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New Races of *Puccinia striiformis* Found in Europe Reveal Race Specificity of Long-Term Effective Adult Plant Resistance in Wheat

Chris K. Sørensen, Mogens S. Hovmøller, Marc Leconte, Françoise Dedryver, and Claude de Vallavieille-Pope

First and second authors: Aarhus University, Faculty of Science and Technology, Department of Agroecology, Research Center Flakkebjerg, DK-4200 Slagelse, Denmark; third and fifth authors: INRA, UR1290 BIOGER-CPP, BP 01, 78850 Thiverval-Grignon, France; and fourth author: INRA, UMR IGEPP, Domaine de la Motte, BP35327, 35653 Le Rheu Cedex, France.

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ABSTRACT

Sørensen, C. K., Hovmøller, M. S., Leconte, M., Dedryver, F., and 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* 104:1042-1051.

Resistance to *Puccinia striiformis* was examined in nine wheat recombinant inbred lines (RILs) from a cross between ‘Camp Rémy’ (resistant parent) and ‘Récital’ (susceptible parent) using an isolate of a strain common to the northwestern European population before 2011 (old) and two additional isolates, one representing an aggressive and high-temperature-adapted strain (*PstS2*) and another representing a virulence phenotype new to Europe since 2011 (new). The RILs carried different combinations of quantitative trait loci (QTL) for resistance to *P. striiformis*. Under greenhouse conditions, the three isolates gave highly contrasting results for infection type, latent period, lesion length, and

diseased leaf area. The *PstS2* isolate revealed *Yr* genes and QTL which conferred complete resistance in adult plants. Six QTL had additive effects against the old isolate whereas the effects of these QTL were significantly lower for the new isolate. Furthermore, the new isolate revealed previously undetected resistance in the susceptible parent. Disease severity under field conditions agreed with greenhouse results, except for Camp Rémy being fully resistant to the new isolate and for two RILs being susceptible in the field. These results stress the need of maintaining high genetic diversity for disease resistance in wheat and of using pathogen isolates of diverse origin in studies of host resistance genetics.

Additional keywords: durable resistance, quantitative resistance, stripe (yellow) rust.

The severity of disease caused by plant pathogens on cereal crops depends on several factors, including the virulence and aggressiveness of the pathogen, resistance in the host, and environmental conditions (12,19). In modern agriculture, resistant host cultivars have often been introduced to control plant disease. However, the level of disease control may vary in time and space if a pathogen population adapts differentially to the resistant cultivars. Therefore, insight into the ability of the pathogen to adapt to host resistance, which may have severe consequences in terms of yield loss and food security, is highly important (12,38). Stripe (yellow) rust of wheat, caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici*, is one of the most important cereal diseases worldwide (23). Historically, *P. striiformis* has mainly been a problem in cool climates but, in recent years, the pathogen has become an increasing problem in areas normally considered too warm for stripe rust epidemics (13,47). Resistance in wheat to stripe rust has often been categorized as either qualitatively or quantitatively inherited (12). Qualitative resistance, in most cases, can be detected at the seedling stage and, therefore, is often referred to as seedling or all-stage resistance. It follows a host–pathogen interaction consistent with the gene-for-gene hypothesis (34). In wheat, 54 genes (*Yr* genes) for qualitative resistance against stripe rust have been named and an additional 35 reported (<http://www.shigen.nig.ac.jp/wheat/komugi/>). Qualitative resistance is very common in European wheat cultivars,

possibly due to its simple inheritance and effective disease control in the absence of virulent pathogen races (20,45). On the other hand, quantitative resistance to stripe rust is mainly expressed at the post-seedling stage, where it tends to become more and more effective as the host plant matures (29). Therefore, it is often referred to as adult plant resistance (APR) and works by delaying pathogen growth (e.g., through an increased latent period [LP] or reduction of lesion growth) (9,15,40). In many cases, APR in wheat has remained effective to control stripe rust even when used over large acreage for several years (i.e., “durable” in sensu Johnson [26]) (7). Recent genetic studies of APR to stripe rust have revealed a number of quantitative trait loci (QTL) with additive and, in some cases, epistatic effects of either minor or major importance (1,44). Durable stripe rust resistance has been observed in a number of commercially important French bread wheat cultivars with different combinations of genes for qualitative and quantitative resistance. In some of these cultivars, the resistance has remained effective for >10 years, even when deployed over large areas (16,30,39). The alleged durability of quantitative resistance has, in most cases, been predicted in a particular epidemiological environment (i.e., not taking into account the potential impact of pathogen isolates of different evolutionary origins). Therefore, the question is whether the emergence of new pathogen races can cause erosion or breakdown of previously effective quantitative resistance. In recent years, a number of atypical *P. striiformis* isolates have been reported in Europe (21) (www.wheatrust.org). This includes isolates of the aggressive and high-temperature-adapted strain *PstS2* which, together with the closely related strain *PstS1*, have played a significant role in the changing epidemiology of stripe rust with severe disease in relatively warm areas (24,37). Isolates of these new strains have spread on a global scale and now almost com-

Corresponding authors: C. K. Sørensen; E-mail address: chris.sorensen@agrsci.dk; and C. de Vallavieille-Pope; E-mail address: pope@grignon.inra.fr.

