Final Report

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An on-farm approach to evaluate the interaction of management and environment on *Fusarium* Head Blight development in wheat

Compiled by:

Christiane Catellier, M.Sc., P.Ag. – Research Associate

Indian Head Agricultural Research Foundation

Collaborators:

Jessica Enns & Kayla Slind, Western Applied Research Corporation

Brianne McInnes & Jessica Slowski, Northeast Agriculture Research Foundation

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ABSTRACT

Fusarium head blight (FHB) has become a substantial management concern for wheat growers in Saskatchewan, affecting both yield and quality of the crop. An integrated approach to *Fusarium* Head Blight (FHB) management is recommended, including the use of resistant varieties, a timely fungicide application, and crop rotation. FHB forecasting tools have been developed to predict the risk of disease development based on environmental conditions. In commercial production, the effectiveness of FHB risk management practices undoubtedly varies with environmental conditions. Improved disease management can potentially be attained with a better understanding of the interactive effects of management and environment on FHB development in wheat. An observational study was conducted on commercial farms, in collaboration with local producers near Indian Head, Melfort, and Scott, Saskatchewan, from 2018-2020. Environmental and agronomic data were collected from several sample sites in several fields throughout the growing season, and management data was provided by producers. Individual effects of management and environmental variables on FHB symptoms in the field (FHB index), *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) level were examined. Then, a multivariate analysis using a multiple regression and competing models approach was conducted to assess the interactive effects of management and environmental variables on FDK. Results showed that the choice of variety and fungicide product were highly influential on FHB development, but that the timeliness of fungicide application was less important. Environmental variables affecting the development of FDK in the crop during pre-anthesis stages were mainly related to temperature, while influential variables during post-anthesis stages were related to moisture. More importantly, results showed that environmental variables were mainly interactive with management practices, and the effects were not additive. As FHB risk management practices are commonly applied in commercial wheat fields, these findings confirm that in order to advance our ability to forecast the risk of FHB infection using predictive models, it will be necessary to more thoroughly evaluate the interactive effects of management and environment. Based on the results of this study, it would be most insightful to compare genetic FHB resistance or effectiveness of different fungicide strategies (products and timing) as a function of various environmental conditions. The study was also useful in demonstrating the potential of on-farm observational studies in agronomic research.

INTRODUCTION

Fusarium head blight (FHB) has become a substantial management concern for wheat growers in Saskatchewan due to changing weather patterns, more intensive farming practices, and the movement of infected seed. FHB infection results in a reduction in yield, but of primary concern is the reduction in grain quality due to the presence of *Fusarium* damaged kernels (FDK) and mycotoxins such as deoxynivalenol (DON), and the resulting reduction in marketing opportunities (Fernando et al. 2021, Gilbert & Tekauz 2000).

Agronomic research to date has contributed significantly to our understanding of the role of important management variables in the development of *Fusarium* head blight and production of mycotoxins. An integrated approach is recommended for FHB management in wheat as disease development is a function of many independently triggered mechanisms, thus no single management practice is individually effective in controlling the disease (Fernando et al. 2021, McMullen et al. 2008). The most important management practices in managing FHB have been: 1) the selection of resistant wheat classes and varieties, 2) a diverse crop rotation, and 3) a timely fungicide application in conjunction with FHB forecasting tools (Fernando et al. 2021, Ye et al. 2017, Gilbert & Haber 2013).

It is important that producers incorporate these management practices to reduce FHB infection and disease incidence and severity in their crops; however, FHB development is also highly dependent on environmental conditions (Fernando et al. 2021, Kriss et al. 2010, Gilbert & Fernando 2004). Additional cultural practices can be used as part of an integrated disease management approach and may minimize the influence of environmental factors (Gilbert & Tekauz 2011, Gilbert & Tekauz 2000). These would include practices that: 1) favour uniform crop development that will lessen the period of susceptibility to infection and aid in making fungicide application more effective, 2) create a canopy configuration and density that is conducive to air movement, and 3) result in vigorous seedlings, such as using seed with low level of infection and using seed treatment.

Still, the effectiveness of management practices applied in commercial fields is not guaranteed or consistent across regions, soil zones, or even farms, where environmental conditions are highly variable. Management recommendations are based on agronomic experiments which are fundamentally designed to isolate the effect of specific treatments through controlled manipulations that factor out the variability resulting from environmental conditions. The interacting effects of environmental variables are rarely explicitly measured or quantified. Yet, in commercial production, the effectiveness of mitigative management practices undoubtedly varies with ambient conditions affecting disease development in the crop. Therefore, better disease management can potentially be attained with a better understanding of how environmental conditions impact the effectiveness of management decisions, and how management decisions can in turn affect the microclimate within the crop at important developmental stages.

In order to advance our understanding of FHB management in wheat production, it will be insightful to examine these inter-correlated factors simultaneously rather than independently, utilizing a multivariate perspective. Thus, the objective of this study is to examine the additive and interactive effects of multiple management and environmental variables on FHB development in wheat under commercial production. This study will fill the gap between agronomic research results and on-farm observations by simultaneously examining the many interacting factors affecting FHB development in wheat, and by explicitly measuring and incorporating environmental data in a multivariate analysis. Fortunately, factors that are influential on FHB development in wheat have been identified through past agronomic research as well as growers' and agronomists' knowledge and experience, and so effort can be focused on these particular variables.

This study utilizes an innovative observational design with data collected directly from producers' fields. By adopting a systems approach to agronomic research, we will attempt to provide a novel perspective of how we can build on the extensive knowledge of individual relationships and advance our understanding of the intercorrelation of relationships within the agricultural system.

METHODOLOGY

Study design

The study design consisted of an observational approach with a multivariate and hierarchical data structure. The study was conducted on commercial farms, in collaboration with local producers in three locations, Indian Head, Melfort, and Scott, Saskatchewan, for three growing seasons, from 2018-2020.

Producers were contacted ahead of seeding in the spring to identify fields which would be planted to wheat. There were no treatments or experimental manipulation; producers managed their fields as usual. The fields chosen for the study were approximately 160 acres in area but could be part of larger management units. The geographical coordinates of 3 or 4 representative sample sites in each field was marked for repeated sampling. The sample sites were located along roads for quick access but at sufficient distance to avoid headlands, and were isolated from each other as much as possible within a field. The replicates were arranged hierarchically, in that sample sites were nested within fields, fields were nested within operations, and the same operations could potentially be included over the 3 years of the study. As each operation had multiple fields of wheat that were seeded successively in the spring, this provided a range of different environmental conditions for each replicate throughout the growth stages of the crop. The number of replicates at each level over the 3 years of the study is summarized in Table 1. Over the 3-year duration of the study, data was collected at 314 sample sites, in 91 fields from 12 different operations in the three locations.

Table 1. Replication at the sample site, field, and operation level in each growing season over the duration of the study.

Year	Location	Producers	Fields	Sample Sites
2018	Indian Head	4	14	52
	Melfort	3	9	27
	Scott	3	9	35
	Total	*10*	*32*	*114*
2019	Indian Head	4	12	41
	Melfort	3	9	30
	Scott	3	9	34
	Total	*10*	*30*	*105*
2020	Indian Head	3	12	39
	Melfort	3	9	32
	Scott	3	8	24
	Total	*9*	*29*	*95*
	Total	12*	91	314

*7 producers x 3 years, 3 producers x 2 years, and 2 producers x 1 year each

Data collection

The geographical location of each sample site was marked so all samples and measurements were taken within an approximately 5-m radius area. The following data were collected at each sample site:

i. Spring soil quality: Soil samples were collected prior to seeding and/or spring fertilizer application, and analyzed for macronutrients (N, P, K, S), micronutrients, organic matter, pH, and cation exchange capacity (CEC). Soil samples from 2020 had not yet been analyzed at the time of data analysis.

ii. Surface residue, before and after seeding: Digital photographs were taken and percent ground cover was assessed digitally using SamplePoint image analysis software (Booth et al. 2006).

iii. Weekly monitoring data: The following measurements were taken at each sample site approximately weekly from the seeding date until crop maturity.
   a. Soil moisture and temperature: several point measurements of soil temperature and of soil volumetric water content were taken using a WaterScout SM 100 sensor attached to a hand-held reader. Measurements were taken before 10:00 am for the large majority of samples, and the time was recorded to be able to control for the effect of sample time on soil temperature.
   b. Precipitation: rain gauges were placed within a 1-mile radius of each sample site. Precipitation was recorded and the gauges were emptied at the time of weekly sampling.
   c. Zadoks growth stage: Starting at approximately 3 weeks after the seeding date, the average growth stage of the crop in the area near the sample site was recorded, as well as the minimum and maximum growth stages observed in the area. Average growth stage was determined using the three values. Standard deviation of the 3 values was calculated to estimate the variability in growth stage ("staginess") of the crop.

iv. Plant density: The number of seedlings in two 1-m sections of crop row was counted at approximately 3 weeks after the seeding date. Plant density per area was determined using producer-reported row spacing.

v. Tiller density: The number of heads in four 0.5-m sections of crop row was counted at the late milk to early dough stage (same time as FHB ratings). Tiller density per area was determined using producer-reported row spacing.

vi. *Fusarium* Head Blight (FHB) ratings: A visual assessment of FHB symptoms was done at the late milk to early dough stage[1].
   a. In 2018, a total of 50 heads (10 heads in 5 different crop rows) were inspected to determine percent incidence of FHB (the proportion of heads showing symptoms of disease), and average severity of infection (the average percent area of heads affected by disease symptoms, of infected plants only). Percent incidence and average percent severity was used to determine the FHB index (percent incidence multiplied by percent severity).

