Special Report

Occurrence and Distribution of Physiological Races of *Exserohilum turcicum* in Ontario, CanadaKrishan K. Jindal,^{1,†} Albert U. Tenuta,² Tsegaye Woldemariam,¹ Xiaoyang Zhu,¹ Dave C. Hooker,³ and Lana M. Reid¹¹ Ottawa Research and Development Centre, Agriculture and Agri-Food, Ottawa ON, K1A 0C6, Canada² Ontario Ministry of Agriculture, Food and Rural Affairs, ON, N0P 2C0, Canada³ University of Guelph, Ridgetown, ON, N0P 2C0, Canada

Abstract

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* is the most common and economically significant fungal leaf disease of corn in Ontario, Canada. During the past 10 years in Ontario, severity and incidence of NCLB have increased, possibly owing to the appearance of new races. Several races have been identified in various parts of the world; however, information on occurrence and distribution of races in Ontario is lacking. In the current study, 677 single conidial isolates of *E. turcicum* were isolated from 687 symptomatic leaf samples collected between 2012 and 2016. These isolates were evaluated for pathogenicity on six corn differential inbreds (A619, A619Ht1, A619Ht2, A619Ht3, A632Hm1, and H102Hm1) under controlled environmental conditions and then grouped into 17 physiological races (0, 1, 2, 3, M, N, 12, 1M, 1N, 3M, 13M, 12N, 13N, 1MN, 12MN, 13MN, 123MN) based on the reaction of the inbreds to infection (resistant or susceptible). Four races (0, 1M, 1N, and 1MN) were most frequent, with an isolation frequency of 13, 10, 12, and 41%, respectively. Seventy-six percent of the isolates were virulent on more than one *Ht* resistance gene, with 2.4% (16 isolates) virulent on all five *Ht* resistance genes used in this study. Further analysis of the distribution of races in four regions over the years revealed that the occurrence and distribution of the races changed with time

in Ontario. Overall, the frequency of virulence of the 677 isolates screened on the differentials with resistance genes *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* varied from 6 to 81% (*Ht1* 81%, *Ht2* 6%, *Ht3* 12%, *Htm1* 64%, and *Htn1* 64%). Virulent isolates produced fewer lesions on the *Htm1* differential, and smaller lesions that were slower and having less sporulation on the *Htn1* differential, compared with infection of the differentials with *Ht1*, *Ht2*, and *Ht3* resistance genes. Virulence frequency also changed within the four geographical regions of Ontario, with fewer isolates virulent on all resistance genes in eastern Ontario compared with southern and western Ontario. Isolates from southern Ontario had greater virulence frequency against *Ht1* and *Htm1*, whereas isolates from western Ontario were more frequently virulent on *Ht1* and *Htn1*. The information generated in this study on the distribution of *E. turcicum* races in Ontario corn will help growers to select appropriate hybrids with required resistance genes and will assist seed companies in deploying resistance genes in corn hybrids across the province or within a particular region.

Keywords: corn, northern corn leaf blight, *Exserohilum turcicum*, physiological races, virulence, distribution

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* (Passerini) Leonard and Suggs (syn. *Setosphaeria turcica* [Luttrell] Leonard and Suggs) (*Er*) is the most common and economically significant leaf disease of corn (*Zea mays* L.) worldwide including the province of Ontario, Canada (Dong et al. 2008; Jindal et al. 2018; Muir et al. 2010; Schechert et al. 1999). Disease epidemics were frequent until the discovery and incorporation of the first NCLB-resistance gene, *Ht1*, into corn hybrids by Hooker in 1963. Since then, many other dominant (*Ht2*, *Ht3*, *Htm1*, *Htn1*, *HtP*, and *HtNB*) and recessive (*ht4* and *rt*) NCLB-resistance genes have been discovered, and some of them have been incorporated into corn hybrids (Carson 1995; Galiano-Carneiro and Miedaner 2017; Gevers 1975; Hooker 1977, 1981; Ogliari et al. 2005; Robbins and Warren 1993; Wang et al. 2012; Welz and Geiger 2000). Among all of these

resistance genes, *Ht1* has been the most utilized in commercial corn breeding programs for NCLB control until virulent populations against this gene were reported (Ferguson and Carson 2004; Jordan et al. 1983; Lipps and Hite 1982). *Ht2*, *Ht3*, and *Htm1* have not been commonly deployed in commercial hybrids owing to variable resistance expression under different environmental temperatures and light intensities (Ferguson and Carson 2004; Leath et al. 1990; Leonard et al. 1989; Thakur et al. 1989b), and the dominant inhibitor gene (*Sht1*) that is often associated with these genes hinders their transfer into elite germplasm used in backcrossing breeding programs (Ceballos and Gracen 1989; Simcox and Bennetzen 1993). The other dominant and recessive resistance genes, *Htm1* (Robbins and Warren 1993), *HtP* and *rt* (Ogliari et al. 2005), *HtNB* (Wang et al. 2012), and *ht4* (Carson 1995), also have limited use in corn breeding.