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pletely replaced previous *P. striiformis* populations in Australia and some regions of North America (33,47). In this study, we examined the impact of isolates representing races new to Europe on the resistance to stripe rust in recombinant inbred lines (RILs) derived from a cross between the French wheat ‘Camp Rémy’ (resistant to stripe rust under field conditions since 1980) and ‘Récital’ (susceptible). The resistance in Camp Rémy is known to be based on a combination of two genes for qualitative resistance (seedling resistance) and seven QTL for APR whereas the susceptible parent, Récital, carries the defeated *Yr6* resistance gene (30). The RILs possessed different combinations of *Yr* genes and QTL derived from the parental cultivars. The lines and the parent cultivars were inoculated with three isolates which had all been collected in France. One isolate belonged to a strain of typical northwestern European origin (17). The second isolate was detected for the first time at multiple locations in northwestern Europe in 2011 and it was of a race not previously detected in Europe (www.wheatrust.org) (22). A third isolate represented the *PstS2* strain of clearly non-European origin (3). Based on the evaluation of a range of epidemiological traits assayed in field and greenhouse, significant race specificity was demonstrated for both seedling resistance genes (*Yr* genes) and QTL for APR. The implications of the results for wheat resistance breeding and disease management strategies are discussed.

MATERIALS AND METHODS

Pathogen isolates and wheat genotypes. Three pathogen isolates were selected based on evolutionary origin, virulence phenotype, and aggressiveness. All three isolates had been collected in France and are part of the stripe rust collection at UR BIOGER, INRA Grignon, France. Isolates were inoculated onto 27 differential wheat genotypes, including 15 differential cultivars from the World and European set (27) for test of virulence and avirulence to the resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *YrSd*, *YrSp*, and *YrSu* (Table 1).

Infection type was evaluated on a 0-to-9 scale (35), where infection type 0 to 4 was considered resistant, infection type 5 and 6 intermediate, and infection type 7 to 9 virulent. Isolate J99198 (old) was first detected in France in 1999 and the race became common in 2002 (17). This isolate is of a strain typical to northwestern Europe at the time of detection (24). The second isolate, J11019 (new), was first detected in northwestern Europe in 2011, and the race was sampled in high frequencies in France in 2011 and 2012 (isolates of a similar race have been designated as the ‘Warrior’ or ‘Ambition’ race in different European countries) (www.wheatrust.org). The third isolate, J04003 (*PstS2*), was detected in France in 2004 and belongs to the aggressive and high-temperature-adapted strain *PstS2* (17). Isolates of the *PstS2* strain were first detected in Europe in 2000 (24,37). The wheat genotypes used in the experiment included nine RILs obtained by single-seed descent derived from a cross between the two French bread wheat cultivars, Camp Rémy and Récital. The lines were developed at UMR IGEPP, INRA Le Rheu, France, as a part of a genetic study of durable resistance in Camp Rémy (30). Camp Rémy possessed a unique combination of genes for qualitative and quantitative resistance, whereas only *Yr6* was detected in Récital. The nine recombinant lines were expected to carry different combinations of QTL for resistance derived from Camp Rémy (Table 2). Among the nine lines were two pairs of isolines selected in F_6 ($\approx 3\%$ heterozygosity). One pair, CRR2178R and CRR2178S, differed with respect to *QYr.inra.2BL.2* and *QYr.inra.2BS*, and another pair, CRR2182R and CRR2182S, differed for *QYr.inra.2BL.2* and *QYr.inra.2DS*. The inclusion of these isolines allowed evaluation of the effect of individual QTL without the confounding effect of genetic background. The seedling resistance genes in the nine RILs and their parents were confirmed by differential tests at the seedling stage, and all QTL had previously been detected by presence of molecular marker alleles of Camp Rémy and inoculated field experiments (30). The viability of spores and isolate aggressiveness (LP) under the considered experimental conditions was assessed on seedlings of

TABLE 1. Virulence phenotype of *Puccinia striiformis* isolates used in the experiment^a

Isolate ID	Common name	Virulence for <i>Yr</i> genes																	Race ^b
		1	2	3	4	5	6	7	8	9	10	17	25	27	32	Sd	Sp	Su	
J04003	<i>Pst</i> S2	–	2	–	–	–	6	7	8	9	–	–	25	27	–	(Sd)	–	–	6E16V9
J99198	Old	1	2	3	4	–	6	–	–	9	–	17	25	–	–	Sd	–	Su	237E141V17
J11019	New	1	2	3	4	–	6	7	–	9	–	17	25	–	32	Sd	(Sp)	Su	239E17t1V17

^a Numbers designate virulence corresponding to specific stripe rust resistance genes; Sd, Sp, and Su refer to resistance in Strubes Dickoff, Spaldings Prolific, and Suwon 92 × Omar, respectively; – indicates avirulence; parentheses indicate an intermediate infection type of 5–6 on a 0-to-9 scale (35).

^b Designation according to Johnson (26).

