Plant Pathology (2016) **65**, 402–411 Doi: 10.1111/ppa.12433

Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region

M. S. Hovmøller[a]*, S. Walter[a], R. A. Bayles[b], A.Hubbard[b], K. Flath[c], N. Sommerfeldt[c], M. Leconte[d], P. Czembor[e], J. Rodriguez-Algaba[a], T. Thach[a], J. G. Hansen[a], P. Lassen[a], A. F. Justesen[a], S. Ali[af] and C. de Vallavieille-Pope[d]

[a]*Department of Agroecology, Aarhus University, Flakkebjerg, DK-4200, Slagelse, Denmark;* [b]*National Institute of Agricultural Botany, CB3 0LE, Cambridge, UK;* [c]*Federal Research Centre for Cultivated Plants, Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland 14532, Kleinmachnow, Germany;* [d]*INRA UMR 1290 BIOGER, BP01, 78850 Thiverval-Grignon, France;* [e]*Plant Breeding & Acclimatization Institute, Radzikow, 05-870 Blonie, Poland; and* [f]*Institute of Biotechnology & Genetic Engineering, the University of Agriculture, Peshawar, Pakistan*

Isolates of recently spreading races of yellow rust from wheat and triticale in Europe were analysed using virulence phenotypic data of 2605 isolates sampled in 12 countries between 2000 and 2014. A subset of 239 isolates was investigated by microsatellite markers. At least three races of non-European origin, termed 'Warrior', 'Kranich' and 'Triticale aggressive', were identified in the post-2011 population. The Warrior race was already present in high frequencies in the first year of detection in most European countries and to a large extent it replaced the pre-2011 European population. In contrast, the two other exotic races were localized to certain regions and/or crop type. The presence already of at least six multilocus genotypes of the Warrior race and five genotypes of the Kranich race in the first year of detection and across large areas is consistent with a hypothesis of aerial spread from genetically diverse source populations. A comparison with reference isolates sampled from six continents suggested that the Warrior and Kranich races originated from sexually recombining populations in the centre of diversity of the yellow rust fungus in the near-Himalayan region of Asia. However, the Triticale aggressive race was most similar to populations in the Middle East/Central Asia. The study illustrated the potential role of sexual *Puccinia striiformis* populations as a reservoir for new races replacing distant clonal populations.

Keywords: host resistance, invasion, population genetics, stripe rust, virulence

Introduction

The wheat yellow (stripe) rust pathogen *Puccinia striiformis* is a biotrophic basidiomycete that has a capacity for long-distance migration by airborne spores (Zadoks, 1961; Hodson, 2011; Hovmøller *et al.*, 2011). A number of invasions originating from distant geographical areas have been reported, either as an incursion to a region where it was previously absent (e.g. North and South America, Australia and South Africa), or as re-emergence of new virulent strains with increased aggressiveness and tolerance to high temperatures in North America, Australia and North Africa (Singh *et al.*, 2004; Hovmøller *et al.*, 2008; Bahri *et al.*, 2009; Milus *et al.*, 2009; Ali *et al.*, 2014). The pathogen has a recombinant population structure and a high diversity in the centre of diversity in the Himalayan and near-Himalayan region, but a clonal population structure in Europe, America and Australia (Wellings & McIntosh, 1990; Hovmøller *et al.*, 2011; Ali *et al.*, 2014).

In the clonal population in Europe, the evolution of virulence to different host resistance genes has been documented through the comprehensive monitoring of *P. striiformis* races (virulence phenotypes) since the 1930s (Gassner & Straib, 1932; Bayles *et al.*, 2000; Hovmøller, 2001; de Vallavieille-Pope *et al.*, 2012). The role of mutation was first noted in the early 20th century (Gassner & Straib, 1932) and later documented to explain single step increase in virulence in European and Australian races (Wellings & McIntosh, 1990; Hovmøller & Justesen, 2007b). The characteristics of recent and significant races of *P. striiformis* in Europe, year of first detection and genetic grouping based on molecular markers are summarized in Table 1.

Most of the predominant races up to 2010 were typical to the NW-European genetic group although races of atypical virulence phenotypes were also observed in several countries and years (Enjalbert *et al.*, 2005; Hovmøller & Justesen, 2007a). The latter often had distinctly different molecular characteristics and were interpreted as 'exotic' to the European population. Most exotic races up to

*E-mail: mogens.hovmoller@agro.au.dk

Published online 20 July 2015

Plant Pathology

Table 1 First year of detection of common races of *Puccinia striiformis* in Europe and their virulence phenotype and genetic grouping

First year of detection	Common name	Virulence phenotype	Number of isolates	Genetic group	Reference
<2000	Brigadier, Audace	1, 2, 3, 9, 17, 25	114	EU[a]	Hovmøller & Justesen (2007a)
<2000	Brigadier, Audace, +V4	1, 2, 3, 4, 9, 17, 25	343	EU	Hovmøller & Justesen (2007a)
<2000	Foreman	1, 2, 3, 4, 9, 25	18	EU	Hovmøller & Justesen (2007a)
<2000	Madrigal, Ornicar	1, 2, 3, 6, 9, 17, 25	54	EU	Hovmøller & Justesen (2007a)
<2000	Madrigal, Ornicar, +V4	1, 2, 3, 4, 6, 9, 17, 25	178	EU	Hovmøller & Justesen (2007a)
<2000	Robigus	1, 2, 3, 4, 9, 17, 25, 32	403	EU	Hovmøller & Justesen (2007a)
<2000	*Pst*S3	2, 6, 7, 8, 25	75	non-EU	Enjalbert *et al.* (2005)
<2000	Robigus, +V7	1, 2, 3, 4, 7, 9, 17, 25, 32	19	EU	Hovmøller & Justesen (2007b) and
<2000	Triticale non-aggressive[b]	None	17	EU/non-EU	present study
2000	Oakley, Solstice	1, 2, 3, 4, 6, 9, 17, 25, 32	134	EU	Present study
2000	Oakley, Solstice, +V7	1, 2, 3, 4, 6, 7, 9, 17, 25, 32	24	EU	Present study
2000	*Pst*S2	2, 6, 7, 8, 9, 25, 27	32	non-EU	Hovmøller & Justesen (2007b)
2006	Triticale aggressive	2, 6, 7, 8, 10	107	non-EU	Present study
2008	Tulsa	3, 4, 6, 25, 32	110	EU	Present study
2011	Warrior	1, 2, 3, 4, 6, 7, 9, 17, 25, 32, Sp	687	non-EU	Present study
2011	Kranich	1, 2, 3, 6, 7, 8, 9, 17, 25, 32	132	non-EU	Present study
Total (16 most common races)			2447		

[a]NW-European population as defined by Hovmøller *et al.* (2008).
[b]Non-aggressive race observed on triticale and barley.

