Fusarium Ratings in Ontario Winter Wheat Performance Trial (OWWPT) Using an Index that Combines Fusarium Head Blight Symptoms and Deoxynivalenol Levels

L. TAMBURIC-ILINCIC[1,2], D. FALK[2] and A. SCHAAFSMA[1,2]

[1]Ridgetown Campus, University of Guelph, Ridgetown, N0P 2C0 Ridgetown, Ontario, Canada;
[2]Department of Plant Agriculture, University of Guelph, N1G 2W1 Guelph, Ontario, Canada;
e-mail: ltamburi@ridgetownc.uoguelph.ca

Abstract: Fusarium head blight (FHB) is one of the most serious diseases of wheat (*Triticum aestivum* L.). FHB reduces grain yield and quality, and the fungus produces mycotoxins, such as deoxynivalenol (DON). The most practical way to control FHB is through the development of resistant cultivars. In addition to exotic sources of resistance (such as cultivars Sumai 3 and Frontana), native sources of resistance are commonly used in winter wheat breeding programs in North America. In 1996, 2000, and 2004 severe epidemics of FHB cost the winter wheat industry in Ontario, Canada combined over $200 million. All wheat grown in Ontario is entered in the Ontario Winter Wheat Performance Trial (OWWPT) and tested every year for Fusarium resistance and DON level in three inoculated FHB nurseries. The objective of this study is to explain how the index that accounts for FHB symptoms and DON level jointly was developed, and how stable the performance of the cultivars grouped to susceptibility classes has been over a number of years. The index is related to Fusarium susceptibility classes (moderately resistant – MR, moderately susceptible – MS, susceptible – S and highly susceptible – HS), robust, stable, open-ended (old cultivars out, new cultivars in) and useful to farmers in making cultivars selection decisions. This information is available to growers and industry through the website www.gocereals.ca.

Keywords: breeding; mycotoxins; resistance

Fusarium graminearum (Schwabe), the anamorph of *Gibberella zeae* (Schw.) Petch., is the principal cause of Fusarium head blight (FHB) in North America, one of the most serious diseases of wheat (*Triticum aestivum* L.). FHB reduces grain yield and quality, and the fungus produces mycotoxins, such as deoxynivalenol (DON) (Dexter *et al.* 1997), which contaminate the grain. The most practical way to control FHB and obtain low level of DON in grain is through the development of resistant cultivars (Koch *et al.* 2006). However, different types of resistance and their polygenic control make breeding for FHB resistance very difficult. Several types of resistance and/or tolerance to FHB have been described. Type I is resistance to initial infection; type II is resistance to the spread of the disease within the spike, and type III is tolerance to DON accumulation (Schroeder & Christensen 1963; Mesterhazy 1995). The major source of FHB resistance used in many breeding programs is Asian source Sumai 3 (Bai & Shaner 2004). Brazilian spring wheat cultivar Frontana is also frequently used as FHB donor parent (Van Ginkel *et al.* 1996). However, in addition to exotic sources of resistance (such as cultivars Sumai 3 and Frontana), native sources

of resistance are commonly used in winter wheat breeding programs in North America.

In 1996, 2000, and 2004 severe epidemics of FHB cost the winter wheat industry in Ontario, Canada combined over $200 million. The wheat industry in Ontario including seed companies, producers and end-user, endorses and supports the formal registration of wheat varieties grown for commercial wheat production. Prior to 1997, agronomic and quality characteristics were the only criteria required for wheat registration. After the 1996 epidemic, the entire industry through the Ontario Cereal Crop Committee (OCCC) mandated that all candidate cultivars considered for registration must be screened for resistance to Fusarium by public and private breeding programs as part of the registration requirements. The OCCC organized multiple FHB inoculated sites across Ontario with designated check cultivar(s) for comparison with the candidate wheat cultivar(s). After registration, all wheat cultivars grown in Ontario are entered in the Ontario Winter Wheat Performance Trial (OWWPT) and tested every year for Fusarium resistance and DON level in three inoculated FHB nurseries. The objective of this study is to explain how the index that accounts for FHB symptoms and DON level jointly was developed, and how stable the performance of the cultivars grouped to susceptibility classes has been over a number of years.

