Fusarium Head Blight and Deoxynivalenol from Barley in the Maritime Provinces

by
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Table of Contents

Abstract

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Fusarium Head Blight (FHB) is one of the most devastating agriculture diseases that affects barley worldwide by reducing crop yields and grain quality. The main causal organism of concern in Eastern Canada is the fungal pathogen, *Fusarium graminearum*. This pathogen inhibits protein synthesis in the seeds, resulting in low yields, and can produce mycotoxins to contaminate the grains. Deoxynivalenol (DON) is the most important mycotoxins as it is toxic to animals and humans. It is important to perform barley disease surveys in the Maritimes as they have ceased in the early 2000s, resulting in a lack of FHB information in these provinces. To further understand the presence of FHB causing species and DON in the Maritime provinces, barley seeds were collected by partners at the Atlantic Grains Council. Seed samples were separated per field to isolate *Fusarium* species, and a second subsample ground for quantitative-PCR (qPCR) and DON analysis. A total of 336 isolates were collected, the majority being *F. graminearum*, other species identified were *F. poae, F. avenaceum,* and *F. sporotrichioides*. DON concentrations ranged from 0 to 15.6 ppm in each field. Nova Scotia presented the highest disease level based on qPCR of *F. graminearum* DNA, isolate numbers and DON levels, with less disease presence in New Brunswick and PEI. *F. graminearum* DNA correlated significantly with DON concentration (R^2=0.92). A virulence assay using *F. graminearum* isolates from each province was performed to observe differences between provincial isolates and to assess visual rating methods. qPCR data did reveal a weak positive correlation with visual severity ratings (R^2=0.48). This qPCR assay revealed a rapid and reliable method to evaluate and quantify FHB in barley to be used in future surveys. Correlation results can be used to increase precision in other agronomic studies that aim to reduce FHB severity.

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Introduction

1.1 Barley Domestication and Production

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world today, it is fourth in quantity produced and area of cereal crops that are cultivated in the world (Zhou 2010). Domesticated barley originated in Fertile Crescent approximately 10,000 years ago and spread across Europe following the path of human migration (Badr et al. 2000). It is thought to be the first domesticated crop and was developed as an essential food to make bread for farmers. While it has remained an important food in many countries, its main uses are now as animal feed and in beer production (Langridge 2018).

Barley has different types, 2-rowed and 6-rowed, hull and hulless barley, with the main difference being their morphology. In two-rowed barley, only the central spike is fertile, whereas in six-rowed barley, all three spikelets are fertile due to a mutation in the *VRS1* (six-rowed spike 1) gene (Komatsuda et al. 2007). Two-rowed barley produces larger kernels that contain more starch, whereas six-rowed contains higher protein and less carbohydrate. Hulless barley is a form of domesticated barley with easy to remove hull, the outer shell on the seed. Hulless barley is used in the diet of non-ruminants that are not able to digest the fibrous hull of hulled barley, making this an advantage of hulless varieties (Kaur et al. 2019).

Barley was originally domesticated to be used for human food, now accounting for only 5% of barley end use, although several studies have indicated that it is one of the healthiest cereals for the human diet (Langridge 2018). Barley is one of the four major feed grains used as feed for livestock animals as it is a major source of energy, protein,

and fiber, with about 85% of Canadian barley consumed is as animal feed (Zhou 2010). Barley is considered to be highly degradable in the rumen, releasing more synchronous energy and nitrogen than corn, thereby improving microbial and host nutrient integration (Nikkhah 2012).

The second largest use of barley is as a fermentable source in beer production as the production of malt from barley is most economically desirable (Langridge 2018). Barley malt is a key material for beer and whiskey brewing, the seeds go through a process of steeping to germinate the seeds, drying, and grinding to extract the malt to be added for beer. This process called malting allows the starches to become sugars that are added to the solvent. With the new trend of craft brewing facilities, barley production for malting purposes has increased (Langridge 2018).

1.2 Major Fungal Diseases in Barley

Cereal crops are vulnerable to many diseases that result in significant yield loss. Barley is no exception and is susceptible to a variety of pathogens that infect the leaves, roots, and kernels. Fungal barley diseases commonly reported in Atlantic Canada include leaf scald *Rhynchosporium secalis* (Oudem.) J. J. Davis caused by *P. teres f. teres* and *P. teres f. maculate*, Common Root Rot *Bipolaris sorokiniana*, Ergot *Claviceps purpurea*, and *Fusarium* head blight (Martin et al. 1982).

Leaf scald of barley is caused by *Rhynchosporium secalis* (Oudem.) J. J. Davis, a haploid, necrotrophic fungal pathogen (Brunner et al. 2007). This fungal pathogen can be spread from season to season within the crop residues left on the soil, its spores can also be carried by rain-splash from these residues or from neighboring plants (Brunner et al. 2007). Once scald has compromised the leaf, its photosynthetic ability is reduced, and it

will often wither away and die. Scald can lead to yield losses of up to 20%, yet good control methods such as crop rotation, field sanitation, foliar fungicides, and crop residue elimination can aid in reducing scald infection (Martin et al. 1982).

Another important leaf disease of barley is Net blotch, caused by *Pyrenophora teres* Desch (Arabi et al. 2003). There are two types of leaf symptoms caused by two different forms of this pathogen, *P. teres f. teres* that causes the net form of the disease has a dark brown reticulate venation pattern, and *P. teres f. maculata* that causes the spot form of blotch, characterized by dark brown circular or elliptical spots with chlorosis surrounding the leaf tissue (Grewal et al. 2008). Net blotch can cause yield losses of 20-30%, with infection negatively affecting malting and feeding quality of the kernels (Grewal et al. 2008).

Common Root Rot in barley and wheat is caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph): *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur. This pathogen is found worldwide and has resulted in yield losses of 10% in the Canadian prairies (Kutcher et al. 1996; Arabi and Jawhar 2013). This disease creates brown to black discoloration of the subcrown internode of the plants root (Arabi and Jawhar 2013). This disease can be caused by other pathogens including *Fusarium* species (Fernandez et al. 2009).

Ergot is mainly caused by the pathogen *Claviceps purpurea* (Fr.) Tul. 1853 in Canadian cereals, it is characterized by the presence of dark-colored sclerotia that replace healthy kernels and are up to 10 times larger than the grain kernels (Tittlemier et al. 2015; Coufal-Majewski et al. 2016). Ergot is found commonly after wet periods and during the flowering stage of the plant's development, as ergot infects the open floret (Coufal-

Majewski et al. 2016). *Ergot sclerotia* produce alkaloid secondary metabolites that are toxic when present in feed, these toxic metabolites cause hallucinations, and vasoconstriction when consumed (Tittlemier et al. 2015).

1.3 Fusarium Head Blight in Barley

Fusarium head blight (FHB) is one of the most economically damaging agricultural diseases affecting cereal crops; there are many species that cause FHB such as *Fusarium poae* (Peck) Wollenw., *Fusarium avenaceum* (Fr.) Sacc. (teleomorph *Gibberella avenaceum* R.J. Cook), *Fusarium sporotrichioides* Sherb., and *Fusarium culmorum* (W.G. Sm.) Sacc., but it is primarily caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch (*F. graminearum)* (Panthi et al. 2014). Epidemics of FHB started in the 1920s in North America, slowing down between 1950-1970, but recurring in the early 1980s in Eastern Canada and Manitoba. FHB has resulted in major yield loss by destroying the grain leading to damaged kernels and negatively affecting the quality for livestock feed, malting, and milling purposes (Ali and Calpas 2019). *F. graminearum* can affect the germination capacity of the grain and increase the protein and nitrogen content therefore, negatively affecting malting processes (Nogueira et al. 2018). This pathogen can produce toxic poisons that are potent to animals and humans, above certain thresholds. This cereal crop disease has caused strain on the Canadian agriculture industry, with economic loss ranging from $50 million to $300 million annually, to combat this agriculture disease, *F. graminearum* was listed under the Albert *Agricultural Pests Act* in 1999 (Agriculture Alberta 2003).

Disease severity and incidence depends on favourable environmental conditions, such as rain fall and humidity. Rainfall creates the optimal environment

for *Fusarium* species to initially infect cereal crops at anthesis, the growth stage at which they are most susceptible to disease infection (Palazzini et al. 2016). *F. graminearum* is capable of destroying starch granules, storage proteins and cell walls during invasion of the grains. This plant pathogen also produces proteolytic enzymes that hydrolyse endosperm proteins during fermentation and can cause losses in yield from 30 to 70% (Pirgozliev et al. 2003; Palazzini et al. 2016).

Fusarium species reproduce asexually and sexually (Fig. 1), being able to reproduce without a partner is beneficial for this pathogen so it can reproduce quickly, and their spores allow it to do so in harsh environments (Schmale III and Bergstrom 2003). The perithecia is the fruiting body, found on crop debris, containing a pore for spore discharge, it will release ascospores that infect the spike, leaf sheath and culm of the wheat and barley head. Sporodochia are stroma that produce the sexual spores that are released for rounds of secondary infection, and finally the perithecia are produced that overwinter to allow the infection cycle for the next year. As the infection progresses, the seeds begin to present pink/blush colour, and shrink and wrinkle (Schmale III and Bergstrom 2003), and the fungal infection will cause the plant to wilt and necrosis to occur (Boenisch and Schäfer 2011).

