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Population Biology

e-Xtra*

The Changing Virulence of Stripe Rust in Canada from 1984 to 2017

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ABSTRACT

Stripe rust, caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici*, is an important wheat disease worldwide. In this study, the *P. striiformis* f. sp. *tritici* population in Canada, representing a time period from 1984 to 2017, was analyzed for virulence diversity and geographical distribution. Virulence of 140 *P. striiformis* f. sp. *tritici* isolates was evaluated on 17 near-isogenic wheat lines in the 'Avocet S' background, each containing a single resistance gene along with an 18th line 'Tyee'. Seedlings were inoculated with a urediniospore/talc mixture and infection types were evaluated on a scale of 0 to 9. In total, 89 races were identified with various combinations of defeated *Yr* genes. Clear changes in pathogen virulence have been observed through time that are confirmed by clustering algorithms. The results showed that the tested *P. striiformis* f. sp. *tritici* isolates remained avirulent on *Yr1*, *Yr5*, and *Yr15*, and have very low frequency of virulence on *Yr76*, but had high frequencies of virulence on *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*. *P. striiformis* f. sp. *tritici* virulence spiked on *Yr7*, *Yr8*, and *Yr9* for the first time in 2000, and on *Yr10* and *Yr27* in 2010. Overall, the predominant races in Canada were very similar to those reported in the United States (PSTv-37, PSTv-41, and PSTv-52), which indicates long-distance migration of *P. striiformis* f. sp. *tritici* from the United States to Canada. Sixty-four races had unique virulence combinations that had not been previously reported in the United States, which suggested that evolution of virulence/avirulence for host resistance by mutation at local scale, is possible. Analysis of diversity between Canadian isolates and races from the United States since 2010 showed that the *P. striiformis* f. sp. *tritici* population in western Canada is similar to that in the western states of the United States, and that the population in eastern Canada is similar to the eastern and/or central regions of the United States, supporting the hypothesis that specific *P. striiformis* f. sp. *tritici* populations in North America travel through different wind trajectories.

Keywords: Canada, *Puccinia striiformis* f. sp. *tritici*, stripe rust, USA, virulence, wheat

Stripe (yellow) rust of wheat is caused by the basidiomycete fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Erikss (in this article, simply called as "*P. striiformis* f. sp. *tritici*"). *P. striiformis* f. sp. *tritici* is an obligate parasite that occurs worldwide in Europe, Australia, New Zealand, the Americas, China, India, and Africa. Generally, *P. striiformis* f. sp. *tritici* is known as a threat in regions with high elevations, where cool and moist conditions during the wheat-growing season favor infection (Line 2002). However, since 2000, the pathogen has been expanding beyond its conventional geographical boundaries into warmer regions, and today the majority of the world's wheat cultivars are susceptible to *P. striiformis* f. sp. *tritici*, with estimated annual losses of USD$ 1 billion (Beddow et al. 2015).

Stripe rust in Canada was first reported in the early 1900s (reviewed in Aboukhaddour et al. 2020), but it was not recognized back then to have any economic impact until the outbreaks in 1981 and 1986 in southern Alberta (AB) (Conner and Kuzyk 1988). These outbreaks caused high yield loss in the soft white spring wheat class, which has late maturity and was produced under irrigated conditions that favored infection. Outbreaks of stripe rust in central AB since the late 1990s have been reported, but were described as "devastating" in 2005, 2006, and 2011 (McCallum et al. 2007b; Puchalski and Gaudet 2011). Since 2000, outbreaks of stripe rust in Canada, particularly in the Pacific Northwest (PNW) regions of AB extending to western Saskatchewan (SK), and from there, to Manitoba (MB), have been reported. These outbreaks all coincided with severe infection in the United States (McCallum et al. 2007a). It is known that stripe rust can overwinter in southern and central AB as dormant mycelia in winter wheat (Aboukhaddour et al. 2018; Xi et al. 2015). The pathogen has been suggested to overwinter in AB since 1927, given enough and continuous snow coverage functioning as a "blanket" to protect the dormant fungus in winter wheat (Sanford and Broadfoot 1929, 1932). Yet, the primary source of inoculum in Canada is believed to be traveling from the more southern regions of North America, from the United States, mainly through PNW wind trajectories. Since 2000, stripe rust was reported in eastern prairie regions in MB and SK, and was likely carried by the wind on the *Puccinia* pathway through the United States- and Canada-shared regions of the Great Plains (reviewed in Aboukhaddour et al. 2020).

Unlike stem and leaf rust, a single urediniospore of stripe rust can produce numerous uredinia, each of which contains thousands of urediniospores, within 12 to 14 days after initial infection. Without mutation, all generated urediniospores would have genotypes identical to the original urediniospore (clonal reproduction). However, single-step mutation from avirulence to virulence or vice versa is the main mechanism to generate different races in *P. striiformis* f. sp. *tritici*. Mutation frequencies have been estimated to range from 1.4×10^{-6} to 4.1×10^{-6} per locus per generation in individual clonal lineages in the northwest European *P. striiformis* f. sp. *tritici* population, with an assumption of an average of 15 generations per year (Hovmøller and Justesen 2007). The majority of *P. striiformis* f. sp. *tritici* populations are clonal, and beside the single-step mutations, the pathogen has evolved its virulence by somatic recombination and parasexuality (Wan et al. 2017). Sexual recombination in *P. striiformis* f. sp. *tritici* has been described in the literature (Jin et al. 2010; Wang and Chen 2013; Zhao et al. 2011, 2013). Earlier studies suggested Transcaucasia as the pathogen center of origin (Hassebrauk

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1965 as reviewed in Chen et al. 2014), where it evolved on grasses, and from there the pathogen spread around the world (reviewed in Chen et al. 2014). Also, Asian regions near the Himalayas are considered as the pathogen's center of origin; this is based on the ability of rust isolates in these regions to produce a high number of telia and this characteristic, combined with the pathogen's known high genetic diversity, indicates a sexually recombining population (Ali et al. 2010; Mboup et al. 2009; Vallavielle-Pope et al. 2012). In North America, the pathogen is believed to reproduce clonally without the sexual cycle, but race incursions from regions outside North America have occurred (Chen et al. 2014).

For the last 85 years, *P. striiformis* f. sp. *tritici* races around the world have been analyzed by determining their infection types (ITs; by host reaction) on a set of host genotypes or single gene lines referred to as a "differential set." Many differential sets have been developed and used around the world (Wan et al. 2017), which can make virulence comparisons between different regions difficult. Additionally, the number of *P. striiformis* f. sp. *tritici* races detected, their virulence spectra, and their aggressiveness has been increasing on a global scale (Wan et al. 2017). *P. striiformis* f. sp. *tritici* exhibits a high degree of variability, and its virulence evolves rapidly, as illustrated by the number of new races that have been reported. For example, in the United States, the number of newly detected races every year since 2000 is almost fivefold higher than before 2000 (Wan and Chen 2014). This trend is not solely because *P. striiformis* f. sp. *tritici* is being investigated more; even in regions with long histories of race identification, more pathotypes and genotypes are being identified (Wan et al. 2017). Selection pressure on *P. striiformis* f. sp. *tritici* populations has become higher with the utilization of more *Yr* genes in breeding programs (Wan et al. 2017), and it appears to take only a few years for the pathogen to evolve virulence by defeating a newly employed *Yr* gene.

In North America, different races were reported in the early 1900s (Humphrey et al. 1924; Hungerford and Owens 1923), with two races being identified in Canada and the United States that were similar to each other (Bever 1934; Newton and Johnson 1936; Newton et al. 1933). Newton and Johnson (1936) differentiated between the North American and European *P. striiformis* f. sp. *tritici* races using 'Chinese 166' as the wheat differential carrying the *Yr1* gene. North American isolates were virulent on *Yr1* and the European races remained avirulent. In the early years, stripe rust on wheat was not considered as devastating a disease as stem or leaf rust (Aboukhaddour et al. 2020), but epidemics in the central plains in Texas and Kansas in 1957 and 1958, and then in the PNW in the early 1960s, necessitated the routine race characterization of *P. striiformis* f. sp. *tritici* in the United States that continues until today. In Canada, there were a few (if limited) numbers of reports on *P. striiformis* f. sp. *tritici* race characterization in the 1930s (Newton and Johnson 1936; Newton et al. 1933). A gap in new reports ensued until Su et al. (2003), followed by reports from western Canada (Brar and Kutcher 2016; Holtz et al. 2013; Kumar et al. 2012). The limited research on stripe rust in Canada before 2000 can be explained by the lack of damage evidenced before that time. Su et al. (2003) evaluated the race identity of 57 different Canadian *P. striiformis* f. sp. *tritici* isolates collected from 1984 to 2002. A collection of isolates is characterized into races in some studies, and these are mainly focused on rust in AB and SK (Brar and Kutcher 2016; Holtz et al. 2013; Kumar et al. 2012).