Heavy infections of NCLB before tasseling cause grain yield losses from 28 to 91% (Ferguson and Carson 2007; Kloppers and Tweer 2009; Nwanosike et al. 2013; Raymundo and Hooker 1981; Wang et al. 2010). Significant losses continue to occur in corn production with planting of highly susceptible inbreds (Mueller et al. 2016). The increased prevalence of NCLB in recent years in the United States and elsewhere has been attributed to many factors, such as changes in the pathogen population (i.e., appearance of new virulent races) (Adipala et al. 1993; Muir et al. 2010; Pierce 2016; Thakur et al. 1989a; Wang et al. 2011); cultivation practices; and climatic conditions (Mallowa et al. 2015; Wise and Mueller 2011). The province of Ontario has also experienced a surge in NCLB in the past 10 years. Results of annual disease surveys of corn in the main growing

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regions of Ontario over the past 10 years have indicated that the severity and incidence of NCLB have increased particularly during the 2015, 2016, and 2017 crop seasons, when the disease was observed in 97, 83, and 82% of the fields surveyed (Jindal et al. 2016, 2017, 2018).

Growing resistant hybrids continues to be the most cost-effective and widely used method for NCLB control (Ayiga-Aluba et al. 2015; Ferguson and Carson 2007; Ishfaq et al. 2014; Vieira et al. 2009). The sources of resistance identified to NCLB are qualitative (Hilu and Hooker 1964; Hooker 1961, 1963) or quantitative (Leonard 1993; Pataky et al. 1986). The use of quantitative resistance is always preferred over qualitative because it is more stable and sustainable (Galiano-Carneiro and Miedaner 2017). Continuous surveillance for races of *Et* even on resistant hybrids with quantitative resistance is important for deploying resistance genes for effective disease management in the field.

The initial identification and designation of *Et* races was performed by Bergquist and Masias (1974) when they found isolates that were virulent on corn lines with *Ht1* in Hawaii. They designated these isolates as race 2 to differentiate them from avirulent isolates on corn lines with *Ht1*, which were designated race 1. Subsequently, isolates virulent on corn with *Ht2*, *Ht3*, and *HtN* from various parts of the United States were designated as race 2, race 3, and race 4 (Smith and Kinsey 1980; Thakur et al. 1989a; Turner and Johnson 1980). Later on, Leonard et al. (1989) proposed a new system for designation of physiological races of *Et* based on symptoms on corn inbreds with resistance genes *Ht1*, *Ht2*, *Ht3*, and *Htm1*. Since then, this system has been followed to characterize various isolates of the NCLB pathogen into physiological races. Several studies have used this method to assess local populations of *Et*, for example, races 0, 1, 2, 23, 2N, and 23N in the United States (Fallah Moghaddam and Pataky 1994; Simcox et al. 1993; Windes and Pedersen 1991); 0, 1, 3, N, 12, 13, 23, 1N, 2N, 3N, 12N, 23N, and 123N in China (Dong et al. 2008); 23, 2N, and 23N in Mexico; 0, 23, and 23N in Zambia; 0, 1, 2, 3, N, and 23N in Uganda (Welz et al. 1993); 0, 1N, 12N, and 123N in Brazil (Gianasi et al. 1996); and 0, 1, 2, 3, N, 12, 13, 23, 3N, 123, 13N, and 23N in Kenya, Germany, and Austria (Muiru et al. 2010). In the most recent study, 20 races (0, 1, 2, M, N, 12, 13, 23, 1M, 1N, MN, 123, 1MN, 2MN, 23M, 23N, 12MN, 23MN, 123M, and 123MN) were identified from the north central United States (Weems and Bradley 2018).

Limited information is available on the occurrence, genetic diversity, and geographical distribution of *Et* race populations in Canada. One report from 2010 identified 23 races of the pathogen recovered from 196 leaf samples with typical NCLB lesions collected in 2005 and 2006, although no individual isolations were made from the leaf samples (Zhu et al. 2010). Because the physiological races of the fungi are closely linked to their ability to cause disease or pathogenicity on the host plant, their identification and distribution across geographical areas are important for disease control and the study of host-pathogen interaction. For example, if the *Ht1* gene confers no resistance to any of the NCLB races found in a particular region, the use of hybrids containing the *Ht1* gene will be ineffective and may result in significant yield losses in the region; therefore, in the current study, a thorough and systematic approach was undertaken to identify and determine the occurrence and geographical distribution of physiological races of *Et* in the province of Ontario and to define the reaction of currently available *Ht* resistance genes to the identified races. An understanding of the occurrence and distribution of *Et* races across Ontario would be useful in plant breeding for screening resistance genes and for developing disease risk management strategies in corn production across the province.

Materials and Methods

Collection of pathogen isolates. A total of 687 leaf samples (each sample composed of two to six leaves collected from a field visited) with typical NCLB symptoms (long, elliptical, 2 to 15 cm, tan or greyish-green necrotic lesions) were collected from corn plants during annual surveys of commercial grain and silage crops, Ontario Corn Committee hybrid performance trials, breeders' nurseries,

commercial demonstration plots, and corn seed production fields, across 27 counties of Ontario in 2012 and in 2014 to 2016. A total of 137 symptomatic leaf samples were collected from 23 counties in 2012, and similarly, 178 from 18 counties in 2014, 186 from 20 counties in 2015, and 168 from 19 counties in 2016. An additional 18 symptomatic leaf samples were collected from the Ottawa Research and Development Center (ORDC) research plots in 2013. Isolations of *Et* from leaf samples collected within each growing season were made during the subsequent winter months. All race-determination work was conducted at ORDC, Agriculture and Agri-Food Canada, Ottawa.