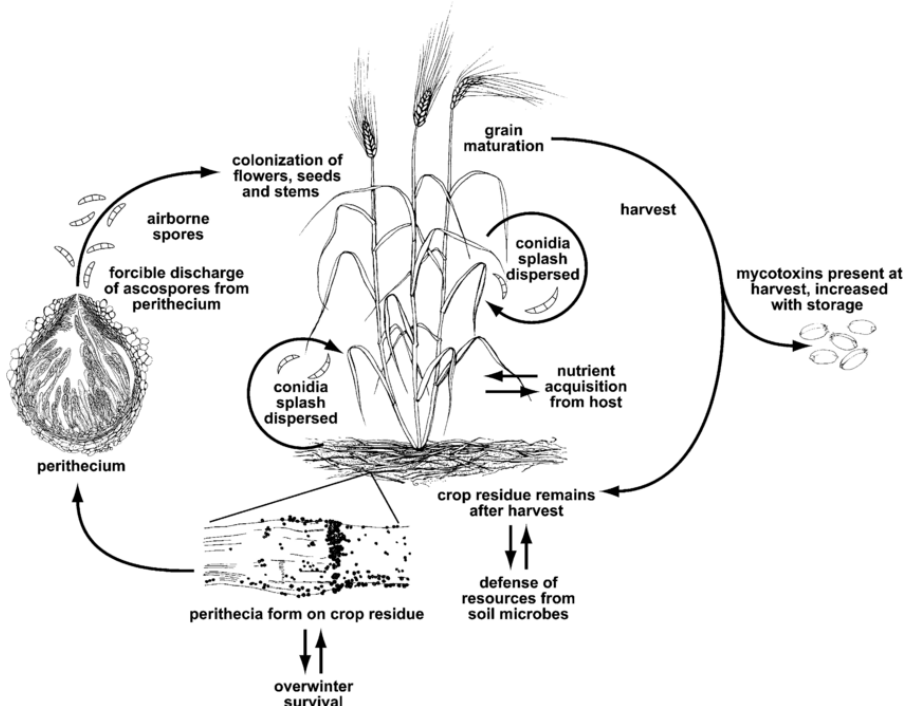

Figure 1. The disease cycle of *Fusarium graminearum* (sexual phase, *G. zeae*), causal agent of FHB on wheat. Spores will disperse by air to colonize the seeds of the grain and secrete mycotoxins; crop residue remains after harvest that contain fungal spores to infect future crops. (Schmale III and Bergstrom 2003).

1.4 Virulence of Fusarium Head Blight

The aggressiveness of *Fusarium* is due to the production of various virulence factors by the fungi that enable and add to their effectiveness such as proteases (proteinases), effectors, and mycotoxins. Proteases act as sharp scissors to catalyze specific proteolytic reactions, regulating the activity of many proteins and contribute to the processing of cellular information, thereby influencing DNA transcription and regulation. There is evidence showing the role of proteases in the pathogenic strategy of fungi (León Rodríguez et al. 2017). *Fusarium* subtilisin-like and trypsin-like proteinases have been shown to degrade major barley storage proteins to negatively affect the quality of the grain for ruminant feed and malting purposes (Pekkarinen et al. 2007).

Effectors are secreted molecules that enable microbes to interact with their hosts and to influence the outcome of the interaction, these molecules are distinguished based on their function within the biological context of an interaction (Uhse and Djamei 2018). *F. graminearum* is known to produce 39 effector proteins, some with the ability to increase transcript abundance of genes involved in the biosynthesis of mycotoxins in wheat tissues. For example, *TRI6* and *TRI10* genes, which regulate DON production and are needed for full virulence, showed dramatic increase in transcription (Brown et al. 2017).

Mycotoxins are natural products produced as secondary metabolites by fungi to induce a toxic response in the host (Bennett 1987; Nelson et al. 1993). *F. graminearum* can produce β-trichothecene mycotoxins that include nivalenol (NIV), deoxynivalenol (DON), its acetylated versions 15-ADON and 3-ADON, and also NX2 (Varga et al. 2015). 3-ADON and 15-ADON are potent phytotoxins that cause symptoms such as wilting and necrosis in the host plant. The toxic effect of DON is due to its ability to bind to the 60S ribosomal unit of eukaryotes, resulting in the inhibition of protein synthesis and apoptosis (Boenisch and Schäfer 2011). DON and NIV can be detoxified by the plant by adding a glucoside, these detoxified products are known as DON-3-O-glucoside & NIV-0-β-D-glucoside. They are known as masked mycotoxins because human and animal stomach microbiota remove this sugar when consumed, bringing it back to its toxic DON and NIV state (Jin et al. 2018). The host genotype plays a role in toxin accumulation, more resistant cultivars will have less mycotoxins being produced, while low FHB severity can still have high DON content.

Some countries have set DON concentration limits, for example, Europe has an upper limit of 1.25 ppm. Canada has regulatory limits for malt, feed, and human

consumption. Some animals are more susceptible than others to DON, 1 ppm is the guideline for dairy cattle, pigs and horses, while beef cattle, and poultry have a guideline of 5 ppm. Malt barley typically has zero tolerance for DON depending on the certain company's regulations (Agriculture Alberta 2003).

Many other *Fusarium* species also produce mycotoxins. *Fusarium poae* is a typical β-trichothecene producer as well, with NIV as the dominant mycotoxin, yet it has been cited to produce type-A trichothecenes (HT-2 and T-2). Type A trichothecenes differ from β toxins by a hydroxyl group, an ester or no substituent at all at C-8, whereas β-trichothecenes carry a keto group at this position (Varga et al. 2015). *Fusarium avenaceum* produces the mycotoxins moniliformin (MON), beauvericin (BEA), and enniatins (ENs). MON is a feed contaminant that is lethal to fowl. BEA and ENs are bioactive compounds that are insecticidal, antimicrobial, antiviral, and cytotoxic (Bottalico and Perrone 2002). *Fusarium sporotrichioides* produces T-2 and HT-2 mycotoxins on barley. T-2/HT-2 and DON show mutual exclusion therefore, high levels of T-2/HT-2 means low DON concentration and vice-versa (Hietaniemi et al. 2016). Table 1 represents the main mycotoxins that are produced by some of the principle *Fusarium* species found around the world.

Table 1. Mycotoxin production by principle *Fusarium* species.

Species	Mycotoxin	Source
Fusarium graminearum	**DON, NIV,** ZEN, MON, NX2	Boenisch and Schäfer 2011; Varga et al. 2015
Fusarium poae	**NIV**, BEA	Nogueira et al. 2018
Fusarium avenaceum	MON	Bottalico and Perrone 2002
Fusarium sporotrichioides	T-2, HT-2	Hietaniemi et al. 2016
Fusarium culmorum	**DON, NIV,** ZEN	Wagacha and Muthomi 2007
Fusarium oxysporum	T-2, HT-2	Mirocha et al. 1989

1.5 Disease Management

There are various methods to manage and control FHB in cereal crops, including barley. Cultural, chemical and biological control methods are the few currently being researched for combating this disease. Cultivation control methods include crop rotation, appropriate fertilizers, and weed control. Crop rotation prevents the build-up of *Fusarium* spores in the residue, making it a very effective method for reducing the disease pressure of *Fusarium graminearum*. For example, wheat sown into the field following non-host crop species, such as oats (*A. sativa)* or alfalfa (*M. sativa)* and soybean (*G. max)*, resulted in reduced infection. While both incidence and severity of FHB were greater when wheat followed another cereal crop and host species of *Fusarium*, corn (*Z. mays)*, (Pirgozliev et al. 2003), and lower when wheat followed soybeans. Other agronomic practices can also be used to reduce FHB disease pressure such as ploughing or removal of crop debris, and controlling fertilizer applications (Pirgozliev et al. 2003).

Application of the fungi *Clonostachys rosea* strain ACM941 has also been proposed as a means of biocontrol for FHB, with development underway at Agriculture

and Agri-Food Canada (AAFC) Ottawa (Xue et al. 2009). This fungal strain is a mycoparasite biopesticide that has been shown to suppress *Fusarium* complexes that cause disease in wheat and soybeans. Through greenhouse and field trials, there is promise for applications against FHB in barley.

Chemical control methods use various fungicides to inhibit growth of *Fusarium* species on cereal crops. Fungicides such as tebuconzaole, inhibit mycelial growth of *F. graminearum* and 3-ADON production in cereals (Pirgozliev et al. 2003). Strobilurin fungicides are modeled after an antifungal substance that is produced by a mushroom called *Strobilurus tenacellus* (Pers.) Singer, the chemicals in these fungicides act by inhibiting energy production in the fungus to prevent growth thereby killing the fungus (Balba 2007).

