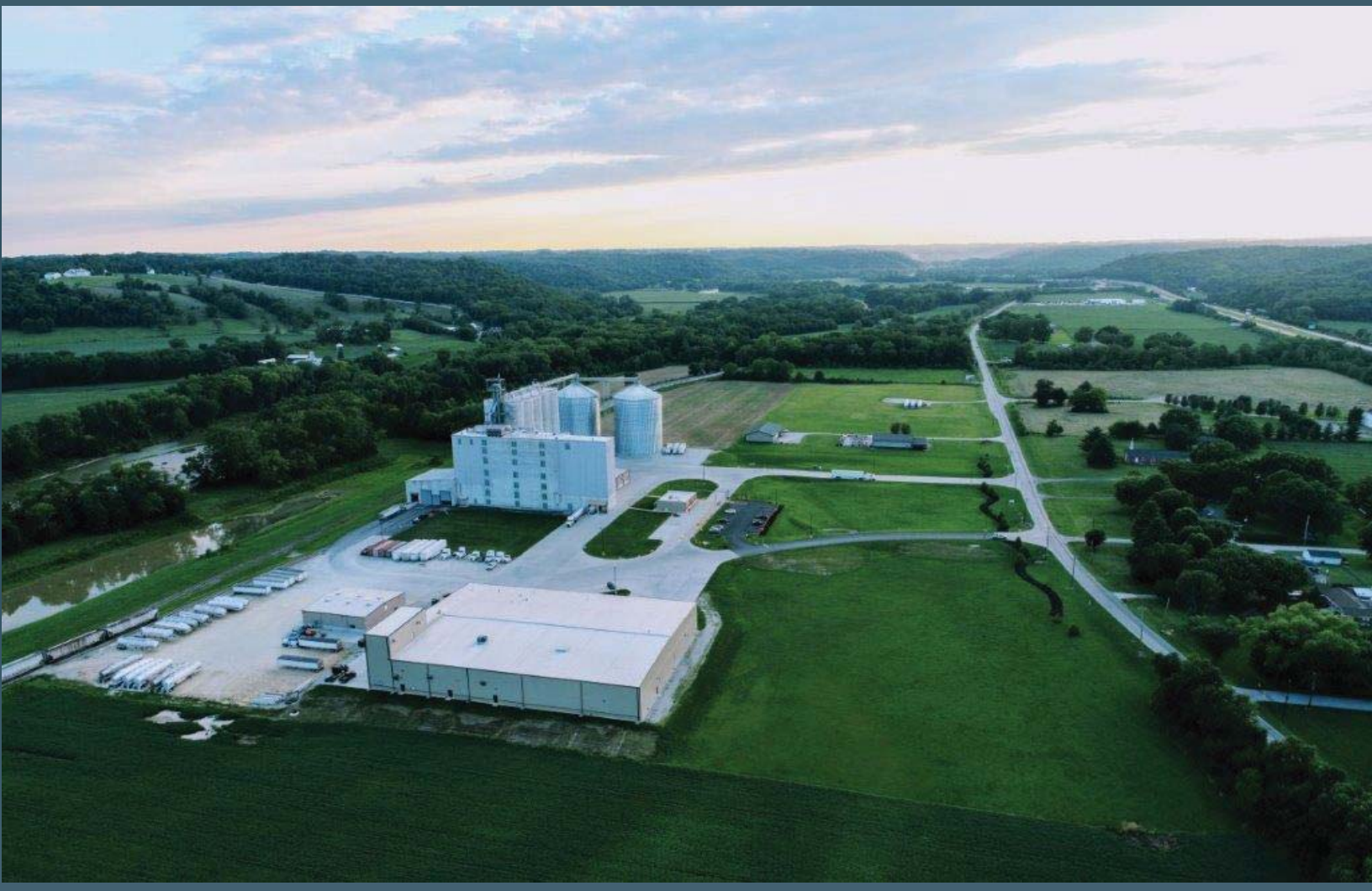
Proceedings of the 2020 National Fusarium Head Blight Forum



Virtual

December 7-11, 2020

Proceedings of the 2020 National Fusarium Head Blight Forum

VIRTUAL

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Proceedings compiled and edited by: S. Canty, A. Hoffstetter and R. Dill-Macky

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BARLEY COORDINATED PROJECT

FUSARIUM HEAD BLIGHT BIOMASS IN SPRING BARLEY COMPARING 2018 TO 2019 IN U.S. NURSERIES

Sidrat Abdullah[1], Eninka Mndowla[1], Suzette Arcibal Baldwin[2], Ellen Kress[1], Ruth Dill-Macky[3], Mark Earl Sorrells[4], Patrick Gross[5], Robert Brueggeman[5], Carl Griffey[6], Joshua Fitzgerald[6], Juliet Marshall[2], Kathy Klos[1] and Thomas Baldwin[5,*]

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ABSTRACT

Selection of Fusarium head blight (FHB) resistance in the field has been complicated by the low correlation between severity ratings and deoxynivalenol (DON), particularly for barley. Measurement of fungal biomass by quantitative PCR may alleviate these selection issues by directly estimating fungal infection and growth. Spring barley samples from 2018 and 2019 national FHB nurseries were evaluated for DON, severity rating and biomass. The coefficient of determination (R^2) was determined for biomass and DON and for severity rating and DON for 2019 and 2018 nurseries. More technician time was required for biomass estimates than for visual severity ratings in Idaho nurseries. However, biomass had higher correlation to DON than did visual severity ratings in both years. The consistency between 2018 and 2019 of line ranking based on severity rating, biomass and DON was evaluated. Comparing a second year of biomass analysis provided support that this measurement is helpful in giving an overall view of infection in the field and determining resistance in spring barley.

DEVELOPMENT OF TR18262 TWO-ROW FEED BARLEY WITH DESIRABLE AGRONOMICS AND DISEASE RESISTANCE INCLUDING LOWER DON ACCUMULATION

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is one of the most devastating diseases of barley due mainly to effects of the mycotoxin deoxynivalenol (DON) on grain quality. Incorporating genetic resistance is a desirable strategy, and has been an important objective of the barley breeding program at Agriculture and Agri-Food Canada's Brandon Research and Development Centre (AAFC-Brandon), Manitoba, Canada since 1996. Combining lower DON content with good agronomic traits including high yield and resistance to other diseases in a commercially acceptable package has been challenging. TR18262 is a promising two-row, hulled feed barley line that was evaluated for two years (2018 and 2019) in the Western Two-row Cooperative Registration Trial. It was bred and selected specifically for FHB resistance from the cross Cerveza/Xena made at AAFC-Brandon. Based on two-years of cooperative testing, it demonstrated to combine a good yield potential with an average to above average combination of disease resistance which includes resistance to the stem rust and surface smuts and moderate resistance to spot blotch and FHB. Overall its DON levels are lower than all the checks included in these trials. In addition, lower DON levels were also recorded for TR18262 when tested in the 2019 North American Barley Scab Evaluation Nursery. With its good yield and low DON levels, TR18262 could offer a good production choice for feed growers especially in areas where FHB is a concern.

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DETERMINING FUSARIUM HEAD BLIGHT RESISTANCE OF SPRING BARLEY IN IDAHO

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ABSTRACT

The impact of Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, has been progressively increasing in Idaho. Unacceptable levels of deoxynivalenol (DON) have been reported in commercial barley production. The majority of released varieties and advanced breeding lines remain vulnerable under irrigated conditions and higher growing degree days. Spring barley lines have been evaluated for FHB resistance since 2014 at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, ID. With additional funding from the U.S. Wheat and Barley Scab Initiative, a second screening nursery was established at the USDA-ARS in Kimberly, ID in 2019. The primary study objective at these two locations is to continue evaluating resistance of spring barley lines to FHB and accumulation of DON. In 2019, screening nurseries were planted on March 21 in Kimberly and May 2 in Aberdeen. Both nurseries were arranged in a randomized complete block design arrangement with two replications. To facilitate FHB development, corn inoculum was applied three weeks before anthesis and conidial inoculum was sprayed at head emergence (Feekes 10.5). In addition to regular irrigation, a supplemental misting system was installed to provide favorable microclimate conditions. Disease evaluations were conducted at soft dough (Feekes 11.2) and DON levels were obtained from samples submitted to University of Minnesota DON Testing Lab. At Kimberly, IND and DON ranged from >0.1 to 39.8 % and 3.6 to 58.3 ppm, respectively. At Aberdeen, IND and DON ranged from 0.2 to 37.4 % and 1.6 to 20.4 ppm, respectively. DON levels tend to be higher in Kimberly (med = 13.2 ppm) compared to Aberdeen (med = 5.5 ppm). Using disease ratings and weather data from the screening nurseries, FHB predictions models will be developed that will aid local growers in managing FHB and DON risk in Idaho.

ACKNOWLEDGEMENT AND DISCLAIMER

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2020 HINDSIGHT ON THE NORTH AMERICAN BARLEY EVALUATION NURSERY (NABSEN)

Thomas Baldwin[1*], Patrick Gross[1], Richard Horsley[1], Kevin Smith[2], Ruth Dill-Macky[2], James Tucker[3], Ana Badea[3], Marie Timmerman[4], Austin Case[4] and Robert Brueggeman[5]

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ABSTRACT

Barley production in the Midwestern U.S. and Prairie provinces of Canada is severely threatened by the decades long issue of Fusarium head blight (FHB) and contamination of the mycotoxin deoxynivalenol (DON). The malt barley industry is particularly impacted by FHB with tolerances for DON below 0.5 ppm. The mandate to address breeding FHB resistance in barley was initiated in 2002 when the first uniform field trial nurseries were established giving rise to the North American Barley Evaluation Nursery (NABSEN). For the past 18 years and counting, NABSEN has provided coordination and insights necessary for malt barley breeders to select for high levels of resistance against FHB and DON in their germplasm. Elite barley lines are planted each year from university cooperators and industry partners. Five misted nurseries comprising NABSEN are located in the state of North Dakota, Minnesota, and the Canadian province of Manitoba providing a broad range of barley growing regions. Lines are planted in short rows (1.2 m long) in a random controlled block design. Each line is planted in three replicates with consistent checks. Corn spawn and/or macroconidia are used as inoculation sources. FHB evaluation is determined at Feekes 11.2 growth stage (soft to mid-dough). Severity and DON are measured using a robust procedure to ensure uniformity over the years. Performance of the NABSEN nurseries varies yearly with dynamic changes in weather conditions and yearly refreshing of barley lines being evaluated. However, a clear trend of increased FHB and DON is observable from the first 9 years (2002-2010) to the second 9 years (2011- 2019). Increase in overall average severity of 41.7% and DON of 18.8% comparing the first and second decades. This trend could be due in part to better strategies for inducing disease, but are also likely impacted by more favorable climate conditions in recent years. Performance of the 10 top and bottom barley lines compared to the resistant and susceptible checks show a trend of increased FHB resistance over the decades. Top 10 lines showed 13.4% low severity and 13.3% low DON compared to the resistant checks over the decades. Bottom 10 lines showed 5.7% lower severity and 18.3% lower DON compared to the susceptible checks over the decades. This is encouraging results despite the increased disease pressure. The complex nature of FHB resistance in barley requires continued coordinated strategies, such as NABSEN, to make progress in breeding resistance. The coordinated program will be dedicated to providing even better FHB evaluation using advanced screening tools, such as biomass and hyperspectral measurements, to aid barley breeders in developing FHB resistance in their elite barley lines well into the future.

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Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

EXPLORING VARIATION FOR FHB RESISTANCE IN NAKED BARLEY

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ABSTRACT

Naked or Hull-less barley is receiving growing interest for malt, feed and food applications. One potential advantage of naked barley compared to covered barley is lower levels of deoxynivalenol (DON) due to the loss of the hull. Studies on covered barley suggest that hull removal can eliminate some amount of DON during processing, but the role of the hull as a DON sink in naked varieties has not been studied extensively. If there is substantial variation for DON in the hull, it may be possible to select for stronger DON sink activity of hulls, reducing kernel DON content and the risks associated with this mycotoxin. The goals of this study are to evaluate a naked barley diversity panel for Fusarium head blight (FHB) resistance and favorable DON distribution and use that data to conduct genome-wide association studies (GWAS). The diversity panel, consisting of 242 naked spring barley lines along with one naked and five covered check varieties, were planted in single row plots in irrigated FHB nurseries in St. Paul and Crookston. The St. Paul nursery was inoculated by spraying a solution containing macroconidia, while the Crookston nursery was inoculated with grain spawn. Data was collected on height, heading date and percent FHB infection in the field. Heads were harvested and separated into hull, kernel and rachis subsamples for analysis of DON concentration. Preliminary results indicate that there is substantial variation for FHB severity in the panel. At the St. Paul location, no strong correlation was observed between percent FHB infection and height or heading date, both of which are disease avoidance factors. A preliminary analysis of five genotypes from the panel grown in the St. Paul nursery for DON concentration showed wide variation (3.9 to 31.1 ppm) in the kernels. Hulls were observed to have similar concentrations of DON and as they comprise an average of 22% of the sample mass they represent a significant opportunity to reduce DON contamination. Overall, naked barley shows potentially useful variation in FHB resistance and toxin distribution. The next steps in this project are to finish collecting DON data and conduct GWAS to characterize the genetic architecture of FHB resistance and DON accumulation.

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QUANTITATIVE TRAIT LOCI ASSOCIATED WITH RESISTANCE TO FUSARIUM HEAD BLIGHT AND DON ACCUMULATION IN BARLEY POPULATIONS DERIVED FROM MODERATELY RESISTANT SIX- AND TWO-ROWED PARENTS

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ABSTRACT

Fusarium head blight (FHB) is the most economically impactful barley disease in the Upper Midwest production region. It has devastated the once-thriving malting barley industry and now threatens production in the western and northeastern United States. Identification and deployment of quantitative trait loci (QTL) that confer resistance to FHB and accumulation of the associated mycotoxin deoxynivalenol (DON) in barley cultivars are essential steps in order to reduce the losses caused by the disease. From field evaluations of over 25,000 *Hordeum* accessions, less than 1% were found to possess a moderate level of FHB resistance. One of the most promising two-rowed sources identified was PI 350725, a landrace from Austria. To characterize the FHB resistance of PI 350725, an advanced backcross population was developed between it and the recurrent parent Quest, a six-rowed Minnesota malting cultivar bred for improved FHB resistance in the Upper Midwest region. From 90 BC_2 families, 161 doubled haploid (DH) lines were generated by the Barley Breeding Program at Oregon State University. In addition to the DH lines, 319 BC_2F_5 recombinant inbred lines (RILs) were generated. Phenotypic evaluations for the DH population were conducted at the University of Minnesota Northwest Research and Outreach Center (NWROC) in Crookston, MN in 2016, 2018, and 2019 and the Minnesota Agricultural Experiment Station (MAES) in Saint Paul, MN in 2017 and 2018. The RIL population was screened at the NWROC in 2018 and 2019 and at the MAES in 2017 and 2018. The 50k Infinium iSelect genotyping array for barley was used on all lines. After quality and redundancy filtering, a total of 1,174 and 478 markers were used for the development of linkage maps in the DH and RIL populations, respectively. In the DH population, six QTL were detected for reduced FHB severity across all environments. PI 350725 contributed the resistance allele for four of these FHB QTL. One FHB QTL was identified in two of five environments, with the resistance allele being contributed by Quest. In the RIL population, four QTL were detected for reduced FHB severity across all environments. PI 350725 contributed the resistance allele for two of these FHB QTL. One FHB QTL was identified in two of four environments, with the resistance allele contributed by Quest. In the DH population, five QTL were detected for reduced DON accumulation across all environments. PI 350725 contributed the resistance allele for three of these DON QTL. One DON QTL was identified in three of five environments, with the resistance allele contributed by Quest. In the RIL population, four QTL were detected for reduced DON accumulation across all environments. PI 350725 contributed the resistance allele for one of these DON QTL. One DON QTL was identified in three of four environments,

with the resistance allele contributed by Quest. In both populations, the major effect QTL explaining the majority of variation were coincident with QTL for height and heading date, suggesting pleiotropic effects of major QTL. Across the two populations, comparison of the physical positions of the markers flanking significant QTL revealed three QTL influencing FHB resistance and/or DON accumulation residing within the same or in overlapping marker intervals. In both populations, transgressive segregants were identified that had both lower FHB severity and DON accumulation than either parent across all environments. A few of these transgressive segregant progeny were found to carry favorable QTL alleles from both parents. Lines carrying potentially novel QTL from PI 350725 and also having desirable agro-morphological traits will be advanced in the breeding program.

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BIOFILM FORMATION IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Biofilms are known to play important roles in bacterial pathogens of plants and animals where the formations help protect cells from defense responses and antimicrobial treatments. Although biofilms in bacterial plant pathogens are well studied, the role in filamentous fungal plant pathogens is virtually unexplored. We are characterizing the formation of biofilms in *Fusarium graminearum in vitro,* which will provide a basis for moving this work to plants. We hypothesize that biofilms impact many steps in the disease cycle of FHB, from initial plant infection, to colonization, to overwintering on crop residues in the field**.** *F. graminearum* grows as fluffy hyphal colonies on most media, however, we observed an altered morphology, with predominantly short, bulbous hyphae under some conditions. We have mapped the full development of *F. graminearum* biofilms *in vitro,* adhered to a polystyrene surface, from the initial adhesion to dispersal of propagules and senescence of biofilms, and are investigating the conditions that promote biofilm formation. Additionally, we have identified components of the complex extracellular polymeric matrix, which is an important protective component of biofilms. From this work, we hope to provide new targets for disease control through biofilm formations in *F. graminearum.*

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FIVE YEARS IN: OUTLOOK FOR BREEDING FOR FHB RESISTANCE IN BARLEY IN NEW YORK

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ABSTRACT

Malting barley (*Hordeum vulgare* L.) has a long production history prior to prohibition in New York state but interest in malting barley was very low until the 2012 New York Farm Brewery bill created demand for locally grown grain for craft brewing. Fusarium head blight (FHB) is a major concern for winter and spring malting barley production in New York. The threshold for deoxynivalenol (DON) in malting quality grain is 1 ppm, making control of *Fusarium* infection critical to malting barley grower success. The Cornell University Small Grains "Born, Bred, and Brewed in New York" program has screened winter and spring malting barley germplasm in misted, inoculated FHB nurseries since 2015. FHB incidence, severity, index, and DON data from winter and spring nurseries from 2015 to 2020 were jointly analyzed within growth habit using mixed models to estimate trait heritability and best linear unbiased predictors (BLUPs). The winter dataset included regional and cooperative malting barley trials as well as naked barley trials. The spring dataset included regional and cooperative malting trials, experimental malting populations, and a European heritage malting panel. Significant differences in FHB symptoms across years and trials were observed and genetic variance was typically much lower than error variance. Spring and winter lines with potential FHB resistance were identified. This analysis highlights the importance of FHB screening over multiple years in barley and identifies germplasm to integrate into future FHB resistance breeding efforts.

DURUM COORDINATED PROJECT

IMPACT ON DURUM WHEAT OF SMALL INTROGRESSIONS FROM WILD *THINOPYRUM* SPECIES CONFERRING EFFECTIVE RESISTANCE TO *FUSARIUM* DISEASES: BREEDING PERFORMANCE AND METABOLIC RESPONSES

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ABSTRACT

Durum wheat (*Triticum durum*, 2n = 4x = 28, DW) covers only 8% of the global wheat surface, yet it represents a strategic commodity for countries spanning many and diversified world areas, primarily the Mediterranean Basin. Recent climate changes have not only contributed to modify the DW conventional distribution areas, but also exposed the crop to unfamiliar pathogens. This is the case for fungal pathogens of the *Fusarium* genus, responsible for some of the most threatening diseases of wheat and other cereals, namely Fusarium head blight (FHB), or scab, and Fusarium crown rot (FCR). For DW, which results more vulnerable to both FHB and FCR than 6x bread wheat (BW), this condition is particularly worrying. In fact, being typically devoted to human consumption, the DW crop greatly suffers, besides yield and quality reduction, the safety problems associated with health-dangerous *Fusarium* mycotoxins, such as deoxynivalenol (DON). Since effective resistant sources are not available in breeding pools, a profitable and sustainable approach to arm the species with appropriate defense means consists of the exploitation of genetic variability present in related gene pools, such as those of the *Thinopyrum* genus. Of specific interest is the distal end of 7L arm of several congeneric species, enriched with genes/QTL for resistance to relevant wheat diseases. Among them, the *Lr19+Sr25* genes (leaf and stem rust resistance, respectively), located on the $7el_1L$ arm of 10x *Th. ponticum*, and also major QTL contributing resistance to both FHB and FCR, namely *Fhb7*, identified on the $7el_2L$ arm of a different *Th. ponticum* accession, as well as its likely ortholog present on 7EL of the 2x *Th. elongatum* (1, 2, 3, 4, and refs. therein). The extraordinary efficacy against FHB of both *Fhb7* QTL (indicated hereafter as $Fhb7el_2$ and *Fhb7E* for the sake of distinction), was initially established in laboratory BW lines, such as Thatcher 7DS·$7el_2L$ centric translocation line, and Chinese Spring (CS) 7E(7D) substitution line. Besides contributing to cytogenetic mapping of *Fhb7* QTL (e.g. 2), we have successfully exploited chromosome engineering strategies to incorporate firstly $Fhb7el_2$ (1) and more recently *Fhb7E* (3) into DW. In both transfer schemes, previously produced DW recombinant genotypes, with $7el_1L$ segments (including *Lr19*, *Sr25* and *Yp* genes) spanning 23% (named R5) and 28% (R112) of their 7AL arms, were used as recipient lines. As donor of $Fhb7el_2$, the 7DS·$7el_2L$ BW-*Th. ponticum* translocation line was used, whereas previously obtained BW 7DS·7DL-$7el_1L$/7EL recombinant types (named R69-9 and R74-10), carrying a terminal 7EL portion, including *Fhb7E*, embedded into a $7el_1L$ *Th. ponticum* segment present on wheat 7DL

(2), were employed. In both cases, transfer of the *Fhb7* QTL was achieved by homologous pairing and recombination between the $7el_1L$ portions shared by donor and recipient recombinant chromosomes in 5x F_1s. Stable DW recombinants with either *Fhb7* QTL combined with valuable $7el_1L$ genes were isolated in BC_{1-2} progenies to the Italian DW cv. Simeto of all cross combinations. Further BCs to the same DW yielded near-isogenic recombinant lines (NILRs, HOM+), whose spikes and seedlings were inoculated with *F. graminearum* and *F. culmorum*, respectively, for assessment of their FHB and FCR phenotypes compared with those of HOM– sibs. Following *F. graminearum* point inoculation, NILRs of both $7el_1L$+$7el_2L$ DW recombinant types, named R216 and R193 (same telomeric $7el_2L$ portion with *Fhb7el*$_2$, but inserted into the $7el_1L$ segments of either R5 or R112, respectively), exhibited an average 75% reduction in FHB severity vs. HOM– controls (1). Similarly, $7el_1L$+7EL DW recombinants, possibly due to some contribution of the CS donor background, showed an even stronger resistance, with > 90% reduction of disease severity (3). Moreover, unlike the case of *Fusarium* spp. resistance QTL native to wheat, both *Fhb7el2* and *Fhb7E* showed to confer tolerance to FCR as well, with disease index reduced by over 50% (3).