In all, 677 isolates were isolated from the 687 leaf samples, which represented 684 individual fields. Isolations were made by cutting a small sample (3 × 4 mm) of the leaf along the advancing margin of the disease lesions and surface sterilizing the sample with 2% sodium hypochlorite solution for 2 min. The leaf tissue samples were washed in sterile distilled water, plated onto potato dextrose agar (PDA) medium (39 g of Difco PDA in 1 liter of water) in 100- × 15-mm polystyrene Petri dishes, and then incubated at a temperature of 23 ± 2°C for 10 to 12 days. A pure culture was obtained by subculturing the leading edge of the fungal colony that grew from the leaf tissue sample onto PDA. To obtain single conidial cultures, the pure culture grew for 10 to 15 days at 25°C until it sporulated. The spores were gently washed from the culture, and a dilute conidial suspension was spread onto water agar (10 g of agar and 1 liter of water) to obtain single-colony isolates. After 12 to 24 h of incubation at 23 ± 2°C, separable germinating conidia were identified and marked using a zoom 20× stereomicroscope (Wild Heerbrugg AG, Switzerland) at a working distance of 25 mm (total magnification 80×). Marked conidia were transferred onto sterilized PDA medium using a sterile inoculation needle and stored on PDA in a refrigerator at 2 to 4°C until further analysis.

Race determination. Six differential lines (A619 [P1587139], A619*Ht1* [Ames 25219], A619*Ht2* [Ames 25220], A619*Ht3* [Ames 25221], H102*Htm1* [PI 550496], and A632*Htm1* [Ames 23469]) obtained from the United States Department of Agriculture, Agricultural Research Service (<https://www.ars-grin.gov/npgs/index.html>) (North Central Regional Plant Introduction Station, Iowa State University, Regional Plant Protection Station, Ames, IA, U.S.A.) were used for the identification of physiological races of *Et*. Plastic planting pots 25 cm in diameter were filled with a mixture of Pro-Mix 'BX' (Premier Horticulture, Riviera-du-Loup, QC, Canada). Three seeds per line of three differentials (total nine seeds) were planted in pot A (A619, A619*Ht1*, and A619*Ht2*) and pot B (A619*Ht3*, H102*Htm1*, and A632*Htm1*). The line A619 served as a positive control to verify successful inoculation and disease development. If all three seedlings emerged in one pot, one plant was removed, and the remaining two plants of each differential line served as two replicates for each *Et* isolate. Two plants of each differential were inoculated with a single *Et* isolate to be characterized. Preliminary studies conducted in an environmentally controlled greenhouse compared NCLB development and plant growth in growth cabinets at two temperatures: 26/22°C day/night and 22/18°C day/night, at light intensities of 35 to 50 Klux. The latter showed the most consistent NCLB development; thus, plant growth cabinets in the present study for screening isolates were maintained 22/18°C day/night with light intensities between 35 to 50 Klux (40 Klux) for 16/8-h day/night similar to that used by Leonard et al. (1989).

Isolates of *Et* to be characterized were multiplied from the first generation of single conidial cultures stored in a refrigerator at 2 to 4°C on PDA. The cultures were grown on PDA at 25°C for 10 to 12 days under 12-h light/dark regimes to induce sporulation, and the conidia were displaced from the surface with a sterile glass slide and removed with sterile water. This conidial suspension was filtered through a double layer of sterile cheese cloth, the conidia were counted with a hemocytometer, and the suspension diluted to 1 × 10⁴ conidia/ml. Two drops of Tween 20 (polyoxyethylenesorbitan monolaurate) were added to the conidial suspension, and 0.2 ml of the conidial suspension was pipetted with micropipettes into the leaf whorl of corn seedlings at V4 to V6 stage. Inoculated plants were

incubated in the plant growth cabinets maintained at 22°C day/18°C night and light intensities between 35 and 50 Klux (16 h daytime and 8 h nighttime), similar to that used by Leonard et al. (1989).

Differentials were examined for NCLB symptoms 14 days after inoculation, and lesions were scored based on their appearance as resistant (narrow chlorotic lesions surrounded by yellowish chlorosis with no sporulation) or susceptible (straw-colored, olive-green, necrotic elliptical lesions without chlorotic border and with sporulation). Lesion size, number of lesions, and sporulation were also recorded to further understand the infection response of the five differentials to inoculation with test isolates. Resistant and susceptible lesions from each differential and replicate were evaluated for length and width of each lesion and sporulation. One 6-mm disk was cut with a paper hole punch from the center of each lesion. Three leaf disks from each differential replicate were placed into 9-cm Petri plates containing 10 ml of cornmeal agar medium without surface sterilization. The leaf disks were then incubated at 24 to 26°C with a 12-h light/dark cycle in a culture room to observe sporulation. After 24 h, 5 ml of distilled water was added to each Petri plate and swirled around the surface to wash out any conidia. The conidia were counted in 1 ml of the resulting conidial suspension on a Spencer Bright-Line improved counting chamber with improved Neubauer 1/10 mm deep slide 13 (U.S. patent no. 2,660,091) under a light microscope. The race of isolates was determined using the race scheme outlined by Leonard et al. (1989) with one additional resistance gene, *Htm1*, as described in Table 1, to further characterize the isolates. Representative cultures of identified races were deposited in the Canadian Collection of Fungal Cultures, ORDC, Ottawa, Canada, for future use.

