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Population Structure, Chemotype Diversity, and Potential Chemotype Shifting of *Fusarium graminearum* in Wheat Fields of Manitoba

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ABSTRACT

Guo, X. W., Fernando, W. G. D., and Seow-Brock, H. Y. 2008. Population structure, chemotype diversity, and potential chemotype shifting of *Fusarium graminearum* in wheat fields of Manitoba. *Plant Dis.* 92:756-762.

This study was to investigate the variation of acetyl ester derivative of DON at 15-position oxygen (15ADON) and acetyl ester derivative of DON at 3-position oxygen (3ADON) chemotypes and potential chemotype shifting of *Fusarium graminearum* based on the population structure of this species in Manitoba. The study was conducted in 15 locations with wheat cvs. Superb and AC Barrie in Manitoba from 2004 to 2005. Percentages of chemotypes 3ADON and 15ADON of *F. graminearum* ranged from 0 to 95.7 and 4.3 to 100%, respectively. The 3ADON chemotype was distributed in the southern part of Manitoba and predominant in Morris and Horndean. The two chemotypes almost shared the same percentage in Portage la Prairie. The 15ADON chemotype was predominant in the other locations. Significant gene flow was found among the populations from Sanford, Portage la Prairie, Hamiota, Plumias, Rapid City, and Virdein; the populations from Cartier, Rivers, Killarney, and Souris; and the populations from Morris, Kenville, and Dauphin. There were no differences between the populations from two wheat cultivars and two chemotypes. The great variation of chemotype likely resulted from the great genetic diversity of *F. graminearum*. Sexual recombination, population age, and cropping system could result in genetic and chemotypic diversities. Wheat seed shipment and long-distance spore transportation of *F. graminearum* potentially caused the genetic migration and chemotype shifting in Manitoba.

Additional keywords: *Fusarium* head blight, trichothecene

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch) and other *Fusarium* spp., is one of the most destructive diseases in wheat worldwide. Since it was first reported in 1884 (11), FHB disease spread quickly over the cereal production areas, including North America, Europe, and East Asia (11). The disease causes wheat yield loss of up to 50% and damages wheat quality (including protein quality, kernel test weight, and color), threatens safety of human food, and causes animal diseases, which results in difficulties for wheat marketing, exporting, and processing (11).

Molecular techniques allowed identification of *F. graminearum* from other species (4) and genetic information of the species populations (3,7,9). Aoki and O'Donnell (1) grouped *F. graminearum* into two separate species, group 1 (*F. pseudograminearum*) and group 2 (*F. graminearum*). O'Donnell et al. (19) proposed nine geographically structured line-

ages of *F. graminearum* based on the DNA sequences of 11 unclustered genes from the isolates collected worldwide. Genetic structures of *F. graminearum* have been characterized in different places in Europe (3), Asia (9), America (6,7,26), and other regions worldwide (15,19).

F. graminearum produces trichothecene mycotoxins. One of the most important toxins is deoxynivalenol (DON), known as vomitoxin, which inhibits protein biosynthesis in eukaryotic organisms (5) and causes feeding refusal, diarrhea, emesis, alimentary hemorrhaging, and contact dermatitis in animals (5). Human illnesses aleukia and Akakabi, and syndromes including nausea, vomiting, anorexia, and convulsion, are associated with *F. graminearum*.

Three chemotypes, which are strain-specific profiles of trichothecene metabolites, were found in *F. graminearum* (5). They produce a C-4 oxygenated derivative of DON, nivalenol (NIV); an acetyl ester derivative of DON at 15-position oxygen (15ADON); and an acetyl ester derivative of DON at 3-position oxygen (3ADON) (5). The biosynthesis pathway of trichothecene toxin has been well characterized in *F. graminearum* (5). The 15ADON chemotype is predominant in North America and the 3ADON chemotype is predominant in some areas in Asia, including China, Australia, and New Zealand (16).

A higher frequency of the 3ADON chemotype isolates was found in North Dakota and Minnesota in recent years than before (8). Recent molecular surveillance showed that chemotype 3ADON was replacing 15ADON from eastern to western Canada (25), though a study found no chemotype 3ADON in Ontario (23). However, genetic evidence to indicate whether there really was a chemotype shift in the above regions has not been studied. Recent studies showed that a 3ADON chemotype isolate produced more DON than a 15ADON chemotype isolate (25). These situations cause increasing concern to the cereal industries.

The objectives of this study were to investigate variations of chemotype of *F. graminearum* and potential chemotype shifting of *F. graminearum* and to understand the reasons for the above by analyzing genetic diversity and gene flow in the populations of *F. graminearum* isolates in Manitoba.

MATERIALS AND METHODS

Isolate collection and DNA extraction.

In total, 291 *F. graminearum* isolates were collected from wheat heads in 17 farmers' fields in 2004 and 22 fields in 2005 in Manitoba. In 2004, nine fields were planted with wheat cv. Superb, moderately susceptible to FHB, and eight fields were planted with AC Barrie, intermediate in resistance to FHB (20). In 2005, 10 fields were planted with Superb and 12 fields were planted with AC Barrie. The position of each farmer's home was considered as one location, and there were 15 locations in all from 2004 to 2005 (Table 1; Fig. 1). The wheat heads were collected 21 days after anthesis and surface sterilized using 1% bleach for 1 min, washed using sterilized distilled water (sd water) for 1 min, and then dried under a flow hood. The spikelets were taken off the wheat heads and put on a potato dextrose agar (PDA; Difco Laboratories, MD) medium and incubated under fluorescent light at room temperature for 7 days. *F. graminearum* colonies were identified initially using the method described by Nelson (18), transferred to a *Fusarium*-specific medium-specific nutrition-poor agar (SNA), and incubated for 7 days for sporulation under the same conditions as above. *F. graminearum* sporodochia in the SNA medium were washed three to four times using a drop of 50 µl of sd water, and the macroconidia

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suspension in sd water was spread over the surface of a water agar (WA) medium. The macroconidia on the WA were incubated under the same conditions for 6 h, and a single macroconidium was transferred to a PDA medium and incubated for 10 days. The mycelium was harvested, lyophilized for 10 h, and stored at -80°C until use.

