The Journal of Microbiology (2012) Vol. 50, No. 1, pp. 97–102
DOI 10.1007/s12275-012-1381-5

Heat- and Cold-Shock Responses in *Fusarium graminearum* 3 Acetyl- and 15 Acetyl-Deoxynivalenol Chemotypes

Vladimir Vujanovic*, Yit Kheng Goh, and Prasad Daida

Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada

(Received August 3, 2011 / Accepted October 7, 2011)

Fusarium graminearum **Schwabe is the primary cause of Fusarium head blight (FHB) in North America. Chemically distinct *F. graminearum* sub-populations can be identified based on the type or composition of deoxynivalenol (DON) mycotoxin derivatives, including 3-acetyl (3-ADON) and 15-acetyl (15-ADON). The evaluation of randomly selected 3-ADON and 15-ADON isolates, collected from spring wheat throughout Canada, was performed using thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), ice-nucleation activity (INA), and heat and cold tolerance tests conducted within a temperature range of -70°C to 65°C. The results indicated that the 3-ADON sub-population, which is responsible for the highest disease severity and has rapidly displaced the 15-ADON sub-population, produces more DON and zearalenone (ZEA) than the 15-ADON sub-population when exposed to heat and cold. Following exposures (1 and 2 h) to extremely high or low temperatures, 3-ADON isolates exhibited faster mycelial growth than 15-ADON isolates. In addition, the warmest temperature at which INA activity occurred was in 3-ADON (-3.6°C) vs. 15-ADON (-5.1°C). Taken together, these features suggest that the newly emerging 3-ADON sub-population is more resilient than the resident 15-ADON sub-population. Overall, the differences between the two sub-populations could provide new insights into FHB epidemiology and if validated under field conditions, may provide important information for predicting future FHB epidemics.**

Keywords: *Fusarium graminearum*, chemotypes, heat-shock, cold-shock, INA activity, mycotoxins

Introduction

Fusarium graminearum strains are responsible for causing Fusarium head blight (FHB), and these strains vary greatly in the ability to cause disease and produce mycotoxins on wheat, barley, maize and other major small grain cereals (Bai and Shaner, 2004; Goswami *et al.*, 2006; Ward *et al.*, 2008). *F. graminearum* is an important producer of type B trichothecenes, such as the toxic deoxynovalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyledeoxynivalenol (15-ADON) derivatives, and zearlenone (ZEA) (Von der Ohe *et al.*, 2010; Gale *et al.*, 2011). In North America, the aggressive 3-ADON chemotype is displacing the 15-ADON chemotype sub-population (Puri and Zhong, 2010) that had been the resident chemotype for many years. Several hypotheses have suggested that the epidemiology and aggressiveness of 3-ADON, as manifested by its rapid growth and reproduction, have a common genetic, biochemical and physiological basis. The climate, notably variations in temperature, can influence *F. graminarium* chemotype occurrence and the production of toxic secondary metabolites in grains (Schaafsma, 2007). Previous studies have demonstrated that *F. graminarium* can readily adapt to different stressful situations with optimal growth and mycotoxin biosynthesis occurring at 25°C (Doohan *et al.*, 2003). Thus, a shift in the values of environmental parameters can affect the amount of mycotoxin production or accumulation (Champeil *et al.*, 2004). Mycotoxin production seems to be an adaptive response to stressful conditions, such as extreme heat and cold. In recent years, much attention has also been given to the ice-nucleation activities (INA) of *F. graminearum* that are triggered by the cold. The ability of 3-ADON and 15-ADON to produce INA has never been properly investigated (Pouleur *et al.*, 1992). In this study, we hypothesized that INA activity and mycotoxin production of the fast-growing 3-ADON chemotype allows 3-ADON to outcompete with the relatively slow-growing 15-ADON chemotype when exposed to the same environmental (heat and cold) stressors. A potential link between thermal stress and differences associated with growth, DON and ZEA production, and INA activity were examined for each of the two *F. graminarium* chemotypes in order to allow rapid differentiation between them, and to assess the adaptability of each under changing environmental conditions. In this study, thin layer chromatography (TLC) based on comparisons to standards was coupled with high-performance liquid chromatography (HPLC) for quantifying DON and ZEA (Vasavada and Hsieh, 1987; Schaafsa *et al.*, 1998).

