Plant Pathol. J. 37(5) : 455-464 (2021)
https://doi.org/10.5423/PPJ.OA.06.2021.0094
pISSN 1598-2254 eISSN 2093-9280

Research Article **Open Access**

The Plant Pathology Journal

Response of Barley Genotypes to Fusarium Head Blight under Natural Infection and Artificial Inoculation Conditions

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(Received on June 8, 2021; Revised on July 28, 2021; Accepted on August 8, 2021)

Forty-eight spring barley genotypes were evaluated for deoxynivalenol (DON) concentration under natural infection across 5 years at Harrington, Prince Edward Island. These genotypes were also evaluated for Fusarium head blight (FHB) severity and DON concentration under field nurseries with artificial inoculation of *Fusarium graminearum* by the grain spawn method across 2 years at Ottawa, Ontario, and one year at Hangzhou, China. Additionally, these genotypes were also evaluated for FHB severity under greenhouse conditions with artificial inoculation of *F. graminearum* by conidial suspension spray method across 3 years at Ottawa, Ontario. The objective of the study was to investigate if reactions of barley genotypes to artificial FHB inoculation correlate with reactions to natural FHB infection. DON concentration under natural infection was positively correlated with DON concentration ($r = 0.47$, $P < 0.01$) and FHB incidence ($r = 0.56$, $P < 0.01$) in the artificially inoculated nursery with grain spawn method. Therefore, the grain spawn method can be used to effectively screen for low DON. FHB severity, generated from greenhouse spray, however, was not correlated with DON concentration ($r = 0.12$, $P > 0.05$) under natural infection and it was not correlated with DON concentration ($r = –0.23$, $P > 0.05$) and FHB incidence ($r = 0.19$, $P > 0.05$) in the artificially inoculated nursery with grain spawn method. FHB severity, DON concentration, and yield were affected by year, genotype, and the genotype × year interaction. The effectiveness of greenhouse spray inoculation for indirect selection for low DON concentration requires further studies. Nine of the 48 genotypes were found to contain low DON under natural infection. Island barley had low DON and also had high yield.

Keywords **:** barley, deoxynivalenol, *Fusarium graminearum*, Fusarium head blight, *Hordeum vulgare* L.

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Handling Editor : Jungkwan Lee

Fusarium head blight (FHB) is a destructive disease of barley (*Hordeum vulgare* L.) in many warm, humid growing regions of the world. It is caused by *Fusarium* species, particularly *F. graminearum.* The fungi produce a range of mycotoxins, including acetyldeoxynivalenol, deoxynivalenol (DON), nivalenol, and zearalenone, which can be harmful to human and animal health (Choo, 2006). In Canada, the maximum tolerance level of DON for swine diets should not exceed 1 mg/kg as recommended by the Canadian Food Inspection Agency (Charmley and Trenholm, 2015). The use of genetic resistance is an environmentally sustainable and the most economical way to mitigate the level of DON contamination. Many breeders and pathologists have established FHB nurseries in the field to screen barley lines for DON accumulation, and many have used

the grain spawn method to artificially inoculate the FHB nursery (Zhou et al., 1991). It is believed that disease development in the FHB nurseries would be close to that under natural infection. To our knowledge, no systematic empirical research exists addressing the question of what is the correlation between DON concentration under grain spawn inoculation and natural infection of *F. graminearum*.

Barley lines can also be screened indoor for DON accumulation. As Geddes et al. (2008) pointed out; indoor screening offers several advantages over field screening. First, indoor screening can be done throughout the calendar year. Second, it can prevent competing organisms (e.g., *Bipolaris sorokiniana* (Sacc.) Shoemaker) from infection. Third, its experimental error can be minimized by controlling the environmental factors and inoculum dosage. Fourth, it requires less time and labor for inoculation. If FHB symptoms are correlated well with DON concentration as reported by Choo et al. (2004), then indoor screening for FHB severity can be used for indirect selection for low DON without resorting to the time-consuming, labor-intensive DON quantification. Geddes et al. (2008) concluded that indoor spray inoculation is most reproducible in comparison with point-inoculation and grain spawn. Empirical experiments are needed to determine if grain spawn inoculation or indoor spray inoculation produces similar results as those under natural infection. The objective of the study was to investigate if reactions of barley genotypes to artificial FHB inoculation with both grain spawn and indoor spray correlate well with reactions to natural FHB infection.