DNA extraction was performed using the method described by Fernando et al. (7). The lyophilized fungal mycelium was ground in 600 μl of TES buffer (100 mM Tris, 10 mM EDTA, and 2% sodium dodecyl sulfate) in a 1.5-ml microcentrifuge tube. Then, 140 μl of 5 M NaCl and 70 μl of 10% cetyltrimethylammonium bromide were added to the tube and vortexed. The mixture was incubated at 65°C for 20 min. Next, 600 μl of a mixture of chloroform and isoamyl alcohol (vol/vol, 24:1) was added and then centrifuged at 10,000 rpm for 15 min. The supernatant was transferred into a new tube. The latter step was repeated. Then, 80 μl of 5 M NaCl and 1,000 μl of 100% ethanol were added to precipitate the DNA, and the solution was centrifuged at 13,000 for 5 min. The DNA pellet was washed using 200 ml of cold 80% ethanol. After drying, the pellet was suspended in 100 μl of warm sd water (37°C). The DNA was quantified using 1% agarose gel and diluted to 10 ng/ μl .

Identification of *F. graminearum* and chemotypes. Identification of *F. graminearum* was performed using the specific polymerase chain reaction (PCR) marker described by Demeke et al. (4). Two primers were used in the PCR, Fg16F (5'-CTCCGGATATGTTGCGTCAA-3') and Fg16R (5'-GGTAGGTATCCGACATGGCAA-3'), which produced a fragment of 450 bp (Fig. 2A). The PCR reaction was performed in a 25- μl volume, containing 20 ng of template DNA, 1.5 mM MgCl_2 , 50 mM KCl, 10 mM Tris HCl (pH 8.0), 0.2 mM each dNTP, 0.4 μM each primer, and 0.75 units of *Taq* DNA polymerase.

F. graminearum chemotype was identified using the multiplex PCR marker developed by Ward et al. (24). The three primers used in the PCR were 3CON (5'-TGGCAAAGACTGGTTTCAC-3'), 3D15A (5'-ACTGACCCAAGCTGCCATC-3'), and 3D3A (5'-CGCATTGGCTAACACATG-3'). They produced a 610-bp fragment for the 15ADON chemotype and a 243-bp fragment for the 3ADON chemotype (Fig. 2B). The PCR reaction was performed in a 15- μl volume containing 20 ng of template DNA, 2.0 mM MgCl_2 , 50 mM KCl, 10 mM Tris HCl (pH 8.0), 0.2 mM each dNTP, 0.4 μM each primer, and 0.75 units of *Taq* DNA polymerase.

Amplification of polymorphisms. The sequence-related amplified polymorphism (SRAP) technique (12) was used for the analyses of genetic diversity and migration of *F. graminearum*. Out of 36 primer pairs, 4 SRAP primer pairs were screened based on efficiency of the primers producing

polymorphic bands. They were ODD9 (5'-AGTTCCTCAGACGCTACC-3') and ODD15 (5'-GCGAGGATGCTACTGGTT-3'), DC1 (5'-TAAACAATGGCTACT

CAAG-3') and RP1 (5'-CATTGTGGA TGGCATCTGA-3'), ODD30 (5'-GCG ATCACAGAAGGAAGGT-3') and EM1(5'-GACTGCGTACGAAATTCAAT-3'), and

Table 1. Percentage of acetyl ester derivative of DON at 15- and 3-position oxygen (15ADON and 3ADON, respectively) chemotypes of *Fusarium graminearum* for different locations and wheat cultivars in Manitoba from 2004 to 2005

Location, cultivar ^a	Total ^b	15ADON (%) ^c	3ADON (%) ^d
SFD	19	73.7	26.3
MRS	23	4.3	95.7
HND	10	40.0	60.0
CTR	11	72.7	27.3
Subtotal	63	42.9	57.1
PLP	52	51.9	48.1
RVS	28	89.3	10.7
PMS	26	65.4	34.6
RC	13	76.9	23.1
KLN	43	72.1	27.9
SRS	12	83.3	16.7
Subtotal	174	67.0	33.0
MAL	11	90.9	9.1
HMT	16	68.8	31.2
VD	19	89.5	10.5
Subtotal	46	82.6	17.4
DPN	3	100.0	0.0
Subtotal	3	100.0	0.0
KNL	5	100.0	0.0
Subtotal	5	100.0	0.0
Superb	126	72.2	27.8
AC Barrie	165	61.8	38.2
Total/average	291	66.3	33.7

^a Fifteen locations consisting of 39 farmers' fields sown to the two wheat cvs. Superb and AC Barrie in Manitoba from 2004 to 2005. SFD = Sanford, MRS = Morris, HND = Horndean, CTR = Cartier, PLP = Portage la Prairie, HMT = Hamiota, RVS = Rivers, PMS = Plumas, RC = Rapid City, KLN = Killarney, SRS = Souris, MAL = McAuley, VD = Virden, DPN = Dauphin, and KNL = Kenville.

^b Total *F. graminearum*.

