al. 2014). 3-ADON has been indicated as the dominant genotype in the Maritimes having replaced the once dominant, but still present, 15-ADON genotype (Ward et al. 2008; Kelly et al. 2015). *Fusarium*

graminearum with the 3-ADON genotype have greater reproductive rates and produce more DON than 15-ADON populations (Ward et al. 2008; Gilbert et al. 2010).

Across Canada, *F. graminearum* is the species of primary concern due to its frequency compared to other causal species of FHB and the toxicity of DON. This species is of concern in the Maritimes as well, however surveillance publications have only reported the severity of FHB until 1996 (Martin and Johnston 1997; Martin 2004). Regional FHB surveillance allows researchers to establish a baseline of disease-causing populations, monitor for and predict changes in species composition, virulence, and fungicide susceptibility over time and as the climate changes. This knowledge also provides valuable insight to cereal producers of the effectiveness of management strategies. Therefore, the objective of the following study was to survey the region to increase knowledge of the composition of *Fusarium* spp. population causing FHB and associated mycotoxins in the Maritimes. The dominant mycotoxin genotype of *F. graminearum* and fungicide susceptibility was also determined.

2.2 Materials and Methods

2.2.1   Grain Sample Collection

Wheat and barley grain samples were provided by the Atlantic Grains Council (AGC) on-farm agronomy sites across the Maritimes each year from 2018 to 2021 (Figure 2.1). At each site, 2-4 five-acre fields were harvested and a single subsample of about 500 g were received from each field (Table 2.1). A total of 175 barley and wheat samples were received from the AGC; however, no wheat was received in 2019. Wheat samples totaled 4, 19, and 20 in 2018, 2020, and 2021, respectively, and barley accounted for the remainder of total samples received.
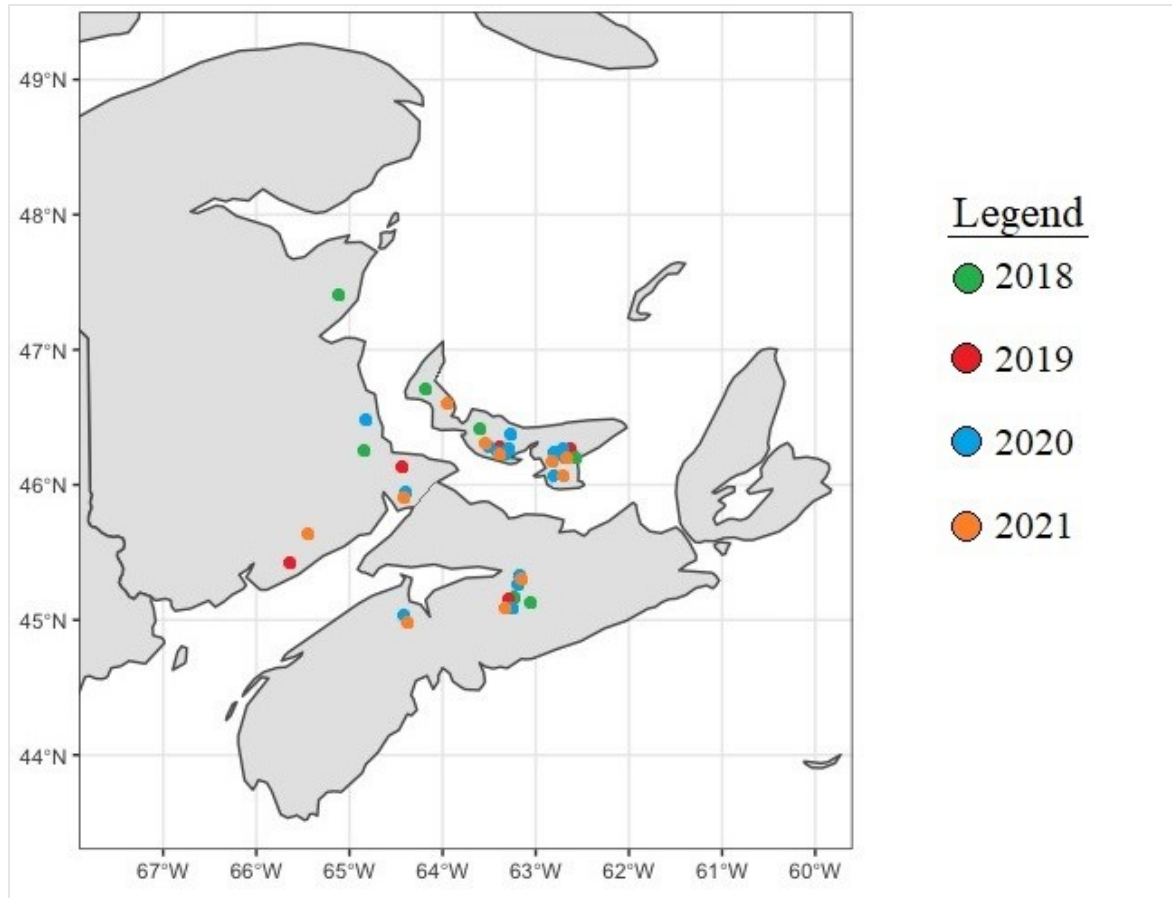
Figure 2.1 Map of the Maritime provinces with approximate AGC sample site locations.

Table 2.1 Total fields and grain samples received from each Maritime province from 2018 to 2021.

Sample information	2018			2019			2020			2021		
	NB	NS	PE	NB	NS	PE	NB	NS	PE	NB	NS	PE
# Sites	2	2	5	2	1	2	2	3	8	2	3	7
# Fields	7	6	13	8	4	11	8	10	25	7	10	25
# Wheat Samples	0	0	4	0	0	0	0	6	13	0	6	17
# Barley Samples	7	12	9	8	8	15	12	8	20	7	4	18
Total Samples	7	12	13	8	8	15	12	14	33	7	10	35

2.2.2 Isolation of Fusarium

In 2018 and 2019 *Fusarium* was isolated according to Halliday (2020) and modified in following years to improve *Fusarium* isolation and prevention of contamination. Seeds from each field sample were surface sterilized in 70% ethanol for 30 s with agitation. Sterilized seeds were placed 10 to a plate with PDA amended with pentachloronitrobenzene (1 mg mL^{-1}), tetracycline (50 µg mL^{-1}), streptomycin (50 µg mL^{-1}), and cefotaxime (250 µg mL^{-1}). A total of 100 seeds were plated per sample in 2020, and 50 seeds per sample in 2021. Plates were incubated at room temperature for 7 d then observed for suspect *Fusarium* growth. Fungal colonies with red, pink, or orange pigmentation and white puffy mycelium were sub-cultured onto fresh PDA plates amended with tetracycline (50 µg mL^{-1}), streptomycin (50 µg mL^{-1}), and cefotaxime (250 µg mL^{-1}). Cultures that maintained *Fusarium* characteristics were again sub-cultured from hyphal tips at the edge of the colony, continuing until a pure isolate was obtained.

2.2.3 DNA Extraction from Culture

Colony DNA was extracted by scraping mycelium from each isolate into a micro centrifuge tube with 50 µL nuclease-free water (Qiagen), then microwaved for 30 s, incubated for 1 min at 99°C, then cooled on ice. This method was modified in 2021 where colony mycelium was instead scraped into a micro centrifuge tube with 200 mL AE buffer (Qiagen) and approximately 40 µg acid-washed silicon dioxide sand (Sigma-Aldrich). Each sample was ground using a Fisherbrand bead mill 24 homogenizer (FisherScientific) for 45 s at 3 m s^{-2} (3 x 15 s with 2 s breaks) then centrifuged at 7100 RPM for 1 min (Eppendorf 5430 R).

2.2.4 Identification of Fusarium Isolates

Isolated *Fusarium* spp. were first identified to species by observing morphological and phenotypic traits as defined in the Fusarium Laboratory Manual (Leslie and Summerell 2006), including mycelium colour, conidiophore, and spore shape. This identification was confirmed by molecular techniques.

In 2018, *Fusarium* spp. were identified by classical molecular techniques according to Halliday (2020). In 2019 and 2020 molecular PCR identification was performed with TEF1α primers to identify *Fusarium* species and ITS primers to confirm genus *Fusarium* when species identification failed (Table 2.2). Reactions consisting of 30 µL Phusion Green Hot Start II High Fidelity master mix (Thermo Scientific) and 2 µL of colony DNA as template were prepared with 1 µL each forward and reverse primer and 16 µL nuclease free water. A SimpliAmp™ thermal cycler (Applied Biosystems™) was preheated and amplification carried out using the following conditions: 2 min denaturation at 98°C, 35 annealing cycles of 10 s at 98°C, 20 s at 58°C and 15 s at 72°C, followed by 1 min extension at 72°C. PCR products were separated on a 1% agarose gel stained with SYBR™ Safe (Thermo Scientific) by electrophoresis then observed with a blue light E-Gel® imager (Thermo Scientific). The remaining PCR products were purified using the Qiagen MinElute kit following manufacturer's recommendations with an elution volume of 40 µL before sequencing by Eurofins Genomics (Toronto, Ontario). Sequencing results were analyzed by NCBI BLAST against fungi type and reference material of the internal transcribed spacer region (ITS) database and translation elongation factor 1 (TEF) databases.

Table 2.2 Primers used in PCR assay to identify *Fusarium* spp..

Primer Name	Target gene	Sequence	Source
ITS1F	ITS1	CTTGGTCATTTAGAGGAAGTAA	White *et al*., 1990
ITS4		TCCTCCGCTTATTGATATGC	
EF1	TEF-1α	ATGGGTAAGGA(A/G)GACAAGAC	O'Donnell *et al*., 1998
EF2		GGA(G/A)GTACCAGT(G/C)ATCATGTT	

In 2021, quantitative PCR (qPCR) was used for molecular identification of isolated *Fusarium*. qPCR allowed for a more rapid identification of *Fusarium* spp. compared to conventional PCR and sequencing methods. Two duplex reactions of 15 µL consisting of 7.5 µL PrimeTime Gene Expression Master Mix (Integrated DNA Technologies) and 3 µL of colony DNA template were prepared with 0.75 µL of each species-specific probe and primers for *F. graminearum* (*sensu stricto*) and *F. sporotrichioides* in the first reaction and *F. avenaceum* and *F. poae* in the second (Table 2.3). Reactions were run in 96-well plates in a Bio-Rad CFX thermal cycler under the following conditions: 3 min denaturation at 95°C, followed by 39 annealing/extension cycles of 5 s at 95°C and 40 s at 60°C. *Fusarium* isolates unresolved to species by qPCR followed PCR and sequencing methods described above.

Table 2.3 Primers used in qPCR assay to identify *Fusarium* spp. and quantify *Fusarium* spp. DNA.

Primer Name	Target organism	Sequence 5'-3'
FgR4-F		TGCGGCTTTGTCGTAATTTTTTYCCC[a]
FgR4-R	*F. graminearum* sensu stricto[1]	AGTGACTGGTTGACACGTGATGATGA
FgR4-Pr		FAM/CAGGCGTCT/ZEN/GCCCTCTTCCCACAAACCA/3IABkFQ
SpoF		TTTTTACGGCTGTGTCGTGA
SpoR	*F. sporotrichioides*[2]	5'-CGGCTTATTGACAGGTG
SpoPr		HEX/TGATAGTGG/ZEN/GGCTCATACCC/3IABkFQ
AveF		GCTTATCTGCACTCGGAACC
AveR	*F. avenaceum*[2]	CGCGTAATCGAAGGGATATT
AvePr		FAM/CGACAAGCG/ZEN/AACCATCGAGA/3IABkFQ
Fp-ACL1-F160		CCATCCCCAAGACACTGAG
Fp-ACL1-R330	*F. poae*[2]	TACAAGTTGCTRCAAGCCC
PoaeACL1_poae1_pr		HEX/GTTCTTCTC/ZEN/AGGACTTTACCCCGAAAGCC/3IABkFQ

[1] Hafez *et al.* (2021)   [2] Zitnick-Anderson *et al.* (2018)
[a] Y = C or T
HEX/ = green fluorescent dye; FAM/ = blue fluorescent dye; /ZEN/ = internal quencher ; /3IABkFQ = Iowa Black quencher

2.2.5   Grain Sample Preparation

A 15-20 g sub-sample of whole grain was ground in an IKA Tube Mill (IKA Works, Inc.) for a total of 30 s (3 x 10 s with 2 s breaks) at 25,000 RPM. Ground grain was required for DNA extraction and mycotoxin analysis.

2.2.6   DNA Extraction from Grain

DNA was extracted from a 100 mg sub-sample of ground grain using Qiagen DNeasy Plant Mini Kit following the manufacturers protocol and recommendations. DNA concentrations were determined using NanoDrop™ One spectrophotometer (Thermo Scientific).

2.2.7 Quantification of *Fusarium* spp. DNA

DNA was extracted (Section 2.2.6) from grain samples (Section 2.2.5) and normalized to 20 ng μL^{-1}. Samples from 2019 were not included in statistical analysis due to poor DNA recovery. qPCR quantification in 2018 followed the procedure of Halliday (2020). In 2020 and 2021, using species specific probes and primers listed in Table 2.3, DNA of each identified *Fusarium* spp. was quantified from 20 µl duplex reactions consisting of 1 µl of each probe and primer and 4 µl DNA template. qPCR conditions are as described in section 2.2.4. DNA standards for qPCR analysis developed from cultures from the Canadian National Mycological Herbarium (DAOM) including *F. graminearum* A-11-1-4 114, *F. sporotrichioides* DAOMC 238880, *F. avenaceum* DAOMC 238866, and *F. poae* A-3-3 35. DAOM cultures were first grown on PDA then sub-cultured to 50 mL PDB for 7 d in culture flasks (VWR) and shaken at 250 RPM at 28°C. DNA was extracted using the Qiagen Mini Plant kit. Four DNA standards of each species were prepared by tenfold serial dilution of 2 ng μL^{-1}, 0.2 ng μL^{-1}, 0.02 ng μL^{-1}, and 0.002 ng μL^{-1}. *Fusarium* DNA abundance was determined by dividing total DNA of each *Fusarium* spp. quantified (ng rxn^{-1}) by the amount of template DNA (ng rxn^{-1}) used in each reaction (% DNA = ng rxn^{-1} quantified/ ng rxn^{-1} template).

2.2.8 Mycotoxin Genotype

The mycotoxin genotype of each *F. graminearum* isolate was determined by PCR using primers targeting the *TRI3* gene in a multiplex reaction (Ward et al. 2002). 20 µL duplex reactions were prepared with 10 µL Phusion and 1 µL each of 3CON and 3D3A to identify the 3-ADON genotype and 3CON and 3D15A in another reaction to identify the 15-ADON genotype (Table 2.4) each with 2 µL template DNA normalized to 20 ng μL^{-1}. Template DNA extraction from culture was modified from Section 2.2.3 where instead of AE buffer, a small amount of

yatalase, 400 µL AP1 buffer, 2 µL RNase A, and 2 µL Reagent DX were added to the grinding tube. After grinding, the DNeasy mini plant kit extraction protocol was used. Amplification occurred under the following PCR conditions: 2 min denaturation at 98°C followed by 35 cycles of 10 s at 98°C, 20 s at 60°C and 5 s at 72°C, followed by 2 min extension at 72°C. Annealing temperature was reduced to 56°C in the 3D3A assay. PCR products were observed with a blue light E-Gel® imager (Thermo Scientific) on a 1% agarose gel stained with SYBR™ Safe (Thermo Scientific) separated by electrophoresis.

Table 2.4 Primers used in PCR assay to assign *TRI3* mycotoxin genotype (Ward et al. 2002).

Primer Name	Target	Sequence	Product size (bp)
3CON	*Tri3*	TGGCAAAGACTGGTTCAC	
3D3A	3-ADON	CGCATTGGCTAACACAT	243
3D15A	15-ADON	ACTGACCCAAGCTGCCATC	610

2.2.9 Mycotoxin Analysis

Each grain sample received from the AGC was subjected to mycotoxin analysis. DON quantification was conducted using Vicam DON-V lateral flow strip tests and quantified by Vicam Virtu lateral flow reader (Waters™ Vicam; Milford, MA, USA) following manufacturer protocol using 5 g ground grain (Section 2.2.5). Samples from 2018 were not subjected to Vicam DON quantification.

Multi-mycotoxin analysis was conducted by Dr. Justin Renaud at AAFC London Research and Development Centre, using liquid chromatography and tandem mass spectrometry (LC/MS-MS) on extracts from ground grain samples. The LC-MS/MS protocol followed Crippin *et al.* (2019). Chemical standards of deoxynivalenol-3-glucoside (D3G), NIV, 4-ANIV (fusarenone-X), 3-ADON, 15-ADON, NX and 3-ANX were used.

2.2.10 Fungicide Sensitivity

A subset of 364 *F. graminearum* isolates collected in 2018 to 2021 were screened for their susceptibility to agricultural fungicides Prosaro® XTR, Folicur®, Miravis® Ace, and Tilt® by adapting the protocol of Anderson *et al*. (2020). The concentration of each fungicide treatment was determined by evaluating growth reduction of 6 random isolates over 5 concentrations of each product. The concentration that achieved ≥ 70 % growth reduction was used. Three isolates from this process were retained throughout experimentation as standards.

The experiment was conducted on 24-well tissue culture plates (VWR). Media were prepared by placing 30 mL molten half strength PDA into sterile centrifuge tubes and cooling to about 60°C in a water bath. Once cooled, the PDA was amended with respective fungicide product and inverted 10 times to thoroughly mix (Table 2.5). Each well of the tissue culture plate received 1 mL amended media. A 2-4 d old *F. graminearum* isolate was then sub-cultured into a single well of each product. The plates were sealed 2x with Parafilm (Bemis) and placed in an incubator (Heracell™, Thermoscientific) at 25°C in the dark for approximately 24 h. The time was noted when plates entered the incubator and again when measurements were taken.

Table 2.5 Concentrations of fungicide active ingredient added to media for fungicide sensitivity screening.

Fungicide Product	Active Ingredient (μg mL^{-1})			
	Prothioconazole	Tebuconazole	Propiconazole	Pydiflumetofen
Prosaro® XTR	0.1	0.1		
Folicur®		0.1		
Miravis® Ace			0.083	0.1
Tilt®			1	

Radial growth of cultures was measured with a digital caliper (VWR), taking two perpendicular measurements, and using the average to calculate % growth reduction (GR) compared to the untreated controls (Eq 2.1). Growth reduction was normalized for statistical analysis by determining mean growth of standards under each treatment and calculating the % GR. The %GR of test isolates was then subtracted from %GR of standards under the respective treatment (Eq 2.2).

$$\% \text{ GR} = \left(100 - \left(\frac{\text{average diameter of treated colony}}{\text{average diameter of control colony}} \right) \right) \times 100 \tag{2.1}$$

$$\text{Normalized \% GR} = \% \text{ GR of standard isolates} - \% \text{ GR of treated isolate} \tag{2.2}$$

2.2.11 Koch's Postulates

Twenty-four *F. graminearum* isolates were selected to verify Koch's Postulates on wheat and barley (25 treatments x 2 species x 3 reps; Byrd and Segre 2016). Isolates from 2020 and 2021 were selected randomly from all three Maritime provinces as well as a 2016 reference isolate. *Fusarium graminearum* was grown on fresh PDA for 4 d then sub-cultured onto Spezieller Nährstoffarmer agar (SNA) and incubated for 7-10 d to encourage sporulation for single spore isolation. SNA plates were then flooded with sterile Milli-Q® (Millipore Sigma) water and agitated with a sterile loop. The solution was then poured through a 70 μm basket filter into a centrifuge tube and spores were counted using a hemocytometer (Arthur H. Thomas). A volume of 50 μL sterile Milli-Q® water was then used to streak 200 spores on a 100 mm plate of 1% water agar. The next day, water agar cultures were observed under the microscope where 3-4 germinating spores were marked for isolation. Each spore was sub-cultured onto a PDA plate.

A single spore isolate of each *F. graminearum* culture was selected for subculture into mung bean (MB) liquid sporulation media. Conical culture flasks (VWR) containing 25 mL MB

cultures were placed in a shaker incubator (New Brunswick Scientific) at 28°C and shaken at 200 RPM. To extract spores from liquid media, MB cultures were shaken vigorously, poured through a 70 µm cell strainer into a sterile centrifuge tube then centrifuged at 7100 RPM for 5-10 min. The supernatant was poured off leaving the pelleted spores in the tube. Spores were re-suspended in 25 mL sterile Milli-Q® water. Total spores of each isolate were counted using a hemocytometer then spray suspensions were diluted to 20k spores mL^{-1}.

'Island' barley and 'AC Walton' wheat were grown in a greenhouse. Four seeds were planted into Pro-Mix BX (Premier Tech) in a 15 cm diameter pot then thinned to a single plant per pot after emergence. Plants were fertilized weekly with quarter strength Hoagland's No. 2 Basal Salt Mixture (Sigma-Aldrich) from two weeks after emergence until boot stage. At barley head emergence and wheat anthesis each plant received an application of approximately 7 mL of a single *F. graminearum* isolate (2 sprays x 4 rotations). FHB severity ratings were taken on a scale of 0-9 (0 = no infection; 9 = entire head infected) at 7 and 14 DAA (days after application). At crop maturity, wheat and barley heads were removed from the plants, surface sterilized for 30 s in 70% ethanol then placed on PDA amended with antibiotics (Section 2.2.2) to confirm infection by *F. graminearum*.

2.2.12 Statistical Analysis

All statistical analyses were performed using JMP version 16.0 (SAS Institute, Cary, North Carolina, USA). Standard least squares ANOVA was used to determine significant effects of year, province, and species on % frequency of *Fusarium* spp., abundance of *Fusarium* spp. DNA, and mycotoxins detected. Tukey's HSD test ($\alpha \leq 0.05$) was used to determine significant differences among means. The analysis was also blocked by host crop where all factors and interactions were significant in the % frequency of *Fusarium* spp. isolated from barley. Effects

could not be determined for wheat due to the loss of degrees of freedom. Percent frequency of *Fusarium* spp. was calculated as the total isolates of each species per 100 seeds. Products used in the fungicide sensitivity experiment were analysed independently and standard least squares ANOVA was used to determine significant effects of isolation year, *TRI3* genotype and host crop on fungal growth in response to fungicide treatment. Tukey's HSD test ($\alpha \leq 0.05$) was used to determine significant differences among means. Correlation analysis was performed to determine the relationship between % frequency of *F. graminearum* and quantity of *F. graminearum*, quantity of *F. graminearum* DNA and DON contamination, as well as DON quantification methods. Pearson's correlations between % frequency of *Fusarium* spp., quantity of *Fusarium* spp. DNA, *TRI3* genotype and mycotoxins detected were clustered. A principal component analysis (PCA) was also performed to further visualize associations between variables.

Prior to analysis, histograms were generated for each variable to observe data distributions. If the distribution was not normal, the most appropriate distribution with the lowest AIC was applied to each y-variable to set the distribution of ANOVA. The beta-binomial distribution was applied to % frequency of *Fusarium* spp., *TRI3* genotype and disease severity ratings. The Sinh-Arcsinh (SHASH) distribution was applied to quantity of *Fusarium spp.* DNA, and LC-MS/MS mycotoxin quantification data. Exponential distribution was applied to DON quantified by the Vicam kit. Outliers were removed from the fungicide sensitivity analysis as the growth reduction means were significantly changed in their presence. After outlier removal a Normal 3 Mixture distribution was applied to the data.

2.3 Results

2.3.1   Causal species of FHB identified in the Maritimes

Fusarium species were identified with a combination of morphological and molecular methods. Each year, *F. graminearum*, *F. sporotrichioides*, *F. avenaceum*, and *F. poae* were the principal species isolated. In 2020, additional species including *F. tricinctum* (4), *F. asiaticum* (1), *F. equiseti* (1), *F. proliferatum* (1) and *F. sambucinum* (1) were isolated. A single isolate of each *F. cerealis* and *F. culmorum* was recovered in addition to the four principal species in 2021. These additional species were grouped together as 'other' in statistical analyses. The total number of *Fusarium* spp. isolates collected and identified from 2018 to 2021 was 322, 93, 479, and 437, respectively. *Fusarium graminearum* was the most frequently isolated species in 2018 and 2021 while *F. poae* was most frequent in 2019 and 2020 (Figure 2.2).
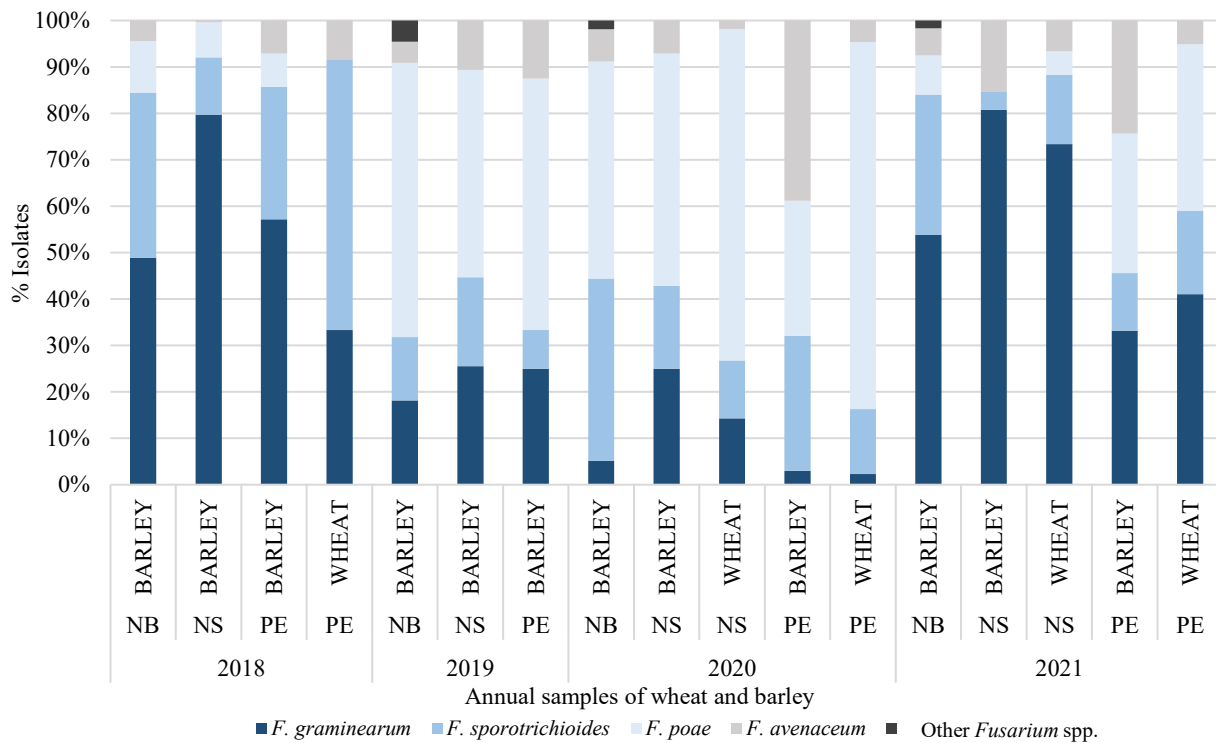
Figure 2.2 Stacked bar representing the proportion of each isolated *Fusarium* spp. each year, from 2018 to 2021 from NS, NB, and PE wheat and barley samples.