MATERIALS AND METHODS

Winter wheat cultivars entered in OWWPT were planted in three locations (Ridgetown, Ottawa and Nairn) in Ontario, Canada from 2002 to 2010. At each location, the plots were planted in a randomized block design with four replications. The plots were single rows, 2 m long, spaced 17.8 cm apart containing 100 seeds each. *F. graminearum* inoculum was produced from single spore cultures of four isolates grown seven to ten days on PDA under 24 h UV light. Two plugs of mycelium (1 cm diameter) of each isolate were added per one flask to a liquid medium to induce sporulation of the pathogen (Tamburic-Ilincic *et al.* 2007). The plots were spray-inoculated with a combined suspension of macroconidia of the isolates at a concentration of 5×10^4 spores/ml. Due to differences in flowering date, each cultivar was sprayed when wheat heads were at 50% anthesis (ZGS 65, Zadoks *et al.* 1974). Nurseries were mist irrigated daily to promote fungal development, until three days after the last inoculation. Each cultivar was assessed for visual symptoms at the early dough stage (ZGS 83), and rated for FHB incidence (percent of spikes infected) and severity (percent of spikelets infected). Disease levels were calculated as Fusarium head blight index (FHBI), which was the product of the percent heads infected and the percent spikelets infected, divided by 100. Harvested mature grain samples were ground 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 analyzed using EZ-Quant® Vomitoxin ELISA kit from Diagnostix (www.diagnostix.ca) with a DON detection limit of 0.5 ppm (Sinha & Savard 1996) using bulked samples from the three replications of each cultivar. Data from individual locations were ranked for FHBI and DON separately, and then ranking for each trait were combined over locations. The overall mean ranking for FHBI and DON were then combined together and the combined ranking value was ranked to give a single value for the combine traits. The varieties were scored as follows: 1 ≥ 1SD below mean (moderately resistant – MR), 2 ≤ 1SD below mean (moderately susceptible – MS), 3 ≤ 1SD above mean (susceptible – S), 4 ≥ 1SD above mean (highly susceptible – HS). Pearson correlation coefficient between traits was calculated using the PROC CORR statement (SAS Institute Inc. 2004).

RESULTS

High FHB index and DON levels were obtained across the cultivars tested in 2009 OWWPT; FHB index ranged from 3.0% to 49.2% and DON from 6.3 ppm to 34.0 ppm (Table 1). Average level for FHB index and DON level was 9.8% and 9.2 ppm; 16.3% and 13.0 ppm; 24.9% and 14.7 ppm; 33.5% and 25.3 ppm for cultivars rated as MR, MS, S and HS, respectively. Nine winter wheat cultivars were rated MR (20.4%), twelve MS (27.3%), fifteen S (34.1%) and eight HS (18.2%). Cultivars 25R51, FT Wonder, Vienna and AC Morley had excellent resistance to FHB and low DON level in the grain, while AC Mackinnon and E1009W had very high FHB index and DON level (Table 1). However, some cultivars had lower DON level than expected based on FHB index (ACS55017) and opposite (Benefit was ranked 5th for FHB index and 35th for DON level). Example of ranking cultivars, from three different inoculated

Table 1. Fusarium head blight incidence, severity, index (%), deoxynivalenol level, ranking and score of winter wheat used for *Fusarium* rating in OWWPT in 2009