There have been improvements in developing resistant wheat cultivars to FHB and DON through the identification of resistance genes (Zhou et al. 2002). There is no source of complete resistance in barley due to the lack of resistance genes (Mesterházy 2001), Schroeder and Christensen (1963) suggested two types of resistance in cereal grains, Type I resistance is that of resistance to initial infection, Type II is resistance to spread of infection of bight symptoms within the spike. Other types of resistance have been proposed based on resistance against kernel infection (Type III), tolerance of the grain to infection and yield loss (Type IV) which is linked with Type III resistance, resistance against toxin accumulation in the grain (Type V), and resistance against alteration of the grain constituents (Type VI) (Martin et al. 2018). Type V is important for grain utilization purposes, such as in beer production, as DON concentrations can hinder the beer quality, evidence has shown a significant correlation between *Fusarium* on

barley grains and low quality of resulting malts (Nogueira et al. 2018). In barley, assessment of resistance has been focused on Type I resistance, as Type II resistance is already strong, and the factors associated with Type III, V, IV, VI resistance in wheat are not yet accurately characterized in barley (Martin et al. 2018). The enzyme superoxide dismutase has been studied for its role in resistance mechanisms in the spikes of resistant and susceptible wheat cultivars, showing higher activity in resistant cultivars after infection (Bai and Shaner 2004). Barley is very susceptible to initial infection and severe disease can be a result of multiple initial infections on the spike (Bai and Shaner 2004). Barley resistance is not well supported and is modest, yet some Chinese cultivars have been identified resistance with low DON content (CI 4196, Zhedar 2, Imperial), all being 2-rowed cultivars (Steffenson 1998). Resistance is also morphologically linked, two-rowed barley is naturally more resistant to FHB than six-rowed barley, and hulled barley is more resistant than hull-less (Takeda 1990; Bai and Shaner 2004). Chevron is a 6-rowed barley cultivar that has the highest resistance to kernel discolouration (Steffenson 1998).

While the application of fungicides and other cultivation control methods can partly reduce FHB, integrating resistant cultivars with residue management and applying foliar fungicides is are a more effective solution (Dweba et al. 2017). Quantitative trait loci (QTLs) are regions of DNA associated with a particular phenotypic trait, and they are mapped by identifying the molecular markers that are correlated with an observed trait. Mapping studies have indicated that many QTLs for FHB resistance co-segregate with the QTL for plant height, heading date, and spike characteristics (Thin et al. 2004).. There are limited sources of FHB resistance in barley, yet several QTLs have been identified for

lower FHB severity, DON content and kernel discoloration (KD) (Bai and Shaner 2004). KD results in reduced market value, malting and brewing companies will reject grain that suffer from KD and presence of DON that could hinder malting quality. Ten QTLs are associated with FHB resistance, 11 QTLs for KD resistance, and 4 QTLs for resistance against DON accumulation (De La Pena et al. 1999), and 3 QTLs have been identified on chromosomes 2H and 7H for low DON levels that are associated with low FHB severity (Canci et al. 2003). The architecture of 2-rowed barley allows it to be more naturally resistant to 6-rowed barley, considering resistant cultivars should be a major part of FHB disease management.

1.6 Maritime Barley Cultivation and Disease

Barley is grown in New Brunswick, Nova Scotia, and Prince Edward Island, and in 2017, PEI had the largest production of barley in the region at 105,000 tonnes (Statistics Canada 2020). Barley is grown in the Maritimes primarily as a feed source but is now being considered for malting purposes for local breweries. Each year between 2000 and 2018, barley production in PEI has surpassed the production in NB and NS. The constant decline in these last years can partially be attributed to the presence of *Fusarium* Head Blight (Atlantic Grains Council 2018).

Current efforts are being made to evaluate the suitability of Western barley to the growing conditions in NB and PE, preliminary results have concluded that it can be possible. This growing opportunity provides brewers with the potential to provide a market that will support local growers and build a locally based chain (Atlantic Grains Council 2013).

FHB has been an issue in Eastern Canada since the 1930s, according to disease surveys published online by the Canadian Phytopathology Society (Canadian Pyhtopathology Society 1937). These surveys provide important information for farmers and the public to acknowledge the dominant diseases affecting barley and other cereal crops. The earliest documented disease survey that included the Maritimes, dates back to 1927, and each year up until the early 2000s, and these surveys note only short observations regarding barley diseases. Crop damage was noted to be severe throughout the 1950s, and 6-rowed barley was noticed to be more susceptible to FHB than 2-rowed during this decade. Through to the 1990s, FHB could be found in all three Maritime provinces (Martin, R.A., Johnston 1994). Yet these disease surveys have ended in the 2000s, leaving little information about the FHB causing species and regional presence of FHB.

Agriculture and Agri-Food Canada (AAFC) collaborates with the Atlantic Grains Council to conduct on-farm agronomy research to test and evaluate best management practices for barley. In 2018, these tests examined nitrogen application studies and examining soil enhancement in large plot trials on farms throughout the Maritime provinces (Atlantic Grains Council 2018). Collaboration with the Atlantic Grains Council has made it possible to conduct an efficient disease survey in the Maritime provinces.

Cereal disease surveys are important to provide information about cereal crops to growers. Collecting and reporting information about the impact of cereal pathogens can allow growers to alter and manage their agricultural methods to account for changes in the environment that allow disease infection and the dominant pathogens to ensue. Knowledge of the dominant cereal crop diseases is also important for

understanding how the pathogen populations are changing throughout Canada, Atlantic Canada in particular. In Atlantic Canada, disease surveys have dated back to the 1920s (Canadian Phytopathology Society) with little information specific to the identification of FHB causing *Fusarium* species. Very few surveys have been performed recently in Atlantic Canada to assess FHB in cereal crops, therefore it is important to provide up to date information concerning FHB incidence and severity in the Atlantic provinces to understand the changing dynamic of *Fusarium* complexes.

Since the environment influences when and how the pathogen infects the crop, it is necessary to monitor the weather patterns each year to understand how climate change is affecting these diseases. Rising carbon dioxide levels, higher temperatures, and altering precipitation modifies plant growth and development, allowing production and survival of pathogens on host plants, as pathogen populations have shown to adapt to rising temperatures (Pangga et al. 2013).

Cereal surveys are important for determining if FHB is getting better or worse, if there are invasive and more virulent isolates, and also in determining if there are differences between *Fusarium* species from different regions. Growers want resistant cultivars and surveys allow researchers to collect isolates to screen for FHB resistance in new barley lines, this ensures the data is relevant to the natural pathogen population. Cereal crop surveys are important for gaining beneficial information about diseases that are drastically affecting the yield of crops for farmers in Canada. With environmental conditions quickly changing, it is important to understand how these changes will and can affect the crop. Without regular surveying of pathogens that distress cereals, there is no way to monitor these changes over time.

1.7 Molecular Identification and Quantitation of Plant Pathogens

Identification methods of fungal pathogens include characterizing based on morphology of the pathogen in real time on the crop, cultured on media, and spore structures. The visual identification of fungi by characterizing morphological components down to species level is difficult for many fungi, including *Fusarium,* which can lead to incorrect identification (Hsuan et al. 2011). Closely related species can also possess similar characteristics such as pigment and spore structures that are difficult to discern. Molecular techniques provide a more sensitive and accurate evaluation, allowing faster conclusions and eliminates the potential of subjective human behavior when assessing the phenotype of a pathogen (Ahmadi et al. 2015). DNA-based identification using polymerase chain reaction (PCR) provides a more reliable mechanism for identification and differentiation of *Fusarium* species.

Quantitative/Real-Time Polymerase Chain Reaction (QPCR) is based on the same principles as PCR but the results can be seen in real-time and detects the expression level of a certain fragment in the organism, making it a quantitative method for identification. QPCR is more sensitive due to its high level of resolution in comparison to PCR, instead of using gel electrophoresis to visualize bands, QPCR uses fluorescent dyes to detect the product (Zitnick-Anderson et al. 2018).

1.8 Purpose

The objectives of this study are to survey the Maritime provinces, New Brunswick, Nova Scotia and Prince Edward Island for FHB and DON contamination, and to determine the relationships between FHB and DON. This study utilized QPCR methods to quantify FHB causing *Fusarium* species in barley seeds collected in a 2018 survey

from grower fields that participated in the on-farm agronomy studies conducted by the Atlantic Grains Council with AAFC through the Canadian Agriculture Partnership (CAP) in the three Maritime provinces. Through this research, a survey of *Fusarium* species and DON content of barley seeds from the fields will establish regional differences in disease pressure of FHB.

Materials and Methods

2.1 Sample Collection

Barley samples were collected from on-farm agronomy research trials conducted throughout the Maritimes by the Atlantic Grains Council. A total of 10 field locations were sampled with 9 sites from PEI, 10 sites from NB and 12 sites from NS were surveyed (Fig. 2). A sub sample of approximately 500 g of seeds was provided from each field site post-harvest and cleaning. Barley seeds were collected and surface-sterilized in 70% ethanol for 5 minutes.

To isolate *Fusarium* species, 100 seeds per field were plated onto potato dextrose agar (PDA) containing streptomycin [50 μg/mL] and tetracycline [50 μg/mL] to prevent bacterial contamination and incubated at room temperature. After 7 days, suspected *Fusarium* isolates were sub-cultured onto PDA supplemented with the same antibiotics, then subcultured from hyphal tips again after 7 to 14 days to obtain pure cultures.