The effects of different years, provinces, and *Fusarium* species on the % frequency of

Fusarium spp. isolated from each field sample were analysed with all factors and interactions

being significant (Table 2.6). Percent frequency of *Fusarium* spp. is the frequency at which

each *Fusarium* species was isolated per 100 seeds. Statistical analysis showed similar

significant effects of year, province, and species on abundance of *Fusarium* spp. DNA and the

% frequency of *Fusarium* spp. (Table 2.7). DNA abundance of the four principal *Fusarium*

species was quantified by qPCR from post-harvest grain samples. Results from 2019 were

omitted due to poor DNA recovery. *Fusarium* species was not a significant factor in the

abundance of *Fusarium* spp. DNA however, species interactions were significant.

Table 2.6 ANOVA probability values for % frequency of *Fusarium* spp. isolated from AGC grain samples. Bold text indicates significant effect at $p \leq 0.05$.

Effect	DF	Sum of Squares	F Ratio	Prob > F
Year	3	1081.2	20.5017	**<.0001**
Province	2	542.091	15.4187	**<.0001**
Fusarium spp.	4	1966.96	27.973	**<.0001**
Year*Province	6	685.028	6.4947	**<.0001**
Year**Fusarium* spp.	12	2729.67	12.94	**<.0001**
Province**Fusarium* spp.	8	1172.4	8.3366	**<.0001**
Year*Province**Fusarium* spp.	24	1828.93	4.335	**<.0001**

Table 2.7 ANOVA probability values for abundance of *Fusarium* spp. DNA quantified in AGC grain samples. Bold text indicates significant effect at $p \leq 0.05$.

Effect	DF	Sum of Squares	F Ratio	Prob > F
Year	2	1.32654	14.3233	**<.0001**
Province	2	2.39224	25.8302	**<.0001**
Fusarium spp.	3	0.24139	1.7376	0.1582
Year*Province	4	1.48596	8.0223	**<.0001**
Year**Fusarium* spp.	6	2.4308	8.7489	**<.0001**
Province**Fusarium* spp.	6	2.5291	9.1026	**<.0001**
Year*Province**Fusarium* spp.	12	3.45467	6.217	**<.0001**

There were significant differences in the % frequency of *Fusarium* spp. isolated and abundance of *Fusarium* spp. DNA in each Maritime province, each year (Figure 2.3). In 2018, significantly less *Fusarium* was isolated from PE grain samples than NB and NS. *Fusarium* was most frequently isolated from NS in 2019 however, there were no significant differences between provinces. In 2020 and 2021, *Fusarium* was significantly more frequent in NB grain samples than NS and PE.

Fusarium was significantly more frequent and DNA most abundant in NB fields in 2021 than any other year (Figure 2.3). From NS fields, *Fusarium* was significantly more frequent in 2018 than 2019 and 2020 but was not significantly different between 2018 and 2021. *Fusarium* spp. DNA was also significantly more abundant in NS in 2018 and 2021 than 2020. *Fusarium* spp. were significantly more frequent in PE fields in 2021 than other sample years, however, no significant difference in DNA abundance among years was observed in PE.

A
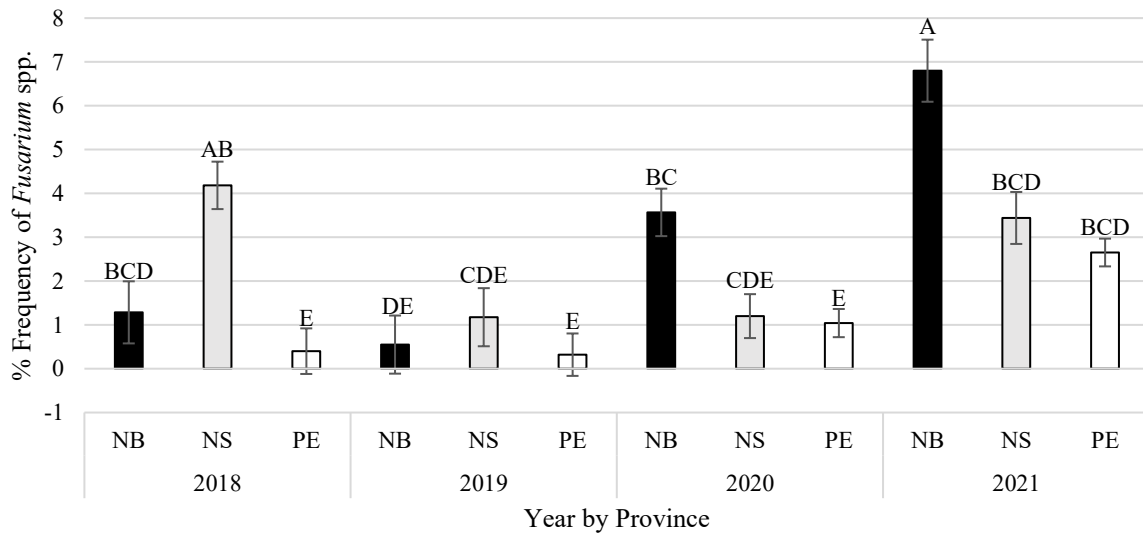
B
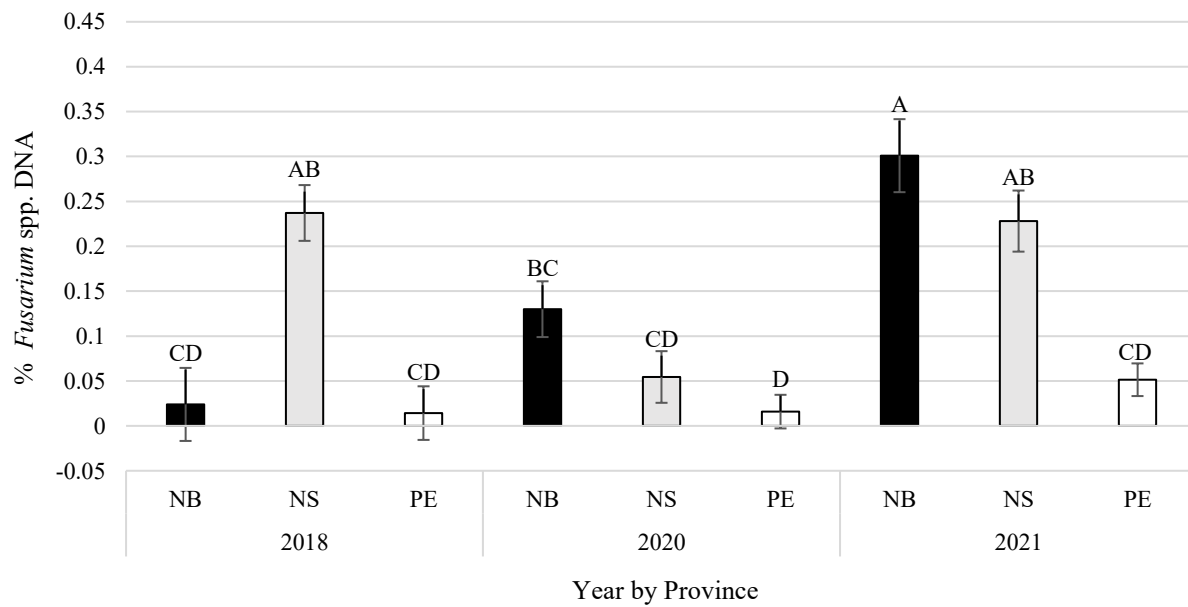
Figure 2.3 (A) Percent frequency of *Fusarium* spp. isolated from each province, each year from 2018 to 2021. (B) Abundance of *Fusarium* spp. DNA in wheat and barley samples from each province, each year. Vertical bars represent standard errors. Bars sharing letters are not significantly different at $\alpha \leq 0.05$ using Tukey's HSD test.

Significant differences in % frequency of *Fusarium* spp. isolated and abundance of *Fusarium* spp. DNA was observed among years and *Fusarium* species. Isolation of *F. graminearum* in 2021 was significantly more frequent than other causal species of FHB from 2018 to 2021 and was significantly more frequent in 2018 and 2021 than 2019 and 2020 (Figure 2.4). Only in 2018 was DNA of *F. graminearum* more abundant than other causal species of FHB. *Fusarium poae* was more frequently isolated than other species except *F. sporotrichioides* in 2020. There were no significant differences in % frequency among species isolated in 2019 or abundance of *Fusarium* spp. DNA in 2020 and 2021.
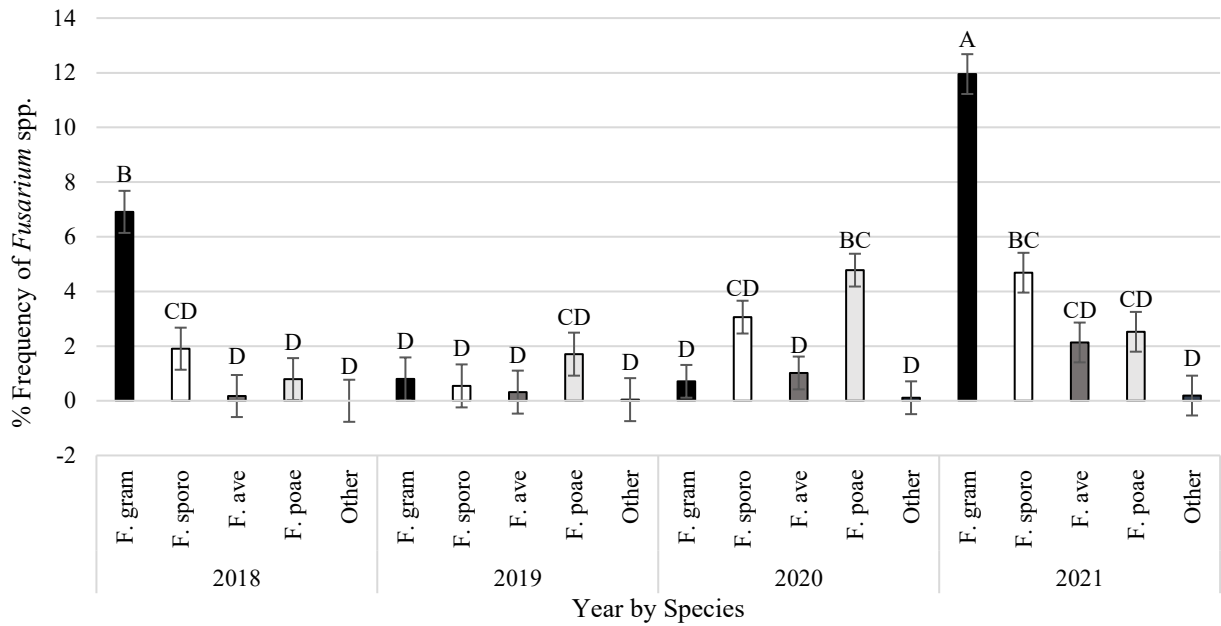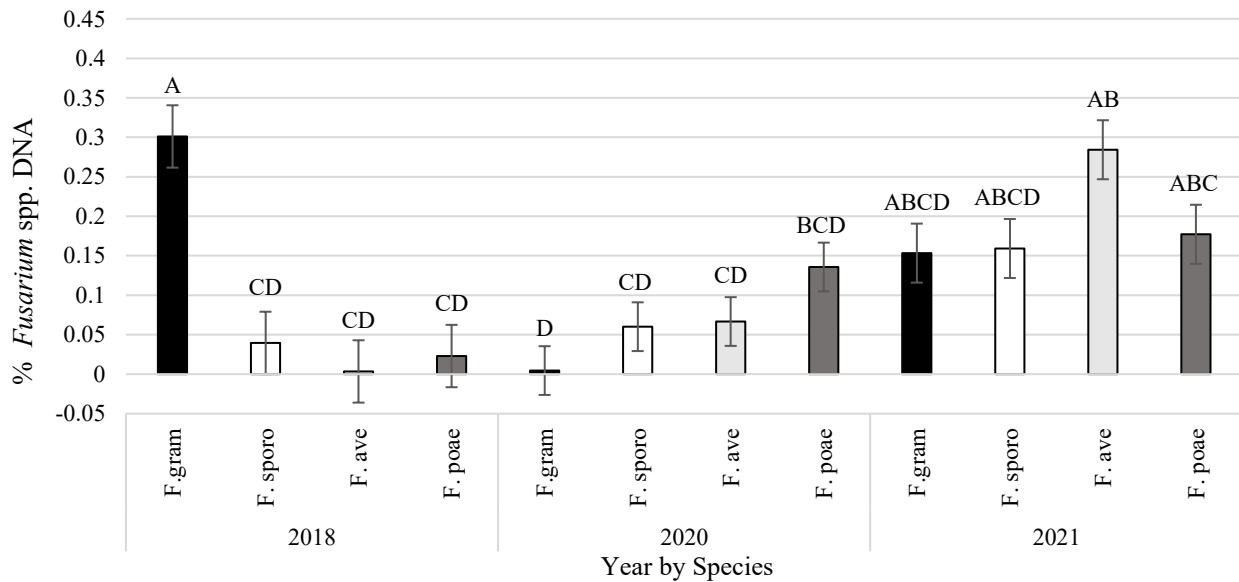
Figure 2.4 (A) Percent frequency of each isolated *Fusarium* spp. each year, from 2018 to 2021. (B) Abundance *Fusarium* species DNA from wheat and barley samples each year. F. gram = *F. graminearum*; F. sporo = *F. sporotrichioides*; F. ave = *F. avenaceum*. Vertical bars represent standard errors. Bars sharing letters are not significantly different at $\alpha \le 0.05$ using Tukey's HSD test.

There were significant differences in % frequency of each *Fusarium* species and DNA abundance among provinces (Figure 2.5). In NB samples, no significant differences were found among the frequencies of *F. graminearum*, *F. sporotrichioides*, and *F. poae* however, significantly less *F. avenaceum* was isolated than *F. graminearum* and *F. sporotrichioides*. Isolation of *F. graminearum* was significantly more frequent in NS than other causal species of FHB and was more frequently recovered in NS and NB than in PE. *Fusarium graminearum* DNA was also most abundant in NS grain samples. No significant differences were among *Fusarium* species for % frequency or DNA abundance in samples from PE.
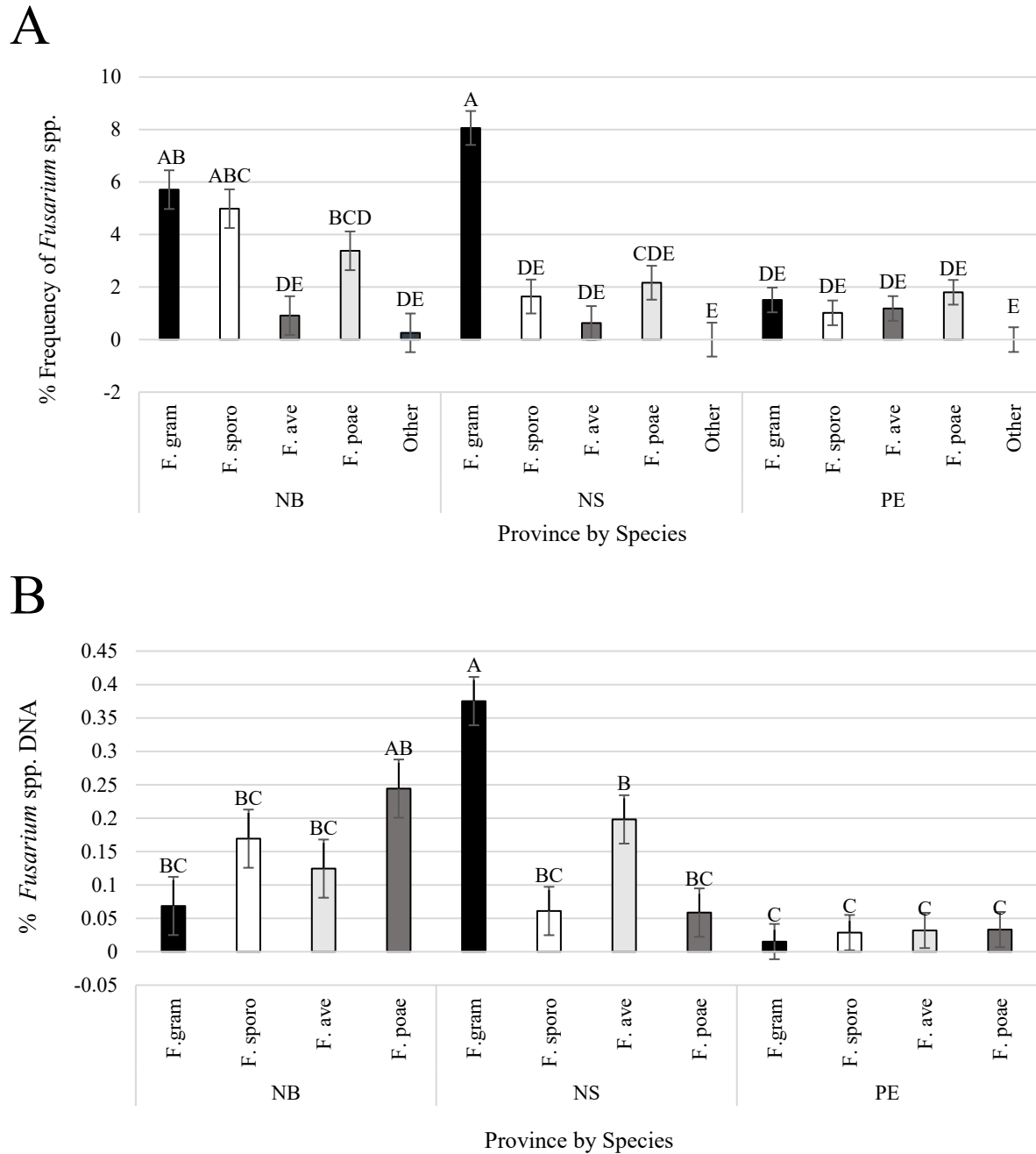
Figure 2.5 (A) Percent frequency of each isolated *Fusarium* spp. from each province. (B) Abundance of Fusarium species DNA from wheat and barley samples from each province. F. gram = *F. graminearum*; F. sporo = *F. sporotrichioides*; F. ave = *F. avenaceum*. Vertical bars represent standard errors. Bars sharing letters are not significantly different at $\alpha \leq 0.05$ using Tukey's HSD test.

2.3.2 Mycotoxin Genotype

A subset of 280 *F. graminearum* isolates collected from 2018-2021 were subject to molecular analysis to determine the *TRI3* genotype. Overall, 3-ADON was the dominant *TRI3* genotype accounting for 71.43% of *F. graminearum* isolates in the Maritime provinces (Figure 2.6). Nova Scotia, however, had the largest proportion *F. graminearum* with the 15-ADON genotype compared to other Maritime provinces.
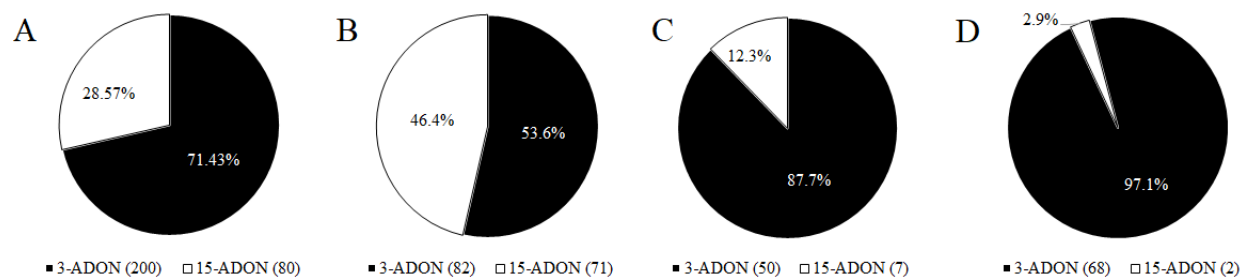
Figure 2.6 Proportion of *TRI3* genotype of *F. graminearum* in (A) the Maritime provinces, (B) NS, (C) NB, and (D) PE.

2.3.3 Mycotoxin Analysis

DON contamination was detected every year from samples across the Maritimes. DON was most frequently detected in NS samples from 2018 to 2020 and most frequently detected in PE samples in 2021. Mean DON contamination in NS was greater than 1 ppm in 2018 and 2021(Table 2.8). In 2018, LC-MS/MS analysis found 13 samples with DON greater than 1 ppm of which 11 were received from Colchester County, NS. The two remaining samples were from Kings and Queens counties, PE. In 2021, two Colchester samples and one Kings, NB sample were above 1 ppm. DON derivative 3-ADON was detected in samples primarily from NS in 2018 and 2021. D3G, the plant detoxified DON product was present each year with a mean value above 1 ppm in 2018. ZEA was detected in samples across the Maritimes except for 2019.

Other Fusarium mycotoxins, T-2 and HT-2 were not frequently detected. 15-ADON, NIV and

NX-2 toxins were not detected in Maritime grain samples.

Table 2.8 Summary of mycotoxins detected by LC-MS/MS by year by province. Mean values and standard deviation are presented in ppm. Bolded text within year indicates significance at $\alpha \leq 0.05$ using Tukey's HSD test. Dash (-) indicates mycotoxin not detected.

YEAR	Province	DON	3ADON	D3G	ZEA	T2	HT2
2018	NB	0.01 ± 0.17	-	-	-	0.13 ± 0.34	**0.28 ± 0.34**
	NS	**5.24 ± 5.04**	**0.18 ± 0.13**	**1.2 ± 1.18**	**0.26 ± 0.37**	0.06 ± 0.2	0.02 ± 0.08
	PE	0.63 ± 0.85	0.04 ± 0.07	0.11 ± 0.39	0.01 ± 0.01	-	-
2019	NB	0.01 ± 0.04	-	0.01 ± 0.03	-	-	-
	NS	**0.06 ± 0.09**	-	0.02 ± 0.03	-	0.17 ± 0.01	-
	PE	-	-	-	-	-	-
2020	NB	-	-	-	0.01 ± 0.02	0.01 ± 0.1	-
	NS	**0.12 ± 0.22**	-	0.03 ± 0.06	-	-	-
	PE	0.01 ± 0.04	-	-	0.07 ± 0.39	-	-
2021	NB	0.28 ± 0.34	-	0.06 ± 0.07	0.03 ± 0.03	-	-
	NS	**1.08 ± 1.6**	0.02 ± 0.04	**0.16 ± 0.18**	0.03 ± 0.03	-	-
	PE	0.26 ± 0.28	0.01 ± 0.03	0.04 ± 0.06	-	-	-

DON was also quantified in grain samples using the Vicam kit. In 2020, this method detected two samples with DON above 1 ppm, both from Colchester County, NS. Half of the grain samples received in 2021 had DON above 1 ppm, with four from sites across NB and seven from Colchester County, NS. The remaining 15 samples were from PE with a single Queens County sample above 1 ppm DON. There was a strong positive correlation between DON quantified by the Vicam kit and LC-MS/MS (*p*-value < 0.0001; Figure 2.7).
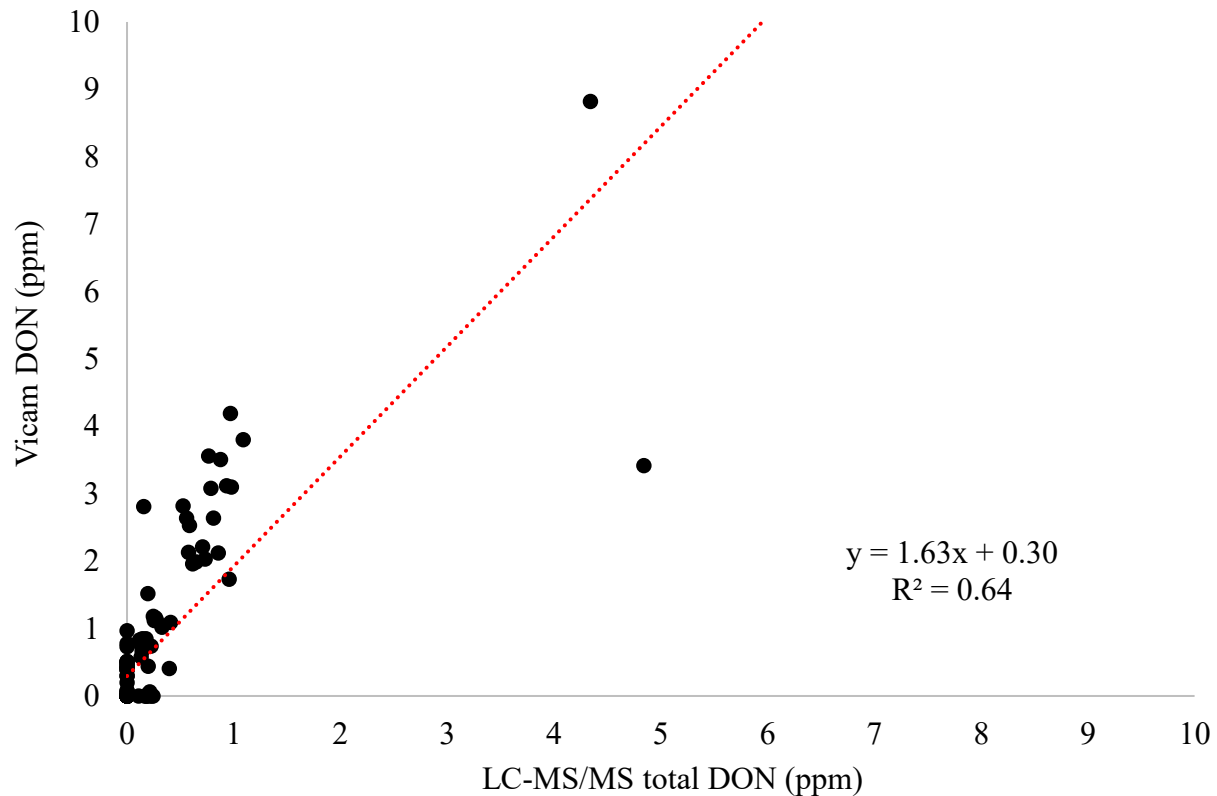
Figure 2.7 Comparison of DON (ppm) quantified by Vicam kit and total DON (ppm) quantified by LC-MS/MS. Linear fit line is dotted. Linear equations and R-squared values are indicted in the figure.

2.3.4   Correlation between % frequency of *Fusarium* spp., DNA and DON

Significant correlation among several variables analysed in this study including % frequency of *Fusarium* spp., amount of *Fusarium* spp. DNA, *TRI3* genotypes and mycotoxins was observed (Table 2.9). *Fusarium graminearum* % frequency was positively correlated with qPCR quantified DNA indicating that the amount of *F. graminearum* DNA can provide insight to the level of infection without isolating the fungi from grain (Figure 2.8 A). There was also a positive correlation between DON quantified by LC-MS/MS and the amount of *F. graminearum* DNA quantified (Figure 2.8 B). Therefore, increased DON contamination can be expected as *F. graminearum* infects and spreads within the grain.

Table 2.9 Correlation matrix of Pearson correlation coefficients. Percent frequency of *Fusarium* spp. are abbreviated as F. gram = *F. graminearum*; F. sporo = *F. sporotrichioides*; F. ave = *F. avenaceum*. DNA quantified for each species (ng rxn^{-1}); Fg = *F. graminearum*; Fs = *F. sporotrichioides*; Fa = *F. avenaceum*; Fp = *F. poae*. Bold text indicates significant correlation at $p \leq 0.05$.