Frequency and distribution of isolates of each race, isolates' virulence complexity (number of *Ht* resistance genes with which an isolate has a susceptible response), virulence frequency of isolates to *Ht* resistance genes, and three commonly used diversity indices (the simple, Gleason, and Shannon indices) were calculated using the formulas presented by Groth and Roelfs (1987) and compared among regions and years.

Results

A total of 677 isolates (14 from central, 64 from eastern, 292 from southern, and 107 from western Ontario) were obtained from the leaf samples collected from 684 fields visited across Ontario between 2012 and 2016. These isolates produced small, pinhead-sized chlorotic lesions 48 to 72 h after inoculation on corn plants. Clear resistant and susceptible reactions became apparent 10 to 12 days after inoculation. Based on the infection reaction (resistant or susceptible) of the isolates on the five differentials, the 677 isolates were divided into 17 physiological races (0, 1, 2, 3, M, N, 12, 1M, 1N, 3M, 12N, 13M, 13N, 1MN, 12MN, 13MN, and 123MN). Resistance responses were observed on all the differential plants with *Ht* resistance genes on inoculation with an avirulent isolate. Resistance responses on differential lines with *Ht1*, *Ht2*, *Ht3*, and *Htm1* were characterized by prominent chlorosis around elongated necrotic lesions of size 3.3 ± 1.3 cm long and 0.4 ± 0.1 cm wide in the center). The *Htm1* resistance

response was characterized by fewer lesions (1.6 ± 0.6 lesion per leaf on the differential with *Htm1* compared with 4.5 ± 1.5 on the differentials with other resistance genes) with almost no prominent chlorosis.

Three-quarters of the 677 isolates belonged to four predominant races: race 1MN (274 isolates, 40.5%), race 0 (85 isolates, 12.6%), race 1N (79 isolates, 11.7%), and race 1M (70 isolates, 10.3%). Sixteen isolates (2.8%) were virulent on all differentials and were consequently designated as race 123MN (Table 2).

Geographic distribution and frequency of occurrence of races of *Et*. The frequencies of identified races were not consistent across the years. Nine races (1, N, 1M, 3M, 13M, 1MN, 12MN, 13MN, and 123MN) were common among all symptomatic leaf samples collected in all years (2012 to 2016). Among these, race 1MN was the most frequently isolated race in four of five years; race 1N was the most frequent in 2015. Race 2 was recovered only in 2015 and 2016, and race 12 was recovered only in 2012 and 2016. The second most common and widespread race was 1M in 2012 and 2014, race 1MN in 2015, and race 0 in 2016 (Table 2).

Race populations also varied within and among each region from 2012 to 2016 (Table 3). Over five years, all 17 races were recovered from the leaf samples collected from southern Ontario, 16 from eastern Ontario, 12 from western Ontario, and 7 from central Ontario. Seven races (1, N, 1M, 13N, 1MN, 13MN, and 123MN) were identified from all four regions. Five races (2, 3, M, 3M, and 13M) were identified only from leaf samples collected from southern and eastern Ontario. Race 123MN was detected in nine inbred leaf samples from southern Ontario seed corn fields. The second most prevalent race was race 0 in eastern and southern Ontario and race 1N in western Ontario. The third most predominant races were 1M, 1N, and 1 in eastern, southern, and western Ontario, respectively. Frequency of occurrence of races also varied within and across counties from 2012 to 2016. Race 0 was found in 12 counties of eastern, southern, and western Ontario, whereas race 1N was found in 15, race 1M in 18, and race 1MN in 23 out of the 27 counties sampled. Race 3 was identified in three counties, race 3M and race 12 in four counties, race 2 in five counties, and race M in six counties sampled (Table 3). The *Et* population was very complex. Seventy-six percent of screened isolates were virulent on multiple *Ht* resistance genes and 52.2% on three or more genes. One-tenth of the isolates were extremely virulent on four or more *Ht* genes screened (Fig. 1). Isolates with virulence to one *Ht* gene were observed as often as race 0.

The population of *Et* recovered from leaf samples collected from the central region of Ontario had the greatest simple diversity (ratio of races to isolates sampled); the southern region had the highest Gleason (indicating richness of races) and the eastern region the highest Shannon indices (indicating evenness of race distribution within a region) of diversity (Table 4). Among years, the simple diversity index was greatest in 2014, whereas the greatest Gleason (indicating race richness) and Shannon indices (indicating evenness of race distribution with a region) of diversity values were observed in 2015.