^c Percentage of *Fusarium graminearum* isolates with 15ADON chemotype over all *F. graminearum* isolates.

^d Percentage of *Fusarium graminearum* isolates with 3ADON chemotype over all *F. graminearum* isolates.

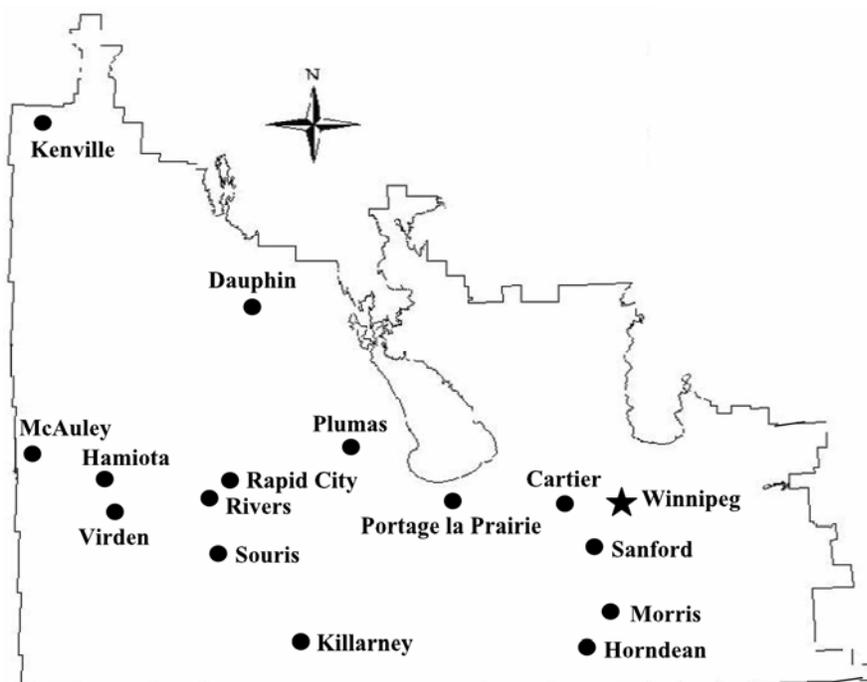


Fig. 1. Map of southern part of Manitoba, Canada. The star symbol represents Winnipeg, the capital city of Manitoba, and solid circles (●) indicate the location of a farmer's home from which wheat heads were collected in this province from 2004 to 2005.

ODD30 and DC1. SRAP PCR reaction was performed in a 15- μ l volume containing 20 ng of template DNA, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 0.1 mM each dNTP, 0.4 μ M each of two primers, and 0.75 units of *Taq* polymerase (Invitrogen Life Technologies, ON, Canada). The PCR products were separated by electrophoresis using 5% polyacrylamide DNA sequence gel with 7.5 M urea for denaturing DNA. Gel fixing, staining, and developing were followed to visualize the DNA bands using the silver-staining kit (Promega Corp., Madison, WI).

Data analysis. Percentage of a chemotype was assessed as the number of the isolates with this chemotype divided by total number of *F. graminearum* isolates from a location, multiplied by 100. An *F. graminearum* population was defined as all isolates collected from one location, wheat cultivar, or chemotype. Polymorphic DNA bands were estimated as two-allele loci with the presence of fragment assigned to 1 and the absence of fragment assigned to 0. Therefore, a binary matrix including the number of *F. graminearum* isolates and DNA polymorphisms was developed. The software POPGENE (version 1.32; Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, Canada) was used for genetic data analysis. Genetic diversity of the populations from different locations, wheat cultivars, and chemotypes (*H*) was estimated using the formula $H = 1 - \sum P_i^2$, where P_i was frequency of allele *i* at the locus (17). Heterozygosity and percent polymorphic loci were assessed for all of the types of populations. Genotypic diversity was estimated using Shannon's

information index (*s*) (21). Fisher's exact test was used for differentiation of different types of populations. Gene flow (genetic migration) was calculated using the formula $N_m = (1 - F_{st}) / (4 \times F_{st})$ (22), where *N* was population size, *m* was migration rate of gene flow, N_m was average number of migrants among the populations, and F_{st} was variance in allele frequencies between the populations (22). The cluster phenograms for different locations and regions were constructed using the unweighted pair-group method with arithmetic average (UPGMA) from Nei's genetic distance with 1,000-replicate bootstrap sampling (17).

RESULTS

Chemotypes of *F. graminearum*. Based on the data collected from the 15 locations from 2004 to 2005, a variation of percent chemotype between the locations was found. Isolates of chemotype 15ADON were predominant in all locations except for Morris and Horndean, in which isolates of chemotype 3ADON accounted for 95.7 and 60.0%, respectively (Table 1). Chemotype 3ADON isolates showed the greatest percentage (57.1%) in the area including Sanford, Morris, and Horndean; and accounted for 33% in the area including Portage la Prairie, Rivers, Plumas, Rapid City, Killarney, and Souris and 17.4% in

Table 2. Genetic diversity of *Fusarium graminearum* populations from different locations in Manitoba

Population	Location ^b	Sequence-related amplified polymorphic data ^a				
		<i>n</i>	<i>g</i> (%)	<i>s</i>	<i>r</i> (%)	<i>H</i>
1	SFD	19	84.2	0.3432	67.96	0.2270
2	MRS	23	82.6	0.4085	46.60	0.2726
3	HND	10	80.0	0.2693	36.89	0.1761
4	CTR	11	81.8	0.2757	40.78	0.1774
5	PLP	52	73.1	0.4038	91.26	0.2584
6	HMT	16	81.3	0.3092	62.14	0.2012
7	RVS	28	75.0	0.2067	39.81	0.1287
8	PMS	26	76.9	0.2980	66.99	0.1910
9	RC	13	92.3	0.2127	39.81	0.1418
10	KLN	43	69.8	0.3432	60.19	0.2131
11	SRS	12	75.0	0.2842	50.49	0.1727
12	MAL	11	81.8	0.1516	35.92	0.0937
13	VD	19	78.9	0.2527	56.31	0.1606
14	DPN	3	66.7	0.1340	11.65	0.0936
15	KNL	5	80.0	0.2991	31.07	0.1965

^a Abbreviations: *n* = population size, *g* = percentage of genotypes in populations, *s* = Shannon's information index, *r* = percentage of polymorphic loci (99% criterion), and *H* = average unbiased proportion heterozygosity.