2010 only had minor impact on wheat in Europe due to the presence of resistance preventing their establishment on most of the deployed host varieties (de Vallavieille-Pope *et al.*, 2012). However, a Triticale aggressive race, first detected in 2006, caused yield losses up to 100% in triticale in Scandinavia, where the epidemics were often difficult to control particularly for organic growers.

In 2011 two new races, termed Warrior and Kranich, were detected on both wheat and triticale in many European countries (www.wheatrust.org). Isolates of the two races had a number of unusual traits compared to typical isolates from European populations, e.g. by causing more disease on adult plants of wheat genotypes carrying long-term effective adult plant resistance and less disease on others, including previously susceptible genotypes (Sørensen *et al.*, 2014). Furthermore, telia were often produced in high quantities on infected leaves (Rodriguez-Algaba *et al.*, 2014), suggesting that the new races may have evolved from a sexual population (Ali *et al.*, 2010). A recent study of yellow rust in the UK in 2013 revealed a significant shift in the population structure and a high diversity compared to isolates collected in the past (Hubbard *et al.*, 2015). In summary, understanding of the recent changes in epidemiology and population structure of *P. striiformis* in Europe is fragmented and additional analyses and documentation are needed.

The present study provided an opportunity to explore the ongoing dynamics of a clonal plant pathogen population at a continental scale. A comprehensive data set was used, consisting of phenotypic and genotypic data from large international collections of *P. striiformis* isolates together with the worldwide data set from Ali *et al.* (2014) as a main reference. This enabled investigation of: (i) to what extent the pre-existing clonal yellow rust population in Europe was replaced, (ii) whether the recently spreading races originated through mutation or recombination within the European population or by migration from outside Europe, (iii) the potential source populations of races if these were exotic to Europe, and (iv) the level of diversity within the new races. The results are discussed in relation to invasion biology of crop pathogens in general and more specifically with respect to the implications for future crop disease management and resistance breeding strategies.

Materials and methods

Yellow rust sample collection, multiplication and virulence phenotyping

Puccinia striiformis isolates were derived from infected wheat leaves, which were submitted to or collected by the national yellow rust virulence survey programmes in France (de Vallavieille-Pope *et al.*, 2012), the United Kingdom (UK Cereal Pathogen Virulence Survey; Bayles *et al.*, 2000), Germany (Dawit *et al.*, 2009), Poland and the Global Rust Reference Centre (GRRC) in Denmark (Hovmøller & Justesen, 2007a). The number of samples submitted varied across years and countries, which reflected the national resources allocated for the survey or the significance of yellow rust disease in different years. A total of 2605 isolates sampled from 2000 to 2014 were virulence phenotyped while a subset of 239 isolates was selected for microsatellite genotyping in an attempt to represent predominant races between 2009 and 2013 in different countries at different locations (Table 2).

Isolate recovery and virulence phenotyping (race identification) was made according to the standard procedures of the respective national laboratories (Bayles *et al.*, 2000; de Vallavieille-Pope *et al.*, 2012), and Danish isolates and reference isolates from France, Germany and the UK, and additional samples from Belgium, the Czech Republic, Poland, Portugal, Slovakia, Spain and Sweden were processed by GRRC according to Hovmøller & Justesen (2007b).

Table 2 Origin and number of *Puccinia striiformis* isolates that have been race phenotyped and single sequence repeat genotyped

Country	Year	No. of isolates genotyped	No. of isolates race phenotyped	Race phenotyping laboratory[a]
Austria	2000–2009	–	26	GRRC
	2012	–	2	GRRC
	2013	–	1	GRRC
Belgium	2014	–	8	GRRC
Czech Republic	2014	–	1	GRRC
Denmark	2000–2009	4	370	GRRC
	2010	2	6	GRRC
	2011	19	30	GRRC
	2012	12	30	GRRC
	2013	47	18	GRRC
	2014	–	80	GRRC
Finland	2012	6	–	GRRC
France	2000–2009	–	666	INRA
	2010	5	49	INRA
	2011	11	201	INRA
	2012	7	244	INRA
	2013	–	156	INRA
Germany	2000–2009	–	167	JKI
	2010	1	8	JKI
	2011	–	12	JKI
	2012	15	4	JKI
	2013	–	4	JKI
	2014	–	34	JKI
Poland	2014	–	19	IHAR/GRRC
Portugal	2013	4	3	GRRC
Slovakia	2014	–	5	GRRC
Spain	2011	–	1	INRA
	2012	13	6	GRRC
	2013	19	8	GRRC
	2014	–	6	GRRC
Sweden	2000–2009	–	45	GRRC
	2010	19	22	GRRC
	2011	20	36	GRRC
	2012	1	11	GRRC
	2013	–	13	GRRC
	2014	–	19	GRRC
UK[b]	2000–2009	–	231	NIAB
	2010	20	12	GRRC
	2011	13	1	GRRC
	2012	1	26	NIAB
	2013	–	24	NIAB
Total		239	2605	

[a]GRRC, Global Rust Reference Centre, Aarhus University, Denmark; IHAR, Instytut Hodowli i Aklimatyzacji Roślin, Poland; INRA, Institute National de la Recherche Agronomique, France; JKI, Julius Kühn Institute, Germany; NIAB, National Institute of Agricultural Botany, UK.
[b]In 2006–2011 NIAB did not race phenotype for all considered virulences and corresponding isolates were removed from the analyses.

Compilation of a common race phenotype data set in a public database

Virulence phenotype data from all national laboratories and GRRC were compiled into a common data set. A core set of 13 virulences, corresponding to host resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr25*, *Yr32* and the resistance in Spaldings Prolific (*Sp*) were considered for the virulence phenotyping, and only isolates that were phenotyped for all of these were included in the study. All data, including incomplete race phenotyping data, are stored, managed and can be displayed via the Wheat Rust Toolbox database (http://wheatrust.org/service/), a data management system developed in the frame of the Borlaug Global Rust Initiative (http://www.globalrust.org/) and hosted by the Global Rust Reference Centre (Hansen & Lassen, 2013).