Frequency of virulence of isolates on specific *Ht* genes. The frequency of virulence to the specific *Ht* genes tested in this study was compared across years and regions for all 677 isolates. Overall, the frequency of virulence of the isolates screened varied from 6 to 81%. A total of 81% of the isolates were virulent on the differential line with the resistance gene *Ht1*. The frequency of virulence of the isolates on the resistance genes *Htm1* and *Htm1* was 64% for both genes. On average, virulent isolates produced $40 \pm 15\%$ fewer lesions on the *Htm1* differential compared with the differentials with *Ht1*, *Ht2*, and *Ht3* resistance genes (data not given). Moreover, the *Htm1* differential infection by virulent isolates resulted in $25 \pm 9.5\%$ smaller lesions that were slower (4 to 5 days) to develop and had $40 \pm 11\%$ less sporulation compared with infection of the differentials with *Ht1*, *Ht2*, and *Ht3* resistance genes. There were fewer isolates (6 and 12%) recovered that were virulent on differentials with the resistance gene *Ht2* and *Ht3*, respectively. The frequency of virulence on *Ht* resistance gene differentials also varied within and across the years. Fewer isolates were virulent on the *Htm1*

Table 1. Designation of physiological races of *Exserohilum turcicum* on the basis of their infection reaction on five differentials with different *Ht* resistance genes

Race designation	Response ^a					
	A619	A619Ht1	A619Ht2	A619Ht3	Ht102Htm1	A632Htm1
0	S	R	R	R	R	R
1	S	S	R	R	R	R
2	S	R	S	R	R	R
12	S	S	S	R	R	R
23	S	R	S	S	R	R
23N	S	R	S	S	R	S
123MN	S	S	S	S	S	S

^a R = resistant (narrow chlorotic lesions surrounded by chlorosis, chlorotic fleck-like lesions with little or no necrosis and sporulation); and S = susceptible (straw-colored, olive-green, necrotic elliptical lesions without chlorotic border and with a lot of sporulation).

differential in 2015 compared with 2012 and 2014, and fewer isolates were virulent on the *Htn1* differential in 2012 compared with 2014 and 2015. Temporal variation was also observed among the number of isolates virulent in all the years, with fewer isolates virulent on the

Ht1, *Htm1*, and *Htn1* differentials in 2016 compared with 2014 (Table 5).

The frequency of the virulence of isolates on the differentials with *Ht* genes also varied within and across four regions of Ontario. The

Table 2. Frequency of *Exserohilum turcicum* isolates of each race in Ontario corn within and across years

Race	Number of isolates					Per race (%)	DAOMC number ^a
	2012–13	2014	2015	2016	All years		
0	0	16	26	43	85	12.6	250602, 250761
1	18	7	12	6	43	6.4	250603, 250762
2	0	0	2	7	9	1.3	251774
3	0	1	4	0	5	0.7	250605, 250783, 250784
M	0	12	1	2	15	2.2	251773
N	1	1	1	4	7	1.0	250787, 250788
12	3	0	0	1	4	0.6	251775
1M	40	20	8	2	70	10.3	250777
1N	0	8	55	16	79	11.7	250604, 250778, 250779
3M	1	1	2	1	5	0.7	250785, 250786
12N	0	1	1	0	2	0.3	250765, 250766
13M	4	1	1	1	7	1.0	250769, 250770, 250771
13N	0	1	3	4	8	1.2	250772, 250773
1MN	70	90	51	63	274	40.5	250780, 250781, 250782
12MN	3	5	2	1	11	1.6	250767, 250768
13MN	7	6	12	12	37	5.5	250674, 250675, 250676
123MN	4	5	3	4	16	2.4	250763, 250764
All races	151	175	184	167	677		

^a Representative samples were submitted to the Department of Agriculture, Ottawa, Mycology Collection (DAOMC) repository.

Table 3. Frequency and distribution of *Exserohilum turcicum* isolates of each race within and across regions of Ontario corn

		Number of isolates																	All races
Region, county	Number of races	0	1	2	3	M	N	12	1M	1N	3M	12N	13M	13N	1MN	12MN	13MN	123MN	
Central Ontario																			
Durham	7	...	1	...	...	...	1	...	2	...	...	...	...	1	3	...	1	1	10
Northumberland	1	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1
Peterborough	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	1
York	1	...	...	...	...	...	...	...	...	...	...	...	...	...	-	...	1	...	1
Victoria	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	1
Total for region	7	...	1	...	...	...	1	...	3	...	...	...	...	1	5	...	2	1	14
Eastern Ontario																			
Frontenac	4	1	...	...	...	...	...	...	2	...	...	...	...	...	1	...	1	...	5
Hastings	3	...	...	1	...	...	...	...	1	...	1	...	...	...	...	...	...	...	3
Lanark	3	...	...	...	...	...	...	1	...	5	...	...	...	...	2	...	...	...	8
Leeds and Grenville	7	2	1	...	...	...	1	...	6	...	...	...	...	3	11	1	...	...	25
Ottawa	13	37	4	5	2	3	...	...	11	13	2	...	2	...	39	1	3	4	126
Prescott and Russell	7	2	1	1	...	...	...	...	4	6	...	...	...	...	5	...	1	...	20
Renfrew	4	...	6	...	...	...	...	...	2	1	...	...	...	...	12	...	...	...	21
SDG	11	7	4	1	...	1	1	...	13	6	...	...	2	...	17	1	3	...	56
Total for region	16	49	16	8	2	4	2	1	39	31	3	...	4	3	87	3	8	4	264
Southern Ontario																			
Chatham-Kent	14	19	9	...	2	6	3	1	8	14	1	...	2	...	56	1	11	5	138
Elgin	10	7	3	...	...	2	...	...	3	1	1	...	1	...	22	...	4	1	45
Essex	4	...	...	...	1	...	...	...	...	4	...	...	...	...	11	...	...	2	18
Lambton	4	...	1	...	...	...	...	...	3	...	...	...	...	...	5	1	...	...	10
Middlesex	10	4	...	...	...	2	...	1	6	6	...	...	...	1	17	2	2	1	42
Norfolk	1	...	...	...	...	...	...	...	...	...	...	...	...	...	4	...	...	...	4
Oxford	9	1	3	1	...	1	...	...	2	3	...	1	...	...	18	1	3	...	34
Simcoe	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	1
Total for region	17	31	16	1	3	11	3	2	22	28	2	1	3	1	134	5	20	9	292
Western Ontario																			
Dufferin	5	1	1	...	...	...	...	...	1	...	...	...	...	...	7	...	1	...	11
Gray	2	...	...	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	2
Huron	8	1	5	...	...	...	...	...	1	2	...	...	...	1	16	1	3	...	30
Perth	7	3	...	...	...	...	1	...	...	11	...	...	...	...	13	1	2	2	33
Waterloo	4	...	2	...	...	...	...	...	3	3	...	...	...	...	7	...	...	...	15
Wellington	8	...	2	...	...	...	...	1	1	3	...	1	...	1	5	1	1	...	16
Total for region	12	5	10	...	...	...	1	1	6	20	...	1	...	3	48	3	7	2	107