[1] After the first year of data collection, it was determined that the number of wheat heads being examined in the field was insufficient and did not provide a representative or adequate sample size, leading to disproportionate estimates of the incidence of FHB symptoms. Thus, in the second year of the study, the method utilized for visually assessing FHB symptoms in the field was modified.

b. In 2019 and 2020, the number of heads showing symptoms of FHB was counted in ten-0.5 m sections of crop row, and the average percent area of the heads affected by the disease was recorded (infected heads only). Tiller density was used to determine the percent incidence. Percent incidence and average percent severity was used to determine the FHB index.

vii. Grain yield: All wheat heads from four representative 0.5 m sections of crop row were manually harvested, dried, threshed, and weighed. Grain yield in kilograms per hectare was calculated using producer-reported row spacing. This was an optional measurement and was only completed in Indian Head in all three growing seasons.

Producers were asked to provide a harvested grain sample from each field to be submitted for laboratory analysis of *Fusarium* damaged kernels (FDK) and percent deoxynivalenol (DON). The following management data was also requested from producers for each field:

i. Seed quality information (percent *Fusarium* spp., percent *Fusarium graminareum*, thousand kernel weight (TKW), percent germination) – if this information was not available, producers were asked to provide a sample of the seed to send in for quality analysis;
ii. Crop rotation (crop type and variety (if wheat) in the previous 3 years);
iii. Seeding date, seeding rate, variety (cultivar), and seed treatment;
iv. Applied fertilizer rate, form, placement and timing;
v. Row spacing, seeding speed, seeding implement type;
vi. Fungicide application date, product, rate, and nozzle type and size, water volume, and sprayer speed or pressure;
vii. Fungicide rotation (fungicide products used in previous 3 years);
viii. Other crop protection product application dates, rates, and products;
ix. Swathing, desiccation, or crop termination date, harvest date and yield.

Management data was not always reported completely or in detail, resulting in missing values or incomplete data for certain variables. In addition, some variables of interest did not have enough replication of certain groups or levels to be included in the analysis. In particular, the wheat class, and the rate of applied micronutrients such as copper did not have replication within groups to be included in the analysis.

Soil characteristics were obtained for each sample site using the Saskatchewan Soil Information System (SKSIS Working Group 2018).

Regional weather data was retrieved from Environment and Climate Change Canada [ECCC]'s online database (ECCC 2020), using the nearest weather stations with complete data over the three growing seasons (Indian Head, Melfort, and Scott). Daily mean temperature and daily precipitation were compiled, and daily growing degree days (GDD) was calculated from the daily mean air temperature, using 5°C as the base temperature. Hourly relative humidity and wind speed were averaged to obtain daily values.

Data management: variable definition and justification

Weekly measurements (soil temperature, soil moisture, precipitation from rain gauges, and minimum, average, and maximum growth stage) were interpolated to obtain daily estimates. Data from all sources were associated by site, year, location (sample site or field), and/or date using relational database software.

A large number of variables were defined and calculated to assess their individual and combined effects on the response variables (FHB index, FDK, and DON). The definition and attributes of all explanatory variables which were included in the analysis are provided in

Table 2Table 3,

Table 4. A description and justification for the inclusion of each explanatory variable follows.

Cropping information reported by producers was utilized to calculate the frequency of wheat and the frequency of cereal (wheat, barley, oats, canaryseed) crops in 4 year rotations, and the number of years since the last wheat and since the last cereal crop. Sporulation of the fungus occurs on infected residue persisting from previous crops, so increased frequency of wheat or other host crops, and recent production of wheat or host crops can contribute to an increased inoculum load (Osborne & Stein 2007, Schaafsma et al. 2005, Dill-Macky & Jones 2000). The amount of residue pre- and post-seeding were also included as variables affecting inoculum potential (Dill-Macky & Jones 2000).

Wheat varieties, or more appropriately cultivars, differ in their level of resistance to FHB infection, and cultivar choice has consistently been shown to be a major factor for FHB management in wheat (Fernando et al. 2021, Ye et al. 2017). Cultivar resistance to FHB is assessed as part of the variety registration process, and cultivars are classified on a five-point scale of susceptible to resistant. The effect of genetic resistance was assessed both at the level of individual cultivars and grouped by resistance rating (moderately susceptible (MS), intermediate (I), or moderately resistant (MR)). Resistance rating was also assessed as a nominal value, representing increasing level of resistance (MS=2, I=3, MR=4).

Good seed quality contributes to quick and even emergence, and subsequently, a uniformly developing crop and vigorous plants that are less vulnerable to pests and adverse conditions (Gilbert & Tekauz 2000). A uniformly developing crop may also contribute to greater fungicide effectiveness as a function of the timing of application in relation to the growth stage of individual plants. Seed source (certified or farm-saved), seed size, contamination with *Fusarium graminareum*, seed treatment, seeding rate, seeding depth, applied fertilizer rates, residual soil nutrients and salts, and other measures of soil texture and quality were all included as variables that could affect crop vigour and uniformity of crop development.

The date of anthesis (Zadoks 65) was determined for each sample site individually. Wheat is susceptible to infection from anthesis up to the soft dough stage but is most susceptible at anthesis (Fernando et al. 2021, Kriss et al. 2010). The probability of higher inoculum load and crop exposure at susceptible stages increase as the growing season progresses (Gorczyca et al. 2018). Therefore, we would expect FHB infection to increase with later seeding dates, and later anthesis dates. This effect is likely dependent on cultivar and growing season conditions, thus we would expect to see interactions between seeding date or anthesis date and other variables. The standard deviation of growth stage was also calculated for each sample site using the minimum, average, and maximum growth stage at anthesis. Staginess of the crop at anthesis could contribute to a reduced fungicide efficacy.

Application of foliar fungicide at or near anthesis has been shown to be an effective FHB management strategy (Bolanos-Carriel et al. 2020, Paul et al. 2018, Paul et al. 2008). Fungicide application at anthesis has been shown to be the most effective for control of FHB, however, post-anthesis fungicide applications have been shown to be equally effective (Bolanos-Carriel et al. 2020, Paul et al. 2018). The timing of fungicide application was calculated in relation to the date of anthesis at each sample site individually. Different fungicide active ingredients have been shown to have varying effectiveness against FHB infection (Bolanos-Carriel et al. 202, Paul et al. 2008), thus the effect of fungicide product, active ingredient(s), and mode of action group were each included separately. Fungicide water volume and application speed were included as they have implications for fungicide coverage. However, these effects would also be a function of nozzle type, size, and pressure, which were not included as they were not sufficiently reported. Reported fungicide rotations were utilized to calculate the frequency of the same fungicide active ingredient and the frequency of the same fungicide mode of action applied to wheat or applied to any cereal crop in four years. Repeated use of the same active ingredient or mode of action could lead to fungicide resistance and reduced efficacy (Betcher et al. 2010).

Plant density, tiller density, and row spacing are measures of crop structure and spatial arrangement that could influence the canopy microclimate for spore production, dispersion, and infection severity (Jensen & Jorgensen 2016). Soil texture and other measures of soil quality can also affect microclimate within the crop.

Daily values of environmental variables (soil temperature, soil moisture, precipitation from rain gauges, mean air temperature, GDD, precipitation, average relative humidity, and average wind speed) were averaged or totalled over specific pre- and post-anthesis intervals (3 days, 7 days, 14 days, and 30 days) for each sample site individually. Rain gauge measurement, precipitation, and GDD were also totalled for the entire season prior to anthesis. Environmental conditions experienced during varying lengths of time around the time of anthesis have been shown to be positively correlated with FHB intensity (Kriss et al. 2010, De Wolf et al. 2003, Hooker et al. 2002). Conditions prior to anthesis affect disease risk as a function of spore production and dispersal (Gilbert et al. 2008, Paul et al. 2007), while conditions experienced after anthesis affect disease risk as a function of fungal infection of wheat spikes and production of mycotoxins (Cowger et al. 2009).

Table 2. Definition and attributes of management variables included in multivariate analysis.

Variable name	Variable type	Definition	Replication
wheatRotation4years	Ordinal	Frequency of wheat in 4-year rotation; two values (1, 2)	Field
yearsSinceWheat	Ordinal	Number of years since last wheat crop; three values (2, 3, 4)	Field
cerealRotation4years	Ordinal	Frequency of cereal crop (wheat, barley, oats, canaryseed) in 4-year rotation; three values (1, 2, 3)	Field
yearsSinceCerealCrop	Ordinal	Number of years since last cereal crop; four values (1, 2, 3, 4)	Field
wheatVariety	Factor	Wheat cultivar; 13 values	Field
FHBresistance	Factor	Varietal FHB resistance rating; three values (MS, I, MR)	Field
FHBresistanceScale	Ordinal	Varietal FHB resistance rating; three values (MS=2, I=3, MR=4)	Field
seedSource	Factor	Seed source; three values (Certified, FarmSaved, Unknown)	Field
seedTKW	Continuous	Thousand kernel weight of seed. Producer- or lab-reported in g per 1000 seeds; range 32 – 53 g 1000 seeds^{-1}	Field
seedTrt	Factor	Seed treatment product brand name; 9 values	Field
percFusGram	Continuous	Percent *Fusarium graminarium* on seed; range 0 – 3%	Field
seedDateJulian	Continuous	Seeding date (julian calendar); range 112 (22 April) – 152 (1 June)	Field
seedRateLbsAc	Continuous	Producer-reported seeding rate; range 105 – 168 lbs ac^{-1}	Field
seedFt2	Continuous	Calculated seeding density, using producer-reported seeding rate and seed TKW; range 24.5 – 49.7 seeds ft^{-2}	Field
rowSpacingInch	Continuous	Producer-reported row spacing; range 9.8 – 12 in	Producer
seedDepth	Continuous	Producer-reported seeding depth; range 0.88 – 1.75 in	Field
fungProduct	Factor	Fungicide product brand/formulation; 9 values	Field
fungActive	Factor	Fungicide active ingredient; 7 values	Field
fungGroup	Factor	Fungicide mode of action group; three values (3, 3&7, 7&11)	Field
fungDaysFromAnthesis	Continuous	Fungicide application date relative to anthesis date; range (-14) – (+8) days	Sample Site
fungWaterVolGalAc	Continuous	Fungicide application water volume; range 7.5 – 18.3 gal ac^{-1}	Field
fungSpeedMph	Continuous	Fungicide application speed; range 10 – 18 mph	Field
sameFungActiveWheat4Years	Ordinal	Frequency of the same fungicide active ingredient applied to wheat in 4 years; two values (0, 1)	Field
sameFungGroupWheat4Years	Ordinal	Frequency of the same fungicide group applied to wheat in 4 years; two values (0, 1)	Field
sameFungActiveCereal4Years	Ordinal	Frequency of the same fungicide active ingredient applied to cereals in 4 years; two values (0, 1)	Field
sameFungGroupCereal4Years	Ordinal	Frequency of the same fungicide group applied to cereals in 4 years; two values (0, 1)	Field
NRateTotalLbsAc	Continuous	Producer-reported, total rate of applied N fertilizer; range 94 – 190 lbs ac^{-1}	Field
PRateTotalLbsAc	Continuous	Producer-reported, total rate of applied P fertilizer; range 20 – 70 lbs ac^{-1}	Field
KRateTotalLbsAc	Continuous	Producer-reported, total rate of applied K fertilizer; range 0 – 32 lbs ac^{-1}	Field
SRateTotalLbsAc	Continuous	Producer-reported, total rate of applied S fertilizer; range 0 – 15 lbs ac^{-1}	Field

Table 3. Definition and attributes of agronomic variables included in multivariate analysis.