^b SFD = Sanford, MRS = Morris, HND = Horndean, CTR = Cartier, PLP = Portage la Prairie, HMT = Hamiota, RVS = Rivers, PMS = Plumas, RC = Rapid City, KLN = Killarney, SRS = Souris, MAL = McAuley, VD = Virden, DPN = Dauphin, and KNL = Kenville.

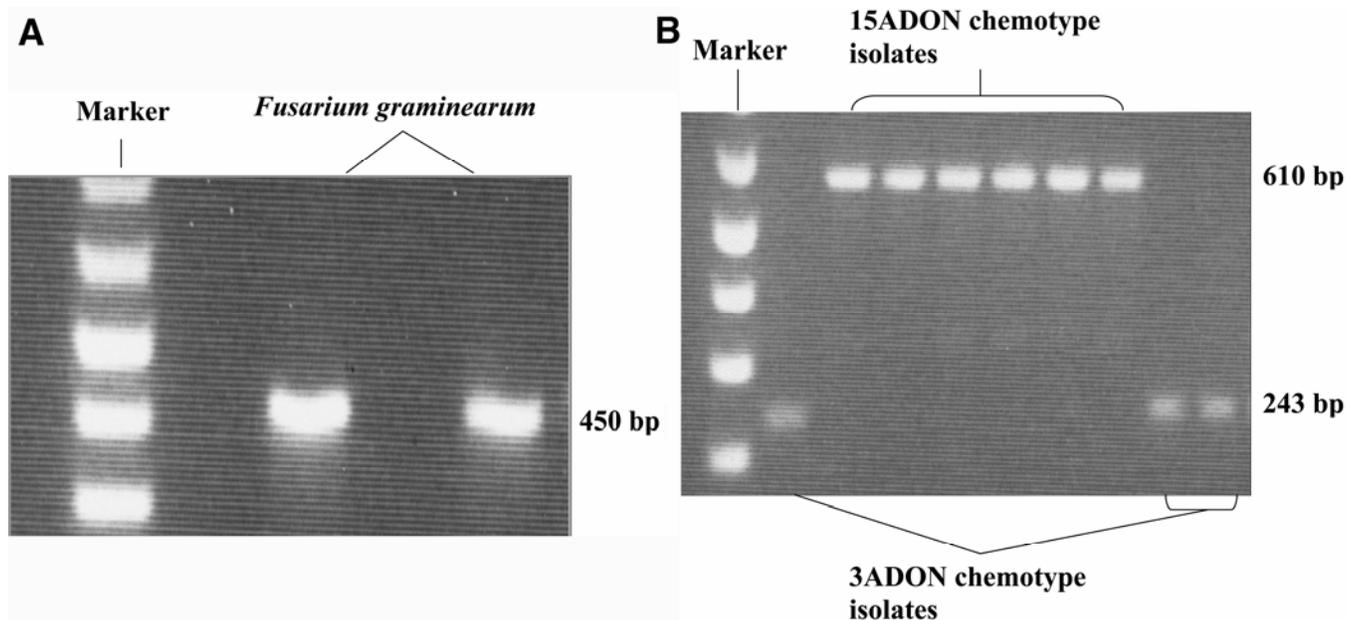


Fig. 2. A, *Fusarium graminearum* DNA amplified using the species-specific polymerase chain reaction (PCR) (4). The 450-bp fragment represented *F. graminearum* species on 2% agarose gel. B, DNA of chemotype isolates amplified using the multiplex PCR (24). The 610-bp fragments represented chemotype acetyl ester derivative of DON at 15-position oxygen (15ADON) and the 243-bp fragments represented chemotype acetyl ester derivative of DON at 3-position oxygen (3ADON) on 2% agarose gel.

the area including McAuley, Hamiota, and Virden. Chemotype 3ADON was not found in Dauphin and Kenville (Table 1). Overall, chemotypes 3ADON and 15ADON accounted for 33.7 and 66.3%, respectively, in Manitoba from 2004 to 2005 (Table 1). Chemotypes 3ADON and 15ADON accounted for 27.8 and 72.2%, respectively, for Superb and accounted for 38.2 and 61.8% for AC Barrie (Table 1).

SRAP variation. Great genetic diversity was found in the 15 populations of *F. graminearum* (Table 2). The percentage of polymorphic loci ranged from 11.65% observed in the population from Dauphin to 91.26% from Portage la Prairie. The number of genotypes in a population showed that the population from Dauphin had the lowest percentage of genotypes (66.7%) and the population from Rapid City had the highest percentage (92.3%), in which almost each isolate was a unique genotype (Table 2). Shannon's information index (*s*) showed that higher levels of genotypic diversity existed in the populations from Morris (0.4085), Portage la Prairie (0.4038), Sanford (0.3432), and Hamiota (0.3092) than in the populations from the other locations (Table 2). The population from Dauphin had the lowest *s* value. Heterozygosity of different populations ranged from 0.0936 (Dauphin) to 0.2726 (Morris), which was consistent

with Shannon's information index (Table 2).