The breeding potential of the various DW recombinant types equipped with either *Fhb7el2* or *Fhb7E* was preliminarily evaluated, on the basis of plant and spike traits, in small-scale field tests carried out during the 2018-19 and 2019-20 seasons at Viterbo experimental site (Central Italy). Natural disease pressure was negligible in both experimental years. Instead, weather conditions were quite different, with 2019-20 being much drier than 2018-19, characterized by a huge amount of rainfall and lower mean temperatures in coincidence with the critical phases of anthesis and grain filling. Whereas an overall absence of negative effects on yield and related traits was observed for all recombinant types (HOM+) vs. their controls (HOM–), the marked between-year difference was helpful at highlighting the best suited recombinant genotypes to the contrasting environmental conditions. Thus, between R216 and R193 ($7el_1L$+$7el_2L$), the 2018-19 season resulted more favourable to the latter, in which the $7el_2L$ portion is part of the R112 longer $7el_1L$ segment compared with R5. The better yield performance of R193 is in line with previous observations from multi-year/environment trials, which suggested R112 to carry in its most proximal $7el_1L$ fraction (retained in R193) genes/QTL for morpho-physiological and agronomical attributes of the aerial and root plant portions that are best expressed in environments with optimal thermo-pluviometric patterns (5). On the other hand, R216 HOM+ had comparable or higher values than R216 HOM– for various yield and fertility traits in the hotter and drier 2019-20 season, thus mimicking the behaviour of its R5 parent (5). As to $7el_1L$+7EL recombinants, assessment of spike traits in R74-10/R112, R74-10/R5 and R69-9/R112 HOM+ genotypes and HOM– controls in the 2018-19 season, showed that presence of any of the three alien segments did not appreciably impair spike fertility and yield. However, R69-9/R112 was the best performing out of the three recombinants, and R74-10/R5 the least productive. This outcome might be associated with a different $7el_1L$/7EL ratio in the various recombinant types, with that of R74-10/R5 (longer 7EL segment than in R69-9, and shorter $7el_1L$ segment than in R112) causing more disturbance to the recipient genome. Thus, the R69-9 derivatives appeared more appropriate for DW breeding, also because their 7EL portion does not replace the $7el_1L$ allele of the *Yp* gene conferring higher semolina yellowness vs. the corresponding 7EL allele (2, 3). Consequently, subsequent work was focused on such recombinant types (R69-9/R5 and R69-9/R112), which exhibited a good performance at the plant and spike levels also in the drier 2019-20 season. Confirming their better adaptability to reduced rainfall, R69-9/R5 plants expressed higher values than their HOM– controls for a number of characters (mainly thousand grain weight and grain yield/spike), while R69-9/R112 was inferior to its HOM– controls for some spike traits. Based on 2019-20 dataset, an overall comparison of all DW-*Thinopyrum* spp. HOM+ recombinants, including R5 and R112 ($7el_1L$ only), as well as DW controls, Simeto and IDYT22 (an ICARDA line involved in

R216 and R193 pedigree), was carried out. ANOVA did not show statistically significant differences in grain yield/plant (GY). However, recombinants containing the $7el_1L$+7EL segment assembly, particularly R69-9/R5, turned out to be the best performing with respect to control varieties and to all other recombinant types. To a minor extent, also R216 and R193 ($7el_1L$+$7el_2L$) showed higher GY than R5/R112 and Simeto, while being comparable to IDYT22. These results indicate that, even disregarding the specific effects of the *Fhb7* QTL introgression, the presence of 7EL or $7el_2L$ chromosomal portions onto $7el_1L$ segments has a favorable impact on yield potential of the recipient DW, even improving the positive contribution known to be associated with the original $7el_1L$ segments (5 and refs. therein).

An additional research activity enabled by the novel recombinant types was undertaken to get insights on the mechanisms underlying the *Fhb7*-mediated resistance. To this aim, the untargeted metabolomic profile of the rachis tissue sampled at 2-4 days following *F. graminearum* (*Fg*) inoculation of spikes of R69-9/R5 (*Fhb7E*) HOM+ NIRLs was recently compared with that of HOM– *Fg*-inoculated sibs and of mock-inoculated plants. Extracted metabolites from the 4 genotype x treatment combinations were analyzed by LC/MS. Preliminary evidence from MetPA (Metabolic Pathway Analysis) shows main changes between HOM+ and HOM– *Fg*-inoculated samples at the level of phenylalanine metabolism, phenylpropanoid and diterpenoid biosynthesis, known to be main routes the plant activates in response to *Fusarium* inoculation. Particular interest arouses the Vitamin B6 metabolism, specifically induced in *Fg*-inoculated *Fhb7E*+ genotypes, which provides supporting evidence for B6 vitamers' role as potent antioxidants in plants subjected to abiotic and also biotic stresses. As a further contributor to the antioxidant capacity of stressed plants, the glutathione (GSH) metabolism was also up-regulated in *Fg*-inoculated *Fhb7E*+ and, to a minor extent, *Fhb7E*– rachises. Of a particular GSH adduct, i.e. a de-epoxidated DON-GSH adduct, whose formation was recently associated with a peculiar, xenobiotic mechanism at the base of *Fhb7* resistance (4), clear proof of exclusive production in the rachis tissue of *Fhb7E*+ plants was obtained by LC-MS/MS analyses. Whereas the GSH-mediated event appears to be a major determinant in DON detoxification brought about in the presence of *Fhb7* genes/QTL, additional detoxifying strategies, such as DON glycosylation, may contribute to an expected composite resistance response. In fact, the DON-3-glucoside (D3G) metabolite was solely detected in *Fhb7E*+ *Fg*-inoculated rachises. In line with this, D3G was four times more abundant in seeds harvested from the latter plants than in *Fhb7E*– sibs. Combined with the nearly 800 times lower DON content found in HOM+ vs. HOM– seeds, these results confirm DW lines carrying *Fhb7E*, and *Fhb7* QTL in general, to be highly valuable also for the crop safety, thus enhancing its market/trade potential.

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IDENTIFICATION AND MOLECULAR MAPPING OF A MAJOR QTL ON CHROMOSOME 2A CONFERRING RESISTANCE TO FUSARIUM HEAD BLIGHT IN EMMER WHEAT

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is one of the most important diseases of durum wheat (*Triticum turgidum* L. ssp. *durum*) and common wheat (*Triticum aestivum* L.) worldwide. Use of resistant wheat cultivars is the most effective and economically sound approach for management of the disease. However, most of durum wheat cultivars currently grown are highly susceptible to FHB and resistant sources in durum wheat are very limited. In the past decades, great efforts have been devoted to screen various tetraploid wheat species for FHB resistance, and some of them have been reported to be resistant to FHB, although the quantitative trait loci (QTL) for FHB resistance are not well characterized. In this study, we aimed to discover new FHB resistance QTL in a population of 186 recombinant inbred lines (RILs) derived from the cross between Divide, an FHB susceptible durum wheat cultivar, and PI 254188, an FHB resistant emmer wheat (*Triticum turgidum* L. subsp. *dicoccum*). The RILs along with their parents were evaluated for reactions to FHB in two greenhouse experiments and one field experiment in 2019 and 2020 and genotyped using the genotyping-by-sequencing (GBS) method. A total of 4,476 single nucleotide polymorphism (SNP) markers with normal segregating ratios were identified in the mapping population, and 1,866 unique SNP markers were used to construct a genetic map of 2656.46 cM. QTL analysis using both phenotypic and genotypic data identified one QTL (*Qfhb.ndwp-2A-2*) on chromosome 2A of PI 254188, which was consistently significant in all greenhouse and field experiments. This QTL explained 23.2, 13.5, and 17.8% of the phenotypic variation in the two greenhouse experiments and one field experiment, respectively. Another QTL (*Qfhb.ndwp-5A-3*) on chromosome 5A was only detected in the field experiment, which explained 20.3% of the phenotypic variation. Identification of the QTL along with the closely linked SNP markers will facilitate the introgression of the FHB resistance from PI 254188 into durum wheat cultivars through marker-assisted backcrossing.

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TOWARD A BETTER UNDERSTANDING OF THE HEXAPLOID WHEAT-DERIVED FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB) resistance exhibits a more complex inheritance pattern in durum wheat than common wheat due probably to the evolutionary divergence of the A and B subgenomes in these two cultivated wheat species and the absence of the D genome in durum. This has limited the deployment of FHB resistance genes in durum germplasm and varieties. In this study, we aim to understand the effect of the durum genomic background and D genome on FHB resistance. We have observed variation in FHB resistance among the durum D-genome chromosome substitution and addition lines, suggesting the D-genome chromosomes play a role in the expression of FHB resistance. In addition, we identified and mapped four new FHB resistance QTL on chromosomes 1A, 3A, 3B, and 7A in addition to the one previously identified on chromosome 5AL (*Qfhb.rwg-5A.2*) by genome-wide QTL analysis for the hexaploid (PI 277012)-derived FHB resistance in a large RIL population (n=234) developed from the crosses of FHB-resistant hexaploid wheat 'PI 277012' with FHB-susceptible 'Langdon' (LDN) durum. However, we did not detect the PI 277012-derived resistance QTL *Qfhb.rwg-5A.1* on 5AS in this tetraploid RIL population. Apparently, the durum genomic background has epistatic effect on FHB resistance. To better understand the resistance QTL on chromosomes 3A, 3B, and 5A, we have performed saturation and fine mapping of the relevant genomic regions. To date, 13 new STARP markers have been mapped to the QTL region on chromosome 5A, 12 to the QTL region on 3B, and 10 to the QTL region on 3A. Three critical RILs that constantly exhibited FHB resistance and contain resistance alleles at these three QTL were selected to cross with LDN to develop large F_2 segregation populations (n > 5,000) for fine mapping of the QTL regions. Embryo rescue was employed to quickly advance generations. A fine map with improved resolution will be constructed for each of these three resistance QTL, positioning the QTL into smaller chromosomal intervals for further epistatic analysis. The STARP markers closely linked to the QTL will be used to assist selection in durum breeding for FHB resistance.

ACKNOWLEDGEMENT AND DISCLAIMER

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UPDATE ON RESEARCH ACTIVITIES FOR *FUSARIUM* RESISTANCE IN DURUM WHEAT FROM BOKU UNIVERSITY - AUSTRIA

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ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of durum wheat, and growing resistant varieties is admittedly the most promising approach for controlling this fungal disease. FHB resistance breeding in durum is however hampered by the limited variation in the elite gene pool and difficulties in efficiently combining the numerous often small-effect resistance QTL in the same breeding line. Introgressing resistance alleles from wild and cultivated relatives is thus a promising approach to broaden the genetic basis for FHB resistance in durum wheat. For this purpose, we evaluated several durum wheat populations that were derived from crosses with such relatives as well as additional collections of tetraploid wheat and investigated the genetic architecture of FHB resistance, the role of morphological and phenological traits as passive resistance factors and the potential to harness the merit of this germplasm by genomic-assisted breeding. A number of 1,000 experimental lines with resistance alleles derived from *T. aestivum*, *T. dicoccoides* and *T. dicoccum*, another panel of 220 elite durum wheat cultivars from an international collection as well as 320 lines of the global durum and tetraploid wheat collections were phenotyped in up to four years for FHB resistance, plant height and anthesis date in artificially inoculated disease nurseries, while a subset of the material was also evaluated for the extent of retained anthers after flowering. Although a lack of highly resistant lines was evident for all populations, a broad variation was found for all investigated traits, including many moderately FHB resistant experimental lines and landraces. Plant height influenced FHB resistance levels and led to co-localization of plant height and resistance QTL, while the height-independent major resistance QTL *Fhb1*, derived from hexaploid wheat, was successfully introgressed into several durum genetic backgrounds. Interestingly, also in the elite durum wheat gene pool a major height-independent QTL mapped close to *Fhb1*, though haplotype analysis highlighted the distinctiveness of both QTL. Apart from plant height, anthers partially trapped within spikelets are a major promoter influencing FHB disease severity. Although the variation and extent of anther extrusion is lower in durum wheat compared to hexaploid wheat, crosses of a durum wheat cultivar exhibiting higher anther extrusion revealed a broad variation for this trait and a significant association with FHB resistance. Anther retention has thus also the potential to serve as a secondary trait in multi-variate genomic prediction models to enable an earlier identification of the most promising lines, which can result in a much faster short-term population improvement, complementing long-term strategies with exotic resistance donors for achieving higher levels of resistance.

DEVELOPING DURUM WHEAT FHB RESISTANT GERMPLASM USING INTERSPECIFIC CROSSES AND PHENOTYPIC SELECTION AT EARLY GENERATIONS

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ABSTRACT

Fusarium head blight (FHB) has frequently caused severe grain yield and quality loss of durum wheat in Northern Great Plains and other major growing areas in the world. Developing FHB resistant cultivars is one of the most effective and environmentally friendly means to mitigate the negative effects of the disease. Due to lack of resistant resources in durum wheat, introgression of resistance genes from hexaploid bread wheat is promising. In addition, due to the complex genetic nature of FHB resistance in wheat as documented by many studies, it is essential to integrate more resistance genes with mediate effects in addition to those major QTL like *Fhb1*. However, it is challenged by low fertility of the progenies from the interspecific crosses, high cost of field phenotypic evaluation, etc. The goal of this project is to use male sterile facilitated interspecific crosses followed by early generations of phenotypic selection to enhance introgression of FHB resistance genes from bread wheat into elite durum wheat. Male-sterile facilitated recurrent selection of FHB resistance has been conducted in a hard red spring wheat population, from which the base population (C0) was developed from the top 10 FHB resistant lines selected from the 439 NDSU HRS wheat breeding lines and 10 elite cultivars. One male-sterile half-sib family with the best FHB resistance was selected from the C0 population. About 40 remnant seeds from the selected family were planted in the greenhouse in 2019 winter and male-sterile plants as female parents were crossed with durum wheat cultivars 'Riveland'. Their F_3 and F_4 plants are being evaluated for FHB severity using single-spike spray method in the greenhouse. The selected F_4 plants will be genotyped with D-genome specific KASP markers to identify the tetraploid progenies. Top 20 $F_{4:5}$ tetraploid lines will be evaluated in the FHB field nursery in 2021 summer. The best half-sib family selected from the C1 population of the hard red spring wheat recurrent selection project is being crossed to 'Riveland', from which another round of phenotypic selection will be conducted to develop $F_{4:5}$ tetraploid lines. From this project, we expect to develop some durum wheat germplasm with improved FHB resistance that can be used for new cultivar development.

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FHB MANAGEMENT

ANALYSIS OF EFFECTIVENESS OF *TRICHODERMA GAMSII* T6085 AS A BIOCONTROL AGENT TO CONTROL THE GROWTH OF *FUSARIUM GRAMINEARUM* AND DEVELOPMENT OF FUSARIUM HEAD BLIGHT DISEASE IN WHEAT

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ABSTRACT

Wheat cultivation has drastically declined in the primary wheat-growing countries during the past decade due to the disease fusarium head blight (FHB). The main contributing agent is *Fusarium graminearum*, which contaminates the wheat kernels with mycotoxins. This contamination directly affects both quality and the quantity of the wheat. Among different strategies used to mitigate the disease, biological control is an environmentally friendly and more sustainable approach. Current research has shown that *Trichoderma gamsii* T6085 could significantly inhibit the growth and mycotoxin production of *F. graminearum* and *F. culmorum*. However, we do not adequately understand the impacts of *T. gamsii* on the broader microbiome of the crop production environment. The objective of this study is to test the efficacy of *T. gamsii* as a biocontrol agent to control FHB in wheat in a field setting, and to analyse the dynamics of the microbiome associated with different plant tissues relevant to FHB development. The field experiments were carried out at the University of Pisa. The experiment was a randomized block design, with four treatments and five replicates. The treatments were: i) application of *T. gamsii* on wheat spikes at anthesis, ii) application of *T. gamsii* on field soil and crop debris at the beginning of stem elongation of the wheat plants, iii) application of *T. gamsii* on soil, on crop debris as in ii, and on wheat spikes at anthesis as in i, and iv) a control treatment with no *T. gamsii* applied. Wheat straw, spike, and kernel samples were collected for microbiome profiling at different times. We generated PCR amplicons of the *16S rRNA* and *ITS2* regions of bacterial and fungal DNA, respectively, and used high throughput sequencing of these marker genes to profile microbiomes. To assess the absolute abundance of *T. gamsii* and *F. graminearum*, we performed quantitative PCR using the same DNA extracts that were used for microbiome profiling. These microbiome profiles reveal the bacterial and fungal taxa that are present in association with wheat plants at our field site, and how these microbial communities are impacted by the application of *T. gamsii* as a biocontrol agent. Our data will be useful in verifying that the application of *T. gamsii* effectively reduces the abundance of *F. graminearum*, and in identifying relationships between success of the pathogen (i.e., *F. graminearum* biomass, or deoxynivalenol concentration) and the relative abundances of other community members. Further experiments will be designed to validate interesting relationships among microbial taxa in response to pathogen presence or application of *T. gamsii*. As with the growing populations there is a need for more sustainable disease management strategies to be implemented. Use of biological control to manage FHB in wheat as an alternative to chemical fungicides will be better and eco-friendly.

PYRACLOSTROBIN SENSITIVITY IN *FUSARIUM GRAMINEARUM* ISOLATED FROM WHEAT IN BRAZIL

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ABSTRACT

In Brazil, *Fusarium graminearum* sensu stricto is the main pathogen causing Fusarium head blight (FHB) in wheat. In the disease-conducive subtropical environment, where wheat crops are mainly grown in the south region of Brazil, sequential sprays of fungicides are common practice. Farmers rely on chemicals to minimize yield losses caused by a complex of foliar and floral diseases, as well as to reduce mycotoxin contamination due to FHB. Usually, a range of active ingredients are used in isolation or dual premixes that include a triazole (DMI) and a quinone-outside inhibitor (QoI). Comprehensive information on the sensitivity/resistance of *F. graminearum* to fungicides is available only for DMIs, while scarce data is available for QoIs. Because of the increasing number of sprays over the last decades, especially the premixes, we hypothesize that *F. graminearum* isolates have become less sensitive to QoI fungicides, particularly in regions where FHB is controlled with two sprays of fungicides, totalling four to five applications during the season. We assessed the sensitivity to pyraclostrobin (Comet®, BASF) using a 50-strain collection of *F. graminearum* isolates representing eight years and two states. Isolates in the Rio Grande do Sul state (RS) subcollection (n = 25 strains) were obtained from 2007 to 2011. Those of the Paraná state (PR) subcollection (n = 25 strains) were obtained from 2011 to 2014. The *in vitro* sensitivity was evaluated based on a conidial germination assay using six concentrations (0, 0.05,0.5,1.0,5.0 and 10 μg/ml). The effective concentration leading to a 50% reduction in the germination rate (EC_{50}) was estimated from the fit of a three-parameter Weibull model to the data combined from two experiments. The EC_{50} values did not differ statistically between the two subcollections and between years within the same region. They ranged from 0.028 to 1.13 (mean = 0.340 μg/ml and median = 0.249 μg/ml) in the RS subcollection and from 0.12 to 0.668 (mean = 0.337 μg/ml and median = 0.289 μg/ml) in the PR. These mean values are greater than the maximum EC_{50} reported in a previous work that used five isolates from each state (EC_{50} < 0.16 μg/ml). Partial sequences of the cytochrome b gene (cyt b) will be analysed to check whether the (least sensitive) isolates harbor point mutations associated with QoI resistance. To better assess whether the sensitivity and the frequency of mutations, if present, are increasing over time, additional 50 isolates collected more recently (post 2014) will be included in the study.

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IS DMI + QOI PREMIX APPLIED DURING FLOWERING WORTH FOR PROTECTING WHEAT YIELDS FROM FUSARIUM HEAD BLIGHT? A META-ANALYSIS

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ABSTRACT

Fusarium head blight (FHB), caused mainly by *F. graminearum*, is mainly controlled with demethylation inhibitor (DMI) fungicides applied during flowering. However, the use of premixes of DMI and quinone outside inhibitor (QoI) fungicides has been encouraged in Brazil given the effects of the latter against foliar diseases that affect wheat yield in Brazil. To check whether the use of premixes are an effective and profitable choice, focusing only on wheat response, data on FHB severity and yield obtained in Brazilian field trials were gathered from both the literature and cooperative trials, totaling 73 trials spanning almost two decades (2000 - 2018) and 25 locations. A meta-analytic approach was used to summarize percent control (efficacy) and yield response; the latter provided estimates for calculating the risk of not offsetting the cost of fungicide. Four DMI+QoI and one tebuconazole treatment, applied mostly twice (full-flowering and 10 days) tested in at least 14 trials and three year each, were selected for analysis. Estimates of efficacy ranged from 44.1% (pyraclostrobin + metconazole applied once) to 64.3% (pyraclostrobin + metconazole); the latter not differing from trifloxystrobin + prothioconazole and tebuconazole (59.9% to 62.6%); but differed from azoxystrobin + tebuconazole and trifloxystrobin + tebuconazole (~58.4%). Yield response (kg/ha) was statistically similar for pyraclostrobin + metconazole and trifloxystrobin + prothioconazole (494.9 to 532.1), and both differed statistically from a group composed of tebuconazole trifloxystrobin + tebuconazole, azoxystrobin + tebuconazole (448.2 to 462) and pyraclostrobin + metconazole applied once (413.7). The two categories of FHB index (7% threshold) and two of yield (3,000 kg/ha), both in the non-treated check did not explain the heterogeneity in the estimates. The probability of not-offsetting the costs (fungicide + applications) was generally lower than 0.45 for scenarios considering two sequential sprays of the low-cost tebuconazole or one spray of pyraclostrobin + metconazole as management choices. The envisioned enhanced economic return, solely based on yield response, from using two sprays of DMI+QoI premixes to control FHB should be seen with caution given the marginal levels of profitability.

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EFFECTS OF GENETIC RESISTANCE AND FUNGICIDE APPLICATION ON FUSARIUM HEAD BLIGHT IN WHEAT AS INFLUENCED BY PRE- AND POST-ANTHESIS RAINFALL

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ABSTRACT

Fusarium head blight (FHB), caused predominantly by the fungus *Fusarium graminearum*, is a devastating disease of wheat and other small grain crops in the United States and other parts of the world. FHB significantly reduces yield and leads to grain contamination with mycotoxins under disease-favorable conditions (moderate to warm temperatures, high relative humidity, and rainfall during pre- and early post-anthesis periods). Thus, fungicides are most warranted for FHB control when wet, rainy conditions occur during and shortly after anthesis. Two field experiments were conducted during 2018 and 2019 in Wooster, OH, to investigate the efficacy of fungicide treatments in combination with genetic resistance against FHB and mycotoxins under the influence of persistent pre- and post-anthesis rainfall. The experimental design was a randomized complete block, with a split-plot arrangement of cultivar (moderately resistant [MR], moderately susceptible [MS], and susceptible [S]) as whole-plot and fungicide treatment as sub-plot. The fungicide treatments consisted of a single application of Prosaro® (prothioconazole+tebuconazole) at 50% early anthesis [PA], or at 3 [P3], 6 [P6], or 9 [P9] days after early anthesis, or PA followed by a single application of Caramba® (metconazole) at 3 [PA+C3], 6 [PA+C6], or 9 [PA+C9] days after early anthesis. Plots were individually inoculated at full flag leaf emergence (Feekes GS 9) with corn kernels colonized by *F. graminearum* and subjected to simulated rainfall during the 8 days immediately before and 12 days immediately after early anthesis. Relative to the susceptible check, percent control of FHB index (IND), deoxynivalenol (DON), zearalenone (ZEA), and percent increase in grain yield and test weight (TW) were highest when the fungicide treatments were applied to the MS or MR cultivar. When only a single fungicide application was made, PA and P3 were more efficacious than P6 and P9. Plots that received a single application of Prosaro at early anthesis followed by a post-anthesis application of Caramba had the lowest mean IND, DON, and ZEA, and highest mean grain yield and TW. PA+C3, PA+C6, and PA+C9 were of comparable efficacy against FHB, DON, and ZEA, and resulted in comparable mean grain yield and TW. Integrated management programs with an MS or MR cultivar treated with Prosaro at early anthesis followed by Caramba after anthesis provided the best results in terms of IND, DON, and ZEA reduction and grain yield and TW increase. Results from formal analyses of the data will be presented.