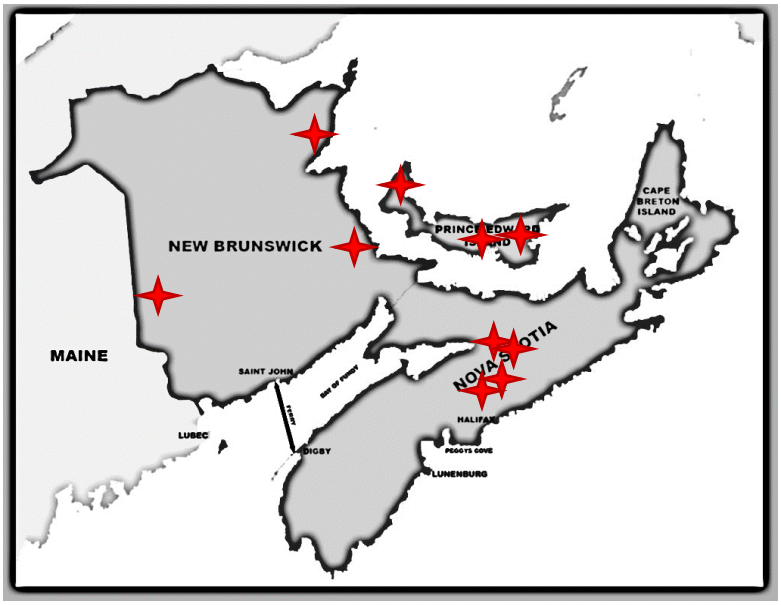

Figure 2. Map of the Maritime provinces involved in the survey, the red points are representing the field site locations where samples were taken. https://www.flickr.com/photos/22711505@N05/38068706042

2.2 Identification of Fusarium Species from Culture

Determination of *Fusarium* species was first performed based on morphological characteristics that are indicative of *Fusarium*. The phenotypes of cultures grown on PDA were characterized based on pigmentation, amount of growth over the incubation period, and by conidiophore identification. Each isolate was sub-cultured on Spezieller Nährstoffarmer Agar (SNA) and incubated for 7-10 days to allow for sporulation; spore structure and shape was examined according to Fusikey by Dr. Keith Seifert (Seifert, K., 1996).

Molecular techniques were performed to further confirm species identification. *Fusarium* specific primers (Table 2) were used to identify *Fusarium* species present in each isolate cultured; *F. graminearum* primers (Nicholson et al. 1998) Fg16 F/R, *F. avenaceum* primers (Turner et al. 1998) Fa F/R, *F. poae* primers (Parry and Nicholson 1996) Fp82 F/R, *F. sporotrichioides* primers (Demeke et al. 2005) AF330109C F/R, were

used for PCR identification. DNA for colony PCR was extracted by scraping mycelium from isolates grown on PDA and placing into 40 μL of sterile nuclease-free water, then microwaving for 30 s and cooling on ice. For PCR 2 μL of the 40 μL DNA stock was used directly in the 20 μL reaction, using Phusion Hot Start II DNA Polymerase (Thermo Scientific™). DNA amplification was performed in an Applied Biosystems Thermocycler (Life Technologies) using an initial 2 min denaturation at 98°C; then 35 cycles of 10 s at 98°C, and 20 s at 60°C, and 5 s at 72°C, followed by a final extension of 2 min at 72°C. Amplification products were separated by electrophoresis in a 1% agarose gel; expected product sizes for each species are shown in Table 2.

Table 2. List of primer names, sequences, expected product sizes, *Fusarium* species, and source of primers.

Primer Name	Sequence (5' to 3')	Product Size (bp)	Target	Source
Fg16F	CTCCGGATATTTCGTCAA	450	*F. graminearum*	Nicholson et al. 1998
Fg16R	GGTAGGTATCCGACATGG CAA	450	*F. graminearum*	
FaF	CAAGCATTGTCGCCACTCT C	920	*F. avenaceum*	Turner et al. 1998
FaR	GTTTGGCTCTACCGGGACT G	920	*F. avenaceum*	
Fp82F	CAAGCAAACAGGCTCTTC ACC	220	*F. poae*	Parry and Nicholson 1996
Fp82R	TGTTCCACCTCAGTGACAG GTT	220	*F. poae*	
AF330109 CF	AAAAGCCCAAATTGCTGA TG	332	*F. sporotrichioides*	Demeke et al. 2005
AF330109 CR	TGGCATGTTCATTGTCACC T	332	*F. sporotrichioides*	

2.3 Molecular Chemotyping of Fusarium Graminearum Isolates

The chemotype of the *F. graminearum* isolates were identified using specific primers for 3-ADON, 15-ADON and NIV chemotypes present in *F. graminearum*. 3CON was used to target the Tri3 gene, 3NA was used to identify NIV, 3D15A was used to identify 15-ADON, and 3D3A was used to identify 3-ADON (Ward et al. 2008). DNA for colony PCR as described for identification by species. PCR conditions were 98°C for 2 mins; and then 35 cycles of 10 s at 98°C, and 20 s at 60°C, and 5 s at 72°C, followed by a final extension of 2 min at 72°C. Amplification products were separated by electrophoresis in a 1% agarose gel, Table 3 shows the expected product size for each chemotype.

Table 3. List of *Fusarium* chemotyping primer names, sequences, product sizes, target and source (Ji et al. 2019).

Primer Name	Sequence	Product Size (bp)	Target/Toxin Name
3CON	TGGCAAAGACTGGTTCAC		Tri3
3NA	GTGCACAGAATATACGAGC	840	NIV
3D15A	ACTGACCCAAGCTGCCATC	610	15-ADON
3D3A	CGCATTGGCTAACACATG	243	3-ADON

2.4 DNA Extraction from Barley Samples

For barley seeds, 25 g was ground in a grinder for 45 s, and 0.3 g was weighed in a 2-mL microcentrifuge tube and used for DNA extraction using the QIAGEN™ Dneasy Plant Mini Kit (QIAGEN). The concentration of the eluted DNA was determined using a Nanodrop One™ (Thermoscientific).

2.5 DNA Extraction for Fusarium Standards

To create *Fusarium* DNA standards for QPCR analysis, *Fusarium* cultures from the DOAM were grown in liquid culture of potato dextrose broth for 1 week at room temperature. The mycelium grown was then filtered and ground with a mortar and pestle in liquid nitrogen, and the DNA was extracted using the QIAGEN™ Dneasy Plant Mini Kit (QIAGEN). The concentration of the eluted DNA was determined using a Nanodrop One™ (Thermo Scientific).

2.6 QPCR of Fusarium Species from Barley Seeds

DNA standards were created by ten-fold dilutions of DNA: 8, 0.8, 0.08, and 0.008 ng/rxn. Sample DNA concentrations were all normalized to 20 ng/μL (80 μg/rxn), then 20 μL multiplex reactions were prepared with 2X PrimeTime MasterMix (Integrated DNA Technologies, Inc), 0.5mM of each primer, 150 nM of each probe, and 4 μL of template DNA. Real-time PCR analysis was performed on a Bio-Rad CFX Thermocycler (Bio-Rad) using the following conditions, 3 min at 95°C, and then 39 cycles of 10 s at 95°C, 10 s at 57°C, and 30 s at 72°C (Zitnick-Anderson et al, 2018). The *F. graminearum* and *F. avenaceum* probes used were labelled with the fluorescent tag FAM, and the *F. poae* and *F. sporotrichioides* probes were labelled with the fluorescent tag

HEX. Table 4 contains a list of primer sets and probes for the four *Fusarium* species used in the quantification analysis.

Table 4. List of primers, probes, and sequences used in real-time PCR analysis. (Zitnick-Anderson et al. 2018).

Primer Name	Sequence	Target
PoaeACL1_poae1_pr	5'-/5HEX/GTTCTTCTC/ZEN/AGGACTTTAC CCCGAAAGCC/3IABkFQ/-3'	*F. poae*
Fp-ACL1-F160	5'-CCATCCCCAAGACACTGAG-3'	*F. poae*
Fp-ACL1-R330	5'TACAAGTTGCTRCAAGCCC-3'	*F. poae*
AvePr	5'-/56-FAM/CGACAAGCG/ZEN/AACCATCGA GA/3IABkFQ/-3'	*F. avenaceum*
AveF	5'-GCTTATCTGCACTCGGAACC-3'	*F. avenaceum*
AveR	5'-CGCGTAATCGAAGGGATATT-3'	*F. avenaceum*
SpoPr	5'-/5HEX/TGATAGTGG/ZEN/GGCTCATAC CC/3IABkFQ/-3'	*F. sporotrichioides*
SpoF	5'-TTTTTACGGCTGTGTCGTGA-3'	*F. sporotrichioides*
SpoR	5'-CGGCTTATTGACAGGTG-3'	*F. sporotrichioides*
GramPr	5'-/56FAM/TCCCACAAA/ZEN/CCATTCCCT GG/3IABkFQ/-3'	*F. graminearum*
GramF	5'-GCGGCTTTGTCGTAATTTTT-3'	*F. graminearum*
GramR	5'-TATTGACAGGTGGTTAGTGACTGG-3'	*F. graminearum*

/HEX/: green fluorescent dye /FAM/: blue fluorescent dye
/ZEN/: internal quencher for TaqMan and QPCR probes
/3IABkFQ/: Iowa Black quencher ideal for use with fluorescein

2.7 Mycotoxin Analysis

F. graminearum samples were sent to Dr. Justin Renaud at AAF London to perform liquid chromatography with tandem mass spectrometry (LC-MS/MS), a multi-mycotoxin analysis. Chemical standards were purchased and isolated from cultures. To prepare the samples, 200mg were removed and extracted, then 1 mL of acetonitrile/water/formic acid 78/20/2 (*v*/*v*/*v*) was added to the 200 mg samples, vortexed for 30s, sonicated for 30 minutes and agitated on a ThermoMixer for 30 min at 1400 rpm. Samples were spun at 4°C at 10,000 rpm for 6 min and 125 μL aliquots were removed and diluted with 125 μL of LC-MS grade H_2O. Each sample had 0.5 ppm of ^{13}C-DON internal standard equivalent added and transferred into polypropylene HPLC vials for LC-MS analysis.