Variables measured	F. gram	F. sporo	F. ave	F. poae	Fg DNA	Fs DNA	Fa DNA	Fp DNA	PCR 3-ADON	PCR 15-ADON	VICAM DON	LC-MS/MS DON	LC-MS/MS 3-ADON	LC-MS/MS D3G	LC-MS/MS ZEA
F. gram	-														
F. sporo	**0.36**	-													
F. ave	0.07	**0.20**	-												
F. poae	0.05	**0.39**	**0.19**	-											
Fg DNA	**0.72**	**0.22**	-0.09	0.03	-										
Fs DNA	**0.23**	**0.40**	0.12	0.02	0.04	-									
Fa DNA	**0.21**	**0.22**	**0.25**	0.07	0.01	**0.30**	-								
Fp DNA	0.12	**0.39**	0.12	**0.29**	-0.02	**0.47**	**0.52**	-							
PCR 3-ADON	**0.86**	**0.37**	**0.16**	0.03	**0.39**	**0.37**	**0.24**	**0.18**	-						
PCR 15-ADON	**0.61**	**0.22**	-0.08	0.04	**0.93**	-0.02	0.00	-0.03	**0.26**	-					
VICAM DON	**0.66**	0.08	**0.21**	**0.20**	**0.57**	-0.01	**0.25**	0.06	**0.56**	**0.35**	-				
LC-MS/MS DON	**0.66**	**0.16**	-0.09	0.02	**0.96**	-0.03	-0.05	-0.07	**0.34**	**0.88**	**0.78**	-			
LC-MS/MS 3-ADON	**0.54**	0.08	-0.09	-0.01	**0.83**	-0.04	-0.02	-0.09	**0.24**	**0.73**	**0.40**	**0.89**	-		
LC-MS/MS D3G	**0.59**	0.15	-0.09	0.02	**0.83**	-0.04	-0.03	-0.06	**0.28**	**0.78**	**0.81**	**0.91**	**0.84**	-	
LC-MS/MS ZEA	**0.29**	0.03	-0.09	0.11	**0.44**	0.00	-0.04	-0.03	0.10	**0.38**	0.00	**0.41**	**0.36**	**0.36**	-

A

y = 24.05x + 2.35
$R^2 = 0.52$

B

y = 8.13x + 0.11
$R^2 = 0.90$
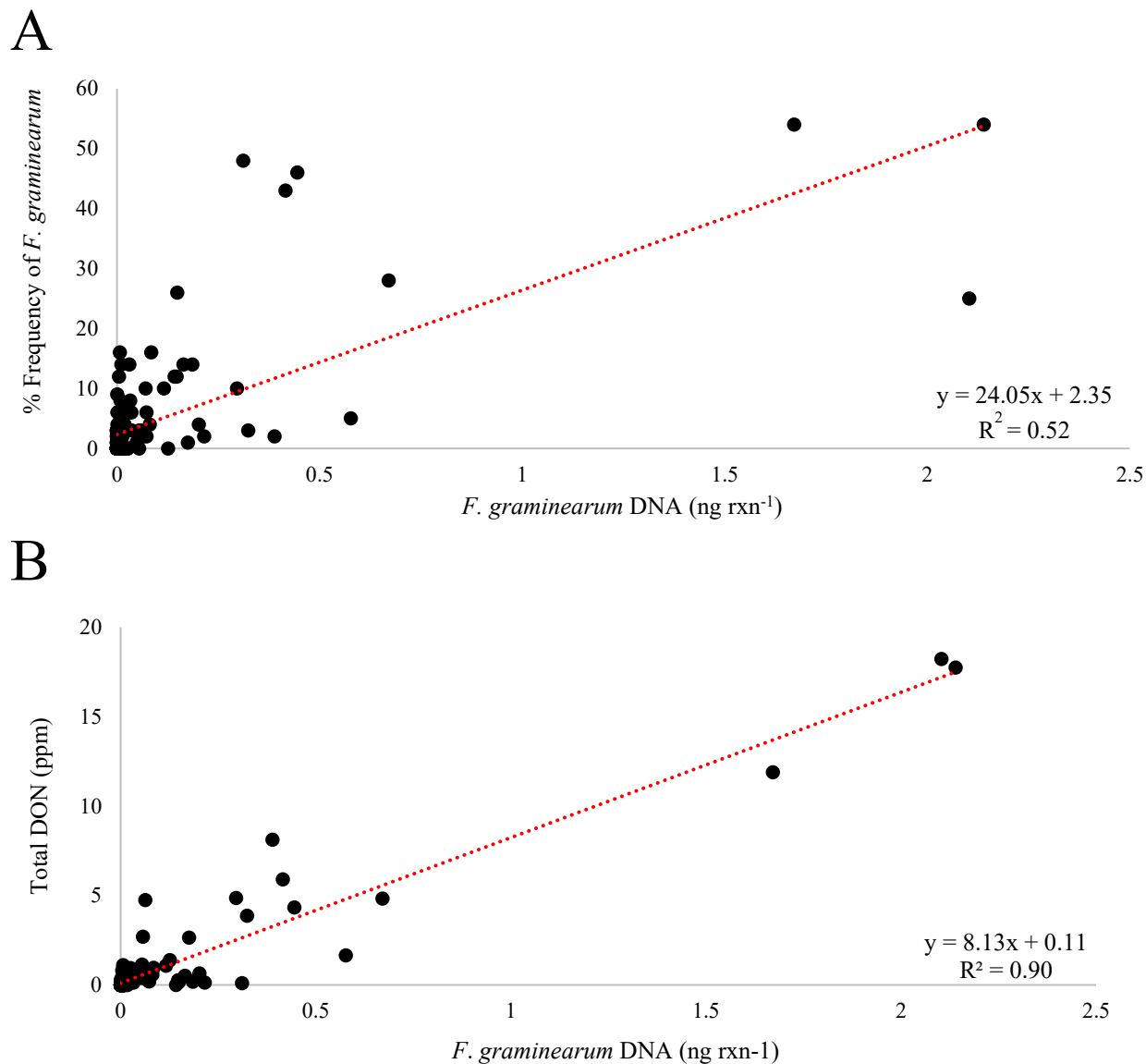
Figure 2.8 (A) Percent frequency of *F. graminearum* in grain samples versus amount of *F. graminearum* DNA quantified from grain samples by qPCR. (B) Total DON quantified by LC-MS/MS versus *F. graminearum* DNA quantified from grain samples by qPCR. Linear fit line is dotted. Linear equations and R-squared values are indicted in the figure.

Percent frequency of *F. graminearum* was positively correlated with % frequency of *F. sporotrichioides* while % frequency of *F. avenaceum* was positively correlated with % frequency of *F. poae*. LC-MS/MS DON was however, weakly correlated with % frequency of *F. sporotrichioides*, consistent with earlier results found in year X species interaction. When *F. graminearum* is dominant, the % frequency of *F. avenaceum* and *F. poae* is reduced. This can be visualized with the clustered Pearson correlation matrix where the % frequency of *F. graminearum*, *F. graminearum* DNA and associated mycotoxins cluster together (Figure 2.9 A). *Fusarium sporotrichioides*, *F. avenaceum* and *F. poae* also cluster with respective DNA away from *F. graminearum*.

A PCA also showed % frequency of *F. graminearum* isolates, *F. graminearum* DNA, *TRI3* genotypes and mycotoxins produced by this species to be associated (Figure 2.9 B). Percent frequency of *F. sporotrichioides*, *F. avenaceum* and *F. poae* and DNA were associated with each other and not with the variables associated with *F. graminearum*. T-2 and HT-2 toxins produced by *F. sporotrichioides* and *F. poae* were not included in multivariate analyses due to infrequent detection.

A
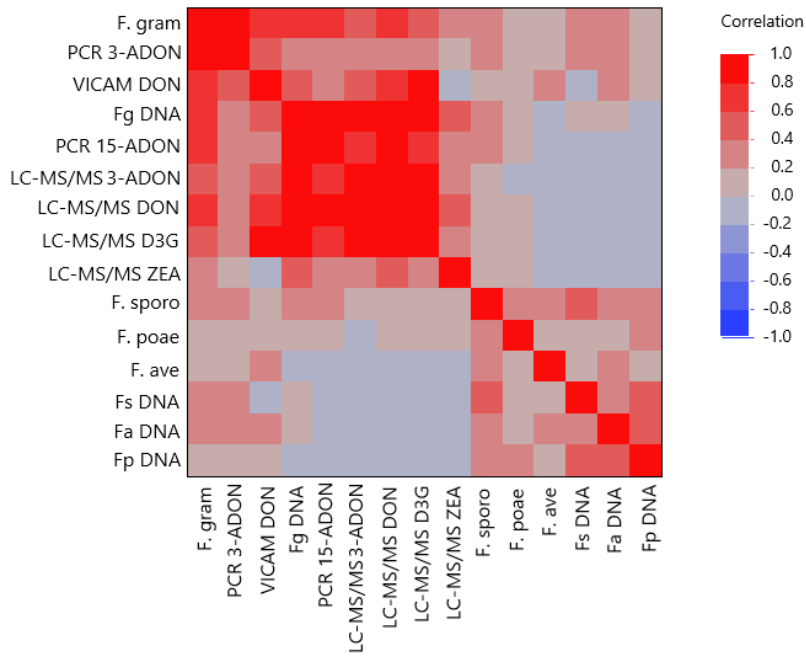
B
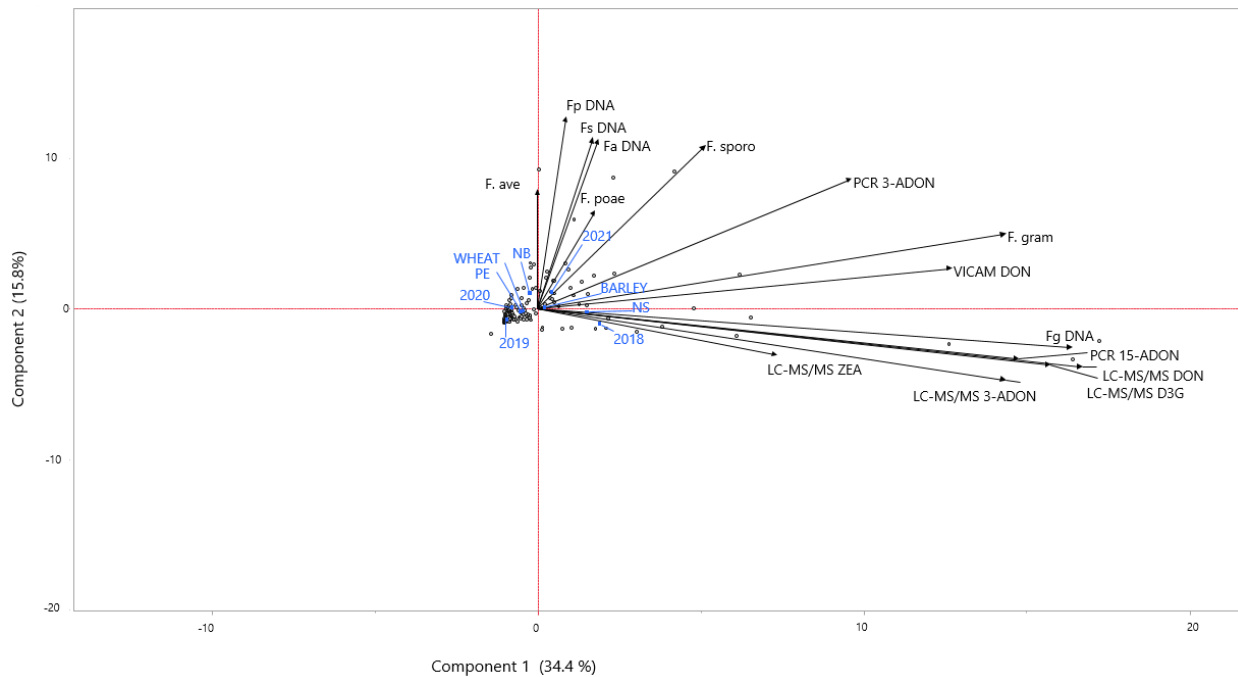
Figure 2.9 (A) Clustered Pearson correlation matrix. (B) Principal component analysis. Percent frequency of *Fusarium* spp. are abbreviated as F. gram = *F. graminearum*; F. sporo = *F. sporotrichioides*; F. ave = *F. avenaceum*. DNA quantified for each species; Fg = *F. graminearum*; Fs = *F. sporotrichioides*; Fa = *F. avenaceum*; Fp = *F. poae*.

2.3.5 Fungicide Sensitivity

Susceptibility of *F. graminearum* isolates to Prosaro® XTR, Miravis® Ace, Tilt® and Folicur® was variable. Some significant effects of province, host crop, and mycotoxin chemotype on *F. graminearum* growth treated with each fungicide product were observed (Table 2.10). *Fusarium graminearum* isolates treated with Miravis® Ace resulted in a lack of red pigmentation characteristic of the species.

Table 2.10 ANOVA probability values for the effects of isolation year, province of origin, host crop, and *TRI3* genotype on *F. graminearum* growth when treated with fungicide. Each fungicide treatment was evaluated independently. Bold text indicates significant effect at $p \le 0.05$.

Product	Effect	DF	Sum of Squares	F Ratio	Prob > F
Folicur®	Isolate Year	3	402.7264	1.6528	0.1807
	Province	2	2152.7188	13.2526	<.0001
	Host	1	799.5302	9.8441	**0.002**
	TRI3 Genotype	1	568.6337	7.0012	**0.0096**
Miravis® Ace	Isolate Year	3	352.79899	0.9311	0.4262
	Province	2	891.61038	3.5296	**0.0314**
	Host	1	551.14551	4.3637	**0.0379**
	TRI3 Genotype	1	501.86454	3.9735	**0.0437**
Prosaro® XTR	Isolate Year	3	1124.0952	3.9854	**0.0087**
	Province	2	350.5612	1.8644	0.1587
	Host	1	646.4464	6.8759	**0.0093**
	TRI3 Genotype	1	254.5452	2.7074	0.106
Tilt®	Isolate Year	3	171.71321	0.8421	0.4719
	Province	2	6.43059	0.0473	0.9538
	Host	1	10.50333	0.1545	0.6946
	TRI3 Genotype	1	560.75059	8.2496	**0.0044**

The year of isolate recovery had a significant effect on *F. graminearum* growth treated with Prosaro® XTR (Figure 2.10). Isolates recovered in 2018 and 2021 were significantly less susceptible to those recovered in 2019. There was no significant difference in growth of isolates recovered in 2020 compared to other recovery years.
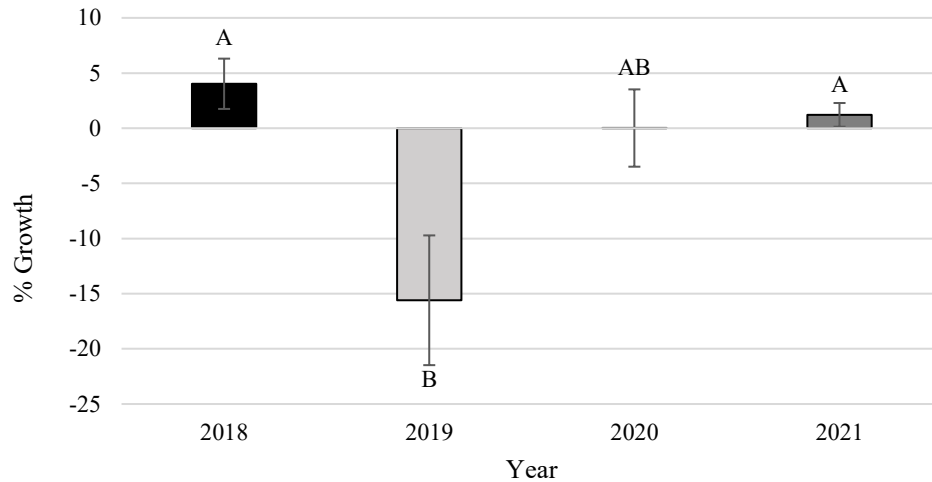
Figure 2.10 Effect of year on normalized growth of *F. graminearum* isolates treated with Prosaro® XTR. Vertical bars represent standard errors. Bars sharing letters are not significantly different at $\alpha \leq 0.05$ using Tukey's HSD test.

The province of origin had a significant effect on *F. graminearum* growth treated with Folicur® and Miravis® Ace (Figure 2.11). Isolates recovered from NB were significantly more susceptible to Folicur® than those from NS and PE but were significantly less susceptible to Miravis® Ace compared to PE isolates. Isolates recovered from NS were not significantly different in their susceptibility to Miravis® Ace when compared to NB or PE.
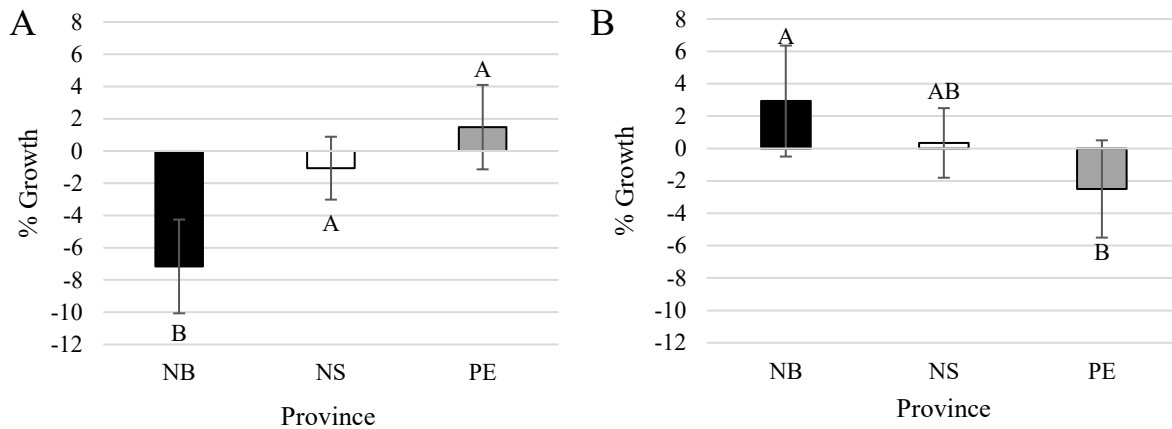
Figure 2.11 Effect of provincial origin on normalized growth of *F. graminearum* isolates treated with (A) Folicur® and (B) Miravis® Ace. Vertical bars represent standard errors. Bars sharing letters within fungicide treatments are not significantly different at $\alpha \leq 0.05$ using Tukey's HSD test.

Host crop had significant effect on *F. graminearum* growth when treated with Folicur®, Miravis Ace® and Prosaro® XTR (Figure 2.12). Under all treatments, isolates recovered from barley were significantly less susceptible than those recovered from wheat.
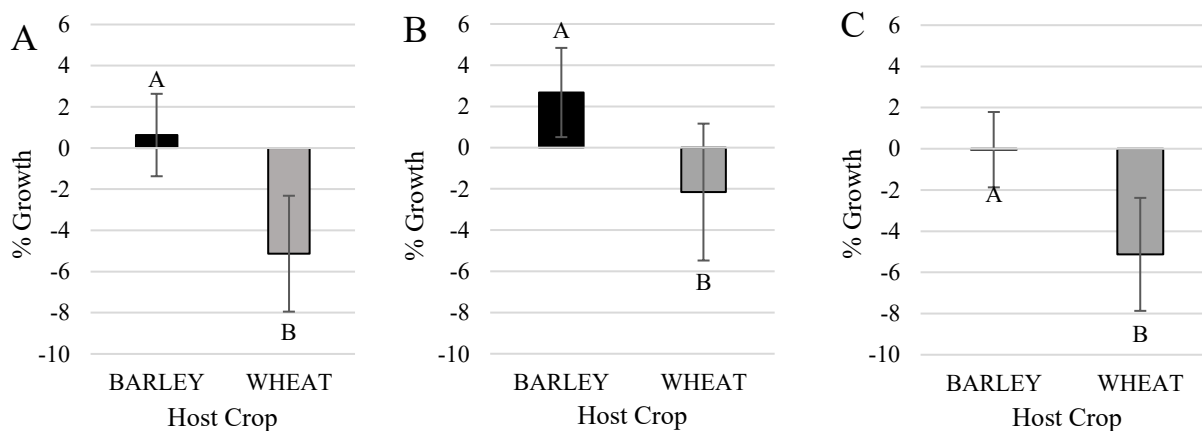
Figure 2.12 Effect of host crop recovery on normalized growth of *F. graminearum* isolates treated with (A) Folicur®, (B) Miravis® Ace and (C) Prosaro® XTR. Vertical bars represent standard errors. Bars sharing letters within fungicide treatments are not significantly different at $\alpha \leq 0.05$ using Tukey's HSD test.

The *TRI3* mycotoxin genotype had a significant effect on *F. graminearum* growth when treated with Folicur®, Miravis® Ace, and Tilt® (Figure 2.13). Isolates with a 15-ADON *TRI3* genotype were significantly less susceptible to these fungicides than those with a 3-ADON *TRI3* genotype.
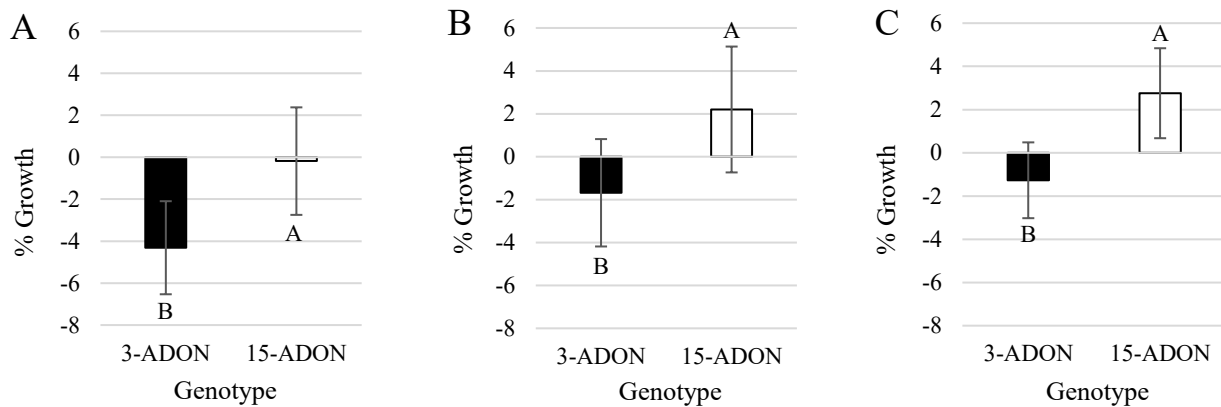
Figure 2.13 Effect of *TRI3* genotype on normalized *F. graminearum* growth of isolates treated with (A) Folicur®, (B) Miravis Ace® and (C) Tilt®. Vertical bars represent standard errors. Bars sharing letters within fungicide treatments are not significantly different at $\alpha \leq 0.05$ using Tukey's HSD test.

2.3.6 Koch's Postulates

Twenty-four isolates of *F. graminearum* were applied to wheat and barley seed heads in triplicate in a greenhouse to verify Koch's Postulates. FHB severity ratings were given at 7 and 14 DAA and seed heads were plated on PDA at maturity and the pathogen was re-isolated from all samples. All isolates caused disease on barley, while all but three caused disease on wheat. There were no similarities of province, host crop, or *TRI3* genotype amongst these three isolates. Symptoms observed among isolates and replicates were variable and is expected that all isolates would cause disease with improved methods. Through Koch's postulates, *F. graminearum* was confirmed to be a causal agent of FHB and symptoms observed on seed heads evaluated in the field were due to FHB.

2.4   Discussion

This study represents the first multi-year report of species causing FHB and examination of *F. graminearum* characteristics in the Maritime provinces of Canada. This work supplements the regional study by Halliday (2020) and recent FHB surveillance reports from PE (Foster and Matters 2020; Johnstone et al. 2021, 2022). FHB in the Maritimes was found to be caused by four principal species, *F. graminearum*, *F. sporotrichioides*, *F. avenaceum* and *F. poae*. The dominant pathogen in 2018 and 2021 when *Fusarium* frequency was greatest was *F. graminearum* (Figure 2.4 A). In 2019 and 2020 when % frequency of *Fusarium* was lowest, *F. poae* was dominant. In Ontario, a 17-year study found the same four principal species caused FHB as well as *F. equiseti*. The predominant causal species of FHB in Ontario were also found to be *F. graminearum* and *F. poae* and *F. graminearum* was associated with epidemic events (Xue et al. 2019). Reports from 2018 to 2021 in the Canadian Plant Disease survey also show the same four principal causal species of FHB of wheat and barley in Ontario, Manitoba, and Saskatchewan. *Fusarium equiseti* and *F. culmorum* were more common in these provinces and the dominant species also varied annually (Canadian Plant Disease Survey, 2019, 2020, 2021, 2022).

Prior to this study, *F. graminearum* was the greatest concern in the Maritimes and the results of this research support that conclusion. Deoxynivalenol and its derivatives were detected annually and above Canadian guidelines of 1 ppm for swine, calf, and lactating dairy animal feed when *F. graminearum* was the dominant causal species of FHB. Samples in 2018 even exceeded the maximum guideline of 5 ppm in cattle and poultry feed. Finished wheat products for human consumption may have a maximum of 2 ppm DON (Charmley and

Trenholm 2017). Mycotoxins produced by *F. sporotrichioides*, *F. poae*, and *F. avenaceum* were infrequently detected.

Deoxynivalenol was quantified by both ELISA and LC-MS/MS methods and some discrepancy was observed in this study. ELISA based tests were reported to detect not only DON, but 3-ADON and D3G, and overestimate the overall DON contamination (Righetti et al. 2017). This overestimation may account for the discrepancy between quantification methods, however, extraction methods may also play a role. The strong positive correlation between DON quantified by the Vicam kit and LC-MS/MS (Figure 2.7) is useful for grain handlers as results from an accessible benchtop kit can be comparable to expensive analytical methods.

The frequency of each FHB causal species appears to be related to weather conditions in the months of June and July (Table 2.11). Optimum environment is not only essential for infection and growth of *Fusarium* within the host but for development of inoculum as well (Manstretta et al. 2016; Manstretta and Rossi 2016). Infection by *F. graminearum* is associated with warm, wet conditions (Osborne and Stein 2007), which were observed in NS in 2018 and across the Maritimes in 2021. Moderate drought conditions were observed across the Maritimes in 2019 and 2020 and were likely responsible for the moderate disease pressure that occurred as inoculum build up was prevented. *F. poae* has been reported to prefer drier conditions (Xu et al. 2008) and was more frequently isolated in these years. Although NS had ample rainfall in June 2019 and July 2020, dry conditions before and after these months could have prevented increased disease development. Further exploration into the role of environmental conditions suitable to the development of severe FHB in Maritime wheat and barley crops would support development of disease risk forecasts.