frequency of virulence for the isolates from the eastern Ontario region was lowest on the *Ht1*, *Ht2*, *Ht3*, and *Htm1* differentials compared with the isolates from the central, southern, and western Ontario regions (Table 6). The frequency of virulence of the isolates on *Ht1* differential was >70% in all the regions, with a maximum frequency of 94% for the western Ontario isolates. In contrast to *Ht1*, the frequency of virulence of the isolates on *Ht2* differential was approximately 6% in all the regions, with a maximum frequency of 7% again for the western Ontario isolates. The frequency of virulence of the isolates on *Ht3* differential ranged from 9% to a maximum of 29% in the central Ontario isolates. The frequency of virulence of the isolates on *Htm1* and *Htm1* differentials was >50% in all the regions, with a maximum frequency of 71% on *Htm1* differential for the southern Ontario isolates and 79% on *Htm1* for the western Ontario isolates (Table 6). Of the isolates tested in this study, 13% were avirulent to all *Ht* genes, 81% were virulent to *Ht1*, 6% were to *Ht2*, 12% were to *Ht3*, 64% were to *Htm1*, and 64% were to *Htm1* differentials.

Discussion

In this study, we identified 17 races from the leaf samples collected from Ontario, with races 0, 1M, 1N, and 1MN being the most prevalent. Zhu et al. (2010) identified 25 races from leaf samples collected from Ontario and Quebec in 2006 and 2007 using the same set of five differentials with, races 0, 1, 3, 23, and 23M being the most prevalent. In this study, five races (N, 12N, 13N, 12MN, and 123MN) were identified for the first time in Canada, and 10 races (13, 23, 2M, 3N, 12M, 23M, 23N, 3NM, 23NM, and 123M) that were previously identified from Ontario and Quebec leaf samples (Zhu et al. 2010) were not detected. Detection of the race 123MN in this study, which was virulent on all five resistance genes, suggests that current

Fig. 1. The complexity of *Exserohilum turcicum* isolates collected from Ontario corn between 2012 and 2016, denoted by the number of differential lines with which a specific isolate produced a susceptible response.

commercial hybrids may eventually become susceptible to changing NCLB populations in Ontario. The predominance of races 0, 1M, 1N, and 1MN, compared with races 2, 3, N, 3M, 12N, 13M, 13N, and 12MN in the current study is similar to earlier studies, which reported that races 0, 1, and 1N were predominant in North America and other races such as 2N and 23N were less prevalent (Bergquist and Masias 1974; Fallah Moghaddam and Pataky 1994; Jordan et al. 1983; Lipps and Hite 1982; Pieczarka 1980; Smith and Kinsey 1980; Thakur et al. 1989a; Windes and Pedersen 1991; Weems and Bradley 2018). Races 1M and 1MN in this study are equivalent to races 1 and 1N found in the earlier studies with four differentials for race differentiation.

The change in race structure over time has also been previously reported in other locations such as Africa, China, Europe, and the United States (Dong et al. 2008; Ferguson and Carson 2007; Ji et al. 2013; Jordan et al. 1983; Krausz et al. 1993; Muir et al. 2010; Pierce 2016; Weems and Bradley 2018). Change in the race structure of the *Et* population has been attributed to changes in cultivation practices, hybrids, and/or climate over time (Mallowa et al. 2015; Wise and Mueller 2011). The change in race structure can also be attributed to the source of leaf samples used for isolation in this study Zhu et al. (2010).

Multiple races being found within and between fields, counties, or regions over the 5 years demonstrates that a high diversity of races are present in the province. Some races found in one county or region were not found in other counties or regions within a year. Multiple races were also found within corn fields. This was observed at the ORDC research farm breeding nursery. Five races were identified from 18 NCLB-symptomatic leaf samples collected from the ORDC research farm in 2013. The population of *Et* in a breeding nursery is likely to be more diverse compared with the population of the pathogen in most commercial fields owing to the large diversity of host genotypes and a predominance of inbreds versus hybrids. Some variation in race diversity among regions and years observed in this study may be the result of isolates collected from different fields within a county or region and the hybrids in each year. These variations in source of isolates (leaf sampling) made it difficult to draw a clear comparison between population diversity across regions and years. However, the high race diversity in this collection does suggest a genetically diverse population within regions and years. This diversity may be attributed to (i) sexual recombination and mutation of existing races (Bunkoed et al. 2014; Ferguson and Carson 2004), (ii) selection pressure linked to physiological races owing to cultivation of different hybrids in different areas or a few hybrids grown in a large area (Galiano-Carneiro and Miedaner 2017), and (iii) a long-distance dispersal of spores between counties (Ferguson and Carson 2004). Others have hypothesized similar reasons to explain the change in race diversity, both temporally and spatially, for other corn growing regions of the world (Carson 2006; Weems and Bradley 2018; Welz and Geiger 2000). The increase in virulence frequency with race complexity (virulence to multiple *Ht* genes) observed in this study suggests possible fitness penalties associated with virulence genes. The diversity indices presented in the current study helped to understand the race population distribution across