DNA extraction and molecular genotyping

DNA was extracted, using modified CTAB protocols, from spores (Justesen *et al.*, 2002) or from infected wheat lesions (Ali *et al.*, 2011). Molecular genotyping was carried out using 16 microsatellite loci i.e. RJN3, RJN4, RJN5, RJN6, RJN8, RJN9, RJN10, RJN11, RJN12, RJN13, RJO4, RJO18, RJO20, RJO21, RJO24, WU-6 (Ali *et al.*, 2011). These represented a subset of 16 of the 20 microsatellite loci used in the worldwide study by Ali *et al.* (2014) and enabled a common analysis of the two data sets. The microsatellites were amplified using the Type-it Microsatellite PCR kit (QIAGEN) in two multiplex reactions (Ali *et al.*, 2011; Rodriguez-Algaba *et al.*, 2014), with PCR product separation carried out with an Applied Biosystems 3730xl DNA Analyzer (Life Technologies Corp.) using the services of Uppsala Genome Centre, Uppsala University, Sweden. The chromatograms were scored with GeneMarker (SoftGenetics).

Population genetic analysis

To investigate the diversity and postulate the source of post-2011 European races, a series of population genetic analyses were made and compared with the reference data set representing worldwide populations described by Ali *et al.* (2014) and microsatellite genotyping results for additional isolates from China collected in the 1980s (Thach *et al.*, 2015). The sufficiency of markers to describe the population structure was assessed through the detection of multilocus genotypes (MLGs) plotted against the number of loci, using GENCLONE (Arnaud-Haond & Belkhir, 2007). An assessment was made of the divergence of these recent races from pre-existing European races and from isolates representing the worldwide genetic groups (Ali *et al.*, 2014). The level of divergence was assessed using pairwise F_{ST} statistics between pairs of populations, using GENETIX v. 4.05.2 (Belkhir *et al.*, 2004).

Nonparametric discriminant analyses of principal components (DAPC) were carried out using the ADEGENET package implemented in the R environment (Jombart *et al.*, 2010), to test the clustering of these recent races with the previously described worldwide populations. The model-based Bayesian assignment with STRUCTURE v. 2.2 (Pritchard *et al.*, 2000) was then used to confirm the results generated by DAPC. STRUCTURE was run for 200 000 iterations with a 100 000 burn-in period, with K ranging from 1 to 10, using the model with admixture and was run in 20 independent replications for each K. CLUMPP was used to process the STRUCTURE outputs, using a G′-statistic greater than 70% to assign groups of runs with a common clustering pattern.

The diversity within the recent races was assessed through inspecting the number of MLGs detected within each race, estimated with GENCLONE (Arnaud-Haond & Belkhir, 2007); gene diversity, number of alleles and allelic richness, estimated with

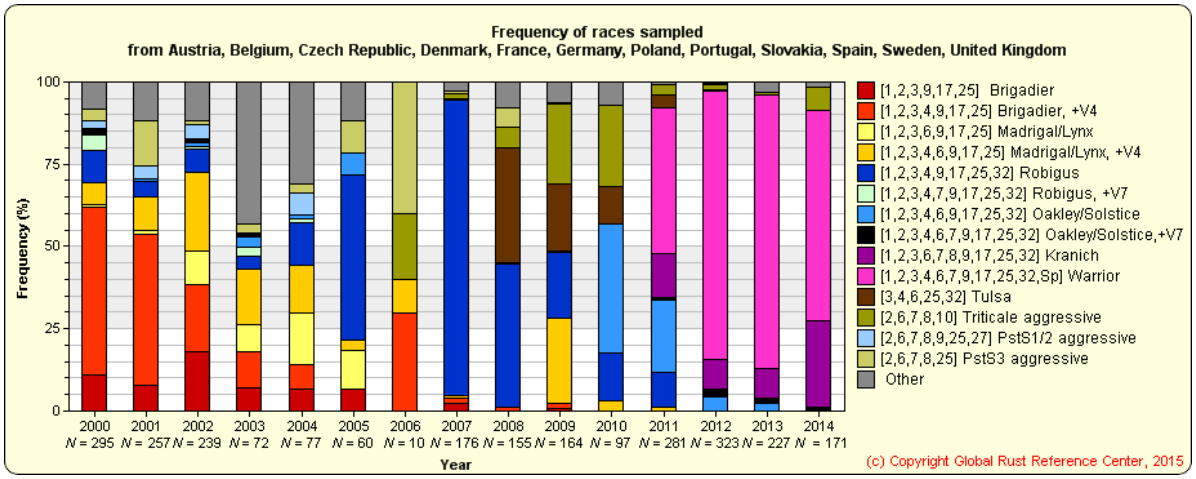

Figure 1 Dynamics of prevalent races of *Puccinia striiformis* sampled from wheat and triticale in Europe between 2000 and 2014. The virulence phenotype (in square brackets) and a common name referring to the commercial wheat variety where the race first caused yellow rust epidemics is shown in the legend to the right.

FSTAT (Goudet, 2001); and genotypic diversity, estimated with MULTILOCUS (Agapow & Burt, 2001). To avoid the effect of epidemic clonality, calculations were performed using both the whole and the clone-corrected data set, in which only one representative of each repeated MLG was considered.

Results

Race dynamics

The dynamics and spatial distribution of the 14 most common races (on average) in Europe between 2000 and 2014 are shown in Figures 1 and 2. A number of significant shifts in the distribution of races between 2000 and 2014 were observed. In 2000, races that were often associated with epidemics on *Yr17*-resistant varieties, such as Brigadier, were prevalent. These races became gradually replaced by races of similar virulence phenotype, but with additional virulence to *Yr6* (termed 'Madrigal/Lynx'), followed by a group of races with additional virulence to *Yr32*, which often caused epidemics on the cultivar Robigus. From 2007 to 2008 onward, these latter races were gradually replaced by the 'Oakley/Solstice' races up to 2010. At least four races of quite different virulence phenotype were observed in the same period, '*Pst*S2' (first detected in 2000), '*Pst*S3' (first detected before 2000), 'Triticale aggressive' from 2006 and 'Tulsa' from 2008.

