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ORIGINAL RESEARCH

A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat

Christiane von der Ohe · Victoria Gauthier · Lily Tamburic-Ilincic · Anita Brule-Babel · W. G. Dilantha Fernando · Randy Clear · Todd J. Ward · Thomas Miedaner

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Abstract Twenty four isolates of *Fusarium graminearum*, half of which were 3-acetyldeoxynivalenol (3-ADON) and half 15-acetyldeoxynivalenol (15-ADON) chemotypes, were tested for their ability to produce deoxynivalenol and to cause Fusarium head blight (FHB) in spring wheat cultivars. The objectives of this study were to determine (1) whether 3-ADON isolates differ in aggressiveness, as measured by the FHB index, and DON production from 15-ADON isolates under field conditions, and (2) whether the performance of resistant host cultivars was stable across isolates. Field tests of all isolates were conducted with three replicates at each of two locations in Canada and Germany in 2008 with three host genotypes differing in FHB resistance level. The resistant host genotype showed resistance regardless of the chemotype or location. The differences between mean FHB indices of 3-ADON and 15-ADON isolates were not significant for any wheat genotype. In contrast, average DON production by the 3-ADON isolates (10.44 mg kg^{-1}) was significantly ($P<0.05$) higher than for the 15-ADON isolates (6.95 mg kg^{-1}) at three of the four locations where moderately resistant lines were tested, and at both locations where susceptible lines were evaluated. These results indicate that 3-ADON isolates could pose a greater risk to food safety. However, as the mean aggressiveness and DON production of 3-ADON and 15-ADON chemotypes was similar on highly resistant lines, breeding and use of highly resistant lines is still the most effective measure of reducing the risks associated with DON in wheat.

Keywords Chemotype · DON · FHB index

C. von der Ohe · T. Miedaner (✉)
State Plant Breeding Institute, Universitaet Hohenheim,
70599 Stuttgart, Germany
e-mail: miedaner@uni-hohenheim.de

V. Gauthier · A. Brule-Babel · W. G. D. Fernando
Department of Plant Science, University of Manitoba,
Winnipeg MB R3T 2N2, Canada

L. Tamburic-Ilincic
Department of Plant Agriculture, University of Guelph,
Ridgetown Ontario N0P 2C0, Canada

R. Clear
Grain Research Laboratory, Canadian Grain Commission,
Winnipeg MB R3C 3G8, Canada

T. J. Ward
Bacterial Foodborne Pathogens and Mycology Research
Unit, United States Department of Agriculture - Agriculture
Research Service,
Peoria IL 61604, USA

Introduction

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is the most common causal agent of Fusarium Head Blight (FHB) in the

world (Goswami and Kistler 2004). Two characteristics of *F. graminearum* are important: the pathogenicity (the ability to cause disease) and the aggressiveness (the amount of disease caused in a background of non-race specific resistance) (Miedaner et al. 2008). FHB of wheat is a far reaching and detrimental disease, directly causing reduced yield and grain quality (Goswami and Kistler 2004). Although the disease has been endemic in many parts of the world including eastern Canada, it has generated increasing interest since the devastating epidemics in 1993 and 1994 in the upper midwest of the USA and Manitoba, and in north-eastern USA and Ontario in 1996 (Paulitz et al. 1999).

Symptoms of FHB in wheat are blighted spike and peduncle tissues, which are brown or tan in colour and senesce prematurely (Osborne and Stein 2007). Wheat is most susceptible to infection at anthesis when the anthers are still able to provide nutrients for the colonizing pathogen and/or stimulate fungal growth (Osborne and Stein 2007). If susceptible host plants are infected during anthesis, the full spike may be entirely bleached. Purple-black perithecia and or pink sporodochia may cover infected spikes especially around the glumes (Osborne and Stein 2007).

Some of the effects of FHB infection include decreased yield, bleached and shrunken kernels, low seed weight, decreased seed quality and vigour, and accumulation of mycotoxins, specifically deoxynivalenol (DON) (Goswami and Kistler 2004). Mycotoxins produced by the pathogen may make the grain unsuitable for flour milling, cereal foods, malting and use as animal feed as well as result in significantly reduced market price and marketability. A decade of significant losses and damage due to FHB has resulted in great efforts to understand the pathogen, the disease and the factors that cause and intensify the disease (Gilbert and Fernando 2004).