Materials and Methods

Plant materials. Forty-eight genotypes of spring barley were used for this study (Table 1). Many of these genotypes have shown some level of FHB resistance in previous studies or in the literature, while Brucefield, Encore, Stander, and Viviane were susceptible checks (Choo et al., 2004, 2014). These barley genotypes originated from nine countries (China, Canada, Ecuador, Japan, Germany, Sweden, Switzerland, Russia, and the United States) and included 34 two-row, two *deficiens* (absence of sterile lateral florets), and 12 six-row type. Six of the genotypes were hulless and 42 were covered. The genotype Birka was specifically selected for this study as its spike was partly enclosed in the upper leaf sheath from awns emergence to maturity in the field (Anonymous, 1982), which may influence infection and disease development. The genotype Kanto Nadate Gold was included because of its cleistogamy (closed flowering) as reported by Sameri et al. (2006).

Natural infection. The 48 genotypes were planted in a randomized complete block design with two replicates at the Harrington Research Farm, Charlottetown Research and Development Centre, Prince Edward Island from 2009 to 2012. Each experimental unit consisted of three rows, 3 m long, and 17.8 cm between rows. Each year, a fertilizer (17-17-17) at a rate of 412 kg/ha (or 70 kg N/ha) was applied to the field before planting. The herbicide MCPA Amine 500 + Refine Extra (a.i. MCPA and thifensulfuron/tribenuron) was sprayed at the recommended rate. Grain yield, heading date, plant height, and DON concentration was determined in each plot. For DON analysis, approximately 20 to 25 g of harvested barley seed was ground to pass through a 0.4 mm screen on a Udy-mill. To prepare extracts, 5 ml of methanol: water (1:9, v/v) were added to 1 g ground samples in 10 ml plastic tubes, which were then subjected to end-over-end mixing for one hour, centrifuged for 5 min at 2,000 rpm. DON analysis was conducted on the filtrate following the enzyme-linked immunosorbent assay (ELISA) procedures described by Sinha et al. (1995). The antibody for this ELISA was developed and produced in-house and has a high affinity for both DON and 15-ADON. The accuracy of the ELISA procedures has been reported to be comparable to that of the gas chromatography method (Sinha and Savard, 1996). The limit of quantitation was 0.1 mg/kg. DON analysis was conducted in the mycotoxin laboratory at the Ottawa Research and Development Centre, Ottawa.

Field grain spawn inoculation. The 48 barley genotypes were seeded in a randomized complete block design with three replicates at Ottawa in 2004 and 2005, and with three replicates at Hangzhou, China in 2005. At Ottawa, the experimental materials were seeded at a seeding rate of 5 g per row.

Each experimental plot consisted of two 1.5-m rows with a row spacing of 15 cm. Three *F. graminearum* isolates (DAOM178148, DAOM212678, and DAOM232369) from the Canadian National Mycological Herbarium, Ottawa Research and Development Centre, were used as inoculum for this study. These isolates were chosen since they are known to be aggressive in Canadian condition (Xue et al., 2004). Equal parts by volume of corn (*Zea mays* L.) and barley grain were mixed and soaked in water for 48 hours. Excess water was removed and the kernel mixture, which was placed in an aluminum pan (53 × 32 × 8 cm^3) and covered with two layers of aluminum foil, was autoclaved for 1 h at 120°C. In each pan, 100 ml of spore suspension of individual isolates at a concentration of 5.5 × 10^4 spores per ml was evenly poured over the surface of the

Table 1. List of barley genotypes used and their spike type, kernel type, pedigree, and references