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RAINFASTNESS OF FUNGICIDES FOR FUSARIUM HEAD BLIGHT MANAGEMENT IN SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB), caused predominantly by the fungus *Fusarium graminearum*, and associated grain contamination with mycotoxins such as deoxynivalenol (DON), are major concerns for the production of wheat and other small grain crops in the United States and other parts of the world. Fungicides are most warranted for FHB control when wet, rainy conditions occur during anthesis and early grain fill. However, rainfall following treatment application may affect efficacy against FHB and DON, and this may vary among fungicides. Here we determined the rainfastness of the fungicides Prosaro® (prothioconazole + tebuconazole), Caramba® (metconazole), and Miravis® Ace (pydiflumetofen + propiconazole) when applied to wheat spikes at anthesis. The experimental design was a randomized complete block, with a split-plot arrangement of simulated rainfall treatment (combinations of durations [0, 15, 30, 60, and 120 min] and start times [0, 15, 30, and 60 min] after fungicide application) as whole-plot and fungicide treatment (Prosaro, Caramba, and Miravis Ace) as sub-plot. The three fungicides were applied to separate plots at their respective label-recommended rates of 6.5 (Prosaro), 13.5 (Caramba), and 13.7 (Miravis Ace) fl. oz./A, with or without the nonionic surfactant Induce (0.125 v/v). Irrigation risers mounted in each whole plot were then used to simulate the desired rainfall treatment at an intensity of 6.5 mm/h. Approximately 24-36 h after the treatments were applied, all plots were spray inoculated with a spore suspension of *F. graminearum*. All fungicide-treated plots had significantly lower mean FHB index (IND) and higher mean grain yield than the untreated check plots, regardless of rainfall treatment. When applied with the surfactant, the efficacy of the fungicides against FHB was not affected by rainfall duration, regardless of when the rainfall began after application. However, plots treated with the fungicides without the surfactant and subjected to 120 min of simulated rainfall immediately after the treatment was applied had numerically higher mean FHB index and lower mean yield that were comparable to the untreated check. These results suggested that the rainfastness of Prosaro, Caramba, and Miravis Ace to the simulated rainfall treatments was largely due to the surfactant. DON results were not available at the time of this report. The experiment will be repeated in 2021, and all data will be analyzed to formally quantify the observed effects.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nº 59-0206-8-187. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

EVALUATION OF ORGANIC COPPER FUNGICIDE APPLICATIONS PLUS CULTIVAR RESISTANCE TO REDUCE FHB AND DON INFECTION OF BARLEY IN VERMONT

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of a conventional and an organic copper fungicide on barley yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) in Vermont.

INTRODUCTION

Public interest in sourcing local foods has extended into beverages leading to a rapid expansion of the northeast malting industry. This has provided farmers with new market opportunities and many of these markets are interested in purchasing certified organic barley. However, all farmers are struggling to produce barley that is not infected with FHB and DON. Hence integrated management strategies are essential for managing yield and quality losses from FHB. Most farmers in New England have experienced significant crop loss from FHB and some farmers have already stopped growing barley. At present, few farmers are specifically selecting varieties for resistance to FHB and even fewer are combining host resistance with fungicide applications. There has been little to no research conducted to evaluate organic approved fungicides. Other regions have shown that the use of a well-timed fungicide is an important management tool when suppressing FHB in barley production. In Vermont during 2017, 2018, and 2019 we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides (organic and conventional) at two timings.

MATERIALS AND METHODS

The trial was conducted in Alburgh, VT during 2017, 2018, and 2019. The soil type was a Benson silt loam soil. The plot size was 5 x 20 ft including seven rows with 7-in spacing. Each year, planting occurred during the last week of April. Main plots were sown with barley at 125 lb ac^{-1} with a Great Plains grain drill (Salinas, KS). The experiment was set up as a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the fungicide treatments as subplots, randomized in four replicated blocks. The two spring barley varieties were 'Robust' (susceptible to FHB) and 'Conlon' (moderately resistant to FHB). Fungicide treatments are shown in Table 1. The first fungicide application (with surfactant at 0.125% V/V) was applied at heading (Feekes growth stage, FGS 10.1).After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) to augment the development of FHB. The second fungicide application occurred four days after heading, and inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. Fungicide and *F. graminearum* treatments were applied with a CO_2 backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Grain was harvested using an Almaco plot combine (Nevada, IA).. Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bushels ac^{-1} at 13.5% moisture. Analysis of DON content in grain was conducted at the University of Vermont Cereal Grain Testing

Laboratory located in Burlington, VT. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test (P = 0.05).

RESULTS AND DISCUSSION

Interactions

There were no year by variety or year by fungicide treatment interactions indicating that the treatments responded similarly regardless of year (Table 2). There was significant variety by fungicide treatment (Table 2). Caramba® and ChampION® applied 4 days after heading resulted in DON concentrations significantly lower than the inoculated control in the variety Conlon. With the variety Robust, Caramba applied 4 days after heading was significantly lower than the inoculated control. However, ChampION applied 4 days after heading did not result in DON concentrations that were significantly lower than the inoculated control.

As expected, years were significantly different in DON concentrations, test weight, and yields (Table 3). Weather conditions in Vermont during the 2017 growing season can be characterized as having higher than normal temperatures in April and lower than average temperatures in May, June, July, and August. Rainfall amounts were higher than average throughout the growing season resulting in 7.39 inches of precipitation more than normal. Higher rainfall throughout the season resulted in the highest DON concentrations observed throughout the project. The 2018 growing season was warmer and drier than average. From April through July, there was an accumulation of 3403 Growing Degree Days (GDDs), 50 GDD above the 30-year average. In 2019, April, May and June were all colder than normal. April and May had higher precipitation than the 30-year average, while June was somewhat drier. July was both hotter and drier than the 30-year average. Overall, drier and warmer conditions resulted in lower DON concentrations, higher grain yields, and test weights.

The barley varieties performed similarly in yield and test weight however as expected differed significantly in DON concentrations (Table 4). The moderately resistant variety had 47% less DON compared to the susceptible variety. When results were combined across cultivars, the fungicide treatments significantly influenced DON concentrations (Table 5). The fungicide treatment Caramba applied at heading resulted in significantly lower DON concentrations than all other fungicide treatments. The certified organic treatment of ChampION applied at heading was statistically similar to the conventional fungicide Caramba applied at heading. The barley yields did respond differently to the fungicide treatments (Table 5).

Even though all of the variety and fungicide+timing treatments resulted in DON concentrations above 1 ppm, it's important to note that Conlon, a moderately resistant variety, had lowest incidence of DON levels, while Robust, a susceptible variety, had DON levels almost double (2.94 ppm) that of Conlon (1.54 ppm). This indicates the importance of selecting resistant cultivars to manage FHB in our region.

The application of the conventional fungicides Caramba, and the organic fungicide ChampION at heading reduced DON concentrations compared to the inoculated control. In general, the fungicide applications at heading resulted in lower DON concentrations than the fungicides applied 4-days after heading. The organic approved copper fungicide appeared to provide some control of FHB but was not as effective as the conventional fungicide applied at heading. Additional research should be conducted to assess the efficacy of multiple applications of copper-based fungicide on FHB.

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a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Fungicide treatments, active ingredients and rates applied.

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
Fusarium graminearum			40,000 spores/ml
Caramba®	BASF Ag Products	Metconazole	14 fl oz ac^{-1} + Induce at 0.125% V/V
Champ ION^{++}	NuFarm	Copper hydroxide	1.5 lbs ac^{-1}

Table 2. Statistical significance of treatment effects on DON, test weight, and yield of barley.

Source of variation	DON	Test weight	Yield
Year	***†	***	***
Variety	***	NS	NS
Fungicide + timing	***	NS	NS
Variety x fungicide + timing	NS	***	NS
Year x variety	***	NS	**
Year x fungicide timing	NS	NS	NS
Year x variety x fungicide timing	NS	NS	NS

†statistical significance - ***, p=0.001; **, p= 0.01; *, p= 0.05; NS, not significant

Table 3. Effect of year on deoxynivalenol (DON) concentration, grain yield, and test weight at Alburgh.

Year	DON	Test weight	Yield
	ppm	lb bu^{-1}	bu ac^{-1}
2017	6.05	44.4	62.7
2018	0.46	48.2	76.3
2019	0.18	46.8	82.5
LSD (p=0.05)	0.32	0.50	5.03

Table 4. Main effect of cultivar on deoxynivalenol (DON) concentration, grain yield, and test weight at Alburgh, VT across years.

Variety	**DON**	**Test weight**	**Yield**
	ppm	**lb bu^{-1}**	**bu ac^{-1}**
Conlon (moderately resistant)	1.55	46.7	71.8
Robust (susceptible)	2.94	46.4	75.9
LSD (p=0.05)	0.25	NS	NS

Table 5. Main effect of cultivar susceptibility on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT.

Fungicide + timing	**DON**	**Test weight**	**Yield**
	ppm	**lb bu^{-1}**	**bu ac^{-1}**
Non-sprayed, non-inoculated control	1.54	46.7	77.0
Inoculated FGS 10.1	2.91	46.4	72.0
Caramba (14 fl oz) at heading	1.81	46.4	71.6
Caramba (14 fl oz) 4 days after heading	2.39	47.0	76.6
ChampION (1.5 lbs) at heading	2.14	46.2	71.8
ChampION (1.5lbs) 4 days after heading	2.60	46.3	74.2
LSD (p=0.05)	0.45	NS	NS

RISING CO_2 MORE SEVERELY IMPACTS FHB MODERATELY RESISTANT HRSW COMPARED TO SUSCEPTIBLE CULTIVARS

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ABSTRACT

Rising carbon dioxide (CO_2) can change the nutritional content of wheat and increase the severity of Fusarium head blight (FHB), a devastating fungal disease of wheat that reduces yield and contaminates grain with harmful mycotoxins. At elevated CO_2, FHB susceptible and moderately resistant hard red spring wheat had disproportionate losses in nutritional content, with moderately resistant cultivars more severely impacted. Decreases in the nutritional content of wheat may provide a pathogenic advantage to the fungus, threatening global food safety and security. Furthermore, moderately resistant wheat had significantly greater increases in plant height at elevated CO_2, which may increase lodging risk. Declining grain quality and diminished efficacy of FHB resistance factors may deter wheat growers from choosing moderately resistant cultivars. Therefore, FHB control strategies should consider the climate resilience of wheat nutritional content.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. This research was supported by the U.S. Department of Agriculture, Agricultural Research Service. (ORCID ID: 0000-0001-8784-6591)

INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT (FHB) AND DON IN WHEAT IN VIRGINIA WITH AN EMPHASIS ON NEW FUNGICIDE, MIRAVIS ACE

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ABSTRACT

Fusarium head blight (FHB), an important disease of small grains caused by *Fusarium graminearum*, significantly reduces yield and quality of grains worldwide. In addition, *F. graminearum* contaminates grain with deoxynivalenol (DON), which impacts both human and animal health. Disease management approaches that integrate planting of moderately resistant varieties and well-timed applications of effective fungicides can reduce FHB and DON and protect grain yields. New wheat varieties and fungicide chemistries are available, and the effectiveness of these for management of FHB and DON needs to be assessed. The objectives of our study were to 1) evaluate the efficacy of fungicide application timings and chemistries with an emphasis on a new fungicide, Miravis® Ace, and 2) assess the integrated effects of applying fungicides and planting new FHB resistant wheat varieties in Virginia. The experiment was conducted at Suffolk, Virginia and three new varieties moderately resistant to FHB ('Hilliard', 'Liberty 5658', and 'AgriMaxx 463') along with an FHB susceptible variety ('Shirley') were planted. Evaluated fungicides included prothioconazole + tebuconazole (Prosaro®, Bayer CropScience), pydiflumetofen + propiconazole (Miravis Ace, Syngenta) and tebuconazole (Folicur®, Bayer CropScience). Fungicide rates per acre and application timings included 1) an untreated control, 2) Prosaro 6.5 fl oz at anthesis, 3) Miravis Ace 13.7 fl oz at anthesis, 4) Miravis Ace 13.7 fl oz at heading, and 5) Miravis Ace 13.7 fl oz at anthesis followed by Folicur 4.0 fl oz 4-6 days after anthesis. The experiment was a factorial randomized block design with four varieties, five fungicide treatments, and four replicates. All plots were inoculated with *F. graminearum* conidia 24 hours after anthesis. Foliar disease and FHB incidence and severity were rated at the soft dough stage, and yield, test weight, percent Fusarium damaged kernels (FDK), and DON concentrations were determined following harvest. Leaf rust (*Puccinia triticina*) was the major foliar disease observed, and severity differed among varieties but not fungicide treatments. Environmental conditions during the flowering stage of the crop were not conducive for FHB development, so overall FHB incidence and severity was low. However, plots treated with Miravis Ace at heading or anthesis had less FHB incidence than the untreated control. Yield, test weight, and FDK did not differ among the fungicide treatments or varieties. DON concentrations were low (≤1.1 ppm) in all treatments including the untreated controls. Results indicate that though the new fungicide Miravis Ace effectively reduces FHB incidence, when environmental conditions are not favorable for the FHB development, fungicide applications have little to no impact on grain yield and quality. In contrast to previous studies in which foliar disease and/or FHB severity were high, wheat varieties with variable levels of disease resistance did not differ in overall grain yield.

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FUSARIUM HEAD BLIGHT MANAGEMENT COORDINATED PROJECT: INTEGRATED MANAGEMENT TRIALS 2018-2020

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INTRODUCTION

Efforts to evaluate integrated management strategies for Fusarium head blight (FHB) and deoxynivalenol (DON) management in wheat and barley continued in 2018, 2019, and 2020. The focus over these three years of the integrated management coordinated project (IM_CP) was Miravis Ace®, a new Succinate Dehydrogenase Inhibitor (SDHI; Adepidyn - Pydiflumetofen) + Demethylation Inhibitor (DMI; Propiconazole) premix fungicide that was recently labeled for managing diseases of wheat, barley, and other small grain crops. Preliminary results from a limited number of trials showed that when applied at early anthesis (Feekes 10.5.1) or within the first 6 days after early anthesis, Miravis Ace was just as effective as Prosaro® and Caramba® (3,4,5). This suggested that like the latter two fungicides, this new fungicide alone will not be sufficient to manage FHB and DON under highly favorable conditions. Based on results from the 2018 and 2019 IM_CP, Miravis Ace® was most effective against FHB and DON when combined with genetic resistance, but the magnitude of the effect varied among trials, particularly when Miravis Ace was applied at early heading. The IM_CP experiment was repeated in 2020 following protocols similar to those used

in 2018 and 2019, with the primary modification being the inclusion of a treatment consisting of the application of Miravis Ace at anthesis followed by tebuconazole 4-6 days later. Again, the overall objective was to evaluate the integrated effects of fungicide programs and genetic resistance on FHB and DON, with emphasis on the new fungicide, Miravis Ace. Results from the last three years are summarized herein.

MATERIALS AND METHODS

To accomplish the aforementioned objective, field experiments were conducted in 22 US wheat-growing states in 2018, 2019 and 2020. The standard protocol consisted of the application of fungicide treatment programs (sub-plot; Table 1) to plots of FHB-susceptible (S), -moderately susceptible (MS), and -moderately resistant (MR) cultivars (whole-plot). Hereafter, the combinations of fungicide programs by cultivar resistance classes will be referred to as: MR_CK (MR untreated), MR_I (MR treated with Prosaro at early anthesis [Feekes 10.5.1]), MR_II (MR treated with Miravis Ace at early anthesis), MR_III (MR treated with Miravis Ace at early heading [Feekes 10.3-5]), and MR_IV (MR treated with Miravis Ace at early anthesis followed by tebuconazole 4-6 days after anthesis [DAA]) for MR the cultivar. When referring to the same fungicide programs applied to the MS and S, the combinations were labelled MS_CK, MS_I, MS_II, MS_III, MS_IV, S_CK, S_I, S_II, S_III and S_IV. The experimental design was a randomized complete block, with at least 4 replicate blocks. In most experiments, plots were spray inoculated with a spore suspension of the fungus *Fusarium graminearum* approximately 24-36 h after the anthesis treatments were applied, with or without mist-irrigation. Trials were naturally infected at some locations. FHB index (IND) was rated or calculated as previously described (2,6) on 60-100 spikes per plot at approximately Feekes 11.2. Plots were harvested and a sample of grain from each experimental unit was sent to a USWBSI-supported laboratory for mycotoxin analysis. Linear mixed models (multi-location) were fitted to the pooled arcsine square root-transformed IND and log-transformed DON data to evaluate the main and interaction effects of fungicide treatment and genetic resistance on IND and DON.

RESULTS AND DISCUSSION

Mean Fusarium head blight index (IND) and deoxynivalenol (DON) grain contamination data from 57 environments (trial x state x year combinations) are shown in Figures 1 and 2, respectively. The environments represent spring and winter wheats from five market classes (durum, hard red spring, hard red winter, soft red winter, and soft white winter).

FHB index: Means varied across the 57 environments and among management combinations within environments as shown by the spread of the data points around the median in Fig 1A. Means ranged from 0 to 80% across management combinations and were more variable across environments on S (interquartile range [IQR] 6 to 22%) and MS (IQR 3 to 10%) cultivars than on MR (IQR 2 to 7%) cultivars. This in part reflects the fact that there were fewer environments with S and MS cultivars than with MR cultivars (Fig. 1A). The susceptible, nontreated check (S_CK) had the highest mean IND (18%), whereas the application of Miravis Ace at anthesis (MR_II) and the Miravis Ace at anthesis followed by tebuconazole 4-5 days later (MR_IV) to a moderately resistant cultivar resulted in the lowest means (2.7 and 2.6%, respectively) (Fig. 2A). For all tested resistance classes, all fungicide programs resulted in significantly lower mean IND (on the arcsine square root-transformed scale) than the nontreated check (Table 2). Pairwise differences between fungicide programs were statistically significant ($P < 0.05$). The only exceptions were for comparisons between Prosaro at anthesis and Miravis Ace at early heading on MS and S cultivars.

Deoxynivalenol: Mean DON ranged from 0 to 59 ppm across environments and management combinations (Fig. 1B). Contrary to what was observed for IND, MS_III (application of Miravis

Ace to MS cultivars at Feekes 10.3-5) had the highest mean level of DON contamination on the raw data scale. However, on the log-transformed scale, S_CK had the highest mean. Sequential application of Miravis Ace at anthesis followed by tebuconazole 4-6 days later had the lowest overall mean DON contamination. For programs with a single fungicide application, management combinations consisting of a Prosaro or Miravis Ace application at anthesis to an MR (MR_I and MR_II) cultivars had the lowest overall mean DON contamination (Fig. 1B and 2B, and Table 2). Within each resistance class, all treatments resulted in significantly lower mean DON (on the log-transformed scale) than the nontreated check, and Miravis Ace at anthesis and Prosaro at anthesis had significantly lower log-transformed DON and the Feekes 10.3-5 application of Miravis Ace. Pairwise differences between fungicide programs were statistically significant, except for comparisons between MS_I and MS_III, MS_I and MS_II, and S_I and S_II.

As additional data become available, a more complete set of analyses will be performed. However, the results summarized herein suggest that while a Feekes 10.3-5 application of Miravis Ace may suppress FHB IND to levels comparable to those achieved with an anthesis application of Miravis Ace or Prosaro, such an early application is considerably less effective than the anthesis applications in terms of DON suppression.

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Table 1. The following core treatments were randomly assigned to experimental units. All fungicide treatments were applied along with a nonionic surfactant.

Treatment[a]	Product	Rate	Timing
1 (CK)	Untreated check	…	…
2 (I)	Prosaro	6.5 fl oz/A	Anthesis
3 (II)	Miravis Ace	13.7 fl oz/A	Anthesis
4 (III)	Miravis Ace	13.7 fl oz/A	Feekes 10.3
5 (IV)[*]	Miravis Ace fb Tebuconazole	13.7 fl oz/4 fl ozA	Anthesis/4-5 DAA

[*]Only tested in 2020, DAA = days after anthesis

Table 2. LS-Mean arcsine square root-transformed FHB index (arcIND) and log-transformed deoxynivalenol (lDON) contamination of wheat grain for different fungicide program x cultivar resistance management combinations. LS-Means with the same letters are not significantly different at α=0.05.

Management combination[*]	**arcIND (%)**		**lDON (ppm)**	
S_CK	0.390	A	1.35	A
MS_CK	0.255	B	1.07	B
S_I	0.249	B	1.07	B
S_III	0.246	B	0.99	BC
MR_CK	0.218	C	0.97	BC
S_II	0.200	CD	0.94	BCDE
MS_I	0.183	DE	0.91	CDEF
MS_III	0.168	EF	0.85	DEF
MR_III	0.155	FG	0.81	EF
MS_II	0.138	GH	0.76	FG
MR_I	0.135	H	0.67	G
MR_II	0.111	I	0.58	H

[*]**S**, **MS**, and **MR** represent susceptible, moderately susceptible, and moderately resistant, respectively, whereas **CK** = nontreated, **I** = treated with Prosaro (6.5 fl. oz.) at Anthesis, **II** = treated with Miravis Ace (13.7 fl. oz.) at anthesis, **III** = treated with Miravis Ace (13.7 fl. oz.) between Feekes 10.3 (early head emergence) and 10.5 (complete head emergence).

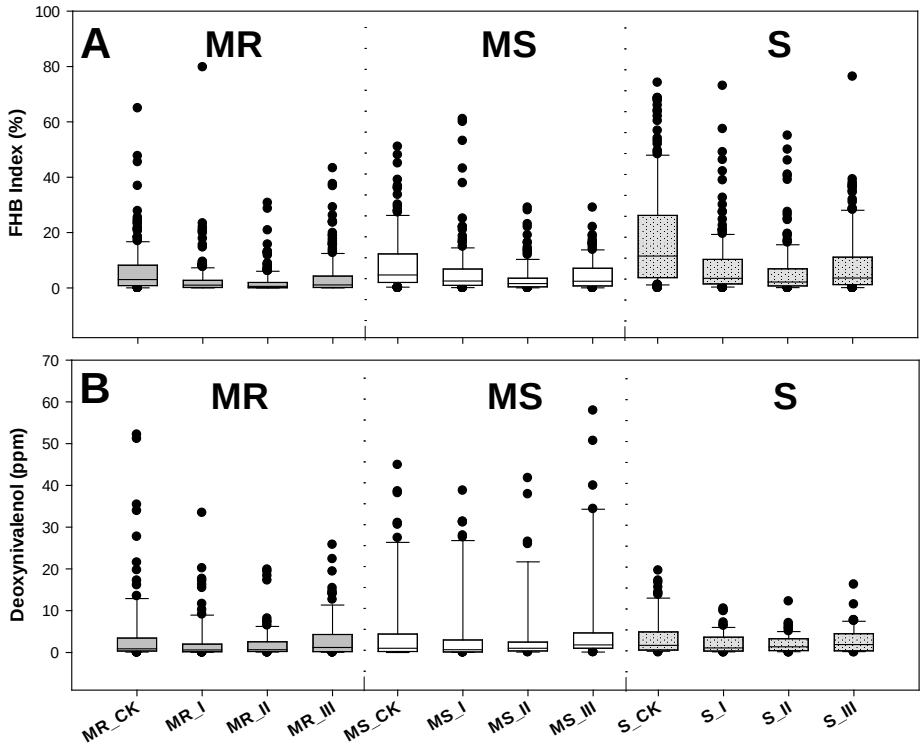

Figure 1. Boxplots showing the distribution of **A**, mean Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide program x cultivar resistance management combinations. **S**, **MS**, and **MR** represent susceptible, moderately susceptible, and moderately resistant, respectively, whereas **CK** = nontreated, **I** = treated with Prosaro (6.5 fl. oz.) at Anthesis, **II** = treated with Miravis Ace (13.7 fl. oz.) at anthesis and **III** = treated with Miravis Ace (13.7 fl. oz.) between Feekes 10.3 (early head emergence) and 10.5 (complete head emergence).