Table 2.11 Monthly weather summary from May to August in 2018 to 2021 compared to 10-year average (2011-2020). Temp °C = Monthly mean temperature °C; Precip (mm) = total monthly precipitation (mm). Missing data from Charlottetown, PE station filled with data from Harrington, PE. Weather data available from: Environment and Climate Change Canada.

Location	Year	May		June		July		August	
		Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)
Moncton, NB	2018	10.3	18	13.5	31.8	21.4	44.2	20.4	55.6
	2019	7.6	41	14.9	43.2	20	42.4	18.8	106.7
	2020	9.8	45.5	16.9	34.3	20.3	16.1	20.2	42.9
	2021	9.7	113.5	18.2	30.2	18	159.6	19.8	68.6
	10 y	9.18	89.2	14.69	110.38	19.41	74.31	18.97	73.47
Halifax, NS	2018	10.7	63.1	13.6	178.1	20.8	65.9	21	58
	2019	7.6	104.8	14.7	166.6	20	48.4	77.1	19.7
	2020	9.6	121.7	16	33.2	19.8	103	19.8	105.8
	2021	10	111.7	17.9	72.4	18.6	123.5	20.2	145.1
	10 y	10.24	96.04	14.75	117.85	19.73	89.56	25.35	65.56
Charlottetown, PE	2018	9.1	61.7	12.7	154	21.1	24.1	20.5	115.7
	2019	6.7	82.5	14.2	152.1	19.1	34.5	19.8	89.3
	2020	8.9	70.3	16.1	29.5	19.3	44.6	20	50.3
	2021	8.5	107.3	17.4	66.8	17.6	134.1	20.5	31.8
	10 y	9.37	81.77	14.24	84.08	19.42	56.8	19.53	95.01

It has been reported that 3-ADON is the dominant *F. graminearum* mycotoxin genotype in the Maritime provinces (Ward et al. 2008; Kelly et al. 2015). The 3-ADON mycotoxin was detected in grain samples, however, no 15-ADON was detected in samples from 2018 to 2021. Overall, the current study found 71.4% of *F. graminearum* to have the 3-ADON genotype in contrast to Kelly *et al* (2015) who reported that the 3-ADON genotype was present in 91% of Maritime isolates collected from 2005 to 2007. Ward *et al* (2008) found exclusively 3-ADON genotypes in PE, however, Kelly *et al* (2015), reported that the 3-ADON genotype accounted for 98% of isolates from PE and 88% and 56% of NB and NS isolates, respectively. This study confirms dominance of the more virulent 3-ADON genotype in the Maritimes with widespread presence of the 15-ADON genotype in NS and shows that these proportions have remained stable for nearly two decades.

Significant differences were found in the susceptibility of *F. graminearum* to commercially available fungicides Prosaro® XTR, Folicur®, Miravis® Ace, and Tilt®. Effects of

year, province of origin, host crop, and *TRI3* genotype on colony growth treated with each fungicide product were compared. However, another major phenotype observation was made as *F. graminearum* isolates treated with Miravis® Ace resulted in a lack of red pigmentation. This lack of pigmentation was also observed by Sun *et al*. (2020). Pydiflumetofen, an SDHI is an active component of Miravis® Ace which blocks the succinate dehydrogenase enzyme's involvement in cellular respiration possibly impacting carotenoid biosynthesis (Gmoser et al. 2017).

Overall, no significant decrease in susceptibility to the evaluated fungicides of *F. graminearum* isolates recovered from 2018 to 2021 was observed. Alternative host crops of *F. graminearum* such as corn in NS and NB as well as potatoes in PE and NB are also treated with triazole fungicide products in disease management programs (Agriculture and Agri-Food Canada 2021; Statistics Canada 2022b). Length of crop rotations and where wheat and barley are placed within a rotation may have an impact on fungicide susceptibility of *F. graminearum* isolates. Fungicides were effective on both 3-ADON and 15-ADON genotypes, but the more aggressive 3-ADON genotype was significantly more susceptible in this study. Amarasinghe *et al.* (2013) reported no significant differences between genotypes and effectiveness of fungicides. In the field however, effectiveness of fungicides is also dependant on weather and application timing as well as disease intensity and cultivar resistance (Mesterhazy et al. 2003; Paul et al. 2010).

Management practices including crop rotation and tillage were not recorded in this study but likely play a role in the development of FHB. Residues of corn and potatoes are alternative hosts of *F. graminearum*. Incidence and severity of FHB in wheat has been found to increase when wheat follows corn in rotation (Dill-Macky and Jones 2000; Schaafsma et al. 2005;

Tillmann et al. 2017). NS has the largest corn acreage of the Maritime provinces (Agriculture and Agri-Food Canada 2021) where *F. graminearum* was most frequently isolated. Tillage practices bury these residues where degradation occurs quickly and prevents development of disease inoculum (Khonga and Sutton 1988; Pereyra et al. 2004).

2.5   Conclusions

There are four primary causal species of FHB in the Maritime provinces of Canada, however, *F. graminearum* is the species of greatest concern. DON and its derivatives, produced by *F. graminearum,* have been detected above Canadian guidelines every year when conditions were favourable for FHB. Each year *F. sporotrichioides*, *F. avenaceum*, and *F. poae* were present but the associated mycotoxins were not detected at levels of concern. The influence of annual environmental conditions on the frequency of *F. graminearum* and DON contamination supports the necessity of FHB forecasting. The Maritime population of *F. graminearum* is sensitive to commercially available fungicides but regular monitoring should be considered as resistance has been detected in other regions (Spolti et al. 2014). *Fusarium* will continue to adapt, and FHB risk will increase as the climate changes (Parikka et al. 2012; Zhang et al. 2014; Valverde-Bogantes et al. 2020). Therefore, the population of *Fusarium* spp. causing FHB and their characteristics should continue to be surveyed in the Maritimes.

Chapter 3 - Evaluating FHB Forecast Models in the Canadian Maritimes

3.0   Abstract

Fusarium head blight (FHB) is an economically important disease of wheat and barley for which epidemic risk varies annually in relation to environment and management practices. Weather-based disease forecasting models have been developed in cereal growing regions to assess in-season FHB risk and support fungicide decision making. Currently no risk assessment tool is available in the Maritime provinces of Canada. This study evaluated nine North American FHB forecasting models to determine which model would best predict epidemics in the Maritimes. Epidemic predictions were compared to 12 years of historical cultivar recommendation data, 10 fungicide trials, and 39 regional FHB surveillance sites over 4 years. Field trials were planted to compare fungicide application with risk assessment. DON contamination ≥ 0.9 ppm was used to determine the occurrence of an epidemic. The evaluation found models developed using 7-day pre-anthesis relative humidity and temperature performed best in the Maritimes for both wheat and barley.

Keywords: *F. graminearum*, Fusarium head blight (FHB), wheat, barley, disease forecasting, fungicides, deoxynivalenol

3.1 Introduction

Fusarium head blight (FHB) is an economically important disease of cereal crops around the world. Of the many *Fusarium* spp. that cause FHB, *F. graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schweinitz) Petch) is most concerning as the fungi imparts a mycotoxin, deoxynivalenol (DON), in the grain. DON may cause severe health effects in humans and livestock when consumed at certain concentrations (Pestka 2007, 2010; McMullen et al. 2012). Canadian guidelines restrict consumption of grain and finished grain products by livestock and humans at levels as low as 1 ppm DON (Charmley and Trenholm 2017). The yield and subsequent grade and market value of the grain may also be reduced with the presence of shrunken, Fusarium damaged kernels (FDK) (Canadian Grain Commission 2020). FHB has negatively impacted producers in the Canadian Maritimes where cereals are important rotational crops. Various efforts may be undertaken by producers to minimize the impacts of FHB.

Management strategies to reduce FHB and mycotoxin contamination include growing resistant cultivars, crop rotation, and fungicide applications. These strategies are most effective when used together in an integrated management plan (Wegulo et al. 2015). Fungicide products in Canada are permitted for a single application to supress *Fusarium* when the crop is most susceptible to infection from early head emergence through anthesis. *Fusarium graminearum* favours warm, humid conditions for spore development, dispersal, and infection (Osborne and Stein 2007). Therefore, understanding weather conditions including temperature, humidity, and rainfall events that have occurred or are forecasted within the weeks surrounding anthesis, is imperative for fungicide application decision making. In addition to environmental conditions, decision making around fungicide application may be further confounded by field history of FHB, cost to apply fungicides and market value of the grain post-harvest (Cowger et al. 2016).

An additional management tool, such as an FHB forecasting system, to predict DON contamination or epidemic risk could support fungicide decision making.

Empirical data, such as historical climate information has been compared to historical occurrences of FHB to easily develop predictive models using methods such as regression. Information on average temperature, rainfall, and humidity at or near anthesis are most often used as model predictors, however, some have integrated cultivar susceptibility and presence of corn residue as predictors. More complicated methods can be employed to comprehensively describe the pathosystem, but these forecasts are difficult to implement in real-world situations (Prandini et al. 2009). FHB forecast model predictions are a simple representation of the pathosystem at a certain time and space and most have established decision-making thresholds, providing support for determining whether a fungicide application is warranted under the current conditions (Prandini et al. 2009; Wegulo et al. 2015). Different accuracy metrics have been used to evaluate FHB forecast models with overall accuracy values ranging from 70 to 90%.

Forecast tools to estimate post-harvest DON contamination or risk of FHB epidemics caused by *F. graminearum* based on field severity or incidence have been developed in many cereal growing regions. In Canada, web-based risk maps are available from the provinces of Alberta, Saskatchewan, and Manitoba (Alberta Climate Information Service 2022; Manitoba Agriculture 2022a, 2022b; SaskWheat Development Commission 2022). A commercial tool predicting DON contamination known as DONcast®, was developed in Ontario and uses proprietary weather monitoring stations (Weather INnovations LP). In the United States, the USWBSI FHB Risk Tool is an extensive web-based risk map currently covering 35 states that the user may customize based on crop susceptibility to disease. FHB forecasting has been

developed in South America (Moschini and Fortungo 1996; Moschini et al. 2001; Moschini et al. 2004; Del Ponte et al. 2005), and Europe (Rossi et al. 2003; Musa et al. 2007; Van Der Fels-Klerx et al. 2010; Landschoot et al. 2013).

Empirical models are often less accurate when used outside the region of origin that they were developed for; hence calibration is required before implementation elsewhere (Prandini et al. 2009). Adjustments to decision thresholds were shown to improve the accuracy of FHB forecasting models when implemented in a new location as found in a study that evaluated and compared FHB forecasting models under the environmental conditions of Quebec (Giroux et al. 2016). Currently, no FHB forecasting tools have been evaluated or developed in the Maritime region while all other cereal producing regions in Canada have either evaluated or implemented them. Therefore, the first objective of this study was to evaluate and compare performance of empirical forecasting models developed in North America to determine which model type and environmental predictors work best in the Maritimes. The second objective was to evaluate the efficacy of the most accurate model(s) to guide fungicide applications. This study presents a baseline of what weather variables and crop damage indicators can best forecast epidemics of FHB to support fungicide decision making in the Maritimes.

3.2   Materials and Methods

3.2.1   Historical Evaluation of FHB Forecasts

Weather variables used in predictive FHB models developed in North America that were found most accurate by Hooker *et al*. (2002), De Wolf *et al*. (2003), Molineros (2007), Shah *et al*. (2013), and Bondalapati *et al*. (2012), were compared to historical weather variables available from Environment and Climate Change Canada (ECCC). FHB forecast models that could be

fulfilled by ECCC data were determined suitable for evaluation in the Maritimes. Current equations from web-based models have not been published however, previous versions of DONcast® and USWBSI Risk Tool were available. A total of nine models from five reports were evaluated in this study and summarized in Table 3.1. The model developed by Hooker *et al*. (2002) predicts DON contamination in harvested grain using a flowchart to determine use between three regression equations at time periods before and after anthesis. The models by De Wolf *et al*. (2003), Molineros (2007), Shah *et al*. (2013), and Bondalapati *et al*. (2012) were developed by logistic regression and use single linear equations to predict probability of an FHB epidemic occurring. Decision thresholds were implemented to deliver a binary response of 1 = epidemic or 0 = no epidemic. Bondalapati *et al*. (2012) developed the only barley specific model while the others were developed for spring and, or winter wheat.

Historical climate data from Harrington, PE were retrieved from ECCC using R statistical language (R Core Team 2022) and the *Weathercan* package (LaZerte and Albers 2018). Missing data were replaced using the nearby Charlottetown, PE weather station. Historical FHB data and head emergence dates from 2010 to 2021 at AAFC Harrington research farm was provided by the Atlantic Recommending Committee for Cereal Crops (ARCCC) Maritime Cereal Cultivars Performance Trial coordinators Alan Cummiskey and Sharon ter Beek with permission of Chair, Dan MacEachern. Recommended cultivars for the Maritimes that were chosen for FHB forecasting analysis were, AAC Mirabel, Island, and Leader barley, AAC Scotia, AC Helena, AC Walton, and Easton spring wheat, and AC Sampson, Pioneer® 25R40, Priesley, and UGRC Ring winter wheat, which varied in their susceptibility to FHB.

Weather data respective to each anthesis or heading date was inputted into each FHB forecast model equation to predict DON contamination or probability of disease epidemic. The

anthesis date of wheat cultivars was estimated by adding 3 d to the head emergence date. When evaluating anthesis models for barley cultivars, head emergence date was used in place of anthesis. The spring wheat models, which incorporated cultivar susceptibility, were conducted at all levels (0-3) and no historical field data from a corn rotation was available; therefore, this variable was not evaluated in the Molineros (2007) winter wheat model. De Wolf *et al*. (2003) Model I was not evaluated prior to 2014 as hourly historical rainfall was not yet available.

Predicted outcomes of each FHB forecast were compared to actual DON contamination (ppm) reported by ARCCC to determine the most accurate model for the Maritimes. DON was used as the crop damage indicator to evaluate forecasts given the subjective nature of visual FHB rating and variability in the relationship between field severity and DON (Paul et al. 2005). Bondalapati *et al*. (2012) chose 0.5 ppm DON as the crop damage indicator because the concentration represents an economic threshold for malting quality. Giroux *et al*. (2016) used multiple thresholds of DON, FDK and FHB index (incidence*severity / 100) to evaluate FHB forecasting models in Quebec and found DON ≥ 1 ppm to be the best indicator. Canadian guidelines limit DON as low as 1 ppm (Charmley and Trenholm 2017) therefore, DON ≥ 0.9 ppm was used as the crop damage indicator in this study to account for analytical error with the Vicam lateral flow kit (Righetti et al. 2017).

Table 3.1 Summary of evaluated FHB forecasting models.

Origin	Author	Method		Predictor Variables	Response Variable	Model Name	Equation	Decision Threshold	Reported Accuracy
Canada	Hooker *et al.* (2002)	Multiple regression - flowchart	RAIN A	# Days with rain > 5mm 4-7 days before head emergence	DON concentration (ppm)	1	DON = exp[-0.30 + 1.84**RAINA** - 0.43(**RAINA**)^2 - 0.56**TMIN**] - 0.1	-	R^2 = 0.73
			RAIN B	# Days with rain > 3mm 3-6 days after head emergence		2	If **RAINB** >0 DON = exp[-2.15 + 2.21**RAINA** - 0.61(**RAINA**)^2 + 0.85**RAINB** + 0.52**RAINC** - 0.3**TMIN** - 1.10**TMAX**] - 0.1	-	
			RAIN C	# Days with rain > 3mm 7-10 days after head emergence					
			TMIN	# Days with temperature < 10°C 4-7 days before head emergence		3	If **RAINB** <0 DON = exp(-.84 + 0.78**RAINA** + 0.40**RAINC** - 0.42**TMIN**) - 0.1	-	
			TMAX	# Days with temperature > 32°C 4-7 days before head emergence					
USA	De Wolf *et al.* (2003)	Logistic Regression	TRH9010	Duration (h) of 15 ≤ T ≤ 30°C **and** RH ≥ 90% 0-10 days after anthesis	Epidemic if field severity >10% epidemic = 1 no epidemic = 0	A	logit (μ) = -3.3756 + 6.8128**TRH9010**	p = 0.36	84%
			T15307	Duration (h) of 15 ≤ T ≤ 30°C 0-7 days before anthesis		B	logit (μ) = -3.2751 + 10.5097**INT3**	p = 0.44	84%
			INT3	Interaction term = T15307 x TRH9010					
			DPPT7	Duration (h) of precipitation 0-7 days before anthesis		I	logit (μ) = –8.2175 + 8.4358**T15307** + 4.7319**DPPT7**	p = 0.5	70%

p = 1/1+exp(-logit(μ))

Table 3.1 Summary of evaluated FHB forecast models (continued).

Origin	Author	Method	Predictor Variables		Response Variable	Model Name	Equation	Decision Threshold	Reported Accuracy
USA	Molineros (2007)	Logistic Regression	H1	Mean RH 1-7 days before anthesis		Spring Wheat 2005	logit (μ) = -16.9369 + 0.23839**H1** - 1.5442**Variety**	$p = 0.5$	78%
			TH2	Duration (h) of 9 ≤ T ≤ 30°C **and** RH ≥ 90% 1-7 days before anthesis					
			T3	Duration (h) of 9 ≤ T ≤ 30°C 1-7 days before anthesis	Epidemic if field severity >10%				
			R2	Duration (h) of measurable rain events >0.01 mm 1-7 days before anthesis	epidemic = 1 no epidemic = 0	Winter Wheat Model 3	logit (μ) = -6.3906 + 0.0746**TH2** + 0.000753***Corn*H1*T3** -0.00244***Corn*T3*R2**	$p = 0.38$	74%
			Variety	Very Susceptible = 0 Moderately Susceptible = 1 Moderately Resistant = 2 Resistant = 3					
			Corn	No corn residue in field = 0 Corn Residue Present = 1					
	Shah *et al.* (2013)	Logistic Regression	TH2	Duration (h) of 9 ≤ T ≤ 30°C **and** RH ≥ 90% 1-7 days before anthesis		Spring Wheat (Updated)	logit (μ) = −11.008 − 0.9578**RESISTC** + 0.1516**H1**	$p = 0.37$	Not reported
			H1	Mean hourly RH% 1-7 days before anthesis	Epidemic if FHB index >10% epidemic = 1 no epidemic = 0	Winter Wheat (Updated)	logit (μ) = −1.7954 + 0.0245**TH2**	$p = 0.23$	
			Cultivar Resistance (Resist C)	Very Susceptible = 0 Susceptible = 1 Moderately Susceptible = 2 Moderately Resistant = 3					

$p = 1/1+\exp(-logit(\mu))$

Table 3.1 Summary of evaluated FHB forecast models (continued).

Origin	Author	Method	Predictor Variables		Response Variable	Model Name	Equation	Decision Threshold	Reported Accuracy
USA	Bondalapati *et al.* (2012)	Logistic Regression	A	1	Epidemic if DON ≥ 0.5 ppm epidemic = 1 no epidemic = 0	Model 3	logit (μ) = -3.85 + 5.16**Wb3**	*p* = 0.56	79%
			B	= E * ((H + 1) / H) * H^(1 / H + 1) * exp((t - F) * G / (H + 1)) / (1 + exp((*t* - F) * G)))					
			C	12					
			D	2.6475					
			E	0.0252					
			F	25					
			G	0.4804					
			H	1.0744					
			t	Mean hourly temperature 0-9 days before head emergence					
			w	# 8-hour periods RH ≥ 90% 0-9 days before head emergence					
			Wb3	Relationship between *t* and *w* expressed by modified Weibull function where B is rate of increase of response with respect to *w*.* = A (1 - exp(-1*(B*(*w* - C)^D)))					

*Full description of all variables available from Bondalapati *et al*. (2012), *p* = 1/1+exp(-logit(μ)

3.2.2 Fungicide Applications for FHB Forecast Evaluation

Field experiments were conducted at Agriculture and Agri-Food Canada's (AAFC) Harrington Research Farm (46°20'34.5" N, 63°09'23" W) on Prince Edward Island (PE) to determine the efficacy of fungicide application for suppression of FHB in wheat and barley when guided by an FHB forecast in 2020 and 2021. Wheat was planted at a rate of 350 seeds m^{-2} and barley was planted at a rate of 300 seeds m^{-2} in 1.5 x 5 m plots. The soil was prepared to a depth of 10 cm with a John Deere 980 field cultivator equipped with rolling basket harrows. Fertilizer was applied to winter wheat as a top-dress with ammonium nitrate-based fertilizer, 27-0-0 9S, at a rate of 50 kg ha^{-1} on 18 Jun 2020 and at 70 kg ha^{-1} N on 16 Apr 2021. The field containing spring cereal trials in 2021 received 2 t ha^{-1} lime on 14 Apr 2021. All spring cereal trials received 17-17-17 fertilizer at a rate of 50 kg ha^{-1} N pre-plant. Broadleaf weeds were managed with an herbicide tank mix of Refine SG (30 g ha^{-1}; FMC of Canada Ltd.) and MCPA Ester 600 (1 L ha^{-1}; ADAMA Agricultural Solutions Canada Ltd.) applied after the 3-leaf crop stage as indicated on manufacturer's label. Fungicide was applied at barley head emergence and wheat anthesis during the crop growth stage window according to the manufacturer's label. Prosaro® XTR (prothioconazole and tebuconazole; Bayer Crop Science Inc.) was applied at a rate of 0.8 L ha^{-1} and Miravis® Ace (propiconazole and pydiflumetofen; Syngenta® Canada) was applied at a rate of 1 L ha^{-1}. All fungicide treatments were applied using bi-directional nozzles ≤ 60 cm above the crop.

In 2020, three independent field trials were conducted in a randomized complete block design with two treatments and four replicates. Pioneer® 25R40 and AC Sampson winter wheat were planted on 20 Sept 2019. The spring cereal trial area was cultivated and fertilized on 28 May 2020. Island barley and Easton spring wheat were planted on 29 May and 11 Jun,

respectively. Herbicide was applied to winter wheat and barley trials on 16 Jun. Applications of Prosaro® XTR were applied to winter wheat on 25 Jun, barley on 14 Jul, and spring wheat on 4 August 2020.

A total of seven independent field trials were conducted in 2021 with one winter wheat trial and three staggered planting dates for each spring wheat and barley trial. All seed was treated with Vitaflo-280 (Chemtura, Ontario, Canada). Winter wheat cultivars AC Sampson and Pioneer® 25R40 were planted on 25 September 2020. The trial was conducted in a randomized complete block design with 4 replications, 2 fungicide treatments and an untreated control (2 cultivars x 3 treatments x 4 replicates). Herbicide was applied to winter wheat on 24 May and fungicides were applied to Pioneer® 25R40 on 18 Jun and to AC Sampson on 23 Jun 2021.

Spring cereal trials were planted on 16 Apr, 4 May, and 25 May 2021 to maximize environmental variability and *Fusarium* infection risk to evaluate forecasting models and are referred to as Trial A, B, and C, respectively. These trials were conducted in a split-plot randomized complete block design with four replications totalling 24 plots per experiment (two cultivars x three treatments x four replicates). The main plot factor was fungicide treatment with three levels: two fungicide products and an untreated control. The split plot factor was cultivar with two levels: AC Walton and Easton wheat or Island and Leader barley. Herbicide was applied to Trials A and B on 24 May and to Trial C on 29 Jun. Fungicide was applied to barley on 26 Jun, 29 Jun, and 17 Jul. Spring wheat trials received fungicide applications on 2 Jul, 11 Jul, and 23 Jul.

Leaf diseases of wheat and barley and were evaluated as they may also impact crop yields. The penultimate and flag leaves of ten random plants in each plot were observed within 24 h prior to fungicide application and again 7-10 d after application. Wheat was observed for

signs and symptoms of powdery mildew (PM) as described by Salgado and Paul (2016) and Septoria blotch (SB) as described by McMullen and Adhikari (2021). These leaf diseases evaluated on a scale of 0 to 9 where 0 = no signs or symptoms of disease and 9 = > 80% of the leaf was covered with pustules (PM) or lesions and pycnidia (SB). Barley was observed for signs and symptoms of net blotch (NB) and scald as described by Neate and McMullen (2005). Barley leaf diseases were evaluated on a scale of 0-9 where 0 = no signs or symptoms of disease and 9 = > 80% of the leaf was covered in lesions.

Signs and symptoms of FHB on wheat and barley in field trials were assessed at early dough (ZGS-80) and again 7 d later (FHB 7 d), each plot was evaluated for severity and incidence on a scale of 0 to 9 (0 = no infection, 9 = head completely infected; whole plot infected). A visual scale by Stack and McMullen (1998) was used to guide the ratings.

Harvested grain from experimental plots was evaluated for quality metrics, including yield, thousand kernel weight (TKW), moisture and protein content, FDK, and DON contamination. Grain from each plot was dried then de-awned and cleaned with an SLN4 sample cleaner (Pfeuffer GmbH, Germany). Samples were then weighed, and yield corrected for 15% moisture. A sub-sample of approximately 150 g was taken for the remaining analysis. TKW was determined by counting 500 seeds with an Old Mill Company (model 850-2) seed counter and multiplying that weight by 2. Near-infrared spectroscopy (NIR) was used to determine moisture and protein content of whole grain (Spectra Star, Unity Scientific). An FDK rating was given on a scale of 0 to 9 (0 = no FDK, 9 = >81% seed shriveled) to each plot by counting 150 seeds and observing for characteristics described by the Official Grain Grading Guide (Canadian Grain Commission 2020). A subsample from each plot as well as AGC samples were subject to DON

quantification to compare with FHB forecast predictions. DON quantification was conducted using a Vicam DON-V lateral flow kit as described in section 2.2.9.

The FHB forecast model found to be most accurate in the Maritimes by historical analysis was used to determine whether fungicides treatments were warranted in 2020 and 2021 fungicide trials. FHB epidemics were determined by DON contamination ≥ 0.9 ppm. Fungicides were considered warranted if an application resulted in DON contamination < 0.9 ppm compared to untreated plots with DON contamination ≥ 0.9 ppm. Fungicides were not considered warranted if untreated plots did not have DON contamination ≥ 0.9 ppm.

3.2.3 Assessment of Best Fit FHB Forecast in the Maritimes

FHB epidemic predictions by the best FHB forecast models determined by historical analysis were also compared to DON contamination of Atlantic Grains Council (AGC) On-Farm Agronomy samples collected in Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PE) from 2018 to 2021 (Section 2.2.1). AGC head emergence and anthesis dates, however, were not available. Linear regression of planting date on head emergence date of barley grown in Harrington ARCCC trials provided an equation to input known AGC planting dates to determine approximate head emergence date. Unknown planting dates were assumed as 28 May. Winter wheat anthesis dates were determined from the average days to anthesis of Pioneer® 25R40 from 2010 to 2021 at each winter wheat ARCCC trial site across the Maritimes. This date was used to determine approximate winter wheat anthesis at the nearest AGC site. As the forecast dates were estimated, each head emergence or anthesis date was assigned to a Julian week then daily risk determined by the model was used to average risk by Julian week. DON contamination of each sample was compared to the weekly average FHB risk forecasted by the model.