Genotype	Spike	Kernel	Pedigree	Reference
AC Alberte	2-row	Hulless	Mimai 114/Rodeo//Rodeo	Choo et al. (2001b)
AC Klinck	6-row	Covered	Cadette/Chapais	Choo et al. (2001a)
AC Minoa	2-row	Covered	Symko/AC Sterling	Choo et al. (2010)
AC Sterling	2-row	Covered	Micmac/K75-10//Rodeo	Choo et al. (1994)
ACCA	6-row	Covered	QB730.2/UL0072//Leger/#32 de 8 IBON	Cooper (1993)
Atahualpa	*deficiens*	Hulless	Sutter/Gloria"S"/Come"S"/3/PI6384/Capuchona	Vivar et al. (1997)
Birka	2-row	Covered	W82-68/W17-68	Anonymous (1982)
Brucefield	6-row	Covered	Maskot/Chapais	Lanoie (pers. comm.)
CDC Freedom	2-row	Hulless	AC Oxbow/SB88579	Rossnagel et al. (1997)
CGB83-46	2-row	Covered	UPBS76/UPBS60//UPBS60/UPBS66	Choo et al. (2003)
CH9419-9	2-row	Covered	AC Queens/Morrison	Choo (unpublished)
CH9423-20	2-row	Covered	AB159-6/Morrison	Choo (unpublished)
CH9507-8	2-row	Covered	DB784/Lester	Choo (unpublished)
CH9520-30	2-row	Covered	DB466/AB79-17	Choo (unpublished)
CH9526-9	2-row	Covered	AC Kings/AC Sterling	Choo (unpublished)
CH9528-10	2-row	Covered	AC Kings/Iona	Choo (unpublished)
CH9625-12	2-row	Covered	AB162-12/Iona	Choo (unpublished)
CH9627-3	2-row	Covered	AB162-12/DB200	Choo (unpublished)
Chapais	6-row	Covered	QB58.14/Beacon//BT904	Anonymous (1991)
Chevron	6-row	Covered	Swiss unimproved cultivar	Shands (1939)
Chief	2-row	Covered	DB145/AB143-3	Choo et al. (2006a)
CI4196	2-row	Covered	Chinese barley	Takeda and Heta (1989)
Encore	6-row	Covered	Cadette/QB198.39	Choo et al. (2006b)
EX645-3-6	2-row	Hulless	(MSG053/B1602/BT213/806F7/CONQUEST/M82/BT946)/GAINER	Badea (pers. comm.)
Frederickson	2-row	Covered	Unknown	Takeda and Heta (1989)
Golozernyj 1	2-row	Hulless	K-920/Odesskij-9//K-900/Kolchoznyj-Golozernyj	McCallum et al. (2004)
Harbin-2r	2-row	Covered	Unknown	Takeda and Heta (1989)
Island	2-row	Covered	CGB83-46/Rodeo//Symko	Choo et al. (2003)
Kanto Nakate Gold	2-row	Covered	Golden Melon/Shikoku//Kinki Shu	Sameri et al. (2006)
Leader	2-row	Covered	AB163-4/DB200	Choo et al. (2009)
M92-513	6-row	Covered	M87-218/Minn M69	Rasmussion (pers. comm.)
Maja	2-row	Covered	Unknown	Takeda and Heta (1989)
Mimai 114	2-row	Hulless	Xiaoshan Ciwangerleng/Xiaoshan Lixiahuang//Aibaiyang	Zhou et al. (1991)
Myriam	6-row	Covered	OB339-1/Bedford	Bastien (pers. comm.)
Niedzica 1	2-row	Covered	Unknown	Takeda and Heta (1989)
OAC Kippen	6-row	Covered	OB141-1/Perth	Falk and Reinbergs (1991)
Orthega	2-row	Covered	Ceb.7931/Pompadour//S.77323/Golf	Buerstmayr et al. (2004)
Peatland	6-row	Covered	Swiss unimproved cultivar	Immer and Christensen (1943)
Primus	2-row	Covered	Unknown	Takeda and Heta (1989)
Rodeo	2-row	Covered	UPBS60/UPBS76	Campbell et al. (1984)
Shyri	*deficiens*	Covered	Lignee 640/Kober//Teran 78	Vivar et al. (1997)
Stander	6-row	Covered	Excel/M80-224	Rasmusson et al. (1993)
Symko	2-row	Covered	Rodeo/Gitane	Ho (1990)
Viviane	6-row	Covered	Maskot/Chapais	Lanoie (pers. comm.)
Zaoshu 3	2-row	Covered	Kanto Nijo 3	Gao (1989)
Zhedar 1	2-row	Covered	Chinese barley	Urrea et al. (2005)
Zhedar 2	2-row	Covered	Chinese barley	Urrea et al. (2005)
Zhenongda 3	2-row	Covered	76-6477/76-20	Ding et al. (1987)

kernel mixture in a laminar flow hood. The kernel mixture was incubated at room temperature for each isolate for at least 4 weeks. Kernels infected with the three isolates were mixed in the same proportion and then broadcast at a rate of 50 g per m^2 once in 2004 (July 5 at late stem elongation) and at a rate of 30 g per m^2 twice in 2005 (June 22 at early stem elongation and June 28 at booting). Shortly after inoculation, the plots were watered with a sprinkler system twice a day (at 11:00-11:30 am and 3:00-4:00 pm) for 20-30 min to increase the humidity.

Heading date, plant height, and FHB incidence (i.e., percentage of heads infected per row) were recorded. DON concentration was determined at Ottawa for all plots using the ELISA method as described earlier.