TABLE 2. Identified seedling and adult plant resistance in the two parental cultivars (‘Camp Rémy’ and ‘Récital’) and the recombinant inbred lines (RILs) used in this experiment^a

Host genotype	Identity ^b	Seedling resistance genes			Adult plant resistance						
		<i>Yr6</i>	<i>Yr7</i>	<i>QYr.inra-2BL.2</i> ^c	<i>QYr.inra-2BL.1</i>	<i>QYr.inra-2BS</i>	<i>QYr.inra-2AL.1</i>	<i>QYr.inra-2AL.2</i>	<i>QYr.inra-2DS</i>	<i>QYr.inra-5BL.1</i>	<i>QYr.inra-5BL.2</i>
Camp Rémy	Parent	–	+	+	+	+	+	+	+	+	+
Récital	Parent	+	–	–	–	–	–	–	–	–	–
CRR2083	RIL	–	+	–	+	–	+	+	–	+	+
CRR2283	RIL	+	–	–	–	–	–	–	–	–	–
CRR2019	RIL	–	–	–	–	–	–	–	+	–	–
CRR2020	RIL	+	–	–	+	+	+	+	+	–	+
CRR2088	RIL	–	–	–	+	–	+	–	+	–	+
CRR2178R	NIL	–	–	+	–	+	–	–	–	+	–
CRR2178S	NIL	–	–	–	–	–	–	–	–	+	–
CRR2182R	NIL	–	–	+	+	+	–	–	+	–	+
CRR2182S	NIL	–	–	–	+	+	–	–	–	–	+

^a Symbols: + indicates presence and – indicates absence of seedling resistance genes and quantitative trait loci (QTL). Designation of *QYr.inra* QTL is based on Mallard et al. (30) and Ma and Singh (29).

^b NIL = near-isogenic line (30,31).

^c *QYr.inra2BL.2* = *Yrsp*, seedling resistance gene according to (30).

'Cartago' and 'Victo', which were susceptible to all isolates used in this study.

Field experiment. Field trials were conducted at INRA Versailles (Ile de France) in 2010, 2011, and 2012. Percent leaf area with sporulating lesions was assessed by visual assessment of the RILs and the parent cultivars inoculated with the isolate J99198 (old) in 3 years (2010 to 2012). In 2010 and 2012, all lines and both parents were evaluated whereas, in 2011, only the four lines CRR2019, CRR2020, CRR2088, and CRR2178R and the two parents were included. In 2012, all genotypes were additionally tested to the multivirulent isolate J11019 (new). By the end of October, 30 seeds of each genotype were planted in 1.2-m rows in a complete randomized block design with two replicates. The two replicates, inoculated with identical isolates, were situated next to each other. In 2012, blocks inoculated with different isolates were located 700 m apart. These blocks were further separated by buildings and five rows of trees $\approx$ 15 to 30 m tall. The isolate J99198 (old) was multiplied on spreader rows of Victo (with no known *Yr* gene), and the isolate J11019 (new) was multiplied on 'Toison d'or' (*Yr32*). Spreader rows were sown at both ends of the blocks, perpendicular to and flanking the rows of the investigated genotypes. Inoculation of spreader plants was done in March with infected seedlings of Victo. Seedlings of Victo had been raised in growth chambers and inoculated at the two-leaf stage. Just before sporulation, these seedlings were planted in the field between plants in the spreader row, one pot per 2-m row. The scorings of average percent infected leaf area (sporulating lesions) on the upper three leaves were done three times during the growing season using the modified Cobb scale (0 to 100%) (42). Control cultivars were used to evaluate the risk of cross infection between blocks inoculated with different isolates. The level of natural infection was assessed by inspection of the fields surrounding the experimental plots.

Greenhouse experiment. Prior to the adult plant experiment, compatibility between pathogen isolates and the wheat genotypes were assessed using 16-day-old seedlings. The isolate J04003 (*PstS2*) showed incompatibility with Camp Rémy and four of the RILs at the seedling stage and, therefore, was only assessed on adult plants of Réctal and the five susceptible lines CRR2019, CRR2020, CRR2178S, CRR2083, and CRR2283. The two other isolates, J99198 (old) and J11019 (new), were used on all 11 wheat genotypes at the adult plant stage. Unlike isolate J04003, J99198 was assessed on adult plants of host genotypes for which it showed incompatibility at the seedling stage. This was done in order to compare results in the greenhouse with those obtained in the field. Isolates were multiplied on seedlings of Victo under identical environmental conditions for three generations in order to standardize the viability of the inoculum. The seedlings were grown in pots filled with standard peat soil (blocking substrate type 5; Gebr. Brill Substrate GmbH & Co. KG, Germany) and incubated in climate-controlled cabinets at temperatures of 20°C (day) and 14°C (night) and a 16-h photoperiod from natural and supplemental light at 300 μ E/m²/s photosynthetically active radiation (PAR). When they were $\approx$ 1-cm high, seedlings were treated with 20 ml of a maleic hydrazide solution (0.25 g/liter) per pot to slow the emergence of the secondary leaves and to increase spore production. The seedlings were inoculated when primary leaves were fully expanded by dusting spores from a pot of previously infected seedlings. Inoculated plants were incubated for 24 h in darkness in a dew chamber at 8°C and 100% relative humidity, after which they were placed in cabinets with conditions as described above. Before the onset of spore release, the infected plants were covered with cellophane bags to prevent cross-contamination. Spores for experimental use were collected $\approx$ 14 days after inoculation and subsequently kept in a desiccator with silica gel at 5°C for 4 days. Spores were additionally stored 3 to 4 days in liquid nitrogen in order to secure inoculum for the time when plants were ready for inoculation.