Genetic and genotypic diversity of *F. graminearum* populations were measured from both wheat cvs. Superb and AC Barrie and chemotype 3ADON and 15ADON strains (Table 3). A high percentage of polymorphic loci was observed in all the populations, ranging from 95.15% (Superb) to 97.09% (AC Barrie) for the cultivars and from 96.15% (15ADON) to 97.09% (3ADON) for the chemotypes (Table 3). High levels of heterozygosity were found in the populations from Superb (0.2385) and AC Barrie (0.2533) and in the populations from 15ADON (0.2264) and 3ADON (0.2827) (Table 3). Shannon's information index and ratio of genotype and population size also showed a similar trend (Table 3).

Population structure. Population structure was determined using genetic distance and identity of populations. Nei's genetic identity index showed that populations from Portage la Prairie and Hamiota (0.9554), Rapid City and Virden (0.9571), Cartier and Rivers (0.9803), and Morris and Kenville (0.9100) were closer to each other than to others (Table 4), which was consistent with genetic distance between the same populations. The results of UPGMA cluster analysis using Nei's genetic distance between populations showed that

the distances varied from 0.0228 (Rivers versus Souris) to 1.3388 (Rivers versus Dauphin) (Table 4). Fifteen populations were clustered into three subgroups, including the populations from Sanford, Portage la Prairie, Hamiota, Plumas, Rapid City, Virden, and McAuley; the populations from Horndean, Cartier, Rivers, Killarney and Souris; and the populations from the other locations (Table 4; Fig. 3). The genetic distance between populations within these individual subgroups ranged from 0.0438 to 0.1127, 0.0199 to 0.0495, and 0.0943 to 0.3088, respectively (Table 4).

The analysis of population differentiation revealed genetic variations among different locations. The populations from Sanford, Portage la Prairie, Hamiota, and Plumas were not significantly different from each other, and a greater gene flow was found among them, ranging from 2.4434 between populations from Sanford and Plumas to 18.9608 between Portage la Prairie and Hamiota (Table 5). There were no significant differences between populations from Rapid City and Virden; from Cartier, Rivers, and Killarney; and from Morris, Kenville, and Dauphin (Table 5). An extensive gene flow was observed among these locations. It was discovered that, except for the population from Morris, no populations showed gene flow with the population from Dauphin, and only the populations from Rapid City and Virden showed gene flow with the population from Kenville. The other population pairs were significantly different from each other (Table 5). It was observed that a gene flow existed among some populations that were significantly different from each other (Table 5). For example, there was gene flow ($N_m > 1$) among the locations Sanford, Portage la Prairie, Hamiota, Plumas, Rapid City, and Virden; however, some of the populations were different from each other, such as the populations from Sanford and Virden (Table 5). Gene flow also was found among the popula-

Table 3. Genetic diversity of *Fusarium graminearum* populations from two wheat cultivars and two chemotypes in Manitoba

Population ^b	Sequence-related amplified polymorphic data ^a				
	<i>n</i>	<i>g</i> (%)	<i>s</i>	<i>r</i> (%)	<i>H</i>
Superb	126	70.6	0.3813	95.15	0.2385
AC Barrie	165	71.5	0.3993	97.09	0.2533
15ADON	193	69.4	0.3634	96.12	0.2264
3ADON	98	74.4	0.4379	97.09	0.2827

^a Abbreviations: *n* = population size, *g* = percentage of genotypes in populations, *s* = Shannon's information index, *r* = percentage of polymorphic loci (99% criterion), and *H* = average unbiased proportion heterozygosity.

^b Populations were defined as groups of isolates from different wheat cultivars, Superb and AC Barrie, and different chemotypes, acetyl ester derivative of DON at 15- and 3-position oxygen (15ADON and 3ADON, respectively).

Table 4. Nei's genetic identity (above the diagonal) and genetic distance coefficients (below the diagonal) among different geographic populations of *Fusarium graminearum* based on the sequence-related amplified polymorphism loci in Manitoba

Pop. ^a	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	...	0.6707	0.7315	0.7608	0.9402	0.9377	0.7559	0.9274	0.8983	0.7609	0.7494	0.8934	0.8835	0.5709	0.7079
2	0.3994	...	0.3178	0.3215	0.6901	0.6906	0.3147	0.6657	0.6497	0.3386	0.3166	0.5847	0.6651	0.8633	0.9100
3	0.3127	1.1462	...	0.9309	0.7986	0.7435	0.9602	0.7347	0.7827	0.9727	0.9623	0.7666	0.7837	0.2927	0.2888
4	0.2734	1.1349	0.0716	...	0.7942	0.7642	0.9803	0.7738	0.8014	0.9676	0.9517	0.7968	0.7580	0.2488	0.3103
5	0.0617	0.3709	0.2249	0.2304	...	0.9554	0.7948	0.9503	0.9490	0.8098	0.7906	0.8994	0.9480	0.6182	0.6811
6	0.0643	0.3702	0.2964	0.2690	0.0456	...	0.7629	0.9378	0.9019	0.7687	0.7618	0.8903	0.9183	0.6017	0.6845
7	0.2799	1.1560	0.0406	0.0199	0.2296	0.2706	...	0.7445	0.7987	0.9789	0.9775	0.7943	0.7672	0.2622	0.2986
8	0.0754	0.4069	0.3083	0.2564	0.0509	0.0642	0.2951	...	0.8940	0.7677	0.7362	0.8825	0.8859	0.5491	0.7118
9	0.1072	0.4312	0.2451	0.2214	0.0524	0.1032	0.2248	0.1121	...	0.7949	0.7849	0.8774	0.9571	0.6531	0.6068
10	0.2733	1.0829	0.0276	0.0330	0.2109	0.2630	0.0213	0.2643	0.2295	...	0.9702	0.7846	0.7838	0.2926	0.3216
11	0.2884	1.1503	0.0385	0.0495	0.2350	0.2721	0.0228	0.3063	0.2422	0.0302	...	0.7758	0.7727	0.2713	0.2913
12	0.1127	0.5367	0.2658	0.2272	0.1060	0.1162	0.2303	0.1250	0.1308	0.2426	0.2538	...	0.8776	0.4985	0.5829
13	0.1238	0.4078	0.2438	0.2770	0.0535	0.0853	0.2650	0.1212	0.0438	0.2436	0.2578	0.1306	...	0.6401	0.6107
14	0.5605	0.1470	1.2287	1.3912	0.4809	0.5080	1.3388	0.5995	0.4261	1.2289	1.3046	0.6961	0.4461	...	0.7343
15	0.3454	0.0943	1.2421	1.1704	0.3840	0.3790	1.2086	0.3400	0.4995	1.1344	1.2335	0.5397	0.4931	0.3088	...