From 2011 onwards, a major shift appeared and two races not reported previously, termed 'Warrior' and 'Kranich', were detected at multiple sites and often in high

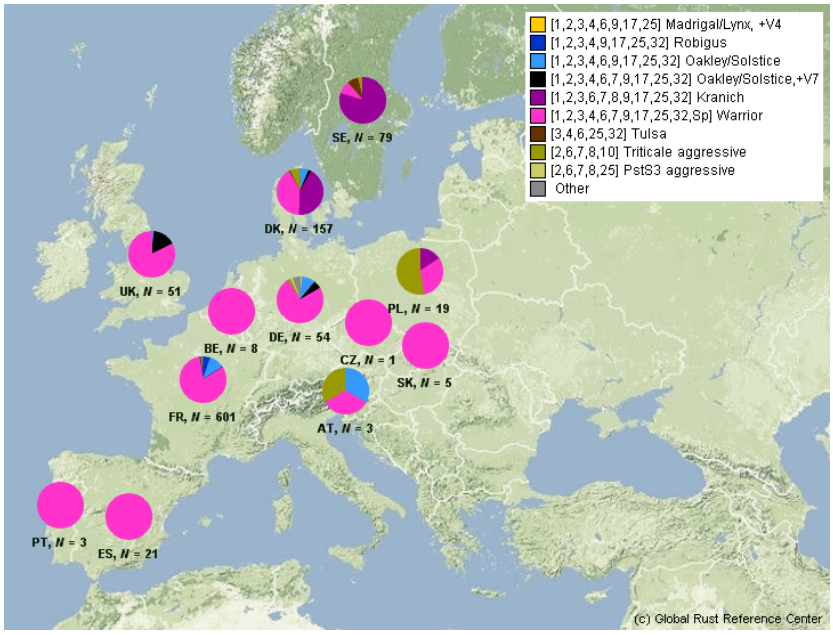

Figure 2 Spatial distribution in Europe of *Puccinia striiformis* races between 2009 and 2013. The virulence phenotype (in square brackets) and a common name referring to the commercial wheat variety where the race first caused yellow rust epidemics is shown in the legend to the right.

Table 3 Divergence (F_{ST}) among common races of *Puccinia striiformis* in Europe

	Warrior	Kranich	Triticale aggressive	Tulsa	Solstice/ Oakley	NW-European races
Warrior	–	0·358	0·514	0·566	0·562	0·508
Kranich	0·000	–	0·518	0·533	0·529	0·473
Triticale aggressive[a]	0·000	0·000	–	0·598	0·616	0·521
Tulsa[b]	0·000	0·000	0·000	–	**0·000**	**0·000**
Solstice/Oakley[b]	0·000	0·000	0·000	**0·304**	–	**0·010**
NW-European races[b]	0·000	0·000	0·000	**0·325**	**0·159**	–

The upper diagonal shows the F_{ST} values and the lower diagonal show the corresponding *P* values. The bold values refer to nonsignificant differentiation.

[a]Aggressive race prevalent on triticale.

[b]Representatives of the old NW-European population on wheat.

frequencies. Isolates of the Warrior race were present in many countries already in the first year of detection (2011), e.g. in Denmark, France, Germany, Spain, Sweden and the UK. The Warrior race was also detected in samples collected between 2012 and 2014 in Austria, Belgium, the Czech Republic, Slovakia, Poland and Portugal (Fig. 2). Isolates of the Kranich race were found in Denmark, Poland, Sweden and Finland, the latter based on interpretation of microsatellite results in the absence of recovered spore samples (data not shown). Generally, the Warrior race increased in frequency after the first year of detection. Additional virulence variants within the Warrior race, based on resistance specificities in supplementary commercial wheat varieties, were also observed. For instance, a variant that contained all the virulence characteristics of the Warrior race, but was avirulent on Ambition, was detected in increasing frequencies from 2012 (data not shown).

Microsatellite diversity

Representative isolates of the Warrior and Kranich races from different countries and years, and of races with similar virulence phenotypes from 2009 to 2010, as well as reference isolates from previous years were selected for microsatellite (SSR) marker analysis (Table 2). The Warrior and Kranich races were clearly different from typical NW-European races based on F_{ST} values around 0·5 or more and they were clearly different from each another (F_{ST} = 0·358; Table 3). A race that was typically sampled from triticale also diverged significantly from races of NW-European origin (F_{ST} = 0·521). In contrast, isolates of Tulsa and Solstice/Oakley races were not significantly different from typical NW-European races (F_{ST} values of 0·01 or below). In summary, very few of the 'old' European races detected between 2000 and 2010 were observed from 2011 to 2014, suggesting a continuing replacement of the pre-existing European clonal population by new races.

Divergence of the post-2011 European races from the worldwide populations

A comparison of the pre- and post-2011 races with six worldwide population genetic groups (G1–G6) revealed a high and significant differentiation from most of these populations (Table 4), except for the typical European races, which were not significantly different from the NW-European genetic group (G6). In contrast, isolates of the three non-European races (cf. Table 3) were distinctly different from all six genetic groups and none of them were resampled. The highest degree of similarity for the three races was observed for isolates of the Triticale aggressive race and Mediterranean-Central Asian genetic group

Table 4 Within-race diversity of recent European races of *Puccinia striiformis* and their divergence (F_{ST}) from the worldwide populations

				Divergence (F_{ST}) from worldwide genetic groups (Ali *et al.*, 2014)					
Race	No. of alleles	Gene diversity	Allelic richness	Near-Himalaya (G1)	Near-Himalaya (G2)	Near-Himalaya (G3)	Middle-East (G4)	Mediterranean-Central Asia (G5)	NW-European (G6)
Warrior	1·813	0·206	1·425	0·145	0·336	0·660	0·427	0·446	0·519
Kranich	1·625	0·223	1·448	0·254	0·349	0·604	0·424	0·443	0·490
Triticale aggressive[a]	1·625	0·283	1·562	0·452	0·362	0·508	0·265	0·115	0·571
Tulsa[b]	1·375	0·188	1·375	0·359	0·444	0·632	0·490	0·504	**0·059**
Solstice/Oakley[b]	1·625	0·204	1·425	0·374	0·463	0·635	0·499	0·540	**0·066**
NW-European races[b]	2·250	0·285	1·704	0·314	0·392	0·588	0·436	0·451	**0·044**

Values in bold represent nonsignificant F_{ST} differentiation.