3.2.4 Statistical Analysis

Statistical analysis of field trial results was performed using JMP version 16.0 (SAS Institute, Cary, North Carolina, USA). Standard least squares ANOVA was used to determine significant effects of treatment and cultivar followed by post-hoc Tukey's HSD test ($\alpha \leq 0.05$) to determine differences between fungicide treatments and cultivar on leaf disease, grain quality, yield, and DON contamination of 2020 and 2021 winter wheat and 2020 spring cereals. Significant effects of fungicide treatment on leaf disease, grain quality, yield and DON contamination of 2021 spring cereals were determined by mixed linear modeling using restricted maximum likelihood (REML) and Tukey's HSD test ($\alpha \leq 0.05$) with replicate and column treated as random effects.

Prior to analysis, histograms were generated for each variable to observe data distributions. If the distribution was not normal, the most appropriate distribution with the lowest AIC was applied to each y-variable to set the distribution of ANOVA. For barley, the Sinh-Arcsinh (SHASH) distribution was applied to DON, and scald of the penultimate leaf, the Lognormal distribution was applied to protein, an Exponential distribution was applied to net blotch and scald of the flag leaf, and a Normal 2 Mixture distribution was applied to net blotch of the penultimate leaf. For spring wheat, the SHASH distribution was applied to DON, TKW, PM of the penultimate leaf, and SB of the flag and penultimate leaf. The Weibull distribution was applied to FDK, and FHB 7 d, a Lognormal distribution was applied to protein, a Normal 2 Mixture distribution was applied to FHB Z80, and Normal 3 Mixture applied to PM of the flag leaf. For winter wheat, the SHASH distribution was applied to DON, PM of the penultimate leaf, and SB of the flag and penultimate leaf, and FHB 7 d. The Lognormal distribution was applied to protein, and PM of the flag leaf, the Weibull distribution was applied to FDK, and an Exponential distribution was applied to FHB Z80.

FHB forecast models were run using R statistical language. Correlation analysis was used to evaluate the relationship between observed DON contamination and DON forecasted by Hooker *et al*. (2002). Accuracy of the model was determined using the root-mean-square error (RMSE) and Lin's concordance correlation coefficient (CCC) using the *DescTools* package (Signorell et al. 2022). RMSE is the standard deviation of residuals, measuring model error, where a value of 0 would indicate a perfect model. CCC evaluates the agreement between two measurements of the same variable and the degree to which they fall on the 45° line of perfect concordance, which intercepts at the origin. The CCC has a value of -1 to 1 (≤ 0 = no concordance 1 = perfect concordance) calculated using the Pearson correlation coefficient (r) and a bias correction factor (C_b) that accounts for under- and over-prediction by the model (Lin 1989). The C_b measures the deviation of the best fit line from the 45° line therefore, if $r = 1$, data deviating from the line 45° line will result in a CCC < 1 (Lin 1989).

The outcomes of classification models were compared to actual epidemics of FHB indicated when DON contamination was ≥ 0.9. True positive (TP), true negative (TN), false positive (FP) and false negative (FN) predictions were calculated as well as accuracy, sensitivity, and specificity. Accuracy is the proportion of predictions that the model correctly classified (Eq 3.1). Sensitivity evaluates the model's ability to predict true positive cases and is known as the true positive rate (Eq 3.2). Specificity evaluates the model's ability to predict true negative controls and is known as the true negative rate (Eq 3.3).

$$\text{accuracy} = \frac{\text{events correctly predicted}}{\text{all events}} \tag{3.1}$$

$$\text{sensitivity} = \frac{TP}{TP+FN} \tag{3.2}$$

$$\text{specificity} = \frac{TN}{TN+FP} \tag{3.3}$$

Receiver operating curve (ROC) analysis was conducted in R using the *PresenceAbsence* package (Freeman and Moisen 2008). ROC analysis graphically presents the relationship between the proportion of true positives and proportion of false positives at all decision thresholds. The area under the ROC curve (AUC) is determined to evaluate model performance, where the AUC of the no information line is 0.5 and the AUC of a perfect model is 1 (Fawcett 2006). An AUC of 0.5 would indicate that a classification model is not better than chance.

Cohen's kappa (κ), an agreement statistic, was also determined using the *PresenceAbsence* package to compare models. Kappa measures the proportion of correctly classified events after accounting for the probability of agreement expected by chance (equation 3.4) and can have a value from -1, no agreement, to 1, perfect agreement, between model predictions and observed values (Cohen 1960). The scale presented by Landis and Koch (1977) was used to judge agreement. This metric was preferred when the number of cases and controls were not balanced.

$$\kappa = \frac{\text{observed proportionate agreement - probability of random agreement}}{1 - \text{probability of random agreement}} \tag{3.4}$$

3.3 Results and Discussion

3.3.1   History of FHB in the Canadian Maritimes

Barley, spring wheat and winter wheat cultivars were chosen for their varied susceptibility to

FHB and popularity in the Maritimes. From 2010 to 2021, all barley and spring wheat cultivars

had at least two years of post-harvest DON contamination ≥ 0.9 ppm (Table 3.2). Weather

conditions in 2010 and 2011 were favourable to the development of FHB with warm

temperatures and high % RH resulting in DON contamination ≥ 0.9 ppm in all cultivars

evaluated. Conditions were again favourable for disease in 2014 and 2015 when spring wheat

cultivars and 6-row barley had DON contamination ≥ 0.9 ppm. Of the selected winter wheat

cultivars, only AC Sampson was planted in 2010 and 2011 epidemic years, which did not allow

for thorough analysis of the FHB forecasting models for this crop.

Table 3.2 Cultivars selected for FHB forecast analysis, number of years evaluated and corresponding epidemics where DON contamination ≥ 0.9 ppm.

Crop	Cultivar	Total Years Evaluated	Epidemics	
			#	Years
Barley	AAC Mirabel	11	4	2010, 2011, 2015, 2021
	Island	11	2	2010, 2011
	Leader	12	2	2010, 2011
Spring Wheat	AAC Scotia	12	3	2010, 2011, 2014
	AC Walton	12	4	2010, 2011, 2014, 2015
	AC Helena	12	6	2010, 2011, 2012, 2014, 2015, 2021
	Easton	8	2	2014, 2015
Winter Wheat	AC Sampson	12	2	2010, 2011
	Pioneer® 25R40	9	0	-
	Priesley	9	0	-
	UGRC Ring	6	0	-

3.3.2 Historical Analysis of FHB Forecasts

A total of nine FHB forecast models from North America with a variety of environmental predictors and objectives were evaluated for predicting FHB epidemics in the Maritime provinces. The epidemic predictions of Model 3 by Bondalapati *et al*. (2012) were compared to historical epidemics of barley and was non-functional in Harrington, PE (data not shown). At the average 10 d temperature associated with barley head emergence, the number of 8-hour periods ≥ 90% RH were often too low; therefore, the model could not calculate a response, or were too high resulting in maximum risk. Of the cultivar years this model was able to calculate a response (21 of 34), it correctly classified 5 of 7 epidemics, but incorrectly classified 10 of 14 non-epidemics. The results of Bondalapati *et al*. (2012) were similar to those of this study where the model over predicted the occurrence of epidemics. Unlike the other FHB forecast models presented, this model was never implemented for producers and was not evaluated further in this study.

The FHB forecast model by Hooker *et al*. (2002) contained three equations used at different time periods around head emergence (Table 3.1). Equation 1 only evaluates conditions 4-7 d before head emergence and can be used up to 12 d before head emergence with a 5 d forecast. Equations 2 and 3 are used at head emergence and include conditions before and 7-10 d after head emergence. Equation 2 is used when rainfall > 3mm occurs 3-6 d after head emergence and Equation 3 is used when rainfall < 3 mm occurs 3-6 d after head emergence. Predictions of each of the three equations were evaluated as well as a composite prediction using the Equations 2 and 3.

Correlation analysis was performed, and accuracy metrics were calculated to determine if DON contamination in cereals grown in the Maritimes could be predicted by the Hooker *et al*.

(2002) forecast model (Table 3.3). The model equations explained less variation in DON

contamination at Maritime trial sites than the originally reported R^2 values of 0.55, 0.79 and 0.56

for Equations 1, 2, and 3, respectively. Hooker *et al.* (2002) found an R^2 value of 0.73 based on

399 winter wheat field sites over 5 years in the composite analysis of Equations 2 and 3. The R^2

value was significant only in the combined use of Equations 2 and 3 for spring wheat. Compared

to the R^2 value for each crop in the current study, model performance was reduced in the

Maritimes. The low concordance indicated by CCC values demonstrates that the Hooker *et al.*

(2002) model could not be used to predict DON contamination in cereals grown in the Maritimes

(Figure 3.1).

Table 3.3 Performance of Hooker *et al.* (2002) FHB forecast model in the Maritime provinces of Canada. Bold text indicates significant correlation at $p \leq 0.05$. RMSE = root mean square error, CCC = Lin's concordance correlation coefficient, C_b = bias correction factor.

Crop	Equation	R^2	*p-value*	RMSE	CCC	C_b
	1	0.00	0.95	2.33	-0.01	0.88
Barley	2	0.07	0.25	4.28	0.20	0.78
	3	0.08	0.34	0.60	-0.09	0.32
	2 and 3	0.10	0.08	3.39	0.24	0.79
	1	0.33	0.24	1.85	0.16	0.90
Spring Wheat	2	0.20	0.08	3.28	0.38	0.85
	3	0.01	0.68	0.56	0.07	0.83
	2 and 3	0.16	**0.01**	2.12	0.38	0.96
	1	0.06	0.15	1.22	-0.09	0.37
Winter Wheat	2	0.05	0.40	1.24	-0.11	0.47
	3	0.07	0.26	0.85	-0.11	0.43
	2 and 3	0.06	0.15	1.04	-0.12	0.47
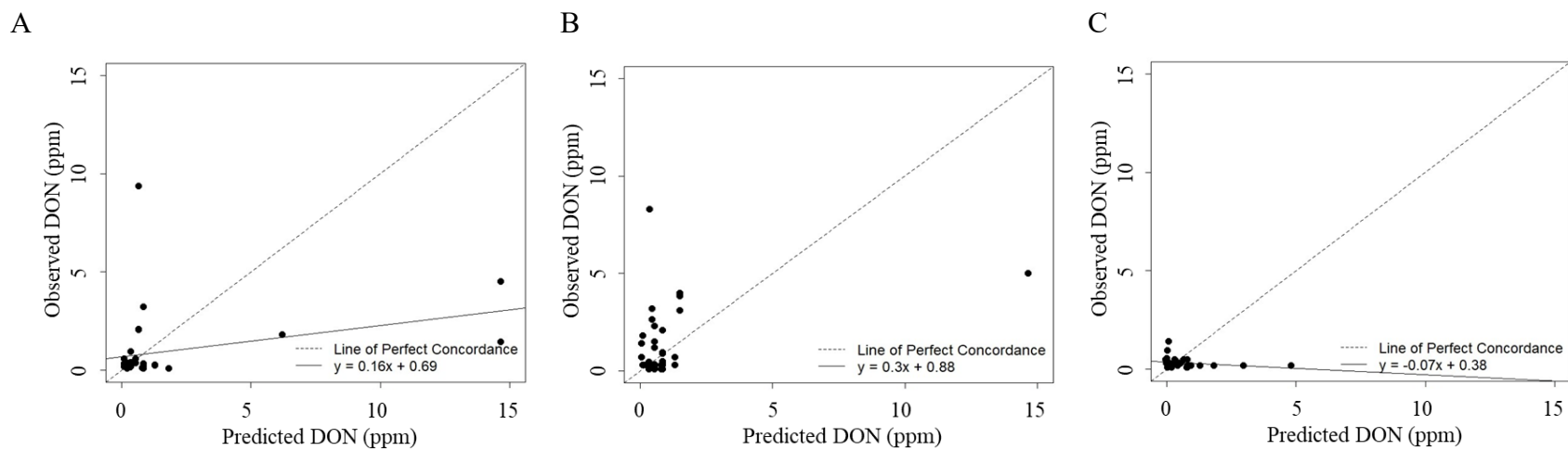
Figure 3.1 Observed versus predicted DON contamination (ppm) for (A) barley, (B) spring wheat, and (C) winter wheat using composite predictions of Hooker *et al*. (2002) Equations 2 and 3 under climatic conditions of the Canadian Maritime provinces.

The Hooker *et al*. (2002) model was the only model evaluated in this study that used head emergence as the critical growth stage for FHB development in winter wheat, which may be too early as wheat is most susceptible at anthesis (Osborne and Stein 2007). The ability of this model to accurately predict DON may also be affected by using daily rainfall amounts rather than % RH used in other models. Rainfall does not adequately capture how long free moisture remains in the environment and prolonged periods of high RH have been shown to increase spore germination and infection (Rossi et al. 2001; Xu et al. 2008; Shah et al. 2013). Shah *et al*. (2019) did not evaluate rainfall as a variable in the development of FHB since rainfall amounts are site specific. The commercial version of the Hooker *et al*. (2002) model, DONcast®, has site specific forecasts available through a proprietary weather network and accuracy has improved (Weather INnovations LP; Pitblado et al. 2007). Nonetheless, ECCC only publishes a rainfall range < 24 h before the event which is not suitable for this version of the Hooker model.

FHB forecasting models by De Wolf *et al*. (2003), Molineros (2007), and Shah *et al*. (2013) are classification models that were once deployed in the USWBSI Risk Tool. These models were evaluated for their accuracy, sensitivity and specificity when predicting epidemic and non-epidemic situations where a well fitted model would have accuracy > 80% and a balance between sensitivity and specificity. The AUC and κ were also calculated to determine whether the classification models performed better than chance or if predictions were in agreement with observed epidemics. In the case of the dataset used to evaluate these models, many TN were observed, inflating the accuracy calculation due to the class imbalance. Therefore, the agreement statistic (κ) provides the best measure of model accuracy.

The performance of FHB forecast models by De Wolf *et al*. (2003) varied. Model I was inferior for all crops as the agreement statistic could not be calculated for winter wheat and

indicated only slight agreement for barley and spring wheat (Table 3.4). Performance of this model declined in the Maritimes compared to its original 70% accuracy, 56% sensitivity, and 78% specificity in wheat (De Wolf et al. 2003). In Quebec, Giroux *et al*. (2016) found further reduction in performance of Model I with an AUC of 0.355, as few epidemics and non-epidemics were correctly classified. The environmental data set for Model I was reduced to only 8 yr as historical hourly rainfall was not available prior to 2014, decreasing the number of epidemics observed. Additional years of data may have improved the performance of Model I. As discussed above, moisture required for FHB development may be better represented by % RH than rainfall for forecasting purposes.

Models A and B by De Wolf *et al*. (2003) were unsuitable for spring and winter wheat in the Maritimes. Although model A had a high AUC and moderate agreement with historical epidemics of spring wheat, sensitivity was only 40% (Table 3.4). Sensitivity of Model B was 53%; however, specificity was reduced from 97% to 72% compared to Model A. The two epidemics observed in winter wheat were misclassified resulting in 0% sensitivity and poor agreement (Figure 3.2).

Table 3.4 Performance of De Wolf *et al*. (2003) FHB forecasting models under climatic conditions of the Canadian Maritime provinces. TP = true positives, TN = true negatives, FP = false positives, FN = false negatives, κ = Cohen's kappa.

Crop	De Wolf model	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC	κ
Barley										
	A	6	26	0	2	94	75	100	0.91	0.82
	B	7	20	6	1	79	88	77	0.85	0.53
	I	1	13	8	1	61	50	62	0.68	0.05
Spring Wheat										
	A	6	28	1	9	77	40	97	0.80	0.42
	B	8	21	8	7	66	53	72	0.73	0.25
	I	7	8	15	1	48	88	35	0.67	0.14
Winter Wheat										
	A	0	29	5	2	81	0	85	0.69	-0.09
	B	0	29	5	2	81	0	85	0.69	-0.09
	I	0	21	8	0	72	*	72	*	*

De Wolf *et al*. (2003) Model A performed best overall for barley in the Maritimes by correctly predicting 6 of 8 epidemics and all non-epidemics (Table 3.4). The AUC and kappa indicated a near perfect model (Figure 3.2). These results are similar to the findings of the Quebec study which found AUC values >0.9 (Giroux et al. 2016). The sensitivity of Model A was reduced, and accuracy and specificity were increased compared to the original performance of 84% accuracy, 83% sensitivity, and 84% specificity (De Wolf et al. 2003). Model B, which correctly predicted 7 of 8 epidemics and 20 of 26 non-epidemics, performed better than chance and had moderate agreement. Sensitivity of Model B was increased, and accuracy and specificity were reduced compared to the original performance of 84% accuracy and specificity and 83% sensitivity (De Wolf et al. 2003).
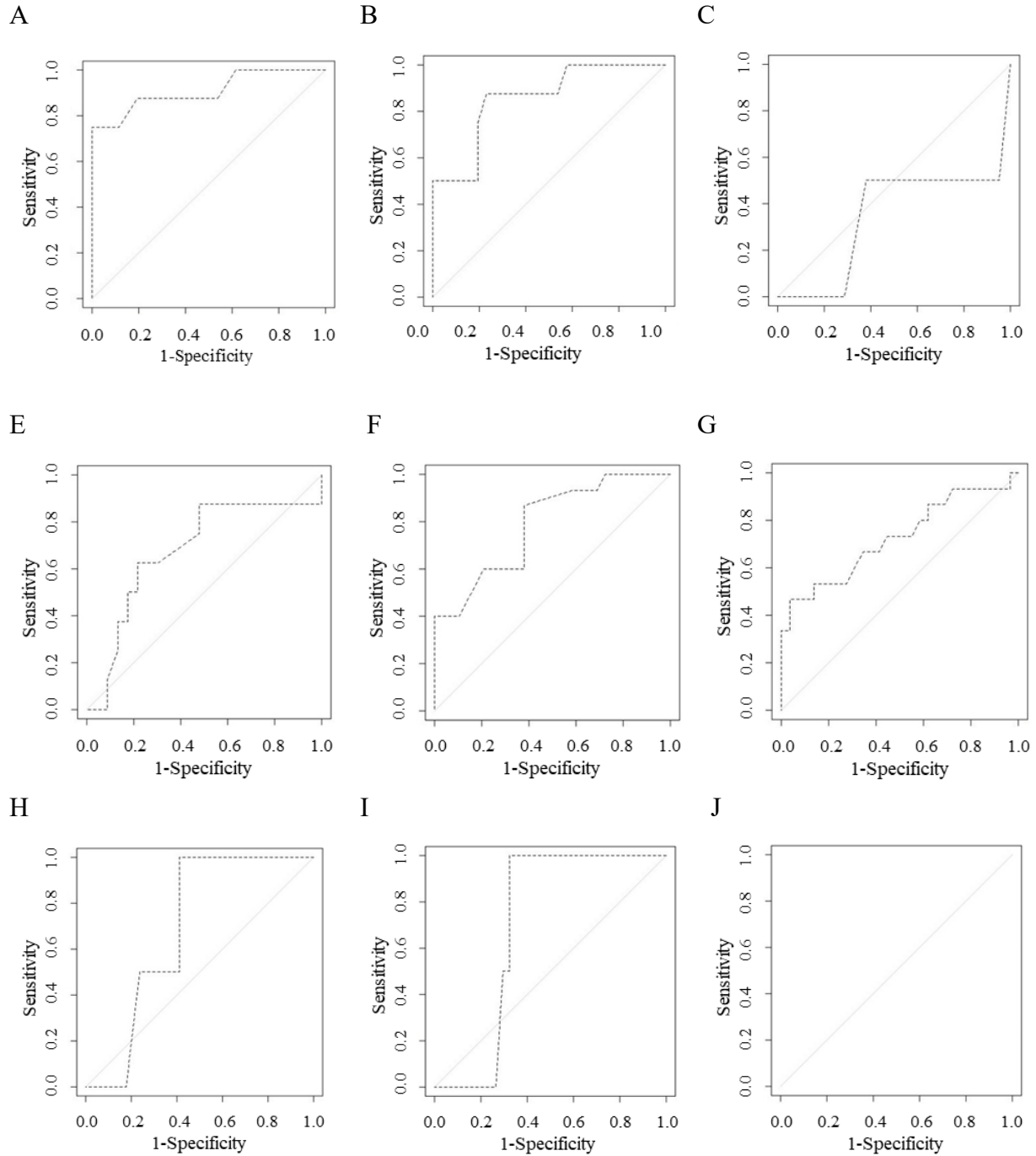
Figure 3.2 ROC curves for De Wolf *et al*. (2003) FHB forecasting models A, B, and I (left to right) of barley (A-C), spring wheat (E-G) and winter wheat (H-J). Solid diagonal represents no information line.

The ideal performance of the De Wolf *et al*. (2003) models for barley relies on a 10-d weather forecast. Model A uses a 0-10 d post-anthesis temperature and humidity variable and Model B uses a 0-7 d pre-anthesis temperature variable in an interaction with the Model A variable. These variables were effective under historical evaluation but not suitable for FHB forecasting in the Maritimes as a 10-d forecast is not available from ECCC. This long-range forecast could, however, have practicality in understanding history of FHB risk, effects of climate change, and development of a barley specific FHB forecast model in the Maritimes.

The FHB forecast models developed by Molineros (2007) integrated cultivar susceptibility in the spring wheat models and presence or absence of corn residue in winter wheat models. These models did not stand out for any crop as the most accurate models did not balance sensitivity and specificity and had poor to fair agreement (Table 3.5). The winter wheat model over predicted epidemics in the Maritimes as it misclassified all epidemics except for two observed in spring wheat. This model incorporates the presence of corn residue as a binary variable (1 = present, 0 = absent) that when present, interacts with temperature and humidity and temperature and rainfall variables. No historical data were available from a field previously planted to corn; therefore, this model was only evaluated in the absence of corn residue. When corn is absent, this model is not adequately sensitive for use in the Maritimes (Figure 3.3).

Table 3.5 Performance of Molineros (2007) FHB forecasting models under climatic conditions of the Canadian Maritime provinces. Susc. = susceptibility, TP = true positives, TN = true negatives, FP = false positives, FN = false negatives, κ = Cohen's kappa, SW = spring wheat, WW = winter wheat.

Crop, Molineros model	Susc. level	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC	κ
Barley										
SW	0:VS	8	8	18	0	47	100	31	0.66	0.17
	1:MS	5	17	9	3	65	63	65	0.66	0.22
	2:MR	1	20	6	7	62	13	77	0.66	-0.11
	3:R	0	26	0	8	76	0	100	0.66	0.00
WW	-	0	24	2	8	71	0	92	0.63	-0.10
Spring Wheat										
SW	0:VS	15	4	25	0	43	100	14	0.72	0.10
	1:MS	9	18	11	6	61	60	62	0.72	0.20
	2:MR	5	26	3	10	70	33	90	0.72	0.26
	3:R	0	29	0	15	66	0	100	0.72	0.00
WW	-	2	29	0	13	70	13	100	0.69	0.17
Winter Wheat										
SW	0:VS	2	10	24	0	33	100	29	0.72	0.04
	1:MS	1	26	8	1	75	50	76	0.72	0.10
	2:MR	1	32	2	1	92	50	94	0.72	0.36
	3:R	0	34	0	2	94	0	100	0.72	0.00
WW	-	0	32	2	2	89	0	94	0.87	-0.06

For barley and spring wheat, the spring wheat model performed better overall than the winter wheat model (Figure 3.3). Of the spring wheat model susceptibility levels, 0:VS overestimated epidemics, and 2:MR and 3:R underestimated epidemics. At level 1:MS fair agreement was met with balanced accuracy, sensitivity, and specificity at or near 65% for barley and 60% for spring wheat. The kappa value was highest for spring wheat at level 2:MR however, only 33% of epidemics were correctly classified. Few epidemic years in winter wheat did not allow for a thorough analysis however, the spring wheat model at the level 2:MR performed best with fair agreement and high specificity. Although the AUC was greater for winter wheat under the winter wheat model, it had poor agreement as both epidemics were incorrectly classified (Figure 3.3).

A   B

C   D

E   F
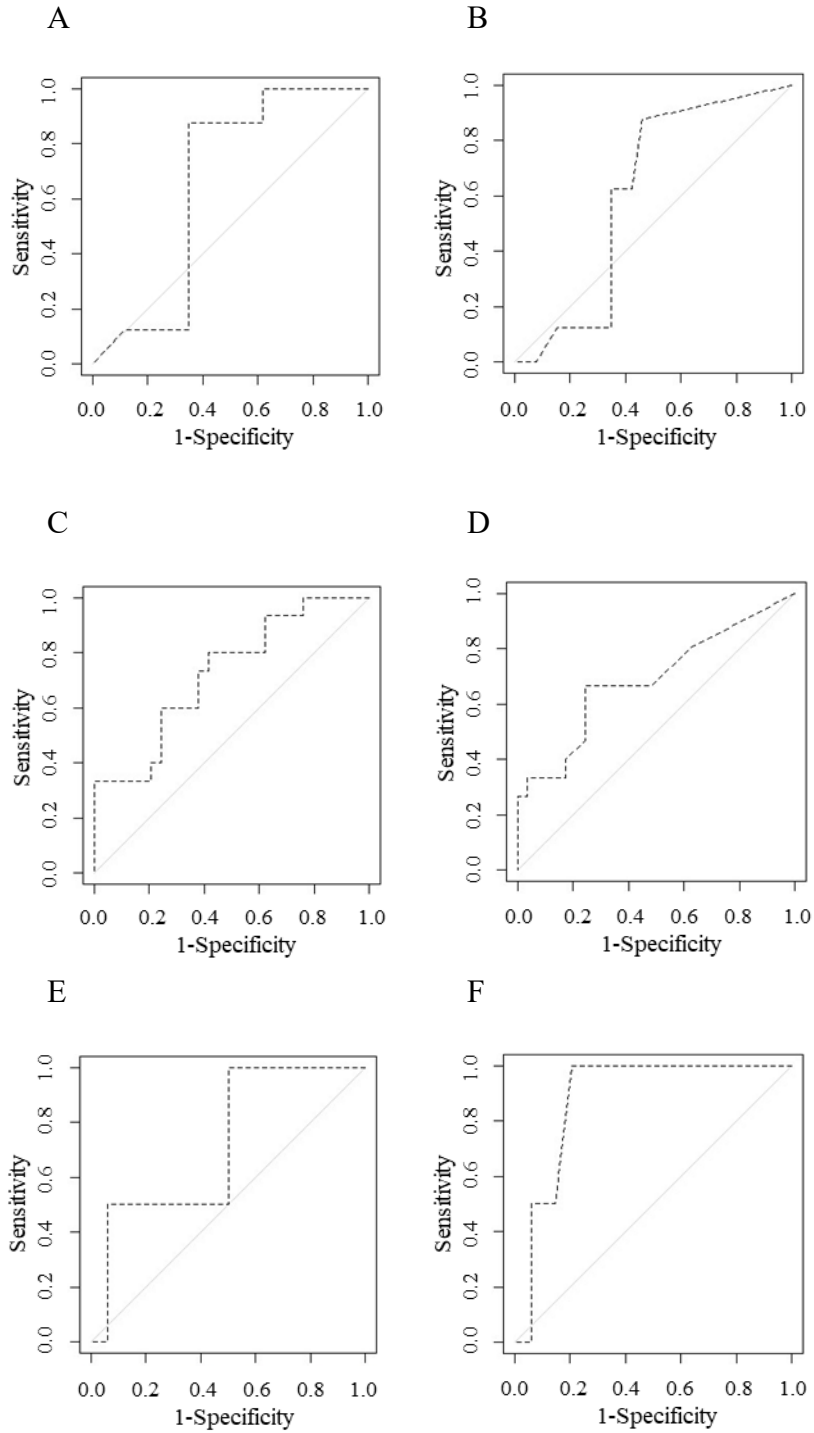
Figure 3.3 ROC curves for Molineros (2007) spring wheat (left)
and winter wheat (right) FHB forecasting models for barley (A,
B), spring wheat (C, D) and winter wheat (E, F). Solid diagonal
represents no information line.

