Fusarium graminearum Chemotype–Spring Wheat Genotype Interaction Effects in Type I and II Resistance Response Assays

Mitra Serajazari, Kerin Hudson, Mina Kaviani, and Alireza Navabi†

Department of Plant Agriculture, University of Guelph, Guelph, ON Canada.

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ABSTRACT

Fusarium head blight (FHB), caused by several *Fusarium* spp., is a worldwide problem that severely impacts cereal grain yield and poses major risks to human and animal health due to production of the mycotoxin deoxynivalenol (DON) and its acetylated forms, 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON). Recent studies suggest an inconsistent effect of *F. graminearum* chemotypes and resistance of wheat (*Triticum aestivum*) genotypes. To gain insight into the interaction effects of *F. graminearum* chemotypes and spring wheat genotypes on FHB resistance response, 10 spring wheat genotypes with varying levels of FHB resistance were inoculated with 10 *F. graminearum* isolates, consisting of 5 3-ADON- and 5 15-ADON-producing isolates and evaluated in type I (spray inoculation) and type II (point inoculation) resistance assays. Wheat genotypes

carrying the resistance allele of the *Fhb1* quantitative trait locus on chromosome 3BS had lower disease in type II evaluations, regardless of *F. graminearum* isolate or chemotype. Isolates of *F. graminearum* were also significantly different for disease aggressiveness. In addition, the 3-ADON-producing isolates were 18% more aggressive than the 15-ADON isolates in type I resistance assays. No difference in aggressiveness of the two chemotypes was observed, when tested in type II resistance assays. There was no interaction effect between *F. graminearum* chemotypes and spring wheat genotypes, suggesting that screening of germplasm for resistance can be performed with limited number of aggressive isolates.

Keywords: 3-ADON, 15-ADON, *Fusarium* head blight, wheat.

In most wheat (*Triticum aestivum* L.)-producing areas of the world, *Fusarium* head blight (FHB, also known as head scab) is a major threat to grain yield and quality, as well as to human health. This fungal disease, caused by several species of *Fusarium*, mainly *Fusarium graminearum* Schwabe in North America, was first reported in the late 19th century in the United States (Arthur 1891; Chester 1890). Every 4 or 5 years, a severe FHB epidemic occurs in the United States, China, European Union, United Kingdom, Africa, and Brazil and the estimated yield loss in the United States between 1990s and 2008 was US\$ 3 billion (Schumann and D'Arcy 2009). In Canada, six FHB epidemics were reported from 1940 to 1980 (Sutton 1982). In addition, the FHB incidence reached 70% in an epidemic year in south-central Manitoba (Shaner 2003). Over a 20-year period, economic losses due to FHB in Canada were estimated at \$50 million to \$300 million annually (Milligan 2014).

Apart from reducing grain yield, *F. graminearum* produces trichothecene mycotoxins such as deoxynivalenol (DON) in the grain. The DON-producing strains of *F. graminearum* can be divided into 3-acetyl-DON (3-ADON)- and 15-acetyl-DON (15-ADON)-producing chemotypes, based on the addition of an acetyl group to C3 or C15 in the trichothecene biosynthesis pathway. Molecular markers detecting 15-O-acetyltransferase (*TRI3*) and a trichothecene efflux pump (*TRI2*) genes have often been used to determine 3-ADON and 15-ADON chemotypes (Starkey et al. 2007). In a recent study, the toxicity of 3-ADON and 15-ADON chemotypes was evaluated using a lethal dose that kills 50% of a mice population (LD50). Oral administration of infected wheat grains containing 3-ADON or 15-ADON to mice revealed significantly higher toxicity of 3-ADON

(LD50 = 34 mg/kg) compared with 15-ADON (LD50 = 49 mg/kg) (Asam et al. 2017). In addition, disease surveys across Canada suggest that the *F. graminearum* 15-ADON chemotype is being replaced by the 3-ADON chemotype (Barker 2009). Analyses of trichothecene genotypes of *Gibberella zeae* (anamorph: *F. graminearum*) collected from winter wheat fields in the eastern parts of the United States revealed an increasing gradient in 3-ADON distribution from south to north and closer to Canada (Schmale et al. 2011). A European database of *F. graminearum* trichothecene isolates indicated that, from 1,147 strains of *F. graminearum* collected from 17 European countries, 15-ADON (82.9%) was the main chemotype, followed by 3-ADON (13.6%) and nivalenol (NIV) (3.5%) (Pasquali et al. 2016).