The purpose of this study is to provide insight into the changes in *P. striiformis* f. sp. *tritici* virulence in Canada over the last 33 years, and to compare these results to what is already known in the United States.

MATERIALS AND METHODS

Fungal isolates and purification. A total of 140 *P. striiformis* f. sp. *tritici* isolates have been characterized at the Lethbridge Research and Development Centre of Agriculture and Agriculture and Agri-Food Canada (Table 1). The authors actively collected and received samples of infected wheat leaves from 2016 and 2017, with the remaining samples being retrieved from liquid nitrogen or −80°C storage. All the isolates tested represent a time period of 33 years (1984 to 2017) and were collected from different provinces in Canada: these are 81 isolates from AB, 27 isolates from British Columbia (BC), 13 isolates from MB, 12 isolates from Ontario (ON), and seven isolates from SK (Table 1).

All the isolates were originally obtained from naturally infected commercial wheat crops during annual disease surveys. The isolates were pooled into three groups based on their sampling time period: period-1 (PI) represents isolates collected between 1984 and 1999; period-2 (PII) represents isolates collected between 2000 and 2009; and period-3 (PIII) represents isolates collected between 2010 and 2017. The reason for such groupings was based on earlier information describing changes in pathogen virulence (mainly to *Yr8* and *Yr9*) since 2000 and then again in 2010 (mainly to *Yr10* and *Yr24*; Chen et al. 2014). We divided the isolates into these groups and performed inoculation with isolates from one period at a time only. (We avoided mixing any isolates from different time periods as a precaution against the remote possibility of cross-contamination.)

Purification and increasing of isolates were done as described by Kumar et al. (2012) and Chen et al. (2010), but with slight modifications. Briefly, for the samples collected from infected leaves, leaf sections bearing a single stripe were placed, with the stripe facing up, in separate petri dishes containing 1% water agar or sterilized, moistened Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, United Kingdom) for 24 h. Fresh urediniospores produced on leaf surfaces were rubbed using sterile Q-tips on the leaves of 12-day-old susceptible wheat cultivar Avocet S (referred to as just Avocet hereafter). Avocet was grown in 10-cm-diameter plastic pots filled with Sunshine potting Mix (W.R. Grace and Co., Fogelsville, PA) with four seeds per pot. Inoculated plants were kept in a dew chamber at 10°C to 12°C for 24 h in the dark and then transferred to a growth chamber with a 16-h photoperiod (18°C day/16°C night) at 180 mmol/liter m^{-2} s^{-1}. Plants inoculated with different samples were covered to isolate them from each other to avoid cross contamination. Two weeks after inoculation, or when prominent sporulation was observed, urediniospores for each isolate were collected using a vacuum spore collector (Porter-Cable, Jackson, TN). The collected spores were dried in desiccators before storing at 4°C for short-term or transferred to liquid nitrogen for long-term storage. These spores were increased again onto Avocet an additional time and a subset of the collected spores was used to inoculate wheat differential lines to identify races. (For spores brought out from the historical collection [isolates before 2000], it was prohibitive to attempt to reisolate and increase each isolate for this study; note that these were presumed to be single stripe isolates as indicated by the inventory data supplied with them.) Ratings across both replicates were consistent, and inoculation with isolates from different time periods was never done on

TABLE 1. Number of isolates and races (parentheses) in each time period in Canada and individual provinces[a]

Time period	Canada	British Columbia	Alberta	Saskatchewan	Manitoba	Ontario
1984 to 1999 (PI)	38 (26)	21 (17)	16 (14)	1 (1)	–	–
2000 to 2009 (PII)	30 (26)	2 (2)	20 (18)	3 (3)	5 (4)	–
2010 to 2017 (PIII)	72 (37)	4 (4)	45 (28)	3 (3)	8 (4)	12 (10)

[a] P, time period.

the same day; as noted above, this was to avoid any chance of cross-contaminations among isolates from different time periods.

Virulence phenotyping on a differential set. A set of 17 near-isogenic lines in the Avocet background (*Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrExp2*, *YrSP*, and *YrTr1*) and an additional wheat differential cultivar Tyee possessing *YrTye* (also known as *Yr76*) were used to classify *P. striiformis* f. sp. *tritici* isolates into races as described in the literature (Chen et al. 2002; Kumar et al. 2012; Wang and Chen 2013). All differentials were planted in 10-cm-diameter plastic pots filled with Sunshine potting Mix (W.R. Grace and Co.). One pot containing five Avocet seedlings was added as a control for each tested isolate. Eppendorf vials containing urediniospores that had been stored in a −80°C freezer or liquid nitrogen were heat-shocked in a 42°C water bath for 2 min before inoculation. The tested differential lines were inoculated at the two-leaf stage with urediniospores from individual single isolates mixed with talcum (Sigma-Aldrich, St. Louis, MO) at a ratio of 1:20 (Chen et al. 2010) using sterilized Q-tips. Inoculated seedlings were transferred to a dew chamber, then grown in a growth chamber as described above. ITs on both first and second leaves were observed and the ITs were recorded on the second leaf at 18 to 21 days after inoculation using a 0 to 9 scale (Supplementary Fig. S1; Line and Qayoum 1992).

An isolate was considered to be "avirulent" to a particular gene if the inoculated leaf exhibited no symptom (IT = 0), or only showed chlorotic to necrotic flecks or blotches with no sporulation (IT = 1 to 2), or only showed chlorotic to necrotic flecks with trace to moderate sporulation (IT = 3 to 6). The isolate causing symptoms with abundant sporulation either with or without chlorotic or necrotic blotches (IT = 7 to 9) was considered virulent. The bioassay for each isolate was repeated independently to ensure consistency of disease ratings.

Data analyses. P. striiformis *f. sp.* tritici *race identification, frequency, and cluster analysis.* Race identity and frequency were determined by employing a custom Python script (Supplementary Appendix) using IT data as the input. The R package, ggplot2, was used to generate a heat-map based on virulence phenotypes of *P. striiformis* f. sp. *tritici* isolates (ordered by date) on the differential lines. The high dimensional nature of 18 factors (i.e., resistance genes) makes principal component analysis (PCA) a useful tool for reducing the dimensionality of this data set and making patterns more readily apparent. The IT data (0 to 9) was used as input for PCA using the "prcomp" function in the R program (v3.4.3). Principal components one (PC1) and two (PC2) were extracted from the output and used as input for two clustering algorithms: *k*-means and hierarchical clustering. Two methods were used in the hope of better identifying appropriate clusters of isolates. The *k*-means analysis was iterated through different values of *k* from 1 to 10, and then a line plot was generated using the number of clusters as the *x* axis and the ratio of sum of squares (between sum of squares/total sum of squares) as the *y* axis (Supplementary Fig. S2A). The lowest number of clusters that maximized the sum of squares ratio (i.e., a hard bend in the curve) was four clusters. For the hierarchical clustering method, the "ward.D2" method was used to construct a dendrogram and the largest branches of the tree were defined as "clusters" (Supplementary Fig. S2B); four clusters were defined and selected for study. Several scatter plots using PC1 as the *x* axis and PC2 as the *y* axis were generated where isolates were grouped by time period, province of isolation, *k*-means clustering, and hierarchical clustering (here, ellipses surrounding data points represent 95% confidence intervals).

Race diversity and distribution. Virulence profiles were used to compare the diversity of *P. striiformis* f. sp. *tritici* isolates within and among Canadian provinces, as well as the main stripe rust-affected cereal regions in the United States using the "vegan" R package in the R environment (Oksanen 2011). To compare indices using populations of different sizes, the number of races were scaled with rarefaction curves using the Analytic Rarefaction program (v.1.3) (Holland 2003). Two ecological diversity indices (α- and β-diversities) were calculated to evaluate the *P. striiformis* f. sp. *tritici* race similarity/dissimilarity. Shannon and Simpson indices (Shannon 1948; Simpson 1949) as well as evenness values were calculated as α-diversity indicators. A β-diversity analysis (Bray-Curtis dissimilarity values) was used to test whether the provinces of AB, MB, and ON or a U.S. state component represented a unique environment. The virulence profile of *P. striiformis* f. sp. *tritici* races in each state were collected from the U.S. Department of Agriculture and the Washington State University, Pullman, WA websites. Because there was a limited number of isolates for PIII 2010 to 2017 from SK, BC, and some U.S. states, these locations were excluded from this analysis.