The published performance of the spring wheat model was 78% accuracy, 86% sensitivity and 69% specificity (Molineros 2007). Accuracy and specificity may have been improved in the Maritimes if susceptibility ratings of each cultivar were available for this analysis. Giroux *et al*. (2016) evaluated the spring wheat model level 0:VS only and found an AUC of 0.6 whereas this study found a greater AUC of 0.66 for barley and 0.72 in spring and winter wheat. Using the original decision threshold, Giroux *et al*. (2016) reported an accuracy of 0.77, 86.7% sensitivity and 73% specificity. The performance of the Molineros spring wheat model was reduced in the Maritimes however sensitivity was improved in the Quebec study (Giroux et al. 2016). The spring wheat model at level 1:MS was the best model developed by Molineros (2007) for barley and spring wheat and at level 2:MR for winter wheat in the Maritimes.

The FHB forecast models by Shah *et al*. (2013) were an updated version of Molineros (2007). The corresponding cultivar susceptibility on a scale of 0-3 was adjusted in the revision by Shah *et al*. (2013) where 0 = very susceptible (VS), 1 = susceptible (S), 2 = moderately susceptible (MS), and 3 = moderately resistant (MR). The same weather variables were used in the spring and winter wheat models, only the coefficients differed, however presence or absence of corn residue was not accounted for in the updated winter wheat model. The resulting AUC of winter and spring wheat models by Shah *et al*. (2013) were similar to the models by Molineros *et al*. (2007; Figure 3.4).
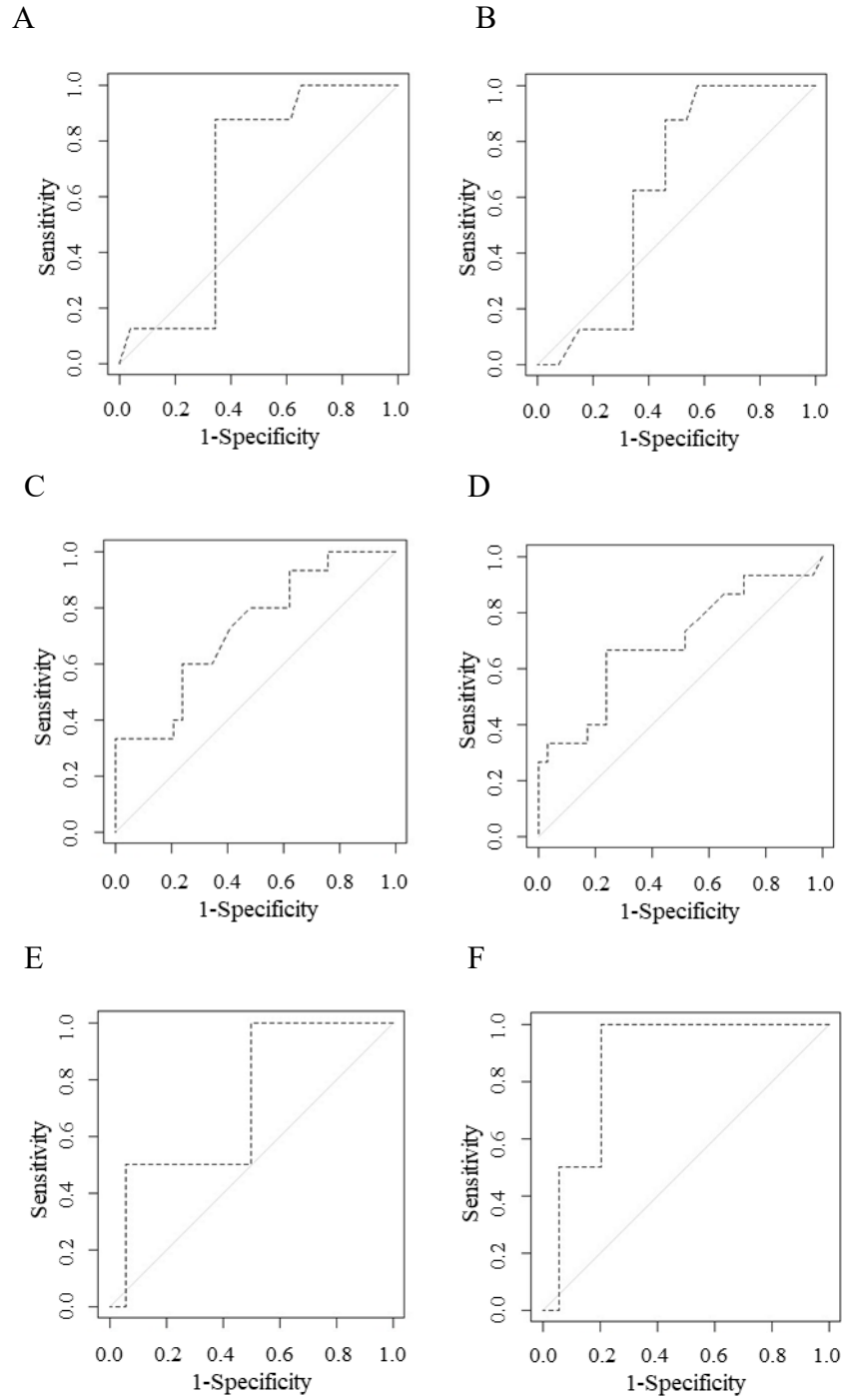
Figure 3.4 ROC curves for Shah *et al*. (2013) (left to right) spring wheat and winter wheat FHB forecasting models for (A, B) barley, (C, D) spring wheat and (E, F) winter wheat. Solid diagonal represents no information line.

Performance of the updated spring wheat model by Shah *et al.* (2013) was similar to that of Molineros (2007) for barley and spring wheat as level 0:VS, over predicted epidemics and at level 2:MS and 3:MR, epidemics were under predicted (Table 3.6). The agreement of the updated spring wheat model was improved at level 1:S and the accuracy was comparable to that of Molineros (2007) level 1:MS, as sensitivity increased to 80% for spring wheat and 88% for barley while specificity was reduced to 52% and 54%. At the susceptibility level 2:MS, performance of the updated model was identical to Molineros (2007) level 2:MR for winter wheat and remained the best model for this crop.

Table 3.6 Performance of Shah *et al.* (2013) FHB forecasting models under climatic conditions of the Canadian Maritime provinces. Susc. = susceptibility, TP = true positives, TN = true negatives, FP = false positives, FN = false negatives, κ = Cohen's kappa, SW = spring wheat, WW = winter wheat.

Crop, Shah model	Susc. level	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC	κ
Barley										
SW	0:VS	8	4	22	0	0.35	1.00	0.15	0.66	0.08
	1:S	7	14	12	1	0.62	0.88	0.54	0.66	0.28
	2:MS	1	17	9	7	0.53	0.13	0.65	0.66	-0.2
	3:MR	1	25	1	7	0.76	0.13	0.96	0.66	0.12
WW	-	7	14	12	1	0.62	0.88	0.54	0.71	0.28
Spring Wheat										
SW	0:VS	15	1	28	0	0.36	1.00	0.03	0.73	0.02
	1:S	12	15	14	3	0.61	0.80	0.52	0.73	0.27
	2:MS	6	23	6	9	0.66	0.40	0.79	0.73	0.20
	3:MR	0	29	0	15	0.66	0.00	1.00	0.73	0.00
WW	-	13	10	19	2	0.52	0.87	0.34	0.69	0.17
Winter Wheat										
SW	0:VS	2	4	30	0	0.17	1.00	0.12	0.72	0.01
	1:S	1	20	14	1	0.58	0.50	0.59	0.72	0.02
	2:MS	1	32	2	1	0.92	0.50	0.94	0.72	0.36
	3:MR	0	32	2	2	0.89	0	0.94	0.72	-0.06
WW	-	2	8	26	0	0.28	1.00	0.24	0.87	0.03

There was considerable improvement in the sensitivity of the winter wheat model by Shah *et al*. (2013) compared to that of Molineros (2007) as the updated model gained sensitivity while maintaining the same level of accuracy although specificity was reduced due to an increase of false positives. The agreement between historical epidemics and the updated winter wheat model increased from poor to fair for barley and poor to slight for winter wheat. The agreement remained the same for spring wheat. The updated winter wheat model over-predicted whereas the previous under predicted epidemics, however, it performed just as well as the spring wheat model at level 1:MS for barley. After the spring and winter wheat model updates by Shah *et al*. (2013), the reported combined performance was about 86% sensitivity and 52% specificity. This study observed a similar performance at the susceptibility level of spring wheat models selected as most accurate as well as the winter wheat model for barley. The sensitivity of the 3:MS level for winter wheat was lower than the original performance, however, few epidemics were observed in this study.

Of the nine FHB forecast models evaluated for use in the Maritimes, the updated models by Shah *et al*. (2013) were the most accurate. Although model performance was similar to Molineros (2007), the updated models represent the most recent epidemiological data. These models use only pre-anthesis weather variables which are available from ECCC unlike those requiring long range post-anthesis forecasts. Percent RH is also used as opposed to rainfall which may improve epidemic prediction.

3.3.3   Fungicide Efficacy Field Trials

Trials were established under natural field inoculation conditions to evaluate the efficacy of fungicide application guided by an FHB forecast. The effect of cultivar and fungicide treatment on yield and DON contamination were compared to untreated controls for barley, spring wheat,

and winter wheat. In 2020, weather conditions did not support the development of FHB.

Significant differences in yield were only observed in barley where an application of Prosaro®

XTR increased yields (Table 3.7). DON contamination was not reduced with fungicide

application in barley and DON was not detected in wheat (Table 3.7). Contamination did not

exceed 0.9 ppm DON; therefore, a fungicide application to reduce FHB was not necessary in

2020.

Table 3.7 Means of crop yield and DON contamination of 2020 fungicide efficacy field trials. Bold text indicates significant effect of fungicide treatment determined using ANOVA at $p \leq 0.05$.

Crop	Cultivar	Fungicide Treatment	Yield (t ha^{-1})	DON (ppm)
Barley	Island	UTC	2.63	0.13
		Prosaro® XTR	**3.04**	0.16
Spring Wheat	Easton	UTC	2.73	0
		Prosaro® XTR	2.85	0
Winter Wheat	AC Sampson	UTC	2.51	0
		Prosaro® XTR	2.39	0
	Pioneer® 25R40	UTC	2.32	0
		Prosaro® XTR	2.18	0

In 2021, cultivar had a significant effect on barley yield in Trial B only (Table 3.8). Yield

of Island barley was 3.59 t ha^{-1}, significantly greater than Leader with a yield of 3.38 t ha^{-1}.

Fungicide treatment had a significant effect on barley yield in Trial C only and no significant

cultivar X treatment interaction effects were observed (Table 3.8). The Miravis® Ace treatment

in Trial C resulted in a significantly higher yield of 4.20 t ha^{-1} compared to the untreated barley

control with 3.69 t ha^{-1}. Cultivar and treatment did not have a significant effect on DON

contamination which was ≤ 0.51 ppm in the barley trials (Table 3.9). Fungicide treatments to

reduce FHB in barley in 2021 were not necessary.

Table 3.8 ANOVA probability values for effects of cultivar, fungicide treatment, and their interaction on barley yield in 2021 fungicide efficacy trials. Bold text indicates significant effect at $p \leq 0.05$.

Barley trial	Effect	DF	F Ratio	Prob > F
	Cultivar	1	0.6081	0.4555
A	Treatment	2	2.0961	0.2576
	Cultivar*Treatment	2	0.2707	0.7688
	Cultivar	1	5.1669	**0.0491**
B	Treatment	2	7.8687	0.0555
	Cultivar*Treatment	2	2.3950	0.1466
	Cultivar	1	2.1562	0.1761
C	Treatment	2	9.844	**0.0300**
	Cultivar*Treatment	2	3.1361	0.0926

Table 3.9 ANOVA probability values for effects of cultivar, fungicide treatment, and their interaction on DON contamination of barley in 2021 fungicide efficacy trials.

Barley trial	Effect	DF	F Ratio	Prob > F
	Cultivar	1	0.8254	0.3873
A	Treatment	2	0.2087	0.8189
	Cultivar*Treatment	2	0.6679	0.5318
	Cultivar	1	0.0113	0.9177
B	Treatment	2	0.4141	0.6817
	Cultivar*Treatment	2	2.926	0.105
	Cultivar	1	0.5395	0.4813
C	Treatment	2	8.2147	0.0761
	Cultivar*Treatment	2	0.3043	0.7449

Cultivar had a significant effect on spring wheat yield in all three trials (Table 3.10). Yields of Easton were significantly greater at 2.98, 2.56, 2.68 t ha^{-1} than yields of AC Walton at 2.58, 2.20, 2.40 t ha^{-1} in Trials A, B, and C, respectively. Fungicide treatment had a significant effect on yield in Trials A and B (Table 3.10) In Trial A, spring wheat treated with Miravis® Ace yielded 2.89 t ha^{-1} and Prosaro® XTR yielded 2.93 t ha^{-1}. Trial A treatments had significantly greater yield than the untreated spring wheat control at 2.51 t ha^{-1}. In Trial B, only the Prosaro®

XTR treatment resulted in significantly higher yield of 2.55 t ha^{-1} than the untreated control with

of 2.18 t ha^{-1}. There was no significant interaction between spring wheat cultivars and treatment.

Cultivar and treatment had a significant effect on DON contamination in harvested spring

wheat of Trial A only (Table 3.11). DON contamination in Easton grain was 1.14 ppm and

significantly less at 0.33 ppm in AC Walton grain. In the untreated spring wheat control of Trial

A, DON contamination was 1.15 ppm and DON was significantly lower at 0.84 and 0.21 ppm

when treated with Prosaro® XTR and Miravis® Ace, respectively. Untreated spring wheat

controls in Trials B and C had 0.31 and 0.49 ppm DON. Spring wheat samples treated with

Miravis® Ace from Trial B were excluded from DON analysis due to a storage issue. Fungicides

were required in spring wheat Trial A and reduced DON below 0.9 ppm, however only Miravis®

Ace resulted in a significant reduction (Table 3.11). Fungicides were unnecessary at Trials B and

C anthesis dates.

Table 3.10 ANOVA probability values for effects of cultivar, fungicide treatment, and their interaction on spring wheat yield in 2021 fungicide efficacy trials. Bold text indicates significant effect at $p \leq 0.05$.

Spring Wheat Trial	Source	DF	F Ratio	Prob > F
A	Cultivar	1	11.7801	**0.0037**
	Treatment	2	5.3293	**0.0178**
	Cultivar*Treatment	2	0.0585	0.9433
B	Cultivar	1	10.5025	**0.0055**
	Treatment	2	3.9141	**0.0429**
	Cultivar*Treatment	2	1.234	0.3191
C	Cultivar	1	26.3848	**<.0001**
	Treatment	2	1.9044	0.43
	Cultivar*Treatment	2	0.3732	0.6937

Table 3.11 ANOVA probability values for effects of cultivar, fungicide treatment, and their interaction DON contamination of spring wheat in 2021 fungicide efficacy trials. Bold text indicates significant effect at $p \leq 0.05$.

Spring Wheat Trial	Source	DF	F Ratio	Prob > F
A	Cultivar	1	8.2571	**0.0116**
	Treatment	2	3.989	**0.0408**
	Cultivar*Treatment	2	1.7078	0.2655
B	Cultivar	1	0.1919	0.6717
	Treatment	1	0.0914	0.7693
	Cultivar*Treatment	1	1.727	0.2213
C	Cultivar	1	0.3527	0.5672
	Treatment	2	1.3545	0.3536
	Cultivar*Treatment	2	0.7222	0.5118

Cultivar significantly affected winter wheat yield while effects of fungicide treatment and cultivar X treatment interaction were not significant (Table 3.12). Pioneer® 25R40 yielded 4.07 t ha^{-1}, which was significantly more than AC Sampson at 3.53 t ha^{-1}. This could be due to differences in plant density resulting from winter kill. Fungicide treatments did not have a significant effect on yield or DON contamination. DON contamination was <0.1 ppm in winter wheat and fungicides for FHB were not necessary.

Table 3.12 ANOVA probability values for effects of cultivar, fungicide treatment, and their interaction on winter wheat yield and DON in 2021. Bold text indicates significant effect at $p \leq 0.05$.

Crop metric, effect	DF	F Ratio	Prob > F
Yield			
Cultivar	1	17.3643	**0.0006**
Treatment	2	0.7216	0.4995
Cultivar*Treatment	2	1.2679	0.3054
DON			
Cultivar	1	1.884	0.1867
Treatment	2	0.494	0.6182
Cultivar*Treatment	2	1.1478	0.3395

While FHB was the focus of this study, severity of leaf disease was evaluated as severe leaf infections may also impact yields. Other than DON, visual FHB, and grain quality metrics such as TKW, protein and FDK may also be impacted by FHB and fungicide application. In 2021, effects of fungicide treatment on visual disease and grain quality were determined by ANOVA. There were no significant effects of fungicide treatments observed in winter wheat. In barley, there were significant reductions in net blotch across trials when treated with fungicides (Table 3.13). Miravis® Ace significantly reduced visual FHB in Trial C and protein was reduced in Trials A and B. TKW of barley was significantly increased with fungicide treatments. In spring wheat trials, fungicide treatments resulted in significant decreases of PM severity, visual FHB, and protein and significant increases in TKW (Table 3.14). FDK were significantly reduced in Trial C only with the Prosaro® XTR treatment.

Table 3.13 Means of leaf disease, visual FHB, and grain quality of barley in 2021 fungicide efficacy trials determined to be significantly affected by fungicide treatment using ANOVA. Mean values by planting date (A) 16 Apr, (B) 4 May, and (C) 25 May 2021 are presented. Bold text indicates mean significantly different than UTC at $\alpha \leq 0.05$ using Tukey's HSD test.

Barley Trial	Treatment	Net blotch Flag	Net blotch Pen	FHB Z80	FHB 7d	TKW (g)	Protein (%)
A	UTC	0.34	0.71	1.00	1.13	45.89	9.45
	Prosaro® XTR	**0.06**	**0.35**	1.00	1.00	**47.81**	9.40
	Miravis® Ace	0.14	0.39	1.00	1.00	47.51	9.06
B	UTC	0.31	0.56	1.00	1.13	48.45	10.37
	Prosaro® XTR	**0.03**	0.35	1.00	1.00	**51.56**	10.27
	Miravis® Ace	**0.03**	**0.20**	1.00	1.00	**53.19**	**9.71**
C	UTC	1.24	3.21	3.00	-	48.32	10.47
	Prosaro® XTR	**0.54**	**0.96**	**1.00**	-	**51.15**	10.27
	Miravis® Ace	**0.55**	**0.95**	**1.00**	-	**53.06**	10.10

UTC = untreated control; Flag = flag leaf; Pen = penultimate leaf; FHB = Fusarium head blight; FHB Z80 = visual FHB evaluation at Zadok's growth stage 80; FHB 7d = visual FHB evaluation 7 d after Z80; TKW = thousand kernel weight.

Table 3.14 Means of leaf disease, visual FHB, and grain quality of spring wheat in 2021 fungicide efficacy trials determined to be significantly affected by fungicide treatment using ANOVA. Mean values by planting date (A) 16 Apr, (B) 4 May, and (C) 25 May 2021 are presented. Bold text indicates mean significantly different than UTC at $\alpha \leq 0.05$ using Tukey's HSD test.

Spring Wheat Trial	Treatment	PM Flag	FHB Z80	FHB 7d	TKW (g)	Protein (%)	FDK
A	UTC	1.23	3.50	18.00	32.08	11.00	1.88
	Prosaro® XTR	1.03	2.88	**7.63**	**35.77**	10.58	1.63
	Miravis® Ace	1.04	**1.25**	**5.63**	**37.36**	**10.39**	1.38
B	UTC	1.36	12.13	45.88	31.75	11.47	3.00
	Prosaro® XTR	**1.01**	**8.63**	**24.00**	**34.76**	**10.97**	2.75
	Miravis® Ace	**0.98**	7.88	**12.75**	**36.38**	**10.94**	2.88
C	UTC	0.84	7.38	41.00	30.02	11.44	2.13
	Prosaro® XTR	0.69	5.63	**26.75**	31.19	**10.75**	**1.38**
	Miravis® Ace	0.66	**4.00**	**14.00**	**34.81**	10.72	1.75

UTC = untreated control; PM = powdery mildew; Flag = flag leaf; FHB = Fusarium head blight, FHB Z80 = visual FHB evaluation at Zadok's growth stage 80; FHB 7d = visual FHB evaluation 7 d after Z80; TKW = thousand kernel weight; FDK = Fusarium damaged kernels.

Fungicides applied to supress FHB and reduce DON are also registered for the control of certain leaf diseases (Bayer CropScience Inc. ; Syngenta Canada Inc. 2021). Net blotch and powdery mildew as well as visual FHB symptoms were reduced with one or both fungicide treatments in this study. Fungicide applications have been found to increase yields due to a delay in flag leaf senescence which may enhance grain fill and increase kernel size and TKW (Entz et al. 1990; Ruske et al. 2003). In the current study, yields were not always increased with a fungicide treatment, however, one or both fungicide treatments increased TKW of barley and spring wheat.

Protein is an important quality parameter for milling wheat and malt barley and was sometimes reduced by fungicide treatment in this study. Significant differences in protein may be accounted for by a cultivar or fungicide x cultivar interaction (Dexter et al. 1996; Ruske et al. 2003) that was not studied here. Other research however, found no effect of fungicide treatment on grain protein content (Blandino et al. 2009; Blandino and Reyneri 2009).

3.3.4 Fungicide Application Guided by FHB Forecasts

The FHB forecast models by Shah *et al*. (2013) determined to be most accurate for cereal crops grown in the Maritimes were evaluated on fungicide trials conducted in 2020 and 2021 to compare model predictions with treated and untreated barley and wheat cultivars. (Table 3.15). No epidemics were observed in barley and neither selected model for this crop performed well. The spring wheat 1:S model misclassified five of seven non-epidemics and the winter wheat model misclassified all non-epidemics of barley.

DON was ≥ 0.9 ppm only in Easton spring wheat with anthesis on Jul 2, 2021, which was correctly classified however, all non-epidemics were misclassified by the spring wheat 1:S model. Non-epidemics of winter wheat were correctly classified by the spring wheat 2:MS model in 2020 and 2021. Favourable conditions for FHB development were observed in 2021 however, moderate drought experienced in PE in 2020 (Agriculture and Agri-Food Canada 2022) may have prevented inoculum buildup for 2021.

Table 3.15 Epidemics observed in 2020 and 2021 in field trials and performance parameters of selected Shah *et al*. (2013) FHB forecasting models. FHB Epidemics are represented by LS mean of DON ≥ 0.9 ppm in untreated control plots. SW = spring wheat, WW = winter wheat.

Crop, model	Epidemic	Non-epidemic	TP	TN	FP	FN	Accuracy	Sensitivity	Specificity	AUC	κ
Barley											
SW 1:S	0	7	0	2	5	0	0.29	0	0.29	*	0
WW	0	7	0	0	7	0	0	0	0	*	0
Spring Wheat											
SW 1:S	1	6	1	0	6	0	0.14	1.00	0	0.92	0
Winter Wheat											
SW 2:MS	0	4	0	4	0	0	1.00	0	1.00	*	*

Several false positive classifications occurred in the analysis, which indicated risk of an epidemic when one did not occur. Fungicide application under this circumstance would add unnecessary expenses in a non-epidemic year. However, up to 74% yield losses have been reported due to FHB epidemics (Wegulo et al. 2015) therefore, greater economic losses arise from crop failure than unnecessary fungicide application (Bondalapati et al. 2012; Giroux et al. 2016). Fungicides have been found effective in reducing *Fusarium* infection and DON contamination and may increase yields when applied appropriately, however, their effectiveness may be reduced when conditions favourable for disease continue post-anthesis (Paul et al. 2008; Paul et al. 2010). As observed in 2021 field trials, when DON was > 1 ppm in spring wheat, fungicide application reduced DON contamination to < 0.9 ppm which would have retained the value of the grain. However, significant yield increases were not always observed when fungicides were applied in this study. The potential yield increase alone may not recuperate the expense of an unnecessary fungicide application and depends on the cultivar susceptibility and market value of harvested grain (Cowger et al. 2016).

3.3.5 Evaluating FHB Forecasting in the Maritimes

Each barley and winter wheat sample received from the AGC was subject to DON analysis. Average DON for each site determined whether an epidemic occurred at DON ≥ 0.9 ppm. Using the approximate week of head emergence and anthesis, epidemics of barley and spring wheat from across the Maritimes were compared to weekly averages of forecast risk. Predictive errors were expected when using estimates of critical growth stage and risk. Future validation work would benefit from accurate head emergence and anthesis recorded in regional survey efforts.

The κ values for the epidemic predictions by Shah *et al.* (2013) models were low, resulting in poor agreement with epidemic occurrences in AGC barley and winter wheat trials

(Table 3.16). The spring wheat 1:S and winter wheat models evaluated for barley had low

accuracy and specificity due to the high number of false positives. However, the winter wheat

model had greater sensitivity as eight of nine FHB epidemics in barley were correctly classified.

In 2018, three of seven barley sites from Colchester, NS and Queens, PE had DON

contamination greater than 0.9 ppm and were misclassified by the spring wheat 1:S model. The

winter wheat model misclassified only the PE barley epidemics. All six barley sites across the

region in 2021 had DON contamination > 0.9 ppm which were correctly classified by both

models.

Table 3.16 Epidemics observed in the AGC survey from 2018-2021 and performance parameters of selected Shah *et al*. (2013) FHB forecasting models. Epidemics are represented by mean DON $\geq$ 0.9 ppm at each survey site. SW = spring wheat, WW = winter wheat.

Crop, model	Epidemic	Non - epidemic	TP	TN	FP	FN	Accuracy	Sensitivity	Specificity	AUC	κ
Barley											
SW 1:S	9	16	6	3	13	3	0.36	0.67	0.19	0.69	-0.12
WW	9	16	8	2	14	1	0.4	0.89	0.13	0.74	0.01
Winter Wheat											
SW 2:MS	1	13	0	13	0	1	0.93	0.00	1.00	0.88	0.00

A Colchester, NS site represented the only epidemic observed in winter wheat from the

AGC survey, which was misclassified by the spring wheat 2:MS model by Shah *et al.* (2013). It

is suspected that corn residue may have been present at the Colchester site, which may have

played a role in this epidemic development. All non-epidemics at winter wheat sites were

correctly classified by the spring wheat 2:MS model. From combined historical analysis,

fungicide trials, and regional survey data, FHB epidemics of barley are best classified in the

Maritimes using the Shah *et al*. (2013) winter wheat model. FHB epidemics of spring and winter

wheat are best classified using the Shah *et al*. (2013) spring wheat model at susceptibility level 1:S and 2:MS respectively, in the Maritimes.