Adult plants were raised in the greenhouse in 1-liter pots filled with standard peat soil, following a vernalization period of 8 weeks at 6°C. Greenhouse temperature was maintained at 14°C at night and a maximum of 20°C during the day. A 16-h photoperiod from natural and supplemental light at 300 μ E/m²/s PAR was kept by the use of sodium vapor lamps. Each pot contained one plant and the number of tillers was reduced to two before inoculation. Adult plants were inoculated when the flag leaf was fully expanded, growth stage 40–48 (49). Seedlings of Victo and Cartago were grown in 7-by-7-cm pots by sowing six seeds per pot, reduced to five uniform plants prior to inoculation. Seedlings were grown under the same conditions as adult plants and inoculated when they were 2 weeks old and the second leaf fully expanded.

Adult plants were kept in high light intensity for at least 4 h before inoculation to favor infection (18). Spores were taken out of the liquid nitrogen and immediately heat shocked at 40°C for 10 min before use. Inoculum was prepared by mixing 5 mg of urediniospores with 25 mg of talcum powder. Inoculum was applied to the leaves on the adaxial side by gently pressing the edge of a plastic label (thickness $\approx$ 1 mm) onto the central part of the leaves to form a narrow band of spores across the leaf. This technique allowed precise measurement of LP and lesion growth rate.

For adult plants, 10 pots, each with two tillers, were used per treatment. On each tiller, both the flag leaf and flag leaf minus one were inoculated. For seedlings of Victo and Cartago, 10 pots were used per treatment. All plants within a pot were inoculated with the same isolate. After inoculation, plants were incubated in a dark dew chamber at 8°C and 100% relative humidity for 24 h to ensure infection. Following incubation, adult plants were set in trays and placed at random in the same greenhouse section. Seedling pots were arranged similarly to adult plants but in a different greenhouse section.

Observations and sampling. Assessment of LP started 8 days after inoculation by examining inoculation sites using a $\times$ 10 hand lens (Eschenbach, Germany). The observations were repeated at 24-h intervals for 17 days. The LP was defined as the time interval (hours) from inoculation until the first visual appearance of spores in uredinia breaking the leaf epidermis. Assessment of lesion growth started 3 days after the LP for individual leaves. Assessment was done by marking the expanding edge of the spore-producing area (lesion) with a waterproof felt-tip pen (Art.nr. 318-3; Staedtler, Germany). This marking was carried out at a 3-day interval on four consecutive dates. After the final marking, all leaves were detached, placed on sheets of blue paper (A4), and digitally scanned using a flatbed scanner.

The image analysis software Fiji (<http://fiji.sc/wiki/index.php/Fiji>) was used for determination of lesion length and size of the spore-producing area for each leaf. The length between consecutive markings and the total length of the lesion was assessed. Disease area was defined as the total leaf area with spores, chlorosis, and necrosis caused by *P. striiformis*. The size of the diseased area was determined for individual leaves by a color threshold using the HSB color space. Separation of the diseased area from the background and healthy parts of the leaf was done for individual leaves by adjustment of hue, saturation, and brightness. The threshold method was set to default.

Statistical analysis. Field data for percentage of sporulating leaf area were analyzed by a general linear model (GLM) based on log-transformed data to obtain variance homogeneity and normal distribution. Variance structure and normal distribution of data were confirmed by plots of model residuals. One model was used to analyze data for isolate J99198 (old) only. This model included year, host genotype, and the interaction between these as fixed effect, together with block nested in year. With this first model, two separate analyses were made, one based on results from 2010 and 2012 and another based on results for the six wheat genotypes that were included in all 3 years (2010, 2011 and

2012). A second model was used for analysis of field data from 2012 for the two isolates J99198 (old) and J11019 (new). This second model included isolate, host genotype, and the interaction between these, together with block nested within isolate. Models were applied using the Proc GLM procedure of SAS statistical software (version 9.2; SAS Institute Inc., Cary, NC).

Data obtained from the greenhouse experiment were analyzed using a linear mixed model. Isolate, host genotype, and leaf were included as fixed effects along with two- and three-way interactions. The effect of pot, the interaction of pot with fixed effects, and the residual effects were treated as random effects. Data for LP and disease area were log transformed before analysis and back transformed for presentation. Due to variance inhomogeneity of lesion length data, separate residual variances were recorded for host-pathogen interactions characterized by low, intermediate, and high infection types. For each variable, the possible models were compared using Akaike's Information Criteria (AIC) (2) and the model with the lowest AIC value was chosen for final analysis. Tests were performed using *F* tests with the denominator according to the theory of mixed models, and degrees of freedom were calculated for the denominator using the principles of Kenward-Rogers (28). Model parameters were estimated with residual maximum likelihood based on which least square means were calculated. Comparisons of treatment were based on least square means for each variable using *t* tests, with no adjustments for multiple comparisons. All analyses were carried out using the MIXED procedure in SAS statistical software (version 9.2; SAS Institute Inc.).

For greenhouse data, the correlation among variables was analyzed separately for each isolate using Pearson's linear correlation. Pot means were calculated and used as experimental unit. Comparisons of coefficients between isolates were performed by Fischer's *z* test after Fischer's *z* transformation of correlation coefficients. Analysis was performed using the procedure CORR of SAS statistical software (version 9.2; SAS Institute Inc.).