At Hangzhou, China, the experimental materials were seeded at a seeding rate of 5 g per row. Each plot consisted of one 1.5-m row with a row spacing of 30 cm. A mixture of *F. graminearum* isolates collected from the cultivar Giu Damai from Hailing, Zhejiang was multiplied on a potato-dextrose-agar (PDA) medium at 28°C for 10 days. Wheat kernels were soaked in water for 24 h, put in 1-l glass bottles, and then autoclaved for 30 min at 120°C. The wheat kernels were inoculated with the *F. graminearum* mycelium at five positions in a glass bottle and incubated at 25°C for 10 days. Infected wheat kernels were broadcast at a rate of 7.5 g per m^2 at the booting stage. At heading, all plots were also sprayed with a *F. graminearum* suspension. There was no supplementary water of the inoculated plots at this location. Heading date, plant height, and FHB incidence (i.e., percentage of heads infected per row) were recorded. DON concentration was determined for all plots at Ottawa using the ELISA method as described natural infection section of this study.

Indoor spray inoculation. Three greenhouse trials were conducted at the Ottawa Research and Development Centre, Ottawa, from January to June each year in 2005, 2006 and 2007, using the same three isolates (DAOM178148, DAOM212678, and DAOM232369) as in grain spawn inoculation. The isolates were cultured on a modified PDA (10 g/l of dextrose amended with 34 mol/l streptomycin sulfate) and incubated at 22-25°C under mixed UV and fluorescent lighting on a 12 h light:12 h dark cycle for 14 days. The modified PDA medium was used to reduce mycelium growth, possible mutation, and poor vigour, and to increase spore production by the pathogen (Xue et al., 2004). To prepare inoculum, 0.5 ml of a concentrated macroconidial suspension (approx. 107 spores/ml), obtained from the above, was spread over the surface of the modified PDA in 9-cm Petri dishes and incubated as described above for 48 h. Ten millilitres of sterile distilled water containing 0.01% Tween 20 (polyoxyethylene sorbitan monolaurate) was then added to each dish, and the surface was scraped gently with a sterile microscope slide to dislodge spores. The resulting conidial suspension was filtered through two layers of cheesecloth and adjusted to a concentration of 5×10^4 spores/ml using a hemocytometer. Separate conidial suspensions were prepared for each isolate. The final suspension used for the greenhouse consisted of 1:1:1 mixture of each of the three *F. grameniarum* isolates.

The seeds of 48 barley genotypes were planted in 15-cm diameter pots containing a mixture of loam soil, sand, and composted cow manure (1:1:1, v/v/v) and maintained at 23-25°C during the day and 18-20°C at night in a greenhouse. Supplemental light was provided by 300 W metal halide lamps to ensure a 16 h photoperiod and a minimum intensity of 360 mol/m^2/s. Plants were fertilized with a 1% solution of 20-20-20 (N-P-K) at the fifth week after planting and once per week thereafter. The 48 barley genotypes were inoculated 10-14 days after heading. Plants were sprayed with the spore suspension at approximately 0.2 ml per spike using a DeVilbiss model 15 atomizer (The DeVilbiss Co., Somerset, PA, USA). After the inoculum dried for 30 min, plants were transferred to a polyethylene humidity chamber in a growth room for 24 h. The growth room was operated at 25°C with 12-h photoperiod at a light intensity of 250 mol/m^2/s. The humidity chamber was maintained at or near 100% relative humidity by the continuous operation of two ultrasonic humidifiers. Air temperature and humidity in the chamber were monitored with a portable datalogger (model 21XL micrologger, Campbell Scientific Canada Corp., Edmonton, AB, Canada). After incubation, plants were returned to the greenhouse bench. For each barley genotype, eight replicate pots were used in 2005 and 2006 and four replicate pots in 2007. Pots were arranged in a completely randomized design in both the humidity chamber and the greenhouse after the inoculation. The severity of FHB was rated as the percentage of infected spikelets 21-23 days after inoculation when plants were at the soft dough stage.

Statistical analysis. Data were analyzed using the MIXED procedure of the SAS software (SAS Institute, 2004). The genotype was considered to be a fixed effect and all of the other terms (year, rep [year], and genotype year) to be random effects. Log-transformed DON data were used to perform the analysis of variance. Means over all of the trials in each experiment were used for calculating Pearson correlation coefficients. Pearson correlation coefficient was calculated to determine (1) if DON concentration was cor-

related with any of the three agronomic traits (yield, height, and heading date) in the natural infection experiment, (2) if DON concentration was correlated with FHB incidence, height, and heading date in the grain spawn experiment, (3) if DON concentration under natural infection was correlated with DON concentration and FHB incidence with grain spawn inoculation, and (4) if FHB severities with indoor spray inoculation were correlated with DON concentration under natural infection and DON concentration and FHB incidence with grain spawn inoculation.