3.3.6 Limitations of the Study and Future Work

The goal of FHB forecasting is to not only confirm necessity of fungicide application when disease risk is elevated but to prevent unnecessary applications when risk is low (Wegulo et al. 2015). In this study, even the best performing models overestimated epidemics which suggests unwarranted fungicide applications. This may have been improved in the current study if accurate dates rather than weekly estimates were used in the analysis of AGC survey samples. It is known however, that forecast models have to be calibrated when used in a new region (Prandini et al. 2009; Giroux et al. 2016). Decision thresholds could be modified, and the environmental dataset could be expanded and used to re-fit forecast models for improved FHB predictions in future Maritime FHB forecasting work.

Environmental variables other than those used in the evaluated models may be considered in a completely new FHB forecast model customized for the Maritimes. Each study from which models were extracted for this study included numerous unused variables that were not correlated to FHB in the region where the models were developed. These additional environmental variables could be evaluated in the Maritimes. There is also an abundance of research on the environmental conditions supportive of FHB development that new variables could be drawn from (Osborne and Stein 2007; Manstretta et al. 2016; Manstretta and Rossi 2016; Shah et al. 2019). Additionally, a barley specific FHB forecast model would be of interest for the region given its far greater economic importance than wheat (Agriculture and Agri-Food Canada 2021).

Cultivar susceptibility and crop rotation are important management considerations when managing FHB and were not evaluated in this study (Wegulo et al. 2015). Susceptibility to FHB varies among cultivars and planting the least susceptible cultivar is important for minimizing impacts of FHB therefore, Molineros (2007) and Shah *et al*. (2013) incorporated cultivar susceptibility in the spring wheat forecast models. ARCCC does not publish FHB susceptibility ratings which did not allow for a complete analysis of the effectiveness of these forecast models in the Maritimes. Ontario (Ontario Cereal Crops Committee 2022) and Quebec (Réseau des grandes cultures du Québec 2022) however, publish their ratings annually.

The presence or absence of corn residue was included in the Molineros (2007) winter wheat model and was not evaluated as crop rotation was not included in this study. NS produces the most corn in the Maritimes while corn production in PE is steadily increasing (Agriculture and Agri-Food Canada 2021). Corn producers may benefit from a forecast model incorporating the effect of corn residue. Access to information on cultivar susceptibility and crop rotation would not only support cultivar choices by producers but aid further development of FHB forecasting in the Maritimes.

3.4 Conclusion

During this study, epidemics of FHB in the Maritimes were sporadic which is characteristic of the disease (McMullen et al. 2012). Many factors contribute to the annual risk of FHB and integrated management strategies remain paramount in reducing impacts of this devastating disease regardless of forecast availability (Pitblado et al. 2007; Wegulo et al. 2015). The best FHB forecast models for the region used a 1-7 d pre-anthesis record of temperature and relative humidity. Additional agronomic data and study of environmental variables associated with elevated FHB risk in the Maritimes would support future work to reduce false positive epidemic

predictions and implement FHB forecasting in the region. No model is or will be perfect in the prediction of FHB epidemics and this study represents the first step in introducing FHB forecasting to the Canadian Maritimes.

Chapter 4 - Conclusion

4.1 Research Summary

This research was conducted to characterize and establish a baseline of the population of causal species of Fusarium head blight (FHB) in the Maritime provinces of Canada and to explore FHB forecasting in the region. Chapter 2 aimed to survey barley and wheat crops in the Maritimes to identify the causal species of FHB and determine the mycotoxin genotype, virulence, and fungicide susceptibility of *F. graminearum*. It was hypothesized that species composition and primary causal agent would vary by year and province. This hypothesis was supported as four principal causal species were identified including *F. graminearum*, *F. sporotrichioides*, *F. avenaceum*, and *F. poae* (Figure 2.2). Year, province, and species were all significant factors affecting the frequency of *Fusarium* spp. isolated (Table 2.6). It was also hypothesized that 3-ADON would be the most abundant mycotoxin genotype of *F. graminearum* in the region and that fungicide sensitivity and pathogen virulence would vary by province. 3-ADON was the dominant *TRI3* genotype accounting for 71.43% of *F. graminearum* isolates (Figure 2.6) and sensitivity to certain fungicide products were affected by year and host (Table 2.10) supporting this hypothesis. The results of the virulence assay were inconclusive but provided an opportunity to perform Koch's postulates.

The objective of Chapter 3 was to evaluate the performance of published North American FHB forecasting models in the Maritime provinces. It was hypothesized that models developed in similar climates or using pre-anthesis weather variables would have superior performance. While none of the models were specifically developed in a Maritime climate, some are applied in the coastal US. The models that performed best in the Maritimes were those using pre-anthesis weather variables, such as temperature and humidity, which are readily available from local

102

weather stations, supporting this hypothesis (Table 3.6). It was also hypothesized that fungicide applications would only be economically viable with elevated risk of disease. The research showed that yields do not always increase with fungicide application. When yield increases were observed, fungicide application expenses may have been recuperated depending on application costs, current selling price for grain including discounts for DON contamination and FDK.

Although four principal causal species were identified in the Maritimes, mycotoxin contamination above regulatory guidelines were only associated with *F. graminearum*. DON contamination was elevated in years and provinces when *F. graminearum* was most frequently isolated (Figure 2.2; Table 2.8). Mycotoxins produced by *F. sporotrichioides*, *F. avenaceum*, and *F. poae* were quantified below regulatory guidelines in this research. Understanding whether *F. graminearum* and DON were the primary concern in the region was an important first step to evaluating FHB forecasting. North American models have been developed to predict epidemics caused by *F. graminearum* and DON accumulation and would not be suitable for the other species identified as their respective temperature and humidity ranges differ from *F. graminearum* (Xu et al. 2008). FHB forecasting could support Maritime cereal producers in understanding annual risk of *F. graminearum* infection and dangerous accumulation of DON, and guide fungicide decision making. This will be a valuable tool for wheat and barley producers in addition to integrated management plans.

4.2 Future Research

The future of this research includes modified methodologies and FHB forecast validation and implementation. Characterization of the FHB causal population could be enhanced with the addition of other FHB susceptible cereal crops to surveys. The population of FHB causal species in the Maritimes is not limited to those hosted by wheat and barley as corn and oat are also

infected by *Fusarium* spp. causing ear rot and FHB (Sutton 1982; Tekauz et al. 2004). Fusarium ear rot of corn in Canada is caused by *F. graminearum* and is often associated with insect damage (Munkvold et al. 1997; Foroud et al. 2014). Grain corn contributed $47.3 million to the Maritime economy in 2021 and is mainly produced in NS however, production in PE has been increasing (Agriculture and Agri-Food Canada 2021; Atlantic Grains Council 2022b). Oat is most often infected with *F. poae* and *F. sporotrichioides* in Canada therefore, NIV, T-2 and HT-2 toxins are of concern in this crop, while also being susceptible to *F. graminearum* and DON contamination (Tamburic-Ilincic 2010; Xue and Chen 2022). Oat contributed $14.1 million to the Maritime economy in 2021 and is mainly produced in NB (Agriculture and Agri-Food Canada 2021; Atlantic Grains Council 2022c). Corn and oat could be added to the regional FHB surveys to understand the full scope of pathogen populations and associated mycotoxins in the Maritimes.

In the current study, the chemotype of *F. graminearum* isolates was inferred by determining the trichothecene *TRI*3 genotype using a PCR assay. In the Maritimes, 3-ADON and 15-ADON genotypes were identified. Genotyping however, is not always predictive of the chemotype and chemical analysis of isolates is suggested (Desjardins 2008). This obstacle was faced when *F. graminearum* isolates genotyped as 3-ADON did not produce DON or NIV (Gale et al. 2007; Gale et al. 2010) and were later found to produce a previously unknown mycotoxin, NX-2 (Varga et al. 2015). *F*usarium *graminearum* producing NX-2 has been isolated from the Maritimes (Kelly et al. 2015; Crippin et al. 2019) however, this toxin was not detected in LC-MS/MS analysis of grain samples from 2018-2021. The limitations associated with LC-MS/MS including sample size or toxin presence below the MQL (method quantification limit) may have impacted this result. Isolates genotyped as 3-ADON, however, may be screened using a PCR

RFLP (restriction fragment length polymorphism) assay to identify a *TRI1* polymorphism that is predictive of NX-2 toxin production (Liang et al. 2014). The suspect NX-2 producers can be grown on GYEP (glucose-yeast extract-peptone) agar to induce toxin production for confirmation by chemical analysis (Crippin et al. 2019).

To characterize virulence, wheat and barley plants were grown in a greenhouse and a conidial suspension of 24 *F. graminearum* isolates were each applied to seed heads. Disease severity varied among replicates and no significant differences were observed among isolates in this study. The experiment may have been improved with enhanced humidity control and light quality. However, a simpler, rapid assay known as 'clip-dipping' developed by Shin *et al*. (2014) could be investigated for virulence work. The method used 10-day-old wheat seedlings where the tip was cut off the coleoptile then dipped in a conidial suspension (Shin et al. 2014). The resultant disease severity evaluations from this method were highly correlated with in field disease development and type II resistance. Understanding whether highly virulent strains are present in the region supports breeding initiatives, disease management, and forecasting.

To support validation of future FHB forecasting in the Maritimes, additional agronomic data and site years are required. Improved survey data including tillage, crop rotation history and plant growth stages, and most importantly head emergence and anthesis dates of surveyed cereal crops would improve future forecast validation efforts. These factors, along with weather, could be analysed for their contribution to FHB risk and integrated into forecasting tools like Molineros (2007) and Shah *et al*. (2013).

Upon implementation, the FHB epidemic risk level associated with head emergence or anthesis of each crop could be added to cultivar registration publications to compare with DON detected each year. Currently, DON is only quantified by the Atlantic Recommending

Committee from registration trials conducted at AAFC Harrington. Additional sites should be considered to provide regional insight into FHB development, which would not only be informative to producers for cultivar selection but to translate risk values to the outcomes of fungicide applications and DON accumulation. The current research presents a first look at FHB forecasting in the Maritimes with opportunities to advance and implement a new management tool for cereal producers.

REFERENCES

Aboukhaddour R., Fetch T., McCallum B.D., Harding M.W., Beres B.L., Graf R.J. 2020. Wheat diseases on the prairies: A Canadian story. Plant Pathol. 69(3):418-432. doi:10.1111/ppa.13147

Agriculture and Agri-Food Canada. 2021. Area, yield, and production of Canadian principal field crops report. [accessed 2022 Nov 8]. https://agriculture.canada.ca/en/sector/crops/reports-statistics?menupos=01.01.01.01.

Agriculture and Agri-Food Canada. 2022. Canadian drought monitor. [accessed 2022 Nov 8]. https://agriculture.canada.ca/en/agricultural-production/weather/canadian-drought-monitor.

Alberta Climate Information Service. 2022. Fusarium disease severity. [accessed 2023 Jan 5]. https://agriculture.alberta.ca/acis/m#!fusarium.

Alexander N.J., Proctor R.H., McCormick S.P. 2009. Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. Toxin Reviews. 28(2-3):198-215. doi:10.1080/15569540903092142

Amarasinghe C.C., Tamburic-Ilincic L., Gilbert J., Brûlé-Babel A.L., Dilantha Fernando W.G. 2013. Evaluation of different fungicides for control of Fusarium head blight in wheat inoculated with 3ADON and 15ADON chemotypes of *Fusarium graminearum* in Canada. Can J Plant Pathol. 35(2):200-208. doi:10.1080/07060661.2013.773942

Anderson N.R., Freije A.N., Bergstrom G.C., Bradley C.A., Cowger C., Faske T., Hollier C., Kleczewski N., Padgett G.B., Paul P. et al. 2020. Sensitivity of *Fusarium graminearum* to metconazole and tebuconazole fungicides before and after widespread use in wheat in the United States. Plant Health Progr. 21(2):85-90. doi:10.1094/PHP-11-19-0083-RS

Atlantic Grains Council. 2022a. Barley. [accessed 2023 Jan 20]. https://atlanticgrainscouncil.ca/newwp/wp-content/uploads/2022/01/Barley-Profile-2021.pdf.

Atlantic Grains Council. 2022b. Corn. [accessed 2022 Nov 15]. https://atlanticgrainscouncil.ca/newwp/wp-content/uploads/2022/01/Corn-Profile-2021.pdf.

Atlantic Grains Council. 2022c. Oats. [accessed 2022 Nov 15]. https://atlanticgrainscouncil.ca/newwp/wp-content/uploads/2022/01/Oats-Profile-2021.pdf.

Atlantic Grains Council. 2022d. Wheat. [accessed 2023 Jan 20]. https://atlanticgrainscouncil.ca/newwp/wp-content/uploads/2022/01/Wheat-Profile-2021.pdf.

Bai G., Desjardins A.E., Plattner R.D. 2002. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia. 153(2):91-98. doi:10.1023/a:1014419323550

Bai G., Shaner G. 2004. Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol. 42:135-161. doi:10.1146/annurev.phyto.42.040803.140340

Bai G., Su Z., Cai J. 2018. Wheat resistance to Fusarium head blight. Can J Plant Pathol. 40(3):336-346. doi:10.1080/07060661.2018.1476411

Bayer CropScience Inc. Prosaro® XTR Booklet Label Online. [accessed 2022 Aug 9]. https://www.cropscience.bayer.ca/-/media/Bayer-CropScience/Country-Canada-Internet/Products/Prosaro-XTR/Prosaro-XTR-Label-EN_01-03-21_ac.ashx?la=en&hash=1FBC7E5479F33A946BE9B7DB84C71B5D1C031059.

Bechtel D., Kaleikau L.A., Gaines R., Seitz L. 1985. The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem. 62(3):191-197. https://www.cerealsgrains.org/publications/cc/backissues/1985/Documents/chem62_191.pdf

Bennett J.W., Klich M. 2003. Mycotoxins. Clin Microbiol Rev. 16(3):497-516. doi:10.1128/CMR.16.3.497-516.2003

Berthiller F., Dall'Asta C., Schuhmacher R., Lemmens M., Adam G., Krska R. 2005. Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J Agric Food Chem. 53(9) doi:10.1021/jf047798g

Birr T., Hasler M., Verreet J.-A., Klink H. 2020. Composition and predominance of *Fusarium* species causing Fusarium head blight in winter wheat grain depending on cultivar susceptibility and meteorological factors. Microorganisms. 8(4):617. doi:10.3390/microorganisms8040617

Blandino M., Haidukowski M., Pascale M., Plizzari L., Scudellari D., Reyneri A. 2012. Integrated strategies for the control of Fusarium head blight and deoxynivalenol contamination in winter wheat. Field Crops Res. 133:139-149. doi:10.1016/j.fcr.2012.04.004

Blandino M., Pilati A., Reyneri A. 2009. Effect of foliar treatments to durum wheat on flag leaf senescence, grain yield, quality and deoxynivalenol contamination in North Italy. Field Crops Res. 114(2):214-222. doi:10.1016/j.fcr.2009.08.008

Blandino M., Reyneri A. 2009. Effect of fungicide and foliar fertilizer application to winter wheat at anthesis on flag leaf senescence, grain yield, flour bread-making quality and DON contamination. Eur J Agron. 30(4):275-282. doi:10.1016/j.eja.2008.12.005

Bondalapati K.D., Stein J.M., Neate S.M., Halley S.H., Osborne L.E., Hollingsworth C.R. 2012. Development of weather-based predictive models for Fusarium head blight and deoxynivalenol accumulation for spring malting barley. Plant Dis. 96(5):673-680. doi:10.1094/pdis-05-11-0389

Bottalico A., Perrone G. 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol. 108(7):611-624. doi:10.1023/a:1020635214971

Bridges A., Lemenager R., Richert B., Schutz M. 2010. Zearalenone concerns in reproducing livestock. Lafayette, Indiana.: Purdue University Extension; [accessed 2022 May 10]. https://www.extension.purdue.edu/extmedia/AS/AS-598-W.pdf.

Broders K.D., Lipps P.E., Paul P.A., Dorrance A.E. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91(9):1155-1160. doi:10.1094/pdis-91-9-1155

Byrd A.L., Segre J.A. 2016. Adapting Koch's postulates. Science. 351(6270):224-226. doi:doi:10.1126/science.aad6753

Canadian Grain Commission. 2019. Identifying wheat and barley seed affected by Fusarium head blight. [accessed 2022 Jan 20]. https://grainscanada.gc.ca/en/grain-quality/grain-grading/grading-factors/identifying-fusarium.html.

Canadian Grain Commission. 2020. Official grain grading guide. 4: Wheat. [accessed Jan 20 2022]. https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/oggg-aug-1-2020-english.pdf.

Canadian Phytopathological Society. n.d. Publications; Canadian Plant Disease Survey. [accessed 2022 Feb 27]. https://phytopath.ca/publication/cpds/.

Canadian Plant Disease Survey 2019 Volume 99: Disease Highlights 2018. 2019. Can J Plant Pathol. 41(sup1):1-197. doi:10.1080/07060661.2019.1619270

Canadian Plant Disease Survey 2020 Volume 100: Disease Highlights 2019. 2020. Can J Plant Pathol. 42(sup1):1-175. doi:10.1080/07060661.2020.1752524

Canadian Plant Disease Survey 2021 Volume 101: Disease Highlights 2020. 2021. Can J Plant Pathol. 43(sup1):S1-S182. doi:10.1080/07060661.2021.1932163

Canadian Plant Disease Survey 2022 volume 102: Disease Highlights 2021. 2022. Can J Plant Pathol. 44(sup1):S1-S187. doi:10.1080/07060661.2022.2076342

CAST. 2003. Mycotoxins: Risks in Plant, Animal, and Human Systems. Task Force Reports Ames, Iowa: Council for Agricultural Science and Technology. No. 139. [accessed 2022 Mar 6.] https://www.cast-science.org/publication/mycotoxins-risks-in-plant-animal-and-human-systems/.

CFIA. 2010. 25R40. Canadian Food Inspection Agency. [accessed 2022 Nov 16]. https://inspection.canada.ca/english/plaveg/pbrpov/cropreport/whe/app00008074e.shtml.

Charmley L.L., Trenholm H.L. 2017. Fact Sheet - Mycotoxins RG-8 regulatory guidance: Contaminants in feed (formerly RG-1. Chapter 7). Canadian Food Inspection Agency. [accessed 2022 Jan 20]. https://inspection.canada.ca/animal-health/livestock-feeds/regulatory-guidance/rg-8/eng/1347383943203/1347384015909?chap=0#s1c1.

Chatterton S., Punja Z.K. 2009. Chitinase and β-1,3-glucanase enzyme production by the mycoparasite *Clonostachys rosea f. catenulata* against fungal plant pathogens. Can J Microbiol. 55(4):356-367. doi:10.1139/w08-156

Choo T.M. 2009. Fusarium head blight of barley in China. Can J Plant Pathol. 31(1):3-15. doi:10.1080/07060660909507566

Choo T.M., Martin R.A., ter Beek S.M., Ho K.M., Caldwell C.D., Walker D., Rodd V., Dion Y., Rioux S. 2003. Island barley. Can J Plant Sci. 83(4):793-795. doi:10.4141/p02-149

Choo T.M., Vigier B., Shen Q.Q., Martin R.A., Ho K.M., Savard M. 2004. Barley traits associated with resistance to Fusarium head blight and deoxynivalenol accumulation. Phytopathology. 94(10):1145-1150. doi:10.1094/phyto.2004.94.10.1145

Cohen J. 1960. A coefficient of agreement for nominal scales. Educ Psychol Meas. 20(1):37-46. doi:10.1177/001316446002000104

Cowger C., Weisz R., Arellano C., Murphy P. 2016. Profitability of integrated management of Fusarium head blight in North Carolina winter wheat. Phytopathology. 106(8):814-823. doi:10.1094/phyto-10-15-0263-r

Crippin T., Renaud J.B., Sumarah M.W., Miller J.D. 2019. Comparing genotype and chemotype of *Fusarium graminearum* from cereals in Ontario, Canada. PLOS ONE. 14(5):e0216735. doi:10.1371/journal.pone.0216735

Dahl B., Wilson W.W. 2018. Risk premiums due to Fusarium head blight (FHB) in wheat and barley. Agric Syst. 162:145-153. doi:10.1016/j.agsy.2018.01.025

De Wolf E.D., Madden L.V., Lipps P.E. 2003. Risk assessment models for wheat fusarium head blight epidemics based on within-season weather data. Phytopathology. 93(4):428-435. doi:10.1094/PHYTO.2003.93.4.428

Del Ponte E.M., Fernandes J.M.C., Pavan W. 2005. A risk infection simulation model for fusarium head blight of wheat. Fitopatol Bras. 30(6):634-642. doi:10.1590/S0100-41582005000600011

Deshaies M., Lamari N., Ng C.K.Y., Ward P., Doohan F.M. 2022. The impact of chitosan on the early metabolomic response of wheat to infection by *Fusarium graminearum*. BMC Plant Biol. 22(1):73. doi:10.1186/s12870-022-03451-w

Desjardins A.E. 2008. Natural product chemistry meets genetics: when is a genotype a chemotype? J Agric Food Chem. 56(17):7587-7592. doi:10.1021/jf801239j

Desjardins A.E., Proctor R.H. 2007. Molecular biology of Fusarium mycotoxins. Int J Food Microbiol. 119(1):47-50. doi:10.1016/j.ijfoodmicro.2007.07.024

Dexter J.E., Clear R.M., Preston K.R. 1996. Fusarium head blight: Effect on the milling and baking of some Canadian wheats. Cereal Chem. 73(6):695-701. https://www.cerealsgrains.org/publications/cc/backissues/1996/Documents/73_695.pdf

Dill-Macky R., Jones R.K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84(1):71-76. doi:10.1094/pdis.2000.84.1.71

El Hadrami A., Adam L.R., El Hadrami I., Daayf F. 2010. Chitosan in plant protection. Mar drugs. 8(4):968-987. doi:10.3390/md8040968

Entz M.H., Van den berg C.G.J., Stobbe E.H., Rossnagel B.G., Lafond G.P., Austenson H.M. 1990. Effect of late-season fungicide application on grain yield and seed size distribution in wheat and barley. 70(3):699-706. doi:10.4141/cjps90-086

Estrada Jr R., Gudmestad N.C., Rivera V.V., Secor G.A. 2010. *Fusarium graminearum* as a dry rot pathogen of potato in the USA: prevalence, comparison of host isolate aggressiveness and factors affecting aetiology. Plant Pathol. 59(6):1114-1120. doi:10.1111/j.1365-3059.2010.02343.x

Eudes F., Comeau A., Rioux S., Collin J. 2001. Impact of trichothecenes on Fusarium head blight [*Fusarium graminearum*] development in spring wheat (*Triticum aestivum*). Can J Plant Pathol. 23(3):318-322. doi:10.1080/07060660109506948

European Union. 2006. Commission Regulation (EC) Setting maximum levels for certain contaminants in foodstuffs. No. 1881/2006. Section 2: Mycotoxins. [accessed 2023 Jan 20]. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1881-20220701.

Faltermaier A., Waters D., Becker T., Arendt E., Gastl M. 2014. Common wheat (*Triticum aestivum* L.) and its use as a brewing cereal – a review. J Inst Brew. 120(1):1-15. doi:10.1002/jib.107

FAOSTAT. 2023. Crops and livestock products. Rome, Italy; : Food and agriculture organization of the United Nations; [accessed 2023 Jan 22]. http://www.fao.org/faostat/en/#data/QC.

Fawcett T. 2006. An introduction to ROC analysis. Pattern Recognit Lett. 27(8):861-874. doi:10.1016/j.patrec.2005.10.010

Fernandez M.R., Zentner R.P., DePauw R.M., Gehl D., Stevenson F.C. 2007. Impacts of crop production factors on Fusarium head blight in barley in eastern Saskatchewan. Crop Sci. 47(4):1574-1584. doi:10.2135/cropsci2006.09.0596

Foroud N.A., Chatterton S., Reid L.M., Turkington T.K., Tittlemier S.A., Gräfenhan T. 2014. Fusarium diseases of Canadian grain crops: Impact and disease management strategies. In: Goyal A, Manoharachary C, editors. Future Challenges in Crop Protection Against Fungal Pathogens. New York, NY: Springer New York; p. 267-316.

Foster A., Matters R. 2020. Fusarium head blight causal species in spring wheat in Prince Edward Island in 2019. Can Plant Dis Surv. 100:94-95. *In*, Can J Plant Pathol.42:sup1. doi:10.1080/07060661.2020.1752524

FRAC. 2022. FRAC Code List ©*2022: Fungal control agents sorted by cross-resistance pattern and mode of action (including coding for FRAC Groups on product labels). Fungicide Resistance Action Committee; [accessed 2022 Jan 16]. https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2021--final.pdf?sfvrsn=f7ec499a_2.

Freeman E.A., Moisen G. 2008. PresenceAbsence: An R package for presence absence analysis. J Stat Softw. 23(11):1 - 31. doi:10.18637/jss.v023.i11

Friskop A., Zhong S., Brueggeman R. 2018. Fusarium head blight (scab) of small grains. Fargo, North Dakota: North Dakota State University; [accessed 2022 Jan 20]. https://www.ndsu.edu/agriculture/sites/default/files/2022-08/pp804.pdf.

Fulcher M.R., Winans J.B., Quan M., Oladipo E.D., Bergstrom G.C. 2019. Population genetics of *Fusarium graminearum* at the interface of wheat and wild grass communities in New York. Phytopathology. 109(12):2124-2131. doi:10.1094/phyto-05-19-0169-r

Gale L.R., Ward T.J., Balmas V., Kistler H.C. 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the Upper Midwestern United States. Phytopathology. 97(11):1434-1439. doi:10.1094/phyto-97-11-1434

Gale L.R., Ward T.J., Kistler H.C. 2010. A subset of the newly discovered Northland population of *Fusarium graminearum* from the U.S. does not produce the B-type trichothecenes DON, 15ADON, 3ADON or NIV. Meeting Abstract. USDA Agriculture Research Service; [accessed 2022 November 11]. https://www.ars.usda.gov/research/publications/publication/?seqNo115=262050.

Gilbert J., Clear R.M., Ward T.J., Gaba D., Tekauz A., Turkington T.K., Woods S.M., Nowicki T., 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(2):146-152. doi:10.1080/07060661003740231

Gilbert J., Fernando W.G.D. 2004. Epidemiology and biological control of *Gibberella zeae* / *Fusarium graminearum*. Can J Plant Pathol. 26(4):464-472. doi:10.1080/07060660409507166

Giroux M.E., Bourgeois G., Dion Y., Rioux S., Pageau D., Zoghlami S., Parent C., Vachon E., Vanasse A. 2016. Evaluation of forecasting models for Fusarium head blight of wheat under growing conditions of Quebec, Canada. Plant Dis. 100(6):1192-1201. doi:10.1094/pdis-04-15-0404-re

Gmoser R., Ferreira J.A., Lennartsson P.R., Taherzadeh M.J. 2017. Filamentous ascomycetes fungi as a source of natural pigments. Fungal Biol Biotechnol. 4(1):4. doi:10.1186/s40694-017-0033-2

Hafez M., Gourlie R., Telfer M., Schatz N., Turkington T., Beres B., Aboukhaddour R. 2022. Diversity of *Fusarium* spp. associated with wheat node and grain in representative sites across the Western Canadian Prairies. Phytopathology. 112(5) doi:10.1094/PHYTO-06-21-0241-R

Halliday E.E. 2020. Fusarium head blight and deoxynivalenol from barley in the Maritime Provinces [Undergraduate (Honours)]. Saint Mary's University.