Principal component analysis (PCA) was done for illustration of the interactions between isolate and host genotype. Pot means were used as experimental units and data for LP and disease area were log transformed. Because LP was not defined for incompatible interactions, input values for these missing data in the PCA were calculated using the equation for the linear relationship between LP and infection type of treatments for which experimental data for LP were obtained. The four variables infection type, LP (log transformed), lesion length, and disease area (log transformed) were reduced to two principal components (PC1 and PC2) explaining 89.7 and 5.8% of the variation, respectively. For each treatment, means $\pm$ standard error of the two components were plotted in a coordinate system with PC1 as the x-axis and PC2 as the y-axis. PCA was done with XLSTAT (XLSTAT version 2012.6.01).

RESULTS

Field experiments. Isolate J99198 (old) gave consistent results over all three experimental years (2010 to 2012) (Fig. 1A). The results for resistant and susceptible genotypes were consistent across years, whereas some variability was observed for host genotypes showing moderate resistance. Results for 2010 and 2012, where all 11 host genotypes were included, revealed an interaction between year and host genotype ($P = 0.022$), which may be ascribed to the relatively large differences for disease on CRR2020 and CRR2182S between the 2 years. The six host genotypes assessed in all 3 years also gave an interaction between year and host genotype ($P = 0.035$). This interaction could be ascribed to the large differences for CRR2020 and CRR2088 between 2010 and 2012.

In contrast, comparisons in 2012 of the isolates J99198 (old) and J11019 (new) revealed highly significant interactions between

host genotype and isolate ($P < 0.001$) (Fig. 1B). In all, 7 of the 11 host genotypes had significant shifts in disease severity depending on the isolate. J99198 (old) resulted in significantly more disease on Réctal and CRR2283 than J11019 (new), and the opposite was the case for the five lines CRR2020, CRR2088, CRR2182R, CRR2178R, and CRR2083. The lines CRR2178R, CRR2182R, and CRR2083, which were resistant to J99198 (old), had a disease severity of 25 to 50% when inoculated with J11019 (new).

Greenhouse experiments. Seedling trials. All host genotypes were susceptible to J11019 (new) at the seedling stage (Table 3). In contrast, Camp Rémy and three of the RILs were resistant to J99198 (old), which indicates the presence of seedling resistance. Réctal and six of the RILs had infection type ≥ 5 (0-to-9 scale) when inoculated with J04003 (*PstS2*) but only three lines were fully susceptible (infection type 8 to 9). The low infection type on CRR2088 for the *PstS2* isolate suggests the presence of additional seedling resistance in the material.

Spore viability was confirmed on the susceptible controls Cartago and Victo. In addition, results for LP verified the higher level of aggressiveness of J04003 (*PstS2*), which had a significantly shorter LP than J99198 (old) on both Cartago ($P = 0.024$) and Victo ($P < 0.0001$). Moreover, a significant interaction between isolate and host genotype was observed ($P < 0.0001$). J11019 (new) had a similar LP as the *PstS2* isolate on Victo but a significantly longer LP than the other two isolates on Cartago ($P < 0.0001$ for both comparisons). This extended LP was associated with the emergence of chlorosis (infection type 6), indicating previously unrecognized resistance in Cartago.

Adult plant trials. Overall, 80% of the inoculations on adult plants resulted in macroscopic symptoms (i.e., infection type of ≥ 1). Line CRR2178S differed from this general trend with an overall infection efficiency of only 43%. Reliable assessment of infection type on CRR2178R and CRR2083 was restricted for low infection types due to the appearance of leaf spots on the leaves.

Adult plants of all the included host genotypes were confirmed resistant to J04003 (*PstS2*) with infection type 0 to 1 for all combinations. For this reason, only the isolates J99198 (old) and J11019 (new) are considered in the following. All five host genotypes susceptible to J99198 (old) (infection type ≥ 5) were less susceptible to J11019 (new) (infection types < 5), and the opposite was the case for the six most resistant host genotypes (infection type ≤ 3) (Table 3). CRR2083 and CRR2178R remained resistant to J11019 (new) at the adult plant stage (infection types < 2), Camp Rémy and CRR2182R were moderately susceptible (infection type 4 to 5) whereas CRR2020 and CRR2088 were susceptible (infection type 6 to 7).

LP was much influenced by both isolate and host genotype with contrasting LPs for the two isolates (Fig. 2A). LP of J99198 (old) was related to the number of QTL previously detected in the host genotypes. LP was shortest for CRR2019 and CRR2178S, with only one identified QTL, and longest for CRR2020, with six identified QTL. For this old isolate, no spore-producing pustules were observed on the four host genotypes with identified seedling resistance. For J11019 (new), lack of spore production was only observed on CRR2083 and CRR2178R and LP was shortest on CRR2088, CRR2019, and CRR2020, with four, two, and six QTL, respectively. The longest LP for this new isolate was observed on the susceptible parent Réctal and the lines CRR2283 (no QTL) and CRR2182S (three QTL).

Lesion length was assessed every 3 days after the first appearance of spores on the surface of individual leaves. The final lesion length 12 days after the end of the LP differed significantly between the two isolates for all host genotypes, thereby following the pattern of results for LP (Fig. 2B).