Variable name	Variable type	Definition	Replication
percLitterPreSeed	Continuous	Pre-seed residue cover (percent of ground cover that is litter (not bare soil)); range 7 – 100%	Sample Site
percLitterPostSeed	Continuous	Post-seed residue cover (percent of ground cover than is litter (not bare soil)); range 0 – 92%	Sample Site
plantsM2	Continuous	Plant density; range 131 – 463 plants m^{-2}	Sample Site
tillersM2	Continuous	Tiller (head) density; range 177 – 894 heads m^{-2}	Sample Site
anthesisJulian	Continuous	Anthesis date (julian calendar date when average growth stage = Zadoks 65); range 182 (1 July) – 213 (1 August)	Sample Site
zadoksSD	Continuous	Standard deviation of growth stage at anthesis, indicates variability in crop stage; range 0.31-11.44	Sample Site
soilQuality	Factor	Soil quality; six values (Calcareous, Gleyed, Orthic, Rego, Solodized, Solonetzic)	Sample Site
soilColour	Factor	Soil colour; four values (Black, DarkBrown, DarkGray, Gray)	Sample Site
soilType	Factor	Soil type; three values (Chernozem, Luvisol, Solonetz)	Sample Site
surfaceTexture	Factor	Soil surface texture; 10 values (e.g. Clay, Loam, Silty Clay, etc.)	Sample Site
soilTexture	Factor	Soil texture; 8 values (e.g. Fine, Moderately Fine, Medium, etc.)	Sample Site
soilTextureGrade	Ordinal	Soil texture grade; 9 values from 1 (Fine) – 9 (Coarse)	Sample Site
agCapabilityGrade	Continuous	Agricultural capability grade; range 1.0-4.4	Sample Site
pH06	Continuous	Soil pH at 0-6 in depth; range 5.0 – 8.3	Sample Site
pH612	Continuous	Soil pH at 6-12 in depth; range 5.5 – 8.5	Sample Site
Omperc	Continuous	Percent soil organic matter; range 0.6 – 9.3%	Sample Site
nitratePPM06	Continuous	Soil nitrate at 0-6 in depth; range 2.5 – 110 ppm	Sample Site
nitratePPM612	Continuous	Soil nitrate at 6-12 in depth; range 1.5 – 100 ppm	Sample Site
POlsenPPM	Continuous	Soil phosphorus content; range 2 – 79 ppm	Sample Site
KPPM	Continuous	Soil potassium content; range 57 – 867 ppm	Sample Site
CaPPM	Continuous	Soil calcium content; range 644 – 8315 ppm	Sample Site
MgPPM	Continuous	Soil magnesium content; range 70 – 1375 ppm	Sample Site
NaPPM	Continuous	Soil sodium content; range 11 – 189 ppm	Sample Site
SPPM06	Continuous	Soil sulfur content at 0-6 in depth; range 4 – 75 ppm	Sample Site
SPPM612	Continuous	Soil sulfur content at 6-12 in depth; range 4 – 75 ppm	Sample Site
CECMeq100g	Continuous	Soil cation exchange capacity; range 4.4 – 50.3 mEq 100 g^{-1}	Sample Site

Table 4. Definition and attributes of environmental variables included in multivariate analysis.

Variable name	Variable type	Definition	Replication
avgSoilMois	Continuous	Volumetric soil moisture, averaged over the specified pre- or post-anthesis time interval; range 30 days (pre): 4.1 – 49.9%; 14 days (pre): 3.1 – 51.6%; 7 days (pre): 1.4 – 55.5%; 3 days (pre): 1.0 – 57.2%; 3 days (post): 1.1 – 57.4%; 7 days (post): 1.2 – 55.5%; 14 days (post): 2.4 – 52.2%; 30 days (post): 2.9 – 45.7%	Sample Site
cumRain	Continuous	Total seasonal rain accumulation at anthesis, based on rain gauge measurement; range 32.3 – 170.5 mm	Sample Site
totalRain	Continuous	Rain accumulation based on rain gauge measurement, totalled over the specified pre- or post-anthesis interval; range 30 days (pre): 11.4 – 114.1 mm; 14 days (pre): 4.6 – 78.1 mm; 7 days (pre): 1.2 – 40.1 mm; 3 days (pre): 0 – 17.2 mm; 3 days (post): 0 – 14.3 mm; 7 days (post): 0 – 29.6 mm; 14 days (post): 0.5 – 49.1 mm; 30 days (post): 0.5 – 91.0 mm	Sample Site
cumPrecipMm	Continuous	Total growing season precipitation at anthesis, based on regional weather data; range 116.5 – 311.4 mm	Sample Site
totalPrecip	Continuous	Precipitation, based on regional weather data, totalled over the specified pre- and post-anthesis intervals; range 30 days (pre): 29.7 – 14 mm; 14 days (pre): 3.4 – 89.3 mm; 7 days (pre): 0.0 – 58.3 mm; 3 days (pre): 0.0 – 47.6 mm; 3 days (post): 0.0 – 47.6 mm; 7 days (post): 0.0 – 58.5 mm; 14 days (post): 1.1 – 90.4 mm; 30 days (post): 7.6 – 96.4 mm	Sample Site
avgSoilTemp	Continuous	Soil temperature, averaged over the specified pre- and post-anthesis intervals; range 30 days (pre): 13.3 – 22.4°C; 14 days (pre): 13.7 – 21.9°C; 7 days (pre): 14.0 – 20.6°C; 3 days (pre): 13.6 – 19.3°C; 3 days (post): 12.1 – 20.1°C; 7 days (post): 12.0 – 19.6°C; 14 days (post): 13.5 – 20.0°C; 30 days (post): 13.5 – 19.4°C	Sample Site
avgMeanT	Continuous	Mean air temperature, based on regional weather data, averaged over the specified pre- and post-anthesis intervals; range 30 days (pre): 15.0 – 18.7°C; 14 days (pre): 15.9 – 20.0°C; 7 days (pre): 15.3 – 20.8°C; 3 days (pre): 14.5 – 22.8°C; 3 days (post): 14.5 – 22.8°C; 7 days (post): 16.1 – 20.8°C; 14 days (post): 16.7 – 20.5°C; 30 days (post): 15.7 – 19.6°C	Sample Site
cumGDD	Continuous	Total seasonal growing degree days accumulation at anthesis; range 582 – 899	Sample Site
totalGDD	Continuous	Growing degree days accumulation, totalled over the specified pre- and post-anthesis intervals; range 30 days (pre): 301 – 412; 14 days (pre): 152 – 210; 7 days (pre): 72 – 111; 3 days (pre): 29 – 53; 3 days (post): 29 – 53; 7 days (post): 78 – 111; 14 days (post): 164 – 218; 30 days (post): 321 – 438	Sample Site
avgRH	Continuous	Relative humidity, averaged over the specified pre- and post-anthesis intervals; range 30 days (pre): 61 – 76%; 14 days (pre): 65 – 78%; 7 days (pre): 66 – 81%; 3 days (pre): 61 – 85%; 3 days (post): 61 – 84%; 7 days (post): 64 – 81%; 14 days (post): 64 – 79%; 30 days (post): 63 – 76%	Sample Site
avgWindSpd	Continuous	Wind speed, averaged over the specified pre- and post-anthesis intervals; range 30 days (pre): 11 – 19 km hr^{-1}; 14 days (pre): 10 – 17 km hr^{-1}; 7 days (pre): 8 – 18 km hr^{-1}; 3 days (pre): 8 – 21 km hr^{-1}; 3 days (post): 8 – 22 km hr^{-1}; 7 days (post): 9 – 19 km hr^{-1}; 14 days (post): 10 – 17 km hr^{-1}; 30 days (post): 11 – 16 km hr^{-1}	Sample Site

Statistical analysis

Multiple regression with mixed-effect models was used to assess the effect of various explanatory variables on the three response variables: FHB index, FDK, and DON. Mixed-effects models appropriately deal with non-homogeneity of variance resulting from the unbalanced and nested data structure. FHB index and FDK were log-transformed to deal with non-normal distribution of model residuals resulting from left-skewed data. A constant was added to all values prior to transforming to deal with values of zero. DON was transformed to a binary response (0 = none detected, 1 = any level of DON detected), to deal with the issue of zero-inflated data.

Data were analyzed with the R statistical program, version 4.0.4 (R Core Team 2021), using the *lme4* package (Bates et al. 2015) for fitting mixed-effects models, and the *lmerTest* package (Kuznetsova et al. 2017) for assessing model fit and effect significance. FHB index was replicated at the sample site level, so the nested random effects were specified as location, year within location, producer within year within location, and field within producer within year within location. For response variables replicated at the field level (FDK, DON), the nested random effects were specified as location, year within location, and producer within year within location. Time of sampling was also included as a random effect in the models for soil temperature. Only linear effects were included in the regression models.