[a]Aggressive race prevalent on triticale.

[b]Recent representatives of the old NW-European population on wheat.

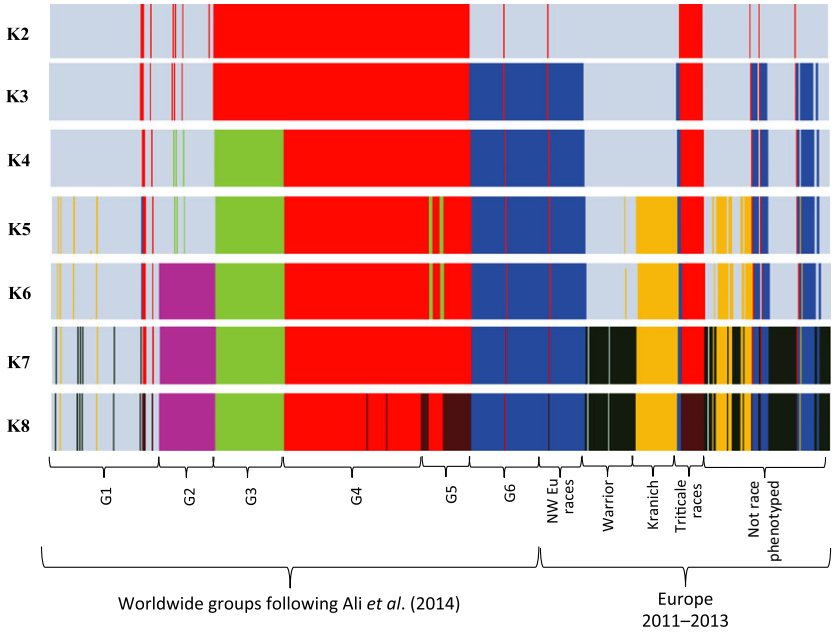

Figure 3 Discriminant analysis of principal components (DAPC) of the recent *Puccinia striiformis* races prevalent in Europe and the worldwide populations. Results shown for *K* values from 2 to 8, where G1 represents the genetic group predominant in China; G2 in Nepal; G3 in Pakistan; G4 in Middle East–East Africa; G5 in Mediterranean region–Central Asia; G6 in NW Europe according to Ali *et al.* (2014).

(G5), F_{ST} = 0·115, and between isolates of the Warrior race and the near-Himalayan G1 group (F_{ST} = 0·145). Gene diversity, number of alleles, allelic richness and genotypic diversity tended to be higher for the three non-European races compared to the NW-European races.

A nonparametric discriminant analysis of principal components (DAPC) was made to assess the clustering of the recent European races in relation to the previously described six worldwide genetic groups (Fig. 3). The analyses made at *K* values ranging from 2 to 4 detected the same genetic groups as previously described, where the non-European races Warrior and Kranich grouped with isolates from the near-Himalayan group G1. At *K* = 5, isolates of the Kranich race were separated whereas isolates of the Warrior race separated at *K* = 7. Isolates of the Triticale aggressive race were assigned to the Mediterranean group/Central Asian group, which appeared at *K* = 8. Additional samples, which had not been recovered from infected wheat leaves and therefore not race phenotyped, were all assigned to existing genetic groups. F_{ST} estimates revealed a significant divergence between the DAPC groups (data not shown). The results of DAPC were compared with the model-based Bayesian assignment method implemented in STRUCTURE, and revealed almost the same assignment results (data not shown).

The number of multilocus genotypes (MLGs) was relatively high for the Warrior and Kranich genetic groups compared to the pre-2011 European population, which displayed very limited diversity. A total of six MLGs were detected in the Warrior race, and five MLGs were detected in the Kranich race (Table 5). MLGW-6 in the Warrior race (representing an Ambition-avirulent variant) differed by at least five SSR alleles from the other groups whereas most of the other groups in the Warrior race differed by one or two alleles only (data not shown). The five MLGs in the Kranich race also differed by one or two alleles only. Most of the MLGs in both races were found from the first year of detection in 2011, often in several countries.

Discussion

This study provides evidence of a gradual replacement of the pre-existing clonal population of the yellow rust

Table 5 Distribution of single sequence repeat multilocus genotypes (MLG) detected within the Warrior and Kranich races

Race	Multilocus genotype	Country	2011	2012	2013	Total
Warrior	MLGW-1	Denmark	2	1	24	27
		France	6	5	–	11
		Germany	–	4	–	4
		Portugal	–	–	3	3
		Spain	–	13	19	32
		UK	6	–	–	6
	MLGW-2	France	1	–	–	1
		Germany	–	1	–	1
		Sweden	1	–	–	1
		UK	4	–	–	4
	MLGW-3	Denmark	–	–	1	1
	MLGW-4	UK	1	–	–	1
	MLGW-5	UK	1	–	–	1
	MLGW-6	Denmark	–	1	–	1
Kranich	MLGK-1	Denmark	4	8	19	31
		Finland	–	3	–	3
		Sweden	13	1	–	14
	MLGK-2	Denmark	–	–	2	2
		Finland	–	2	–	2
	MLGK-3	Sweden	2	–	–	2
	MLGK-4	Finland	–	1	–	1
	MLGK-5	Sweden	1	–	–	1
Total			41	41	68	150

fungus in Europe by races of exotic origin. Such a replacement of clonal populations by exotic incursions has previously been observed in the USA (Milus *et al.*, 2009) and in Australia (Wellings, 2007). However, the replacement population in these countries differed from the current European population by having a very limited molecular diversity after the first detection in 2000. In the present study, the new exotic races showed a significantly higher diversity than races of the pre-existing clonal population. These results emphasize the fact that, despite the continuous development and deployment of host genotypes with resistance to the prevalent pathogen population, invasions may initiate new disease epidemics at a continental scale (Brown & Hovmøller, 2002).