*For correspondence. E-mail: vladimir.vujanovic@usask.ca; Tel.: +306-966-5048; Fax: +306-966-8898

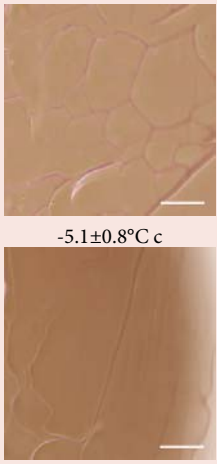
Materials and Methods

Fungal isolates and growth

In this study, eight different *F. graminarium* isolates (four 3-ADON chemotypes and four 15-ADON chemotypes) were randomly selected from Canadian fungal collections (Table 1), as previously described by Puri and Zhong (2010).

Table 1. Survival and growth of *F. graminearum* chemotypes challenged at different temperatures (-70, -20, 45, and 65°C) for 1 and 2 h with room temperature as a control. Collective measurements on ice-nucleation activity (INA) of four 3-ADON and four 15-ADON *F. graminearum* chemotypes (each isolate was in triplicate) with *F. avenaceum* as a control.

F. graminearum chemotype	Isolates	Fungal growth (cm)*						Ice-nucleation† activity (INA) and crystals†
		Incubation time/Temp.	-70°C	-20°C	23°C	45°C	65°C	
3-ADON	M2-06-1	1 h	3.03±0.06 b	1.67±0.12 c	3.57±0.15 a	3.03±0.06 b	0.33±0.14 d	-3.6±0.4°C b
		2 h	2.97±0.06 b	1.17±0.1 c	3.57±0.15 a	2.47±0.38 b	0 d	
	S3-06-1	1 h	3.0±0.1 a	1.93±0.12 c	2.6±0.14 b	1.87±0.32 c	1.47±0.35 c	
		2 h	2.27±0.68 a	0.62±0.07 b	2.6±0.14 a	2.57±0.2 a	0 c	
	SMCD 2243	1 h	3.07±0.12 a	3.07±0.06 a	2.78±0.48 a	3.03±0.25 a	2.83±0.26 a	
		2 h	2.7±0.26 a	1.1±0.25 b	2.78±0.48 a	2.27±0.25 a	0 c	
	wrs 2070	1 h	0.67±0.15 c	2.97±0.11 a	2.28±0.04 b	2.73±0.76 b	2.7±0.25 b	
		2 h	2.03±0.06 c	3.23±0.13 a	2.28±0.04 b	1.23±0.23 d	0 e	
15-ADON	M2-06-2	1 h	2.38±0.19 b	3.1±0.2 a	3.37±0.15 a	0 c	0 c	-5.1±0.8°C c
		2 h	1.23±0.25 c	2.02±0.07 b	3.37±0.15 a	0 d	0 d	
	M8-06-2	1 h	2.9±0.1 b	3.1±0.1 b	3.5±0.1 a	0 c	0 c	
		2 h	2.47±0.06 c	2.77±0.21 b	3.5±0.1 a	0 d	0 d	
	ON-06-17	1 h	2.2±0.36 a	1.97±0.17 ab	2.83±0.67 a	0.97±0.47 b	0.63±0.32 b	
		2 h	1.08±0.38 b	1.43±0.29 b	2.83±0.67 a	0 c	0 c	
	Q-06-10	1 h	2.17±0.35 b	1.98±0.42 b	2.83±0.15 a	0.97±0.21 c	0.63±0.15 c	
		2 h	2±0.87 a	1.43±0.29 b	2.83±0.15 a	0 c	0 c	
F. avenaceum	SMCD 2242	NA	NA	NA	NA	NA	NA	-2.7±0.2°C a



* Numbers in each row represent the mean fungal growth diameter±standard deviation. Each incubation time for separate *F. graminearum* chemotype isolates was analyzed separately. Means within each row of the incubation times for each *F. graminearum* isolate at five different treatments that are followed by the same letter are not significantly different at P=0.05 after ANOVA-LSD test.
† Polarized light micrographs. Magnification bar=50 µm