First, single-variable models were fitted to examine the individual effect of each explanatory variable on FHB Index, FDK, and DON. For each of the response variables, separate mixed-effect models were fitted with each explanatory variable as a single fixed effect. Then, based on the results of the univariate models, FDK was chosen for the multivariate analysis. For the multivariate analysis, a competing models approach was chosen to assess additive and interactive effects with combinations of two explanatory variables. This approach allows us to evaluate the relative influence of each variable and each combination of variables by comparing the goodness of fit of a set of candidate models. The competing models approach was chosen because of the relatively low level of replication in relation to the large number of explanatory variables, and the high level of inter-correlation among the explanatory variables.

RESULTS AND DISCUSSION

Fusarium occurrence and distribution

Measurements of FHB index, FDK, and DON were variable between locations and years. FHB Index was similar among locations in 2018 and 2019, but differed among locations in 2020, where FHB was higher in Melfort and Scott but remained low in Indian Head (Figure 1). FDK showed greater variability, with no observable pattern within either year or location (Figure 2). DON values appeared to be highest in 2018 and lowest in 2019 in all locations (Figure 3). FHB index and DON appeared to have similar patterns across years and locations, indicating that the two may be correlated or influenced by the same variables. FDK did not appear to be correlated with either FHB index or DON. Environmental conditions appeared to have had a larger influence on FHB index and DON than FDK.

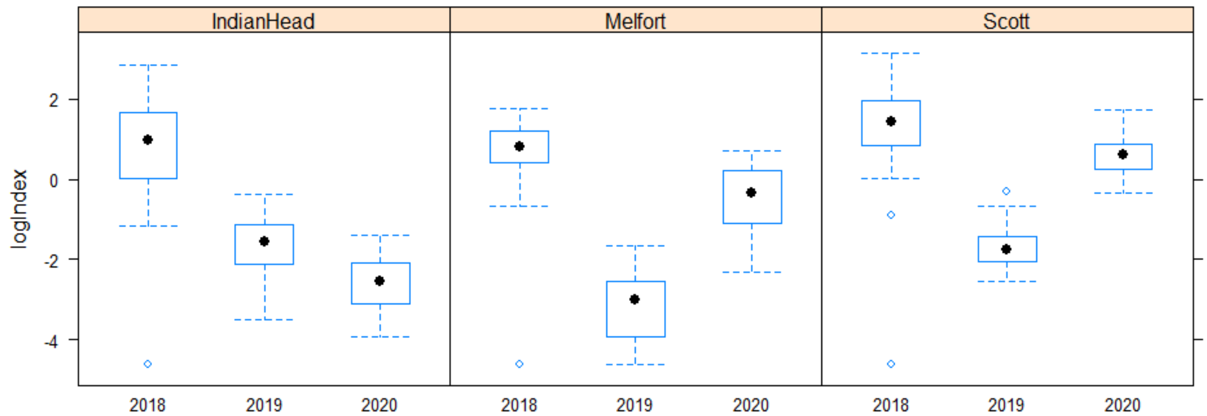

Figure 1. The distribution of *Fusarium* Head Blight (FHB) Index (log-transformed values) within years and locations. Upper and lower limits of the boxes indicate the first and third quartiles, and whiskers indicate the range of values outside the quartiles, with extreme values shown as single points. The centre point indicates the mean.

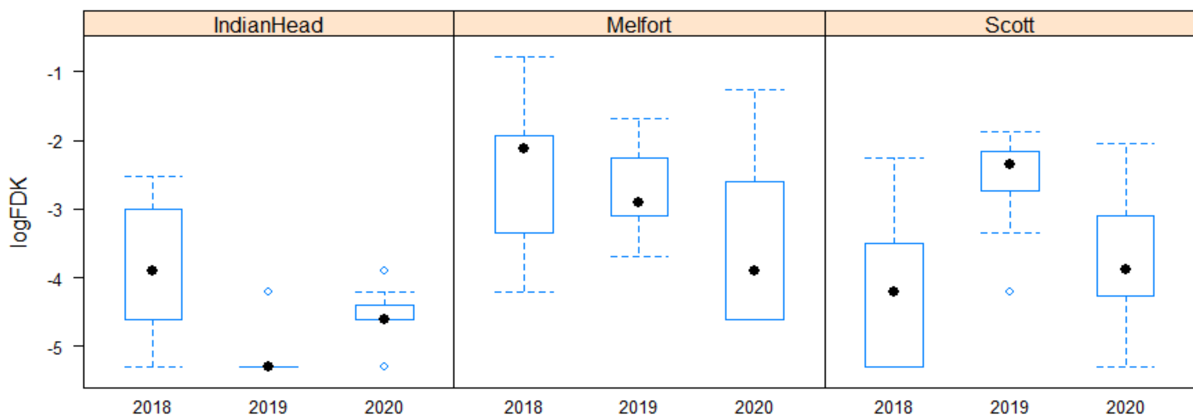

Figure 2. The distribution of *Fusarium* damaged kernels (FDK, log-transformed values) within years and locations. Upper and lower limits of the boxes indicate the first and third quartiles, and whiskers indicate the range of values outside the quartiles, with extreme values shown as single points. The centre point indicates the mean.

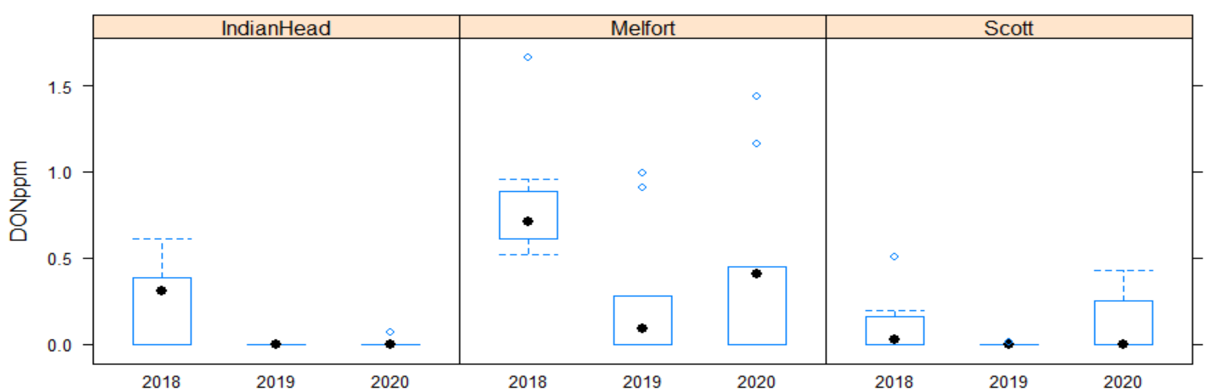

Figure 3. The distribution of deoxynivalenol (DON) level in parts per million (ppm) within years and locations. Upper and lower limits of the boxes indicate the first and third quartiles, and whiskers indicate the range of values outside the quartiles, with extreme values shown as single points. The centre point indicates the mean.

Single-variable models

Significance tests for the individual effect of each explanatory variable on the three response variables are shown in

Table 5, Table 6Table 7. The three response variables did not have similar or consistent responses to many of the same agronomic and environmental variables, further suggesting that the response variables were not correlated, and that assessment of FHB infection using all three variables was not redundant. Correlations among FHB index, FDK, and DON have been shown to be dependent on genotype and environment (Goral et al. 2019, Del Ponte et al. 2007, Paul et al. 2006, Mesterházy et al. 2005). FHB index had fewer significant relationships than either FDK or DON, reflecting the greater subjectivity of in-field assessments, especially in consideration of the smaller scale of replication, at the sample site level.

In an observational study with a multivariate data set, results of univariate models should be interpreted with caution as they could be misleading. In this case, the inclusion of random effects as part of the mixed models should control for a large portion of confounded or unmodeled effects. Unmodeled effects become increasingly unimportant as more variables are added to the same model in a multiple regression. Further, as per the objectives of this study, we expect that significant interactions will be revealed as additional variables are included in the models, which could affect the significance of individual variables or direction of the relationships. Therefore, significant univariate relationships will be highlighted briefly, but interpretation of results will be focused on the multiple regression analysis which follows.

Overall, visual symptoms of FHB in the field were positively associated with 1) row spacing, 2) pre-anthesis regional precipitation (total cumulative) and wind speed (7 and 14 days), and 3) post-anthesis air temperature (3, 7, and 30 days) and GDD (3, 7, and 30 days). FHB index was negatively associated with 1) the number of years since the last wheat crop, 2) residual soil nitrate, potassium (K), and sodium (Na), 3) pre-anthesis wind speed (30 days), and 4) post-anthesis relative humidity (14 days) and wind speed (30 days).

The level of *Fusarium*-damaged kernels (FDK) differed significantly between wheat varieties, fungicide product, fungicide active ingredients, fungicide mode of action groups, soil types, and soil textures. FDK was positively associated with 1) number of cereal crops in 4-year rotation, 2) seed contamination with *Fusarium graminarium*, 3) seeding density, 4) coarseness of soil texture, 5) residual magnesium (Mg), 6) pre-anthesis rain gauge precipitation (3, 7, 14, 30 days, and total cumulative), regional precipitation (7 and 30 days), and relative humidity (14 days), and 7) post-anthesis soil moisture (7 and 14 days), rain gauge precipitation (3, 7, and 14 days), and relative humidity (3, 7, and 14 days). FDK was negatively associated with 1) seed size, 2) seeding date, 3) fungicide application timing (negative values prior to anthesis, thus FDK decreases as application date approaches and passes anthesis), 4) repeated use of the same fungicide group on previous wheat crops, 5) anthesis date, 6) subsoil pH, 7) residual sulfur (S), 8) pre-anthesis soil temperature (3 and 7 days), air temperature (3, 7, 14, and 30 days), and GDD (3, 7, 14, 30 days, and total cumulative), and 9) post-anthesis soil temperature (3 and 7 days), air temperature (3 days), and GDD (3 days).