Health Canada. 2020. Health Canada's maximum levels for chemical contaminants in foods. [accessed 2022 Jan 20]. https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/maximum-levels-chemical-contaminants-foods.html.

Hooker D.C., Schaafsma A.W., Tamburic-Ilincic L. 2002. Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. Plant Dis. 86(6):611-619. doi:10.1094/pdis.2002.86.6.611

Horsley R., Pederson J., Schwarz P., McKay K., Hochhalter M., McMullen M. 2006. Integrated use of tebuconazole and Fusarium head blight–resistant barley genotypes. Agron J. 98(1):194-197. doi:10.2134/agronj2005.0094

Hosack P., Miller L. 2017. Preventing and Managing Plant Diseases. University of Missouri Extension; [accessed 2022 June 27]. https://extension.missouri.edu/mg13.

Johnstone E., Matters R., Foster A. 2021. Causal species of Fusarium head blight of spring wheat and winter wheat in Prince Edward Island in 2020. Can Plant Dis Surv. 101:92-94. *In*, Can J Plant Pathol. 43:sup1. doi:10.1080/07060661.2021.1932163

Johnstone E., Matters R., Foster A. 2022. Survey of Fusarium head blight and leaf diseases of spring wheat on Prince Edward Island in 2021. Can Plant Dis Surv. 102:100-102. *In*, Can J Plant Pathol. 44:sup1. doi:10.1080/07060661.2022.2076342

Karlsson I., Persson P., Friberg H. 2021. Fusarium Head Blight From a Microbiome Perspective. Front Microbiol. 12:628373. doi:10.3389/fmicb.2021.628373

Keller M.D., Bergstrom G.C., Shields E.J. 2014. The aerobiology of *Fusarium graminearum*. Aerobiologia. 30(2):123-136. doi:10.1007/s10453-013-9321-3

Kelly A.C., Clear R.M., O'Donnell K., McCormick S., Turkington T.K., Tekauz A., Gilbert J., Kistler H.C., Busman M., Ward T.J. 2015. Diversity of Fusarium head blight populations and trichothecene toxin types reveals regional differences in pathogen composition and temporal dynamics. Fungal Genet Biol. 82:22-31. doi:10.1016/j.fgb.2015.05.016

Khonga E.B., Sutton J.C. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Can J Plant Pathol. 10(3):232-239. doi:10.1080/07060668809501730

Köhl J., Kolnaar R., Ravensberg W.J. 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front Plant Sci. 10:845. doi:10.3389/fpls.2019.00845

Kuiper-Goodman T., Scott P.M., Watanabe H. 1987. Risk assessment of the mycotoxin zearalenone. Regul Toxicol Pharmacol. 7(3):253-306. doi:10.1016/0273-2300(87)90037-7

Landis J.R., Koch G.G. 1977. The measurement of observer agreement for categorical data. Biometrics. 33(1):159-174. doi:10.2307/2529310

Landschoot S., Waegeman W., Audenaert K., Van Damme P., Vandepitte J., De Baets B., Haesaert G. 2013. A field-specific web tool for the prediction of Fusarium head blight and deoxynivalenol content in Belgium. Comput Electron Agric. 93:140-148. doi:10.1016/j.compag.2013.02.011

LaZerte S.E., Albers S. 2018. Weathercan: Download and format weather data from Environment and Climate Change Canada. J Open Source Softw. 3(22) doi:10.21105/joss.00571

Legrand F., Picot A., Cobo-Díaz J.F., Chen W., Le Floch G. 2017. Challenges facing the biological control strategies for the management of Fusarium head blight of cereals caused by *F. graminearum*. Biol Control. 113:26-38. doi:10.1016/j.biocontrol.2017.06.011

Leplat J., Friberg H., Abid M., Steinberg C. 2013. Survival of *Fusarium graminearum*, the causal agent of Fusarium head blight. A review. Agron Sustain Dev. 33(1):97-111. doi:10.1007/s13593-012-0098-5

Leslie J.F., Summerell B.A. 2006. The Fusarium Laboratory Manual. 1st ed. Ames (IA): Blackwell Publishing.

Lewandowski S.M., Bushnell W.R., Evans C.K. 2006. Distribution of mycelial colonies and lesions in field-grown barley inoculated with *Fusarium graminearum*. Phytopathology. 96(6):567-581. doi:10.1094/phyto-96-0567

Liang J.M., Xayamongkhon H., Broz K., Dong Y., McCormick S.P., Abramova S., Ward T.J., Ma Z.H., Kistler H.C. 2014. Temporal dynamics and population genetic structure of *Fusarium graminearum* in the upper Midwestern United States. Fungal Genet Biol. 73:83-92. doi:10.1016/j.fgb.2014.10.002

Lin L.I.-K. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics. 45(1):255-268. doi:10.2307/2532051

Ma L.J., Geiser D.M., Proctor R.H., Rooney A.P., O'Donnell K., Trail F., Gardiner D.M., Manners J.M., Kazan K. 2013. Fusarium pathogenomics. Annu Rev Microbiol. 67:399-416. doi:10.1146/annurev-micro-092412-155650

Mallett J. 2014. Malt: A Practical Guide from Field to Brewhouse. Boulder, Colorado: Brewers Publications.

Manitoba Agriculture. 2022a. Fusarium head blight risk map. Seasonal Reports; [accessed 2022 Sep 1]. https://www.gov.mb.ca/agriculture/crops/seasonal-reports/fusarium-head-blight-report.html.

Manitoba Agriculture. 2022b. Fusarium head blight risk maps - archives. Seasonal Reports; [accessed 2023 Jan 5]. https://www.gov.mb.ca/agriculture/crops/seasonal-reports/fusarium-head-blight-risk-forecast-archives.html.

Manstretta V., Morcia C., Terzi V., Rossi V. 2016. Germination of *Fusarium graminearum* ascospores and wheat infection are affected by dry periods and by temperature and humidity during dry periods. Phytopathology. 106(3):262-269. doi:10.1094/phyto-05-15-0118-r

Manstretta V., Rossi V. 2016. Effects of temperature and moisture on development of *Fusarium graminearum* perithecia in maize stalk residues. Appl Environ Microbiol. 82(1):184. doi:10.1128/aem.02436-15

Martin R.A. 2004. Fusarium head blight of cereals in Atlantic Canada. Government of New Brunswick; [accessed 2022 May 11]. https://www2.gnb.ca/content/dam/gnb/Departments/10/pdf/Agriculture/FieldCrops-GrandesCultures/2004FHB_AGC.pdf.

Martin R.A., Johnston H.W. 1997. Diseases of cereal crops in the Atlantic Provinces, 1996. Can Plant Dis Surv. 77(1):59-60. https://phytopath.ca/wp-content/uploads/2014/10/cpds-archive/vol76/CPDS_Vol_76_No_1_(73-90)1996.pdf

McCormick S.P., Stanley A.M., Stover N.A., Alexander N.J. 2011. Trichothecenes: from simple to complex mycotoxins. Toxins (Basel). 3(7):802-814. doi:10.3390/toxins3070802

McMullen M., Adhikari T., Friskop A., Zhaohui L. 2021. Fungal leaf spot diseases of wheat: tan spot, Septoria/*Stagonospora nodorum* blotch and Septoria tritici blotch. NDSU Extension. Extension document No.: PP1249. [accessed 2022 Oct 28.] https://www.ndsu.edu/agriculture/sites/default/files/2022-08/pp1249.pdf.

McMullen M., Bergstrom G.C., De Wolf E.D., Dill-Macky R., Hershman D., Shaner G., Van Sanford D. 2012. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. Plant Dis. 96(12):1712-1728. doi:10.1094/pdis-03-12-0291-fe

McMullen M., Halley S., Schatz B., Meyer S., Jordahl J., Ransom J. 2008. Integrated strategies for Fusarium head blight management in the United States. Cereal Res Commun. 36:563-568. http://www.jstor.org/stable/90003287

Mesterházy A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breed. 114(5):377-386. doi:10.1111/j.1439-0523.1995.tb00816.x

Mesterhazy A., Bartok T., 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(9):1107-1115. doi:10.1094/PDIS.2003.87.9.1107

Molineros J.E. 2007. Understanding the challenges of Fusarium head blight forecasting [dissertation]. University Park (PA): Pennsylvania State University.

Moschini R.C., Carranza M.R., Carmona M.A. 2004. Meteorological-Based Predictions of Wheat Head Blight Epidemic in the Southern Argentinean Pampas Region. Cereal Res Commun. 32(1):45-52. doi:10.1007/BF03543279

Moschini R.C., Fortungo C. 1996. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur J Plant Pathol. 102(3):211–218. doi:10.1007/bf01877959

Moschini R.C., Pioli R., Carmona M., Sacchi O. 2001. Empirical predictions of wheat head blight in the northern Argentinean Pampas Region. Crop Sci. 41(5):1541–1545. doi:10.2135/cropsci2001.4151541x

Mourelos C.A., Malbrán I., Balatti P.A., Ghiringhelli P.D., Lori G.A. 2014. Gramineous and non-gramineous weed species as alternative hosts of *Fusarium graminearum*, causal agent of Fusarium head blight of wheat, in Argentina. Crop Prot. 65:100-104. doi:10.1016/j.cropro.2014.07.013

Mueller D.S. 2006. Fungicides: Triazoles. Iowa State University.; [accessed 2022 June 27]. https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=2274&context=cropnews.

Munkvold G.P., Hellmich R.L., Showers W.B. 1997. Reduced Fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology. 87(10):1071-1077. doi:10.1094/phyto.1997.87.10.1071

Munkvold G.P., Proctor R.H., Moretti A. 2021. Mycotoxin production in *Fusarium* according to contemporary species concepts. Annu Rev Phytopathol. 59(1):373-402. doi:10.1146/annurev-phyto-020620-102825

Musa T., Hecker A., Vogelgsang S., Forrer H.R. 2007. Forecasting of Fusarium head blight and deoxynivalenol content in winter wheat with FusaProg. Bull OEPP. 37(2):283-289. doi:10.1111/j.1365-2338.2007.01122.x

Neate S., McMullen M. 2005. Barley Disease Handbook. North Dakota State University. [accessed 2022 Oct 28.] https://www.canr.msu.edu/uploads/236/78697/barley_disease_handbook_-_NDSU.pdf.

Newman C.W., Newman R.K. 2006. A brief history of barley foods. CFW Plex. 51(1):4-7. doi:10.1094/cfw-51-0004

Nikkhah A. 2012. Barley grain for ruminants: A global treasure or tragedy. J Anim Sci Technol. 3(1):22. doi:10.1186/2049-1891-3-22

Novozymes BioAg Limited. 2021. Taegro 2™ Biofungicide. [accessed 2022 May 25]. https://biosolutions.novozymes.com/sites/default/files/file_download/CA-Taegro_label.pdf.

O'Donnell K., Kistler H.C., Cigelnik E., Ploetz R.C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America. 95(5):2044-2049. doi:10.1073/pnas.95.5.2044

OMAFRA. 2017. Agronomy guide for field crops. Toronto, Canada: Ministry of Agriculture, Food and Rural Affairs © Queen's Printer for Ontario; [accessed 2022 May 25]. http://www.omafra.gov.on.ca/english/crops/pub811/pub811.pdf.

OMAFRA. 2021. Wheat for animal feed. Ontario Ministry of Agriculture, Food and Rural Affairs; [accessed 2022 May 25]. http://www.omafra.gov.on.ca/english/livestock/beef/facts/wheat.htm.

Ontario Cereal Crops Committee. 2022. Ontario Cereal Crops Committee. [accessed 2022 Oct 31]. https://www.gocereals.ca/index.php.

Osborne L.E., Stein J.M. 2007. Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol. 119(1):103-108. doi:10.1016/j.ijfoodmicro.2007.07.032

Parikka P., Hakala K., Tiilikkala K. 2012. Expected shifts in *Fusarium* species' composition on cereal grain in Northern Europe due to climatic change. Food additives & contaminants Part A, Chemistry, analysis, control, exposure & risk assessment. 29(10):1543-1555. doi:10.1080/19440049.2012.680613

Parry D.W., Jenkinson P., McLeod L. 1995. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 44(2):207-238. doi:10.1111/j.1365-3059.1995.tb02773.x

Paul P., Lipps P., Hershman D., McMullen M., Draper M., Madden L. 2008. Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: A multivariate meta-analysis. Phytopathology. 98(9):999-1011. doi:10.1094/phyto-98-9-0999

Paul P., Lipps P., Madden L. 2005. Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: A meta-analysis. 95(10):1225-1236. doi:10.1094/phyto-95-1225

Paul P., McMullen M., Hershman D., Madden L. 2010. Meta-analysis of the affects of triazole-based fungicides on wheat yield and test weight as influenced by Fusarium head blight intensity. Phytopathology. 100(2):160-171. doi:10.1094/phyto-100-2-0160

Pereyra S.A., Dill-Macky R. 2008. Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to Fusarium head blight inoculum. Plant Dis. 92(5):800-807. doi:10.1094/pdis-92-5-0800

Pereyra S.A., Dill-Macky R., Sims A.L. 2004. Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis. 88(7):724-730. doi:10.1094/pdis.2004.88.7.724

Pestka J.J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim Feed Sci Technol. 137(3):283-298. doi:10.1016/j.anifeedsci.2007.06.006

Pestka J.J. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Archives of Toxicology. 84(9):663-679. doi:10.1007/s00204-010-0579-8

Pestka J.J., Smolinski A.T. 2005. Deoxynivalenol: Toxicology and potential effects on humans. J Toxicol Environ Health B Crit Rev. 8(1):39-69. doi:10.1080/10937400590889458

Pitblado R., Hooker D.C., Nichols I., Danford R., Schaafsma A.W. 2007. DONcast: Seven years of predicting DON in wheat on a commercial scale. National Fusarium Head Blight Forum; Kansas City, Missouri; [accessed 2023 Jan 20]. https://scabusa.org/pdfs/forum07_proc_complete.pdf.

Prandini A., Sigolo S., Filippi L., Battilani P., Piva G. 2009. Review of predictive models for Fusarium head blight and related mycotoxin contamination in wheat. Food Chem Toxicol. 47(5):927-931. doi:10.1016/j.fct.2008.06.010

Proctor R.H., Hohn T.M., McCormick S.P. 1995. Reduced Virulence of Gibberella zeae Caused by Disruption of a Trichothecene Toxin Biosynthetic Gene. Mol Plant Microbe Interact. 8(4):593-601. doi:10.1094/MPMI-8-0593

R Core Team. 2022. R: A language and environment for statistical computing: R Foundation for Statistical Computing, Vienna, Austria. [accessed 2023 Jan 20]. https://www.R-project.org/.

Réseau des grandes cultures du Québec. 2022. Céréales. [accessed 2022 Oct 31]. https://rgcq.ca/cereales/.

Righetti L., Galaverna G., Dall'Asta C. 2017. Group detection of DON and its modified forms by an ELISA kit. Food Addit Contam Part A. 34(2):248-254. doi:10.1080/19440049.2016.1265671

Rocha O., Ansari K., Doohan F.M. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: A review. Food Addit Contam. 22(4):369-378. doi:10.1080/02652030500058403

Rossi V., Giosuè S., Pattori E., Spanna F., Del Vecchio A. 2003. A model estimating the risk of Fusarium head blight on wheat. EPPO Bulletin. 33(3):421-425. doi:10.1111/j.1365-2338.2003.00667.x

Rossi V., Ravanetti A., Pattori E., Giosuè S. 2001. Influence of temperature and humidity on the infection of wheat spikes by some fungi causing Fusarium head blight. J Plant Pathol. 83(3):189-198. http://www.jstor.org/stable/41998061

Rudd J.C., Horsley R.D., McKendry A.L., Elias E.M. 2001. Host plant resistance genes for Fusarium head blight: sources, mechanisms, and utility in conventional breeding systems. Crop Sci. 41(3):620-627. doi:10.2135/cropsci2001.413620x

Ruske R.E., Gooding M.J., Jones S.A. 2003. The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning, remobilization and grain N accumulation in winter wheat cultivars. J Agric Sci. 140(4):395-407. doi:10.1017/S0021859603003228

Salgado J.D., Paul P.A. 2016. Powdery mildew of wheat. Ohioline, Ohio State University Extension; [accessed 2022 Oct 28]. https://ohioline.osu.edu/factsheet/plpath-cer-11.

SaskWheat Development Commission. 2022. Fusarium Resources. [accessed 2023 Jan 5]. https://saskwheat.ca/fusarium-resources.

Schaafsma A.W., Tamburic-Ilincic L., 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(2):217-224. doi:10.1080/07060660509507219

Schisler D.A., Khan N.I., Boehm M.J., Slininger P.J. 2002. Greenhouse and field evaluation of biological control of Fusarium head blight on durum wheat. Plant Dis. 86(12):1350-1356. doi:10.1094/pdis.2002.86.12.1350

Schmale D.G., Bergstrom G.C. 2003. Fusarium head blight (FHB) or scab. The Plant Health Instructor. doi:10.1094/phi-i-2003-0612-01

Schöneberg T., Musa T., Forrer H.-R., Mascher F., Bucheli T.D., Bertossa M., Keller B., Vogelgsang S. 2018. Infection conditions of *Fusarium graminearum* in barley are variety specific and different from those in wheat. Eur J Plant Pathol. 151(4):975-989. doi:10.1007/s10658-018-1434-7

Shah D.A., De Wolf E.D., Paul P.A., Madden L.V. 2019. Functional data analysis of weather variables linked to Fusarium head blight epidemics in the United States. Phytopathology. 109(1):96-110. doi:10.1094/phyto-11-17-0386-r

Shah D.A., Molineros J.E., Paul P.A., Willyerd K.T., Madden L.V., De Wolf E.D. 2013. Predicting Fusarium head blight epidemics with weather-driven pre- and post-anthesis logistic regression models. Phytopathology. 103(9):906-919. doi:10.1094/phyto-11-12-0304-r

Shewry P.R., Hey S.J. 2015. The contribution of wheat to human diet and health. Food Energy Secur. 4(3):178-202. doi:10.1002/fes3.64

Shin S., Kim K.-H., Kang C.-S., Cho K.-M., Park C.S., Okagaki R., Park J.-C. 2014. A Simple Method for the Assessment of Fusarium Head Blight Resistance in Korean Wheat Seedlings Inoculated with *Fusarium graminearum*. Plant Pathol J. 30(1):25-32. doi:10.5423/PPJ.OA.06.2013.0059

Signorell A., Aho K., Alfons A., Anderegg N., Aragon T., Arachchige C., Arppe A., Baddeley A., Barton K., Bolker B. et al. 2022. DescTools: Tools for Descriptive Statistics. [accessed 2023 Jan 20]. https://cran.r-project.org/web/packages/DescTools/index.html.

Spolti P., Del Ponte E.M., Dong Y., Cummings J.A., Bergstrom G.C. 2014. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. 98(5):607-613. doi:10.1094/pdis-10-13-1051-re

Stack R. 2000. Return of an old problem: Fusarium head blight of small grains. Plant Health Prog. doi:10.1094/php-2000-0622-01-rv

Stack R., McMullen M. 1998. A visual scale to estimate severity of Fusarium head blight in wheat. North Dakota State University. [accessed 2022 Oct 28.] https://library.ndsu.edu/ir/bitstream/handle/10365/9187/PP1095_1998.pdf?sequence=1.

Statistics Canada. 2022a. Table 32-10-0359-01 Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units. 2022 Dec 02. [accessed 2023 Jan 20]. https://www150.statcan.gc.ca/t1/tbl1/en/cv.action?pid=3210035901.

Statistics Canada. 2022b. Table: 32-10-0358-01 Area, production and farm value of potatoes. [accessed 16 Oct 2022]. https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210035801.

Summerell B.A. 2019. Resolving Fusarium: Current status of the Genus. Annu Rev Phytopathol. 57:323-339. doi:10.1146/annurev-phyto-082718-100204

Sun H.-Y., Cui J.-h., Tian B.-h., Cao S.-l., Zhang X.-x., Chen H.-G. 2020. Resistance risk assessment for *Fusarium graminearum* to pydiflumetofen, a new succinate dehydrogenase inhibitor. Pest Manag Sci. 76(4):1549-1559. doi:10.1002/ps.5675

Sun Z.-B., Li S.-D., Ren Q., Xu J.-L., Lu X., Sun M.-H. 2020. Biology and applications of *Clonostachys rosea*. J Appl Microbiol. 129(3):486-495. doi:10.1111/jam.14625

Sutton J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can J Plant Pathol. 4(2):195-209. doi:10.1080/07060668209501326

Syngenta Canada Inc. 2021. Miravis ® Ace Fungicide [accessed 2022 Jan 20]. https://assets.syngenta.ca/pdf/ca/labels/Miravis_Ace_33573_en_pamphlet.pdf.

Tamburic-Ilincic L. 2010. Fusarium species and mycotoxins associated with oat in southwestern Ontario, Canada. Can J Plant Pathol. 90(2):211-216. doi:10.4141/cjps09139

Tekauz A., McCallum B., Ames N., Fetch J.M. 2004. Fusarium head blight of oat — current status in western Canada. Can J Plant Pathol. 26(4):473-479. doi:10.1080/07060660409507167

Tekauz A., McCallum B., Gilbert J. 2000. Review: Fusarium head blight of barley in western Canada. Can J Plant Pathol. 22(1):9-16. doi:10.1080/07060660009501156

Tillmann M., von Tiedemann A., Winter M. 2017. Crop rotation effects on incidence and diversity of *Fusarium* species colonizing stem bases and grains of winter wheat. Plant Dis Prot. 124(2):121-130. doi:10.1007/s41348-016-0064-6

Ullrich S.E. 2011. Chapter Significance, Adaption, Production, and Trade of Barley. Barley: Production, Improvement and Uses. Ames (IA): Wiley-Blackwell; p. 3-13.

USWBSI. 2022. Fusarium Risk Tool. US Wheat and Barley Scab Initiative; [accessed 2023 Jan 5]. https://www.wheatscab.psu.edu/.

Valverde-Bogantes E., Bianchini A., Herr J.R., Rose D.J., Wegulo S.N., Hallen-Adams H.E. 2020. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can J Plant Pathol. 42(3):315-329. doi:10.1080/07060661.2019.1680442

Van Der Fels-Klerx H.J., Burgers S.L.G.E., Booij C.J.H. 2010. Descriptive modelling to predict deoxynivalenol in winter wheat in the Netherlands. Food Additives & Contaminants: Part A. 27(5):636-643. doi:10.1080/19440040903571762

van Maanen A., Xu X.M. 2003. Modelling plant disease epidemics. Eur J Plant Pathol. 109(7):669-682. doi:10.1023/a:1026018005613

Varga E., Wiesenberger G., Hametner C., Ward T.J., Dong Y., Schöfbeck D., McCormick S., Broz K., Stückler R., Schuhmacher R. et al. 2015. New tricks of an old enemy: isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. Environ Microbiol. 17(8):2588-2600. doi:10.1111/1462-2920.12718

Villafana R.T., Ramdass A.C., Rampersad S.N. 2019. Selection of Fusarium trichothecene toxin genes for molecular detection depends on TRI gene cluster organization and gene function. Toxins (Basel). 11(1):36. doi:10.3390/toxins11010036

Ward T.J., Bielawski J.P., Kistler H.C., Sullivan E., O'Donnell K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc Natl Acad Sci U S A. 99(14):9278-9283. doi:10.1073/pnas.142307199

Ward T.J., Clear R.M., Rooney A.P., O'Donnell K., Gaba D., Patrick S., Starkey D.E., Gilbert J., Geiser D.M., Nowicki T.W. 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 Genet Biol. 45(4):473-484. doi:10.1016/j.fgb.2007.10.003

Weather INnovations LP. Data driven decision agriculture. [accessed 2023 Jan 5]. http://weatherinnovations.com/.

Wegulo S.N., Baenziger P.S., Hernandez Nopsa J., Bockus W.W., Hallen-Adams H. 2015. Management of Fusarium head blight of wheat and barley. Crop Prot. 73:100-107. doi:10.1016/j.cropro.2015.02.025

Wegulo S.N., Bockus W.W., Nopsa J.H., De Wolf E.D., Eskridge K.M., Peiris K.H.S., Dowell F.E. 2011. Effects of integrating cultivar resistance and fungicide application on Fusarium head blight and deoxynivalenol in winter wheat. Plant Dis. 95(5):554-560. doi:10.1094/pdis-07-10-0495

White T., Bruns T., Lee S., Taylor J., Innis M., Gelfand D., Sninsky J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J et al., editors. PCR Protocols: A Guide to Methods and Applications. Academic Press Inc., New York; p. 315-322.

Wong L.S.L., Abramson D., Tekauz A., Leisle D., 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(1):261-267. doi:10.4141/cjps95-047

Xu X.M., Nicholson P., Thomsett M.A., Simpson D., Cooke B.M., Doohan F.M., Brennan J., Monaghan S., Moretti A., Mule G. et al. 2008. Relationship between the fungal complex causing Fusarium head blight of wheat and environmental conditions. Phytopathology. 98(1):69-78. doi:10.1094/phyto-98-1-0069

Xue A.G., Chen Y. 2022. Diseases of Oat in Ontario in 2021. Can Plant Dis Surv *In* Can J Plant Pathol. 102(44):2.

Xue A.G., Chen Y., Seifert K., Guo W., Blackwell B.A., Harris L.J., Overy D.P. 2019. Prevalence of *Fusarium* species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. Can J Plant Pathol. 41(3):392-402. doi:10.1080/07060661.2019.1582560

Xue A.G., Chen Y., Voldeng H.D., Fedak G., Savard M., Längle T., Zhang J., Harman G.E. 2014. Concentration and cultivar effects on efficacy of CLO-1 biofungicide in controlling Fusarium head blight of wheat. Biol Control. 73:2-7. doi:10.1016/j.biocontrol.2014.02.010

Xue A.G., Voldeng H.D., Savard M.E., Fedak G., Tian X., Hsiang T. 2009. Biological control of Fusarium head blight of wheat with *Clonostachys rosea* strain ACM941. Can J Plant Pathol. 31(2):169-179. doi:10.1080/07060660909507590

Yan L., Loukoianov A., Blechl A., Tranquilli G., Ramakrishna W., SanMiguel P., Bennetzen J.L., Echenique V., Dubcovsky J. 2004. The wheat *VRN2* gene Is a flowering repressor down-regulated by vernalization. 303(5664):1640-1644. doi:doi:10.1126/science.1094305

Zhang X., Halder J., White R.P., Hughes D.J., Ye Z., Wang C., Xu R., Gan B., Fitt B.D.L. 2014. Climate change increases risk of Fusarium ear blight on wheat in central China. Ann Appl Biol. 164(3):384-395. doi:10.1111/aab.12107

Zitnick-Anderson K., Simons K., Pasche J.S. 2018. Detection and qPCR quantification of seven *Fusarium* species associated with the root rot complex in field pea. Can J Plant Pathol. 40(2):261-271. doi:10.1080/07060661.2018.1429494