At least three exotic races of *P. striiformis* were detected in this study. The Triticale aggressive race (from 2006 onwards) and the Warrior and Kranich races (from 2011 onwards) all became prevalent over large areas within one or a very few years. The Warrior and Kranich races resulted in a continuing replacement of the races that were typical of the European population before 2011. The strong divergence of the exotic races from pre-2011 European races (Hovmøller *et al.*, 2002; Enjalbert *et al.*, 2005) excludes mutation or recombination within the pre-2011 European population as a possible origin. Furthermore, their close relatedness with isolates previously sampled in the near-Himalayan region (Ali *et al.*, 2014), including several alleles that are unique to this region and absent in the pre-2011 population in Europe, suggests an origin from within this area, i.e. the centre of diversity of the yellow rust fungus. This is consistent with a recent study in the UK based on samples collected in 2013, which concluded that isolates of the Warrior race showed much higher genetic diversity than races in the past and that they were very dissimilar to previous European races (Hubbard *et al.*, 2015). The emergence of the Warrior and Kranich races in many European countries in the same year (2011), and the presence already of at least 5–6 MLGs within each race in the first few years after detection, despite limited sampling efforts, suggests large-scale aerial dispersal from a diverse source population rather than a spread from a single point source. An influx from diverse populations in the near-Himalayan region to Europe, via one or more consecutive jumps, could explain their origin in the pathogen's centre of diversity. The two races may have established in Europe after the 2010 wheat cropping season, where yellow rust epidemics on wheat were scarce or absent in many wheat growing areas. This provided an open niche in such areas for invasions from outside Europe. It is well documented that *P. striiformis* may spread long distances through wind dispersal of spores (Zadoks, 1961; Brown & Hovmøller, 2002). During the subsequent establishment on wheat in Europe, the original diversity among aerial spores may have been reduced by selection (Hovmøller *et al.*, 1997; Stukenbrock & McDonald, 2008). For example, selection exerted by resistance genes which are often present in wheat grown in Europe (Bayles *et al.*, 2000; Hovmøller, 2007; de Vallavieille-Pope *et al.*, 2012) will reduce the frequency of races carrying the corresponding avirulences, and additional selection in favour of increased aggressiveness may further reduce diversity, as reported previously (Milus *et al.*, 2009). Isolates of the Warrior race were characterized by many virulences and preliminary results suggest a similar level of aggressiveness as *Pst*S1/*Pst*S2 spreading worldwide (Sørensen *et al.*, 2014). Drift resulting from a limited number of immigrants being originally established would also result in a lower genetic diversity than expected for a recombining source population (McDonald & Linde, 2002). Indeed, the post-2011 races in Europe exhibited relatively higher diversity than the pre-2011 European populations (Hovmøller *et al.*, 2002; Enjalbert *et al.*, 2005; Hubbard *et al.*, 2015), but the diversity was much lower than in the recombinant populations in the Himalayan and near-Himalayan regions of China, Pakistan and Nepal (Ali *et al.*, 2014).

The Triticale aggressive race, in Europe first detected in 2006 on the island Bornholm in the Baltic Sea, became common on widely grown triticale varieties in Germany and Scandinavia in the following years (Hovmøller *et al.*, 2011). It was detected in France in 2012 and 2013 at low frequency. The race was unusual by initiating resistance or intermediate resistance reactions on generally susceptible wheat varieties such as Avocet S, Anja and Morocco, and it was never detected on European winter wheat varieties in this study, probably also due to the presence of resistance genes in these varieties. Another race, which was avirulent on all wheat genotypes carrying any of the named *Yr* genes (often referred to as 0E0), was observed sporadically on triticale and barley over several locations and years, but it never became predominant in the population. The relatively few isolates of this race sampled in 2009–2010 were assigned to the NW-European group, whereas isolates of the same race sampled from triticale in 2003–2004 were clearly of non-European origin (Hovmøller & Justesen, 2007a), suggesting that this race may represent genetically very diverse isolates. Based on the authors' experience, resampling of identical races of different genetic background is not unusual. In fact, the Warrior race had a virulence phenotype that differed only by *Sp* virulence from a previous European race (cf. Table 1).

The infestation of a huge geographical area by a new race and the detection of several genotypes already within the first year are unusual for Europe. In the past, new races emerging by mutation have often been detected only at local or regional scales in the first year of detection and have spread to neighbouring countries in subsequent years. This was the case for *Yr17*-virulent races in the 1990s, which were first detected in the UK in 1994, and 3 years later in Denmark, France and Germany (Bayles *et al.*, 2000). In contrast, races of non-European origin, of which at least five have been detected since 2000 (cf. Table 1), have often been detected at multiple sites 1–2 years after first detection. The aggressive, high temperature-adapted strain *Pst*S2, which was avirulent on many European wheat varieties

(Hovmøller, 2007), was detected between 2000 and 2004 in several European countries but often at low frequencies (Hovmøller *et al.*, 2008; de Vallavieille-Pope *et al.*, 2012). Another high temperature-adapted strain, *Pst*S3 (also often referred to as 6E16), carrying a low number of virulences, was mainly present in southern Europe and only occasionally in the north (Enjalbert *et al.*, 2005; de Vallavieille-Pope *et al.*, 2012). *Pst*S2 and *Pst*S3 may have remained at low frequencies in Europe due to the presence of resistance genes such as *Yr1*, *Yr3*, *Yr17* or *Yr32* in European wheat varieties (cf. Hovmøller, 2007) that provided an effective control of these races (Hovmøller *et al.*, 2008; Mboup *et al.*, 2012; de Vallavieille-Pope *et al.*, 2012). In contrast, *Pst*S1/*Pst*S2 prevailed in many other areas of the world where susceptible wheat varieties were grown (Hovmøller *et al.*, 2008; Bahri *et al.*, 2009).

Isolates of the Warrior and Kranich races were also unusual compared to isolates sampled in Europe up to 2010 in that they produced telia in high quantities on infected leaves of both seedlings and adult plants; under experimental conditions the telia were able to produce basidiospores that successfully infected the alternate host, *Berberis vulgaris* (Rodriguez-Algaba *et al.*, 2014). However, this does not necessarily imply an immediate risk of the onset of sexual reproduction of *P. striiformis* under natural conditions in Europe. Successful recombination in nature would require a synchrony in the prevalence, susceptibility and phenology of *Berberis* spp., the alternate host, as well as the primary cereal host; this was not the case in the US Pacific Northwest (Wang & Chen, 2015) and it is most probably not the case in Europe, where *P. striiformis* has so far not been reported on *Berberis* spp. (Berlin *et al.*, 2013).