Two chemotypes of *F. graminearum* were first identified by Ichinoe et al. (1983), one of which produced DON and predominantly 3-acetyldeoxynivalenol (3-ADON) and another forming DON and predominantly 15-acetyldeoxynivalenol (15-ADON) (Miller et al. 1991). A third chemotype was later characterized as a nivalenol (NIV) producer. Research done by Goswami and Kistler (2005) showed that all strains of *F. graminearum* had the ability to produce detectable quantities of trichothecenes, although disease-causing ability was not influenced by the type of trichothecenes produced. A significant correlation between DON content and aggressiveness was observed.

DNA sequence analysis, multilocus genotyping assays, variable number tandem repeat (VNTR) analyses as well as mycotoxin and pathogenicity analyses demonstrated that the 3-ADON chemotype is quickly displacing the 15-ADON chemotype in *F. graminearum* populations from western Canada (Ward et al. 2008). Phenotypic analysis in the greenhouse showed that on average 3-ADON isolates produced a higher DON concentration than 15-ADON isolates, but no significant differences in aggressiveness were identified between chemotypes (Ward et al. 2008). Each year ascospores can be distributed to new areas through wind from infected wheat and corn fields (Fernando et al. 2000). In Manitoba the 3-ADON isolates are concentrated in the Red River Valley, and moving westwards (Guo et al. 2008) most likely replacing 15-ADON isolates, which is cause for concern to the grain industry.

The objectives of this paper were to determine (1) whether twelve randomly collected 3-ADON producing isolates tested were more aggressive (i.e. had higher mean FHB index value) than twelve 15-ADON isolates tested by evaluating mean and terminal FHB index, and comparing DON in four locations, two in Canada and two in Germany, and (2) whether spring wheat cultivars with different resistance levels perform similarly across chemotypes.

Materials and methods

Locations

Four experiments were conducted in 2008, two in Germany and two in Canada. In Southern Germany, the locations were Hohenheim near Stuttgart and Eckartsweier near Offenburg. In Canada, trials were conducted in Ridgetown, Ontario and Winnipeg, Manitoba.

Genotypes

Spring wheat (*Triticum aestivum* L.) genotypes with known levels of FHB resistance were used. Adapted wheat lines were used in each location to minimize

the genotype x environment interactions and to avoid extreme differences in flowering time. The trials in Germany used a resistant (R) line from KWS-LOCHOW GmbH, Bergen, Germany, AABB, incorporating the *Fhb1* and the Q*fhs.ifa.5A* loci (Miedaner et al. 2006), moderately resistant (MR) cultivar Taifun from the same company and the susceptible (S) cultivar Tybalt from W. von Borries-Eckendorf GmbH, Leopoldshöhe, Germany. Taifun and Tybalt are registered spring wheat cultivars in Germany. The trials in Winnipeg used 93FHB37 incorporating *Fhb1* (R), AC Cora (MR), and CDC Teal (S). AC Cora and CDC Teal are registered spring wheat cultivars in Canada. The trials in Ridgetown used two Canadian registered spring wheat cultivars, Norwel (MR) and Quantum (S).

Isolates

Each location used 24 Canadian *F. graminearum* isolates (Table 1). Half of the isolates were 3-ADON producers and the remainder were 15-ADON producers. All of the isolates were from Fusarium-damaged wheat kernels collected from across Canada in 2006 (Table 1). Isolates were chosen to provide a representative sample from as many Canadian provinces as possible. In regions where both chemotypes were present, a sample of each chemotype was chosen. Sampling within a province was geographically spaced to provide as random a sample as possible. The levels of aggressiveness of the isolates were unknown. The isolates were accessioned into the Agricultural Research Service (ARS) Culture Collection, United States Department of Agriculture, Peoria, IL, and are identified by NRRL numbers. A previously published multilocus genotyping assay (Ward et al. 2008) was used to identify each of the isolates as *F. graminearum* sensu stricto (O'Donnell et al. 2004) and to assess the trichothecene chemotype of each isolate (Table 1).

Inoculum preparation

Inoculum production and inoculation methods were performed in accordance with the best practice at each location. Identical methods were used at both locations in Germany. The inoculum preparation methods are summarized in Table 2. Although some aspects of the inoculum preparation protocols differed, all inocula was produced from single spore cultures grown under 24 h UV light in each location's culture medium (Table 2). Following 7 to 10 days under 24 h UV light, the solid culture medium was then cut up and added to a liquid medium to induce sporulation of the pathogen (please refer to Table 2 for further explanation). After 7 to 10 days in liquid medium, the concentration of the inoculum was determined. The Canadian locations adjusted the spore concentration to 50 000 spores ml^{-1} for inoculations, whereas the German locations used 500 000 spores ml^{-1}. The inoculum concentration was measured on a per plot basis supplementing the inoculum stock with distilled water and 2 ml Tween 20 per litre.