All of the isolates were maintained on PDA plates for one week at room temperature (~21°C). Then, all cultures were incubated in the dark for one and two hours at -70, -20°C (Global Cooling, B.V., deep quick freezer model, USA), 45 and 65°C (Precision Thermo Scientific, incubator model 3522, Canada). The growth differences between the isolates was studied, and the fungal mycelia of each isolate was transferred to new plates of PDA medium enriched with 4% (v/v) corn steep liquor (Pestka *et al.*, 1985) and incubated at 21°C (Precision Thermo Scientific, incubator model 3522, Nepean, Ontario, Canada) for 72 h (Abdellatif *et al.*, 2010). This experiment was repeated three times with three replicates.

Mycotoxins extraction

Approximately 50 mg of mycelia were cut from each isolate and transferred into sterile 2 ml tubes containing 500 µl of sterile distilled water. Samples were then sonicated on ice prior to mycotoxin extraction. DON and ZEA mycotoxins from the PDA plates enriched with corn steep liquor (Pestka *et al.*, 1985) were extracted from the sonicated mixture using three 500 ml volumes of ethyl acetate as outlined in Vasavada and Hsieh (1987), with slight modifications. Samples were shaken vigorously and allowed to stand for 5 min at 23°C for the separation of phases. The organic phase was siphoned off and passed through sodium sulfate to remove the water. The solvent was allowed to evaporate at room temperature for 24 h. The residue was then re-dissolved in 2 ml of acetonitrile for TLC analysis.

Thin layer chromatography (TLC) analysis

Twenty microliters of crude extracts of DON and ZEA were spotted onto 0.25 mm thick silica gel G-25 UV_{254} TLC 20×20 cm plates (Brinkmann Instruments, Inc.) using a 100 µl micropipette device. The samples were developed in an ethyl acetate: toluene (3:1) solvent liquid chromatography system for 40 min. TLC plates were then air-dried and sprayed with 20% aluminum chloride in absolute ethanol, after which they were charred at 110°C for 10 min according to the method used by Vasavada and Hsieh (1987). The presence of DON and ZEA mycotoxins in the crude extracts was identified by comparison to the spots generate by pure DON and ZEA toxins (Sigma-Aldrich). Pure DON and ZEA mycotoxins were dissolved in acetonitrile and diluted to 12.5, 25, 50, 100, and 200 µg/ml to produce band intensities for each concentration of mycotoxin standards on the TLC plates. Standard intensities for respective mycotoxins were used as guidelines to generate five different scales (outlined in Fig. 1) for determining the different concentrations of DON and ZEA on the TLC plates. The standards and the extractions were conducted daily. The band intensities for each mycotoxin type produced by *F. graminearum* 3- and 15-ADON on PDA plates enriched with corn steep liquor were compared to the standards (Abdelatif *et al.*, 2010) and recorded. All tests were conducted in triplicate and repeated twice.

High performance liquid chromatography (HPLC) analyses

Standard trichothecene mycotoxins of DON, ZEA, 3-ADON, and 15-ADON were purchased from Sigma (HPLC grade - Oakville, Ont.). Mycotoxins extracted from different treatments were analyzed with a Waters 2695 HPLC system with 250×4.60 mm, Luna 5 micron C_{18} (2) 100A column (Phenomenex, USA) and a photodiode-array detector was