Q-Exactive™ Quadrupole Orbitrap mass spectrometer (Thermo Scientific, MA, USA) coupled to an Agilent 1290 high-performance liquid chromatography system was used to collect all MS data, and the mycotoxins were resolved on a Zorbax Eclipse Plus RRHD C18 column (2.1× 100 mm, 1.8 μm; Agilent Technologies, CA, USA) maintained at 35°C. The fungal secondary metabolites were analyzed by MS and MS/MS (Renaud).

2.8 Fusarium Head Blight Virulence Assays

Twenty isolates of *F. graminearum* were randomly chosen from the isolates that were collected in the survey. Each isolate was sub-cultured into 50 mL of liquid broth, this was made by boiling 20g/L of mung beans for 20 mins, then filtering to remove any solids. The volume was adjusted to 1L and supplemented with 15g of sodium chloride (NaCl). The solution was autoclaved at a liquid 20 min cycle and allowed to cool before aliquoting 50 ml into 250 mL tissue culture flasks. Using a sterile probe, each flask was inoculated with 5 small plugs from the agar cultures. The liquid cultures were shaken at

room temperature at 50 rpm for 7-10 days. Liquid cultures were then filtered through cell strainer into 50 mL falcon tubes, spun down for 10 min at 4500 rpm, the supernatant was poured off and the pellet was washed with 20 mL of sterile filtered water. 10 μL of spore suspension was loaded onto a hemocytometer (VWR) for spore counting using a haemocytometer; the number of spores per mL was calculated and all spore suspensions were normalized to final concentration of 50,000 spores/mL in 40mL of sterile water. Two isolates, Fg. 213 and 304, had low spore production, therefore only 30,000 spores/mL solutions could be generated.

To determine if isolates were virulent barley was grown until full head emergence (ZGS stage 58), approximately 7 mL of spore suspension was sprayed on 3 barley heads that were then covered with a plastic bag and sealed around the heads for 4 days. The spray bottles were washed using 70% ethanol, then rinsed with sterile filtered water between each isolate before the next inoculation to prevent cross contamination. This was repeated for each isolate and a water control for 4 replications. The two isolates with lower spore production had two replications with six barley heads in each. After 9 days, the heads were removed from the field to document disease severity in the laboratory. Each repetition was photographed, and the infected barley heads were rated according to a 0-9 scale of increasing disease severity (Figure 3).

Figure 3. Examples of different levels of barley FHB virulence observed on inoculated Synergy 2-rowed barley. The numbers on the photos represent the severity rating between 0 and 9 that can be assigned to assess FHB severity. 0 rating means no infection and a rating of 9 represents full head infection. Ratings are estimations of amount of seeds within the barley head that are infected by FHB.

The barley heads from each isolates repetition were frozen using liquid nitrogen then ground with a mortar and pestle, then DNA was extracted from the ground sample using the QIAGEN™ Dneasy Plant Mini Kit (QIAGEN). The concentration of the eluted DNA was determined using a Nanodrop One™ (Thermo Scientific), and all DNA concentrations were normalized to 20 ng/μL.

A QPCR assay was performed to quantify the amount of *F. graminearum* in the inoculated barley heads. The same *F. graminearum* primers and probes were used for the reaction as in Table 4, and the same QPCR conditions were used for amplification as described for barley seeds.

2.9 Statistical Analysis

Using Genstat 64-bit Release 18.1, linear regression analyses were performed for the number of *F. graminearum, F. poae, F. avenaceum,* and *F. sporotrichioides* isolates versus amount of *Fusarium* DNA (ng/rxn). Linear regression analyses were also performed to compare total DON (ppm) and quantity of *Fusarium* DNA (ng/rxn), and to calculate the R-squared value. General linear ANOVA with a post hoc LSD test was

performed to analyze infection severity for the virulence assay test, with linear regression performed to compare severity scores and QPCR data (DNA (ng/rxn).

Results

3.1 Identification of Fusarium Species from Culture

Morphological identification of *Fusarium* species in culture were based on colony pigmentation and the aerial mycelium produced by the isolate; these cultures were isolated for molecular identification. Conidiophores from each of the four *Fusarium* species of interest were examined and characterized into species using FusiKey (Seifert 1996). Figure 4 shows eaxmples of different condia observed from *Fusarium* species isolates.

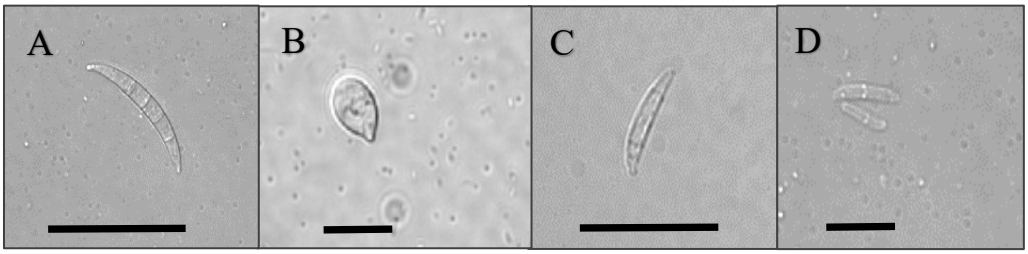

Figure 4. Conidia from *Fusarium species* isolated from barley seeds. *F. graminearum* (A), *F. poae* (B), *F. sporotrichioidies* (C), *F. avenacium* (D). The spores for each species were visualized on a microscope slide with 20 μL of water and 10 μL of liquid culture that was made to produce spores. Photos were captured on a Zeiss Confocal microscope. Bar represents 20 μm.

A total of 336 *Fusarium* isolates were cultured and identified to species by PCR. Each species-specific primer set amplified the expected DNA fragments size in all positive reactions. Of the 336 isolates, 236 were identified as *F. graminearum,* 7 as *F. avenaceum*, 59 as *F. sporotrichioides*, and 25 as *F. poae*. *F. culmorum* and *F. pseudograminearum* primer sets were used but no cultures were identified as those species.

In New Brunswick, there were 44 *Fusarium* isolates cultured. In Nova Scotia, there were 253 *Fusarium* isolates cultured, with the 79% being *F. graminearum*. In PEI, there were 26 *Fusarium* isolates cultured. Figure 5 represents the number of *Fusarium* isolates found in each site location of the provinces surveyed.

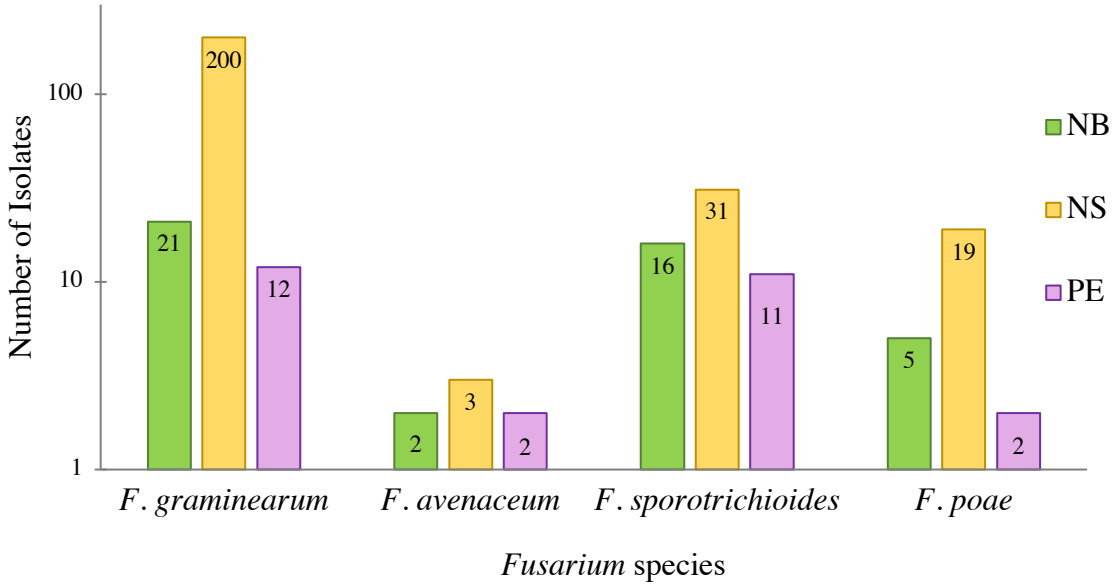

Figure 5. The number of *Fusarium species* isolated in New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PE), using species-specific PCR primers (Table 2) to identify the four principle FHB causing species from plated cultures. The numbers exhibited inside the bars represent the quantity of each *Fusarium* species identified in each province.

3.2 Chemotyping Fusarium Isolates by PCR

F. graminearum isolates showed expected product sizes for 3-ADON (243bp), 15-ADON (610bp) chemotypes, with some isolates presenting as both 3-ADON and 15-ADON chemotypes. 141 of the *F. graminearum* isolates were of the 3-ADON chemotype, and 117 were of the 15-ADON chemotype, and 46 isolates presented as both 3-ADON and 15-ADON.

Nova Scotia presented 121 isolates with 3-ADON chemotype, and 108 isolates with 15-ADON chemotype. New Brunswick presented 10 isolates with 3-ADON chemotype, and 6 isolates with 15-ADON chemotype. PEI presented 6 isolates with 3-ADON chemotype, and 2 with 15-ADON chemotype, as presented in Figure 6.