Name	Incidence	Severity	FHBI	Rank FHBI*	DON (ppm)	Rank Combined DON**, ***		Rank	Score	Rating
	(%)									
AC Morley	61.3	22.3	13.5	13	6.3	1	14	4	1	MR
Superior	75.0	33.0	24.8	31	13.3	17	48	23	3	S
AC Mackinnon	77.5	45.8	35.4	41	25.7	42	83	41	4	HS
AC Mountain	62.5	31.3	19.7	23	17.2	34	57	31	3	S
Maxine	70.0	31.3	21.6	28	15.8	31	59	34	3	S
Warthog	60.0	24.0	13.8	15	11.3	11	26	10	2	MS
Harvard	66.3	31.3	21.8	27	15.5	30	57	31	3	S
Carlisle	82.5	27.0	22.9	29	24.5	39	68	37	4	HS
Vienna	52.5	20.5	10.0	7	11.3	11	18	6	1	MR
FT Wonder	46.3	22.3	10.5	6	8.8	5	11	3	1	MR
AC Sampson	57.5	41.5	24.1	30	14.3	23	53	29	3	S
25R47	88.8	30.0	26.6	32	11.4	14	46	22	3	S
D8006W	61.3	32.5	21.5	24	11.2	9	33	15	2	MS
Emmit	52.5	27.0	15.2	14	13.9	22	36	18	2	MS
E1007R	72.5	41.3	31.4	37	13.8	21	58	33	3	S
R045	62.5	32.5	22.6	26	11.2	9	35	16	2	MS
Huntley	81.3	34.3	28.4	35	14.6	26	61	35	3	S
Becher	60.0	30.0	18.9	21	9.6	6	27	11	2	MS
Ava	26.3	20.5	6.0	2	11.7	15	17	5	1	MR
Wentworth	72.5	37.3	26.5	33	19.0	38	71	38	4	HS
ADV Dyno	75.0	27.0	19.4	25	10.2	7	32	14	2	MS
R055	77.5	37.3	29.6	36	13.4	19	55	30	3	S
E1009W	78.8	49.5	40.0	43	30.5	43	86	44	4	HS
25R56	87.5	37.3	32.7	39	14.3	23	62	36	3	S
Ninja	60.0	32.5	20.8	22	15.2	29	51	25	3	S
Branson	57.5	30.0	17.5	19	16.2	32	51	25	3	S
Benefit	50.0	20.0	10.0	5	17.9	35	40	19	2	MS
Stanford	65.0	24.0	15.2	16	7.5	4	20	7	1	MR
E0028W	75.0	45.8	34.1	40	34.0	44	84	42	4	HS
VA03W-409	85.0	57.0	49.2	44	24.6	40	84	42	4	HS
25R51	15.0	20.0	3.0	1	6.5	2	3	1	1	MR
HY 116-SRW	55.0	22.3	12.5	10	13.3	17	27	11	2	MS
25W36	90.0	34.3	30.8	38	25.3	41	79	40	4	HS
25W43	70.0	22.3	16.2	17	17.9	35	52	28	3	S
Princeton	40.0	19.3	7.9	3	7.2	3	6	2	1	MR

Table 1 to be continued

Name	Incidence	Severity	FHBI	Rank FHBI*	DON (ppm)	Rank DON**, ***	Combined	Rank	Score	Rating
	(%)									
CM153	55.0	29.5	18.8	18	16.6	33	51	25	3	S
Carnaval	37.5	22.3	9.0	4	12.6	16	20	7	1	MR
Surge	60.0	29.5	20.9	20	14.3	23	43	21	2	MS
OTF013-081	55.0	24.0	13.1	11	11.3	11	22	9	1	MR
25R39	65.0	21.0	13.7	12	15.1	28	40	19	2	MS
ACS55017	77.5	49.8	39.4	42	10.8	8	50	24	3	S
Palmer	37.5	32.5	14.0	9	14.6	26	35	16	2	MS
CM3534	72.5	38.3	29.0	34	18.8	37	71	38	4	HS
RO85	50.0	23.5	13.2	8	13.6	20	28	13	2	MS
Mean	63.2	31.0	21.0		15.0					
SD	16.4	9.1	9.9		6.0					