The level of deoxynivalenol (DON) contamination differed significantly between wheat varieties, FHB resistance groups, soil types, and soil textures. DON was positively associated with 1) number of years since the last wheat crop or cereal crop, 2) seeding density, 3) topsoil pH, 4) topsoil residual nitrate, calcium (Ca), and magnesium (Mg), 5) soil cation exchange capacity, 6) pre-anthesis soil moisture (14 days), rain gauge precipitation (3, 7, 14, and 30 days), air temperature (3 and 7 days), GDD (3 and 7 days), and wind speed (3, 14, and 30 days), and 7) post-anthesis regional precipitation (3, 7, and 14 days) and relative humidity (3, 7, and 14 days). DON was negatively associated with 1) number of wheat crops in 4-year rotation, 2) genotypic resistance, 3) fungicide application speed, 4) repeated use of the same fungicide active ingredient or group on previous wheat or cereal crops, 5) amount of pre-seed residue, 6) anthesis date, 7) residual phosphorus (P), 8) pre-anthesis relative humidity (7, 14, and 30 days), and 9) post-anthesis air temperature (3, 7, and 14 days) and GDD (3, 7 and 14 days).

For DON especially, the direction of the relationships were not all as would be expected, suggesting the possibility of spurious correlations. DON levels were all generally very low; 56% of samples had zero or undetectable levels of DON, and 96% of samples were lower than 1 ppm. The variable was converted to a binary response (0=undetected, 1=detected) for the analysis to deal with the issue of zero-inflation, and so predictability may have been affected. DON was not included in the multivariate analysis.

Table 5. Significance tests for the individual effect of each management variable on the three response variables. Each variable was included as a single fixed effect in separate mixed effects models with random effects as stated in the text. Factor variables result in ANOVA-type models, showing F-test results, while continuous and ordinal variables result in regression type models, showing t-test results. P-values <0.05 are bolded for emphasis, and for regression models, the sign of the regression co-efficient is shown in brackets to indicate the direction of the relationship.

Explanatory variable	FHB index	FDK	DON
wheatRotation4years	0.940	0.150	**<0.001 (-)**
yearsSinceWheat	0.694	0.161	**0.002 (+)**
cerealRotation4years	0.135	**0.015 (+)**	0.567
yearsSinceCerealCrop	**0.031 (-)**	0.719	**0.002 (+)**
wheatVariety	0.971	**<0.001**	**0.003**
FHBresistance	0.337	0.639	**<0.001**
FHBresistanceScale	0.833	0.853	**<0.001 (-)**
seedSource	0.856	0.103	0.360
seedTKW	0.427	**0.001 (-)**	0.778
seedTrt	0.530	0.358	0.108
percFusGram	0.583	**0.035 (+)**	0.112
seedDateJulian	0.952	**<0.001 (-)**	0.479
seedRateLbsAc	0.662	0.150	0.231
seedFt2	0.183	**<0.001 (+)**	**0.038 (+)**
rowSpacingInch	**0.026 (+)**	0.659	0.392
seedDepth	0.559	0.648	0.386
fungProduct	0.513	**0.004**	0.270
fungActive	0.892	**0.001**	0.157
fungGroup	0.773	**0.002**	0.603
fungDaysFromAnthesis	0.095	**0.001 (-)**	0.989
fungWaterVolGalAc	0.237	0.218	0.921
fungSpeedMph	0.862	0.825	**0.011 (-)**
sameFungActiveWheat4Years	0.960	0.755	**0.001 (-)**
sameFungGroupWheat4Years	0.813	**0.031 (-)**	**<0.001 (-)**
sameFungActiveCereal4Years	0.748	0.581	**0.002 (-)**
sameFungGroupCereal4Years	0.737	0.263	**<0.001 (-)**
NRateTotalLbsAc	0.505	0.637	0.718
PrateTotalLbsAc	0.237	0.330	0.911
KrateTotalLbsAc	0.674	0.847	0.857
SrateTotalLbsAc	0.123	0.844	0.426

Table 6. Significance tests for the individual effect of each agronomic variable on the three response variables. Each variable was included as a single fixed effect in separate mixed effects models with random effects as stated in the text. Factor variables result in ANOVA-type models, showing F-test results, while continuous and ordinal variables result in regression type models, showing t-test results. P-values <0.05 are bolded for emphasis, and for regression models, the sign of the regression co-efficient is shown in brackets to indicate the direction of the relationship.

Explanatory variable	FHB index	FDK	DON
percLitterPreSeed	0.088	0.627	**0.019 (-)**
percLitterPostSeed	0.057	0.301	0.134
plantsM2	0.112	0.790	0.195
tillersM2	0.801	0.371	0.650
anthesisJulian	0.167	**0.002 (-)**	**0.009 (-)**
zadoksSD	0.924	0.859	0.547
soilQuality	0.411	**0.003**	0.057
soilColour	0.630	0.485	0.997
soilType	0.804	**0.021**	**0.014**
surfaceTexture	0.859	**<0.001**	**0.005**
soilTexture	0.648	**<0.001**	**0.013**
soilTextureGrade	0.616	**0.024 (+)**	0.105
agCapabilityGrade	0.156	0.470	0.087
pH06	0.544	0.066	**<0.001 (+)**
pH612	0.061	**<0.001 (-)**	0.764
Omperc	0.590	0.304	0.919
nitratePPM06	**0.010 (-)**	0.168	**0.029 (+)**
nitratePPM612	**0.011 (-)**	0.180	0.155
POlsenPPM	0.539	0.912	**0.022 (-)**
KPPM	**0.033 (-)**	0.813	0.817
CaPPM	0.801	0.387	**0.005 (+)**
MgPPM	0.642	**0.013 (+)**	**0.003 (+)**
NaPPM	**0.028 (-)**	0.496	0.259
SPPM06	0.902	0.111	0.988
SPPM612	0.920	**0.024 (-)**	0.513
CECMeq100g	0.987	0.188	**0.002 (+)**

Table 7. Significance tests for the individual effect of each environmental variable on the three response variables. Each variable was included as a single fixed effect in separate mixed effects models with random effects as stated in the text. Factor variables result in ANOVA-type models, showing F-test results, while continuous and ordinal variables result in regression type models, showing t-test results. P-values <0.05 are bolded for emphasis, and for regression models, the sign of the regression co-efficient is shown in brackets to indicate the direction of the relationship.

Explanatory variable	FHB index	FDK	DON
avgSoilMois30daysPre	0.434	0.794	0.054
avgSoilMois14daysPre	0.224	0.261	**0.010 (+)**
avgSoilMois7daysPre	0.569	0.919	0.169
avgSoilMois3daysPre	0.706	0.381	0.188
avgSoilMois3daysPost	0.416	0.111	0.115
avgSoilMois7daysPost	0.457	**0.025 (+)**	0.180
avgSoilMois14daysPost	0.232	**0.033 (+)**	0.152
avgSoilMois30daysPost	0.136	0.297	0.202
cumRain	0.160	**<0.001 (+)**	0.285
totalRain30daysPre	0.503	**<0.001 (+)**	**<0.001 (+)**
totalRain14daysPre	0.565	**<0.001 (+)**	**<0.001 (+)**
totalRain7daysPre	0.758	**<0.001 (+)**	**0.002 (+)**
totalRain3daysPre	0.256	**<0.001 (+)**	**0.001 (+)**
totalRain3daysPost	0.234	**<0.001 (+)**	0.228
totalRain7daysPost	0.453	**<0.001 (+)**	0.905
totalRain14daysPost	0.679	**<0.001 (+)**	0.398
totalRain30daysPost	0.964	0.473	0.106
cumPrecipMm	**0.043 (+)**	0.256	0.864
totalPrecip30daysPre	0.386	**0.013 (+)**	0.134
totalPrecip14daysPre	0.854	0.725	0.884
totalPrecip7daysPre	0.093	**0.044 (+)**	0.462
totalPrecip3daysPre	0.666	0.164	0.251
totalPrecip3daysPost	0.137	0.903	**<0.001 (+)**
totalPrecip7daysPost	0.185	0.732	**0.020 (+)**
totalPrecip14daysPost	0.063	0.653	**0.017 (+)**
totalPrecip30daysPost	0.178	0.515	0.359
avgSoilTemp30daysPre	0.634	0.503	0.752
avgSoilTemp14daysPre	0.981	0.808	0.472
avgSoilTemp7daysPre	0.622	**0.017 (-)**	0.538
avgSoilTemp3daysPre	0.412	**<0.001 (-)**	0.974
avgSoilTemp3daysPost	0.112	**0.006 (-)**	0.675
avgSoilTemp7daysPost	0.085	**0.018 (-)**	0.623
avgSoilTemp14daysPost	0.197	0.065	0.694
avgSoilTemp30daysPost	0.308	0.965	0.689
avgMeanT30daysPre	0.239	**<0.001 (-)**	0.425
avgMeanT14daysPre	0.135	**0.009 (-)**	0.083
avgMeanT7daysPre	0.994	**0.001 (-)**	**0.027 (+)**
avgMeanT3daysPre	0.842	**<0.001 (-)**	**0.026 (+)**
avgMeanT3daysPost	**0.042 (+)**	**0.003 (-)**	**0.001 (-)**
avgMeanT7daysPost	**<0.001 (+)**	0.256	**0.023 (-)**
avgMeanT14daysPost	0.211	0.919	**0.004 (-)**
avgMeanT30daysPost	**0.006 (+)**	0.417	0.112
cumGDD	0.070	**<0.001 (-)**	0.298
totalGDD30daysPre	0.215	**<0.001 (-)**	0.565
totalGDD14daysPre	0.108	**0.010 (-)**	0.065
totalGDD7daysPre	0.992	**0.001 (-)**	**0.037 (+)**
totalGDD3daysPre	0.840	**<0.002 (-)**	**0.029 (+)**
totalGDD3daysPost	**0.040 (+)**	**0.003 (-)**	**0.002 (-)**
totalGDD7daysPost	**<0.001 (+)**	0.282	**0.023 (-)**
totalGDD14daysPost	0.223	0.979	**0.002 (-)**
totalGDD30daysPost	**0.011 (+)**	0.326	0.079
avgRH30daysPre	0.701	0.136	**0.025 (-)**
avgRH14daysPre	0.075	**0.012 (+)**	**0.027 (-)**
avgRH7daysPre	0.060	0.074	**0.016 (-)**
avgRH3daysPre	0.435	0.772	0.338
avgRH3daysPost	0.247	**0.010 (+)**	**<0.001 (+)**
avgRH7daysPost	0.135	**<0.001 (+)**	**0.004 (+)**
avgRH14daysPost	**0.001 (-)**	**<0.001 (+)**	**0.006 (+)**
avgRH30daysPost	0.075	0.490	0.154
avgWindSpd30daysPre	**0.044 (-)**	0.055	**0.013 (+)**
avgWindSpd14daysPre	**0.001 (+)**	0.411	**0.003 (+)**
avgWindSpd7daysPre	**0.001 (+)**	0.253	0.448
avgWindSpd3daysPre	0.067	0.257	**0.001 (+)**
avgWindSpd3daysPost	0.204	0.904	0.528
avgWindSpd7daysPost	0.746	0.852	0.089
avgWindSpd14daysPost	0.266	0.136	0.356
avgWindSpd30daysPost	**0.016 (-)**	0.639	0.695