RESULTS

***P. striiformis* f. sp. *tritici* races and virulence profiles.** The ITs of the 140 tested *P. striiformis* f. sp. *tritici* isolates were visualized using a heat-map (Fig. 1). No change in the IT was observed between first and second leaf inoculated by same isolate. The IT scores were generally either low (0 to 2) or high (7 to 9), with 45% of ITs rated 0 to 2, 44% of ITs rated 7 to 9, and only 11% of the ITs rated 3 to 6.

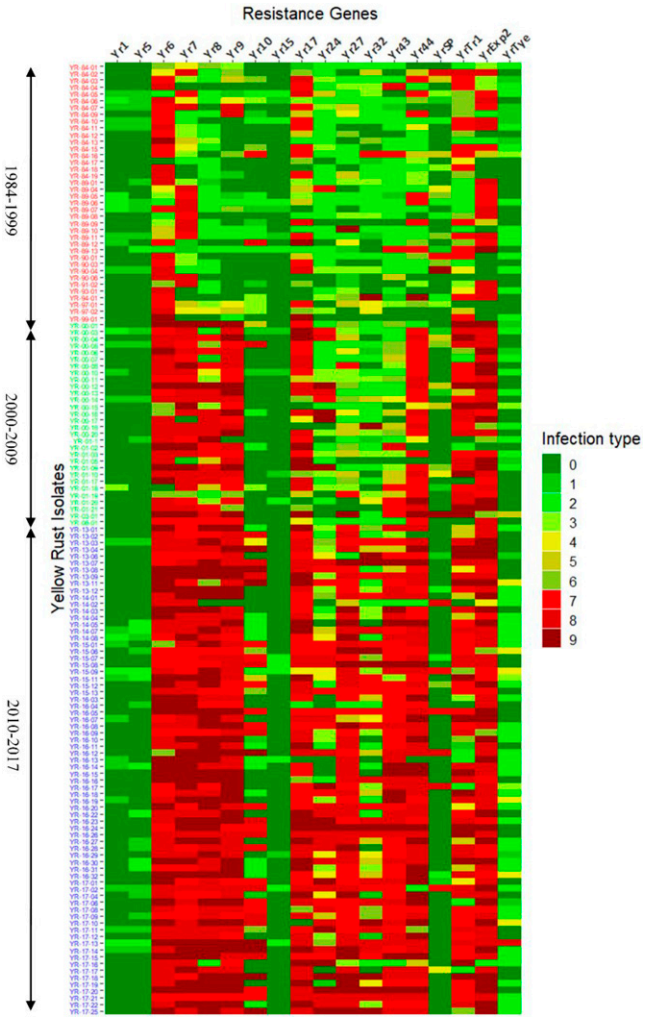

Fig. 1. Heat map based on virulence phenotype of *Puccinia striiformis* f. sp. *tritici* isolates on a set of 17 single *Yr* gene lines and an additional cultivar possessing *YrTye*. Infection types (ITs) were recorded at 18 to 21 days after inoculation based on a 0 to 9 scale. An isolate was considered as *avirulent* on each differential line if there were no symptoms (IT = 0), if only chlorotic to necrotic flecks or blotches were present (IT = 1 to 2), or if only trace-to-moderate sporulation on a two-stage leaf was observed (IT = 3 to 6). An isolate was considered as *virulent* if an isolate caused symptoms with an abundance of sporulation either with, or without, chlorotic or necrotic blotches (IT = 7 to 9).

Canadian isolates in this study were designated into races and coded with a "C" followed by the number of the race (-#); this coding was used to compare with the U.S. races designated as "PSTv" followed by a number (-#), when applicable. In total, 89 races with different virulence phenotypes were identified (Tables 1 and 2). Twenty-five races (67 isolates) were identical to previously characterized *P. striiformis* f. sp. *tritici* races in the United States, and 64 races (73 isolates) were unique to Canada. Before 2010, 69% of the isolates were considered potentially unique to Canada, but after 2010, only 36% were found to be unique to Canada. More importantly, none of the Canadian *P. striiformis* f. sp. *tritici* races defeated any *Yr* gene that had not already been defeated in the United States.

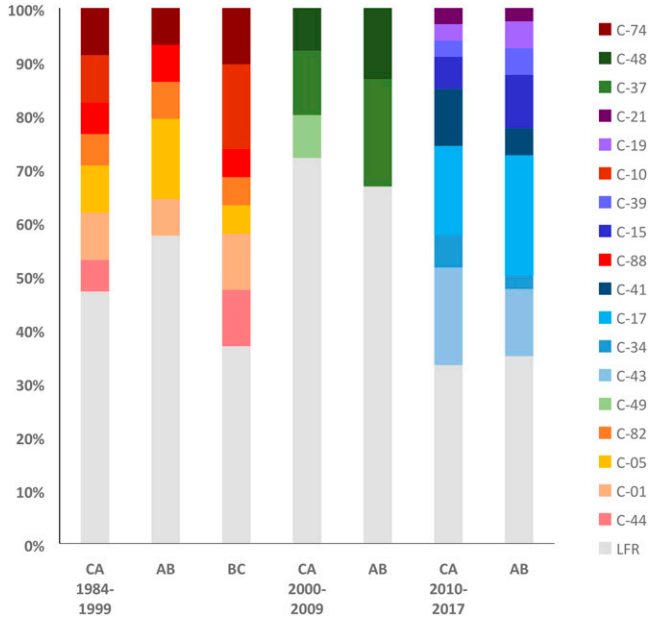

Fig. 2. Frequency of most dominant wheat stripe rust races in Canada (1984 to 2017) based on virulence phenotype of *Puccinia striiformis* f. sp. *tritici* isolates on 18 single *Yr*-gene lines. CA = Canada; AB = Alberta; BC = British Columbia; LFR = low frequency races ≤2%.

Virulence and race frequency. The number of defeated *Yr* genes by any given isolate in this study varied from 0 to 14 genes. Ninety-two percent of the isolates collected before 2000 defeated combinations of four *Yr* genes (*Yr6*, *Yr17*, *YrTr1*, and *YrExp2*) or fewer, and 91% of the isolates collected during and after 2000 defeated combinations of seven *Yr* genes or more. Only two isolates, collected in 1984, were rated "avirulent" on all the tested *Yr* genes, and before 2000, only one isolate defeated seven *Yr* genes. None of the isolates collected in 2000 or after defeated fewer than four *Yr* genes.

The predominant races in Canada have varied over time (Fig. 2). For PI, which is 1984 to 1999, races C-1 (virulent on *Yr6*), C-5 (virulent on *Yr6* and *Yr17*), C-10 (virulent on *Yr6*, *Yr17*, *YrTr1*, and *YrExp2*), and C-74 (virulent on *Yr6* and *YrExp2*) were the most prevalent races at a frequency of 8% each (Table 2; Fig. 2). For PII, which is 2000 to 2009, races C-37 (virulent on *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr24*, *Yr44*, *YrTr1*, and *YrExp2*), C-48 (*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr44*, and *YrExp2*), and C-49 (*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr44*, *YrTr1*, and *YrExp2*) were the most prevalent at frequencies of 10, 7, and 7%, respectively. For PIII, which is 2010 to 2017, races C-43 (*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*), C-17 (*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*), and C-41 (*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, and *YrExp2*) predominate at a frequency of 12, 11, and 7%, respectively (Table 2; Fig. 2).