Breeding for resistance against FHB is very complicated due to different types of resistance against the pathogen. Studies of FHB resistance in wheat have determined five types of resistance mechanisms (He et al. 2016; Mesterházy et al. 2015). These consist of resistance to initial infection (type I), resistance to *Fusarium* sp. spread in spikes (type II) (Schroeder and Christensen 1963), resistance to accumulation of mycotoxins (type III), resistance to kernel infection (type IV), and resistance to yield loss (type V) (He et al. 2016; Lemmens et al. 2005; Mesterházy 1995, 2003; Miller and Arnison 1986). In practice, type I resistance can be measured following spray inoculation with *F. graminearum* based on the infected heads per plots under the field environment (Bai et al. 2018; Buerstmayr and Buerstmayr 2015) or based on the number of infected spikelets per head under controlled environment (Li et al. 2008; Schroeder and Christensen 1963; Shin et al. 2014; Yan et al. 2011; Yu et al. 2008). Yu et al. (2008) evaluated type I resistance only 7 days after spray inoculation prior to spread of the infection to neighboring florets (type II resistance).

Type II resistance is often screened for following point inoculation of spikelets based on the ratio or percentage of infection in one head to total number of spikelets over time (Bai et al. 1999). Type III and type IV resistances are examined postharvest and can be evaluated by measuring DON contents (type III) in the kernels and counting *Fusarium*-damaged kernels (FDKs) (type IV) (He et al. 2016).

†Corresponding author: A. Navabi; E-mail: anavabi@uoguelph.ca

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Genetic studies have identified several quantitative trait loci (QTL), which are effective in resistance to FHB (Buerstmayr et al. 2009) and in reduction of FDK and DON content; however, most of them appeared to be population specific (Mesterházy et al. 2015). The most nonspecific QTL with large effect are 3BS (*Fhb1*) and 5AS (*Qfhs.ifa-5A*) (Mesterházy et al. 2015). These two QTL together confer type I and II resistance to spray inoculation (Mesterházy et al. 2015). The spring wheat variety Sumai 3 from China, which carries the FHB-resistance QTL (*Fhb1*) on chromosome 3BS, has been a source of FHB resistance for many years and in many breeding programs (Bernardo et al. 2012). The *Fhb1* QTL confers resistance to *Fusarium* spp. spread in wheat spikes (type II resistance). Recently, a Kompetitive allele-specific (KASP) marker was developed to detect *Fhb1* in wheat lines (Bernardo et al. 2012). The *Qfhs.ifa.5A* QTL, which confers resistance to *Fusarium* initial infection (type I resistance), was mapped to chromosome 5A using an F₁-derived doubled-haploid (DH) population, resulting from a cross between CM-82036 (resistant) and Remus (susceptible) (Buerstmayr et al. 2003).

For the purpose of this study, we define the main effect of the pathogen isolates as the aggressiveness of a set of given isolates, measured quantitatively as the ability of each isolate to colonize plant tissue. We define the main effect of the host genotype as resistance, measured quantitatively as the ability of the host plant to reduce colonization by the pathogen. We also define the host genotype–pathogen isolate interaction effect as the differential response of wheat genotypes against a given set of pathogen isolates. We are specifically concerned with interaction effect, when it is associated with genotypic rank change, following inoculation with a given set of pathogen isolates.

Previous studies attempting to examine the main and interaction effects of *F. graminearum* chemotypes with wheat genotypes have revealed conflicting results. For example, a study of chemotype aggressiveness, following artificial inoculations in the field across Canada and Europe, reported no significant difference between aggressiveness of the 3-ADON and 15-ADON chemotypes (von der Ohe et al. 2010). Another study of chemotype aggressiveness and the interaction between DON derivatives and NIV-producing isolates on a range of wheat lines with different responses to FHB found stable resistance to FHB in highly resistant wheat lines carrying the 3BS QTL, regardless of *F. graminearum* chemotype (Foroud et al. 2012).

A transcriptome analysis of Sumai 3, following inoculation with 3-ADON and 15-ADON chemotypes (two florets of each wheat head with 10 µl of 1 × 10⁵ conidia/ml), found seven putative

defense-related genes involved in *Fusarium* sp.–wheat interaction (Al-Taweel et al. 2014). This study also suggested that chemotype plays an important role in FHB resistance. Defense-related genes (e.g., UDP-glucosyltransferase, thaumatin-like protein, glucosidase, chitinase III, and peroxidase) were expressed in plants inoculated with 3-ADON and 15-ADON 48 to 144 h after inoculation but the quantity of the expression was higher, when inoculated with 15-ADON. The authors hypothesized that higher expression of defense-related genes plays a role in the suppression of virulence in 15-ADON chemotypes, which may explain some of the reports of higher aggressiveness of 3-ADON isolates.