Results

Natural infection. There was a significant ($P < 0.05$) variation among genotypes for all four traits (Table 2). DON contamination was greatest in 2009 and 2010, intermediate in 2011, and low in 2012 and 2013. The mean yield was similar from year to year. Plant height was tall in 2009, 2010, and 2011, but short in 2012 and 2013. The heading date was early in 2009, 2011, and 2012, but late in 2010 and 2013 (Table 2). There was a significant genotype-year interaction for DON concentration, yield, plant height, and heading date (Table 3). The genotype-year interaction can be attributed to a change in magnitude of mean DON concentration in different years. All of these barley genotypes were contaminated with DON (Table 4). DON concentration of these genotypes was either positively correlated or not correlated between the 5 years. Positive correlation was detected between 2009 and 2010 ($r = 0.69$, $P < 0.01$), between 2009 and 2011 ($r = 0.76$, $P < 0.01$), between 2009 and 2012 ($r = 0.41$, $P < 0.01$), between 2010 and 2011 ($r = 0.60$, $P < 0.01$), and between 2011 and 2012 ($r = 0.43$, $P < 0.01$). Two-row genotypes, in general, contained less DON than six-row genotypes except Zhenongda 3, Zaosho 3, Mimai 114, CH9528-10, and Kanto Nakate Gold (Table 4). The two *deficiens* genotypes contained an average amount of DON. Hulless genotypes generally contained less DON than covered genotypes with the exception of Mimai 114. Only nine genotypes (Island, Zhedar 1, Golozernyj 1, Harbin 2r, CDC Freedom, Zhedar 2, Maja, CH9507-8, and Niedzica 1) from 2-row barley contained less than 1 mg DON kg-1, which is the recommended maximum tolerance level of DON for swine diets in Canada. These nine genotypes were generally tall (ranging from 77 cm to 96 cm) and late in the heading date (ranging from 60 to 64 days) (Table 4). Of the nine genotypes that had low DON,

Table 2. Mean of heading date, height, DON, fusarium head blight severity and incidence and yield in natural infection and artificial infection with *Fusarium graminearum* in five and three environments averaged across 48 barley genotypes

Natural infection					Field artificial inoculation					Indoor spray inoculation	
Environment	Heading (day)	Height (cm)	DON (mg/kg)	Yield (t/ha)	Environment	Heading (day)	Height (cm)	Incidence (%)	DON (mg/kg)	Environment	Severity (%)
Harrington 2009	55 D	92 A	3.19 A	2.92 A	Ottawa 2004	53 B	78 C	42 A	6.3 A	Ottawa 2005	47.42 A
Harrington 2010	68 A	86 B	3.47 A	3.09 A	Ottawa 2005	50 C	85 B	35 B	7.5 A	Ottawa 2006	40.51 A
Harrington 2011	54 E	92 A	2.09 B	2.94 A	Hangzhou 2005	141 A	100 A	7 C	1.6 B	Ottawa 2007	23.15 B
Harrington 2012	57 C	76 C	0.30 C	3.29 A	-	-	-	-	-	-	-
Harrington 2013	64 B	70 D	0.61 C	2.43 A	-	-	-	-	-	-	-

DON, deoxynivalenol.

Table 3. Analysis of variance of heading date, height, DON, Fusarium head blight severity and incidence and yield in natural infection and artificial infection with *Fusarium graminearum* in five and three environments

Source	Natural infection					Field artificial infection					Indoor spray	
	DF	Heading	Height	log(DON)	Yield	DF	Heading	Height	Incidence	log(DON)	DF	Severity
Environment	4	3,855**	8,937*	91.11**	9.25	2	366,715**	18,597**	47,400**	63.7**	2	35,008**
Rep (Environment)	5	14**	999**	0.42**	6.26**	6	10	341**	340*	1.6**	17	677
Genotype	47	104**	648**	2.20**	5.63**	47	171**	536**	902*	4.5**	47	2,388**
Environment × Genotype	182	5**	47**	0.59**	0.48**	87	26**	125**	508**	1.2**	91	1,062**
Error	228	1	30	0.12	0.19	263	5	45	123	0.3	718	507.9

DON, deoxynivalenol.
*, **, and ***, significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 4. Mean of heading date, height, DON, Fusarium head blight severity and incidence and yield of 48 barley genotypes grown under natural infection at Harrington, Prince of Edward Island (2009-2013), artificial infection with grain spawn method at Ottawa (2004 and 2005) and Hangzhou, China (2005) and indoor spray method at Ottawa (2004 to 2006)