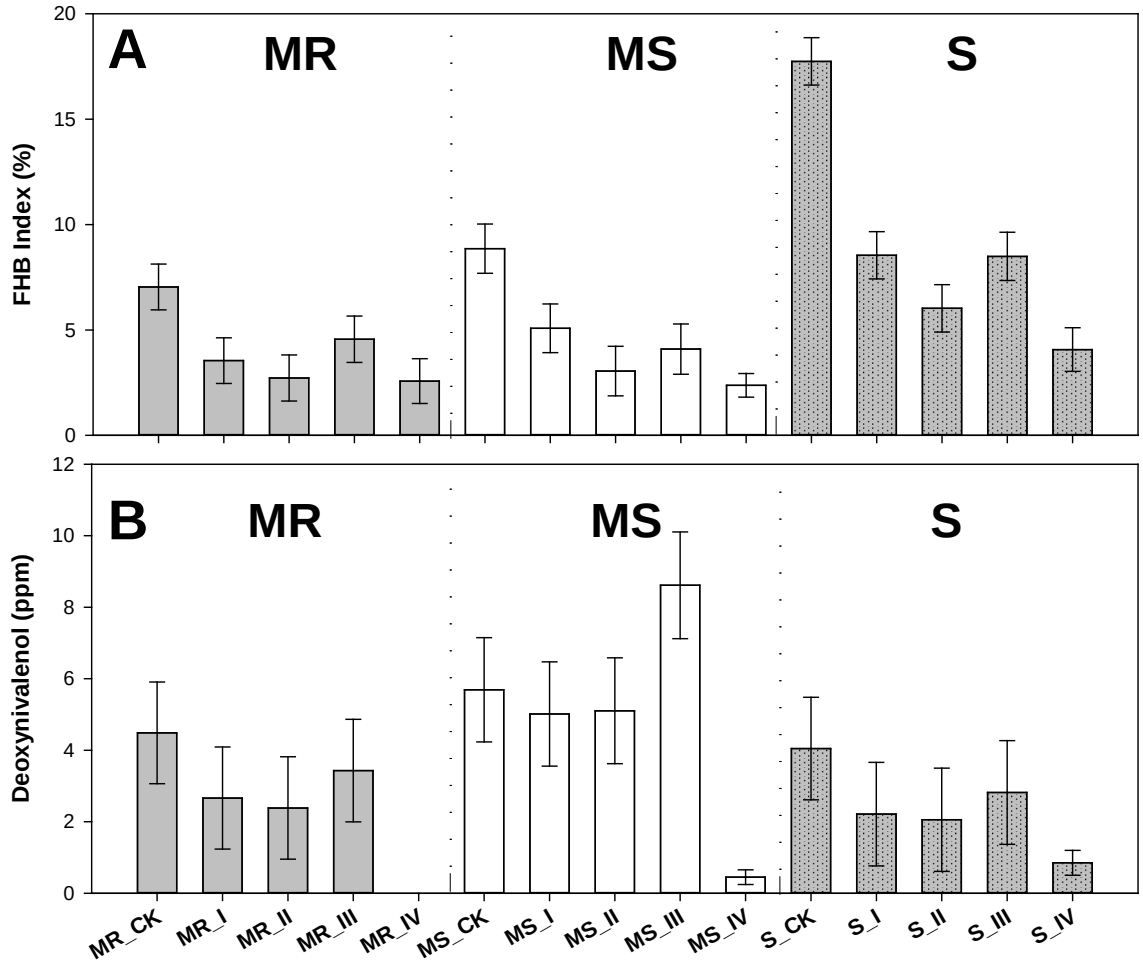

Figure 2. Arithmetic mean **A**, Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide program x cultivar resistance management combinations. **S**, **MS**, and **MR** represent susceptible, moderately susceptible, and moderately resistant, respectively, whereas **CK** = nontreated, **I** = treated with Prosaro (6.5 fl. oz.) at Anthesis, **II** = treated with Miravis Ace (13.7 fl. oz.) at anthesis, **III** = treated with Miravis Ace (13.7 fl. oz.) between Feekes 10.3 (early head emergence) and 10.5 (complete head emergence), and **IV** = treated with Miravis Ace (13.7 fl. oz.) at anthesis followed by tebuconazole (4 fl. oz.) at 4-5 days after anthesis. Errors bars are standard error of the mean.

FUSARIUM HEAD BLIGHT MANAGEMENT COORDINATED PROJECT: UNIFORM FUNGICIDE TRIALS 2018-2020

Jane Marian Luis[1], Sin Joe Ng[1], Gary Bergstrom[2], Kaitlyn Bissonnette[22], Kira Bowen[5], Carl Bradley[4], Emmanuel Byamukama[6], Martin Chilvers[11], Alyssa Collins[23], Christina Cowger[8], Heather Darby[13], Erick DeWolf[21], Ruth Dill Macky[12], Paul Esker[7], Andrew Friskop[9], Nathan Kleczewski[3], Alyssa Koehler[14], David B. Langston[16], Laurence Madden[1], Juliet Marshall[15], Hillary Mehl[16], Wanderson Moraes[1], Martin Negelkirk[11], Nidhi Rawat[10], Damon Smith[18], Darcy Telenko[19], Stephen Wegulo[24], Heather Young-Kelly[20] and Pierce A. Paul[1*]

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INTRODUCTION

Uniform fungicide trials (UFT) were conducted over the last years (2018, 2019, and 2020) to compare the efficacy of Miravis Ace®, a new Succinate Dehydrogenase Inhibitor (SDHI; Adepidyn/Pydiflumetofen) + Demethylation Inhibitor (DMI; Propiconazole) fungicide, when applied at, before, or after anthesis, or sequentially with a DMI fungicide to that of a standard anthesis-only application of Prosaro® or Caramba®. Miravis Ace was recently labeled for management of diseases of wheat, barley, and other small grain crops, and preliminary results from a limited number of trials showed that when applied at early anthesis (Feekes 10.5.1) or within the first 6 days after anthesis, it was just as effective as Prosaro and Caramba (2,3). However, one of the primary questions addressed in the UFTs was whether Miravis Ace was just as effective when applied at Feekes 10.3 (early heading). If it is, this will extend the application window to as many as 10 days, allowing greater flexibility in terms of application timing. In addition, having a new, effective fungicide, particularly one of a different chemistry, and a wider application window will create opportunities for evaluating two-treatment fungicide programs for FHB and DON

management. Several two-treatment programs were evaluated in this study. Results from the three years are summarized herein.

MATERIALS AND METHODS

To accomplish the aforementioned objective, field experiments were conducted in 22 US wheat-growing states in 2018, 2019 and 2020. The standard protocol consisted of the application of fungicide treatments (**Table 1**) to plots of a susceptible cultivar. The experimental design was a randomized complete block, with at least 4 replicate blocks. In all experiments, plots were artificially inoculated with either *F. graminearum*-colonized grain spawn or a spore suspension of the fungus applied approximately 24-36 h after anthesis. Plots were mist-irrigated during and shortly after anthesis in some experiments to enhance inoculum production and infection. FHB index (IND) was rated or calculated as previously described (1) on 60-100 spikes per plot at approximately Feekes growth stage 11.2. Grain was harvested and samples were sent to a USWBSI-supported laboratory for mycotoxin analysis. Linear mixed models (multi-location) were fitted to the pooled arcsine square root-transformed IND and log-transformed DON data to evaluate treatment effects. Efficacy of fungicide treatment was estimated using percent reduction in IND and DON relative to the nontreated check.

RESULTS AND DISCUSSION

Mean Fusarium head blight index (IND) and deoxynivalenol (DON) contamination data from 42 environments (trial x state x year combinations), representing different wheat market classes, are summarized for different fungicide treatments in Figures 1 and 2. FHB IND ranged from 0 to 69% and DON from 0.07 to 39 ppm across all environments. For both responses, the nontreated check has the highest means, whereas treatments that consisted of an early anthesis (Feekes 10.3.1) application of Miravis Ace followed by an application of Prosaro, Caramba, or tebuconazole 4-6 days later had the lowest means (**Fig. 1** and **2**).

FHB index: Means varied across treatment and environments, as shown by the distribution of data points around the median in Figure 1. All treatments resulted in significantly lower mean FHB IND (on the arcsine square root-transformed scale) than the nontreated check (**Fig. 2A**). Single fungicide treatments applied at anthesis reduced mean IND by 57 (Caramba), 58 (Prosaro), and 70% (Miravis Ace), relative to the untreated check (**Fig. 1A, 2A**). A single application of Miravis Ace applied at early heading (Feekes 10.3) or 4-6 days after anthesis reduced mean IND by 59 and 67%, respectively. The greatest reduction in mean IND were observed from the sequential applications of Miravis Ace and a DMI, with percent control ranging from 77 (Miravis Ace followed by Prosaro) to 88% (Miravis Ace followed by tebuconazole). Miravis Ace followed by tebuconazole is not significantly different (on the arcsine square root-transformed scale) from Miravis Ace followed by Caramba. Sequential treatments consisting of Miravis Ace followed by Prosaro and Miravis Ace followed by Caramba were also not significantly different from each other.

Deoxynivalenol: All treatments resulted in significantly lower mean DON contamination of grain (on the transformed scale) than the nontreated check (**Fig. 2B**). All treatments with an application at anthesis and/or within the first six days after anthesis resulted in significantly lower mean DON than the early heading application of Miravis Ace (**Fig 2B**). Among the treatments with a single application at anthesis, Miravis Ace resulted in the highest percent reduction in mean DON (44%), followed by Prosaro (33%), and Caramba (31%). Treatments with sequential applications of Miravis Ace followed by a DMI had lower mean DON (2.0 to 2.5 ppm) than a single application of Miravis Ace at early head emergence (5.4 ppm), anthesis (3.4 ppm), or post-anthesis (3.3 ppm). Relative to the check, sequential treatments reduced mean DON by 58 (Miravis Ace followed by Caramba), 59 (Miravis Ace followed by Prosaro), and 67% (Miravis Ace followed by tebuconazole).

As additional data become available, a more complete set of analyses will be performed. However, the results summarized herein suggest that an application of Miravis Ace at Feekes 10.3 may suppress FHB IND to levels comparable to those achieved with an anthesis application, but such an early application is considerably less effective than a single anthesis or post-anthesis application in terms of DON suppression. More effective control is achieved when Miravis Ace is applied sequentially with a DMI fungicide.

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Table 1. The following treatments were randomly assigned to experimental units. All fungicide treatments were applied along with a nonionic surfactant.

	Treatment - product, rate and timing
Core	
1	Nontreated check
2	Prosaro at 6.5 fl oz/A at anthesis
3	Caramba at 13.5 fl oz/A at anthesis
4	Miravis Ace at 13.7 fl oz/A at Feekes 10.3
5	Miravis Ace at 13.7 fl oz/A at anthesis
6	Miravis Ace at 13.7 fl oz/A at anthesis followed by Prosaro at 6.5 fl oz/A 4-6 days after
7	Miravis Ace at 13.7 fl oz/A at anthesis followed by Caramba at 13.5 fl oz/A 4-6 days after
Optional	
8	Miravis Ace at 13.7 fl oz/A at anthesis followed by tebuconazole at 4 fl oz/A 4-6 d after
9	Miravis Ace at 13.7 fl oz/A at 4-6 days after anthesis

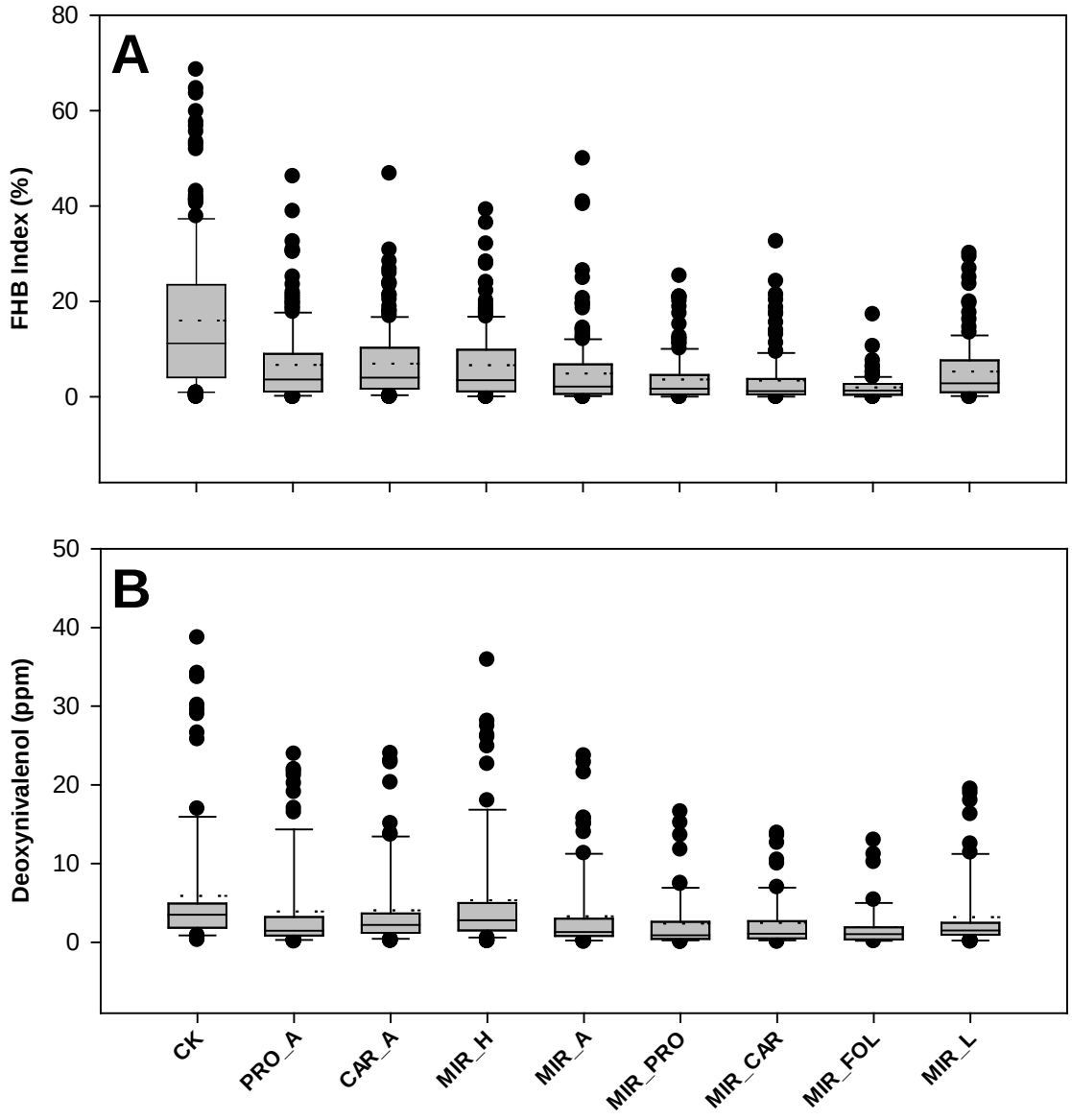

Figure 1. Boxplots showing the distribution of **A**, mean Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide treatments. **PRO_A** = Prosaro at 6.5 fl. oz applied at anthesis, **CAR_A** = Caramba at 13.5 fl. oz applied at anthesis, **MIR_H** = Miravis Ace at 13.7 fl. oz applied at Feekes 10.3-5, **MIR_A** = Miravis Ace at 13.7 fl. oz applied at anthesis, **MIR_PRO** = Miravis Ace at anthesis followed by Prosaro 4-6 days later, **MIR_CAR** = Miravis Ace at anthesis followed by Caramba 4-6 days later, **MIR_FOL** = Miravis Ace at anthesis followed by Tebuconazole (4 fl. oz) 4-6 days later, and **MIR_L** = Miravis Ace applied at 4-6 days after anthesis.

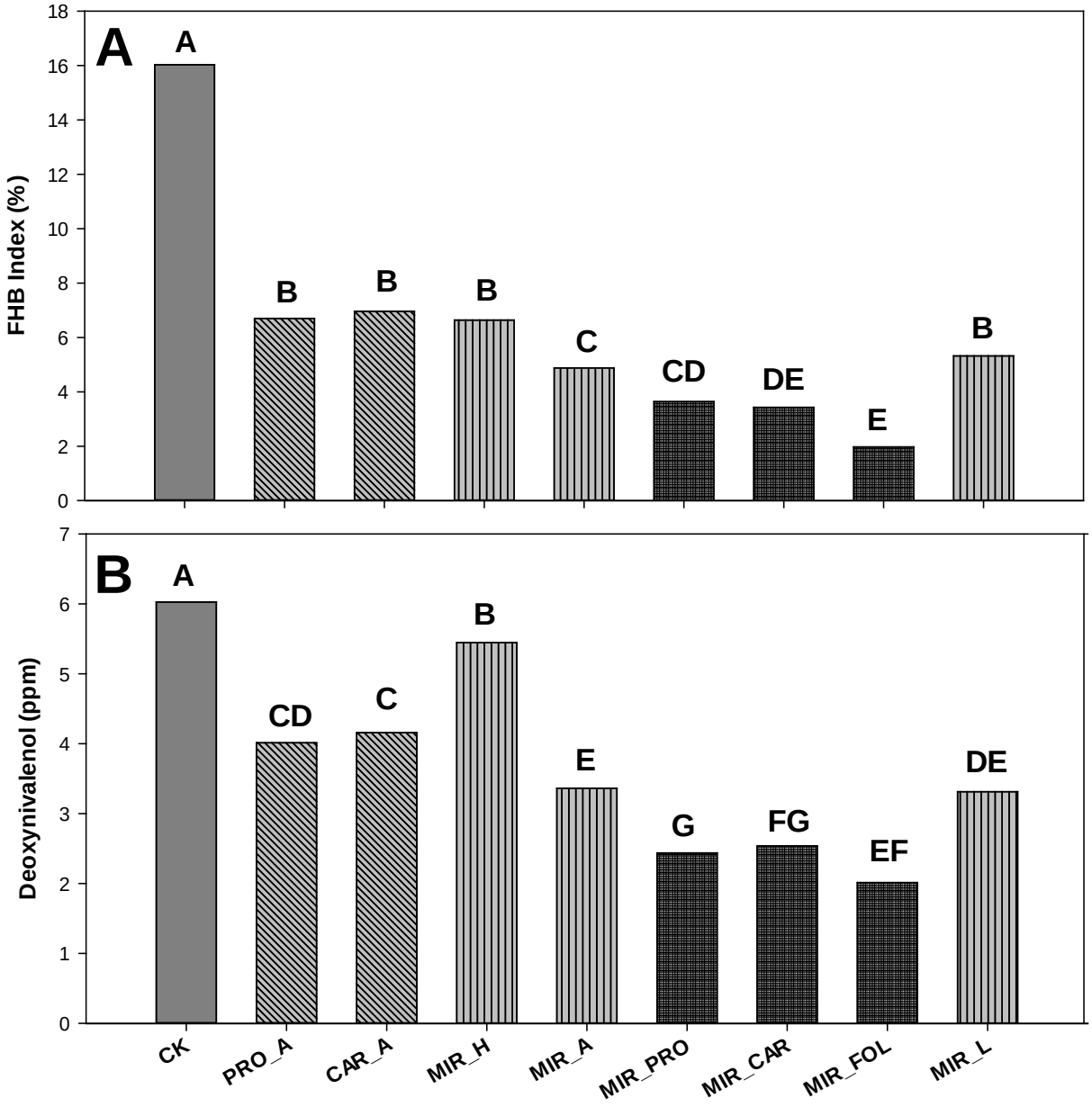

Figure 2. Mean **A**, Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide treatments. **PRO_A** = Prosaro at 6.5 fl. oz applied at anthesis, **CAR_A** = Caramba at 13.5 fl. oz applied at anthesis, **MIR_H** = Miravis Ace at 13.7 fl. oz applied at Feekes 10.3-5, **MIR_A** = Miravis Ace at 13.7 fl. oz applied at anthesis, **MIR_PRO** = Miravis Ace at anthesis followed by Prosaro 4-6 days later, **MIR_CAR** = Miravis Ace at anthesis followed by Caramba 4-6 days later, **MIR_FOL** = Miravis Ace at anthesis followed by Tebuconazole (4 fl. oz) 4-6 days later, and **MIR_L** = Miravis Ace applied at 4-6 days after anthesis. Mean differences were based on arcsine square root-transformed IND and log-transformed DON data but graphs are shown in raw data for convenience.

EFFICACY OF ALTERNATIVE OIL-BASED FUNGICIDES ON THE MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SPRING WHEAT

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum*, is one of the most important diseases affecting wheat. It results in grain yield loss and seed quality reduction and accumulation of mycotoxins. Synthetic fungicides normally used against *F. graminearum* are from mainly one class of fungicides, the triazoles. Therefore, there is a need for implementing new alternatives for managing FHB. The objective of this study was to evaluate the efficacy of essential oils (EOs) and petroleum oils (POs) in the management of FHB. The study was conducted *in vitro* using inhibitory disk diffusion and conidia germination assays and *in vivo* under greenhouse and field conditions. Sixteen alternative oil products were tested and the Prosaro® fungicide (Prothioconazole + Tebuconazole) and untreated check were control checks. Percent mycelial growth inhibition and percent spore germination were calculated. The POs treatments showed a 90 to 100 % mycelial growth inhibition, in the poisoned plate technique, and a 95 to 100 % spore germination inhibition. EOs showed limited inhibitory properties against F. *graminearum* except for three treatments that exhibited 50 to 60 mycelial growth inhibition in the poisoned plate technique and 100 % spore germination inhibition. For both greenhouse and field studies, spring wheat spiklets of select variety at anthesis were inoculated, by spraying *F. graminearum* spore suspension and were then treated with Eos and Pos after two days. Spikelets were then evaluated 21 days after inoculation for FHB incidence and severity and FHB disease index (DI) was calculated. Plants treated with POs had a significantly lower FHB DI ($P < 0.05$) and higher grain yield ($P < 0.05$) relative to the non-treatment check. This study provides useful information for FHB management using alternative oil fungicides in wheat.

DEVELOPING RISK MODELS TO MITIGATE FUSARIUM HEAD BLIGHT IN WESTERN CANADIAN CEREAL PRODUCTION

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ABSTRACT

Fusarium Head Blight (FHB) is a destructive disease that affects small-grain cereal crops in Canada, especially when weather conditions are favorable. In recent years, changes in the Fusarium species complex have been reported in Canada. Thus, existing models that were developed for predicting FHB risk many years ago may no longer be representative. Therefore, this study aims to develop and validate FHB index, *Fusarium* damaged kernel (FDK), and deoxynivalenol (DON) weather-based risk models for spring wheat, winter wheat, barley, and durum across three Canadian Prairie Provinces. Data collected from 15 sites in western Canada during 2019 were categorized into epidemic and non-epidemic using a 10% FHB index threshold for FHB and 0.8% for FDK to develop logistic weather-based predictions of FHB infections in various cereal crop types. Stepwise logistic regression using weather conditions for 8 days up to and including mid-anthesis (8 days+A) and 15 days up to and including mid-anthesis (15 days+A was run to identify combinations of temperature, relative humidity, precipitation, and solar radiation as potential predictor variables. The model sensitivity, specificity, and receiver operating characteristic (ROC) curve analysis were used to define model usefulness for prediction. Three of the FHB index models selected for three different crop types had a sensitivity range of (54-78%) and a specificity range of (76-98 %), while the FDK models selected for the same crop types had a sensitivity range of (31-92%) and specificity range (92-99%). Fusarium head blight pressure was low in 2019, most likely due to unfavorable weather conditions. Average temperature, mean daily RH, and precipitation ranged from 14.5 to 20.6^{O}C, 62.5 to 82.6%, and 0.12 to 4.6 mm respectively across sites for 15 days+A and ranged from 15.1 to 21.7^{O}C, 60 to 86.4%, and 0.2 to 8.5 mm for 8 days+A. The weather variables selected in the FHB index models were RH and air temperature at 8 days+A, and the weather variable selected in the FDK models was RH at 8 and 15 days+A. Increased predictive accuracy is expected as data for 2020 and 2021 is added to the logistic regression dataset for model development.