In contrast, a chemotype aggressiveness study using 10 isolates of 3-ADON and 15-ADON chemotypes on resistant cultivar ND 2710, moderately resistant Steele-ND, and susceptible Grandin found no significant difference between the 3-ADON and 15-ADON in terms of their aggressiveness, when tested for type II resistance (Puri et al. 2016). Analysis of type I and type II resistance by inoculating susceptible cultivar Roblin and the moderately resistant 5602 HR with 58 *F. graminearum* isolates of 3-ADON or 15-ADON chemotypes, collected from Manitoba, Saskatchewan, and Alberta in Canada, found no significant difference between aggressiveness of 3-ADON and 15-ADON (Gilbert et al. 2010). Also, quantification of DON and ADON following point inoculation of Roblin and 5602-HR with 40 *F. graminearum* isolates of 3-ADON or 15-ADON chemotypes, collected from Manitoba, revealed a significant interaction between wheat variety and chemotype for DON ($P \leq 0.05$) but not for ADON content (Gilbert et al. 2010).

Given the importance of an understanding of the main and interaction effects of the *F. graminearum* chemotypes and wheat genotypes and to resolve the discrepancies indicated above, we hypothesized that different spring wheat genotypes, with varying levels of resistance against FHB, would respond differently, when inoculated with a diverse set of 3-ADON- and 15-ADON-producing isolates of *F. graminearum* from across Canada. Therefore, the objectives of this research were to (i) examine the aggressiveness of 3-ADON- and 15-ADON-producing isolates, (ii) examine the resistance response of wheat genotypes with and without the *Fhb1* QTL, and (iii) study the differential responses of wheat genotypes against 3-ADON- and 15-ADON-producing isolates of *F. graminearum* following point and spray inoculation. Such interactions can have implications in designing selection strategies for breeding FHB-resistant varieties.

MATERIALS AND METHODS

Plant materials and growth conditions. Based on prior field FHB responses, 10 spring wheat genotypes were selected for experiments (Table 1). Seeds were planted in a mixture (1:1) of Sunshine LA4 (Sungrow Horticulture; Seba Beach, AB, Canada) and coarse Turface (Turface Athletics; Buffalo Grove, IL, U.S.A.) in 1.5 by 8.25 in. cones (SC10; Greenhouse Megastor, Danville, IL, U.S.A.). Plants were raised in a controlled-environment growth room at the University of Guelph, ON, Canada, with a photoperiod of 16 h of light and 8 h of darkness, a temperature of 20 and 18°C, and a relative humidity of 60% prior to inoculation. After inoculation, growth conditions were a photoperiod of 16 h of light and 8 h of darkness, a temperature of 26 and 24°C, and a relative humidity of 80%.

Preparation of *F. graminearum* inoculum. A collection of 10 *F. graminearum* isolates was obtained from the Canadian Grain Commission (Winnipeg, MB, Canada). This collection, which consists of five 3-ADON and five 15-ADON chemotypes (Table 2), has been isolated from Ontario, Alberta, Saskatchewan, and Manitoba from 2006 to 2010. The isolates were cultured on potato dextrose agar for 7 days and a 5-mm agar disc was transferred to a 250-ml flask containing a sterilized mixture of wheat straw (5 g) and dH₂O (125 ml). The inoculated flasks were placed on a shaker (120 rpm) at 25°C in the dark for 10 days. Macroconidia were

TABLE 1. Spring wheat genotypes used for type I and II resistance experiments

Wheat genotype	Reaction ^a	Source
AAC Brandon	MR	Cuthbert et al. 2016
AAC Scotia	MR	Ontario Spring Cereal Performance Trials 2014
Carberry	MR/MS	DePauw et al. 2011; Ontario Spring Cereal Performance Trials 2014, 2015
Frontana	R	Bai and Shaner 2004
Norwell	MS	Ontario Spring Cereal Performance Trials 2014
Peace	MS	Humphreys et al. 2014
Sable	HS	Ontario Spring Cereal Performance Trials 2014
SS Blomidon	S	Ontario Spring Cereal Performance Trials 2015
Sumai 3	R	Bai and Shaner 2004
Wilkin	S	Ontario Spring Cereal Performance Trials 2014

^a Field reaction to *Fusarium* head blight: MR = moderately resistant, MS = moderately susceptible, R = resistant, HS = highly susceptible, and S = susceptible.

harvested by passing the culture through three layers of cheesecloth. Macroconidia concentration was adjusted to 50,000 macroconidia/ml. Each plant head was inoculated with a single chemotype.