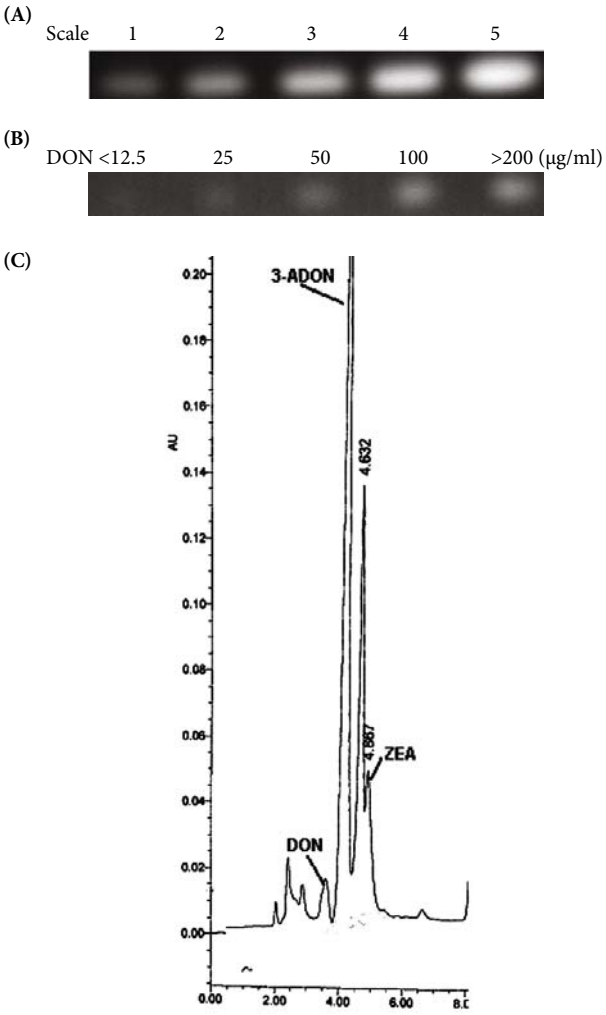


Fig. 1. TLC standards for pure (A) zearalenone (ZEA) and (B) deoxynivalenol (DON) mycotoxins at the concentrations of below 12.5, 25, 50, 100 and more than 200 µg/ml used to generate scales that ranged from 1 to 5, respectively, in ascending order of mycotoxin concentrations. (C) HPLC spectrum of DON and ZEA mycotoxins showing the average amounts produced by the *F. graminearum* 3-ADON chemotype after 7 days at 23°C.

used with an isocratic solvent system (methanol: water-methanol containing 5% [v/v] [90:10]) ratio. The PDA detector measured the UV spectrum (190–500 nm). Samples were dissolved in acetonitrile and 10 µl was loaded onto the column using an automatic injector. Mycotoxins were eluted with solvent or mobile phase at a rate of 0.75 ml/min for 25 min. Standard curves for respective mycotoxins were generated based on five different concentrations of pure toxins and absorbances were obtained from the HPLC analyses.

Ice-nucleation activity (INA)

All eight *F. graminearum* chemotype isolates and one *F. avenaceum* SMCD 2242 were evaluated for INA at -6.0°C according to the methods used by Pouleur *et al.* (1992) using the Lauda refrigerating circulator bath (model RC-6; Brinkmann Instrument Co., Canada). Polarized light micrographs of INA-crystals were acquired using a Nikon Eclipse E400 light microscope equipped with a Nikon DS-FiL color camera and a long working distance 10× and 40× lens and condenser with a resolution of 2560 by 1920. Samples were cooled at -1°C/min to -6°C using a temperature-controlled stage (LTS 120 and PE94 temperature controller (Linkam, UK).

Statistical analyses

Differences in the fungal growth diameter for eight different *F. graminearum* isolates from two incubation periods challenged with five different temperatures were analyzed using ANOVA-LSD (least significant difference) test at $P<0.05$ (SPSS 1990). To compare differences between two incubation time points at respective temperature shock treatments, the *t*-test ($P<0.05$) was performed to analyze the dataset. Survival rates combined with growth data for all *F. graminearum* 3-Acetyl-DON and 15-Acetyl-DON chemotype isolates treated at -70°C for 1 or 2 h, -20°C for 1 or 2 h, 45°C for 1 or 2 h, and 65°C for 1 or 2 h were analyzed using the Dice similarity coefficient. The resulting similarities in the matrix were further processed by employing the SAHN (UPGMA) cluster program in the NTSYSpc software package (Exeter Software, USA) to create a dendrogram (Vujanovic *et al.*, 2009).