Disease area, which were assessed 25 days after inoculation, reflected the final outcome of the host-pathogen interactions. The two isolates resulted in significantly different disease areas on 8 of the 11 host genotypes (Fig. 2C).

Isolate–host genotype interactions were highly significant for all of the variables assessed on adult plants in the greenhouse (Table 4). There was no effect of leaf position for three of the variables. Only disease area revealed a significant difference ($P = 0.0005$), which was difficult to interpret due to higher order interactions.

High correlation coefficients were found between all variables for both isolates and all correlations were highly significant ($P < 0.0001$) (Table 5). The correlation between infection type and disease area and between lesion length and disease area was significantly higher for J99198 (old) than for J11019 (new). Based on these correlations, a PCA provided an overall summary of results for the individual epidemiological parameters. The first

two components of the PCA accounted for 95.5% of the original variation. The first component explained 89.7% of the variation and the four original variables contributed almost equally to this component (Table 6). LP was inversely correlated with the first component. The second component was mainly correlated with LP and disease area and explained 5.8% of the original variation. The second component primarily explained the variation caused by some isolate–host combinations having a relatively short LP combined with a relatively low disease area or vice versa.

The four host genotypes with seedling resistance to J99198 (old) were the most resistant in the PCA (Fig. 3). The highest level of quantitative resistance to this old isolate was found in CRR2020 (six OTL) and CRR2088 (four QTL). CRR2182S, with