Field experiments

A summary of the field experiments is shown in Tables 3 and 4. Each trial was designed as a split plot with three replicates.

Disease evaluation

In Winnipeg and Ridgetown, disease severity and incidence ratings were measured visually to determine FHB index. Disease severity was measured as the number of infected spikelets per spike (Type II resistance, according to Schroeder and Christensen (1963)) using the scale: 0 = no spikelets infected, 100 = all spikelets infected. Disease incidence was measured as the number of infected spikes per plot (Type I resistance), using the scale: 0 = no spikes infected, 100 = all spikes infected. FHB Index was calculated as the product of incidence and severity divided by 100. In Winnipeg, disease incidence and severity were measured at time intervals of 15, 18, 24 and 27 days post inoculation until natural spike senscence. In Ridgetown, the FHB index was calculated on a whole plot basis 14, 17 and 20 days after inoculation.

In Germany, the FHB index was measured at time intervals of 17, 20, 23 and 26 days after inoculation of each single genotype by visually rating severity and incidence on a whole plot basis (0–100%). For resistance comparison, the arithmetic mean of the individual ratings was used (Mean FHB index). For all locations, the terminal FHB index was defined as the last rating before senescence.

Table 1 Isolate codes, origin in Canada, type of DON production verified by molecular analysis and analysis by Gas Chromatography-Mass Spectrometer (GC-MS in % of DON concentration) tested across two locations (Hohenheim, Eckartsweier) in 2008

NRRL	Official code	Origin	Chemotype	GC-MS (% of DON)	
				15-ADON	3-ADON
44635	A2-06–1	Alberta	3-ADON	0.1	2.4
44884	A4-06–5	Alberta	3-ADON	0.0	3.2
44886	A6-06–1	Alberta	3-ADON	0.0	2.6
44358	M2-06–1	Manitoba	3-ADON	0.0	2.1
44512	M8-06–5	Manitoba	3-ADON	0.0	2.0
44963	NB-06–17	New Brunswick	3-ADON	0.0	2.5
45038	NS-06–2	Nova Scotia	3-ADON	0.0	2.4
45100	Q-06–11	Quebec	3-ADON	0.0	2.1
45112	Q-06–23	Quebec	3-ADON	0.0	2.8
44096	S1A-06–3	Saskatchewan	3-ADON	0.0	1.8
44187	S3BS-06–1	Saskatchewan	3-ADON	0.0	3.1
44274	S8A-06–1	Saskatchewan	3-ADON	0.0	2.6
Average				0.0	2.5
44613	A1-06–1	Alberta	15-ADON	1.0	0.4
44359	M2-06–2	Manitoba	15-ADON	1.0	0.0
44509	M8-06–2	Manitoba	15-ADON	0.8	0.5
44964	NB-06–18	New Brunswick	15-ADON	1.0	0.6
45039	NS-06–3	Nova Scotia	15-ADON	0.9	0.5
43897	ON-06–17	Ontario	15-ADON	0.9	0.2
45099	Q-06–10	Quebec	15-ADON	1.1	0.5
45111	Q-06–22	Quebec	15-ADON	0.9	0.0
45123	Q-06–34	Quebec	15-ADON	1.0	0.3
44097	S1A-06–4	Saskatchewan	15-ADON	1.0	0.8
44174	S3AN-06–1	Saskatchewan	15-ADON	1.1	0.4
44278	S8A-06–5	Saskatchewan	15-ADON	0.9	0.7
Average				1.0	0.4

Measurement of DON content

The plots were harvested by hand or with a small combine at full ripening and threshed carefully using a low fan speed to reduce loss of Fusarium damaged kernels. Samples from Canada were evaluated for DON concentration using the Enzyme Linked Immunosorbent Assay (ELISA) method. Samples were ground to a particle size of about 1 mm using a Romer mill (Model 2A, Romer Labs, Inc. Union, MO). DON was extracted from a 10 g subsample of ground grain in 50 ml deionized water. Quantification of the DON was done using the EZ-Quant® Vomitoxin ELISA kit from Diagnostix (www.diagnostix.ca) with a DON detection limit of 0.5 mg kg^{-1} (Sinha and Savard 1996).