Table 4. Diversity indices of *Exserohilum turcicum* populations across regions and years

Region/year	Number of counties	Number of isolates	Number of races	Indices of diversity ^a		
				Simple	Gleason	Shannon
Central region	5	14	7	0.500	2.27	1.72
Eastern region	8	264	16	0.061	2.69	2.03
Southern region	8	292	17	0.058	2.82	1.93
Western region	6	107	12	0.112	2.35	1.78
2012–13	23	151	10	0.066	1.79	1.51
2014	18	175	15	0.086	2.71	1.76
2015	20	184	16	0.087	2.88	1.96
2016	19	167	15	0.090	2.74	1.88
Total	27	677	17	0.025	2.45	2.01

^a Diversity indices calculated using the formulas given in Groth and Roelfs (1987).

regions and years; however, these are affected by the number and distribution of fields sampled and by the isolates obtained from each region and year; thus, the distribution should be considered carefully. In the current study, sampling more fields and testing more isolates from within a region or within a year resulted in observation of more races. For example, the southern region had the largest sample size and maximum number of races.

Races 1N and 1MN (1N in earlier studies) were the most frequently observed and widely distributed races across Ontario, which is similar to observations reported by Ferguson and Carson (2007), who recovered a single race (1N) from most NCLB-symptomatic leaf samples collected from the eastern states of the United States. Race 1 (1 and 1M in our studies) was the second most frequently isolated race in Ontario, and this concurs with previous reports from other corn growing countries (Ferguson and Carson 2007; Galiano-Carneiro and Miedaner 2017; Leonard 1993; Raymundo and Hooker 1981; Turner and Johnson 1980; Welz and Geiger 2000). The widespread distribution of races 1 and 1N in the current study is most likely the result of selection pressure owing to a greater area being planted to corn hybrids with *Ht1* and *Htm1* resistance genes in Ontario, similar to observations reported from the United States by Ferguson and Carson (2007). Population of race 0 (0 and M in our studies) declined from 22% in 2006–07 (Zhu et al. 2010) to 12% in this study (2012 to 2016), possibly because of the more frequent use of *Ht1* and *Htm1* genes in the commercial hybrids (Galiano-Carneiro and Miedaner 2017). Because nine races (2, 3, 12, 3M, 12N, 13M, 13N, 12MN, and 123MN) were less frequently recovered, this suggests the resistance genes *Ht2* and *Ht3* may not have been widely deployed in the commercial hybrids in Ontario, resulting in less selection pressure, less mutation, and less sexual recombination. Similarly, Henekamp et al. (2014) reported a low frequency of races 2, 12, 3, 23, 3N, 123, and 23N in Central Europe.

Fewer isolates (6 to 12%) from the *Et* population tested were virulent on *Ht2* or *Ht3* compared with *Ht1*, *Htm1*, and *Htn1* (64 to 81%) across all four regions (Table 6). Low frequency of races with virulence on *Ht2* and *Ht3* has also been reported from the United States and elsewhere (Dong et al. 2008; Fallah Moghaddam and Pataky 1994; Ferguson and Carson 2004; Ji et al. 2013; Jordan et al. 1983; Lipps and Hite 1982; Weems and Bradley 2018). It is difficult to explain the low frequency of isolates virulent on these *Ht* genes despite their presence across years and regions sampled. One possible explanation is that *Ht2* and *Ht3* have not been widely deployed in

commercial hybrids because the gene (*Sht1*) that is often associated with *Ht2* is known to suppress the transfer to elite germplasm through backcrossing programs (Ceballos and Gracen 1989) and because of variation in resistance expression under different environmental temperatures and light intensities (Ferguson and Carson 2004; Leath et al. 1990; Thakur et al. 1989b).

Similar symptoms on the corn plants with *Ht2* or *Ht3* resistance genes in this study confirm the earlier finding of Welz (1998), who reported that it can be difficult to distinguish the phenotype of the plants with genes *Ht2* or *Ht3* because the virulence to these genes appears to be inherited by the same single gene. The presence of virulence to *Ht2* without virulence to *Ht3* or vice versa reported in this study has also been reported in earlier studies (Dong et al. 2008; Fallah Moghaddam and Pataky 1994; Muir et al. 2010; Ogliari et al. 2005). *Ht2* and *Ht3* resistance genes have been mapped and found on different parts of the chromosome, *Ht2* mapped on the long arm of chromosome 8 (bin 8.06) (Zaitlin et al. 1992) and *Ht3* on bin 7.04 (Zhang et al. 2014), which shows that virulence to *Ht2* and *Ht3* may not be inherited by the same single gene.