FHBI – Fusarium head blight index; SD – standard deviation; MR – moderately resistant; MS – moderately susceptible; S – susceptible; HS – highly susceptible; ***combined rank is sum of FHBI rank (*) and DON rank (**)

Table 2. Fusarium head blight index ranking and deoxynivalenol (DON) level ranking from three inoculated nurseries (Nairn, Ridgetown and Ottawa), combined ranking and score of moderately resistant-MR (1) and moderately susceptible-MS (2) cultivars entered in Ontario Winter Wheat Performance Trial (OWWPT) in 2010

Cultivar	FHBI rank				DON rank				Combined rank*	Rank**	Score
	Nairn	Ridg	Otta	mean	Nairn	Ridg	Otta	mean			
Ava	3	1	10	4.7	2	3	14	6.3	11	1	1
AC Morley	1	4	6	3.7	9	1	13	7.7	11	2	1
Stanford	15	2	16	11.0	2	2	1	1.7	13	3	1
25R51	2	3	36	13.7	1	4	27	10.7	24	6	1
SC-06215R	11	6	32	16.3	2	5	2	3.0	19	4	1
RCDH-19/21	5	15	1	7.0	23	12	16	17.0	24	5	1
TW271 × 099	14	5	6	8.3	23	8	18	16.3	25	7	2
Benefit	12	12	12	12.0	30	6	2	12.7	25	8	2
SC-07201R	17	20	24	20.3	9	14	25	16.0	36	12	2
25R39	6	8	17	10.3	2	41	23	22.0	32	11	2
ACS56113	21	14	14	16.3	23	11	28	20.7	37	13	2
SC-07203R	25	7	28	20.0	18	13	2	11.0	31	9	2
E1007R	28	11	4	14.3	23	22	30	25.0	39	16	2
25W36	10	23	9	14.0	29	19	26	24.7	39	15	2
Carnaval	8	17	29	18.0	2	38	2	14.0	32	10	2
Huntley	21	40	11	24.0	9	21	12	14.0	38	14	2

Ridg = Ridgetown, Otta = Ottawa; *combined rank is sum of FHBI mean rank and DON mean rank; **rank of combined rank

Table 3. Fusarium score of winter wheat cultivars over eight years (Ontario Winter Wheat Performance Trial, 2002–2009)

Name	2002	2003	2004	2005	2006	2007	2008	2009	Mean score	Rating
AC Morley	1	1	1	1	1	1	1	1	1.0	MR
Superior	3	3	3	3	3	3	3	3	3.0	S
AC Mackinnon	4	4	4	4	4	4	4	4	4.0	HS
AC Mountain	2	2	2	2	3	3	3	3	2.5	MS
Maxine	3	3	3	3	4	4	3	3	3.3	S
Warthog	2	2	2	2	2	2	2	2	2.0	MS
Harvard	3	3	3	3	3	3	4	3	3.1	S
Carlisle	2	2	2	2	2	2	2	4	2.3	MS
Vienna	1	1	1	1	1	1	1	1	1.0	MR
FT Wonder	1	1	1	1	1	1	1	1	1.0	MR
AC Sampson	3	3	3	3	4	4	4	3	3.4	S

MR – moderately resistant; MS – moderately susceptible; S – susceptible; HS – highly susceptible

nurseries, for FHBI and DON level first and then combining them for scoring the most resistant cultivars (MR and MS) entered in OWWPT in 2010 is presented in Table 2. The lowest ranking for FHBI and DON level across all locations had AC Morley and Stanford, respectively (Table 2). Out of eleven cultivars that remained in OWWPT for eight years, three were rated as MR, three as MS, four as S and one as HS (Table 3). MR and HS cultivars did not change score during each of the eight years, while some of the MS cultivars (AC Mountain, Carlisle) and S cultivars (Harvard, Maxine, AC Sampson) changed the scores in some years (Table 3). Correlation coefficient between FHB index and DON in 2009 was $r = 0.45$ ($P = 0.0024$, Figure 1). Higher correlation in 2009 was detected between FHB index and severity ($r = 0.91$, $P = 0.0001$) than between FHB index and incidence ($r = 0.76$, $P = 0.0001$) (Figures 2 and 3). Biplot of mean ranking across sites and combined ranking in 2010 is presented in Figure 4.