This changing pattern of prevalent races can be observed in AB over the last 30 years (Fig. 2). In AB, races C-1, C-5, C-74, C-82, and C-88 were more prevalent in PI, races C-37 and C-48 were prevalent in PII, and races C-15, C-17, and C-43 were more prevalent in PIII. In BC, races C-01, C-10, C-44, and C-74 were prevalent in PI, and based on the limited number of isolates, no conclusion could be made on prevalent races in PII and PIII (Fig. 2). Similarly, no comparison could be made for the change in virulence in the rest of the

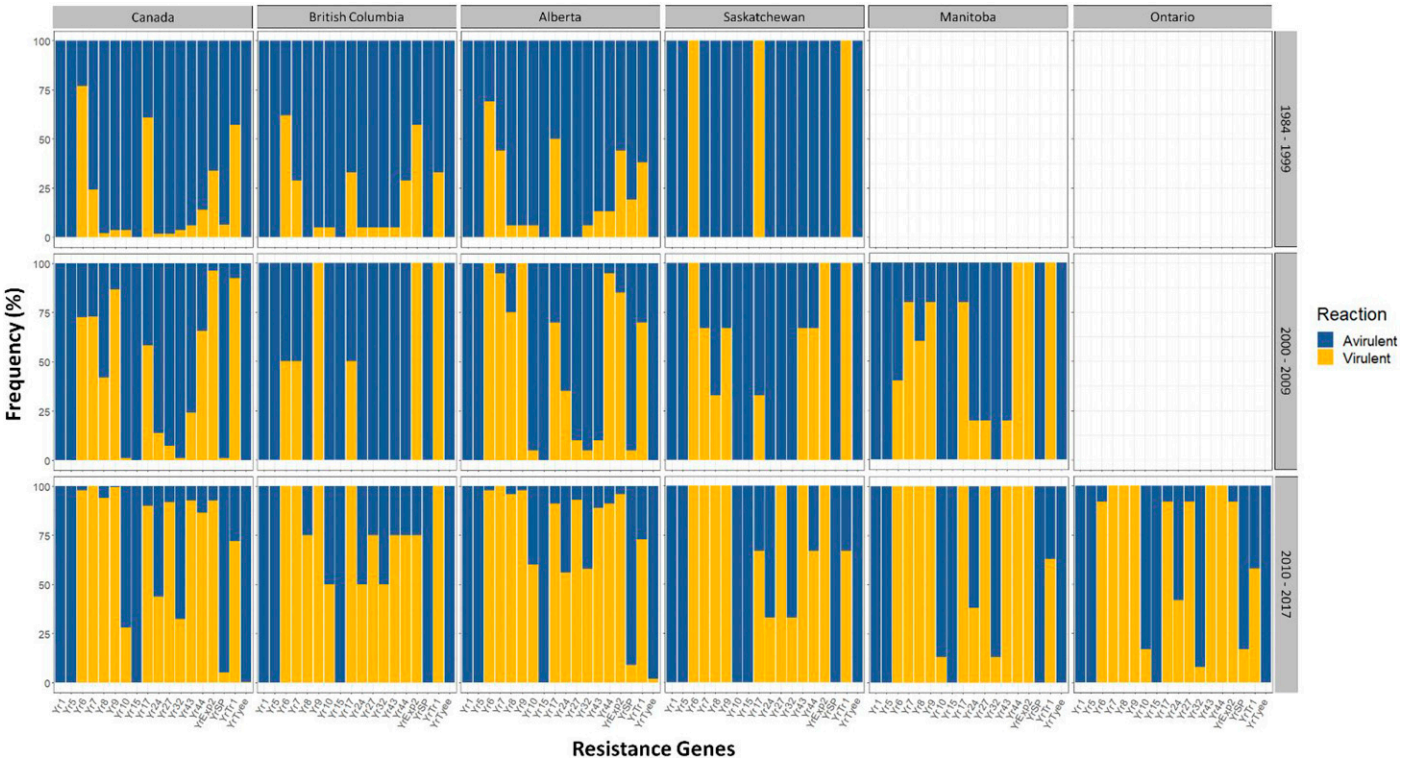

Fig. 3. Frequency of *Puccinia striiformis* f. sp. *tritici* virulence on each *Yr* gene in three time periods and provinces in Canada.

provinces because of the absence of *P. striiformis* f. sp. *tritici* isolates from these provinces (Table 1).

Virulence frequency in relation to the *Yr* genes. There is a clear increase in *P. striiformis* f. sp. *tritici* virulence in Canada through time as illustrated by Figures 1 and 3 and Supplementary Table S1. The tested pathogen population has not defeated the resistance genes *Yr1*, *Yr5*, and *Yr15*. In addition, *YrSp* and *Yr76* are still effective against the majority of the races, and *Yr10*, *Yr27*, and *Yr32* remained resistant to >90% of the pathogen population in PI and PII. For PI, *Yr6*, *Yr7*, *Yr17*, *YrTr1*, and *YrExp2* were defeated by the pathogen population with a virulence frequency of 77, 24.3, 61, 57, and 33.7%, respectively (Fig. 3). *Yr7* and *Yr44* were partially defeated in PI by 24 and 14% of isolates, respectively (Figs. 1 and 3). The remaining *Yr* genes were defeated by <7% of the tested isolates in PI. In PII, from 2000 to 2009, *YrTr1* and *YrExp2* were defeated by >90% of the isolates. Additionally, *Yr6*, *Yr7*, and *Yr9* were defeated by >70% of isolates, while *Yr8*, *Yr17*, and *Yr44* were defeated by >40% of the isolates in PII (Figs. 1 and 3). In PIII, there was a large increase in the number of isolates that were able to overcome *Yr* genes, and a spike in virulence frequency, where >90% of isolates defeated *Yr7*, *Yr9*, *Yr6*, *Yr8*, *Yr43*, *Yr27*, and *YrExp2*. Frequency of virulence exceeded to 70% on *Yr44* and *YrTr1* in PIII, and there was a spike in the proportion of isolates defeating *Yr10*, *Yr24*, *Yr27*, and *Yr33* in this period (Figs. 1 and 3).

Principal component and cluster analysis. To verify if virulence phenotype differences were related to time, IT data for all the isolates on all resistance genes was analyzed using PCA with no prior assumptions made on groupings (i.e., time period was not used as a variable). PC1 accounted for 33% of the total variation, while PC2 accounted for 11% of the variation (total 44%; Supplementary Fig. S3; Supplementary Table S2). The genes *Yr7*, *Yr8*, *Yr9*, *Yr27*, *Yr24*, *Yr43*, *Yr44*, and *YrExp2* contributed the most to PC1, and genes *Yr1*, *Yr5*, *Yr6*, *Yr15*, *YrTye*, and *YrExp2* contributed the most to PC2.

Plotting PC1 and PC2, then coloring isolates by their time period showed a separation between PI and PIII isolates, with PII isolates placed in between (Fig. 4A). Coloring by province showed no major trends, although there is some subtle separation between AB and BC isolates (Fig. 4B). Clustering by *k*-means and hierarchical clustering produced identical cluster groups (Fig. 4C and D). From hereafter, any reference to cluster number is solely referring to the clusters defined by *k*-means. The majority of PI and PIII isolates have been sorted into distinct clusters, with both PI and PIII having some overlap with PII isolates. Cluster-3 (blue in Fig. 4C) contained 79% of isolates from PI, 10% from PII, and 1% of isolates from PIII. Cluster-4 (purple in Fig. 4C) contained 77% of isolates from PII, 1% from PI, and 11% from PIII. Cluster-2 (green in Fig. 4C) contained 81% of isolates from PIII, 7% from PII, and no isolates from PI. A fourth cluster, cluster-1 (red in Fig. 4C), contains a few isolates from all three times of 18, 7, and 7% for PI, PII, and PIII, respectively.

Cross reference of the main contributors of PC1 (Supplementary Table S2) with the virulence heat-map (Fig. 1) shows that the defeat of resistance genes *Yr7*, *Yr8*, *Yr9*, and *Yr44* around the year 2000 is the main reason for the separation of cluster-3 (majority PI) and cluster-4 (majority PII). Similarly, the defeat of genes *Yr24*, *Yr27*, and *Yr43*, and around 2010 is the primary contributor to the separation of cluster-4 (majority PII) and cluster-2 (majority PIII). Cluster-1, which contains isolates from all periods, was largely influenced by the extremely negative correlations of *Yr1*, *Yr5*, and *Yr15* in PC2 (Supplementary Table S2). In this cluster, any nonzero infection types for these genes heavily influenced an isolates position within PC2 (i.e., the *y*-axis). Note that none of these lines rated above IT = 2.

Race diversity and distribution. Two ecological diversity indices (α- and β-diversities) were calculated to evaluate the *P. striiformis* f. sp. *tritici* race similarity/dissimilarity within and among Canadian provinces and states of the United States since 2010. The Shannon and Simpson indices' calculated values for AB were lower or similar in PIII compared with PI and PII, and the evenness values increased in PIII (Table 3), which indicates that race diversity in most Canadian provinces from 1984 to 2017 has declined. No comparison could be made for rest of the provinces through time because of the limited number of isolates (Table 1). The Bray-Curtis dissimilarity from the β-diversity analysis showed that 2010 races in AB were similar to those found in the U.S. PNW from the states of Utah, Idaho, Montana, and California; and 2010 races in MB and ON were similar to those found in the eastern and central United States from the states of Nebraska, Oklahoma, Louisiana, and Virginia (Fig. 5).