Results

Fungal survival and growth

Fungal survival and growth were assessed for the eight different *F. graminaerum* isolates on potato dextrose agar (PDA) at two different incubation time points (1 and 2 h) and these results are summarized in Table 1. All *F. graminearum* 3-ADON chemotype isolates were observed to grow after heat (45 and 65°C) and cold- (-70 and -20°C) shocks for 1 h. In contrast, two *F. graminearum* 15-ADON chemotype M2-06-2 and M8-06-2 isolates died after heat-shock at 45 and 65°C for 1 h (Table 1). Moreover, none of the *F. graminearum* 15-ADON chemotype isolates survived the 45°C heat-shock for 2 h (Table 1). In general, fungal growth was significantly slower when treated at -70, -20, 45, and 65°C for 2 h ($P<0.05$ with *t*-test).

Mycotoxin production

Scales based on five different concentrations of deoxynivalenol (DON) and zearalenone (ZEA) were defined as 1 (<12.5 µg/ml), 2 (25 µg/ml), 3 (50 µg/ml), 4 (100 µg/ml) and 5 (>200 µg/ml), with the respective spot intensity or mycotoxin expression levels (Figs. 1A and B). These scales were used for the identification and comparison of DON and ZEA mycotoxin concentrations for all of the *F. graminearum* isolates in seven different temperature treatments from -70°C to 65°C. Deoxynivalenol (DON) and zearalenone (ZEA) profiles were assessed on TLC plates and fold changes in the quantity of DON and ZEA are presented in Fig. 2A for 3-ADON and Fig. 2B for 15-ADON. The values obtained for each if the tested mycotoxins were confirmed by HPLC (Fig. 1C). In general, the cold treatments conducted

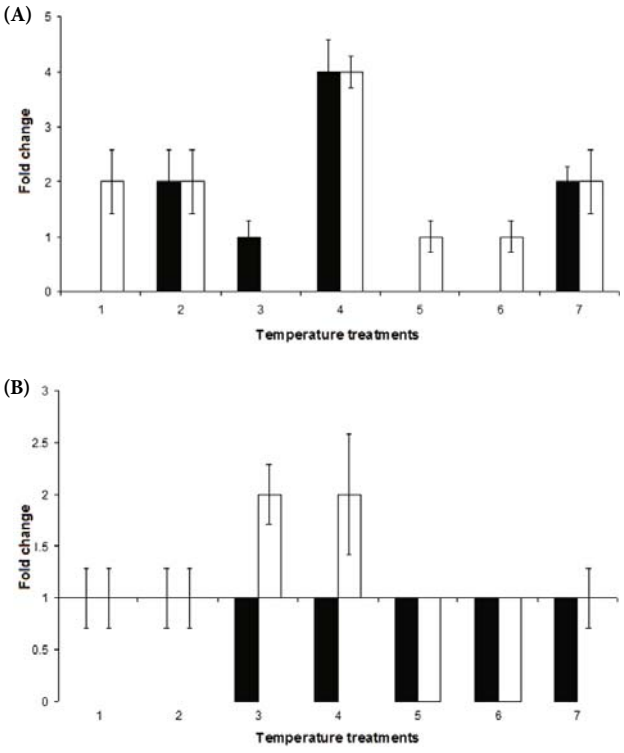


Fig. 2. Thin layer chromatography (TLC) qualitative comparison based on fold changes in two different mycotoxins, (■) zearalenone (ZEA) and (□) deoxynivalenol (DON) produced by two *Fusarium graminearum* chemotypes at different temperatures treatments: (A) the 3-ADON isolates that survived, and (B) the 15-ADON isolates that survived (see Table 1.) throughout 1-7 temperature treatments compared with a control (23°C): 1, at -70°C for 1 h; 2, -70°C for 2 h; 3, -20°C for 1 h; 4, -21°C for 2 h; 5, 45°C for 1 h; 6, 45°C for 2 h; and 7, 65°C for 1 h.

at -70°C (1 h and 2 h) and -20°C (2 h), and the heat-shocks (65°C) were observed to considerably induce the production of DON in the *F. graminearum* 3-ADON chemotype isolates (Fig. 2A). For the treatments at -70°C (1 h), -20°C (1 and 2 h) and 65 °C (1 h), the *F. graminearum* 3-ADON isolates showed higher amounts of ZEA compared to the *F. graminearum* 15-ADON isolates (Figs. 2A and B). However, in most cases, the production of DON and ZEA in the *F. graminearum* 15-ADON chemotype isolates was suppressed if the isolate was exposed to both heat and cold shocks (Fig. 2B).