The DON and 3-ADON quantification test used on the samples from Germany was a commercially available enzyme immunoassay (RIDASCREEN™ FAST DON, R-Biopharm GmbH, Darmstadt, Germany). The 5 g grain samples were ground to a particle size of about 1 mm, mixed and stored at −20°C until analysis. The assay used detects DON and 3-ADON, but has no cross reactivity to other trichothecenes such as 15-ADON, triacetyl DON, nivalenol, triacetyl nivalenol and fusarenon-X. The test plates were measured at 405 nm with a microtitre plate spectrometer (TECAN SLT Lab Instruments,

Table 2 Inoculum preparation at locations

Location	Culture media used	Liquid media used	Conidia production	Spore conc.
Eckartsweier, Hohenheim, Germany	SNA	Nutrient poor media (Nirenberg 1981)	1 l flask filled with 600 ml media on a shaker Table for 7 days at 100 rpm under 24 h UV light at 23°C	500 000 spores ml^{-1}
Ridgetown, Ontario, Canada	Maintained on CLA, subcultured on PDA	Modified Bilay's media (Tamburic-Ilincic et al. 2007)	Shaker Table for 10 days under diffuse sunlight	50 000 spores ml^{-1}
Winnipeg, Manitoba, Canada	PDA	CMC (Cappellini and Peterson 1965)	External air supply used to agitate 2 l flask filled with 1.5 l liquid media for 7 to 10 days under 24 h light bank at 21°C	50 000 spores ml^{-1}

PDA Potato Dextrose Agar (Difco); *SNA* Speziel Nährstoffarmer Agar (Synthetic Nutrient—Poor Agar) (Nirenberg 1981); *CLA* Carnation Leaf Agar (Burgess et al. 1988); *CMC* Carboxy-Methyl Cellulose (Cappellini and Peterson 1965)

Crailsheim, Germany) and DON content was calculated by using a manufacturer owned software package. Five standard solutions in water (0, 0.222, 0.666, 2 and 6 mg kg^{-1}) provided by the immunoassay kit per plate were used for calculation. DON detection limit was 0.222 mg kg^{-1}.

Gas Chromatography-Mass Spectrometer (GC-MS)

In order to confirm chemotype assignments based on molecular data, gas chromatography with ion-trap mass spectrometer detection using acetonitrile chemical ionization was used according to standard procedures of Schollenberger et al. (1998). The samples were milled using the same procedure as described for the ELISA test. Samples from the MR genotype, Taifun, from two locations in Germany with each of two replicates were analyzed by GC-MS to measure 3-ADON and 15-ADON plus total DON in each sample. The advantage of GC-MS is that it measures the different chemotypes, and has a lower detection limit than the ELISA. The GC-MS analyses were also run at Laboratory Services at the University of Guelph in Guelph, Ontario, Canada, with a quantification limit of 0.1 mg kg^{-1} for DON, 15-ADON and 3-ADON. In brief, milled samples were extracted by shaking with acetonitrile/water. The extract from the milled sample was purified using a

Table 3 General field experiment set up

Locations	Experimental design	Plantings	Seeding density (kernels m^{-1})
Eckartsweier, Hohenheim, Germany	Split plot (Main plot = genotype of wheat; sub plot = isolate)	3 rows per plot 0.208 m spacing 1 m length (chessboard-like design[1] with triticale)	40
Ridgetown, Ontario, Canada	Split plot (Main plot = genotype of wheat; sub plot = isolate)	1 row plots 17.8 cm spacing 2 m length; buffer plots between main plots	50
Winnipeg, Manitoba, Canada	Split plot (Main plot = isolate; sub plot = genotype of wheat)	6 row plots 0.17 m between rows 1.5 m between plots (centre to centre)	67

[1] = wheat plots bordered by four plots of triticale in north, south, east, west orientation

Table 4 Inoculation and irrigation set up

Locations	No. of inoculations; time	Amount inoculum applied	Inoculations	Misting system frequency
Eckartsweier, Hohenheim, Germany	1 inoculation (100% anthesis)	100 ml plot^{-1}	Hand-held sprayer (machine-driven air gun) at 3 bar	None
Ridgetown, Ontario, Canada	2 inoculations (50% anthesis; 3 days later)	50 ml plot^{-1}	CO_2 backpack sprayer at 2.1 bar	8 sec burst every min for 6 h (Approx. 7.5 mm water daily)
Winnipeg, Manitoba, Canada	2 inoculations (50% anthesis; 3 days later)	1.0 l plot^{-1}	2.26 kg CO_2 R&B backpack sprayer at 2.1 bar with nozzles of 80 degree fan at 7.57 l per minute and 0.32 mm screens, the boom was 6 nozzles at 20 cm spacing	1 hr after each inoculation for 10 min every hour for 10 h

column containing adsorbents, charcoal and celite. After an evaporation step, the residue was derived using trifluoracetic anhydride with dimethylamino-purine. Trifluoroacyl derivatives of the mycotoxins in question were quantified using gas chromatography with ion-trap detection using an acetonitrile chemical ionization (Schollenberger et al. 1998).