RAPID ASSESSMENT OF WHEAT FUSARIUM HEAD BLIGHT USING COLOR IMAGING AND DEEP LEARNING

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ABSTRACT

The development of resistant cultivars is one means of ameliorating the devastating effects of Fusarium head blight (FHB), but the breeding process requires the evaluation of hundreds of lines each year for reaction to the disease. In this study, mask region convolutional neural network (Mask-RCNN) allowed for reliable identification of wheat spikes and the corresponding diseased areas in images. Images with annotated spikes and sub-images of individual spikes with labelled diseased areas were used as ground truth data to train Mask-RCNN models for automatic image segmentation of wheat spikes and FHB diseased areas, respectively. The feature pyramid network (FPN) based on ResNet-101 network was used as the backbone of Mask-RCNN for constructing the feature pyramid and extracting features. After generating mask images of wheat spikes from full-size images, Mask-RCNN was performed to predict diseased areas on each individual spike. This protocol enabled the rapid recognition of wheat spikes and diseased areas with the detection rates of 77.76% and 98.81%, respectively. This study demonstrates the feasibility of rapidly determining levels of FHB in wheat spikes, which will greatly facilitate the breeding of resistant cultivars.

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IDENTIFICATION OF FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN WINTER WHEAT POPULATION USING ADAPTED, HIGH YIELDING WINTER WHEAT CULTIVARS 'MARKER' AND 'UGRC RING'

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ABSTRACT

Fusarium head blight (FHB) is one of the most serious diseases of wheat. FHB reduces grain yield and quality, and *Fusarium graminearum* (FG) produces mycotoxins, such as deoxynivalenol (DON). Adapted wheat cultivars are commonly used as FHB sources of resistance in North America, with the goal to develop wheat resistant to FHB, without yield and quality penalties. Winter wheat is mainly grown in Eastern Canada (Ontario and Quebec), Manitoba and Alberta. A soft red doubled-haploid (DH) winter wheat population (Ca14-19) was screened for resistance to FHB in Ridgetown, Ontario and Carman, Manitoba in 2019. The parents were the high yielding winter wheat cultivars, 'Marker' and 'UGRC Ring', which are commercially grown in Eastern Canada (www.gocereals.ca). At anthesis, progenies (n=101), parents and check cultivars were spray inoculated with a mixture of FG isolates. FHB symptoms were recorded as incidence and severity, and a FHB index was calculated. FHB incidence, severity and index at Carman were 16.6%, 37.0% and 7.2%, respectively. At Ridgetown, FHB levels were 58.7%, 45.4% and 26.9% for incidence, severity and index, respectively. Line Ca 14-019-161 had the lowest FHB index at both locations (0.3% at Carman and 9.6% at Ridgetown). Transgressive segregants, with a higher level of FHB resistance than both parents, were identified and will be screened for DON level, yield and quality performance. Progenies with good FHB resistance are identified faster by using DH technology and adapted sources of FHB resistance. We expect that new cultivar(s) will be developed from this population.

EVALUATION OF WINTER WHEAT VARIETIES AND SELECTIONS FOR FHB RESISTANCE IN SOUTHEAST IDAHO

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (*Fg*), is a destructive fungal disease of wheat that causes significant yield losses and quality reduction by producing mycotoxins, mainly deoxynivalenol (DON). Forty-one hard winter and forty-six soft white winter wheat varieties and selections obtained from various breeding programs were evaluated for FHB resistance using artificial inoculation under field conditions in Kimberly, Idaho. A mixture of 10 *Fg* isolates collected in Idaho were used to produce *Fg* inoculum (corn spawn and conidial suspension). Each entry was planted in two head-rows in two replications. Corn spawn was spread in the field when plants were at tiller stage in the spring. Additional inoculation of the trial was conducted by spraying the conidial suspension (100,000 spores/ml) at early anthesis. The trial field was irrigated and a supplemental misting system installed to create a conducive environment for disease infection and development. FHB incidence and severity were recorded at the soft dough growth stage from 30 heads of each entry. Disease index was calculated from incidence and severity data. DON content of each variety was determined at the University of Minnesota. FHB mean incidence, severity and index ranged from 0 – 80%, 0 – 70.5% and 0 – 56.4%, respectively. Varieties UIL17-64 CL+, WB4623 CLP and OR2130118H were among the varieties and selections with the lowest FHB incidence, severity and index. On the other hand, UIL17-65 CL+, Caledonia and LCS Ghost (LCS74143) had the highest FHB incidence, severity and index. Only one variety, 'Deloris,' had DON content of <1 ppm (0.79 ppm). All the remaining varieties and selections had DON content of higher than 3 ppm, which is above the minimum threshold level for human consumption. The variety with the highest DON content was WB1529 with 39.4 ppm. The results suggested that there is a need to vigorously evaluate more varieties to identify some that support low DON content under the southeast Idaho conditions in the event FHB starts to spread and become a problem in the region.

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FOOD SAFETY AND TOXICOLOGY

CLOVE OIL-IN-WATER NANOEMULSION MITIGATES GROWTH OF *FUSARIUM GRAMINEARUM* AND TRICHOTHECENE MYCOTOXIN PRODUCTION DURING THE MALTING OF FUSARIUM INFECTED BARLEY

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ABSTRACT

Fusarium and mycotoxin contamination in malting barley is of great concern because it may carry over or even be produced during the malting process, thus representing consumer health issue. Our recent study found that clove oil nanoemulsions can act in vitro as highly efficient agents against the growth of *Fusarium graminearum*, sporulation, and mycotoxin synthesis. As such, we explored the efficacy of clove oil nanoemulsions on *Fusarium* growth and inhibition of mycotoxin production during malting process. The impact of emulsifier types (Tween 80, BSA and quillaja saponins) on the formation and stabilization of clove oil nanoemulsion was evaluated, and the mitigation effects on mycotoxin levels, fungal biomass of malt process subsamples (steeping, germination and kilning), the clove oil flavor residues on final malts were measured. We observed that clove oil nanoemulsions at a concentration of 1.5 mg clove oil/g nanoemulsion showed a negligible influence on germinative energy of barley, while still efficiently eliminated the DON levels and toxicogenic fungal biomass as quantified by Tri5 DNA content. Among the three emulsifiers, Tween 80-stablized clove oil nanoemulsion displayed higher mycotoxin inhibitory activity and less flavor impact on the final malt. The results showcase the huge application potential of essential oil nanoemulsion as an effective antifungal agent as well as novel malt flavors in the malting industry.

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EFFECTS OF *FUSARIUM* INFECTION TIMING ON THE PRODUCTION OF DEOXYNIVALENOL IN BARLEY GRAIN AND MALT

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ABSTRACT

The current study investigated the effect of Fusarium infection timing on the production of deoxynivalenol (DON) in barley grain and malt. The impact of grain storage time and temperature, prior to malting was investigated as a secondary objective. Barley was inoculated with *F. graminearum* using a grain spawn method, and mist irrigated to promote the development of FHB (Fargo, ND 2019). Barley was seeded on May 10 and the grain spawn was applied June 26. The timing of infection was controlled by bagging individual spikes. Control samples were bagged from June 25 until harvest. Late infection was simulated by bagging spikes until July 22. For early infection, the spikes were not bagged, and were harvested at two different dates: first harvest August 6-8) and second harvest August 10-11 (after a heavy rain).

DON levels were lowest in the control (0.30±0.28 μg/g) and late infection samples (3.26±2.34 μg/g). Levels were highest in early infections samples (26.71±4.86 and 27.20±1.57 μg/g first and second harvest, respectively). Over 90% of barley kernels in both the control and late infection samples had DON < 1.0 μg/g. However, in the early infection samples only 72% of kernels had DON <1.0 μg/g, and 6% of kernels actually had DON levels > 100 μg/g. Fungal hyphae were mostly observed in the husk tissues of kernels with DON < 1.0 μg/g, but were also observed in the aleurone, endosperm, and embryo tissues of kernels with DON > 10 μg/g. The results indicated that the early infection in the field caused internal infection with Fusarium, which produced much higher levels of DON in a portion of kernels and the bulk barley sample.

Malting was first performed three months after harvest, with the samples being stored at 20 ℃. A second malting was conducted after another six months of storage, on two sets of samples that were stored at either 20 ℃ or 4 ℃. Following the first malting, malt DON levels of late infection and early infection (first harvest) samples were ±20% higher than those of the unmalted samples. However, the malt DON levels of early infection (second harvest) increased to 153% of that observed in the barley. Following the second maltings at eight months, malt DON levels of late infection samples were significantly lower than observed at threeo months. In contrast, the malt DON levels of the early infection samples didn't change significantly when compared to the first malting. All control malt samples had DON levels <0.20 μg/g. In terms of DON distribution on kernels, the ratio of control kernels with DON < 1.0 μg/g didn't change following malting, but the amount of late infection kernels with DON > 1.0 μg/g increased from 10% to 22% following the malting. With early infection samples the amount of kernels with >1.0 μg DON/g increased from 28% to 73%. The fungal hyphae were observed in the interior of steeping-out kernels with high DON levels, and the hyphae grew much heavier in the malt kernels.

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NON-DESTRUCTIVE DETECTION OF DEOXYNIVALENOL IN BARLEY KERNELS USING HYPERSPECTRAL IMAGING AND MACHINE LEARNING

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ABSTRACT

Due to these health concerns, barley used for malting, food or feed is routinely assayed for deoxynivalenol (DON) levels. In this study, we explored the feasibility of using hyperspectral imaging (382–1030 nm) to develop a rapid and non-destructive protocol for assaying DON in barley kernels. Full-wavelength locally weighted partial least squares regression (LWPLSR) achieved high accuracy with the coefficient of determination in prediction (R2P) of 0.728 and root mean square error of prediction (RMSEP) of 3.802. Competitive adaptive reweighted sampling (CARS) was used to choose potential feature wavelengths, and these selected variables were further optimized using the iterative selection of successive projections algorithm (ISSPA). The CARS-ISSPA-LWPLSR model developed using 7 feature variables yielded R2P of 0.680 and RMSEP of 4.213 in DON content prediction. Based on the 7 wavelengths selected by CARS-ISSPA, partial least square discriminant analysis (PLSDA) discriminated barley kernels having lower DON (<1.25 mg/kg) levels from those with higher levels (including 1.25–3 mg/kg, 3–5 mg/kg, and 5–10 mg/kg), with Matthews correlation coefficient in cross-validation (M-RCV) of as high as 0.931. The results demonstrate that hyperspectral imaging have potential for accelerating non-destructive DON assays of barley samples.

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GENE DISCOVERY AND ENGINEERING RESISTANCE

THE BARLEY UDP-GLYCOSYLTRANSFERASE *UGT13248* IS REQUIRED FOR DEOXYNIVALENOL CONJUGATION AND TYPE 2 RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB) of *Hordeum vulgare* (barley) can cause significant grain yield reduction and quality deterioration by contamination with trichothecene mycotoxins including deoxynivalenol (DON). Glycosylation of DON to DON-3-glucose (D3G) is thought to be catalyzed by UDP-glucosyl transferases (UGTs). Barley *UGT13248*, was previously shown to convert DON to D3G in yeast, *Arabidopsis* and wheat. In wheat, expression of *UGT13248* decreased disease severity of FHB. The Function of *UGT13248* in barley has not been previously investigated. To explore the natural genetic diversity of *UGT13248*, we re-sequenced *UGT13248* from a collection of 28 barley accessions with varying degree of FHB resistance and identified six protein variants. A broader survey of the *UGT13248* sequence from exome capture sequencing data of 34 elite barley lines, 182 wild barley accessions and 317 barley landraces identified seven non-synonymous changes. Accessions carrying any of these *UGT13248* protein variants did not show altered sensitivity to DON on seedling root growth assays. This suggests that mutations in UGT13248 are rare and that *UGT13248* is highly conserved. To analyze the function of *UGT13248* in more detail we generated barley lines overexpressing *UGT13248* and identified two independent TILLING lines carrying mutations in close proximity to the UDP-sugar binding site, *UGT13248* (T368I) and UGT13248 (H369Y). The *UGT13248* (T368I) and *UGT13248* (H369Y) mutants showed hypersensitivity to DON root growth inhibition in seedlings and strongly impaired conjugation of DON to D3G in barley spikes. Constitutively expressing *HvUGT13248* in a susceptible barley cultivar provided resistance to the inhibitory effect of DON on root growth and increased conjugation of DON to D3G in spikes. Field test of TILLING mutants showed increased FHB disease severity, suggesting that DON to D3G conversion contributes to FHB resistance. Point inoculation experiments showed increased FHB disease severity and spread of FHB across the spikes of TILLING plants, suggesting that *UGT13248* contributes to Type 2 resistance in barley.

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MAPPING QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE AND AGRONOMIC TRAITS IN A MUTANT FROM A WHEAT CULTIVAR JAGGER

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ABSTRACT

Wheat Fusarium head blight (FHB) causes significant yield losses and is one of the most destructive diseases of wheat. To explore new sources of resistance, we identified one line (1095EMSMut) with moderate type II resistance after phenotypically screened a EMS mutant population developed from an FHB-susceptible Jagger for FHB resistance. In the greenhouse experiments, 1095EMSMut (PSS = 0.40) showed significantly lower FHB severity than Jagger (PSS = 0.81). We hypothesized that the reduction of FHB susceptibility in the mutant was due to loss-of-function mutations in FHB susceptible gene(s) of Jagger. Also, 1095EMSMut differed from Jagger in several agronomic traits including plant height, spikelet number and heading date. To identify the quantitative trait loci (QTLs) for FHB resistance and the agronomic traits in the mutant, we developed 156 recombinant inbred lines (RILs) of 1095EMSMut x Jagger using the single seed decent method. The RIL population was evaluated for FHB resistance and the agronomic traits in four greenhouse experiments. Both parents and the F_5 RIL population were genotyped using single nucleotide polymorphisms (SNPs) generated by genotyping-by-sequencing (GBS). SNPs was called using reference-based GBS bioinformatics pipeline in Tassel. A total of 3,757 GBS-SNPs across 156 RIL lines were mapped on 21 wheat chromosomes. Using the GBS-SNP map and the greenhouse phenotyping data, we detected one major QTL for FHB resistance on chromosome 4B, one major QTL for plant height on 4B, two QTLs for spikelet number on 2B and 5D, and two QTLs for tiller number on 4B and 2B. We mapped two QTLs for type II FHB resistance on chromosomes 5D and 2B when the population was evaluated for FHB resistance in both 2019 and 2020 field trials at Rocky Ford, Manhattan, Kansas, QTLs for plant height on 4B and for spikelet number on 2B were also identified in the same field trials. In the greenhouse experiments, the FHB type II resistance QTL on chromosome 4B overlapped with the plant height QTL, but this QTL was not significant in the field trials. The correlation coefficient between percentage of symptomatic spikelets in a spike (PSS) and plant height in the greenhouse experiments was positively significant (0.43 at $p < 0.01$), indicating taller plants had higher PSS. However, the correlation coefficient was negative (-0.31 at $p < 0.01$) in the two field experiments. These results lay solid foundation for further fine mapping and map-based cloning of these QTLs for FHB resistance and other important agronomic traits.

EVALUATION FOR NEW SOURCES OF FUSARIUM HEAD BLIGHT RESISTANCE IN HEXAPLOID AND TETRAPLOID WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of bread (*Triticum aestivum* L.) and durum (*Triticum durum* L.) wheat, which can cause significant grain yield losses, reduce wheat quality, and result in the accumulation of harmful mycotoxins in the grain. The deployment of resistant cultivars is the most effective, economical and environmentally friendly means to control the disease. However, breeding for FHB resistance in Canadian wheat breeding programs has been challenging due to limited sources of resistance. The objective of this study was to identify new sources of FHB resistance for use in wheat improvement. We evaluated 107 genotypes of hexaploid wheat including common wheat as well as *Triticum* species: *spelta*, *macha*, *spherococcum*, *compactum* plus *T. aestivum* synthetics, and 106 accessions of tetraploid wheat, including *Triticum durum* and species *turgidum*, *dicoccon*, *polonicum* and *carthlicum*, in an artificially inoculated field FHB nursery in 2017 and 2018. Results indicated significant variation among entries for FHB reaction. FHB index, *Fusarium* damaged kernel (FDK) and deoxynivalenol (DON) content ranged from 1.5 to 78.6%, 2.4 to 61.7% and 1.6 to 139 ppm, respectively. Pearson correlation coefficients ($P < 0.0001$) were 0.63, 0.42 and 0.57 between DON and FDK, DON and FHB index, and FHB index and FDK, respectively. We identified 19 accessions with DON content as low as the resistant check 'Sumai3'. This group consisted of 15 hexaploid genotypes including *Triticum spelta* accession 'CN1849' and four tetraploid wheat accessions, and are promising germplasm for improvement of FHB resistance in wheat breeding programs.

FUNCTIONAL CHARACTERIZATION OF *TAHRC* IN REGULATING FHB RESISTANCE IN WHEAT

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ABSTRACT

Epidemics of Fusarium head blight (FHB) cause a serious reduction in grain yield and quality of wheat and result in significant economic losses to wheat producers. The most sustainable and effective approach to curb FHB is to grow resistant varieties. Many quantitative trait loci (QTLs) for FHB resistance have been reported to date. Among them, *Fhb1* from Sumai 3 and its derivatives from China show a major effect on type II resistance. Recently, we have cloned *Fhb1* as a histidine-rich calcium-binding protein (*TaHRC*) and demonstrated that the wide-type allele of *TaHRC* is FHB susceptible (*TaHRC_S*) and a large deletion in the start codon region of the mutant allele increased FHB resistance. To investigate the molecular mechanisms of *TaHRC* on FHB resistance, we conducted yeast two-hybrid screening (Y2H) against the wheat cDNA expression libraries using *TaHRC_S* as bait and identified four candidate proteins that interacted with *TaHRC_S*. Bimolecular fluorescence complementation (BiFC) assays in *Nicotiana benthamiana* leaf cells revealed the interactions between TaHRC_S and its interacting proteins and confirmed that a wheat homolog CAX interacting protein 4 (TaCXIP4) can strongly interact with *TaHRC_S*. Calcium suppression assays and co-expression analyses with calcium-sensing receptor genes demonstrated that *TaHRC_S* can directly bind TaCAXIP4 to suppress the expression of TaCAX1 that activates calcium transport activity and affect calcium signaling transduction during FHB infection. Reactive oxygen species (ROS) assays further showed that *TaHRC_S* may suppress chitin-triggered plant immune responses during FHB infection by direct binding to TaCAXIP4 to maintain FHB susceptibility. Subcellular colocalization assays revealed the interaction between TaCXIP4 and *TaHRC_S* occurred in the nucleus. Taken together, *TaHRC_S* may interact with TaCAXIP4 to inhibit calcium-mediated defense responses and facilitate pathogen spread in a wheat spike to maintain FHB susceptibility. This work provides further insights into molecular mechanisms of *TaHRC* in regulating FHB resistance in wheat.

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CRISPR-EDITING OF BARLEY SUSCEPTIBILITY GENES TO IMPROVE FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* (*Fg*) is an important disease of wheat and barley, resulting in significant yield loss and reduced grain quality due to mycotoxin contamination. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 nuclease) gene editing technology was used in this study to knock-out (KO) several host genes involved in conditioning *F. graminearum* susceptibility. Our studies with the model plant *Arabidopsis* have shown that CRISPR-mediated knock-outs of the *At2OGO* (*DMR6*) gene, which encodes a putative 2-oxoglutarate Fe(II)-dependent oxygenase, results in enhanced expression of plant defense genes, and an augmented *Fusarium* resistance. CRISPR-mediated knock-outs of the *Arabidopsis AtEIN2* (*ethylene insensitive 2*) gene also enhanced *F. graminearum* resistance. Complementation of *At2OGO*-KO and *AtEIN2*-KO plants with the barley *Hv2OGO* and *HvEIN2* genes restored susceptibility of these plants to *F. graminearum*, indicating that these two genes are functionally related and similarly involved in conditioning barley to *F. graminearum* infection. Another *F. graminearum* susceptibility gene identified in *Arabidopsis* is *AtHSK*, which encodes a homoserine kinase. Both cDNAs and genomic DNAs encoding *Hv2OGO*, *HvEIN2* and *HvHSK* were cloned from cv. Conlon and Genesis. We also cloned the promoter for cv. Morex *HvUGT*, which encodes UDP-glucosyltransferase in order to study the contribution of *HvUGT* gene expression to the detoxification of *Fusarium* mycotoxins. We developed protocols for regeneration and transformation following gene gun and *Agrobacterium*-mediated transformation of barley cultivars Conlon, Genesis and Morex. Target sites within the *Hv2OGO*, *HvEIN2* and *HvHSK* genes and *HvUGT* promoter have been identified and both transient and integrating CRISPR-gene editing vectors have been constructed using the barley, rice or wheat U3 or U6 promoter to drive transcription of guide RNAs. Transgenic barley plants produced using the integrating CRISPR-editing vectors targeting *Hv2OGO*, *HvEIN2* and *HvHSK* genes and *HvUGT* promoter have been produced and, in several cases, the nature of the resulting edited mutant plants verified.

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DISCOVERING GENE EXPRESSION CHANGES LINKED TO PHENYLPROPANOID-BASED FHB RESISTANCE

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ABSTRACT

Our goal is to identify genes or pathways whose activity is altered in response to *Fusarium graminearum* infection, and its production of the mycotoxin deoxynivalenol (DON), in experimental lines exhibiting increased resistance to Fusarium head blight (FHB). Spring wheat (cv. 'CB037') was transformed with constructs constitutively expressing one of four genes from sorghum monolignol biosynthesis, which produces the monomers polymerized into lignin: the SbMyb60 transcriptional activator, and three enzymes in monolignol biosynthesis. Two lead events for each construct, CB037, and the resistant check, 'Sumai 3', were screened in the field at UMore Park, Rosemount, MN, using spray inoculations (initiation of infection or Type I resistance) during 2018 and 2019. FHB Disease Index (DI), proportion of *Fusarium* damaged kernels (FDK) and DON levels were determined. Across both years, transformed lines and CB037 all had DI similar to Sumai 3. Sumai 3 had FDK less than all other lines, but a line constitutively expressing SbMyb60 had significantly lower FDK than CB037. Transgenic lines had DON levels similar to or greater than CB037 but a line constitutively expressing the enzyme coumaroyl shikimate 3-hydroxylase (*SbC3H*) had levels not significantly different from Sumai 3. Greenhouse assays were conducted by inoculating lines with constitutive expression of the enzyme SbC3H or caffeoyl coenzyme A 3-*O*-methyl transferase (*SbCCoAOMT*), CB037, Sumai 3 and the susceptible check, Wheaton, using single-floret and spray inoculations. The area under the disease progress curve (AUDPC) and proportion of FDK were assessed. Although no increased resistance was observed following spray inoculations, lines constitutively expressing *SbC3H* and *SbCCoAOMT* had AUDPC and FDK significantly less than that of CB037 following single-floret inoculation, indicating increased Type II resistance. We combined *SbC3H* and *SbCCoAOMT* constitutive expression constructs in the CB037 background and incorporated the *SbC3H* construct into moderately resistant backgrounds, Sumai 3 and 'Alsen'. Global gene expression analysis will be used to identify genes and pathways associated with increased resistance to FHB from lines exhibiting increased resistance.