DISCUSSION

In this study, we provide clear evidence for a shift toward increased virulence in Canadian stripe rust populations from 1984 to 2017. This is the only study in which a Canadian collection, representing *P. striiformis* f. sp. *tritici* populations sampled over the past 30 years, has been tested for virulence under same experimental conditions. The results provided useful information on *P. striiformis* f. sp. *tritici* virulence evolution over the last three decades in Canada and allowed comparison with the United States. A spike in *P. striiformis* f. sp. *tritici* virulence worldwide occurred in 2000 that is well documented, and it is believed to be accompanied by multiple events of race incursions (Ali et al. 2014; Brar et al. 2018; Hovmøller et al. 2008). Over the last decades, stripe rust outbreaks in Canada have been more frequent because of the emergence of new races in North America overall (Aboukhaddour et al. 2018; Andrivon and Vallavieille-Pope 1995; Chen 2005; Conner and Kuzyk 1988; Kumar et al. 2012; Kutcher et al. 2012; Line 2002; McCallum and Fetch 2001; McCallum et al. 2007a, b; Ontario Ministry of Agriculture, Food and Rural Affairs 2009; Rioux et al. 2015; Xi et al. 2015).

Our results showed that major changes in *P. striiformis* f. sp. *tritici* virulence over the last 30 years have occurred twice—around the year 2000, and again around 2010. Over the past decades, the Canadian *P. striiformis* f. sp. *tritici* population has expanded its ability to defeat at least 13 additional *Yr* genes from the 17 genes tested, and this confirm the evolution of *P. striiformis* f. sp. *tritici* toward a wider spectrum of virulence.

Genetic resistance is the most preferred strategy for controlling stripe rust. In North America, the cultivation of certain cultivars over a wide area, over a certain time period, created a selection pressure on the pathogen to evolve new virulence, resulting in its defeating the deployed resistance (Chen et al. 2014). Stripe rust can overwinter in Canada, but the wind-borne urediniospores that travel from the United States to Canada are believed to be the main source of infection. In Canada, spring wheat is the most prevalent type grown on the prairie and the cultivars are known to carry combinations of *Yr6*, *Yr7*, and *Yr27* (reviewed in Wang and Chen 2017). This may explain the high virulence toward these genes in our results. Since the 1970s, many of the spring wheat cultivars were selected to carry the adult plant resistance genes *Yr18*/*Lr34*, as most cultivars were selected to have the leaf rust resistance gene *Lr34* before stripe rust became an issue in Canada (McCallum et al. 2007a). On the other hand, the Canadian winter wheat cultivars carry combinations of *Yr10* and *Yr17* (Holtz et al. 2013).

This study showed that before 2010, only 31% of the isolates were designated into already identified U.S. races, but since 2010, most Canadian isolates (64%) matched races present in the United States. Most of the 'newer' races were very similar to previously described races, but with a higher virulence. Except for the breakdown of *Yr8* and *Yr9* in PII that was linked to race incursions as explained below, these results suggest the evolution of virulence/avirulence loci for host resistance by mutation at a more local Canadian scale than previously thought. The pathogens can overwinter in AB as dormant mycelia in winter wheat (Aboukhaddour et al. 2018; Xi et al. 2015), and may gain the ability to evolve locally. However, until more detailed information about the presence of *Yr* genes in Canadian wheat become available, it is not possible to predict, with certainty,

TABLE 2. Virulence profile, distribution, and frequencies of *Puccinia striiformis* f. sp. *tritici* races in Canada from 1984 to 2017

C[a]	PSTv[b]	Virulence on *Yr* gene[c]	Location[d]	Year	Number of isolates	Frequency (%)
C-01	PSTv-019	*6*	AB, BC	1984, 1997	3	2.1
C-02	–	*27,44,Tr1,Exp2*	MB	2001	1	<1
C-03	–	*43,44,Tr1,Exp2*	BC	1989	1	<1
C-04	–	*6,10,17,Exp2*	BC	1989	1	<1
C-05	PSTv-021	*6,17*	AB, BC	1984, 1990, 1997	3	2.1
C-06	–	*6,17,43,Tr1*	AB	1984	1	<1
C-07	–	*6,17,44,Tr1*	BC	1984	1	<1
C-08	–	*6,17,SP*	AB	1990	1	<1
C-09	–	*6,17,Tr1*	SK	1999	1	<1
C-10	–	*6,17,Tr1,Exp2*	BC	1984	3	2.1
C-11	–	*6,32,44,Tr1,Exp2*	BC	1994	1	<1
C-12	PSTv-145	*6,7*	AB	1990	1	<1
C-13	–	*6,7,17,SP*	AB	2014	1	<1
C-14	–	*6,7,8,43,44,Tr1,Exp2*	SK	2001	1	<1
C-15	PSTv-072	*6,7,8,9,10,17,24,27,32,43,44,Exp2*	AB	2016, 2017	4	2.9
C-16	–	*6,7,8,9,10,17,24,27,32,43,44,SP,Tr1,Exp2*	AB	2016	1	<1
C-17	PSTv-041	*6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2*	AB, BC, MB	2013, 2015, 2016, 2017	11	7.9
C-18	–	*6,7,8,9,10,17,24,27,32,43,SP,Tr1,Exp2*	AB	2017	1	<1
C-19	–	*6,7,8,9,10,17,24,27,43,44,Tr1,Exp2*	AB	2015, 2016	2	1.4
C-20	–	*6,7,8,9,10,17,24,27,44,Exp2*	AB	2013	1	<1
C-21	–	*6,7,8,9,10,17,27,32,43,44,Tr1,Exp2*	AB, BC	2013, 2015	2	1.4
C-22	–	*6,7,8,9,10,17,27,32,43,44,Tr1,Exp2,Tye*	AB	2017	4	2.9
C-23	–	*6,7,8,9,10,17,27,43,44,Tr1*	ON	2016	1	<1
C-24	PSTv-039	*6,7,8,9,10,17,27,43,44,Tr1,Exp2*	AB	2017	1	<1
C-25	PSTv-359	*6,7,8,9,10,17,27,44,Tr1,Exp2*	AB	2013	1	<1
C-26	–	*6,7,8,9,10,17,27,Exp2*	AB	2013	1	<1
C-27	PSTv-040	*6,7,8,9,10,24,27,32,43,44,Tr1,Exp2*	AB	2017	1	<1
C-28	–	*6,7,8,9,10,24,27,43,44,Tr1,Exp2*	AB	2017	1	<1
C-29	–	*6,7,8,9,17,24,27,32,43,44,Tr1*	AB	2015	1	<1
C-30	–	*6,7,8,9,17,24,27,32,43,44,Tr1,Exp2*	SK	2015	1	<1
C-31	–	*6,7,8,9,17,24,27,32,43,Tr1,Exp2*	AB	2017	1	<1
C-32	–	*6,7,8,9,17,24,27,43,44,Exp2*	ON	2016	1	<1
C-33	–	*6,7,8,9,17,24,27,43,44,SP,Exp2*	ON	2016	1	<1
C-34	PSTv-038	*6,7,8,9,17,24,27,43,44,Tr1,Exp2*	AB, MB, ON	2016, 2017	4	2.9
C-35	–	*6,7,8,9,17,24,43,44,Tr1,Exp2*	ON	2016	1	<1
C-36	–	*6,7,8,9,17,24,44,Exp2*	AB	2000	1	<1
C-37	–	*6,7,8,9,17,24,44,Tr1,Exp2*	AB	2000, 2001	3	2.1
C-38	–	*6,7,8,9,17,27,32,43,44,Exp2*	AB	2014	1	<1
C-39	PSTv-296	*6,7,8,9,17,27,32,43,44,Tr1,Exp2*	AB	2013, 2014	2	1.4
C-40	PSTv-292	*6,7,8,9,17,27,32,44,Tr1,Exp2*	AB	2015	1	<1
C-41	PSTv-052	*6,7,8,9,17,27,43,44,Exp2*	AB, MB, ON	2013, 2014, 2016, 2017	7	5
C-42	–	*6,7,8,9,17,27,43,44,SP,Tr1*	AB	2003	1	<1
C-43	PSTv-037	*6,7,8,9,17,27,43,44,Tr1,Exp2*	AB, BC, MB, ON, SK	2013, 2014, 2016, 2017	12	8.6
C-44	PSTv-018	–	BC	1984	2	1.4
C-45	PSTv-034	*6,7,8,9,17,27,44,Tr1,Exp2*	AB	2000	1	<1
C-46	–	*6,7,8,9,17,32,43,44,Tr1,Exp2*	AB	2013	1	<1
C-47	–	*6,7,8,9,17*	AB	2001	1	<1
C-48	–	*6,7,8,9,17,44,Exp2*	AB	2000, 2001	2	1.4
C-49	PSTv-031	*6,7,8,9,17,44,Tr1,Exp2*	MB	2000, 2001	2	1.4
C-50	–	*6,7,8,9,17,Tr1,Exp2*	AB	2000	1	<1
C-51	–	*6,7,8,9,24,44,Tr1,Exp2*	AB	2000	1	<1
C-52	PSTv-325	*6,7,8,9,27,32,43,44,Tr1,Exp2*	ON	2016	1	<1
C-53	–	*6,7,8,9,27,43,44,SP,Exp2*	AB	2015	1	<1
C-54	–	*6,7,8,9,27,43,Exp2*	SK	2016	1	<1
C-55	–	*6,7,8,9,32,44,Tr1*	AB	2000	1	<1
C-56	PSTv-284	*6,7,8,9,43,44,Exp2*	AB	2016	1	<1
C-57	PSTv-272	*6,7,8,9,44,Exp2*	AB	2006	1	<1
C-58	PSTv-030	*6,7,8,9,44,Tr1,Exp2*	AB	2001	1	<1
C-59	–	*6,7,9,10,17,24,27,43,44,Tr1,Exp2*	AB	2013	1	<1
C-60	PSTv-366	*6,7,9,10,17,44,Tr1,Exp2*	AB	2000	1	<1
C-61	–	*6,7,9,17,24,44,Tr1,Exp2*	AB	2000	1	<1
C-62	–	*6,7,9,17,24,Tr1*	BC	2017	1	<1
C-63	PSTv-028	*6,7,9,17,44,Exp2*	AB	2000	1	<1
C-64	–	*6,7,9,17,44,Tr1,Exp2*	AB	2000	1	<1
C-65	–	*6,7,9,17,Exp2*	AB	1984	1	<1