^a Populations: 1 = Sanford, 2 = Morris, 3 = Horndean, 4 = Cartier, 5 = Portage la prairie, 6 = Hamiota, 7 = Rivers, 8 = Plumas, 9 = Rapid City, 10 = Killarney, 11 = Souris, 12 = McAuley, 13 = Virden, 14 = Dauphin, and 15 = Kenville.

tions from Cartier, Rivers, Killarney, and Souris (Table 5). This was consistent with the results of genetic distance and identity.

Population structure also was determined for the populations from wheat cultivars and chemotypes (Table 6). Genetic distance and identity for the populations from cultivars were 0.0089 and 0.9911, respectively, and 0.0246 and 0.9757 for the populations of chemotypes (Table 6). This indicates that there are close relationships within the populations from wheat cultivars and chemotypes. The analyses of population pairwise comparison and genetic migration revealed a gene flow between the populations of two wheat cultivars and between those of the two chemotypes, which were not significantly different from each other (Table 7).

DISCUSSION

This study reported the distribution of *F. graminearum* chemotypes 15ADON and 3ADON and the potential chemotype shifting in Manitoba. This was the first study undertaken in Manitoba with a large popu-

lation over a 2-year period. This study exhibited a large variation in percentage of chemotypes among the *F. graminearum* populations from different locations. Although chemotype 15ADON is predominant in Manitoba, chemotype 3ADON has recently emerged and showed an increasing trend in southern Manitoba (25). Our study showed that 3ADON was distributed in the southern part of this province and was predominant in the Morris and Horndean areas. Possible reasons for variation in percentage of chemotypes include introduction of chemotypes into a new area with seed or stubble; however, this study focused on population structure and genetic migration accounting for this variation. Because it is difficult to find identical farmers' fields between different years to trace chemotype changes, our study investigated the genetic analysis for the *F. graminearum* populations from different locations to study potential chemotype shifting.

The variation in percentage of chemotypes in different locations was associated

with the degree of genetic diversity of *F. graminearum* populations from the corresponding locations (Table 2), as well as different chemotypes and cultivars (Table 3). Sexual reproduction of the pathogen is one of the most important factors causing genetic diversity. The pathogen is a homothallic fungus but it can be outcrossed both in culture (2) and in nature (6,14). Dusabenyagasani et al. (6) reported that there was a high frequency of sexual recombination in Ontario and Quebec, Canada. Our study revealed great genetic diversities of *F. graminearum* populations from some locations (Table 2). In Sanford, out of 19 *F. graminearum* isolates, 16 isolates were unique genotypes, the genetic diversity index (*H*) was 0.2270, and polymorphic loci accounted for 67.96%. In Morris, 19 different genotypes were found from 23 isolates; genetic diversity index and percentage of polymorphic loci were 0.2726 and 46.6%, respectively. The isolates from Portage la Prairie exhibited the highest percentage of polymorphic loci. This suggests that the frequencies of sexual recombination of the pathogen were higher in these three locations, in which isolates with the 3ADON chemotype were predominant. Sexual recombination within *F. graminearum* populations likely leads to the large variation of chemotypes among different locations, though progeny segregation of chemotypes has not been reported. In Germany, Cumagun et al. (3) reported a large genetic variation of the pathogen derived from a cross between two DON-producing isolates; significant segregation of DON production and pathogenicity was found in the progenies. However, the parents and progenies were not identified for chemotypes.

The age of a population from a certain location is likely another important factor for genetic diversity. The longer the history of the population, the more chances the pathogen isolates have for sexual recombination or mutation and the greater the genetic diversity in this population. Thus, the populations from locations Sanford, Morris, and Portage la Prairie could be older than other locations.

Tillage systems affect genetic diversity of the population as well. Zero tillage or minimum tillage leaves a greater amount of wheat stubble on the soil surface than conventional tillage, which could be a large reservoir of perithecia because perithecial formation needs light (10). Therefore, zero tillage or minimum tillage likely causes a greater genetic diversity than conventional tillage. With the introduction of zero- and minimum-tillage practices in the prairies in the past 18 years, the chances for the genetic diversity of the pathogen populations to increase would have been greater.