Chemotype and genotype classifications using molecular markers. Genomic DNA of *F. graminearum* isolates were extracted from 50 mg of mycelia using DNeasy Plant Mini Spin Columns (Qiagen, Germantown, MD, U.S.A.). Chemotype-specific primers were used in a multiplex polymerase chain reaction (PCR) to identify 3-ADON and 15-ADON chemotypes as previously described by Starkey et al. (2007). The PCR products were size fractionated and visualized using the QIAxcel Advanced System (QIAGEN GmbH, Hilden, Germany).

A single-nucleotide polymorphism marker for *Fhb1*, snp3BS-8, was used to identify whether the *Fhb1* resistance allele was present and was analyzed to verify the *Fhb1*-resistant allele (Bernardo et al. 2012). The KASP assay was used to detect and distinguish resistant and susceptible alleles for *Fhb1*. Polymorphism was identified using the LGC Genomics (<https://www.lgcgroup.com>) KASP system fluorescent assay with two allele-specific primers, susceptible primer (5'-CACATGCATTTGCAAGGTTGTTATCC-3') and resistant primer (5'-CACATGCATTTGCAAGGTTGTTATCG-3'), and one common (reverse) primer (5'-CAAAGCAGCCTTAGGTCATAGTTTGAAA-3') (Bernardo et al. 2012). PCR was run according to the LGC instructions using a reaction volume of 10 µl, which consisted of 5 µl of 2× KASP reaction mix, 0.14 µl of 72× assay mix, and 5 µl of template DNA (10 ng µl⁻¹). Endpoint genotyping was conducted in QuantStudio 6 Flex System (Applied Biosystems, Foster City, CA, U.S.A.).

Evaluation of wheat genotypes for type II resistance. Two experiments were conducted to evaluate wheat genotypes for type II resistance. A preliminary experiment (experiment 1) was performed to determine the efficiency of point inoculation in our experimental system (see "Plant materials and growth conditions"). In this preliminary experiment, Sumai 3, Frontana, Agriculture Adaptation Council (AAC) Brandon, AAC Scotia, Carberry, Peace, Sable, and Wilkin were planted in four replications. Later, in the second experiment, the same spring wheat genotypes as well as SS Blomidon and Norwell were planted in eight replications. At anthesis, each genotype was separately point inoculated with each one of the 10 *F. graminearum* isolates, of which 5 isolates were 3-ADON- and 5 were 15-ADON-producing chemotypes. For point inoculation, the right floret of the middle spikelet was injected with 10 µl of a macroconidia suspension (50,000 macroconidia/ml) using a micropipette. After inoculation, the spikes were immediately covered with plastic bags and the plants were transferred to a growth room with a photoperiod of 16 h of light and 8 h of darkness, a temperature of 26 and 24°C, and a relative humidity of 80%. The number of infected spikelets in each head was recorded 7, 14, and 21 days after inoculation (dai) in the first experiment and 5, 10, and 15 dai in the second experiment. The area under the disease progress curve (AUDPC) was calculated according to the following equation, where Y_i is disease level at time t_i .

$$AUDPC = \sum_{i=1}^a \left[\left\{ \frac{(Y_i + Y_{(i+1)})}{2} \right\} (t_{(i+1)} - t_i) \right]$$

Evaluation of wheat genotypes for type I resistance. To determine type I resistance, experimental plants, *F. graminearum* isolates, and growth conditions were similar to the second experiment for type II resistance, except that each plant head was sprayed with 2 ml of a suspension of macroconidia (50,000 macroconidia/ml) at anthesis. The inoculated heads were covered with plastic bags for 5 days. Plants were kept in a growth room with a photoperiod of 16 h of light and 8 h of darkness, a temperature of 26 and 24°C, and a relative humidity of 80%. Resistance to initial infection (type I resistance) under a controlled environment was

measured according to Yan et al. (2011); Yu et al. (2008), and Schroeder and Christensen (1963) by counting the number of infected spikelets in each head only one time 5 days after spray inoculation prior to spread of the pathogen to neighboring spikelets.