Combined analysis

Cluster tree analysis based on the combined data on the growth and survival of the eight *F. graminearum* (3- and 15-ADON) isolates that were grown on PDA and treated at -70°C for 1 or 2 h; -20°C for 1 or 2 h; 45°C for 1 or 2 h; and 65°C for 1 or 2 h are summarized in Fig. 3. Based on the cluster analysis, *F. graminearum* 3-ADON chemotype isolates formed a single "A" clade (Fig. 3). However, *F. graminearum* 15-ADON chemotype isolates were located in two separate "B" and "C" clades (Fig. 3). The 3-ADON and 15-ADON clades were supported with 88% and 84% similarity, respectively (Fig. 3)

Ice-nucleation activity (INA)

The *Fusarium graminearum* 3-ADON chemotype isolates were observed to have significantly higher ice-nucleation activity (-3.6°C) compared to the *F. graminearum* 15-ADON chemotype isolates (-5.1°C) as determined by the ANOVA-LSD test ($P<0.05$) (Table 1). Both *F. graminearum* chemotypes were found to have lower ice-nucleation activity than the control, *F. avenaceum* (-2.7°C) (Table 1). This was expected, in accordance with the data reported by Pouleur *et al.* (1992) and based on the prevalence of the *F. avenaceum* population in the northern climatic agroecozones (Abdellatif *et al.*, 2010).

Discussion

Abiotic and biotic factors, such as climatic conditions, temperature, water availability, and competition with other fungi have significant effects on the survival, growth, dispersal, and spread of the *Fusarium graminearum* population (Doohan *et al.*, 2003; Goh *et al.*, 2009). Temperature seems to be one of the most important factors in determining growth and mycotoxin formation in *F. graminearum* isolates. Optimal growth conditions for *F. graminearum* isolates on PDA were found to be approximately 24–28°C

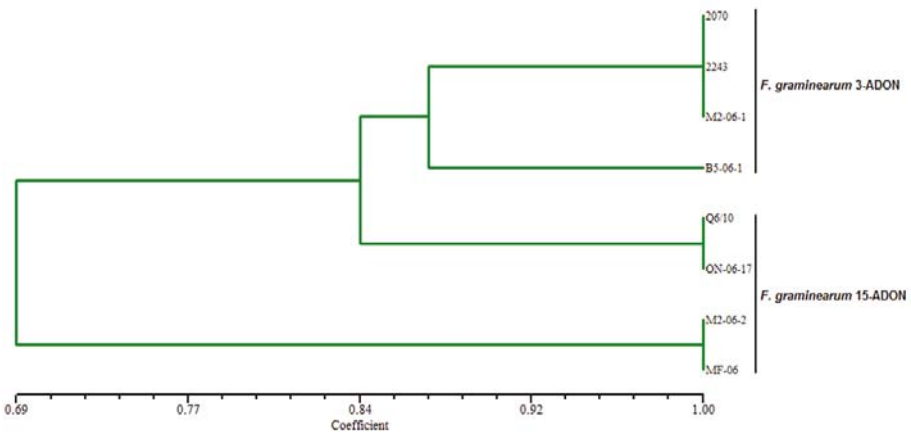


Fig. 3. Numerical (UPGMA) clustering based on growth or survival of eight different *F. graminearum* chemotypes (3- and 15-ADON) on PDA treated at -70°C for 1 or 2 h; -20°C for 1 or 2 h; 45°C for 1 or 2 h; and 65°C for 1 or 2 h.

(Cook and Christen, 1976; Doohan *et al.*, 2003); however, favorable temperatures for the production of *F. graminearum* B trichothecene, deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (ADON), and zearalenone (ZEA) were reported to be between 25–28°C and between 17–28°C (Doohan *et al.*, 2003). This paper explores the effects of cold- and heat-shock on the survival, growth, and mycotoxin synthesis of *F. graminearum* 3- and 15-ADON chemotypes at -70, -20, 45, and 65°C (for 1 to 2 h) in an attempt to explain the displacement of the *F. graminearum* 15-ADON chemotype by the 3-ADON chemotype in North America.