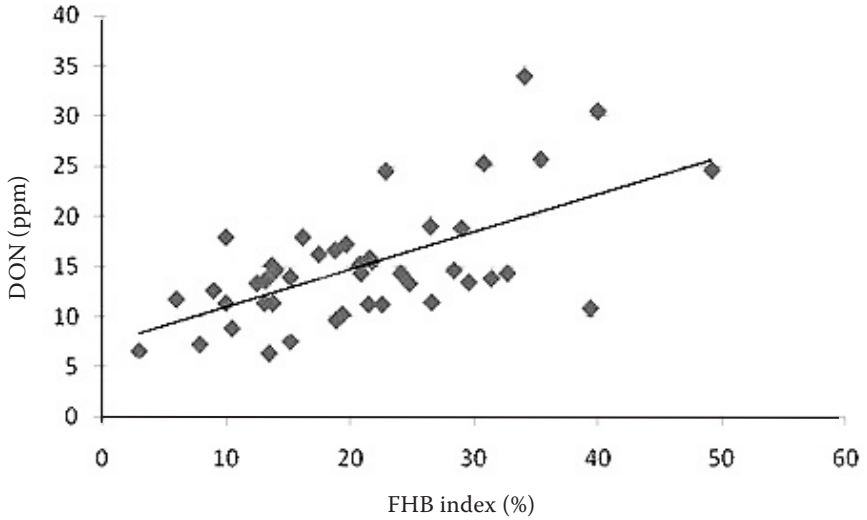


Figure 1. Relationship between FHB index (%) and DON level (ppm) after inoculation with *Fusarium graminearum* in OWWPT in 2009 ($r = 0.45$, $P = 0.0024$)

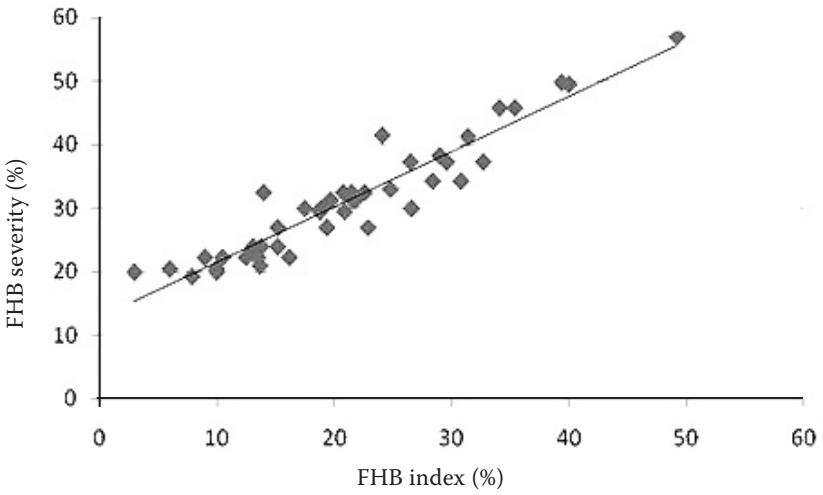


Figure 2. Relationship between FHB index (%) and FHB severity (%) after inoculation with *Fusarium graminearum* in OWWPT in 2009 ($r = 0.91$, $P < 0.0001$)