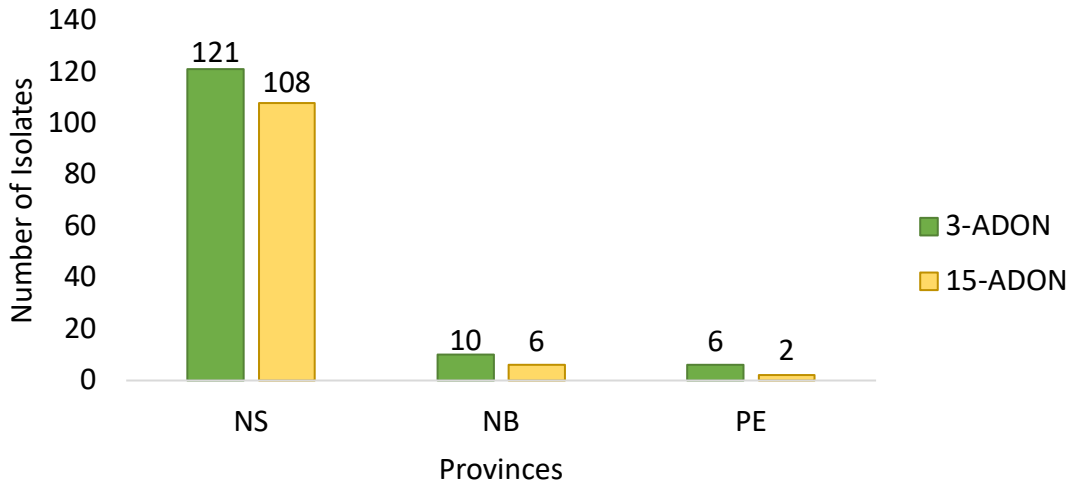

Figure 6. The number of *F. graminearum* isolates that showed 3-ADON (3-acetyldeoxynivalenol) and 15-ADON (15-acetyldeoxynivalenol) chemotypes from Nova Scotia (NS), New Brunswick (NB), and Prince Edward Island (PE) using species specific primers listed in Table 3. PCR was performed from ground barley seed samples and DNA was extracted using the DNeasy Plant Mini Kit from QIAGEN.

3.3 Quantification of Fusarium DNA from Barley

F. graminearum DNA was normalized to 20 ng/μL, used for a 10-fold dilution series for QPCR and generated consistent cycle threshold values for a standard curve. Table 5 represents the significant DNA concentrations revealed by QPCR analysis. The most *F. graminearum* DNA extracted from barley was found in Nova Scotia; field NSTFB101a contained the most *F. graminearum* DNA within the NS fields examined. The most *F. poae* DNA extracted was from New Brunswick; field NBWJ0112 contained the most *F. poae* DNA within NB. Nova Scotia was also found to have the highest

amount of *F. avenaceum* and *F. sporotrichioides* DNA extracted, with fields NSGAB101b and NSGAR103b having the highest quantities.

Table 5. The locations of the highest quantity of DNA measured for each *Fusarium* species per qPCR reaction.

Target	Location	DNA (ng/rxn)*
F. graminearum	NS	8.29
F. poae	NB	0.733
F. avenaceum	NS	0.053
F. sporotrichioides	NS	0.612

*80 ng of barley DNA used per reaction

3.4 Contamination of Deoxynivalenol in Maritime Provinces

The mycotoxins were identified and quantified using LC-MS/MS techniques. Significant total DON concentrations were noted in Nova Scotia with a total of 70.3ppm, New Brunswick total DON was found at a concentration of 4.79ppm, and in Prince Edward Island, total DON concentration was at 8.42ppm. 15-ADON and 3-ADON chemotypes were identified in Nova Scotia and PEI, at concentrations of 5.25ppm and 2.15ppm, 4.13ppm and 0.47ppm, respectively. Only 15-ADON chemotype was found in NB at a concentration of 3.28ppm. Figure 7 represents the average concentrations of DON that were found in each province per field in parts per million, figure 8 represents DON concentrations in Nova Scotia fields. Supplemental Table 1 contains concentrations of DON and its derivatives in each field in each province.

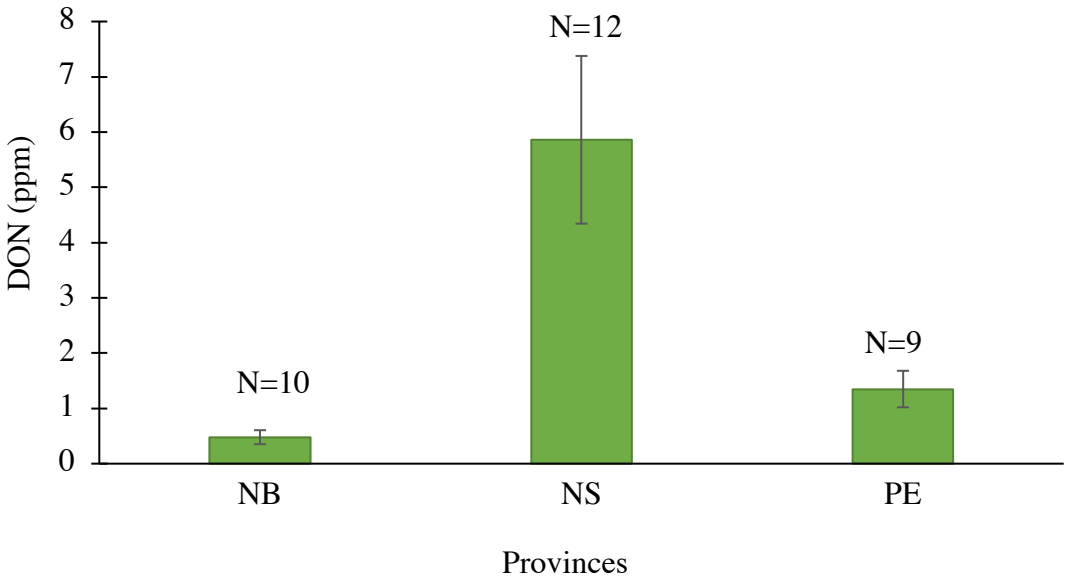

Figure 7. The average level of deoxynivalenol (DON) in parts per million in each Maritime province using LC-MS/MS in London by Justin Renaud, from 1g of ground barley seeds in New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PE). The error bars represent standard error and 'N' represents the sample size.

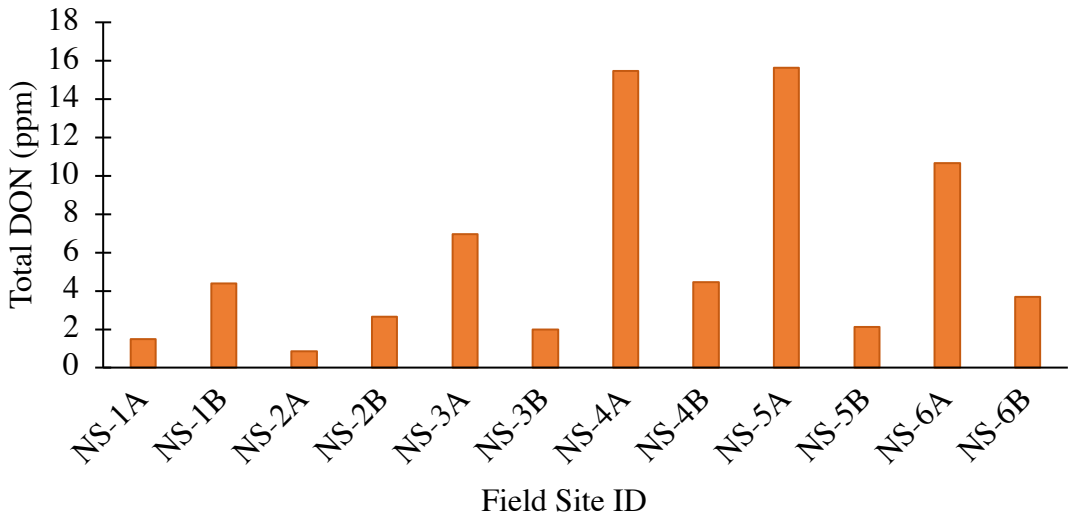

Figure 8. The concentrations of deoxynivalenol (DON) in parts per million in Nova Scotia fields, analyzed by LC-MS/MS from 1g of ground barley seed samples. A and B samples come from the different field plots (min 1 acre) at the same location.

A linear regression analysis was performed comparing *F. graminearum* DNA with total DON concentrations, and an R-squared value was calculated at 0.9272. This value supports the hypothesis that the detection of DNA of *F. graminearum* in barley seeds positively correlates to an increase DON concentration. Figure 9 represents the QPCR analysis versus DON (ppm).