Genotype	Natural infection				Field artificial infection				Indoor spray
	Heading (day)	Height (cm)	DON (mg/kg)	Yield (kg/ha)	Heading (day)	Height (cm)	Incidence (%)	DON (mg/kg)	FHB severity (%)
Island	60	85	0.4	3,750	77	92	29	3.6	23.7
Zhedar 1	62	86	0.5	1,730	87	98	24	1	21.9
Golozernyj 1	60	94	0.6	2,100	83	91	15	2	23.3
Harbin-2r	61	96	0.6	2,790	85	99	18	1.1	27.4
CDC Freedom	62	87	0.7	2,340	85	86	14	2.1	18.3
Zhedar 2	63	88	0.7	2,150	88	92	20	1.5	13.4
Maja	63	89	0.8	2,450	86	85	23	4.3	22.0
CH9507-8	61	91	0.9	3,750	80	88	29	2.7	25.3
Niedzica 1	64	77	0.9	2,600	87	87	17	1.7	24.7
EX645-3-6	59	92	1	2,950	85	87	18	1.6	29.2
Leader	58	88	1.1	3,760	78	85	30	4	28.7
CH9526-9	61	84	1.1	3,990	80	86	36	3.2	27.5
Symko	62	74	1.1	3,350	80	88	34	3.4	16.5
CH9419-9	61	93	1.1	3,770	80	88	27	4.2	27.8
Primus	62	95	1.1	1,930	88	101	22	3.3	19.5
Rodeo	61	78	1.1	2,790	84	85	16	2.4	34.8
Frederickson	62	91	1.1	2,050	87	104	21	1.3	27.2
Chief	59	90	1.2	3,450	80	90	30	3.8	28.1
Birka	63	76	1.2	3,300	85	75	33	2.1	23.2
Orthega	63	63	1.2	3,030	86	69	18	4.5	12.4
Atahualpa	60	68	1.2	1,890	86	78	14	2.5	26.9
CH9520-30	59	88	1.2	3,470	79	84	34	5	8.9
AC Minoa	59	86	1.3	3,790	79	87	22	1.7	21.0
CI4196	69	83	1.3	1,150	92	99	17	2.1	17.9
AC Sterling	61	83	1.3	3,450	81	85	23	3	11.8
AC Alberte	60	80	1.3	2,700	80	86	15	2.7	14.3
CH9423-20	59	83	1.3	3,790	79	85	34	2.9	12.4
CGB83-46	64	66	1.4	2,450	83	68	38	7	23.8
CH9627-3	59	87	1.5	3,920	79	85	25	2.7	9.0
CH9625-12	59	84	1.6	3,480	81	90	34	2.5	21.4
Chevron	56	98	1.9	2,840	75	104	13	1.6	18.0
M92-513	60	94	2.1	3,100	78	96	12	2.4	14.3
Kanto Nakate Gold	57	70	2.1	1,900	77	77	52	25.8	9.2
Myriam	57	83	2.3	3,360	77	89	40	6.8	23.6
OAC Kippen	58	81	2.3	3,370	76	92	28	4.8	22.8
AC Klinck	61	86	2.3	3,520	80	90	27	4.3	21.1
CH9528-10	58	87	2.7	3,750	78	87	35	3.5	20.1
Shyri	58	84	2.7	2,400	81	92	41	5.7	26.0
Chapais	56	72	2.9	3,140	77	80	43	11.7	12.0
Zhenongda 3	50	68	3	1,670	70	73	41	40.7	29.4
Zaoshu 3	50	72	3.2	1,740	71	75	39	3.9	18.5
ACCA	58	85	3.8	3,740	79	86	41	3.1	31.2
Encore	62	86	3.8	3,450	82	92	38	7.2	21.3
Viviane	60	86	3.9	3,460	83	90	44	9.6	28.3
Stander	58	80	4.6	3,410	75	74	48	15.5	6.8
Mimai 114	55	74	5.4	1,440	77	82	34	15.2	12.9
Brucefield	56	80	5.8	3,480	76	83	46	14.3	14.5
Peatland	57	87	5.9	2,760	77	94	26	5.2	3.5

DON, deoxynivalenol.

Island and CH9507-8 had high yield (3,700 kg/ha) while the others had low yield (<2,800 kg/ha) (Table 4). The correlation coefficient between DON concentration and grain yield for all genotypes was not significant at the 0.05 level (Table 5). Plant height was not correlated with DON concentration, but heading date was negatively correlated with DON concentration (Table 5). Heading date remained negatively correlated with DON concentration even when the two earliest heading dates (at least 5 days earlier under natural infection) genotypes (Zhenongda 3 and Zaoshu 3) were excluded from the correlation analysis.