(*Continued on next page*)

[a] "C-" is a nomenclature system for *P. striiformis* f. sp. *tritici* isolates collected from Canada.

[b] "PSTv" is a nomenclature system for *P. striiformis* f. sp. *tritici* isolates collected from the United States or Mexico.

[c] Virulence profile of isolates based on the reaction of 140 *P. striiformis* f. sp. *tritici* isolates on 18 *Yr* single-gene differentials, which are as follows: 1 = AvSYr1NIL (Yr1); 2 = AvSYr5NIL (Yr5); 3 = AvSYr6NIL (Yr6); 4 = AvSYr7NIL (Yr7); 5 = AvSYr8NIL (Yr8); 6 = AvSYr9NIL (Yr9); 7 = AvSYr10NIL (Yr10); 8 = AvsYr15NIL (Yr15); 9 = AvSYr17NIL (Yr17); 10 = AvSYr24NIL (Yr24); 11 = AvSYr27NIL (Yr27); 12 = AvSYr32NIL (Yr32); 13 = AvS/IDO377s (F3-41-1; Yr43); 14 = Avs/Zak (1-1-35-line 1; Yr44); 15 = AvSYrSPNIL (YrSP); 16 = AvSYrTres1NIL (YrTr1); 17 = Avs/Exp 1/1-1 Line 74 (YrExp2); and 18 = AvSYr76NIL (Yr76 = YrTye).

[d] AB = Alberta; BC = British Columbia; MB = Manitoba; SK = Saskatchewan; and ON = Ontario.

TABLE 2. (*Continued from previous page*)

C[a]	PSTv[b]	Virulence on *Yr* gene[c]	Location[d]	Year	Number of isolates	Frequency (%)
C-66	–	*6,7,9,43,44,Tr1,Exp2*	SK	2001	1	<1
C-67	–	*6,7,9,44,Tr1,Exp2*	AB	2000	1	<1
C-68	–	*6,7,Exp2*	BC	1989	1	<1
C-69	–	*6,8,10,17,32,43,Tr1*	AB	1984	1	<1
C-70	–	*6,8,9,24,44,Tr1,Exp2*	AB	2000	1	<1
C-71	–	*6,9,17,Tr1,Exp2*	BC	2001	1	<1
C-72	–	*6,9,17,Tr1,Exp2*	SK	2001	1	<1
C-73	–	*6,9,44*	BC	1984	1	<1
C-74	–	*6,Exp2*	AB, BC	1984, 1989, 1993	3	2.1
C-75	–	*6,SP,Tr1*	AB	1990	1	<1
C-76	–	*6,Tr1*	AB	1984	1	<1
C-77	PSTv-025	*7,17,44,Exp2*	AB	1984	1	<1
C-78	–	*7,17,44,Tr1*	BC	1989	1	<1
C-79	–	*7,17,Tr1,Exp2*	AB	1989	1	<1
C-80	–	*7,24,Exp2*	BC	1989	1	<1
C-81	–	*7,27*	BC	1989	1	<1
C-82	PSTv-024	*7,44,Exp2*	AB, BC	1989	2	1.4
C-83	–	*7,8,9,10,17,24,27,32,43,44,Tr1,Exp2*	AB	2015	1	<1
C-84	–	*7,8,9,10,17,24,27,43,44,SP,Exp2*	ON	2016	1	<1
C-85	–	*7,8,9,17,24,44,Tr1,Exp2*	MB	2000	1	<1
C-86	–	*7,9,17,43,44,Tr1,Exp2*	MB	2001	1	<1
C-87	–	*7,9,Tr1,Exp2*	BC	2000	1	<1
C-88	PSTv-063	*7,Exp2*	AB, BC	1989, 1991	2	1.4
C-89	–	*7,SP,Tr1,Exp2*	AB	1984	1	<1

the role of selection pressure imposed by local cultivars on the *P. striiformis* f. sp. *tritici* population. Since 2010, new races have emerged—notably C-17, C-41, and C-43, which are the most common races in both Canada and the United States. These races were likely to have migrated to Canada from the United States, with virulence, on combinations of *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Y10*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*. In the United States, since 2010, more races with increased virulence continue to be identified (Wan et al. 2017). In this study, it was found that the number of races per time period (PI, PII, and PIII) has not changed greatly, but that the accompanying virulence spectrum has increased/expanded.

In PII, the race/isolate ratio is slightly increased compared with PI, but in PIII, a slight decrease is observed. This is likely resulting from the prevalence of few highly virulent races since 2010. Additionally, many of the *Yr* genes tested have been defeated by the majority of isolates, and therefore the ability to differentiate isolates is becoming limited. The diversity indices of *P. striiformis* f. sp. *tritici* races in AB indicated a decrease in race diversity from PI to PIII, which may be reflective of the prevalence of more aggressive races since 2000. Based on the limited number of isolates from the eastern provinces, it is hard to draw a valid conclusion on race diversity between western and eastern Canada, despite the fact that our data showed ON exhibits higher diversity indices than AB (Table 3). *P. striiformis* f. sp. *tritici* races in AB, MB, and ON since 2010 showed that isolates from AB were closely related to those from the PNW of the United States, whereas isolates from the eastern Prairies and eastern Canada, MB and ON, were closely related to isolates from the eastern and/or central regions of the United States (Fig. 5). This supports the previously proposed hypothesis that there are two migration routes for stripe rust in North America—one through the PNW and the second through the traditional "*Puccinia* pathway" (reviewed in Aboukhaddour et al. 2020). Such comparisons could not be made for SK and BC, given the limited number of isolates in PIII from these provinces. However, PCA showed a separation between isolates from AB and BC (Fig. 4B), and such separation is based on either the time period or a physical barrier (i.e., the Rocky Mountains keeping populations separate), so more data from BC would be needed to better confirm this hypothesis.