The pre-2011 *P. striiformis* population was strongly clonal and mainly dependent on mutation and intracontinental dispersal with little impact from sexual recombination (Hovmøller *et al.*, 2002). In a clonal population, mutations and subsequent selection would generate new races with virulence to the deployed host resistance genes (Linde *et al.*, 2002; de Vallavieille-Pope *et al.*, 2012), although virulences corresponding to undeployed resistance genes have also been reported (Hovmøller & Justesen, 2007b; Wellings, 2007). Invasions from outside, on the other hand, could result not only in the introduction of new virulences, but in new strains with completely different characteristics of fitness and/or aggressiveness (Smart & Fry, 2001; Milus *et al.*, 2009). Thus, the replacement of the population by a genetically more diverse population may increase the adaptive potential of the pathogen (McDonald & Linde, 2002). Future resistance breeding efforts should therefore consider testing new breeding materials using a wide array of pathogen races under quarantine conditions, or expose the breeding materials under field conditions in areas with high pathogen diversity.

The centre of diversity has been suggested to serve as an important source of new invasions of plant pathogens, e.g. *Phytophthora infestans* causing late blight on potato (Smart & Fry, 2001) and *Cryphonectria parasitica* causing chestnut blight (Dutech *et al.*, 2012). The current study has documented invasions from the pathogen's centre of diversity, which replaced a pre-existing clonal population of *P. striiformis* in Europe. This may have implications for disease management and resistance breeding. Future investigations would be required to detect further variability and the potential onset of pathogen sexual recombination in new areas. The study has also demonstrated the need to identify the worldwide population structure for crop pathogens having long distance dispersal capacities in order to assess future invasion risks and their consequences to ecology and crop production. The Europe-wide collaboration and compilation of data into a single data set in a common database proved to be a major advancement. It enabled the identification of current invasions at an early stage, and the assessment of their implications at the field level in many countries. The compilation of a single data set revealed some general challenges in merging data from different laboratories, countries and years. For instance, in the present study, a substantial number of almost complete race phenotype data from the United Kingdom between 2006 and 2011 were omitted because isolates were generally not investigated for all considered virulences, e.g. virulences to *Yr10* and/or the resistance in Spaldings Prolific. However, the WheatRustToolbox (www.wheatrust.org) now offers flexible access to race phenotype data for yellow rust worldwide, including spatial distribution of frequencies of individual virulences and virulence combinations, which can be selected by tick boxes as appropriate (Hansen & Lassen, 2013). Therefore, isolates with incomplete race phenotype data, e.g. where information about a single virulence restricts the calculation of a complete virulence phenotype, have also been included in the Toolbox database. Future collaborative efforts should be continued and strengthened, not only at the European scale but on a worldwide scale, for timely, early-warning of potential invasions by new variants of important crop pathogens with the capacity to spread over long distances within a very short period of time.

Acknowledgements

The authors are grateful to all their colleagues who contributed to the extensive sampling of isolates throughout the years. They especially thank the technical staff Ellen Jørgensen, Sarah Adams, Steen Meier, Laurent Gérard, Nathalie Galet and Frauke Gerken. This research has received funding from the Strategic Danish Research Council (grant no. 11-116241, RUSTFIGHT); the Danish Ministry of Food, Agriculture and Fisheries (grant no. 34120902567); the European Framework Programme 6 (grant no. 513959, BIOEXPLOIT); the European Framework Programme 7 (grant no. 265865, PURE); and CIMMYT and ICARDA supported the GRRC activities for race phenotyping of isolates sampled in Africa and Asia.

References

Agapow PM, Burt A, 2001. Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* **1**, 101–2.

Ali S, Leconte M, Walker A-S, Enjalbert J, de Vallavieille-Pope C, 2010. Reduction in the sex ability of worldwide clonal populations of *Puccinia striiformis* f. sp. *tritici*. *Fungal Genetics and Biology* **47**, 828–38.

Ali S, Gautier A, Leconte M, Enjalbert J, de Vallavieille-Pope C, 2011. A rapid genotyping method for an obligate fungal pathogen, *Puccinia striiformis* f. sp. *tritici*, based on DNA extraction from infected leaf and Multiplex PCR genotyping. *BMC Research Notes* **4**, 240.

Ali S, Gladieux P, Leconte M *et al.*, 2014. Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici*. *PLoS Pathogens* **10**, e1003903.

Arnaud-Haond S, Belkhir K, 2007. GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes* **7**, 15–7.

Bahri B, Leconte M, Ouffroukh A, de Vallavieille-Pope C, Enjalbert J, 2009. Geographic limits of a clonal population of wheat yellow rust in the Mediterranean region. *Molecular Ecology* **18**, 4165–79.

Bayles RA, Flath K, Hovmøller MS, de Vallavieille-Pope C, 2000. Breakdown of the *Yr17* resistance to yellow rust of wheat in northern Europe. *Agronomie* **20**, 805–11.

Belkhir KBP, Chikhi L, Raufaste N, Bonhomme F, 2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations: Laboratoire Génome, Populations, Interactions, CNRS UMR 5000: Université de Montpellier II, Montpellier (France). [http://kimura.univ-montp2.fr/genetix/]. Accessed 1 July 2015.

Berlin A, Kyaschenko J, Justesen AF, Yuen J, 2013. Rust fungi forming aecia on *Berberis* spp. in Sweden. *Plant Disease* **97**, 1281–7.

Brown JKM, Hovmøller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537–41.

Dawit W, Flath K, Weber WE, Schumann E, Kosman E, 2009. Virulence and diversity of *Puccinia striiformis* f. sp *tritici* in Ethiopia. *Canadian Journal of Plant Pathology* **31**, 211–9.

Dutech C, Barrès B, Bridier J, Robin C, Milgroom MG, Ravigné V, 2012. The chestnut blight fungus world tour: successive introduction events from diverse origins in an invasive plant fungal pathogen. *Molecular Ecology* **21**, 3931–46.

Enjalbert J, Duan X, Leconte M, Hovmøller MS, de Vallavieille-Pope C, 2005. Genetic evidence of local adaptation of wheat yellow rust (*Puccinia striiformis* f. sp. *tritici*) within France. *Molecular Ecology* **14**, 2065–73.

Gassner G, Straib W, 1932. Über Mutationen in einer biologischen Rasse von *Puccinia glumarum tritici* (Schmidt) Erikss. und Henn. *Zeitschrift für Induktive Abstammungs -und Vererbungslehre* **63**, 154–80.