Multiple regression and model selection

The competing models approach presented by Symonds & Moussalli (2011) was followed, in consideration of the issues introduced via the inclusion of interactions (Grueber et al. 2011). This method utilizes the Akaike's Information Criterion (AIC) to compare and rank multiple candidate models and to estimate which of them best describes the response variable. The AIC is a measure of the goodness of fit of a statistical model and is a function of the maximum likelihood estimate of a model and the number of fitted parameters. As the maximum likelihood is dependent on the number of observations (rows of data) in a data set, all of the models being compared must be based on the same data set, so observations with missing values must be omitted. In order to maximize both the number of explanatory variables and the number of observations included in the analysis, first, the variables with whole years or locations of missing values were omitted (all variables based on soil sample analysis). Then, from the remaining variables with missing values, fungicide application timing was chosen as a high priority variable, and all observations with missing values for this variable were removed from the data set. Many of the missing values from other variables were from the same rows of data, leaving a total of 266 observations from the original 314 in the data set. Any other variables that still had missing values were then also omitted (seed size, percent *F. Graminarium* on seed, seeding depth, fungicide water volume, fungicide application speed, all fungicide rotation variables, applied fertilizer rates, percent litter post-seed, and all rain gauge variables). Using all the remaining explanatory variables, the candidate set of models was comprised of all combinations of two-variable models and their interactions, as well as all single-variable models, for a total of 3916 possible candidate models. In order to assess interactive effects but also equalize the AIC calculation across models, all two-variable models included an interaction term, whether or not an interaction was likely or expected.

Models with combinations of variables that had issues of non-convergence, rank-deficiency, or singularity were also omitted from the candidate set. When the models were run with FHB index as the response variable, a large majority of the models presented these issues, while only a small proportion of models had such issues with FDK as the response variable. Consequently, competing models analysis was not completed for FHB index. These issues were all likely resulting from insufficient replication, especially for factor variables with many grouping levels, or with highly correlated variable combinations. For the same reasons, models with greater than two variables could not be included in the candidate set for FDK. The final candidate set for FDK included 3286 models.

The candidate models were chosen in consideration of the high level of inter-correlation among explanatory variables. Comparing and ranking all possible combinations of explanatory variables justifies the inclusion of highly correlated variables in the same model. We expect the predictive power of variables to be similar to the extent that they are correlated; however, it is important to include each of these variable combinations because their effects may be independent beyond the extent to which they are correlated. Thus, models that differ only in the substitution of correlated variables will be ranked similarly if effects of the correlated variables are similar. The small number of models that were omitted because of issues with model fit should have minimal effect on the rankings as the candidate set is large, and because those models likely had poor fit or the variables were very highly correlated.

The AIC of each candidate model was obtained, the models were ranked from lowest to highest AIC, and the difference in AIC from the highest ranked model (ΔAIC) was calculated for each model. To assess the relative strength of each candidate model, the Aikaike weight (w_i) was calculated for each model. The

Akaike weight is a value between 0 and 1, with the sum of Akaike weights of all models in the candidate set being 1. The Aikaike weight indicates the probability that the model represents the most accurate description of the response variable, relative to other models in the candidate set. The twenty top-ranked models are listed along with their Aikaike weights in

Table 8. The single top-ranked model had an Aikaike weight of 0.99, indicating that there was a 99% probability that the combination of cultivar and average soil temperature 14 days post-anthesis was the most influential on the development of FDK in the crop. All other models had Aikaike weights less than 0.1. Variety was included in the top eight models, mainly in combination with soil temperature variables, both pre- and post-anthesis. The second ranked model included a significant interaction with soil moisture 3 days pre-anthesis. The soil temperature variables were likely all highly inter-correlated, but apart from soil moisture, no other environmental variable was ranked highly in combination with variety. This indicates a strong overall moderating effect of soil temperature on FDK development among different varieties.

Forward-selection was used to confirm the additive and interactive effects of the combinations of variables included in the top-weighted models. Forward selection begins with the null model, which includes only the random effects. The addition of fixed effects to the null model is justified if the more complex model has significantly better fit than the null model (χ^2 (chi-square) test, $P<0.05$). All of the top-ranked models had significant χ^2 tests, confirming that each combination of variables significantly affected FDK (Table 8). The first variable in the top-ranked models was always a management factor (variety/cultivar, cultivar resistance group, fungicide product, group, or active ingredient) or static agronomic factor (soil texture), while the second variable was a non-static environmental variable or other continuous variable that would be a function of environmental conditions (seeding date, anthesis date). F-test results of the individual fixed effects in each model also showed that in most cases, only the first variable and the interaction were significant, while the second variable was rarely significant as an individual fixed effect. This strongly suggests that management practices are in fact highly effective for FHB management, however their effectiveness is also highly moderated by environmental conditions. Thus, in producers' fields, where efforts to manage FHB are always in place, the effects of environment do not appear to be additive, but rather entirely interactive with management.

Table 8. Top ranked models out of the candidate set of 3286 competing models for *Fusarium* Damaged Kernels (FDK). Models included the two single fixed effects plus their interaction, and random effects as specified in text. ΔAIC is the difference in AIC between each model and the top ranked model. The Akaike weight (w_i) indicates the probability that the model represents the most accurate description of the response variable, relative to other models in the candidate set. The χ^2 test indicates whether the model fit is significantly better than the null model with no fixed effects. F-test results indicate the significance of each fixed effect.

Model rank	Variable 1	Variable 2	ΔAIC	w_i	χ^2 (vs null)	P(>F) Variable 1	P(>F) Variable 2	P(>F) Interaction
1	wheatVariety	avgSoilTemp14daysPost	0	0.999	142.1, P<0.001	<0.001	0.471	<0.001
2	wheatVariety	avgSoilMois3daysPre	14.1	0.001	189.6, P<0.001	<0.001	0.603	<0.001
3	wheatVariety	avgSoilTemp30daysPost	17.3	<0.001	118.9, P<0.001	<0.001	0.738	<0.001
4	wheatVariety	avgSoilTemp3daysPost	17.4	<0.001	123.8, P<0.001	<0.001	0.556	<0.001
5	wheatVariety	avgSoilTemp3daysPre	20.4	<0.001	117.9, P<0.001	<0.001	0.426	<0.001
6	wheatVariety	avgSoilTemp7daysPre	33.4	<0.001	100.8, P<0.001	<0.001	0.611	<0.001
7	wheatVariety	avgSoilTemp14daysPre	34.3	<0.001	100.0, P<0.001	<0.001	0.359	<0.001
8	wheatVariety	avgSoilTemp30daysPre	35.6	<0.001	98.3, P<0.001	<0.001	0.406	0.001
9	fungProduct	avgRH14daysPost	42.0	<0.001	75.2, P<0.001	<0.001	0.351	<0.001
10	FHBresistance	seedDateJulian	44.0	<0.001	66.6, P<0.001	<0.001	0.155	<0.001
11	fungGroup	avgRH14daysPost	44.8	<0.001	47.1, P<0.001	0.010	0.118	0.011
12	fungGroup	avgMeanT30daysPre	45.6	<0.001	37.6, P<0.001	0.036	0.007	0.035
13	fungActive	avgRH14daysPost	46.1	<0.001	62.1, P<0.001	0.010	0.336	0.010
14	soilTexture	anthesisJulian	46.5	<0.001	82.5, P<0.001	0.006	0.711	0.006
15	FHBResistance	avgMeanT14daysPre	49.3	<0.001	48.6, P<0.001	<0.001	<0.001	<0.001
16	FHBResistance	avgSoilMois3daysPre	49.3	<0.001	66.3, P<0.001	<0.001	0.001	<0.001
17	soilTexture	avgSoilTemp3daysPre	50.6	<0.001	65.5, P<0.001	0.086	0.663	0.085
18	fungProduct	avgMeanT14daysPre	50.9	<0.001	47.1, P<0.001	0.011	0.402	0.009
19	FHBResistance	avgSoilMois3daysPost	51.1	<0.001	64.8, P<0.001	<0.001	<0.001	<0.001
20	fungGroup	avgRH7daysPost	51.8	<0.001	43.8, P<0.001	0.012	0.020	0.014

The combination of variety and soil temperature clearly had an overwhelmingly greater influence on the response variable, relative to all other combinations of variables. As the effect of variety was individually significant, differentiation in the level of FDK by variety, over all years and locations, is shown in Figure 4. Starting with the interaction model and using stepwise model simplification, the initial 12 wheat varieties were combined into 4 statistically similar response types, with no significant decrease in model fit (full vs combined model, χ^2=18.9, P=0.167). The remaining 4 groups differed significantly in their interaction with soil temperature. The interaction was examined graphically using the parameter estimates of the final model (Figure 5). Interestingly, the response types did not correspond to the varieties' FHB resistance ratings. Furthermore, variety was more influential than resistance rating in predicting FDK development (Table 8), indicating that the greater specificity of grouping provided better differentiation of the level of FDK in the crop. The level of FDK was consistently low in Type 1 varieties (Brandon, Cameron, Utmost, Elie, Alloy, Hughes) and did not appear to be affected by soil temperature post-anthesis. Type 2 varieties (Cardale, Titanium, Paramount) had higher levels of FDK, but were also unaffected by post-anthesis soil temperature. Type 3 varieties (Redberry, Stettler) had a negative relationship with post-anthesis soil temperature, while type 4 (Landmark) had FDK levels that increased with post-anthesis soil temperatures.