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GENETIC DISSECTION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE, GRAIN PROTEIN CONTENT AND AGRONOMIC TRAITS IN THE PERICENTROMERIC REGION OF CHROMOSOME 6H IN BARLEY

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ABSTRACT

Resistance to Fusarium head blight (FHB), kernel discoloration (KD) and deoxynivalenol (DON) accumulation, and grain protein concentration (GPC) are important traits for breeding barley varieties used for malting and brewing. Ideally, malting barley varieties should have moderate GPC and low levels of FHB, KD and DON. Previous work mapped a Chevron-derived QTL associated with FHB resistance to the pericentromeric region on chromosome 6H, coinciding with QTL for KD resistance and GPC. The Chevron allele reduced FHB and KD and increased GPC. Whether these trait correlations are caused by tightly linked loci or pleiotropy is undetermined. To study the question of linkage and pleiotropy, a fine mapping approach was used to genetically dissect the QTL underlying these quality and disease resistance traits as well as other agronomic traits. Two populations, referred to as Gen10 and Gen10/Lacey, containing 1,941 and 2,082 individuals, were genotyped and 79 and 26 recombinant near-isogenic lines (rNILs), respectively, were identified and phenotyped for FHB and KD severity, DON accumulation, GPC, senescence and other agronomic traits. Sixteen QTLs for all the traits studied were identified in the Gen10 population and seven QTLs were identified in the Gen10/Lacey population. Three FHB, two DON and two KD QTL were identified. One of the three FHB QTLs, one DON QTL, and one KD QTL were coincident with the GPC QTL which contains the *Hv-NAM1/ Hv-Gpc1* locus which effects senescence and grain protein accumulation. The Chevron allele at the GPC QTL increased GPC and FHB severity and decreased DON accumulation and KD score. The other two FHB QTLs and the other DON and KD QTL were identified in the regions flanking the *Hv-NAM1* locus and the Chevron alleles at these loci decreased FHB, DON and KD. Our results suggested that FHB, KD, DON, and GPC in the pericentromeric region of 6H might be controlled by both pleiotropy and tightly linked loci, suggesting that the genetics of resistance might be more complex than expected. The rNILs exhibiting low FHB severity and moderate GPC may be useful materials for breeding malting barley cultivars.

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TARGETING WHEAT GENES ASSOCIATED WITH SUSCEPTIBILITY TO *FUSARIUM GRAMINEARUM* FOR ENHANCING FHB RESISTANCE

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ABSTRACT

Fusarium head blight, which is a serious disease of wheat and small cereal grains caused by *Fusarium graminearum* (*Fg*), adversely affects grain yield and quality. *Fg*-infected grains accumulate mycotoxins. *Fg* can also infect *Arabidopsis*. 9-lipoxygenases (9-LOXs), which catalyze the first step in the synthesis of oxylipins, act as susceptibility factors in wheat and *Arabidopsis* interaction with *Fg*. Knock-down of 9-LOX encoding genes in *Arabidopsis* confers enhanced resistance against *Fg*, which can be complemented by the wheat *Lpx3* gene. Also, knock-down of *Lpx3* in wheat cv. Bobwhite by RNA-interference (RNAi) strategy conferred enhanced resistance against *Fg*. Fungal infection was largely confined to the inoculated spikelet of *Lpx3*-RNAi plants. To develop a non-GMO approach for FHB resistance, wheat TILLING lines with nonsense and/or missense *Lpx3* variants in the hexaploid Cadenza and tetraploid Kronos were identified and characterized for their response to *Fg*. FHB disease severity and DON accumulation was reduced in some of the non-sense *Lpx3* variants. The *Fg*-resistant TILLING lines have been backcrossed to wild type to clear out unwanted mutations at other loci. Simultaneously, crosses have been made to generate lines with non-sense mutations at multiple *Lpx3* homeologs. Molecular approaches are being used to distinguish between mutants with SNPs and wild type. It is anticipated that knockdown of multiple *Lpx3* homeolog(s) will confer higher levels of resistance to FHB.

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ENHANCING WHEAT RESISTANCE TO *FUSARIUM GRAMINEARUM* VIA HOST-INDUCED GENE SILENCING (HIGS) OF THE FUNGAL VIRULENCE GENE *FGL1*

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ABSTRACT

A secretory lipase encoded by *FGL1* is a virulence factor that acts on plant membrane lipids to release polyunsaturated fatty acids like linoleic (18:2) and linolenic (18:3) acids, which promote *Fusarium graminearum* (*Fg*) invasiveness. In this study we aim to silence *FGL1* through host-induced gene silencing (HIGS) for enhancing resistance to Fusarium head blight (FHB) and *Fusarium* seedling blight. HIGS targets the transcript of the gene in the fungus by expressing a double stranded RNA (dsRNA) corresponding to a fungal gene. The small RNAs generated from these dsRNAs in the plant are expected to be taken up by the fungus and thus destabilize the target gene. We used *Cauliflower mosaic virus* (CaMV) *35S* promoter and maize *Ubiquitin* (*Ubi*) gene promoter for expressing the dsFGL1 RNA in *Arabidopsis* and wheat, respectively. The HIGS strategy was found effective in conferring resistance to *Fg* in *Arabidopsis* and wheat. Since plant lipoxygenases (LOXs) synthesize oxylipins from 18:2 and 18:3 fatty acids, and the wheat lipoxygenase-encoding *Lpx3* is a susceptibility factor associated with FHB, our future goal is to study the relationship between *Lpx3* and *FGL1* in facilitating *Fg* infection in wheat.

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TRANSCRIPTOMIC ANALYSIS OF A LOW-DON, *IN VITRO* SELECTED, TWO-ROW MALTING BARLEY VARIETY IN RESPONSE TO INFECTION BY MULTIPLE CHEMOTYPES OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB) caused by the hemi-biotrophic pathogen *Fusarium graminearum* Schwabe, is the most destructive disease of barley (*Hordeum vulgare* L.), with economic loss primarily related to quality degradation from the associated mycotoxin deoxynivalenol (DON). Two-row barley generally displays an intermediate level of FHB resistance, in contrast to more-susceptible six-row. Major breeding efforts have been made to reduce FHB and lower DON, however breeding with exotic resistance sources has often been problematic due to linkage of undesirable characters, particularly poor malting quality. Alternatively, Agriculture and Agri-Food Canada's Brandon Research and Development Centre has used *in vitro* selection (*IVS*) as a breeding method to reduce DON, where doubled-haploid tissue culture was paired with selection on growth media containing trichothecene mycotoxins. To date, little is known regarding the genetic mechanisms underlying resistance of *IVS* barley. RNA-Sequencing has proven a useful tool for investigating the genetic basis of disease resistance. A study was conducted that contrasted 'Norman' with its parental line 'CDC Kendall' under treatment of different chemotypes (15ADON, 3ADON, NIV) and mock control over two time points (72, 96 hours post infection) associated with the switch to necrotrophy and elevated DON production. Highest disease and DON content was observed in the 3ADON treatment. Both varieties displayed induced defense response in phenylpropanoid pathway and gene categories (CYP450, GST, UGT, ABC transporters), which were highly expressed in 3ADON treatment and especially in 'CDC Kendall'. In the 15ADON treatment, 'Norman' displayed an early onset of defense genes associated with xylanase inhibitors, alpha-amylase inhibitors, serpin proteins (serine protease inhibitors), thionin 2.1, late embryogenesis abundant (LEA) proteins, hordeins and negative regulatory elements. Such genes were also differentially expressed in 'Norman' in the 3ADON treatment. Lower levels of receptor kinase genes and induced defense response were observed in the NIV treatment at earlier time point, which increased subsequently. RNA-Seq has identified sets of differentially expressed genes targets, which may be useful for breeding FHB resistant barley cultivars.

CHARACTERIZATION OF QUANTITATIVE TRAIT LOCI (QTLS) FOR WHEAT RESISTANCE TO FUSARIUM HEAD BLIGHT USING A JAGGER MUTANT

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ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases in wheat and barley worldwide. Many FHB resistant resources have been identified, but most of them are wheat landraces and alien species with poor adaptation, which hampers progress in improvement of FHB resistance in wheat. To Explore novel FHB resistant resources, we used mutagenesis to create new genetic variations for FHB resistance. A mutant population was developed by mutagenizing a susceptible hard winter wheat cultivar 'Jagger' with EMS. After screening for FHB resistance in greenhouses, we identified one mutant line (JagR1097) with significantly improved FHB resistance, also taller plant height and later heading date compared to Jagger. An F_5 recombinant inbred line (RIL) population (JagR1097/Jagger) was developed by crossing this mutant to Jagger. The RIL population was inoculated with *Fusarium graminearum* to evaluate their FHB resistance and genotyped using GBS markers to identify novel QTLs for FHB resistance, plant height and heading date in the population. Three QTLs were detected for Type II FHB resistance on chromosomes 4A, 5D, 6A and explained 14%, 8% and 7.4 % of the phenotypic variation, respectively. The 4A and 5D QTLs were overlapped with plant height and heading date. Regression analysis showed a positive correlation between FHB resistance and heading date. These results indicated that morphological traits may play a significant role on FHB resistance in wheat.

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This material is based upon work supported by the U.S. Department of Agriculture. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

DEVELOPMENT OF DIAGNOSTIC MARKERS FOR *FHB7*

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease worldwide. FHB is mainly incited by *Fusarium graminearum* Schwabe and can cause great losses in grain yield and quality, which significantly impacts global wheat production. A major FHB resistance gene *Fhb7* has been identified from chromosome 7E of *Thinopyrum ponticum* and confers broad resistance to *Fusarium* species. Recently *Fhb7* gene has been cloned as a glutathione S-transferase (GST). However, markers for high-throughput screening of the gene are not available for marker-assisted selection. To develop such markers, a high-density linkage map of 130.84 cM was constructed for chromosome 7E, and contains 459 single nucleotide polymorphism (SNP) markers generated from genotyping-by-sequencing (GBS). A set of polymorphic Kompetitive Allele Specific PCR (KASP) markers was mapped in the *Fhb7* region, which were designed based on the sequence of GST and closely linked GBS-SNPs to *Fhb7*. To validate the usefulness of the KASP markers of *Fhb7* in marker-assisted selection (MAS), two gene markers can diagnose the presence of *Fhb7* in both biparental and natural populations, however, they both are dominant markers because GST homolog is absent in planta. To convert them into codominant-like markers, we added a wheat marker together with *Fhb7* gene KASP marker in the same PCR and the markers successfully separate *Fhb7* from wheat sequence, thus they will facilitate deployment of *Fhb7* in wheat breeding programs to improve FHB resistance.

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HARD WINTER WHEAT COORDINATED PROJECT

FUNGICIDE EFFICACY FOR CONTROL OF FUSARIUM HEAD BLIGHT UNDER FIELD NURSERY CONDITIONS

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ABSTRACT

The primary control method for Fusarium head blight (FHB) is fungicide application. Common fungicide modes of action (MOA) used for FHB include demethylation inhibitors (DMI), succinate-dehydrogenase inhibitors (SDHI), and quinone outside inhibitors (QoI). A *Fusarium graminearum* disease nursery was planted to determine the efficacy of commercially available fungicides. The plots were planted with the scab susceptible cultivar, 'Larry', in a randomized complete block design with 5 replications. Plots were scored seven times between heading and maturity. Fungicide treatments were made using a backpack CO_2 sprayer equipped with flat-fan nozzles (8002 VS) with 20-in. spacing at 30 psi at 20 gal/A. Comparisons were made against an untreated check for disease severity using area under the disease progress stairs (AUDPS) and yield. The healthy check, which consisted of three applications of Miravis® Ace, and a single treatment of Miravis Ace yielded the highest and had statistically indifferent AUDPS ratings. The fungicides Prosaro and Caramba performed significantly poorer than the health check but better than Topguard EQ, Lucento, and the experimental treatment, which were not statistically different than the untreated check. Yield results correlated with AUDPS results and ranged from 34.41 bu/A to 63.41 bu/A. All fungicides with the exception of Caramba and Prosaro contain two MOA. The most successful fungicide, Miravis Ace, used a DMI and a SDHI. However, the least successful commercial fungicide also used a DMI and a SDHI. This indicates that MOA alone does not make the result. This research suggests that there is a wide range of fungicide efficacy for controlling FHB and that effective control methods are available.

BREEDING FOR *FUSARIUM* HEAD BLIGHT RESISTANCE OF WHEAT (*TRITICUM AESTIVUM*) BY MARKER-ASSISTED SELECTION AND GENOMIC SELECTION

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ABSTRACT

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is a devastating disease of wheat (*Triticum spp.*) and barley (*Hordeum vulgare* L.). Improvement of host resistance is an effective strategy to mitigate yield and quality losses. The goal of this research is to develop adapted wheat cultivars and improve selection accuracy in the breeding programs for FHB resistance by using marker-assisted selection and genomic selection (GS). Backcross populations were developed using Overland_Fhb10 (a moderately resistant hard winter wheat cultivar with *Fhb1*) as a donor parent and six elite Nebraska breeding lines (NE14696, NE14421, NE16562, NE15624, NE14434 and NE10478-1 (recently licensed to Limagrain Cereal Seeds and marketed as LCS Valiant)) as recurrent parents. A diagnostic KASP marker tagged to *Fhb1* locus was used to identify backcross lines with the *Fhb1* locus in BC_1 to BC_3 generations. The phenotypic evaluations of these lines will be conducted in the greenhouse this winter. Additionally, GS is being explored for improving native resistance. A training dataset for two FHB resistance related traits, severity (SEV) and incidence (INC), was put together for 1199 winter wheat lines planted in 2015 to 2019. The SEV and INC were evaluated in replicated and misted disease nurseries located at Mead or Lincoln, Nebraska. The heritability (H^2) varied through years with an overall H^2 of 0.53 for SEV and 0.82 for INC. The breeding lines were genotyped using genotyping-by-sequencing and 62,478 high-quality SNPs were identified. The genomic prediction accuracy tested using cross-validations ranged from 0.22 to 0.42 for SEV and 0.39 to 0.67 for INC. The breeding progress for FHB resistance is being augmented and enhanced with phenotypic trials conducted by Dr. Clay Sneller at Ohio State University beginning in 2020. The marker-assisted selection with *Fhb1* locus and GS for SEV and INC is aiding in developing adapted cultivars and advancing breeding lines with FHB resistance and good agronomic traits.

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PATHOGEN BIOLOGY & GENETICS

CHARACTERIZING BACTERIAL COMMUNITY DIFFERENCES AMONG WHEAT LINES THAT DIFFER IN FHB RESISTANCES

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (*sensu stricto*), is a devastating disease that affects major cereal grains in the US including wheat and barley. Losses from this disease extended from reductions in yield to increased mycotoxin content from the casual pathogen. Traditionally, managing FHB relied on breeding programs and the use of fungicides. However, increasingly researchers are investigating biocontrol measures for mitigating FHB. Stable biocontrol measures will rely on interactions with the existing microbial communities and the pathogen. Thus, understanding wheat-microbiome-*Fusarium* interactions is needed to advance these approaches. Towards this goal, the bacterial community from two wheat varieties for each genetic resistance type, susceptible, moderate resistant, and resistant, grown under field conditions favorable for FHB infection were investigated using Illumina sequencing of the bacterial 16S rRNA gene. Our results showed that, among mature grain heads, bacterial community diversity significantly changes among resistance types but not across varieties. At the genus level, *Massilia, Hymenobacter, Novosphingobium, Spirosomaand* and *Pseudomonas* had the highest relative abundance in susceptible genotype while in resistant genotype *Pedobacter, Sphingomonas, Massilia, Hymenobacter, Novosphingobium, Roseomonas and Spirosoma* showed the highest relative abundance. Taking all this data together, we can conclude with caution, that Fusarium-wheat-phytomicrbiome interactions impact bacterial community structure. To further understand how these interactions take place we will characterize phytomicrobiome differences among same wheat lines at metagenomic level. This will allow us to capture the interaction network landscape and identify microbial gene nodes that interact with *Fusarium*.

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GENOMICS COMBINED WITH ENTOMOPATHOGENIC FUNGI TOOLS FOR CONTROLLING OF FUSARIUM HEAD BLIGHT IN WHEAT IN MONTANA

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ABSTRACT

Fusarium head blight (FHB) is a devastating fungal disease affecting wheat and barley across the world. This disease has cost U.S. farmers more than $3 billion since 1990. Our main goal is to control of the most devastating problem in wheat using new breeding and genomics tools combined with endophytic entomopathogenic fungi. Selected endophytic fungi induce systemic disease resistance when colonizing plants. The research combined classical assays of induced resistance as assayed by pathogenicity related proteins and with genomic response of the plant to select fungal strains. This research will lead to both fundamental new knowledge in plant response to endophytic colonization and disease resistance as well as development of an effective control of FHB. Novel approaches to manage FHB are sorely needed as to assure sustainable and sufficient wheat yields and end use quality traits.

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CHITIN-TRIGGERED IMMUNE RESPONSES IN WHEAT AND BARLEY

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ABSTRACT

Fusarium graminearum is the primary causal agent of Fusarium head blight (FHB) on wheat and barley. FHB reduces grain yield and contaminates grain with various mycotoxins including deoxynivalenol (DON). DON acts as a virulence factor that helps the fungus pass the wheat rachis node that is a critical barrier for FHB resistance. The production of reactive oxygen species (ROS) is one of the earliest defense responses during plant and pathogen interactions. Chitin, a main component of fungal cell wall, can trigger plant immunity including ROS burst. Prior studies demonstrated that hydrogen peroxide, a key player of ROS, induces DON production in *F. graminearum*. Our recent study showed that *F. graminearum* effectors can suppress ROS when transiently expressed in *Nicotiana benthamiana* leaves. However, the complex roles of ROS during Fusarium and host interactions remain unclear. In this study, we investigated ROS triggered by chitin in FHB resistant and susceptible wheat and barley. We discovered that no ROS burst was detected in chitin-treated wheat leaves, in contrast, ROS were triggered by chitin in barley leaves. We further examined ROS production in different wheat and barley floral tissues. ROS burst was induced by chitin in wheat rachises and rachis nodes. ROS induction was observed in rachis nodes of FHB susceptible and moderately resistant wheat varieties; however, no correlation was found between ROS level and FHB severity. Gene expression analyses revealed plant immunity marker genes were upregulated in wheat rachis nodes after chitin treatments. The genes involved in chitin-mediated signaling may serve as a new target to develop FHB and mycotoxin control strategies by boosting plant immunity.

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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF SECRETED EFFECTOR PROTEASES FROM *FUSARIUM GRAMINEARUM*

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ABSTRACT

Effector proteins secreted by plant pathogens play a vital role in plant pathogenesis by suppressing host immune responses and promoting virulence. Though the effector repertoires of bacterial and oomycete pathogens have been well-characterized, our knowledge of fungal effector proteins is still quite limited. *Fusarium graminearum*, which causes Fusarium head blight (FHB) in barley and wheat, is predicted to secrete hundreds of effector proteins during infection. However, the functional role of effector proteases in plant pathogenesis remains unknown. Here, we identified ten candidate effector proteases from *F. graminearum* using transcriptome data sets obtained from detailed spatial and temporal analyses of the early symptomatic and asymptomatic phases of Fusarium colonization of wheat heads focusing on rachis segments and spikelets. The expression of each of these candidate *F. graminearum* effector proteases (named FgECs) is highly to moderately induced *in planta* compared to when growing *in vitro*. Moreover, each of the FgECs are well-conserved among *F. graminearum* isolates from the United States, Brazil, and Australia, further suggesting these proteases may have a functional role in plant pathogenesis. Immunoblot analysis of super Yellow Fluorescent Protein (sYFP)-tagged FgECs in *Nicotiana benthamiana* revealed that eight of the ten FgECs accumulate protein in leaf cells at detectable levels. Additionally, none of the FgECs suppressed cell death in *N. benthamiana* induced by the auto-active disease resistance protein RPS5^{D266E}. Future work will address whether the FgECs are translocated into host cells during infection using the split-GFP strand system as well as identify host interactors using the miniTurbo-catalyzed proximity-dependent labeling (PDL) approach.

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IDENTIFICATION OF *FUSARIUM GRAMINEARUM* TRAITS THAT RELATE TO MALT QUALITY DEFECTS AND *FUSARIUM* SUCCESS WITHIN THE MALT MICROBIOME

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of barley which negatively impacts malting quality. Unlike most other commodities that are affected by FHB, in malting barley there can also be new pathogen growth and new production of mycotoxins after harvest, due to the suitable conditions afforded by the malting environment. A field study was conducted in 2018 and 2019 at Brandon, MB using barley varieties of different FHB resistance levels: AAC Goldman (moderately resistant) and Newdale (moderately resistant to moderately susceptible). Barley plants were grown under irrigation and plots were inoculated with single strain conidial suspensions using 7 different *Fusarium graminearum* strains, plus a non-inoculated control. The study demonstrated differential response between the varieties, with AAC Goldman displaying lower FHB severity in both years. Results from 2019 indicated that AAC Goldman also presented lower fungal load and lower deoxynivalenol content, compared to Newdale. The *F. graminearum* isolates will be categorized according to hydrophobin production type and production rate. The potential of these hydrophobins to induce gushing in beer will be examined. Barley harvested from the field experiment, infested individually with each pathogen strain, will be micro malted. In the final malt, the *F. graminearum* density, deoxynivalenol concentration, hydrophobin content, and malt quality parameters will be assessed, and the microbiome will be profiled. We expect that the results from this project will lead to the development of new approaches to mitigating *Fusarium* growth during malting.

EVALUATING THE *FUSARIUM* SPECIES DIVERSITY AND CHEMOTYPE DISTRIBUTION OF WHEAT IN WESTERN CANADA

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OBJECTIVES

1. Detect the presence of *Fusarium* species and the chemotype mycotoxin diversity in the 2019-2020 growing seasons in Manitoba, Saskatchewan, and Alberta farmers' fields.

2. Evaluate the presence of *Fusarium* in non-chaff and chaff samples of spring wheat.

INTRODUCTION

Fusarium Head Blight (FHB) disease affects both the quality and quantity of small grain cereal crops such as wheat, oat, corn, and barley (Leonard et al., 2003). The disease is caused by various *Fusarium sp,* which produce mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV) that negatively affect animal and human health (Amarasinghe et al., 2015). The infected cereal grain is not suitable for human consumption or to feed livestock. *Fusarium graminearum* (teleomorph *Gibberella zeae*) is the primary causative agent associated with FHB of wheat (McMullen et al., 1997). The primary inoculum of FHB is ascospores, which may survive in crop debris from previous growing seasons. These spores are airborne and infect the emerging wheat flowers to start a new disease cycle (Sutton, 1982). The visible symptoms of FHB appear about three weeks after floret infection, which includes premature bleaching and partially shattered heads. Florets of the crop will develop a pink coloration near their base due to the presence of fungal mycelia. The affected seeds do not fill correctly and appear shrunken, bleached, and may contain black colored perithecia (Parry et al., 1995). Although FHB disease incidence and progression is higher in hot and humid environments (Gilbert and Fernando, 2004), cereal crop production in Canada is negatively affected by FHB because summer weather conditions favor disease development. Due to the high involvement of environmental conditions, FHB is considered difficult to manage disease (Bai and Shaner, 1994). Hence, FHB forecasting models are essential in disease management. A forecasting system will provide precise data on disease progression to wheat farmers in a reliable and timely manner, reducing yield loss and mycotoxin contamination (De Wolf et al., 2003).