Grain spawn inoculation. On average, these 48 genotypes at Ottawa, Ontario had higher DON, higher FHB incidence, and shorter and headed earlier than those at Hangzhou, China (Table 2). Variation for all four traits among genotypes was significant, so were environment x genotype interactions for all four traits (Table 3). The nine resistant genotypes contained low DON, ranging from 1.0 to 4.3 mg/kg with grain spawn inoculation (Table 4). Two genotypes, Kanto Nakate Gold (25.8 mg/kg) and Zhenongda 3 (40.7 mg/kg), contained an extremely high concentration of DON and they were considered far outliers (Table 4). When these two outliers (Kanto Nakate Gold and Zhenongda 3) were excluded from the correlation analysis, the correlation coefficient for DON concentration was highly positive between the three environments, i.e., between Ottawa 2004 and Ottawa 2005 ($r = 0.79$, $P < 0.01$), between Ottawa 2004 and Hangzhou 2005 ($r = 0.44$, $P < 0.01$), and between Ottawa 2005 and Hangzhou 2005 ($r = 0.43$, $P < 0.01$). In this experiment, DON concentration was positively correlated with FHB incidence, but it was negatively correlated with height and heading date (Table 5). Heading date negatively correlated with DON concentration even when the two earliest heading genotypes (Zhenongda 3 and Zaoshu 3) were excluded from the correlation analysis.

Indoor spray inoculation. FHB severity varied from year to year and from genotype to genotype (Table 2). There were significant interactions between year and genotype (Table 3). The nine resistant genotypes were relatively low in FHB severity, ranging from 24% to 39%, but early heading genotypes such as Zhenongda-3, Zaoshu-3, Mimai-114, and Kanto Nakate Gold had even lower FHB severity (8% to 18%) (Table 4). DON concentration and FHB incidence with grain spawn inoculation were positively correlated with DON concentration obtained from natural infection (Table 5). When the two outliers (Kanto Nakate Gold and Zhenongda-3) were excluded from the correlation analysis the correlation coefficient for DON concentration between natural infection and grain spawn increased from $r = 0.47$-0.76. FHB severity with indoor spray inoculation was not correlated with DON concentration ($r = 0.12$) under natural infection, and similarly, FHB severity with indoor spray inoculation was not correlated with DON concentration ($r = –0.23$) and FHB incidence ($r = 0.19$) under grain spawn (Table 5).

Table 5. Pearson correlation coefficients between FHB parameters (FHB incidence, FHB severity, and DON content) and additionally investigated traits under natural infection, artificial grain spawn method and indoor spray conditions

Traits	Correlation coefficient
Natural infection DON and natural infection yield	0.01
Natural infection DON and natural infection height	–0.21
Natural infection DON and natural infection heading	–0.53**
Grain spawn DON and grain spawn FHB incidence	0.59**
Grain spawn DON and grain spawn height	–0.46**
Grain spawn DON and grain spawn heading	–0.52**
Natural infection DON and grain spawn DON	0.47**
Natural infection DON and grain spawn FHB incidence	0.56**
Natural infection DON and indoor spray FHB severity	0.12
Grain spawn DON and indoor spray FHB severity	–0.23

FHB, Fusarium head blight; DON, deoxynivalenol.

Discussion

To our knowledge, this is the first report on the FHB relationships between artificial inoculation and natural infection in barley. Our results showed that reactions to DON accumulation with grain spawn inoculation were positively correlated with those under natural infection. Despite that grain spawn inoculation was conducted in Ottawa, Ontario, and Hangzhou, China which is far away from Harrington, DON data from grain spawn inoculation were positively correlated between the three environments and also with those under natural infection. This suggests that grain spawn inoculation can be conducted outside the epidemic area and can still effectively identify lines with low DON. In contrast, FHB severity in indoor spray inoculation showed no correlation with DON accumulation under natural infection and no correlation with DON accumulation and FHB incidence with grain spawn inoculation. Some of the early genotypes such as Zhenongda 3 and

Mimai 114 were low in FHB severity with indoor spray inoculation, but they were very susceptible under natural infection and with grain spawn method in artificial inoculation. On the other hand, Rodeo was relatively low in DON concentration under natural infection but it was one of the most susceptible to FHB with indoor spray inoculation. In the indoor spray inoculation, data for FHB severities were collected 21-23 days after inoculation. This short time may not allow for the complete determination of the full impact of the spread of infection within a plant. Some early genotypes could be susceptible to fungal spread (dense spike); while Rodeo and others could be susceptible to initial infection, but they could be resistant to fungal spread. The results suggest that the effectiveness of indoor spray inoculation for indirect selection for low DON requires further studies.