Goudet J, 2001. FSTAT, a program to estimate and test gene diversities and fixation indices v. 2.9.3. [http://www2.unil.ch/popgen/softwares/fstat.htm]. Accessed 1 July 2015.

Hansen JG, Lassen P, 2013. Managing global crop disease data. In: *Proceedings of the EFITA-WCCA-CIGR Conference: Sustainable Agriculture Through ICT Innovation, 2013*. Turin, Italy: EFITA-WCCA-CIGR. [http://www.cigr.org/Proceedings/uploads/2013/0302.pdf]. Accessed 1 July 2015.

Hodson DP, 2011. Shifting boundaries: challenges for rust monitoring. *Euphytica* **179**, 93–104.

Hovmøller MS, 2001. Disease severity and pathotype dynamics of *Puccinia striiformis* f. sp. *tritici* in Denmark. *Plant Pathology* **50**, 181–9.

Hovmøller MS, 2007. Sources of seedling and adult plant resistance to *Puccinia striiformis* f. sp. *tritici* in European wheats. *Plant Breeding* **126**, 225–33.

Hovmøller MS, Justesen AF, 2007a. Appearance of atypical *Puccinia striiformis* f. sp. *tritici* phenotypes in north-western Europe. *Australian Journal of Agricultural Research* **58**, 518–24.

Hovmøller MS, Justesen AF, 2007b. Rates of evolution of avirulence phenotypes and DNA markers in a northwest European population of *Puccinia striiformis* f. sp. *tritici*. *Molecular Ecology* **16**, 4637–47.

Hovmøller M, Ostergard H, Munk L, 1997. Modelling virulence dynamics of airborne plant pathogens in relation to selection by host resistance in agricultural crops. In: Crute IR, Holub EB, Burdan JJ, eds. *The Gene-for-Gene Relationship in Plant–Parasite Interactions*. Wallingford, UK: CAB International, 173–90.

Hovmøller MS, Justesen AF, Brown JKM, 2002. Clonality and long-distance migration of *Puccinia striiformis* f. sp. *tritici* in north-west Europe. *Plant Pathology* **51**, 24–32.

Hovmøller MS, Yahyaoui AH, Milus EA, Justesen AF, 2008. Rapid global spread of two aggressive strains of a wheat rust fungus. *Molecular Ecology* **17**, 3818–26.

Hovmøller MS, Sørensen CK, Walter S, Justesen AF, 2011. Diversity of *Puccinia striiformis* on cereals and grasses. *Annual Review of Phytopathology* **49**, 197–217.

Hubbard A, Lewis CM, Yoshida K *et al.*, 2015. Field pathogenomics reveals the emergence of a diverse wheat yellow rust population. *Genome Biology* **16**, 23.

Jombart T, Devillard S, Balloux F, 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**, 94.

Justesen AF, Ridout CJ, Hovmøller MS, 2002. The recent history of *Puccinia striiformis* f. sp. *tritici* in Denmark as revealed by disease incidence and AFLP markers. *Plant Pathology* **51**, 13–23.

Linde CC, Zhan J, McDonald BA, 2002. Population structure of *Mycosphaerella graminicola*: from lesions to continents. *Phytopathology* **92**, 946–55.

Mboup M, Bahri B, Leconte M, de Vallavieille-Pope C, Kaltz O, Enjalbert J, 2012. Genetic structure and local adaptation of European wheat yellow rust populations: the role of temperature-specific adaptation. *Evolutionary Applications* **5**, 341–52.

McDonald BA, Linde C, 2002. Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* **40**, 349–79.

Milus EA, Kristensen K, Hovmøller MS, 2009. Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp. *tritici* causing stripe rust of wheat. *Phytopathology* **99**, 89–94.

Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–59.

Rodriguez-Algaba J, Walter S, Sørensen CK, Hovmøller MS, Justesen AF, 2014. Sexual structures and recombination of the wheat rust fungus *Puccinia striiformis* on *Berberis vulgaris*. *Fungal Genetics and Biology* **70**, 77–85.

Singh RP, William HM, Huerta-Espino J, Rosewarne G, 2004. Wheat rust in Asia: meeting the challenges with old and new technologies. In: *Proceedings of the 4th International Crop Science Congress*. [http://www.cropscience.org.au/icsc2004/symposia/3/7/141_singhrp.htm]. Accessed 1 July 2015.

Smart CD, Fry WE, 2001. Invasions by the late blight pathogen: renewed sex and enhanced fitness. *Biological Invasions* **3**, 235–43.

Sørensen CK, Hovmøller MS, Leconte M, Dedryver F, de Vallavieille-Pope C, 2014. New races of *Puccinia striiformis* found in Europe reveal race specificity of long-term effective adult plant resistance in wheat. *Phytopathology* **104**, 1042–51.

Stukenbrock EH, McDonald BA, 2008. The origins of plant pathogens in agro-ecosystems. *Annual Review of Phytopathology* **46**, 75–100.

Thach T, Ali S, Justesen AF, Rodriguez-Algaba J, Hovmøller MS, 2015. Recovery and virulence phenotyping of the historic 'Stubbs collection' of the yellow rust fungus *Puccinia striiformis* from wheat. *Annals of Applied Biology*. doi: 10.1111/aab.12227.

de Vallavieille-Pope C, Ali S, Leconte M, Enjalbert J, Delos M, Rouzet J, 2012. Virulence dynamics and regional structuring of *Puccinia striiformis* f. sp. *tritici* in France between 1984 and 2009. *Plant Disease* **96**, 131–40.

Wang M, Chen X, 2015. Barberry does not function as an alternate host for *Puccinia striiformis* f. sp. *tritici* in the US Pacific Northwest due to

teliospore degradation and barberry phenology. *Plant Disease*. doi: 10.1094/PDIS-12-14-1280-RE.

Wellings CR, 2007. *Puccinia striiformis* in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979–2006. *Australian Journal of Agricultural Research* **58**, 567–75.

Wellings CR, McIntosh RA, 1990. *Puccinia striiformis* f. sp. *tritici* in Australasia: pathogenic changes during the first 10 years. *Plant Pathology* **39**, 316–25.

Zadoks JC, 1961. Yellow rust on wheat studies in epidemiology and physiologic specialization. *Tijdschrift over Plantenziekten* **67**, 69–256.