Statistical analyses

All data were computed on a plot basis. Split plot analyses of variance (ANOVA) were performed for each experiment and location using software PLAB-STAT (Utz 2008). The factor isolates was considered as random and the genotypes and chemotypes as fixed factors. The effect of chemotypes, isolates within chemotypes and chemotype by genotype interaction were analyzed by ANOVA (Table 5).

Results

Analysis of 3-ADON and 15-ADON concentration by GC-MS of grain from the two German trials confirmed chemotype assignments based on molecular data for all isolates (Table 1). The 3-ADON and 15-ADON contents of the respective chemotypes were minimal accounting on average for 2.5 and 1.0% of the total DON content (Table 1). However, most samples of the 15-ADON isolates also had small amounts between 0 and 0.7% of 3-ADON, but not vice versa.

The variance component for chemotype was not significant for either the mean FHB index or terminal FHB index (Table 5). Differences among isolates within chemotypes were significant ($P<0.01$) at both locations in Germany for mean FHB index and terminal FHB index. In Canada, no significant differences among isolates within chemotype were detected for mean FHB index and only Winnipeg showed significant differences among isolates for terminal FHB index. The genotypic variances due to cultivar differences were the largest variance components and were always significant ($P<0.01$) for both mean and terminal FHB index, while the genotype x chemotype interaction was not significant.

For DON content, the chemotypes were significantly different at two locations (Eckartsweier and Ridgetown). Differences among isolates within chemotypes were significant ($P<0.01$) at the two locations in Germany and Ridgetown. Variances due to the host genotype were the largest component and significant ($P<0.01$) at all locations except Ridgetown, whereas the genotype x chemotype interaction was significant only in Eckartsweier. Error variances were small compared to the genotypic component.

Mean FHB index was the lowest at Ridgetown, followed by Hohenheim, Eckartsweier and Winnipeg as indicated by the susceptible wheat genotype (Table 6). The resistant host genotypes had the lowest mean and terminal FHB indices at all four locations. The highest mean and terminal FHB indices were observed on a susceptible host genotype in Winnipeg. In Eckartsweier the susceptible genotype was less infected by FHB than the moderately resistant genotype due to poor emergence followed by an unfavorable microclimate. However, the moderately resistant genotype was less infected by FHB than the

Table 5 Variance components of mean and terminal FHB index (0–100%) and DON content (mg kg^{-1}) across twelve 3-ADON and 15-ADON isolates inoculated at four locations in 2008

Location[a]	Chemotype[b] (C)	Isolates within C	Genotype (G)	GxC	Error
Mean FHB index (%)					
EWE	–[c]	3.94**	248.02**	–[c]	12.62
HOH	–[c]	17.12**	221.45**	0.17	13.84
RID	–[c]	0.34	15.32**	–[c]	5.47
WIN	–[c]	21.55	685.18**	–[c]	47.98
Terminal FHB index (%)					
EWE	–[c]	13.93**	799.17**	2.48	65.30
HOH	–[c]	61.71**	838.81**	5.11	59.06
RID	–[c]	0.25	163.98**	–[c]	54.45
WIN	0.57	27.69**	1045.98**	0.59	81.13
DON content (mg kg^{-1})					
EWE	48.29**	15.03*	439.50**	87.12**	74.02
HOH	1.78	12.25**	92.52**	2.45	5.49
RID	0.20**	0.12[+]	–[c]	–[c]	0.67
WIN	0.14	0.68	18.66**	0.22	6.61

[+], *, **, Significant at $P<0.1$, 0.05 and 0.01, respectively

[a] *EWE* Eckartsweier near Offenburg in Germany, *HOH* Hohenheim near Stuttgart in Germany, *RID* Ridgetown Ontario in Canada, *WIN* Winnipeg Manitoba in Canada

[b] Estimate of chemotype should be read as variance of estimated effects (fixed factor)

[c] Negative estimate

susceptible genotype at the other locations. The resistant genotype did not show any significant ($P>0.1$) differences for 3- or 15-ADON chemotypes for any trait at any of the locations. The moderately resistant genotypes showed a significantly ($P<0.05$) higher terminal FHB index for 15-ADON isolates in Hohenheim and for 3-ADON isolates in Winnipeg. However, no consistent differences in FHB indices were observed between chemotypes.