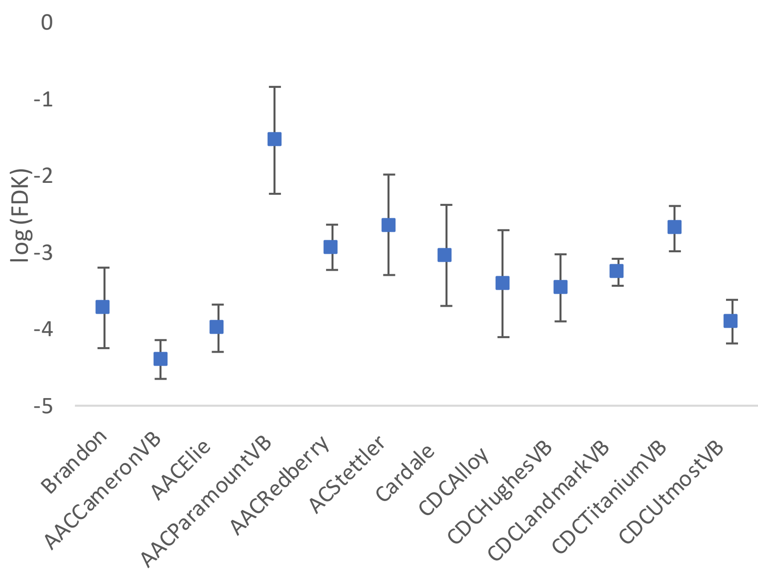

Figure 4. Level of *Fusarium* damaged kernels (FDK) by variety over all years and locations. Error bars indicate the standard error. Log-transformed values are shown to better illustrate the level of differentiation.

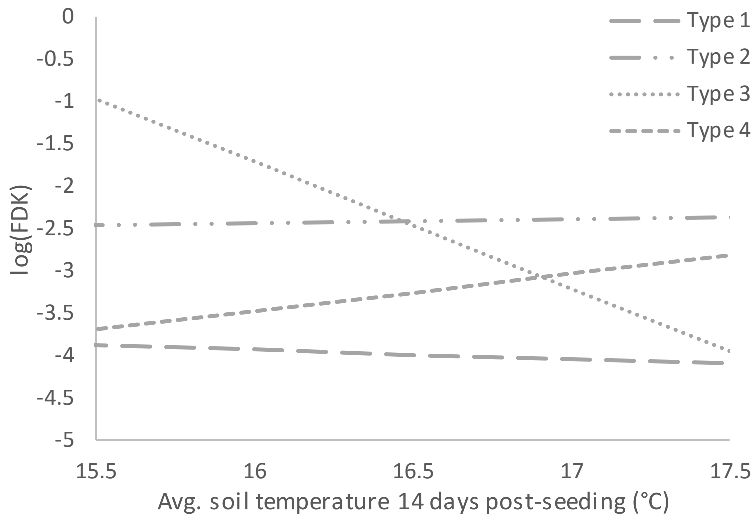

Figure 5. The interaction of variety and post-anthesis soil temperature on the level of *Fusarium*-Damaged Kernels (FDK). The 12 wheat varieties were combined into 4 response types which differed significantly in their interaction with post-anthesis soil temperature (Type 1 = Brandon, Cameron, Utmost, Elie, Alloy, Hughes; Type 2 = Cardale, Titanium; Type 3 = Redberry, Stettler; Type 4 = Landmark). The range of soil temperatures shown represent the first to the third quartiles.

To compare the relative effect of the other variables more equitably, all the models which included variety were removed from the candidate set and the model weights were re-calculated (

Table 9). Models initially ranked 9th to 18th (Table 8) made up the ten top-ranked models. The models were more equally weighted, with greater uncertainty in the ranking. Because of greater model uncertainty, predictor weights for each variable were also calculated by adding the Akaike weights from all the models in which that variable was included (

Table 10). Predictor weights indicate the probability that the variable is the most influential on the response variable, relative to the other variables in the candidate model set. The highest weighted predictor was an environmental variable, average relative humidity 14 days post-anthesis, and was included in three of the ten top-ranked models. Top-ranked variables representing pre-anthesis environmental conditions were mainly related to temperature, while top-ranked post-anthesis environmental conditions were related to moisture. However, it should again be noted that the second variable in each model was often only significant as an interactive effect with the first variable (Table 8), and so the effects of environmental variables were not examined individually but only as interactions.

Table 9. Top ranked models out of the candidate set of competing models for *Fusarium* Damaged Kernels (FDK), not including models with variety as a predictor. Models included the single variables as specified, plus their interaction, and random effects as specified in text. ΔAIC is the difference in AIC between each model and the top ranked model. The Akaike weight (w_i) indicates the probability that the model represents the most accurate description of the response variable, relative to other models in the candidate set. Refer to Table 8 for χ^2 test for model fit and F-test results of fixed effects.

Variable 1	**Variable 2**	**ΔAIC**	**w_i**
fungProduct	avgRH14daysPost	0	0.524
FHBresistance	seedDateJulian	2.00	0.193
fungGroup	avgRH14daysPost	3.65	0.084
fungGroup	avgMeanT30daysPre	4.14	0.066
fungActive	avgRH14daysPost	4.48	0.056
soilTexture	anthesisJulian	7.28	0.014
FHBresistance	avgMeanT14daysPre	7.37	0.013
FHBresistance	avgSoilMois3daysPre	8.63	0.007
soilTexture	avgSoilTemp3daysPre	8.90	0.006
fungProduct	avgMeanT14daysPre	9.07	0.006

Table 10. Predictor weights (*W*) for the top-weighted explanatory variables included in the candidate competing models set for *Fusarium* Damaged Kernels (FDK), not including models with variety as a predictor. Predictor weights indicate the probability that the variable is the most influential on the response variable, relative to the other variables in the candidate model set.

Variable	***W***
avgRH14daysPost	0.668
fungProduct	0.530
FHBresistance	0.220
seedDateJulian	0.196
fungGroup	0.155
avgMeanT30daysPre	0.068
fungActive	0.057
soilTexture	0.024
avgMeanT14daysPre	0.023
anthesisJulian	0.014
avgSoilTemp3daysPre	0.007
avgSoilMois3daysPre	0.007
avgRH7daysPost	0.004
avgSoilMois3daysPost	0.004
avgMeanT3daysPre	0.003

A few of the top ranked models were plotted as examples of the interactive effects of the combination of variables on FDK in the crop. The highest-ranked model included a significant interaction between fungicide product and average relative humidity 14 days post-anthesis. Fungicide product had a significant effect on the level of FDK individually, and the fungicide products were differentially influenced by post-anthesis average relative humidity (Figure 6). Using stepwise model simplification, the initial 7 fungicide products were combined into 3 groups, with no significant decrease in model fit (full vs combined model, χ^2=11.4, P=0.181). The different groups differed significantly in their interaction with post-anthesis relative humidity. Interestingly, the model's grouping did not correspond to fungicide groups or fungicide active ingredients. Furthermore, product was ranked higher than either fungicide group or fungicide active ingredient (Table 10), indicating that this grouping provided better differentiation of the level of FDK in the crop. All but two of the products were grouped together and had lower values of FDK, while significantly higher levels of FDK were seen with Priaxor. Prosaro XTR was the only product to appear to have a significant interaction with post-anthesis relative humidity; FDK was very low with lower relative humidity post-anthesis but increased significantly with higher relative humidity.

The next highest ranked model had a significant interaction between FHB resistance group and seeding date (Figure 7). The effect of FHB resistance rating was individually significant (Table 8), however the effect is difficult to interpret graphically. The resistance rating groups were differentially influenced by seeding date. Moderately resistant cultivars had consistently low levels of FDK, while cultivars with intermediate resistance had marginally higher FDK that decreased significantly with later seeding dates. Moderately susceptible cultivars had higher FDK levels overall that increased with later seeding dates. Level of FDK was similar among resistance rating groups at early seeding dates.

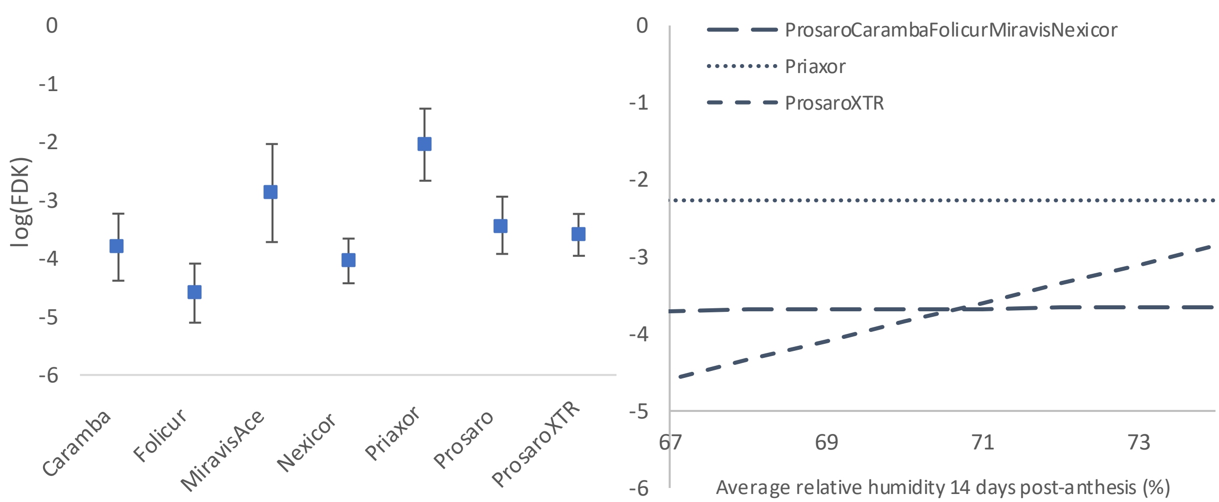

Figure 6. The effect of fungicide product on the level of *Fusarium* damaged kernels (left) and showing the interaction with post-anthesis average relative humidity (right). Error bars indicate standard error. The seven fungicide products were combined into 3 groups which differed significantly from each other in their effect on development of FDK in the crop. The range of relative humidity shown represents the first to third quartiles.