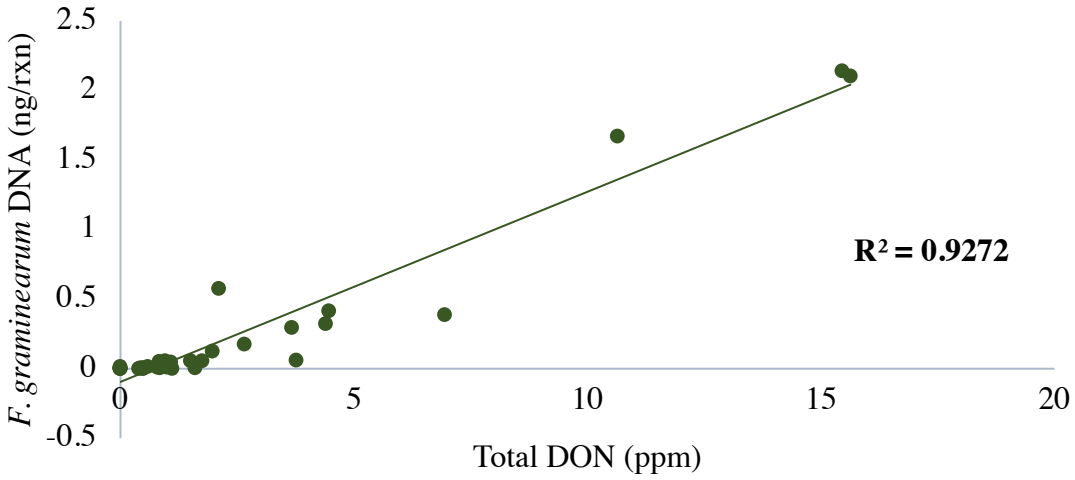

Figure 9. Comparison of *F. graminearum* DNA (ng/rxn) and total DON concentration (ppm). A qPCR assay using species specific primers was used to quantify *F. graminearum* DNA from ground barley seed samples. LC-MS/MS identified and quantified the concentration of DON from 1g of ground barley seed samples. A linear regression analysis was performed to determine the relationship between *F. graminearum* DNA and DON in the Maritime provinces and revealed a strong positive correlation value of 0.9272.

3.5 Fusarium Head Blight Virulence Assay

From the in-field spraying of barley with *Fusarium graminearum* spores, the heads were visually rated for severity, and the QPCR assay was performed. *F. graminearum* DNA was quantified from each isolate used in the virulence assay. To evaluate the relationship between visual severity scores and QPCR data, Figure 10 shows the severity

that was exhibited by each isolate from the randomly chosen fields, and their associations with each other.

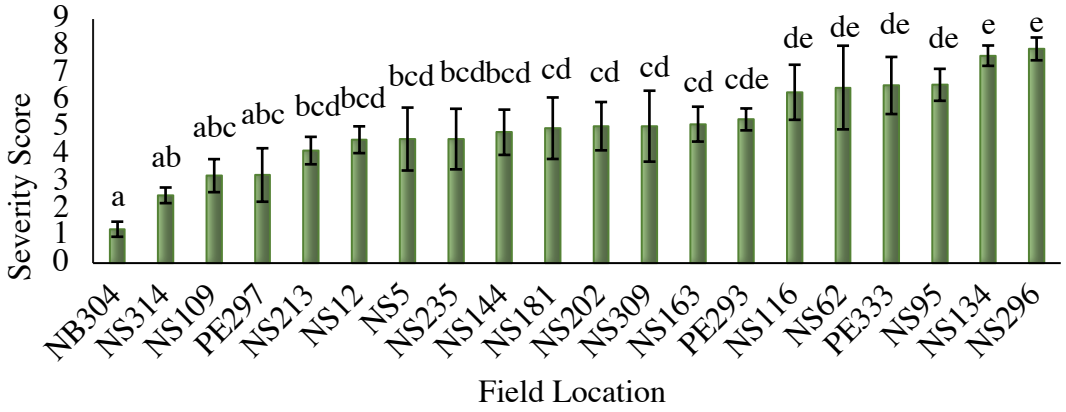

Figure 10. Severity caused by each *F, graminearum* isolate from the virulence assay based on a visual severity rating score from 0 to 9. Each isolate is represented by its field ID labelled on the x-axis. An ANOVA with an LSD test at $p<0.05$, with standard error bars was performed revealing various differences between the isolates and their severity scores. The different letters mean the isolates were significantly different.

A linear regression analysis was performed comparing the visual severity scores and the quantitative measurements of DNA (Figure 11), this regression analysis revealed an R-squared value of 0.4866, with correlation coefficient at $p < 0.05$. This value supporting the hypothesis that visual rating is subjective and not well correlated with quantitative measurements. Figure 11 represents the QPCR analysis versus visual severity ratings.

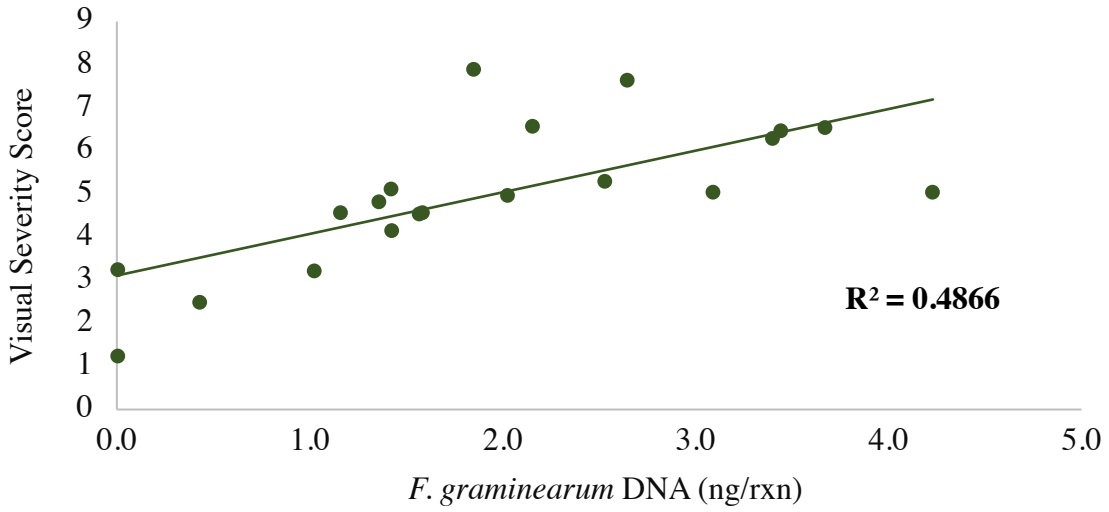

Figure 11. qPCR analysis of *F. graminearum* DNA (ng/rxn) versus visual severity rating (0-9). A linear regression analysis was performed comparing the visual severity scores from the virulence assay on barley heads and the quantitative measurements of *F. graminearum* DNA from the qPCR assay. This regression analysis revealed a weak positive correlation value of 0.4866, with correlation coefficient at $p < 0.05$

Discussion

4.1 Fusarium Species in the Maritimes

This is the first disease survey of FHB in the Maritime provinces since 2000 and provides us with a growing knowledge of FHB presence. With a goal of identifying and quantifying the dominant FHB causing species, the results of the PCR based identification assay confirmed that *F. graminearum* is the principal species isolated that caused FHB of barley in the Maritime Provinces in 2018. This shows that while *Fusarium* species can be more important, *F. graminearum* was the most distressing species causing FHB in 2018. This is supported by the finding that it was the species of most abundance in each of the three provinces surveyed with the largest number of *F. graminearum* isolates found in Nova Scotia, New Brunswick and Prince Edward Island. *F. graminearum* is considered the main causal species of FHB in Eastern Canada and some

countries around the world, according to Ali and Calpas (2019), Bai and Shaner (2004), and Palazzini et al. (2016).

The other species isolated were *Fusarium poae, Fusarium sporotrichioides,* and *Fusarium avenaceum*. In Nova Scotia, the number of isolates for each *Fusarium* species was higher than the other provinces, this larger presence could be attributed to the area of NS that the seed samples were taken, but also the climate in NS during the 2018 growing season, such as persistent rainfall, could account for the difference. In July of 2018, Halifax (NS) experienced a total of 65.9 mm of precipitation with four days having over 5 mm and one day had a total of 36 mm of rain. Moncton (NB) had 44.2 mm with four days above 5 mm and one day with 25.8 mm, the same day that NS had its highest rainfall, NB only received 1.8 mm. Charlottetown (PE) had a total of 24.1 mm, three days above 5 mm with the highest daily rainfall at 11.8 mm (Environment Canada 2019). Pangga et al. (2013) have noted that rising temperatures can increase pathogen infection in hosts. Documenting the different environmental conditions in each province could be included in the next disease surveys to investigate associations between weather and species isolation. By investigating the climate's role in FHB disease severity, a forecast model can be developed to predict FHB severity to assess risk of disease development for Maritime barley growers.

It is significant to note the more severe level of FHB infection in NS, as the rest of the provinces had lower disease. The absence of information between 2000 and 2018 makes it difficult to conclude that the disease severity has increased in each province, though we can conclude the FHB continues to be a major issue in Maritime provinces. By

combining previous FHB information with future surveys, we will be able to gain further understanding of FHB causing species that dominate the Maritime provinces.

4.2 Contamination of DON and Derivatives

The Canadian Food Inspection Agency under the Government of Canada has outlined legislated maximum tolerated levels of mycotoxins in some foodstuffs, feedstuffs, and dairy products. The concentration levels for DON for uncleaned soft wheat for human consumption is 2ppm, the maximum level for grains and grain by-products for dairy cattle and poultry is 5ppm, and grains and by-products for swine, young calves, and lactating dairy animals is 1ppm (Canadian Food Inspection Agency 2017). The DON concentration levels for New Brunswick and PEI were low in comparison to that found in Nova Scotia. This high average level for DON in Nova Scotia can be attributed to 2 fields that were abnormally highly contaminated with DON, their levels were at 15 ppm. Barley seeds that exceed the DON level guidelines can be managed by soaking, using high velocity air cleaning of the kernels, or dehulling the grain to reduce to concentration of DON to be suitable for all livestock (House et al. 2003). The rainy environment in July of 2018 in Nova Scotia can be a factor associated with this high contamination level. Previous data for DON levels in each province would be helpful to understand if mycotoxin contamination is becoming progressively worse each year.