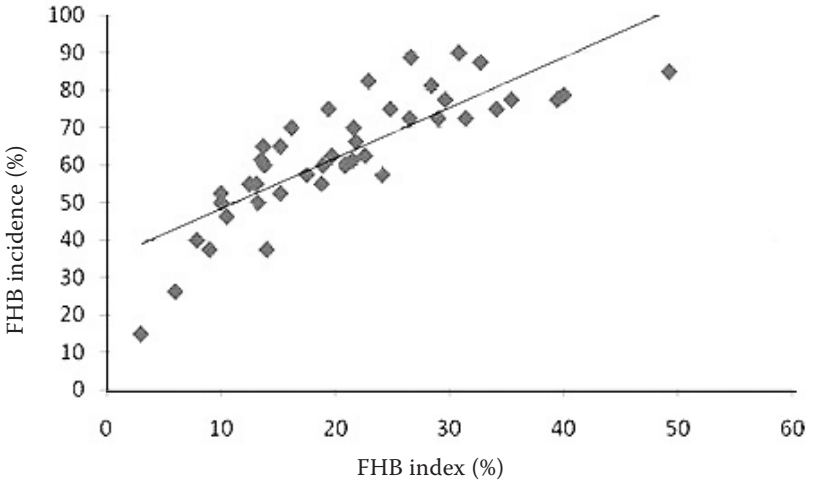
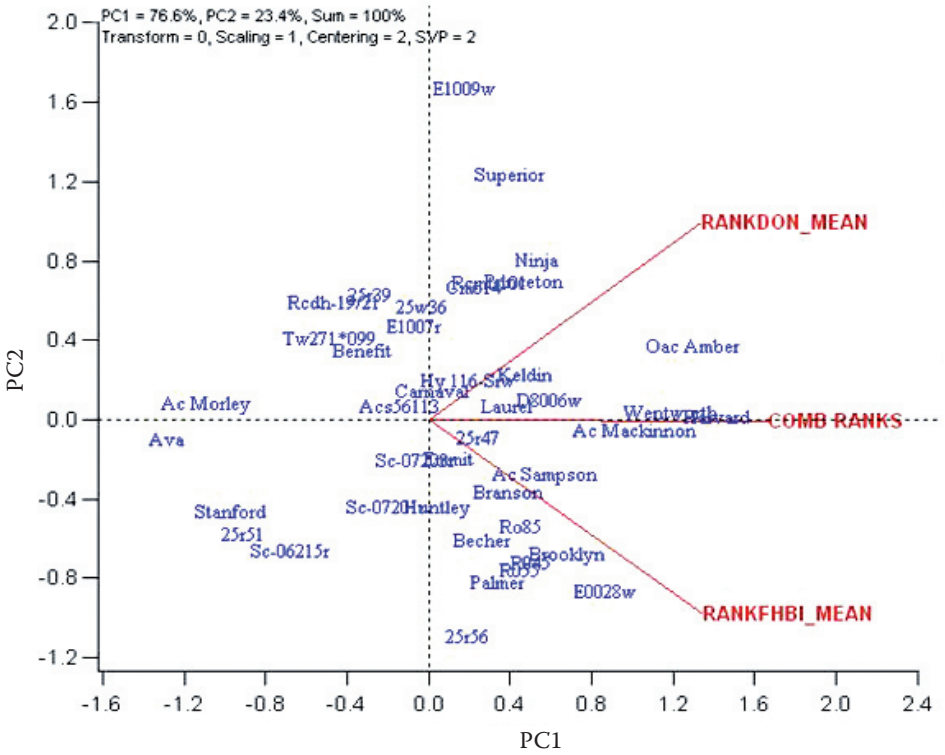


Figure 3. Relationship between FHB index (%) and FHB incidence (%) after inoculation with *Fusarium graminearum* in OWWPT in 2009 ($r = 0.76$, $P < 0.0001$)