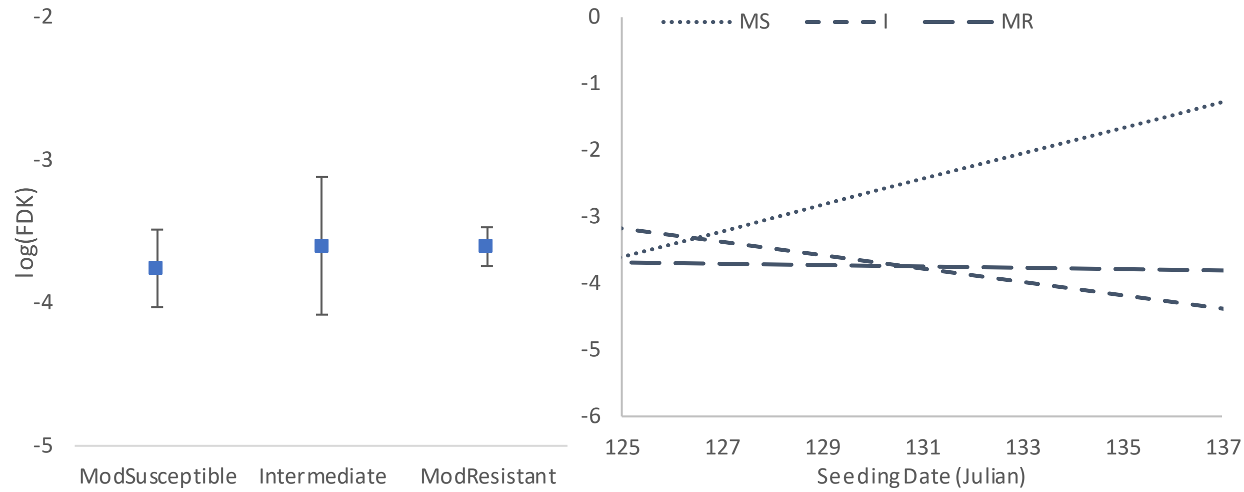

Figure 7. The effect of varietal FHB resistance rating on the level of *Fusarium* damaged kernels (left) and showing the interaction with seeding date (right). Error bars indicate the standard error. The three resistance rating groups differed significantly from each other in their effect on FDK development in the crop. The range of seeding dates shown represents the first to third quartiles.

Discussion

The multivariate and observational design of this study fundamentally leads to a more explorative, as opposed to confirmatory, analytical approach with the objective of identifying associations worthy of further investigation. The competing models analysis allowed us to look at the relative influence of management variables and environmental variables simultaneously, and whether the effects were additive or interactive. The method followed was most appropriate considering the low level of replication in relation to the large number of explanatory variables as well as the high level of intercorrelation among explanatory variables. The inclusion of random effects with mixed effects modeling also helped to address these issues to a certain degree. Importantly, the direction of the effect (positive or negative) as identified with single variable models was fairly consistent with what would be expected based on previous research and experience, which helps to validate the study design and demonstrate the potential for extension of this study or future studies with similar methodology.

In this study, the three response variables (*Fusarium* Head Blight (FHB) index, *Fusarium* damaged kernels (FDK), and level of deoxynivalenol (DON)) were not associated with the same explanatory variables. This is consistent with previous research that has shown that correlations between these variables are dependent on environmental conditions. Goral et al. (2019) found there was no significant correlation between FDK and DON overall, but the two were significantly correlated when environments were analyzed separately. Variability in environmental conditions was desirable in this study as these variables were of interest and were not controlled for. Random effects were specified such that there would be some differentiation of environments by year, location, producer, field, and sometimes sample site, but these groupings were mainly included to account for unbalanced data within these groups. Thus, we would not expect the response variables to be correlated in this study. Furthermore, Del Ponte et al. (2007) found that DON accumulation was not correlated with FHB index or FDK when infection occurred in the late stages of wheat development, which would suggest that the variables would be responding to different environmental triggers that would vary with the timing of infection. The differential influence of timing of infection on the three response variables was accounted for by averaging or totalling the variables over several pre- and post-anthesis intervals. Ultimately, more could have been deduced regarding the relationship between FHB index, FDK, and DON if the same multivariate analysis was conducted for all three variables with the same data set. In consideration of the greater level of subjectivity in assessing FHB index and the very low values of DON observed in this study, more replication would be necessary to conduct the multivariate analysis with these two variables.

For management variables, it is encouraging that the most often and highly recommended practices were shown to have the greatest influence on *Fusarium* infection in this study. Cultivar/variety was the most influential management variable on FDK, and FHB resistance rating was also highly influential. Previous research has frequently shown that the use of FHB resistant varieties is a key factor for management of FHB, especially as part of an integrated management strategy (Fernando et al. 2021, Ye et al. 2017, Wegulo et al. 2011). Fungicide product, mode of action group, and active ingredient were top-ranked variables affecting FDK, however timing of application was not a significantly ranked variable. Timing of application is usually emphasized as a greater priority than fungicide product for FHB management in wheat. In the single variable models, there was a significant negative relationship between fungicide timing and FDK, indicating that the level of FDK decreased as the fungicide application date approached anthesis, as a large majority of fungicide applications were done at the recommended stage, prior to anthesis. Fungicide application timing also had a marginally significant effect on the FHB index, but no effect on DON accumulation. Crop rotation is also often recommended as key factor for integrated management of FHB, however it was not a top-ranked variable. In the single variables models, only one of the crop rotation variables had a significant relationship with FDK; FDK significantly increased with the number of cereal crops in a 4 year rotation. Soil texture was a top-ranked agronomic factor affecting FDK, and could influence residue turnover rate, however the amount of residue, pre- or post-seeding, was not a highly ranked variable, and did not have a significant association with FDK. The effect of soil texture was more likely related to its role in regulating the microclimate within the crop.

Top-ranked environmental variables affecting the development of FDK in the crop during pre-anthesis stages were mainly related to temperature, while top-ranked variables during post-anthesis stages were related to moisture. This is consistent with what is known about conditions required for spore production prior to anthesis (Paul et al. 2007, Gilbert et al. 2008), and infection and accumulation of mycotoxins after anthesis (Kriss et al. 2010, Cowger et al. 2009). More importantly, it was deduced that the effects of environmental variables were not additive, but largely only interactive with management. Predictive models for forecasting FHB risk have focused on the effect of environmental conditions on FHB development in the absence of FHB management. FHB risk management practices are commonly applied in commercial wheat crops, thus, these findings confirm that in order to advance our ability to forecast the risk of FHB infection, it will be necessary to more thoroughly evaluate the interactive effects of management and environment. Based on the results of this study, it would be most insightful to compare genetic resistance to FHB or effectiveness of different fungicide strategies (products and timing) as a function of variable environmental conditions.

The analysis shown in this report is fairly explorative but illustrates the potential that could be achieved with this type of observational study, utilizing on-farm data collection. With greater replication and expansion of this data set, it would be statistically conceivable to explore relationships among several management and environmental variables simultaneously, and to utilize more confirmatory multivariate analytical approaches such as structural equation modeling. Additional replication would also justify the inclusion of variables with missing values in the multivariate analysis; at this point, including those variables would result in an unacceptable level of data loss. Overall, an extension of the study to provide additional replication, with a focus on particular variables of interest, would be beneficial.

A secondary objective that was achieved during this study was the development of relationships between research organizations and producers to facilitate future research collaborations and to help applied research organizations transition to more field-scale agronomic trials. The present study has also demonstrated the usefulness of new and different study designs that have been more common in ecological research, where experimental manipulations can be challenging. Observational studies eliminate the requirement for producers to implement and maintain field trials which are time-consuming and logistically demanding.

CONCLUSIONS AND RECOMMENDATIONS

The management practices most often recommended for FHB risk management in commercial fields include the use of resistant varieties and a timely fungicide application. Results of this study suggest that the choice of variety and fungicide product are highly influential on FHB development, but that the timeliness of fungicide application was less important. Environmental conditions have been shown to be highly influential on the development of FHB, FDK, and DON, and the use of forecasting tools for predicting FHB development in a crop is also highly recommended. Results indicate that environmental variables affecting the development of FDK in the crop during pre-anthesis stages were mainly related to temperature, while influential variables during post-anthesis stages were related to moisture. However, results of this study have also shown that environmental variables are mainly interactive with FHB risk management practices, and the effects are not additive. Yet, the effects of environmental variables are usually isolated from the effects of management in the development of predictive models. As FHB risk management practices are commonly applied in commercial wheat fields, these findings confirm that in order to advance our ability to forecast the risk of FHB infection, it will be necessary to more thoroughly evaluate the interactive effects of management and environment. Based on the results of this study, it would be most insightful to compare genetic FHB resistance or effectiveness of different fungicide strategies (products and timing) as a function of various environmental conditions.

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EXTENSION AND ADMINISTRATION

Extension

Results have not been presented to the public at this time but will be communicated via oral presentations, and online and printed reference material and publications in the near future. Media representatives have been in contact and will be involved in sharing the results of the study with the public.

Financial Report

Provided in a separate document.