Five out of nine location-by-genotype combinations showed that the 3-ADON isolates accumulated significantly ($P<0.05$) more DON than the 15-ADON isolates (Table 6). Specifically, DON accumulation by 3-ADON isolates was significantly greater on moderately resistant wheat genotypes in three of the four locations (Hohenheim, Eckartsweier and Ridgetown), and on susceptible wheat genotypes in both locations in Canada. DON accumulation was not examined on susceptible wheat genotypes in Germany. While average DON production by 3ADON isolates on resistant genotypes was slightly greater than for 15ADON isolates, these difference were not statistically significant.

The high stability of the resistant host genotype was confirmed by the small range of mean and terminal FHB index and DON accumulation for all isolates regardless of chemotype (Fig. 1). The 15-ADON isolates tested on moderately resistant and susceptible genotypes showed a slightly wider range of mean FHB index compared to 3-ADON isolates. The range of terminal FHB index of the 3-ADON isolates tested on susceptible genotypes was smaller with one outlier. The DON content of 3-ADON isolates on moderately resistant lines had a wider range and a considerably higher median than that of 15-ADON isolates across three locations.

Discussion

The aim of this study was to compare FHB development and DON production of 3-ADON and 15-ADON isolates under field conditions. Ward et al. (2008) found differences in incidence of 3-ADON and 15-ADON isolates on a gradient from eastern to western Canada, and demonstrated that the 3-ADON

Table 6 Mean and terminal FHB index (0–100%) and DON content (mg kg^{-1}) of three wheat genotypes (*R* = resistant, MR = moderately resistant, S = susceptible) averaged across twelve 3-ADON and 15-ADON isolates, respectively, at four locations in 2008

Location[a]	R			MR			S		
	3-ADON	15-ADON	Sign.[b]	3-ADON	15-ADON	Sign.[b]	3-ADON	15-ADON	Sign.[b]
Mean FHB index (%)									
EWE	3.14	2.92	n.s.	34.72	34.32	n.s.	17.75	19.43	n.s.
HOH	0.67	0.69	n.s.	18.11	21.33	n.s.	30.11	30.29	n.s.
RID	nd[c]	nd[c]	–	2.75	2.73	n.s.	8.21	8.35	n.s.
WIN	8.59	7.82	n.s.	34.81	32.25	n.s.	59.86	56.64	n.s.
Terminal FHB index (%)									
EWE	4.78	4.39	n.s.	59.16	57.46	n.s.	44.44	49.31	n.s.
HOH	1.69	1.67	n.s.	34.81	42.22	s.	59.47	58.65	n.s.
RID	nd[c]	nd[c]	–	6.98	7.10	n.s.	23.65	23.53	n.s.
WIN	21.75	21.17	n.s.	66.09	59.70	s.	86.37	84.15	n.s.
DON content (mg kg^{-1})									
EWE	3.27	2.78	n.s.	45.48	25.75	s.	nd[c]	nd[c]	–
HOH	1.30	0.94	n.s.	17.02	12.78	s.	nd[c]	nd[c]	–
RID	nd[c]	nd[c]	–	1.54	0.85	s.	1.68	1.02	s.
WIN	2.75	2.54	n.s.	5.19	4.81	n.s.	11.93	10.14	s.

[a] *EWE* Eckartsweier near Offenburg in Germany, *HOH* Hohenheim near Stuttgart in Germany, *RID* Ridgetown Ontario in Canada, *WIN* Winnipeg Manitoba in Canada

[b] Least significant difference between 3- and 15-ADON chemotype at $P<0.05$, with *n.s.* not significant and *s.* significant

[c] *nd* not determined

chemotype frequency in western Canada increased more than 14-fold between 1998 and 2004. These results suggested that selection was driving the rapid spread of an introduced pathogen population with a 3-ADON chemotype. This corroborates well with a study by Guo et al. (2008) that showed a shift from 15-ADON to 3-ADON chemotypes from south-east Manitoba to the north-west Manitoba. It may well be that the 3-ADON chemotype has within its genetic make-up a competitive advantage that is expressed without changes to the environment or agricultural practices, but is due to a general fitness advantage, as indicated by the higher growth rates and greater spore production observed by Ward et al. (2008). Changes in *F. graminearum* populations are not unusual due to their high variation in haplotype as known by analysis with selection-neutral markers (Miedaner et al. 2008). In the Netherlands, a shift in the species composition of FHB isolates from *F. culmorum* to *F. graminearum* occurred in the mid-1990s, which was potentially due to increased maize production (Waalwijk et al. 2003). Gale et al. (2002) proposed fungicides as a selection force towards changes to resistance in *F. graminearum* clade populations in China. In Germany, 3-ADON and 15-ADON chemotypes are both present (Schollenberger et al. 2006), but nothing is known about their predominance or distribution.