DISCUSSION

In the present study, we explained use and development of an index that combine FHB index and DON level together. The index is related to Fusarium susceptibility classes (MR, MS, S, HS), up dated every year with results from three FHB inoculated nurseries and presented to OCCC, industry and farmers. A significant correlation between DON content and aggressiveness was observed in some studies (Miedaner *et al.* 2004), but low or no correlation was reported in another study (Gosman *et al.* 2007). This could be explained by level of resistance in material tested, aggressiveness of isolates used for inoculation, inoculation techniques and different environments. Resistance to FHB is horizontal and non-species specific (Van Eeuwijk *et al.* 1995). To obtain good differentiation among tested cultivars, we have been using a mixture of four highly DON-producing *F. graminearum* isolates and spray inoculation method with daily mist irrigation to promote fungal development. High FHB index and DON level was obtained across the cultivars tested. Among the most FHB resistant cultivars in OWWPT in 2009 were 25R51, FT Wonder, Vienna and AC Morley. 25R51 and FT Wonder had Sumai 3 and Frontana in their pedigrees, respectively. Both Sumai 3 and Frontana are widely used FHB resistance sources with known quantitative trait loci (QTL) regions or resistance genes (Van Ginkel *et al.* 1996; Bai & Shaner 2004; Steiner *et al.* 2004; Cuthbert *et al.* 2006). In adition, it has been reported that Frontana can degrade DON (Miller & Arnison 1986). The introduction of exotic FHB resistance sources into adapted winter wheat material requires several backcross generations to overcome the genetic background associated with poor quality and yield. We developed line RCATL33 with pyramided genes from both Sumai 3 and Frontana in the pedigree, low FHB symptoms and DON level, but modest yield potential (Tamburic-Ilincic *et al.* 2006). However, 25R51 has major QTL 3BS for FHB resist-



Relationship among testers

Figure 4. Biplot of mean ranking across the sites and combined ranking used in OWWPT in 2010

ance (known as *Fhb1)* from Sumai 3, good yield and quality characteristics. In 2010, 25R51 was ranked as 1st and 2nd for FHB and 3rd and 4th for DON level in Nairn and Ridgetown, but did not perform as expected in Ottawa (Table 2). Ranking used in the present study decreases the influence of environment on both traits (FHB index and DON level) and across environments; 25R51 was still overall ranked as 6th cultivars in 2010 and rated as MR. Opposite to exotic sources of resistance, Vienna and AC Morley have native sources of FHB resistance. They also showed stable performance across the years (Table 3). Few cultivars remain in the Performance testing system more than five years. Out of eleven cultivars that remained in OWWPT for eight years, three were rated as MR, three as MS, four as S and one as HS (Table 3). MR and HS cultivars did not change score during each of the eight years, while some of the MS cultivars and S cultivars changed the scores in some years. This could be explained by lower stability in cultivars performance in MS and S classes compared with the most resistant (rated as MR) and the most susceptible ones (rated as HS) under different environments. However, MR cultivars Vienna and FT Wonder were not entered in OWWPT in 2010; they could not stand competition with higher yielding cultivars.

In the present study, higher correlation (0.91 vs. 0.76) was obtained between FHB index and severity (the percent spikelets infected) than between FHB index and incidence (the percent heads infected). Correlation coefficient between FHB index and DON was moderate (r = 0.45). This shows that rating cultivars for both FHB incidence and FHB severity and analyzing harvested grain for DON level is important. To simultaneously combine results and make information more acceptable for industry and farmers, an index that combines FHB index and DON level together was developed and used in OWWPT. The index is robust, stable, open-ended (old cultivars out, new cultivars in) and useful to farmers in making cultivars selection decisions. This information is available to growers and the industry through the website www.gocereals.ca which is maintained by the OCCC. In addition, the OCCC provides objective agronomic and yield data on the performance of cultivars in the major wheat production regions of the province. Significant progress has been made in public and private breeding programs in Ontario, Canada winter wheat, with respect to FHB resistance in the past ten years, but there is still plenty of room for improvements. Our ultimate goal is to have resistant genotypes in OWWPT in the near future.