MATERIALS AND METHOD

Infected wheat spikes were collected from a 50 x 50m area (fungicide-free) of selected farmers' fields located in Manitoba, Saskatchewan, and Alberta. All fields were cultivated and maintained according to normal farmer practices used for wheat production. Two spore traps were set up in each field during the flowering weeks to monitor the inoculum availability. Each spore trap was a rectangular-shaped foam based with Vaseline covered 2-sided tapes on all four sides to which airborne spores could adhere. Spore counts were taken by counting spores in a 1 cm^2 of the Vaseline tape under the light microscope and multiplying by the whole area of the Vaseline tape on the

spore trap. Two independent counts were used to calculate the mean spore count. Wheat spikes were processed to collect grains and chaff from each field (Table 1). The DON content in grain and chaff samples was analyzed by an independent lab and the University of Guelph, ON. *Fusarium* colonies were isolated from both grains and chaff samples. DNA was extracted from each isolate's single spore cultures using the CTAB DNA extraction procedure (Amarasinghe et al., 2015). Species identification was performed by amplifying the extracted DNA with species-specific PCR primers (Table 2). PCR was carried in a total of 25 µL reaction mix containing PCR buffer (1.5 mM MgCl2, 50 mM KCl, 10 mM Tris. HCl, pH 8.0), 0.2 mM of each dNTP, 0.4 µM of each primer, 0.75 U Taq DNA polymerase and 20 ng of template DNA. Amplification was done with the following PCR cycle; initial denaturation at 95 °C for 3 min; with 35 cycles of 30 s at 95 °C, 30 s at annealing temperature (Tm, Table 2), 1 min at 72°C and a final extension of 72°C for 5 min. Gel electrophoresis was used to confirm positive amplification. Multiplex PCR markers were used to determine the chemotype of *F. graminearum* and *F. culmorum* (Ward et al., 2002). The length polymorphism of amplified bands was used to identify the chemotype, where a 610 bp fragment indicates the 15ADON, and a 243 bp fragment indicates the 3ADON.

RESULTS AND DISCUSSION

The grain analysis for deoxynivalenol revealed that the DON levels for spring wheat were <0.5 ppm to 2.4 ppm, <0.5 ppm to 8.9 ppm and <0.5 ppm to 2.9 ppm in Manitoba, Saskatchewan and Alberta, respectively, while it was <0.5 ppm for all winter wheat samples in three provinces. The chaff collected from the same spring wheat heads showed higher DON content compared to the grain. The chaff from moderately susceptible spring wheat contained the highest DON content than moderately resistant and intermediate resistant cultivars (Figure 1). The results suggested the importance of growing moderately resistant cultivars to reduce the DON content. Further, removal of chaff during processing may reduce the DON impact on grain. The spore counting indicated the differences in available inoculum during the flowering season. In 2019, Manitoba and Saskatchewan spring wheat fields got a higher spore influx than Alberta (Figure 2A). However, Saskatchewan winter wheat received a high number of spores (Figure 2B). The availability of inoculum and disease initiation significantly depends on environmental parameters such as temperature and relative humidity. We will later use these environmental parameters to explain disease progression in the three provinces. Seed culturing followed by PCR with specific markers also showed a higher *Fusarium* infection in Manitoba and Saskatchewan's spring wheat samples compared to Alberta. However, FHB was less in the winter wheat in 2019 (Figure 3A). Most samples collected were infected by *F. graminearum* with the highest *F. graminearum* percentage recovered from the spring wheat from Manitoba. However, *F. avenaceum* (45% in grains and 30% in chaff) was highest in Alberta (Table 3). Most of the *F. graminearum* isolates contained the 3ADON chemotype. The percentage of 15ADON was significantly lower in Alberta, showing that the 3ADON is becoming the dominant chemotype in FHB disease epidemics because Alberta was the last of the three provinces to see significant FHB infection over the past two decades (Figure 3B). Phenotypic identification of spore shapes was conducted to confirm the genotypic data (Figure 4). Results will be correlated with macroclimatic and crop phenological data. The experiment will be repeated for samples collected in 2020. The results obtained from this study will evaluate candidate FHB risk assessment models that predict future epidemics in Western Canada.

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Table 1. Variety composition grown in participating farmer's fields – 2019.

Province	Spring Wheat			Total	Winter Wheat				Total
	MR	I	MS/S		R	MR	I	MS/S	
Manitoba	16	5	0	21	2	0	4	0	6
Saskatchewan	7	4	3	14	1	3	3	2	9
Alberta	5	3	2	10	0	1	2	4	7

R: Resistant, MR: Moderately resistant, I: Intermediate, MS/S: Moderately susceptible or susceptible

Table 2. Species-specific primers used for *Fusarium* species identification.

Fusarium spp.	Forward primer (5'-3')	Reverse primer (5'-3')	Tm (°C)	Reference
Fusarium genus	ATGGGTAAGGARGACAAGAC	GGARGTACCAGTSATCATGTT	55	O'Donnell et al., 2004
F. graminearum	CTCCGGATATGTTGCGTCAA	GGTAGGTATCCGACATGGCAA	55	Demeke et al., 2005
F. culmorum	ATGGTGAACTCGTCGTGGC	CCCTTCTTACGCCAATCTCG	58	Nicholson et al. 1998
F. avenaceum	AACATACCTTAATGTTGCCTCGG	ATCCCCAACACCAAACCCGAG	58	Mishra et al., 2003
F. sporotrichioides	CTTGGTGTTGGGATCTGTGTGCAA	ACAAATTACAACTCGGGCCCGAGA	68	Kulik et al. 2004
F. poae	CAAGCAAACAGGCTCTTCACC	TGTTCCACCTCAGTGACAGGTT	62	Parry and Nicholson, 1996
F. proliferatum	CGGCCACCAGAGGATGTG	CAACACGAATCGCTTCCTGAC	69	Jurado et al., 2006
F. pseudograminearum	CGGGGTAGTTTCACATTTCYG	GAGAATGTGATGASGACAATA	55	Aoki and O'Donnell, 1999
F. cerealis	CTCAGTGTCCACCGCGTTGCGTAG	CTCAGTGTCCCATCAAATAGTCC	62	Nicholson et al., 2004

Table 3. Fusarium species diversity found in grains and chaff samples from Western Canada.

Fusarium species	Wheat type	Manitoba		Saskatchewan		Alberta	
		Grains	Chaff	Grains	Chaff	Grains	Chaff
F. graminearum	Spring	87%	74%	65%	48%	27%	21%
	Winter	4%		2%		-	
F. avenaceum	Spring	-	11%	2%	25%	45%	30%
	Winter	-		2%		4%	
F. sporotrichioides	Spring	3%	5%	21%	22%	12%	9%
	Winter	-		2%		-	
F. culmorum	Spring	-	-	3%	-	4%	24%
	Winter	1%		2%		-	
F. poae	Spring	5%	-	-	-	-	-
	Winter	-		-		-	
Other	Spring	-	10%	1%	5%	4%	16%
	Winter	-		-		4%	

Other: unidentified isolates

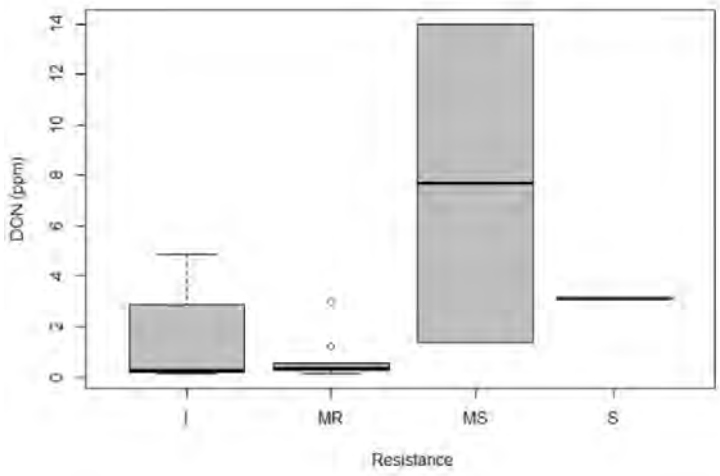

Figure 1. The box plot of DON content found in the chaff of spring wheat according to th resistant category (I: Intermediate resistant, MR: Moderately resistant, MS:

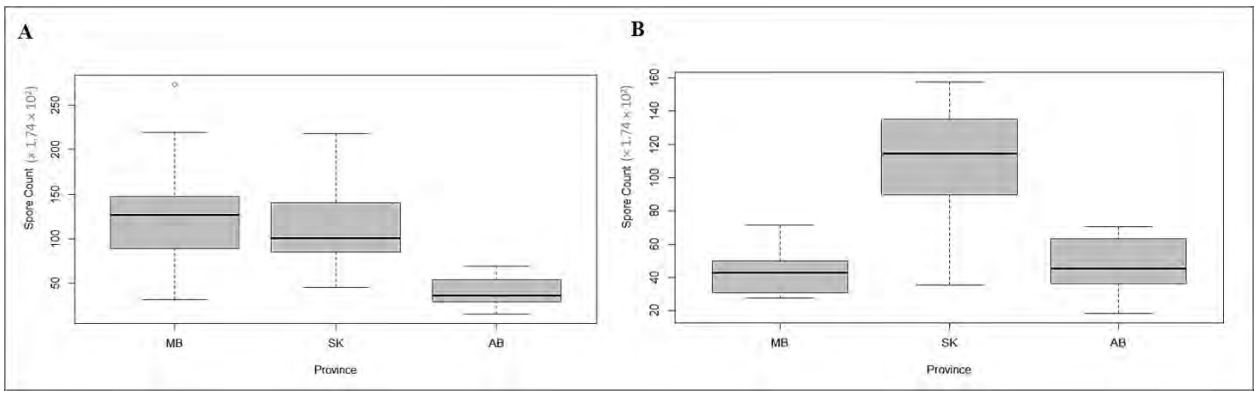

Figure 2. The box plots of mean spore counts observed during the flowering week of 2019 growi season in the farmers' fields of Manitoba (MB), Saskatchewan (SK) and Alberta (AB). The mean spore counts in spring wheat fields, and B: The mean spore counts in winter

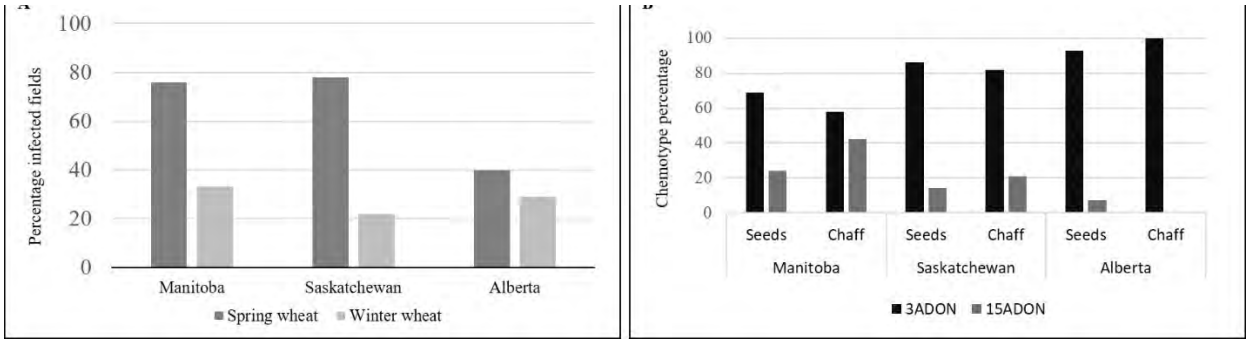

Figure 3. Sample analysis from spring wheat and winter wheat fields from 2019 A: Percentage infected fields and B: Chemotype percentage found in *F. graminearum* and *F. culmoru* isolates.

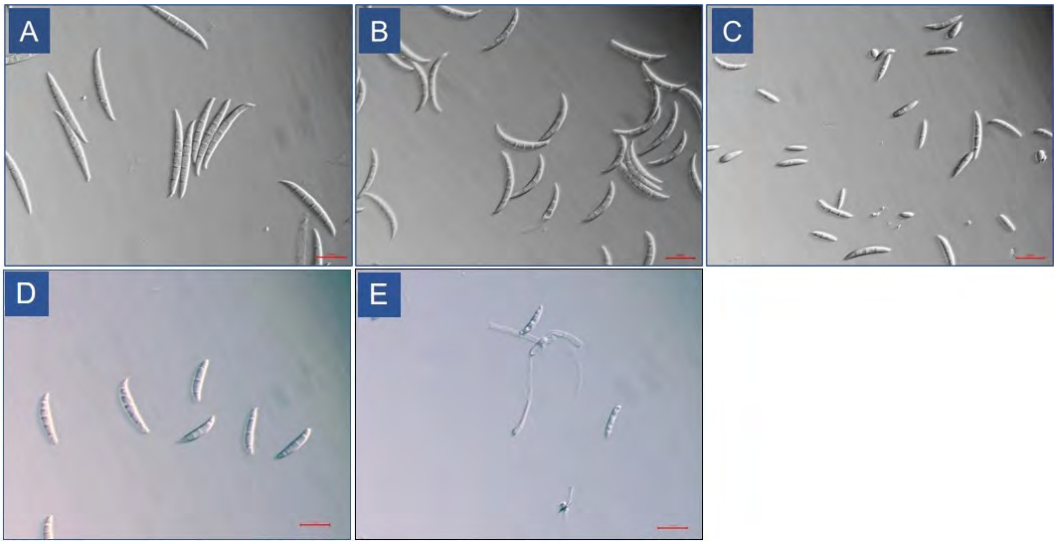

Figure 4. Spore morphology of identified *Fusarium* species, A: *F. graminearum*, B: *F. avenaceum* and C: *F. sporotrichioides*, D: *F. culmorum* and E: *F. poae* (Magnification ×400). Scale bar indicates 20 µm. (Laxco SeBaView 3.7).

DIVERSITY AND AGGRESSIVENESS OF *FUSARIUM GRAMINEARUM* IN ILLINOIS

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ABSTRACT

Understanding the genetic diversity and underlying population structure of a pathogen is critical for monitoring disease outbreaks and developing precision disease management. Changes in *F. graminearum* population diversity can have a huge impact on agriculture, with the emergence of new pathogen populations with potentially higher resistance to fungicides, greater aggressiveness, or increased mycotoxin production. Such changes have critical implications for growers, breeders, and food safety specialists, making the monitoring of pathogen population structure essential. We surveyed the diversity of the Fusarium head blight (FHB) causal agent on soft red winter wheat (*Triticum aestivum*) in Illinois in 2016. We collected naturally infected heads from five wheat lines with different levels of resistance in five locations. Ten percent of the isolates causing FHB were not *F. graminearum*. Other species already known to cause the disease were found, such as *F. acuminatum*, and others not previously known to cause the disease, such as *F. armeniacum*. With a selected number of isolates from different varieties, we found that those from resistant or intermediate lines had variable aggressiveness levels; however, isolates from the susceptible wheat line were all aggressive. We used field pathogenomics to rapidly establish the population structure of the selected isolates and explore the differences in transcription on wheat lines with different resistance levels. The population structure of isolates from different resistance level sources was the same, all belonging to the NA1 population. However, we found differential gene expression among strains causing disease on wheat lines with different resistance levels. Several candidate genes were identified for both, *F. graminearum* infection specific to the level of host resistance, and candidate genes always required for wheat infection. Current efforts are targeted at studding differences in aggressiveness on a larger population of *F. graminearum* isolates.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-011. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

GENETIC STRUCTURE AND MYCOTOXIN PROFILE OF *FUSARIUM GRAMINEARUM* POPULATIONS FROM CANADA AND NORTH EASTERN UNITED STATES

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ABSTRACT

Fusarium head blight (FHB) is a serious concern for wheat production in Canada and the USA. *Fusarium graminearum* is the principal etiological agent, producing mainly the trichothecene mycotoxin, deoxynivalenol (DON) and its acetyl derivatives [(15-acetyl deoxynivalenol (15ADON) and 3-acetyl deoxynivalenol (3ADON)]. Knowledge of the pathogen's population structure and genetic variation is important in developing effective management tools. Despite the availability of information on population dynamics of *F. graminearum* in western Canada, genetic structure studies of isolates collected recently from has not been investigated on a large scale. In this study, we analyzed a total of 588 isolates of *F. graminearum* collected from wheat. A total of 465 isolates collected from five geographic regions in the provinces of Saskatchewan and Manitoba, Western Canada in 2018 and 2019 was analyzed for population structure and diversity using 10 variable number of tandem repeats (VNTR) markers and compared with 90 isolates from the province of Ontario, Eastern Canada and 33 isolates from the north-eastern region of the U.S. About 80% of the isolates collected in Saskatchewan and 73% in Manitoba were of the 3ADON type, suggesting an increase in 3ADON of about 400% in Saskatchewan and 50% in Manitoba within the past 15 years. Interestingly, all isolates from Ontario and north- eastern U.S were of the 15ADON chemotype. Additionally, the 3ADON isolates from all five geographic regions across the two provinces revealed a composition of at least 70%. Analysis of the Saskatchewan and Manitoba isolates revealed a high gene diversity (0.795 – 0.848) and high gene flow (4.971 – 12.213) among regions, indicating no genetic differentiation among the geographic regions. However, Bayesian clustering model analyses of trichothecene chemotype subpopulations divided the populations into two clusters, which was correlated with trichothecene types. In addition, the 3ADON populations had a greater admixture compared to the 15ADON type suggesting this may play a role in increased virulence of 3ADON isolates. Comparative population genetic analysis of all Canadian and U.S isolates is currently on-going. The population genetic structure and mycotoxin profile will assist resistant wheat cultivar development and mycotoxin risk assessment in Canada.

IDENTIFICATION OF A BACTERIAL-FUNGAL ASSOCIATION THAT SILENCES *FUSARIUM GRAMINEARUM* VIRULENCE

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ABSTRACT

Fusarium graminearum (*Fg*) is the primary fungal pathogen responsible for Fusarium head blight (FHB), a devastating disease of wheat and barley worldwide. Bacterial-fungal associations can have considerable influence on the pathogenicity of fungi and shape the outcomes of plant-fungal interactions. Genome sequencing analysis revealed a *Fg* strain, 47556+, heavily contaminated with a bacterial symbiont that was identified as *Paenibacillus illinoisensis*. The bacteria appeared to have an ectosymbiotic association with the fungus because the rod-shaped bacterial cells were visible on the *Fg* 47556+ hyphae. Furthermore, the bacterial association could be eliminated by antibiotic treatment or repeatedly washing the fungal hyphae in a dilute tween solution over a filter large enough to allow bacteria cells through but not the hyphae. In comparison to the cured *Fg* strain (47556cured), 47556+ caused on average 70% less FHB symptoms and produced 80% less DON on wheat (cultivar Apogee). However, the association and phenotype of reduced pathogenicity and DON production could not be reconstituted by simply mixing the bacteria back with the cured *Fg* strain.

ACKNOWLEDGEMENT AND DISCLAIMER

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A STATUS UPDATE ON FUSARIUM HEAD BLIGHT IN CANADIAN WHEAT

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ABSTRACT

The Microbiology Program at the Canadian Grain Commission performs annual surveys of *Fusarium* Damaged Kernels (FDK) of wheat. Harvest samples are received from across Canada and are screened for FDK. Individual kernels are then tested for fungal species and genes that differentiate the toxins that they produce. We perform high-throughput genotyping of 5-10 kernels from ~1,200-1,500 grain samples using a set of 24 PCR-based DNA markers. The markers we use can differentiate *Fusarium* species (i.e. *F. graminearum*, *poae*, *avenaceum*, and etc.) and their trichothecene chemotype (i.e. 15-ADON, 3-ADON, and NX-2). The fungal survey can track the dynamics of the fungal species and chemotypes in different geographic locations in Canada across multiple years. A status update on *Fusarium* in Canadian wheat grain will be presented.

HETEROTHALLIC MUTANTS OF *FUSARIUM GRAMINEARUM* AND THEIR POTENTIAL USE FOR GENETIC ANALYSES OF FUNGAL PATHOGENICITY AND TOXIGENICITY

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ABSTRACT

Fusarium graminearum is a homothallic perithecial Ascomycete. Self-fertility is regulated by the MAT1 locus encoding two idiomorphic compatibility genes called MAT1-1-1 and MAT1-2-1. Previous studies have demonstrated that deletion of the MAT1-1-1 or MAT1-2-1 genes of *F. graminearum* produces obligately heterothallic strains. We set out to produce a MAT knockout (KO) strain for use in heterothallic test matings with wild type (WT) strains, in order to facilitate our genetic analyses of traits of interest including pathogenicity or toxigenicity. We produced multiple independent KOs of MAT1-1-1, and MAT1-2-1 in the PH-1 strain of *F. graminearum* and screened them to confirm high levels of fertility in matings, and normal pathogenicity and aggressiveness. All KOs lost their ability to produce asci and ascospores. As previously reported, MAT1-1-1 KOs produced sterile perithecia, whereas most of the MAT1-2-1 KO strains did not. When MAT1-1-1 KOs were paired with MAT1-2-1 KOs, fertile perithecia containing asci and ascospores were produced, and molecular markers segregated 1:1 among the progeny, confirming that the KOs are heterothallic. Individual transformants varied widely in their levels of female fertility and heterothallic interfertility. One of the MAT1-2-1 KOs produced sterile perithecia like the MAT1-1-1 KOs. Individual transformants also varied in their levels of toxigenicity and pathogenicity to wheat and maize. Most of the MAT1-2-1 KOs were significantly reduced in these traits on susceptible varieties, whereas most of the MAT1-1-1 KOs did not differ from the WT. High levels of fertility were not associated with high levels of pathogenicity. The large amount of variation among different KO transformants in fertility, toxin production, and pathogenicity may be due to off-site mutations that occurred during the transformation process. We will analyze segregation patterns among progeny from crosses of the various KO strains to test this hypothesis. By using backcrosses, our intention is to clear the MAT mutants of these off-site mutations, in order to produce a suitable test-mate strain for our future genetic studies. Our results demonstrate the importance of confirming that fertility levels are normal, and that phenotypes of interest are unaffected, in heterothallic KO strains chosen for use in genetic studies.

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VARIETY DEVELOPMENT & HOST RESISTANCE - COORDINATED PROJECTS (VDHR-CPs)

Northern Soft Winter Wheat, Southern Soft Red Winter Wheat and Spring Wheat Region

MULTIVARIATE GENOMIC PREDICTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a devastating fungal disease of wheat and barley. Resistance to FHB is quantitatively inherited and requires intensive field evaluation. Plant breeders regularly evaluate multiple correlated FHB resistance traits including days to heading (DH), disease incidence (INC), severity (SEV), *Fusarium* damaged kernels (FDK) and dexoynivalenol content (DON) which opens an avenue for using multiple traits in genomic prediction models. In this study, we evaluated the potential of multi-trait (MT) genomic prediction models for predicting DON using INC, SEV, and FDK as secondary traits among two different diversity panels of soft red winter wheat (SRWW), one from Illinois (IL) and one from Purdue (PU). Prediction accuracies of MT genomic prediction models were evaluated using four different cross-validation methodologies which simulated different phenotyping scenarios (MT-CV1, MT-CV2, MT-CV2_0.5b, and MT-CV2_0.5u) and compared to single trait (ST) prediction models. Among the four cross-validation methods, the one which included correlated traits on the training and validation sets achieved highest increment in prediction accuracies with all the secondary traits in comparison to ST prediction model from 0.64 to 0.79 and 0.42 to 0.52 in IL and PU panel respectively. In addition, we compared the prediction accuracies of all the combinations of secondary traits to predict DON. Interestingly, bivariate model consisting FDK and DON as response variable performed similarly (0.76 and 0.52 for IL and PU respectively) to MT prediction model with all secondary traits (0.79 and 0.52 for IL and PU respectively). Our study suggests that using MT genomic prediction provides opportunities to accelerate genetic gains by increasing accuracy of selection while reducing the need to phenotype multiple correlated traits.