Statistical analysis. All experiments were performed using a randomized complete block design. Data were analyzed in the PROC MIXED procedure in SAS (V 9.4; SAS Institute Inc., Cary, NC, U.S.A.) for each test separately, in which the main effects of isolate and genotype and the genotype-isolate interaction were considered fixed effects and block was considered random effect. The main effect of pathogen isolate and wheat genotype examines the aggressiveness of the pathogen and resistance response of the host, while the interaction effect examines the isolate-specific response of the genotypes. Random and independent distribution of residuals were tested by plotting the studentized residuals against predicted values, while normality of the residuals were tested using the Shapiro-Wilks test in the PROC UNIVARIATE procedure in SAS. Data for type I and type II responses were transformed using square root and logarithmic transformations, respectively. Least-squared means were estimated using the LSMEANS statement in PROC MIXED. Pairwise comparisons were conducted using the PDIF statement. The least-squared means were then back-transformed. Single-degree of freedom contrasts were performed to test the hypotheses that the 3-ADON and 15-ADON isolates are similar for aggressiveness and that the genotypes with and without *Fhb1* are similar for disease severity for type II or disease damage for type I resistance. For each test, a genotype-isolate matrix was constructed using the back-transformed least-squared mean values. Each matrix was then subjected to a principal component (PC) analysis in PRINCOMP followed by PRINQUAL procedures in SAS. The biplot of the PC1 and PC2 eigenvalues was constructed and the proportion of variance accounted for by each PC was estimated for each test. Different chemotypes and genotypes with and without *Fhb1* were color coded in the biplots.

RESULTS

Classification of genotypes and chemotypes using molecular markers. Ten *F. graminearum* isolates used in the experiments were grouped into 3-ADON- and 15-ADON-producing chemotypes using *TRI3* and *TRI2* markers (Table 2). The KASP assay identified the presence of an *Fhb1* resistant allele in Sumai 3, Carberry, and AAC Brandon.

Evaluation of type II and type I resistance. In experiments 1 and 2 (type II resistance), the main effect of wheat genotypes (resistance) as well as the difference between genotypes with and without the *Fhb1* marker were significant ($P < 0.0001$). The main effect of *F. graminearum* isolates (aggressiveness) was only significant in experiment 2. However, the differences between the 3-ADON and 15-ADON chemotypes and the isolate-wheat genotype interaction effects were not significant (Table 3). Evaluation of type I response, on the other hand, revealed a significant difference ($P < 0.0001$) in the wheat genotype main effect, *F. graminearum*

TABLE 2. The collection of 3-acetyl-deoxynivalenol (ADON)- and 15-ADON-producing *Fusarium graminearum* isolates used for type I and II assays

Isolate ID	Province	Year	Isolate	Chemotype
ON-06-146	Ontario	2006	<i>F. graminearum</i>	3-ADON
ON-06-211	Ontario	2006	<i>F. graminearum</i>	3-ADON
A7-10-011	Alberta	2010	<i>F. graminearum</i>	3-ADON
S4B-10-007	Saskatchewan	2010	<i>F. graminearum</i>	3-ADON
Grass 10-3	Manitoba	2010	<i>F. graminearum</i>	3-ADON
ON-06-007	Ontario	2006	<i>F. graminearum</i>	15-ADON
ON-06-052	Ontario	2006	<i>F. graminearum</i>	15-ADON
A6-10-007	Alberta	2010	<i>F. graminearum</i>	15-ADON
S4A-10-002	Saskatchewan	2010	<i>F. graminearum</i>	15-ADON
Grass 13-4	Manitoba	2010	<i>F. graminearum</i>	15-ADON

isolates, as well as the aggressiveness of the 3-ADON and 15-ADON chemotypes. The isolates of the 3-ADON chemotype were found to be more aggressive than 15-ADON, causing 18% higher final disease damage (Table 4). This difference between the aggressiveness of the 3-ADON and 15-ADON chemotypes did not hold, when the wheat genotypes were evaluated for type II resistance (Tables 3 and 4).

In experiment 1, wheat genotypes containing the *Fhb1* marker showed 60 and 50% fewer disease symptoms, when inoculated with

3-ADON- and 15-ADON-producing isolates, respectively. In experiment 2, these reductions in disease symptoms were 50 and 30%, respectively (Table 4). As expected, the effect of the *Fhb1* marker on type I response evaluation was not significant regardless of isolate chemotype. Also, in all three experiments, Wilkin was the most susceptible and Sumai 3 was the most resistant line (Fig. 1).