In North America, the rise of *P. striiformis* f. sp. *tritici* isolates defeating both the *Yr8* and *Yr9* genes around 2000 was attributed to the incursions of new, more aggressive races that caused infection under higher temperatures than had been experienced in past decades (Milus et al. 2009). We have reconfirmed the rise of these new races defeating both *Yr8* and *Yr9* since 2000. Similarly, Su et al. (2003) investigated the local *P. striiformis* f. sp. *tritici* virulence in Canada before the year 2000, and the results showed that since 2000, the new races detected in Canada were virulent on both 'Compair' (*Yr8* and *Yr19*) and 'Clement' (*Yr9* and *YrCle*). Before 2000, Su et al. (2003), reported the presence of races that were virulent on 'Compair' from BC and AB in 1991 and 1993, and one race that was virulent on 'Clement' in 1984. The samples we tested from PI (1984 to 1999) are likely the same or at least part of the same samples from the collection used by Su et al. (2003), but the published coding from that study could not be historically matched to the actual samples we used, as we found that isolates, once placed in liquid N2 storage, had been given different individual codes than originally used in the study of Su et al. (2003). In our results, we found two isolates from 1984 that were found to be virulent on *Yr9*. Additional tests were then repeated using different sources of *Yr9* isogenic lines; these were obtained from two different labs (X. M. Chen at the U.S. Department of Agriculture, and T. Fetch of Agriculture and Agri-Food Canada, as provided to him through C. R. Wellings at the University of Sydney, Australia) and virulence on both sources confirmed virulence to *Yr9* before 2000. Interestingly, similar to Su et al. (2003), few of the PI isolates were virulent on *Yr8*. However, races defeating both *Yr8* and *Yr9* before 2000 were not reported in the study of Su et al. (2003), nor in any previous study in Canada or the United States.

Virulence on *Yr9* has been established in northwest Europe since the 1980s, but was not associated with virulence to *Yr8* (Hovmøller 2001). In this study, most isolates collected since 2000 in western and eastern Canada defeated these two genes, and similar results were found in central AB, southern AB, and SK (Brar and Kutcher 2016; Holtz et al. 2013). In the United States, new races with virulence on *Yr8* and *Yr9* were reported for the first time in 2000 in the states east of the Rocky Mountains (Chen et al. 2002), which was unusual as stripe rust was not common in regions outside the PNW. These new races continued to be widespread in the central United States in subsequent years, causing severe epidemics; they are adapted to cause infection at higher temperature than the old races (Milus et al. 2009). Most likely these are the same races that have reached Canada since 2000. *Yr9* originated from rye chromosome arm 1RS (translocation segment 1BL.1RS), which also carries the

genes *Lr26*, *Sr31*, and *Pm8* for resistance to leaf rust, stem rust, and powdery mildew (reviewed in Wang and Chen 2017). This translocation segment is not common in Canadian wheat cultivars (Hiebert et al. 2011), but was widely adopted by International Maize and Wheat Improvement Center (CIMMYT) lines worldwide and in some winter wheat in the midwestern United States (Brar et al. 2019). The virulence on *Yr8* and *Yr9* since 2000 in the United States was also accompanied by virulence on 'Express' (*YrExp1* and *YrExp2*), and in our study, the majority of isolates since 2000 defeated *YrExp2*, *Yr8*, and *Yr9*. More than 40% of the isolates in AB and BC before 2000 had defeated *YrExp2*, but since 2000, this percentage has become >80%.

Virulence toward resistance genes *Yr6* and *Yr17* was present in the majority of tested isolates since 1984, and today, virulence to *Yr6* and *Yr7* seems to be fixed in Canadian and U.S. *P. striiformis* f. sp. *tritici* populations, with such virulence now known worldwide (Sharma-Poudyal et al. 2013). *Yr6* is a common gene in cultivars of South American origin, and has been introduced in many CIMMYT cultivars. *Yr7* is a common gene in North America, Europe, Australia, and New Zealand; *Yr7* can be traced back to the durum cultivar Iumillo and was later transferred to wheat cultivar Thatcher (Johnson and Dyck 1984), and similar to our results, this gene was defeated by most of the U.S. isolates since 2000 (Liu et al. 2017). *Yr17* is widely used in many wheat breeding programs in Europe and throughout the world, including the winter wheat-breeding programs in the United States and Canada (reviewed in Brar et al. 2017; Wang and Chen 2017; Xi et al. 2015). *Yr17* is part of the *Aegilops ventricosa* 2NS/2AS translocation, and its prevalence in commercial cultivars (particularly those developed by CIMMYT) might increase, given that it also confers resistance to the wheat blast pathogen (Cruz et al. 2016). The *Yr17* gene confers resistance to both rust and eyespot pathogens (McIntosh et al. 1995), but its ability to prevent disease is influenced by temperature and light intensity (Milus et al. 2015). Virulence on *Yr17* and *Yr27* is considered the major cause of stripe rust epidemics in many parts of the world during the past 25 years (Wan et al. 2017). Pre-2000, reports on *Yr17* in Canada were missing, as a differential line containing this gene was not included in the testing. Virulence on *Yr17* in the United States has been detected every year since 1968, but at frequencies <50%, and has increased steadily to >80% by 2009 (Liu et al. 2017). In this study, we showed that virulence to *Yr17* has been present in Canada since 1984 at a frequency of 50% in AB in PI (1984 to 1999), and has increased through time to be present in most Canadian isolates.

Races virulent on *Yr24* have been identified at low frequencies in the United States and Australia, but in our results, this gene in PII (2000 to 2009) was defeated by 35% (seven isolates out of 20) in AB; and since 2010 (PIII), virulence on *Yr24* was found to be present in >50% (25 isolates out of 45) in AB. In central AB, *Yr24* was defeated by 21% of the isolates from 2007 to 2008, as reported by Kumar et al. (2012). Virulence to *Yr24* was also positively correlated with virulence to *Yr10* and *Yr32* in the United States (Wan et al. 2017), but based on our results, this association is not confirmed for Canadian isolates, as virulence to *Yr10* and *Yr32* before 2010 was very low.

An increase in virulence toward *Yr10*, *Yr27*, *Yr32*, and *Yr43* since 2010 was also noted. *Yr10* was defeated by >50% of isolates in AB, but virulence was either absent or at a low frequency before 2010. *Yr10* was defeated at low frequencies since 1989 in Creston, BC,

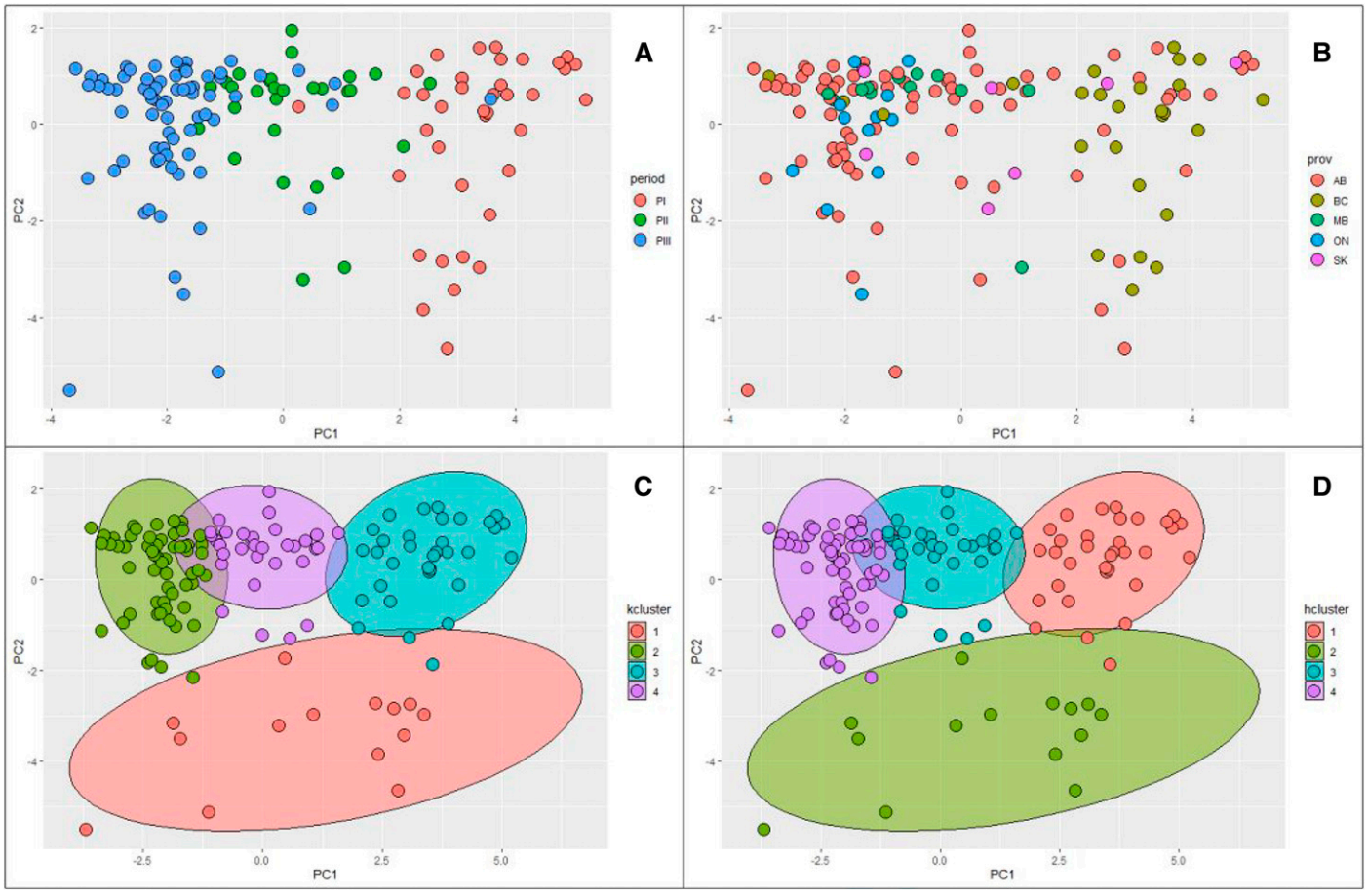

Fig. 4. Infection types of 140 *Puccinia striiformis* f. sp. *tritici* isolates on 18 resistance genes analyzed by principal component analysis (PCA). PC1 and PC2 (principal components one and two) were plotted and isolates were grouped as **A,** time period; **B,** province; **C,** *k*-means clustering algorithm; **D,** hierarchical clustering algorithm. Ellipses represent 95% confidence intervals for the groupings.