Acknowledgements. Financially supported by OCCC, Grain Farmers of Ontario (GFO) and the University of Guelph under contract with the Ontario Ministry of Agriculture, Food and Rural Affairs. The authors thank site coordinators, technicians and students from Nairn, Ridgetown and Ottawa who helped in conducting the field trials and disease evaluations.

References

Bai G., Shaner G. (2004): Management and resistance in wheat and barley to Fusarium head blight. Annual Review of Phytopathology, **42**: 135–161.

Cuthbert P.A., Somers D.J., Thomas J., Cloutier S., Brule-Babel A. (2006): Fine mapping *Fhb1*, a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, **112**: 1465–1472.

Dexter J.E., Marachylo B.A., Clear R.M., Clarke J.M. (1997): Fusarium head blight: effecton semolina and pasta-making quality of durum wheat. Cereal Chemistry, **74**: 519–525.

Gosman N., Bayles R., Jennings P., Kirby J., Nicholson P. (2007): Evaluation and characterization of resistance to Fusarium head blight caused by *Fusarium culmorum* in UK winter wheat cultivars. Plant Pathology, **56**: 264–276.

Koch H.J., Pringas C., Maerlander B. (2006): Evaluation of environmental and management effects on Fusarium head blight infection and deoxynivalenol concentration in the grain of winter wheat. European Journal of Agronomy, **24**: 357–366.

Mesterhazy Á. (1995): Types and components of resistance against Fusarium head blight of wheat. Plant Breeding, **114**: 377–386.

Miedaner T., Heinrich N., Schneider B., Oettler G., Rohde S., Rabenstein F. (2004): Estimation of deoxynivalenol (DON) content by symptom rating and exoantigen content for resistance selection in wheat and triticale. Euphytica, **139**: 123–132.

Miller D.J., Arnison P.G. (1986): Degradation of deoxynivalenol by suspension cultures of the Fusarium head blight resistant wheat cultivar Frontana. Canadian Journal of Plant Pathology, **8**: 147–150.

SAS Institute Inc. (2004): SAS OnlineDoc® 9.1.2. SAS Institute Inc., Cary.

Schroeder H.W., Christensen J.J. (1963): Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology, **53**: 831–838.

Sinha R.C., Savard M.E. (1996): Comparison of immunoassay and gas chromatography methods for the detection of the mycotoxin deoxynivalenol in grain samples. Canadian Journal of Plant Pathology, **18**: 233–236.

Steiner B., Lemmens M., Griesser M., Scholz U., Schondelmaier J., Buerstmayr H. (2004): Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theoretical and Applied Genetics, **109**: 215–224.

Tamburic-Ilincic L., Schaafsma A.W., Falk D.E., Laskar B., Fedak G., Somers D. (2006): Registration of winter wheat germplasm line RCATL33 with Fusarium head blight resistance and reduced deoxynivalenol accumulation. Crop Science, **46**: 1399–1400.

Tamburic-Ilincic L., Schaafsma A.W., Falk D.E. (2007): Indirect selection for lower deoxynivalenol (DON) content in grain in a winter wheat population. Canadian Journal of Plant Science, **87**: 931–936.

Van Eeuwijk E.A., Mesterhazy A., Kling C.I., Ruckenbauer P., Saur L., Buerstmayr H., Lemmens M., Keizer L.C.P., Maurin N., Snijders C.H. (1995): Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European stains of *Fusarium culmorum*, *F. graminearum* and *M. nivale* using a multiplicative model for interaction. Theoretical and Applied Genetics, **90**: 221–228.

Van Ginkel M., Van Der Schaar W., Zhuping Y., Rajaram S. (1996): Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Disease, **80**: 863–867.

Zadoks J.C., Chang T.T., Konzak C.F. (1974): A decimal code for the growth stages of cereals. Weed Research, **14**: 415–421.