In PC analyses (Fig. 2), separately conducted for the three experiments, PC1 accounted for 54 to 64% of the variation and PC2 accounted for 13 to 17% of the variation, resulting in the biplots of

TABLE 3. Mixed-model analysis of variance of type I and type II response of 10 wheat genotypes against 10 isolates of 3-acetyl-deoxynivalenol (ADON)- and 15-ADON-producing *Fusarium graminearum*

Fixed effects	Type I		Type II			
			Experiment 1		Experiment 2	
	F value	P value	F value	P value	F value	P value
Isolate	5.79	<0.0001	1.11	0.359	4.75	<0.0001
3A- versus 15-ADON	20.50	<0.0001	0.48	0.4905	0.01	0.9064
Genotype	8.51	<0.0001	13.35	<0.0001	11.05	<0.0001
<i>Fhb1</i> + versus <i>Fhb1</i> -	3.22	0.0734	69.54	<0.0001	55.99	<0.0001
Isolate × genotype	1.06	0.3569	0.81	0.828	1.1	0.2691

TABLE 4. Least-squared means of the disease severity of 10 spring wheat genotypes for type II and disease damage for type I resistance following inoculation with 10 isolates of 3-acetyl-deoxynivalenol (ADON)- and 15-ADON-producing *Fusarium graminearum*

Genotypes	<i>Fhb1</i>	Type I		Type II			
		3-ADON	15-ADON	Experiment 1		Experiment 2	
				3-ADON	15-ADON	3-ADON	15-ADON
AAC Brandon	+	8.5	8.3	12.8	15.7	21.9	24.9
Carberry	+	11.0	9.2	16.3	13.2	15.9	19.4
Sumai 3	+	7.5	5.8	5.6	6.9	15.9	21.9
AAC Scotia	...	8.6	6.7	22.7	18.2	29.7	30.9
Frontana	...	8.1	7.3	24.4	21.3	27.4	23.5
Norwell	...	10.5	9.7			47.9	37.0
Peace	...	9.8	7.3	25.0	26.0	32.6	35.0
SS Blomidon	...	9.8	7.0			23.0	23.9
Sable	...	10.1	8.5	30.5	26.3	36.2	33.8
Wilkin	...	12.0	11.4	44.1	29.9	46.4	48.3
Mean	...	9.6	8.1	22.7	19.7	29.7	29.9
Standard error							
3-ADON vs. 15-ADON	...		0.28		0.20		0.14
<i>Fhb1</i> + vs. <i>Fhb1</i> -	...		1.27		0.64		0.62

Fig. 1. Disease severity for type II and disease damage for type I resistance of spring wheat genotypes inoculated with 3-acetyl-deoxynivalenol (ADON) (Y axis) and 15-ADON (X axis) chemotypes. **A**, Type I resistance, **B**, type II resistance in experiment 1, and **C**, type II resistance in experiment 2. Dark gray/red dots represent genotypes carrying the resistant allele of the *Fhb1* QTL. Light gray/blue dots represent genotypes lacking the resistant allele of the *Fhb1* QTL.

PC1 and PC2 accounting for 73 to 78% of total variation. In both type II experiments (experiment 1 and experiment 2), PC1 separated the main effect of wheat genotypes, with those carrying the *Fhb1* marker falling on the left side of the origin and genotypes without the *Fhb1* marker on the right side of the origin. In both experiments, except for one isolate, PC2 separated the main effect of the *F. graminearum* isolate. The only meaningful clustering that was found in the biplots of experiments 1 and 2 was clustering of the genotypes carrying the *Fhb1* marker, known to contribute to type II resistance. No clustering was found in these biplots to indicate that wheat genotypes interact differently with the two chemotypes. In the biplot of the type I evaluation, PC1 mainly separated the effect of genotypes and PC2 separated the effect of isolates. However, no clustering of genotypes, based on their *Fhb1* genotype, or clustering of isolates, based on their DON chemotypes, was observed. Results of the three biplots did not point to any meaningful interaction between DON chemotype and wheat genotype.

In all three biplots, *F. graminearum* isolates had positive relationships except for one isolate in type I evaluation and one isolate in experiment 2 of type II evaluation (Fig. 2A, B, and D). In experiment 2, the 15-ADON-producing isolate ON-06-007 had a negative relationship with S4A-10-002, ON-06-52, and A7-10-011. All other isolates had positive relationships (Fig. 2C). For type I resistance, ON-06-211 had a negative relationship with Grass-13-4, Grass-10-3, and ON-06-52.

DISCUSSION

We examined a set of 3-ADON- and 15-ADON-producing isolates of *F. graminearum* on a set of wheat genotypes with different levels of resistance response in order to study variation in aggressiveness of the pathogen as it relates to chemotype, as well as isolate- or chemotype-specific reaction of the host genotypes.