Despite all the available information, it is hard to explain the exact cause of genetic variation in *Et* populations over time and space and their virulence without knowing the degree of deployment of *Ht* resistance genes in commercial hybrids. Unfortunately, the information on the deployment of resistance genes is not available for most of the commercial hybrids developed and marketed by private companies; however, there are a few general reports available on the use of *Ht1* and *Htm1* for NCLB management in North America, which has presumably led to the appearance of virulent races against these genes (Galiano-Carneiro and Miedaner 2017; Welz and Geiger 2000).

The increasing incidence of NCLB across the Ontario Corn Belt in last 10 years may be owing to changes in pathogen population, agronomic practices, climate, and reduced host resistance in the currently cultivated hybrids, as had been reported earlier for other corn growing regions of the world (Galiano-Carneiro and Miedaner 2017; Munkvold et al. 2008; Wise and Mueller 2011). This study has shown that the race structure of *Et* populations varies considerably (more frequent and widespread *Ht1*, *Htm1*, and *Htn1* virulent isolates compared with *Ht2* and *Ht3*), and consequently both quantitative resistance and qualitative resistance should be used for the effective management of NCLB in Ontario. Qualitative resistance is preferred because it is easier and faster to deploy in a breeding program by backcrossing. Quantitative resistances need to be accumulated by population improvements followed by inbred selections, which takes much more time, land, and economic resources. However, qualitative resistance is often not effective over a long period in environments with high disease severity, because new virulent races develop quickly (Welz and Geiger 2000). Different methods such as the use of three-way or double-cross hybrids with inbreds having different resistance genes (McDonald and Linde (2002), pyramiding several *Ht* genes in the same cultivar (Galiano-Carneiro and Miedaner 2017), recurrent selection (Ayiga-Aluba et al. 2015; Carson 2006; Ceballos et al. 1991; Ribeiro et al. 2016), and regular analyses of race distribution/abundance using available molecular markers (Mideros et al. 2018) to select genes that are still effective in the target area have been suggested to improve and prolong the durability of qualitative resistance. Currently available *Ht* resistance genes *Ht2*, *Ht3*, *HtP*, *HtNB*, *ht4*, and *rt*, which have not been widely used, may be

Table 5. Frequency of *Exserohilum turcicum* isolates virulent on each *Ht* resistance gene within and across years^a

Year	Number of isolates	Number of virulent isolates on each <i>Ht</i> gene				
		<i>Ht1</i>	<i>Ht2</i>	<i>Ht3</i>	<i>Htm1</i>	<i>Htn1</i>
2012–13	151	149 (99)	10 (7)	16 (11)	129 (85)	85 (56)
2014	175	144 (82)	11 (6)	15 (9)	140 (80)	117 (67)
2015	184	148 (80)	8 (4)	25 (14)	80 (43)	128 (70)
2016	167	110 (66)	13 (8)	22 (13)	86 (51)	104 (62)
All years	677	551 (81)	42 (6)	78 (12)	435 (64)	434 (64)

^a The number in parentheses is the percentage of total isolates virulent on each *Ht* resistance gene.

Table 6. Frequency of *Exserohilum turcicum* isolates virulent on each *Ht* resistance gene within and across regions^a

Region	Number of isolates	Number of virulent isolates on each <i>Ht</i> resistance gene				
		<i>Ht1</i>	<i>Ht2</i>	<i>Ht3</i>	<i>Htm1</i>	<i>Htn1</i>
Central Ontario	14	13 (93)	1 (7)	4 (29)	11 (79)	10 (71)
Eastern Ontario	264	196 (74)	16 (6)	24 (9)	152 (58)	138 (52)
Southern Ontario	292	241 (83)	18 (6)	38 (13)	206 (71)	201 (69)
Western Ontario	107	101 (94)	7 (7)	12 (11)	66 (62)	85 (79)
All regions	677	551 (81)	42 (6)	78 (12)	435 (64)	434 (64)

^a The number in parentheses is the percentage of total isolates virulent on each *Ht* resistance gene.

introgressed by multiple backcrosses into new elite germplasm for use in breeding programs to develop new hybrids for NCLB management (Galiano-Carneiro and Miedaner 2017; Welz and Geiger 2000). It is difficult to predict whether the use of currently available *Ht* resistance genes could be helpful to improve management of NCLB for the long term; however, there is potential that this may advance disease management. There is a need to follow a comprehensive approach including the search for new resistance genes, new methods to introgress them into breeding material, and identifying molecular markers to study the race distribution to deploy the genes that are effective in the target area.

It is important to understand the occurrence and distribution of *Et* races across Ontario in plant breeding for screening resistance genes and also for developing disease risk management strategies in corn production. Based on the information generated in the current study, growers, potentially, may be able to select hybrids with multiple *Ht* resistance genes to reduce the risk of disease, if it was known what resistance genes were contained in the hybrids. In the current study, races 1, 1N, 1M, and 1MN were more frequent compared with other identified races. This means that hybrids with *Ht1*, *Htm1*, and *Hm1* genes may have a greater risk of NCLB across the province; therefore, these three genes should be used less frequently in NCLB resistance breeding. More work to introgress other *Et* resistance genes in the breeding program will increase the diversity and improve and prolong the durability of qualitative resistance.

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