Results from the PCR based assay to chemotype DON derivatives as 3-ADON or 15-ADON from cultured isolates revealed a fairly even distribution of both DON chemotypes throughout each province. LC-MS/MS results yielded no 3-ADON quantified from New Brunswick, and 15-ADON was the predominant genotype quantified in Nova

Scotia and PEI. This could be explained by the study by Puri and Zhong 2010, whose evidence showed the ability of 3-ADON types to produce more DON than 15-ADON types. The apparent absence of 3-ADON in some NB fields could be attributed to the production of DON from 3-ADON (Tian et al. 2016). The absence of 3-ADON in other NB fields, as well as the low total DON levels, could be attributed to the disease management practices performed that lead to lower *Fusarium* presence and mycotoxin contamination in the grain. Limitations of the PCR method could also account for the differing results, Aitken et al. 2019 stated the insufficiency associated with molecular chemotyping and that chemical analyses should be required to accurately identify and quantify mycotoxins produced by *Fusarium* species, as PCR only has identification potential and LC-MS/MS can sensitively identify and quantify toxins.

Changes in agricultural methods such as fungicide application can affect development of disease and the rate of mycotoxin secretion, which in turn can change DON producing *Fusarium* populations (Edwards et al. 2001). Research studying the difference in toxicity of the two genotypes is limited, yet further understanding of the differences could also investigate their role in resistance. It is important to do surveys to understand and identify *Fusarium* population changes that can occur, especially if new DON derivatives are produced that have increased toxic effects.

4.3 Association Between Fusarium Head Blight and Deoxynivalenol

The correlation value obtained through the linear regression to compare *F. graminearum* DNA to DON concentrations reveals a significant result as any association between these variables is not known in the Maritimes. Noting this result is important evidence to support that the increase of *F. graminearum* infection is indicative of an

increase in DON concentration levels, as it follows results found by previous research in 2019 by Góral et al. 2019 in Poland, who showed positive correlations between *F. graminearum* DNA and DON. In Canada, research in Manitoba by Demeke et al. 2010, also showed positive correlations between *Fusarium* DNA and DON using QPCR techniques. Another survey of Finnish cereals demonstrated a highly significant correlation between *F. graminearum* DNA levels and DON (Hietaniemi et al. 2016). These results demonstrate that this QPCR assay can be effective for quantifying *F. graminearum* in barley in the Maritimes, while not the first study to utilize QPCR assays to quantify *Fusarium* species, this assay can be used in future disease surveys on various important cereal crops that are grown in all provinces. This assay also opens up opportunities in Western provinces for evaluating FHB and the *Fusarium* species that are producing DON without the time-consuming act of culturing fungi onto agar.

The results from performing linear regression analyses to compare *Fusarium* DNA and DON content could be useful in predicting DON contamination. Multiple studies have shown the positive correlation between *Fusarium* and DON to support using QPCR to predict DON contamination (Demeke et al. 2010, Palazzini et al. 2015, Okorski et al. 2017).

4.4 Visual Severity Ratings Versus Quantitative Measurements

Visual ratings have been considered to be the best method for assessing FHB incidence and severity in cereal crops (Jones 2000) as it is rapid for farmers to perform, yet this study revealed flaws in this technique. However, the subjectivity of visually rating FHB severity introduces bias and inconsistency due to the potential of human error. We were able to demonstrate a weak positive correlation between visual severity rating

and QPCR data to support this statement. This is significant since despite the assay being performed under the same conditions as FHB would normally infect and thrive, and documenting infection efficiently and consistently, the correlation to QPCR data was only 48%. Previous studies have discussed that visual ratings of plants diseases is one of the best methods for evaluating disease, yet the relationship with quantitative data is not usually as strong as the relationship between quantitative data and DON (Paul et al. 2005), which is the result observed in this study. Results from Edwards et al. 2001 also noted no correlation between visual assessments and DON content in grain.

QPCR can be used not only to quantify the amount of *Fusarium* species present but can be used to improve precision of various scientific studies such as fungicide evaluations and biological control studies for studying FHB. Testing various chemical fungicides to evaluate the difference in infection pre-application versus post-application can be valuable to measure the effect of the fungicide to determine which chemical, and in what volume, is most effective and efficient to reduce FHB severity. A disadvantage of QPCR is the cost of *Taq*Man probes (Smith and Osborn 2009), the requirement of special equipment and personnel that are highly trained.

Factors to consider in our method includes the inexperienced rating of the infected barley heads, yet this can support the role of human error in visual assessment methods as no head could be rated the same by each person assessing infection. It would be useful to do more research into the relationship between visual ratings and quantitative measurements, while visual ratings are a good presumptive method, further research could uncover more accurate visual ques that correlate to FHB presence at high levels.

Conclusion

The purpose of this survey was to identify and quantify four main FHB causing species, and DON from barley in the Maritime provinces in 2018. This study also determined the association between DNA and DON concentration levels. Populations of *Fusarium* species in the Maritimes were identified as *F. graminearum, F. poae, F. avenaceum,* and *F. sporotrichioides.* The most common species found in 2018 was *F. graminearum,* noting it was especially prevalent in Nova Scotia compared to New Brunswick and PEI.

The DON concentration levels were found to be highest in Nova Scotia, with DON derivatives relatively evenly distributed throughout NS, NB, and PEI. A highly significant correlation was found between *F. graminearum* DNA levels and DON content after performing the QPCR assay. This result shows that QPCR is a valuable technique to reliably quantify the presence of *Fusarium* species in Maritime barley and can be helpful to predict the level of DON contamination. The weak correlation found between visual severity ratings of FHB and *F. graminearum* DNA shows the unreliability of visual assessment. Visual rating can be a good presumptive method yet cannot replace quantitative measurement methods. The potential of human error cannot be overlooked when evaluating FHB as increasing mycotoxin contamination is at risk.

Climate change is bringing warmer temperatures and possible increased rainfall that can increase disease pressure to result in changes to *Fusarium* populations and the production of mycotoxins in Eastern Canada. Barley grown in the Maritimes is an integral ingredient to feed livestock, and the potential for malting barley to be produced for local breweries could be hindered by FHB infection. The continued surveillance of FHB to

identify and quantify FHB causing species and their mycotoxins will be beneficial for Maritime farmers to understand the effect of FHB on their crops. The *Fusarium* species found in each province produce mycotoxins with toxic effects that make animals sick and degrade the quality of the seed. The lack of FHB information in the Maritimes is threatening to farmers, this study introduced a QPCR assay as a survey method that is reliable and accurate to evaluate the presence and abundance of FHB causing species and associated toxins. Furthermore, this assay has provided room to develop disease management practices such as fungicide application, and weather forecast models to predict FHB severity, to help farmers alter and manage current techniques to effectively control FHB.

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Supplemental Tables

Supplemental Table 1. Representing the concentrations of DON, 3-ADON, and 15-ADON quantified by LC-MS/MS found each location with their site ID and province.

Site ID	Province	Site Location	Location ID	15ADON	3ADON	DON
NBFMB012	NB	NBFMB	NB-1	0.49	-	-
NBFMB013	NB	NBFMB	NB-1	0.74	-	0.37
NBFMB014	NB	NBFMB	NB-1	0.49	-	-
NBWJ0111	NB	NBWJ	NB-2	-	-	0.83
NBWJ0112	NB	NBWJ	NB-2	-	-	-
NBWJ0113	NB	NBWJ	NB-2	-	-	-
NBWJ0114	NB	NBWJ	NB-2	-	-	-
NBWJB101	NB	NBWJB	NB-3	0.48	-	-
NBWJB102	NB	NBWJB	NB-3	0.43	-	-
NBWJB103	NB	NBWJB	NB-3	0.65	-	0.31
NSGAB101A	NS	NSGAB	NS-1	-	-	1.5
NSGAB101B	NS	NSGAB	NS-1	0.51	0.18	3.7
NSGAB102A	NS	NSGAB	NS-1	0.46	-	0.4
NSGAB102B	NS	NSGAB	NS-1	-	0.16	2.5
NSGAB103A	NS	NSGAB	NS-1	0.62	0.22	6.1
NSGAR103B	NS	NSGAR	NS-2	0.57	-	1.4
NSTFB101A	NS	NSTFB	NS-3	0.61	0.34	14.5
NSTFB101B	NS	NSTFB	NS-3	0.46	0.21	3.8
NSTFB102A	NS	NSTFB	NS-3	0.61	0.42	14.6
NSTFB102B	NS	NSTFB	NS-3	0.45	0.16	1.5
NSTFB103A	NS	NSTFB	NS-3	0.45	0.3	9.9
NSTFB103B	NS	NSTFB	NS-3	0.51	0.16	3
PEJRB101	PE	PEJRB	PE-1	0.43	-	0.57
PEJRB102	PE	PEJRB	PE-1	0.4	-	-
PEJRB103	PE	PEJRB	PE-1	0.53	-	0.31
PEPBB101	PE	PEPBB	PE-2	0.48	-	0.31
PEPBB102	PE	PEPBB	PE-2	0.6	0.15	1
PEPBB103	PE	PEPBB	PE-2	0.4	-	0.68
PETCB101	PE	PETCB	PE-3	0.49	0.16	0.95
PETCB102	PE	PETCB	PE-3	0.4	-	0.56
PETCB103	PE	PETCB	PE-3	0.4	0.16	3.2