The main effect of wheat genotype was always significant. In type II evaluations, the major contributor to this difference seems to be the presence or absence of *Fhb1* QTL, regardless of *F. graminearum* chemotype. Molecular marker analysis showed that *Fhb1* QTL is involved in type II resistance, which is in agreement with previous reports (Bai et al. 1999; Zhou et al. 2002). In our experiments, the resistant lines carrying the *Fhb1* marker showed resistance to both 3-ADON- and 15-ADON-producing chemotypes. It was shown that plants carrying *Fhb1* QTL are able to detoxify DON by producing DON-glycoside (Lemmens et al. 2005), resulting in higher resistance in plants with lower DON (Mesterházy et al. 2015). Recently, mutation analysis, gene

silencing, and transgenic overexpression showed that a pore-forming toxin-like (*PFT*) gene located in *Fhb1* QTL causes resistance to FHB (Rawat et al. 2016). Foroud et al. (2012) evaluated two DH populations resulting from crosses between Superb (no resistance QTL) and CM82036 (3BS and 5A QTL) against type II resistance with point inoculation. Their results showed that DH populations were resistant to all chemotypes (3-ADON, 15-ADON, and NIV). They also showed that susceptible lines with no resistance QTL (Superb, Crystal, and CIMMYT 11) accumulated higher trichothecene compared with resistant lines and, interestingly, they accumulated 3-ADON more than 15-ADON and NIV (Foroud et al. 2012). We conducted type I evaluations only once, 5 days after spray inoculation. Evaluation of type I resistance in this manner prevented confounding type I and type II resistance (Schroeder and Christensen 1963; Yan et al. 2011; Yu et al. 2008). This evaluation is different than type I evaluation under field conditions, where it is measured based on the number of infected heads per plot, as described by Bai et al. (2018). In our type I evaluation, the presence and absence of the *Fhb1* marker was not the major determinant and wheat genotypes had a range of reactions to *F. graminearum* isolates regardless of their chemotype. Moreover, 3-ADON isolates were more aggressive compared with 15-ADON. von der Ohe et al. (2010) and Gilbert et al. (2010) found no significant difference between the aggressiveness of 3-ADON and 15-ADON related to the field resistance.

In all experiments, Wilkin was the most susceptible and Sumai 3 was the most resistant genotype (Fig. 1). Sable was previously reported as highly susceptible under field inoculation (Table 1) but it was less susceptible than Wilkin in our experiments. This difference in susceptibility of Wilkin may be due to the experimental conditions. In this regard, artificial inoculations under Ontario field conditions were conducted using a mixture of *F. graminearum* isolates (Table 1), whereas our experiments were conducted using a single chemotype for artificial inoculation under controlled-environment growth rooms. Also, FHB field experiments are subjected to a genotype–environment effect. This interaction effect under field conditions cannot be compared with that under our controlled-environment condition. Our study indicates a lack of an overall significant interaction or specificity between wheat genotypes and isolates or DON chemotypes. In type II evaluations, for example, genotypes that carry some level of type II resistance (mainly due to the presence of *Fhb1* QTL) are resistant regardless of the isolate or chemotype they are being tested with, which is in agreement with the study conducted by Foroud et al. (2012). The same results were found for type I response, except for one isolate

Fig. 2. Biplots of PC1 and PC2 in principal component (PC) analyses of type I and type II resistance following inoculation with 10 isolates of 3-acetyl-deoxynivalenol (ADON)- and 15-ADON-producing *Fusarium graminearum*. **A**, Type I resistance, **B**, type II resistance in experiment 1, and **C**, type II resistance in experiment 2. Genotypes with blue font carry the resistance allele of the *Fhb1* QTL. Chemotypes with red font are 15-ADON-producing isolates and others are 3-ADON-producing isolates.

(ON-06-211) (Fig. 2A). The susceptible genotypes, on the other hand, and especially for type II resistance, showed some level of rank change, when tested with different isolates even though the overall interaction was not significant. Regardless of these findings, no conclusion can be made that genotypes interact differentially with the two chemotypes. The significance of this finding in the breeding programs is that, with a lack of genotype–chemotype interaction, evaluation of germplasm for resistance against *F. graminearum* can be done with a number of aggressive local isolates, regardless of their chemotype, and that any resistance found can be expected to have resistance against a broad range of *F. graminearum* isolates.