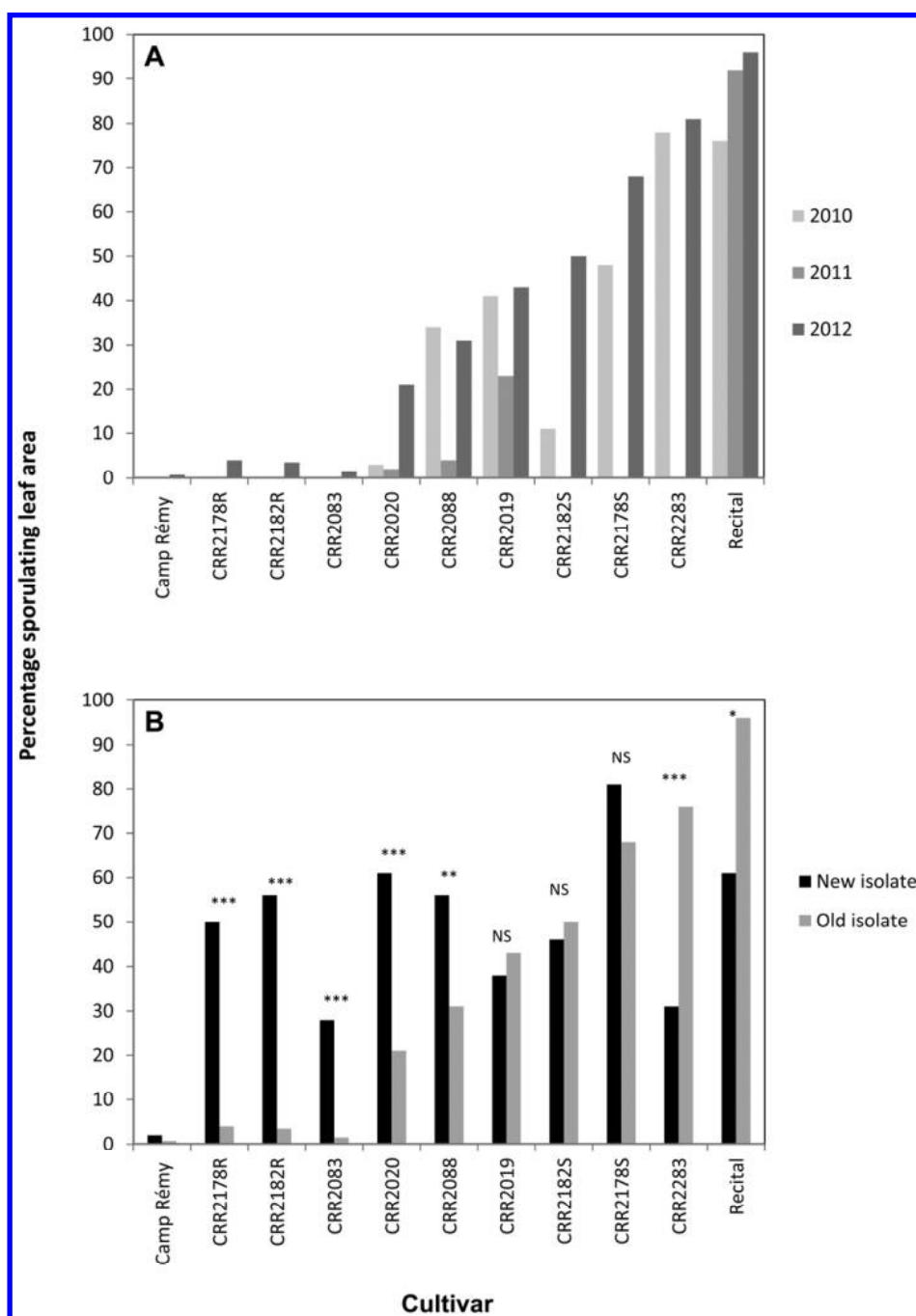


Fig. 1. Means of percent sporulating leaf area for nine recombinant inbred wheat lines (RILs) and two parental cultivars ('Camp Rémy' and 'Récital') inoculated with *Puccinia striiformis* isolates J99198 (old) and J11019 (new). Assessments were done on the upper three leaves (growth stage DC79) under field conditions. **A**, Results based on the old isolate in 2010, 2011, and 2012; note that CRR2182R, CRR2083R, CRR2182S, CRR2178S, and CRR2283 were absent in 2011. **B**, Results based on the old and the new isolates in 2012. Asterisks above bars indicate the level of significance for comparison of isolates within host genotype: *, **, and *** indicate $P < 0.05$, 0.001 , and 0.0001 , respectively; NS = nonsignificant at $\alpha = 0.05$.

three QTL, was moderately susceptible, whereas CRR2019 (one QTL), CRR2178S (one QTL), and CRR2283 (none) were almost as susceptible as Récital (susceptible parent). The resistance to J11019 (new) gave very different results which did not correspond to the number of previously identified QTL. CRR2020 and CRR2088, which had the highest level of quantitative resistance to the old isolate, were susceptible to the new isolate (Fig. 3, solid circles). Camp Rémy and CRR2182R, which carried seedling resistance to the old isolate, changed from resistant to moderately susceptible. In contrast, Récital and CRR2283 changed from highly susceptible against the old isolate to moderately resistant to the new (Fig. 3, broken circles). CRR2019 and CRR2178S were also less susceptible to the new isolate. CRR2178R and CRR2083 were resistant to both isolates and they were more resistant to the new isolate than both of the parents. This may suggest the presence of resistance components derived from both parents.

Field versus greenhouse. There was a high degree of consistency between results when comparing relative disease area in the greenhouse and disease severity in the field. Only three major discrepancies were observed. Camp Rémy remained resistant in the field despite being moderately susceptible in the greenhouse to the new isolate. In contrast, CRR2178R and CRR2083 were susceptible in the field despite being resistant to the new isolate in the greenhouse.

DISCUSSION

Much effort has been invested in identifying new sources of durable resistance to stripe rust and, in recent years, several components of APR providing high levels of disease control have been identified in European wheat cultivars (16,25,30,36). APR based on several genes with minor individual effects is often considered to maintain effective disease control (26,46), in contrast to resistance based on major or race-specific *Yr* genes, which may become ineffective after a few years of large-scale deploy-

ment (6,17). This study addressed the possible impact of *P. striiformis* isolates representing new races in Europe on the susceptibility of wheat genotypes diverging with respect to the number of QTL for resistance expressed mainly at the adult plant growth stages. The results revealed highly significant interactions between isolates representing old and new *P. striiformis* races in Europe and quantitatively expressed QTL for resistance to *P. striiformis* derived from Camp Rémy and Récital French wheat.

Seedlings of Camp Rémy and the two RILs CRR2178R and CRR2182R had effective resistance against the *PstS2* isolate, probably due to the QTL *QYr.inra-2BL.2*. In addition, this isolate revealed previously unidentified seedling resistance in CRR2088. At the adult plant stage, all evaluated host genotypes were resistant to *PstS2*. A previous study using susceptible adult plants showed that isolates of the *PstS2* strain had increased aggressiveness compared with isolates of European origin (37). Here, this was confirmed on susceptible seedlings, where *PstS2* was more aggressive than the old European isolate. However, *PstS2* only appeared in low frequencies in Europe despite being widespread in other parts of the world (e.g., the United States and Australia) (www.wheatrust.org) (21,24,33,48). This may be explained by the lack of virulence in *PstS2* corresponding to *Yr1*, *Yr3*, *Yr4*, *Yr17*, and *Yr32*, which are common in northwestern European cultivars (17,20), and by APR in European wheat cultivars, as indicated in the present study. The old isolate representing a typical northwestern European strain showed a consistent interaction with the resistance components derived from Camp Rémy. Seedling tests revealed that both the seedling stage QTL *QYr.inra-2BL.2* and the *Yr7* resistance were effective against this isolate. Therefore, a possible effect of the APR QTL against this old isolate was masked in host genotypes with these genes. In host genotypes with no effective seedling resistance, the effect of the QTL derived from Camp Rémy were additive when evaluated with this isolate (i.e., additional numbers of QTL led to higher levels of resistance under both greenhouse and field conditions). The results obtained for this typical northwestern European isolate

TABLE 3. Infection types (0-to-9 scale) on seedlings and adult plants of the nine recombinant inbred lines and two parental cultivars in the greenhouse when inoculated by three isolates of *Puccinia striiformis*

Host genotype	<i>P. striiformis</i> isolates ^a					
	Seedlings			Adult plants		
	Old	<i>PstS2</i>	New	Old	<i>PstS2</i>	New
Camp Rémy	2-3	3	8-9	0-1	NA	4-5
Récital	8-9	5-7	8-9	7-9	0-1	2-4
CRR2083	2-3	5	8-9	0-1	0	2-3
CRR2283	8-9	6-7	8-9	6-7	0-1	2-4
CRR2019	8-9	8-9	8-9	7-8	0-1	4-6
CRR2020	8-9	8-9	8-9	2-4	0-1	6-7
CRR2088	8-9	2-3	8-9	2-4	NA	6-8
CRR2178R	2-3	3	8-9	0-1	NA	1-2
CRR2178S	8-9	8-9	8-9	7-9	0	4-5
CRR2182R	2-3	2-3	8-9	0-1	NA	3-5
CRR2182S	8-9	6	8-9	4-6	NA	2-4

^a NA = not possible to assess infection type due to physiological leaf spots.