then in southern AB since 2000 and in MB, ON, and SK in 2016, suggesting an eastward expansion in virulence. Since 2010, it was found that the majority of isolates in southern AB and BC have defeated *Yr10*; however, Su et al. (2003) had reported the defeat of Moro (*Yr10* and *YrMor*) in Creston, BC, only. In the United States, virulence to *Yr10* was restricted to the PNW and had inconsistent frequency, although it never exceeded 30% in a given year. The low occurrence of virulence on *Yr10* since 2009 has encouraged the deployment of *Yr10*-carrying cultivars in the PNW of the United States and Canada. The increase in *Yr10* virulence in western Canada may be explained by the release of the winter wheat cultivar AC Radiant in 2009; as a carrier of the *Yr10* gene, 'AC Radiant' may have imposed a selection pressure on the *P. striiformis* f. sp. *tritici* population to overcome *Yr10* resistance (Brar et al. 2019; Holtz et al. 2013).

The two resistance genes *Yr27* and *Yr43* had been defeated by the majority of isolates in AB, BC, MB, and ON since 2010. *Yr27* has been commonly used in wheat-breeding programs, and was deployed widely in the Near East, eastern Africa, and India; previously known as the 'Selkirk' gene, it can be traced back to the Canadian cultivar McMurachy, which was used to breed for stem rust resistance in Canada (reviewed in Aboukhaddour et al. 2020; McDonald et al. 2004). Virulence on *Yr27* is widespread in many countries (Sharma-Poudyal et al. 2013). In the United States, virulence on *Yr27* traced back to 1971, increasing to >50% after 2005 (Liu et al. 2017). Virulence to *Yr43* was rare before 2000, but has been commonly observed since 2005 in the United States and in many other countries in Asia, Europe, South America, and the Near East, and the *Yr43* gene is present in many U.S. cultivars derived from IDO337s in the PNW (Wan et al. 2016).

TABLE 3. Temporal comparison of *Puccinia striiformis* f. sp. *tritici* race diversity in Canada[a]

Location	Year	Shannon index	Simpson index	Evenness
Alberta	1984 to 1999 (PI)	1.78	0.82	0.46
Alberta	2000 to 2009 (PII)	1.57	0.78	0.48
Alberta	2010 to 2017 (PIII)	0.67	0.48	0.69
British Columbia	1984 to 1999 (PI)	1.57	0.78	0.48
Manitoba	2010 to 2017 (PIII)	0.68	0.49	0.70
Ontario	2010 to 2017 (PIII)	1.06	0.64	0.58

[a] P, time period.

Our results showed that *Yr32*, also known as *YrCV*, was defeated by >50% of *P. striiformis* f. sp. *tritici* isolates in AB since 2010, and that this gene was also defeated by all *P. striiformis* f. sp. *tritici* isolates in central AB tested by Holtz et al. (2013). However, in the United States, *Yr32* has remained effective against the majority of *P. striiformis* f. sp. *tritici* isolates since the 1960s (Liu et al. 2017).

The *Yr1* gene was not defeated by any tested isolate in this study; however, a single race from Bow Island in AB was found to have defeated this gene in 1990 (Su et al. 2003), and in 2011, virulence on *Yr1* was reported at a very low frequency in AB (Brar and Kutcher 2016; Holtz et al. 2013). Races virulent on 'Chinese 166' (*Yr1*) were first detected in Canada (Newton and Johnson 1936; Newton et al. 1933) and the United States (Bever 1934); the ability to defeat 'Chinese 166' separated the North American population from the European (Wan et al. 2017). The North American *P. striiformis* f. sp. *tritici* races in the 1930s could be differentiated by *Yr1* and *Yr6*; in the United States, virulence to these two genes was also found in the 1960s collection (Wan et al. 2017). Virulence to *Yr1* has increased since 2000 in the United States (Liu et al. 2017), and this gene was also found to separate the U.S. isolates collected from 1968 to 2015 into two distinct groups that had very distinct molecular profiles (Wan et al. 2017). In the United States, *Yr1* is believed to play an important role in *P. striiformis* f. sp. *tritici* evolution and it was found to be positively correlated with *YrTye*, a gene present in many wheat cultivars in the PNW (Wan et al. 2016; Xiang et al. 2016).

In this study, virulence toward Tyee (*Yr76*) was present at very low frequencies in AB, and the lack of virulence on *Yr1* and *YrTye* in Canada compared with the United States remains unexplained. Virulence to *YrSP* was present at a low frequency, and no virulence was detected on *Yr5* and *Yr15*. The genes *YrSP*, *Yr5*, and *Yr15* have not been deployed in common cultivars, and may explain the lack of virulence toward these genes.

Overall, our results showed that *P. striiformis* f. sp. *tritici* in Canada has evolved to have a higher and wider spectrum of virulence over the last 33 years, and the pathogen population in Canada is similar to that in the United States, but the pathogen may evolve new combinations of virulence at a local scale. The erosion of seedling-resistance to this pathogen necessitates the adoption of new sources of resistance; adult-plant resistance is needed to provide long-term protection from *P. striiformis* f. sp. *tritici*.

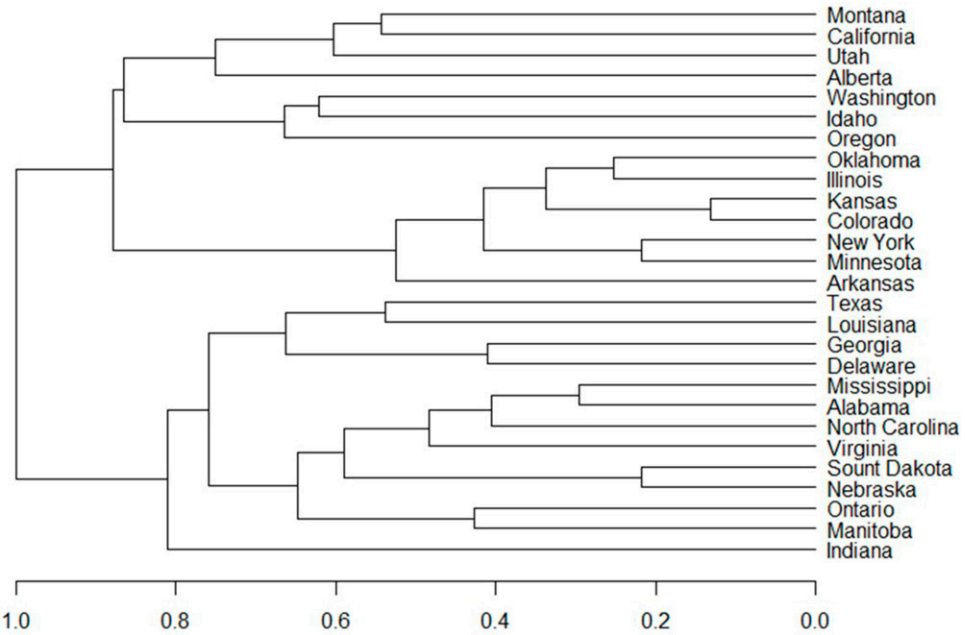

Fig. 5. Dendrogram showing the β-diversity of stripe rust isolates collected since 2010 among each of the Canadian provinces of Alberta (AB), Manitoba (MB), and Ontario (ON), and states in the United States, where data were available.

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