Heat treatments of 60, 70, and 80°C have been proposed for the eradication of *F. graminearum* seed-borne pathogenic isolates (Clear *et al.*, 2002; Gilbert *et al.*, 2005). In the present study, *F. graminearum* 3-ADON isolates survived and grew after being exposed to temperatures of 45°C for 2 h and of 65°C for 1 h, whereas 15-ADON did not, demonstrating that the 15-ADON isolates are less tolerant to heat (Table 1). On the other hand, the -70 and -20°C treatments did not completely inhibit the *F. graminearum* 3- and 15-ADON chemotypes (Table 1), indicating that both sub-populations are tolerant of cold temperatures. This could be due to the fact that these *F. graminearum* isolates originated from cooler Canadian agro-regions. Based on survival and growth after heat- and cold-treatments at two different incubation times (Fig. 3), 3-ADON formed a separate clade from the 15-ADON isolates (coefficient 0.86 under UPGMA analysis), possibly due to the ability of *F. graminearum* 3-ADON to grow, reproduce, and maintain high aggressiveness (Ward *et al.*, 2008) even after exposure to shock temperatures. The *F. graminearum* isolates were also found to have ice-nucleation activities at relatively high temperatures (Table 1). The *F. graminearum* 3-ADON chemotype showed the highest INA activity, even under warm temperatures, which is a possible measure of virulence (Lee *et al.*, 1995). The *F. graminearum* 3-ADON isolates had a faster initiation of INA, explaining in part its greater aggressiveness as compared to 15-ADON in nature. This phenomenon would be especially pronounced in cooler regions in the northern hemisphere, which seem to favor members of the genus Fusarium with higher INA activity (Pouleur *et al.*, 1992). Therefore, we speculate that *F. graminearum* 3-ADON will follow a similar path as *F. avenaceum*, which is known to be a powerful INA producer and plant colonizer in Northern ecozones (Abdellatif *et al.*, 2010).

Cold-shock and heat-shock treatments, especially at -20°C and 45°C, were found to cause more drastic shifts in both deoxynivalenol (DON) and zearalenone (ZEA) production for *F. graminearum* 3-ADON as compared to treatments at other temperatures (Fig. 2A). Interestingly, these two temperatures were registered as the most frequent, cardinal values in Canadian agro-regions. Hence, these temperatures may be associated with changes in the physiological state of *F. graminearum*, related to hibernation (at -20°C) or estivation (at 45°C). These two cyclic and natural phenomena allow for the thermoregulation of eukaryotic cells escaping from cold, warm or dry conditions (Berger and Clutter, 1978).

Heat-shock treatments showed a higher suppression of DON and ZEA production in the *F. graminearum* 15-ADON versus 3-ADON isolates (Fig. 2B), perhaps further explaining *F. graminearum* 3-ADON aggressiveness in replacing the 15-ADON sub-population in northern regions. Although more research should be performed to determine the real mechanisms at the basis of the gradual shift in dominance of *F. graminearum* populations, the conclusions of this study can be used in the differential diagnosis of *F. graminearum* 3-ADON versus 15-ADON chemotypes in North America. A better taxonomical discrimination of the two chemotypes, as well as the ability to better predict FHB epidemiology would contribute to effective disease management, and would provide insights relevant for the breeding or production of FHB-resistant wheat and barley cultivars.

Acknowledgements

This research was financially supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) discovery grant to VV. The authors would like to thank Dr. Tekauz (AAFC) and Dr. Ilincic-Tamburic (U of Guelph) for different *Fusarium graminearum* isolates and M. Ben Mansour for technical assistance.

References

Abdellatif, M., Fernandez, R., Bouzid, S., and Vujanovic, V. 2010. Characterization of virulence and PCR-DGGE profiles of *Fusarium avenaceum* from western Canadian Prairie Ecozone of Saskatchewan. *Can. J. Plant Pathol.* **32**, 468–480.