Genetic identity and migration of *F. graminearum* populations within the sub-grouped locations, and the populations

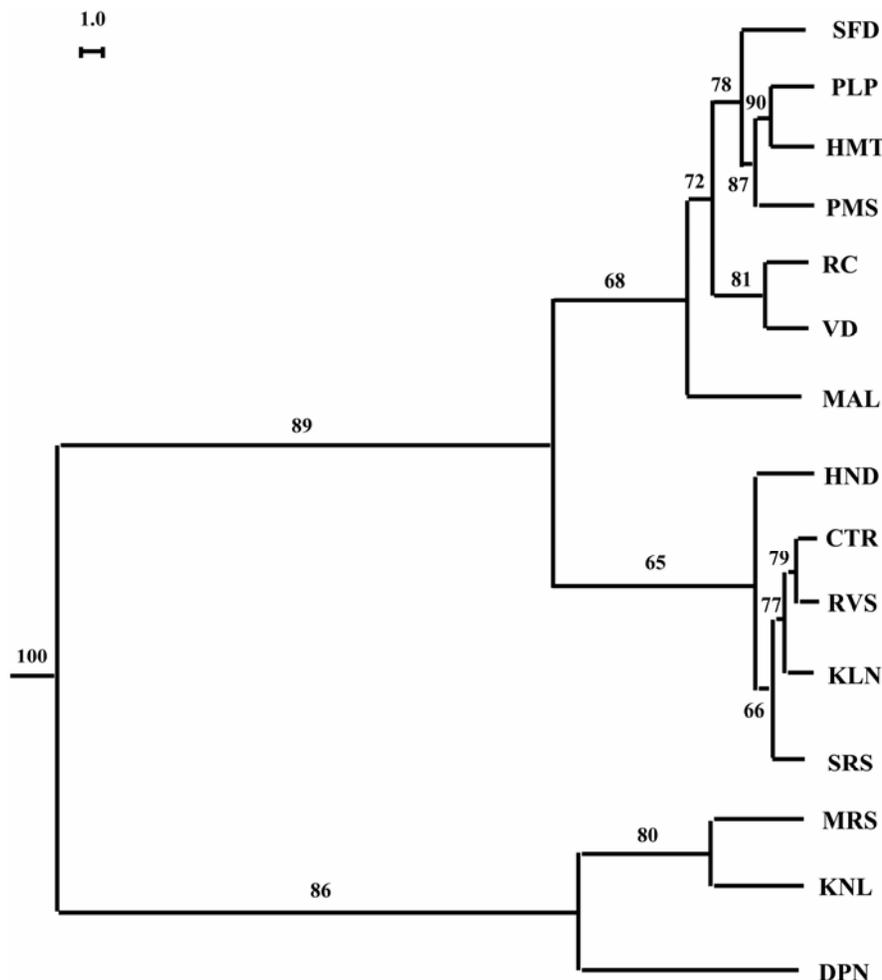


Fig. 3. Cluster phenogram of Nei's genetic distance between the populations of *Fusarium graminearum* from 15 locations in Manitoba. SFD = Sanford, MRS = Morris, HND = Horndean, CTR = Cartier, PLP = Portage la Prairie, HMT = Hamiota, RVS = Rivers, PMS = Plumas, RC = Rapid City, KLN = Killarney, SRS = Souris, MAL = McAuley, VD = Virden, DPN = Dauphin, and KNL = Kenville. The numbers at branches indicated the percentage of occurrence of the cluster in a 1,000-bootstrap sampling.

within the chemotypes and wheat cultivars, revealed potential chemotype shift. However, it was found that some populations that were not geographically close to each other, including Portage la Prairie and Hamiota, Cartier and Rivers, and Morris and Kenville or Dauphin, were similar to each other (Tables 4 and 5; Fig. 3). Wheat seed shipment was likely one reason for the genetic migration between these locations. A study revealed the capability of ascospore survival during long-distance transportation through the planetary boundary layer (13), and a great genetic diversity and gene flow of the pathogen existed spatially and temporally. Therefore, this is another possibility for the genetic migration between populations located far from each other.

Generally, a high level of gene flow results in a low level of genetic diversity among populations. In our study, great variations of chemotypes were found within the subgroups of locations, in which significant gene flows existed. Nevertheless, our study found that the greatest difference in percentage of chemotype 3ADON was 21.8% among locations Sanford, Portage la Prairie, Hamiota, and Plumas; 17.9% between locations Cartier, Rivers, Killarney, and Souris; and 95.7% between locations Morris, Killarney, and Dauphin (Table 1; Fig. 3). This suggests that there were likely greater genetic diversities and variations of chemotypes in the earlier years.

Our study found that the population from Dauphin had no gene flow with the populations from other locations except Morris, and the population from Kenville showed only low gene flow with the populations from Rapid City and Virden. Furthermore, only 15ADON isolates were found in Dauphin and Kenville. Alternatively, no 3ADON shift was detected from other locations. However, there were only three *F. graminearum* isolates collected from Dauphin and three from Kenville due to the lower FHB disease incidence in the two locations than the others (*data not*

shown). With this small number of isolates, chemotype 3ADON may not be detected. Therefore, 3ADON would be expected to account for less than approximately 33% if this chemotype existed in the two locations. This situation likely occurred because the population from the 3ADON-predominant location Morris had a strong gene flow with the populations from Dauphin and Kenville. Although the populations from Dauphin and Kenville had gene flow with the population from Morris, the 95.7% of 3ADON chemotype isolates from Morris might not contaminate the 15ADON-predominant populations in the two locations. The populations from Dauphin and Kenville had a lower percentage of polymorphic loci than others (Table 2), suggesting that the populations from the two locations could have many distinct alleles. However, with the same gene flow

rate, the populations from Dauphin and Kenville could be contaminated by 3ADON in the future.