Previous studies in several cereal crops have reported the complex effects of trichothecenes on *F. graminearum* virulence as being more host specific but less chemotype specific (Maier et al. 2006). The effect of trichothecenes on *F. graminearum* virulence was investigated by disrupting the trichodiene synthase (*Tri5*) gene, the first gene in the trichothecene pathway, using the disruption vector *pTri5-ko*. The isolates with $\Delta Tri5$ were used to inoculate wheat, barley, and maize. In wheat, the $\Delta Tri5$ isolates produced symptoms but there was no spread of symptoms in wheat heads compared with the wild-type isolate. In barley, there was no difference between the $\Delta Tri5$ and wild-type isolates for producing symptoms. In maize, isolates with $\Delta Tri5$ produced the same disease symptoms compared with the wild-type isolates but isolates with disruption in the NIV-producing enzymes caused less disease compared with the wild type (Maier et al. 2006). Their results showed that Munk, a wheat variety susceptible to FHB, is different from barley and maize in response to $\Delta Tri5$ isolates and that trichothecenes play an important role in the spread of infection in the wheat head. To investigate the differences among wheat cultivars against FHB chemotypes, we tested a range of wheat cultivars with different ranges of resistance response. Our results showed a significant difference between wheat genotypes (host) in disease damage against 3-ADON and 15-ADON but not any interaction between wheat genotypes and chemotypes.

A recent study hypothesized that 3-ADON isolates are more aggressive than 15-ADON isolates due to faster growth and reproduction (Vujanovic et al. 2012). This hypothesis was based on the finding that 3-ADON-producing isolates from several regions of Canada grew faster than 15-ADON isolates, when exposed to extremely high or low temperatures. Therefore, the 3-ADON-producing isolates can withstand harsher environmental fluctuations than 15-ADON-producing isolates. Our results show that, under a controlled environment with no environmental fluctuations, the 3-ADON-producing isolates are still more aggressive than 15-ADON-producing isolates for type I resistance (resistance against initial infection) (Table 4) but not for type II resistance (resistance against the spread of the infection within spike). This suggests that the aggressiveness of the 3-ADON chemotype is not related to environmental effects and may rather depend on higher toxicity of this chemotype. Apart from withstanding harsher environmental fluctuations, other characteristics of 3-ADON-producing isolates may be responsible for spreading in larger areas of Canada and replacing 15-ADON-producing isolates (Ward et al. 2002).

In contrast to the results reported by Vujanovic et al. (2012), a recent study of the 3-ADON-, 15-ADON-, and NIV-producing isolates of *F. graminearum* from China did not find a significant difference in growth rates of these isolates at 15°C, although the 15-ADON-producing isolates released more ascospores (Liu et al. 2017). At 20 and 25°C, however, 15-ADON-producing isolates grew faster than 3-ADON- and NIV-producing isolates. In all temperatures examined, the 15-ADON-producing isolates generated more perithecia 2 to 3 days earlier than the two other chemotypes. This study also found no difference in aggressiveness between 3-ADON and 15-ADON chemotypes, when type II resistance was evaluated on a moderately resistant genotype

(Yangmai 158) but more DON was found in seed infected with the 3-ADON-producing isolates. This study suggested that the spread of *F. graminearum* chemotypes depends on their biological characteristics. In agreement with these results, our study of 10 wheat genotypes also found no significant difference between 3-ADON- and 15-ADON-producing isolates for type II resistance (Table 3). Moreover, our evaluation of type I resistance revealed that 3-ADON-producing isolates were more aggressive than 15-ADON-producing isolates. We suggest that testing for type I resistance in the controlled environment will provide a better indication of resistance to *F. graminearum* chemotypes under natural wheat field conditions. If the aggressiveness of 3-ADON-producing isolates was related to their higher toxicity, then we would have expected higher aggressiveness of this chemotype for type II resistance. Indeed, we did not find a significant difference between chemotypes for type II resistance. Our results further suggest that 3-ADON production is an important factor for causing initial infection but this may be related to higher expression of defense-related genes against 15-ADON-producing chemotypes (Al-Taweel et al. 2014) rather than to higher toxicity. Mechanisms that are involved in the type I resistance are unidentified and not well defined (Mesterházy et al. 2015). Although several studies found negative correlation between anther extrusion and plant height with type I susceptibility to FHB (He et al. 2016; Lu et al. 2013; Mao et al. 2010; Skinnies et al. 2010), this type of resistance can be considered more than a morphological barrier against *Fusarium* sp. penetration.

In conclusion, the only significant difference between the 3-ADON- and 15-ADON-producing isolates was higher aggressiveness in type I resistance, and there was no interaction between genotypes and chemotypes in both types of resistance. Type I resistance might not be considered just a physical barrier against *Fusarium* head blight. The more extensive spread of the 3-ADON chemotypes may be related to higher expression of defense genes against 15-ADON (Al-Taweel et al. 2014) apart from its toxicity or tolerance to environmental fluctuations. Further experiments are required to elucidate different reactions of wheat genotypes to 3-ADON and 15-ADON chemotypes at the molecular levels.

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