In our study, we compared each of 12 3-ADON and 15-ADON isolates using the same isolates, but different spore concentrations and host genotypes at each location. This should not affect the outcome of the study, because no host genotype x isolate interaction exists in this pathosystem (van Eeuwijk et al. 1995; Mesterhazy et al. 1999) and multiple inoculation systems have led to reproducible results in several studies (van Eeuwijk et al. 1995; Buerstmayr et al. 2008). Similar mean FHB indices between the two chemotypes were detected at the different locations in Canada and Germany. Mean and terminal FHB index for the chemotypes differed between locations depending on the host and climatic differences. However, mean temperatures and precipitation during pathogenesis were similar at all locations except for Ridgtown which had higher precipitation.

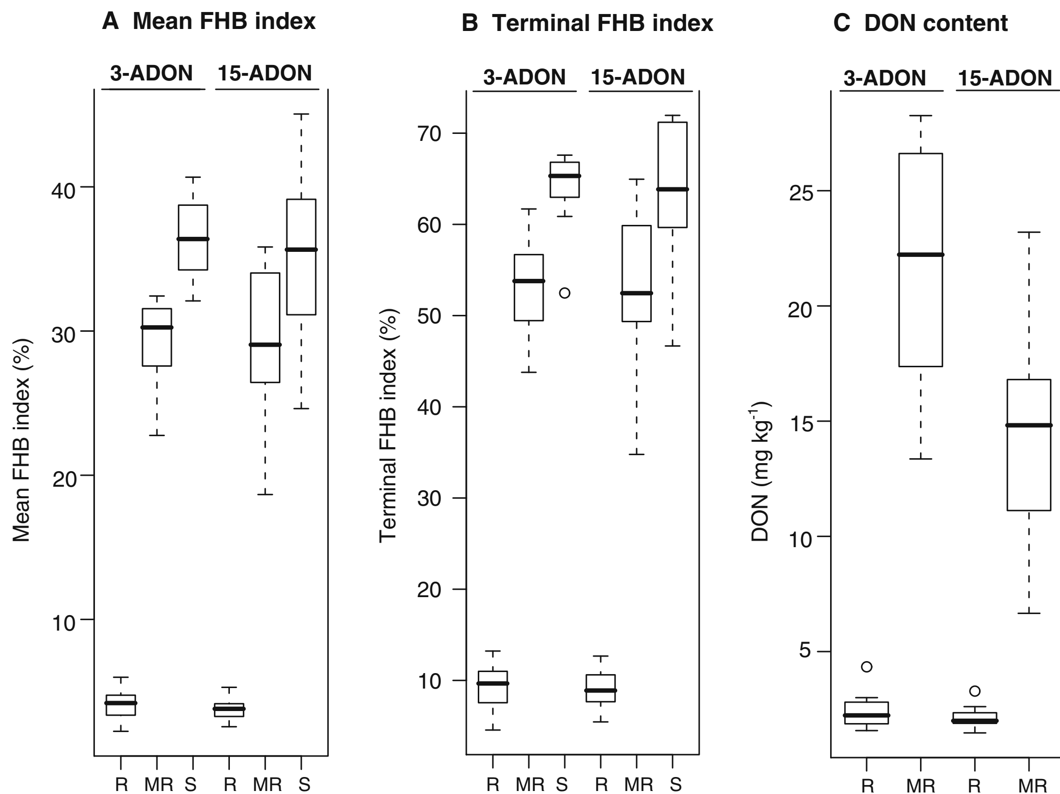

Fig. 1 Boxplots of mean (**a**) and terminal FHB index (0–100 %) (**b**) and DON content (mg kg^{-1}) (**c**) for each of twelve 3-ADON and 15-ADON isolates tested on three, respectively, two wheat genotypes (*R* = resistant, MR = moderately resistant, S = susceptible) averaged across three locations (Eckartsweier, Hohenheim, Winnipeg) in 2008. Boxes indicate the median (*solid line*), 25 and 75 percentiles, respectively, *dash line* show the maximum and minimum, respectively, of the distribution and *circles* refer to outlying data points

In Canada, environmental conditions were modified by using a misting system to create an artificially humid environment. High mean FHB index and DON contents in Eckartsweier were due to favourable weather conditions for the pathogen in the lower Rhine valley as found earlier by Miedaner and Reinbrecht (2001). The second German location, Hohenheim, also showed high mean FHB index and DON content, likely due to the humid climate in Southern Germany in 2008. The high mean FHB index in Winnipeg emphasized the risk of *Fusarium* epidemics in the eastern prairies of Canada (Clear and Patrick 2008). These results showed that Canadian *F. graminearum* isolates can cause high mean FHB indices and elevated DON levels even in non-local environments, such as Germany.