A greater percentage of genotype was found in the populations from the cultivars (70.6 to 71.5%) and chemotypes (69.4 to 74.4%), and there were no differences between the populations from the cultivars and chemotypes. It suggests that the populations from two cultivars and two chemotypes could be differentiated from each other and had low genotypic diversity in earlier times. With the effects of gene flow between the populations and sexual recombination within the populations, the populations became more similar to each other and the levels of genotypic diversity in the populations increased. It has been reported that there was no relationship between chemotype difference and genotype change (24).

Table 6. Pairwise comparison of Nei's genetic identity (above the diagonal) and genetic distance coefficients (below the diagonal) of *Fusarium graminearum* populations from two wheat cultivars and two chemotypes in Manitoba, based on the sequence-related amplified polymorphism loci

Population ^a	Cultivar		Chemotype	
	Superb	AC Barrie	15ADON	3ADON
Superb	...	0.9911
AC Barrie	0.0089
15ADON	0.9757
3ADON	0.0246	...

^a Populations were defined as groups of isolates from different wheat cultivars, Superb and AC Barrie, and different chemotypes, acetyl ester derivative of DON at 15- and 3-position oxygen (15ADON and 3ADON, respectively).

Table 7. Estimates of gene flow (N_m) between populations of *Fusarium graminearum* (above the diagonal) and probability of population differentiation in Manitoba (below the diagonal) using the exact test

Population ^a	Cultivar		Chemotype	
	Superb	AC Barrie	15ADON	3ADON
Superb	...	11.1760
AC Barrie	0.3243
15ADON	5.6021
3ADON	0.1997	...

^a Populations were defined as groups of isolates from different wheat cultivars, Superb and AC Barrie, and different chemotypes, acetyl ester derivative of DON at 15- and 3-position oxygen (15ADON and 3ADON, respectively).

Table 5. Estimates of gene flow (N_m) between populations of *Fusarium graminearum* (above the diagonal) and probability of each pairwise comparison (below the diagonal) using the exact test in Manitoba

Pop. ^a	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	...	0.1546	1.2388	0.6267	7.3327	4.0053	0.5720	2.4434	2.0137	1.1995	0.9006	0.7276	2.8982	0.9697	0.4689
2	0.0455	...	0.6191	0.3788	1.3570	1.1614	0.3617	0.8986	1.0820	0.6805	0.5081	0.4214	1.2092	1.0619	12.4258
3	<0.0001	<0.0001	...	0.5373	1.1861	0.9461	0.7062	0.7954	1.4651	1.5091	1.4363	0.8012	0.6584	0.6835	0.4834
4	<0.0001	<0.0001	<0.0001	...	1.1024	1.7582	16.8342	1.7163	1.8412	1.7154	1.0560	2.5140	0.3617	0.1624	0.4217
5	0.0727	<0.0001	<0.0001	<0.0001	...	18.9608	1.0678	6.2791	1.7117	2.9218	1.2138	1.0850	2.5565	0.8753	0.9236
6	0.0573	<0.0001	<0.0001	<0.0001	0.5546	...	1.1235	3.5684	2.4536	2.3199	1.0391	1.1510	1.6402	0.4113	0.8725
7	<0.0001	<0.0001	<0.0001	0.2909	<0.0001	<0.0001	...	1.2727	2.1194	3.9229	1.4108	1.8616	0.3859	0.1994	0.3996
8	0.0546	<0.0001	<0.0001	<0.0001	0.0573	0.8273	<0.0001	...	4.1013	0.7107	1.0496	1.2576	1.4072	0.3538	0.9097
9	0.0182	<0.0001	0.0091	0.0182	0.0182	0.0291	0.0182	0.0346	...	1.0032	1.7241	1.5271	11.3205	0.4426	1.3240
10	<0.0001	<0.0001	<0.0001	0.0701	<0.0001	0.0182	0.1123	<0.0001	0.0436	...	1.6272	1.6847	0.7722	0.4406	0.9382
11	<0.0001	<0.0001	0.0091	<0.0001	<0.0001	<0.0001	0.0091	<0.0001	0.0091	<0.0001	...	1.1709	0.5716	0.2498	0.4533
12	<0.0001	<0.0001	<0.0001	0.0364	<0.0001	<0.0001	0.0302	<0.0001	0.0273	<0.0001	0.0182	...	0.4822	0.2220	0.4822
13	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2654	<0.0001	<0.0001	<0.0001	...	0.3350	1.3095
14	0.0091	0.0664	0.0364	0.0182	0.0091	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0091	...	4.4318
15	<0.0001	0.1273	<0.0001	<0.0001	0.0182	<0.0001	<0.0001	0.0455	0.0273	0.0091	<0.0001	<0.0001	0.0364	0.0882	...

^a Populations: 1 = Sanford, 2 = Morris, 3 = Horndean, 4 = Cartier, 5 = Portage la prairie, 6 = Hamiota, 7 = Rivers, 8 = Plumas, 9 = Rapid City, 10 = Killarney, 11 = Souris, 12 = McAuley, 13 = Virden, 14 = Dauphin, and 15 = Kenville.

In all, the variations of percentage of chemotypes could result from genetic diversity of *F. graminearum* populations in Manitoba, which could be associated with sexual recombination, age of populations, and tillage system. The significant gene flows in southern Manitoba likely caused potential chemotype shift; however, the direction of the shift is difficult to measure. Wheat seed shipment and long-distance spore transportation between different locations likely contributed to the gene flow observed in this study.

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