Berger, R.J. and Clutter, M.E. 1978. Dormancy and developmental arrest: experimental analysis in plants and animals. Academic Press, New York, USA.

Bai, G. and Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium head blight. *Ann. Rev. Phytopath.* **42**, 135–161.

Champeil, A., Dore, T., and Fourbet, J.F. 2004. Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. *Plant Sci.* **166**, 1389–1415.

Clear, R.M., Patrick, S.K., Turkington, T.K., and Wallis, R. 2002. Effect of dry heat treatment on seed-borne *Fusarium graminearum* and other cereal pathogens. *Can. J. Plant Pathol.* **24**, 489–498.

Cook, R.J. and Christen, A.A. 1976. Growth of cereal root rot fungi as affected by temperature-water potential interactions. *Phytopathology* **66**, 193–197.

Doohan, F.M., Brennan, J., and Cooke, B.M. 2003 Influence of climatic factors on *Fusarium* species pathogenic to cereals. *Eur. J. Plant Pathol.* **109**, 755–768.

Gale, L.R., Harrison, S.A, Ward, T.J., O'Donnell, K., Milus, E.A., Gale, S.W., and Kistler, H.C. 2011. Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana. *Phytopathology* **101**, 124–134.

Gilbert, J., Woods, S.M., Turkington, T.K., and Tekauz, A. 2005. Effect of heat treatment to control *Fusarium graminearum* in wheat seed. *Can. J. Plant Pathol.* **27**, 448–452.

Goh, Y.K., Daida, P., and Vujanovic, V. 2009. Effects of abiotic factors and biocontrol agents on chlamydospore formation in *Fusarium graminearum* and *Fusarium sporotrichioides*. *Biocontrol Sci. Technol.* **19**, 151–167.

Goswami, R.S., Xu, J.R., Trail, F., Hilburn, K., and Kistler, H.C. 2006. Genomic analysis of host-pathogen interaction between *Fusarium graminearum* and wheat during early stages of dis-

ease development. *Microbiology* **152**, 1877–1890.
Lee, R.E., Warren, G.J., and Gusta, L.V. 1995. Biological Ice Nucleation and Its Applications. APS Press, St. Paul, M.N., USA.
Pestka, J.J., E1-Bahrawy, A., and Hart, L.P. 1985. Deoxynivalenol and 15-monoacetyl deoxynivalenol production by *Fusarium graminearum* R6576 in liquid media. *Mycopathologia* **91**, 23–28.
Puri, K.D. and Zhong, S. 2010. The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology* **100**, 1007–1014.
Pouleur, S., Richard, C., Martin, J.-G., and Antoun, H. 1992. Ice nucleation activity in *Fusarium acuminatum* and *F. avenaceum*. *Appl. Environ. Microbiol.* **58**, 2960–2964.
Schaafsma, A.W. 2007. Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. *Int. J. Food Microbiol.* **119**, 116–125.
Schaafsma, A.W., Nicol, R.W., Savard, M.E., Sinha, R.C., Reid, L.M. and Rottinghaus, G. 1998. Analysis of *Fusarium* toxins in maize and wheat using thin layer chromatography. *Mycopathologia* **142**, 107–113.
Vasavada, A.B. and Hsieh, P.H. 1987. Production of 3-acetyldeoxynivalenol by *Fusarium graminearum* R2118 in submerged cultures. *Appl. Microbiol. Biotechnol.* **26**, 517–521.
Von der Ohe, C., Gauthier, V., Tamburic-Ilincic, L., Brule-Babel, A., FerJnando, W.G.D., Clear, R., Ward, T.J., and Miedaner, T. 2010. A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. *Eur. J. Plant Pathol.* **127**, 407–417.
Vujanovic, V., Vidovic, S., Fernandez, M., Daida, P., and Korber, D. 2009. Whole-cell protein and ITS rDNA profiles as diagnostic tools to discriminate *Fusarium avenaceum* intraspecific variability and associated virulence. *Can. J. Microbiol.* **55**, 117–125.
Ward, T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M., and Nowicki, T.W. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations in driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genet. Biol.* **45**, 473–484.