In the field tests, the 3-ADON and 15-ADON chemotypes caused similar mean and terminal FHB indices, but the analysis of DON content showed significant ($P<0.05$) differences between 3-ADON and 15-ADON chemotypes for five out of six moderately resistant and susceptible lines. In Winnipeg, the differences were less pronounced. Higher DON production by 3-ADON isolates was also reported by Ward et al. (2008) after inoculating 18 isolates per chemotype in one greenhouse test, but DON production was only significantly higher on the susceptible cultivar and not on a moderately resistant or resistant wheat cultivar. Ward et al. (2008) concluded, in accordance with our findings, that significantly higher DON accumulation was not correlated with higher aggressiveness. As such, it appears that the DON production was not the only factor for the aggressiveness shown by the isolates. This is in accordance with results from Miedaner et al. (2004), who reported that even isolates with similar DON concentration had differences in fitness within isolate mixtures.

In the present study, the 3-ADON and 15-ADON concentrations accounted on average for 2.5 and 1.0% of the total DON content, respectively. Similar results were obtained in a greenhouse study where the 3-ADON level was 1.5%, while the 15-ADON level was 3.7% of the total DON content (Tamburic-Ilincic et al. 2008). The occurrence of small 3-ADON and 15-ADON concentrations compared to the large total DON content is in accordance to the fact that both are precursors of DON (Schollenberger et al. 2006). In wheat, most ADON was converted to DON prior to harvest. Although the derivates of DON are likely not important to food safety due to their low levels in wheat, the possibility of higher production of DON by 3-ADON isolates is a concern, particularly if the 3-ADON chemotype continues to increase in proportion across Manitoba (Guo et al. 2008) and western Canada (Ward et al. 2008).

The spring wheat lines were significantly ($P<0.01$) different in sensitivity to FHB in each environment (Table 5). Small ranges in mean FHB index for the isolates within chemotypes on the highly resistant host genotype (Fig. 1) proved the stability of resistance. Buerstmayr et al. (2008) and Mesterhazy (1995) also found high stability of their resistant genotypes against FHB disease. Both resistant genotypes contained the *Fhb1* QTL located on chromosome 3BS, which is widely used in resistance breeding all over the world. The resistance due to *Fhb1* is independent of *F. graminearum* and *F. culmorum* (Mesterhazy 1995). In our study host resistance considerably lowered the mean FHB index to less than 5% at three locations. In Winnipeg, where the resistant wheat line still had a FHB index of 9%, when averaged across multiple ratings, the DON content was still low for the resistant genotype. However, moderately resistant or susceptible lines contained significantly ($P<0.05$) higher DON content for the 3-ADON isolates at five out of six locations (Table 6).

It is possible that highly resistant host genotypes deployed on a large cultivated area could result in selection pressure towards highly aggressive DON producers in the long run (Miedaner et al. 2008). However, good resistance will likely remain effective even if the proportion of 3-ADON chemotype increases, as resistance seems to be independent of the chemotype. This is illustrated by the fact that only one significant interaction, but no significant cross-over chemotype-by-host genotype interaction was detected in this study or in other studies with differing isolates (van Eeuwijk et al. 1995). As a consequence, for breeding programs it is important to select for highly resistant varieties, which are environmentally stable for FHB resistance. Previous population genetic and chemotype analyses refuted the hypothesis of a homogeneous *F. graminearum* population in North America that could be represented by local inocula in breeding programs at least for the chemotypes (Ward et al. 2008). However, results of the current study suggest that the performance of resistant host genotypes is stable across Canadian *F. graminearum* isolates with different chemotypes.

Our results indicate that shifts in *F. graminearum* populations from 15-ADON to 3-ADON chemotypes are unlikely to result in higher aggressiveness in terms of mean FHB index, but it may be accompanied by higher DON levels in wheat grain if moderately resistant or susceptible genotypes are grown. These results emphasize that breeding should focus on the development of highly resistant varieties to reduce the risk of elevated DON concentrations in grain.

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