Previously, Geddes et al. (2008) evaluated 19 barley genotypes for FHB responses with three inoculation methods (point or spray inoculation in the greenhouse and grain spawn in a field nursery). Significant genotypic differences for FHB symptoms were detected for all three inoculation methods; a significant difference for DON concentration, however, was detected only in one, point-inoculation, experiment. They concluded that indoor spray inoculation was the most reproducible method for phenotypic evaluation because it mimics natural infection. No evaluation of the 19 genotypes was conducted under natural infection in Geddes et al. (2008) study. This study indicated that indoor spray showed a different response to FHB than under natural infection.

In this study, nine out of the 48 barley genotypes were found to have low DON, with an average of <1 mg DON/kg under natural infection. Of the nine low-DON genotypes, CH9507-8 and Island yielded significantly higher than the other seven genotypes. Choo et al. (2003) were the first to report low DON in Island barley. Choo et al. (2004) again reported that Island, along with AC Alberte and Chevron, were found to be most resistant, as they were consistently low in FHB incidence and DON concentration. Geddes et al. (2008) has also reported that Island was one of the genotypes with a low FHB rating and low DON in their 5-year study with the grain spawn method. All of these results indicate that Island can be used to mitigate DON contamination in Eastern Canada. Island has been on the cultivar recommended list for Atlantic Canada since 2004 and for Quebec since 2006.

The pedigree of Island is CGB83-46/Rodeo//Symko, in which CGB83-46 and Symko are each half related to Rodeo (Table 1). Therefore, Island is genetically very close to Rodeo as its ancestors are either Rodeo or Rodeo-related genotypes. Another most resistant genotype CH9507-8 is also related to Rodeo because one of its parents Lester was derived from a UPBS60/UPBS66//Rodeo cross. AC Alberte was relatively low in DON concentration (Choo et al., 2004) and it was derived from a backcross of Mimai-114/Rodeo//Rodeo. The resistance of AC Alberte to DON accumulation must have come from Rodeo because Rodeo was quite resistant to DON accumulation while Mimai-114 was very susceptible to DON accumulation (also see Urrea et al., 2005). Other Rodeo-derived genotypes (including AC Minoa, AC Sterling, and CH9526-9) contained low DON in response to FHB infection under natural infection. These suggest that resistance to DON accumulation of Island mainly originates from the genotype Rodeo.

The nine genotypes that contained low DON include two hulless barley, Golozernyj 1 and CDC Freedom. But the yield of these two hulless genotypes was very low (2,100-2,300 kg/ha). It has been suggested that *deficiens* barley is associated with FHB resistance (Vivar et al., 1997). The two *deficiens* genotypes in this study, however, were contaminated with DON considerably. These results do not support the notion that *deficiens* barley is associated with FHB resistance. The cleistogamous genotype Kanto Nakate Gold was susceptible to DON accumulation under both natural infection and grain spawn inoculation. Yoshida et al. (2007) pointed out that cleistogamous cultivars are resistant to FHB infection at anthesis but susceptible at 10 days after anthesis. Birka was relatively low in DON concentration in both natural infection and grain spawn inoculation. Maybe its upper leaf sheath did provide some protection from FHB infection as its spike was partially enclosed in the upper leaf sheath from awn emergence to maturity in the field.

In conclusion, artificial inoculation with the grain spawn method produced a similar response to DON accumulation as natural infection, and thus it can be used to effectively screen for low DON. In contrast, artificial inoculation with indoor spray inoculation produced a profile of FHB severities that had no relation with the profile of DON concentration under natural infection and had no relation with the profiles of DON concentration and FHB incidence with grain spawn inoculation. Further studies are required to determine the DON relationship between indoor spray inoculation and natural infection. Nine barley genotypes were found to contain low DON under natural infection. Among these, Island barley not only had low DON but also had high yield.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

The authors were grateful to Professor K. Takeda (Research Institute for Bioresources, Japan), Dr. T. Makino (National Agriculture Research Centre, Japan), Professors S. Ding and S. Xu (Zhejiang Agricultural University, China), Professor D. Rasmussion (University of Minnesota, USA), and Dr. Bill Legge (Brandon Research and Development Centre, Canada) for providing seeds of barley genotypes, and to Yuanhong Chen, Sally Buffam, and Sharon ter Beek for their assistance in conducting this study.

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