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PHENOLOGICAL AND ENVIRONMENTAL INFLUENCES ON THE GENOMIC PREDICTION OF FHB RESISTANCE

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ABSTRACT

Environmental factors like temperature and humidity are presumed to be greatly influencing Fusarium head blight FHB infections in wheat. Anther retention AR, on the other hand, is a morphologically neutral trait with a shared genetic basis with FHB resistance. In this study our aims were (i) to evaluate two types of corrections over the FHB severity scores, namely method-1 via linear regression on flowering time (FT) and method-2 via a best-subset multiple linear regression analysis comprising FT plus accumulate thermal time variables and (ii) to assess the performance of multi-trait genomic selection (MT.GS) models for FHB severity assisted by AR. Training (TS) and validation (VS) sets summed respectively 853 and 143 winter wheat genotypes evaluated in four trials spanned between 2015 and 2018. The validation scenarios where GS models were exclusively trained with the previous trial averaged 0.24, 0.29 and 0.32 in prediction ability (PA) for FHB severity scores uncorrected, corrected by method-1 and method-2 respectively. Likewise, PA for GS models trained with a combination of trials averaged 0.17, 0.20 and 0.23 for FHB severity scores uncorrected, corrected by method-1 and method-2 respectively. FHB severity scores free from the influences of both environment and phenology seemed to be the most efficient trait to be predicted across different seasons. In those scenarios, PA increments of 1.9 fold on average were obtained for the MT.GS models evidencing the feasibility of using AR as an assisting trait on predictions independent from undesirable indirect effects in cases with no FHB records available like early breeding stages.

ACKNOWLEDGEMENT AND DISCLAIMER

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EVALUATION OF COMMERCIALLY GROWN ONTARIO WINTER WHEAT FOR FUSARIUM HEAD BLIGHT RESISTANCE AND DEOXYNIVALENOL LEVEL

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ABSTRACT

Development of wheat resistant to Fusarium head blight (FHB), while improving yield and maintaining quality requirements is important in Ontario, Canada. FHB is a serious disease of wheat and deoxynivalenol (DON) is the most common mycotoxin produced by *Fusarium graminearum* (FG). All wheat commercially grown in Ontario is entered in the Performance Trials and tested for agronomy traits (with and without fungicides application) and for FHB resistance in nurseries inoculated with FG. Wheat cultivars are grouped in several categories, based on FHB visual symptoms and DON level, using historical data and the most recent data (www.gocereals.ca). FHB and DON categories are: moderately resistant (MR), moderately susceptible (S), susceptible (S) and highly susceptible (HS). OCCC soft red and white winter wheat checks are: MR ('Marker' and 'Ava'), MS ('CM614' and '25R46'), S/HS ('DS572SRW','25R40'), while hard red winter wheat checks are: MR ('AC Morley'), MS ('Princeton' and 'Priesley'), S/HS ('AC Sampson'). All check cultivars had stable FHB performance over last seven years. Some cultivars have different category for FHB index and DON level. For example, 'Marker', a soft red winter wheat developed by Ridgetown Campus breeding program is MR to both FHB symptoms and DON level (the best FHB category in Ontario), while our 'UGRC Ring' is in MS category for FHB symptoms, and in MR category for DON level. Information for both FHB related traits is important to growers and industry. Phenotyping and development of new cultivars, with increased level of FHB resistance (resistant category) and higher yield is in progress.

ACKNOWLEDGEMENT

This material is based on data collected by Ontario Cereal Crop Committee (OCCC).

SELECTIVE BREEDING UNDER RAPID GENERATION ADVANCEMENT TO INCREASE RESISTANCE IN WINTER WHEAT TO FUSARIUM HEAD BLIGHT IN MICHIGAN

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ABSTRACT

The use of resistant cultivars in wheat breeding is an effective means to controlling Fusarium head blight. With the use of segregating populations, early generation selection can increase the levels of FHB resistance in derived inbred lines. F_2 populations where selected from the MSU wheat breeding program and grown to maturity in a greenhouse setting using the "minibulk" method. At flowering experimental lines were inoculated with a 1x 10^5 concentration of conidia for a total of three cycles to account for maturity differences. Disease was assessed 21 days after inoculation and heads were pulled if they showed infection greater than 50%. The number of heads removed and remaining were recorded for each population. After selection, resistant heads are grown to maturity and harvested. The same protocol was applied in the F_3 generation. Comparisons will be made between inbred lines derived from populations that have undergone selection vs. controls. Selection for resistance under rapid generation advancement can lead to an increases frequency of FHB resistance among inbred wheat lines derived from segregating populations.

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GENOME-WIDE ASSOCIATION ANALYSIS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN ELITE SOFT RED WINTER WHEAT LINES

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ABSTRACT

Sporadic outbreaks of Fusarium head blight (FHB) in the southeast United States in recent years has necessitated searching for novel sources of resistance as a sustainable and cost-effective means of disease management, while minimizing reduced wheat grain yield and quality associated with toxigenic mycotoxins. The objective of this study was to identify and characterize novel quantitative trait loci (QTL) associated with FHB resistance in a panel of 236 elite soft winter wheat lines. A genome-wide association study (GWAS) was conducted using 27,466 single nucleotide polymorphisms (SNPs) obtained from genotyping-by-sequencing. The panel was phenotyped for seven FHB and phenological traits including FHB incidence, FHB severity, FHB index, *Fusarium*-damaged kernel (FDK), deoxynivalenol (DON), thousand kernel weight, and days to heading in two different environments (greenhouse and field) in 2019 and 2020. A total of 42 significant marker-trait associations (MTAs) were identified at the uniform cut-off value of $-\log_{10}p=4.00$ to all seven traits with the maximum number of MTAs observed for FDK and DON. We observed stable MTAs for FHB traits on chromosomes 2B, 6B, and 7A along with several pleiotropic MTAs across the wheat genome. Additional data analyses are ongoing including all traits from the second year. Validation of identified QTL will be essential for future use of these valuable genetic sources to enhance FHB resistance in soft red winter wheat breeding programs.

THE 2020 UNIFORM SOUTHERN SOFT RED WINTER WHEAT SCAB NURSERY

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ABSTRACT

The Uniform Southern Soft Red Winter Wheat Scab Nursery provides breeders in the public and private sectors the opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties. Valuable data are provided on resistance to other important fungal and viral diseases, resistance to Hessian fly, milling and baking quality and agronomic characteristics. Genotypic analyses identify major QTL alleles present at numerous important loci. In addition, we provide Genomic Estimated Breeding Values (GEBV) for resistance traits in nursery entries to research the utility of genomic selection approaches to breeding for FHB resistance. These were estimated from a training population of nursery entries from 2011 to 2019. A combined mixed model analysis of the phenotypic data from 2011 to 2019 was performed and BLUEs for each genotype were estimated in R using the lme4 and emmeans packages. The number of SNP markers utilized was 19,442. The genotypic selection model utilized Ridge Regression BLUP through the R-package RR-BLUP (ver. 4.6) to predict GEBVs for individuals in the 2020 nursery. GS model accuracy was evaluated by Pearson correlation between GEBVs and best linear unbiased estimate (BLUE) for the 2020 entries. Correlation varied between 0.69 for FHB Rating to 0.64 for FDK and 0.36 for DON.

The 2020 Uniform Southern Soft Red Winter Wheat Scab (FHB) Nursery was distributed to cooperators in fall, 2019. The nursery comprised 42 advanced generation breeding lines and seven check cultivars. “Ernie”, “Bess”, and “Jamestown” were the moderately resistant checks and “Coker 9835” “SS 8641” and “P26R46’ were the susceptible checks.

Five U.S. public programs (Arkansas, Georgia, Louisiana, North Carolina, and Virginia), and one private companies (KWS) submitted entries. Field data were returned from up to seven locations. In addition, three USDA-ARS laboratories conducted evaluations for Hessian fly resistance, milling and baking quality and marker genotypes.

Copies of the full report will be available at the 2020 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: https://scabusa.org/.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-209. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Phenotypic means across locations, correlations between GEBV and phenotypic means and genotypic content of regions associated with FHB resistance.

	Cultivar/ Designation	FHB Rating		FHB Incidence		FHB Severity		FHB Index		FDK		ISK		DON	
			Rank		Rank		Rank		Rank		Rank		Rank		Rank
1	ERNIE	3	14	50	18	60	36	36	31	11	15	38	23	4	19
2	COKER9835	7	46	87	46	80	45	70	46	48	47	69	47	8	46
3	BESS	2	4	52	20	42	11	22	12	12	17	31	14	2	2
4	JAMESTOWN	3	14	54	24	45	18	32	24	16	29	37	21	3	9
5	SS 8641	7	46	88	47	85	47	75	47	58	48	71	48	18	48
6	Pioneer 26R46	7	46	105	48	87	48	82	48	47	46	65	46	15	47
7	NC11546-14	2	4	53	21	48	23	26	18	13	22	37	21	3	9
8	NC12642-81	2	4	58	28	44	17	27	20	10	12	35	18	2	2
9	NC15-21834	2	4	56	26	50	25	32	26	13	22	38	23	5	33
10	NC15-21835	2	4	53	21	58	34	39	34	13	22	39	25	5	33
11	NC16-19449	4	32	45	11	42	11	25	17	22	37	33	17	4	19
12	NC11363-25	3	14	50	18	47	20	27	21	13	22	36	20	4	19
13	NC12164-94	3	14	58	28	52	27	31	22	12	17	39	25	3	9
14	NC12193-49	2	4	31	4	31	4	10	2	6	3	23	2	2	2
15	AR12002-0024N	4	32	63	32	54	30	38	32	12	17	40	29	6	41
16	AR12486-2417N	3	14	68	36	47	20	32	25	12	17	39	25	4	19
17	AR12486-2419N	3	14	64	33	51	26	33	28	16	29	44	35	4	19
18	AR12486-2420N	3	14	68	36	48	23	33	27	16	29	43	31	3	9
19	AR15V21-09-2089N	2	4	43	10	35	6	17	5	8	6	26	5	2	2
20	AR15V31-26-2285N	2	4	50	18	38	10	21	11	6	3	29	7	2	2
21	AR15V41-10-2443N	1	1	47	14	42	11	23	13	9	8	30	11	1	1
22	ARNCDH12753-103-1536M	3	14	85	45	67	39	53	39	27	45	53	42	7	44
23	GAFHBMAS14031-201	3	14	51	19	43	16	24	15	7	5	31	13	3	9
24	GA151313-LDH127-19E36	5	41	73	38	71	43	55	41	25	42	55	44	4	19
25	GA151313-LDH224-19E38	5	41	55	25	52	27	31	23	18	32	39	25	5	33
26	GA10457-19LE13	3	14	75	39	62	37	44	38	12	17	48	38	3	9
27	GANC12642-12-19LE16	3	14	35	5	42	11	21	10	11	15	30	11	4	19
28	GA15328-18E52F	5	41	77	40	82	46	63	45	19	33	51	39	5	33
29	KWS216	4	32	80	43	70	42	57	44	26	43	55	44	6	41
30	KWS238	3	14	77	40	68	40	54	40	20	34	52	41	5	33
31	KWS246	3	14	59	31	62	37	42	36	21	35	43	31	4	19
32	KWS263	3	14	77	40	72	44	57	43	22	37	54	43	7	44
33	KWS333	1	1	47	14	42	11	24	18	13	22	31	14	2	2
34	LA13045D-173	3	14	36	8	30	3	11	3	10	12	25	4	4	19
35	LA13045D-160	5	41	57	27	54	30	33	29	24	41	43	31	3	9
36	LA13181D-151A	4	32	66	35	56	33	38	33	13	22	44	35	4	19
37	LA15166LDH296	3	14	81	44	68	40	56	42	26	43	51	39	6	41
38	LA15203LDH075	4	32	58	28	55	32	39	35	21	35	43	31	5	33
39	LA15VDH-FHB-MAS10-16	2	4	35	5	37	7	20	7	8	6	29	7	3	9
40	LANC9337-63-4	4	32	30	1	32	5	17	6	13	22	26	5	3	9
41	15VDH-FHB-MAS22-14	2	4	30	1	27	2	17	4	5	1	24	3	4	19
42	15VDH-FHB-MAS38-01	3	14	30	1	20	1	7	1	9	8	18	1	3	9
43	15VDH-FHB-MAS17-23	5	41	42	9	45	18	21	9	10	12	29	7	4	19
44	15VDH-FHB-MAS34-18	3	14	45	11	47	20	26	19	5	1	35	18	4	19
45	16VDH-SRW01-120	4	32	45	11	37	7	23	14	9	8	32	16	4	19
46	16VDH-SRW09-025	4	32	65	34	52	27	35	30	23	39	42	30	5	33
47	16VDH-SRW06-131	4	32	53	21	59	35	42	37	23	39	44	35	5	33
48	VA18FHB-58	1	1	35	5	37	7	20	8	9	8	29	7	2	2
	Mean	3		55		50.0		33		17		37		5	
	LSD (0.05)	2		26		25		28		20		19		5	
	CV%	30.8		23.2		24		41.9		59.8		24.4		51.8	
	Correlations with Predic.	.		.		0.67		.		0.64		.		0.36	

Table 1. Continued

	Cultivar/ Designation	Head. Date		Plant Ht.		Flour Yield %		Softness Equiv. %		Hessian Fly		Fhb1	Fhb Massey 3BL	Jamestown 1B	Bess 2B	Bess 3B	NC-Neuse 1A	NC-Neuse 6A
			Rank		Rank		Rank		Rank	Bio. L	H13							
1	ERNIE	106	13	36	16	65.8	45	57.0	22	0-21	no	no	yes	no	no	no	het	yes
2	COKER9835	109	28	34	6	67.6	18	65.0	1	0-18	no	no	no	no	no	no	no	no
3	BESS	110	32	39	40	66.9	29	56.7	26	0-19	no	no	no	yes	yes	yes	yes	no
4	JAMESTOWN	101	2	34	7	66.1	41	56.4	29	0-18	no	no	no	yes	no	no	yes	no
5	SS 8641	106	13	36	21	67.1	24	56.8	24	0-17	no	no	no	no	no	no	no	no
6	Pioneer 26R46	105	9	38	32	71.1	3	56.3	31	0-17	no	no	no	no	no	no	het	no
7	NC11546-14	110	32	37	24	66.8	32	58.6	13	18-1	yes	Fhb1	no	yes	no	no	yes	no
8	NC12642-81	108	22	36	19	67.8	14	59.5	10	17-1	yes	het	het	het	no	no	yes	no
9	NC15-21834	112	43	40	46	65.9	44	56.0	33	0-15	fail	no	fail	fail	no	fail	yes?	fail
10	NC15-21835	113	47	40	45	67.1	24	57.2	20	0-17	no	no	no	no	no	no	yes	no
11	NC16-19449	109	28	38	33	66.6	35	53.7	46	0-17	no	no	no	yes	no	no	yes	yes
12	NC11363-25	110	32	36	22	66.0	43	59.0	11	0-19	no	no	no	het	no	no	yes	yes
13	NC12164-94	108	22	38	36	66.7	33	54.2	45	0-17	no	Fhb1	no	yes	no	no	no	het
14	NC12193-49	107	16	35	14	68.2	10	55.7	38	17-2	het	het	no	het	no	no	no	het
15	AR12002-0024N	108	22	41	48	71.5	1	53.1	47	0-17	no	no	no	yes	no	no	no	het
16	AR12486-2417N	111	41	37	30	67.4	20	55.4	40	0-20	no	no	no	no	no	no	no	no
17	AR12486-2419N	110	32	37	28	68.6	7	56.7	26	0-17	no	no	no	no	no	no	no	yes
18	AR12486-2420N	110	32	36	23	67.8	14	56.3	31	0-19	no	no	no	het	no	no	het	no
19	AR15V21-09-2089N	110	32	35	12	67.9	12	56.4	29	0-19	no	Fhb1	no	no	no	no	no	no
20	AR15V31-26-2285N	108	22	40	43	67.1	24	57.0	22	0-17	no	Fhb1	no	no	no	no	no	no
21	AR15V41-10-2443N	107	16	41	47	62.6	48	50.4	48	0-19	no	Fhb1	no	no	no	no	no	no
22	ARNCDH12753-103-1536M	112	44	37	29	67.8	14	57.4	19	0-23	no	no	no	yes	no	no	het	fail
23	GAFHBMAS14031-201	103	5	34	10	66.6	35	55.8	36	0-19	no	het	no	no	no	no	het	no
24	GA151313-LDH127-19E36	108	22	36	20	66.6	35	59.0	11	20-0	yes	no	no	yes	no	no	no	no
25	GA151313-LDH224-19E38	105	9	36	17	67.2	23	55.6	39	19-0	yes	no	no	yes	no	no	no	no
26	GA10457-19LE13	109	28	33	3	69.0	5	57.7	17	19-2	yes	no	no	no	no	yes	no	yes
27	GANC12642-12-19LE16	107	16	36	18	66.1	41	62.6	2	0-23	no	Fhb1	no	yes	no	no	yes	no
28	GA15328-18E52F	108	22	37	31	68.6	7	55.8	36	19-0	het	het	no	no	no	no	no	no
29	KWS216	110	32	40	44	68.9	6	56.8	24	11-0	yes	no	no	no	no	no	no	no
30	KWS238	112	44	38	39	66.2	40	57.1	21	0-16	no	no	no	no	no	no	no	fail
31	KWS246	112	44	38	34	68.1	11	58.2	14	0-16	no	no	no	no	no	no	no	no
32	KWS263	110	32	39	41	69.1	4	59.7	8	0-18	no	no	no	no	no	no	no	no
33	KWS333	111	41	37	25	67.0	28	59.7	8	0-21	no	no	no	no	no	no	no	fail
34	LA13045D-173	105	9	38	37	67.9	12	55.1	41	0-16	no	Fhb1	no	no	no	no	no	fail
35	LA13045D-160	105	9	38	38	67.4	20	55.1	41	0-17	no	Fhb1	no	no	no	no	no	no
36	LA13181D-151A	107	16	37	26	67.7	17	61.0	5	0-15	no	Fhb1	no	no	no	no	no	het
37	LA15166LDH296	113	47	35	13	71.3	2	62.3	3	22-0	yes	no	no	yes	no	no	yes	no
38	LA15203LDH075	110	32	38	35	66.7	33	57.9	15	0-15	no	no	no	yes	no	no	yes	no
39	LA15VDH-FHB-MAS10-16	103	5	39	42	64.3	47	54.3	44	0-13	no	Fhb1	no	yes	no	no	no	no
40	LANC9337-63-4	101	2	32	2	66.3	38	56.0	33	0-16	no	no	no	yes	no	no	yes	no
41	15VDH-FHB-MAS22-14	104	7	33	4	66.9	29	56.0	33	0-19	no	Fhb1	yes	no	no	no	no	het
42	15VDH-FHB-MAS38-01	101	2	31	1	67.3	22	56.5	28	21-0	yes	Fhb1	no	yes	no	no	no	no
43	15VDH-FHB-MAS17-23	100	1	35	15	65.4	46	60.1	6	0-17	no	Fhb1	no	no	no	no	no	no
44	15VDH-FHB-MAS34-18	107	16	34	9	68.4	9	60.0	7	18-0	yes	Fhb1	no	no	no	no	no	het
45	16VDH-SRW01-120	107	16	33	5	67.1	24	55.0	43	0-20	no	Fhb1	no	no	no	no	no	het
46	16VDH-SRW09-025	109	28	37	27	66.9	29	62.2	4	0-15	no	no	no	yes	no	no	no	no
47	16VDH-SRW06-131	106	13	34	8	67.5	19	57.9	15	0-14	no	no	no	yes	no	no	no	no
48	VA18FHB-58	104	7	34	11	66.3	39	57.7	17	0-14	no	Fhb1	no	no	no	no	no	no
	Mean	108		36		67.3		57.3										
	LSD (0.05)	6.0		2		.		.										
	CV%	3		3		.		.										

MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN NC13-20076 SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) infection causes yield loss, quality degradation, and the production of hazardous mycotoxins in common bread wheat (*Triticum aestivum* L). Genetic resistance to FHB is environmentally ethical and effective; therefore, it is in the best interest of the wheat breeding community to identify quantitative trait loci (QTL) and implement them in marker assisted selection. NC13-20076 (Jamestown//GA951231-4E28/NCAG11G/AGS2026) has demonstrated high levels of FHB resistance over two seasons in the Uniform Southern Soft Red Winter Wheat Scab Nursery. Nevertheless, it does not contain previously identified FHB resistance QTL found in Southern US germplasm. A population of 185 double haploid lines from the cross of GA06493-13LE6, a susceptible line, and NC13-20076 was phenotyped in three misted and inoculated nurseries in North Carolina and Virginia during the 2018-19 and 2019-20 season. *Fusarium* damage on the head was recorded on a 0 to 9 scale during grain filling; *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) accumulation were estimated post-harvest. Heading date (HD) was recorded at Feekes 10.3 and plant height (PH) was recorded between Feekes 10.54-11.1. A linkage map consisting of 2158 single nucleotide polymorphic markers on 21 linkage groups was constructed. Many QTL were identified for FDK on chromosomes 1A, 1D, 2A, 2B, 3A, 3B, 4A, 5A, 5B, and 6B; FHB rating on chromosomes 1A, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, and 5B; and DON on 1A, 2A, 2B, 3A, 3D, 4A, 5A, 6B, 7A, and 7D. Three QTL were found for HD on chromosomes 4A, 5A, and 7B. Three QTL were identified for PH on 4A, 6A, and 7D. Overlapping credible intervals for Resistance QTL were found on 1A, 1D, 2A, 2B, 3A, 4A, 4B, 5A, 5B, 6B, 7A. QTL for FHB, FDK, and DON accounted for 0.40-7.8, 0.90-10.47, and 1.21-9.31 percent of the total additive variation, respectively. The average estimated percent additive variation for resistance QTL on 4A, 5A, and 7A were above three percent, and they are candidate loci for use in MAS. Kompetitive allele specific PCR (KASP) markers are currently being developed for these resistance loci and their efficacy will be tested on an independent population sharing parentage with NC13-20076.

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LEVERAGING CONSECUTIVE BREEDING POPULATIONS TO TRAIN GENOMIC PREDICTION MODELS FOR FHB RESISTANCE IN WHEAT

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ABSTRACT

Genomic selection (GS) has enabled breeders to make selection decisions based on predicted genotypic values instead of observed phenotypes. GS becomes even more important when it is impossible or costly to obtain phenotypes, and the marker effects in the training population (TP) have been estimated when phenotypic observations were meaningful. In the University of Minnesota wheat breeding program, 500 F_5 lines are used as a TP to predict the performance of nearly 2,000 unphenotyped F_5 lines of which selected lines are advanced to the preliminary yield stage. In this study, we examined the possibilities of using advanced breeding lines phenotyped in previous years to predict the performance of all F_5 lines in a future F_5 cohort that is genotyped but not phenotyped, effectively bypassing field testing at the F_5 stage. To do this, we used lines in the preliminary and advanced yield (PY and AY) stages to train a prediction model that was used to predict the performance of the unselected F_5 lines from the 2017 - 2019 TP. In our breeding program, lines selected from the F_5 stage are advanced to the PY stage. Therefore, the unselected F_5 lines from the 2017 – 2019 TP that have been genotyped and phenotyped served as our new validation population (VP) while lines in the 2017 – 2019 PY and AY stages served as our new TP. A total of 1,138 PY and AY lines across three previous cohorts was used as the TP while the VP comprised of 1,231 unselected lines from 2017 – 2019 F_5 cohorts. Additionally, a total of 29,945 markers were used in this study. In this presentation, we will present prediction accuracies when previous breeding cohorts are used to predict succeeding cohorts.

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