TABLE 4. Main effects and interaction of *Puccinia striiformis* isolate, host genotype, and leaf position on infection type, latent period, lesion length, and disease area for greenhouse tests

Source	df	Infection type		Latent period ^a		Lesion length		Disease area ^a	
		<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value	<i>F</i> value	<i>P</i> value
Isolate	1	0.36	0.5476	45.55	<0.0001	22.46	<0.0001	7.28	0.0078
Host	11	378.97	<0.0001	50.03	<0.0001	17.15	<0.0001	11.07	<0.0001
Isolate × host	11	265.62	<0.0001	51.95	<0.0001	77.36	<0.0001	27.55	<0.0001
Leaf	1	0.64	0.4227	2.16	0.1435	2.48	0.1164	12.31	0.0005
Isolate × leaf	1	0.92	0.3390	0.19	0.6661	0.01	0.9213	0.18	0.6684
Host × leaf	11	6.31	<0.0001	4.66	<0.0001	3.73	0.0008	5.75	<0.0001
Isolate × host × leaf	11	3.83	<0.0001	0.68	0.6893	2.58	0.0146	4.20	0.0002

^a Data were log transformed before analysis.

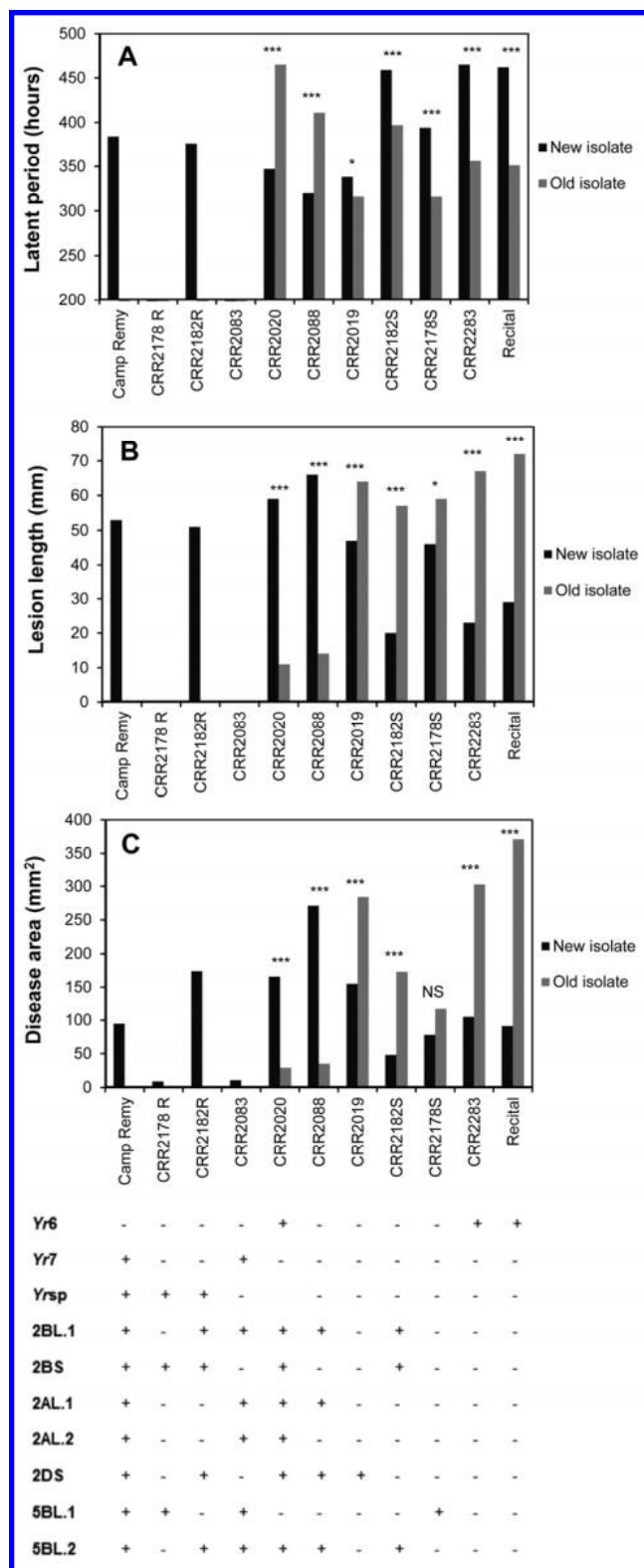


Fig. 2. Interactions between two isolates of *Puccinia striiformis* and nine recombinant inbred lines and two parental cultivars with different components of seedling and adult plant resistance, measured by **A**, latent period; **B**, lesion length; and **C**, disease area. Note that data for latent period, lesion length, and disease area are absent for incompatible interactions: the old isolate showed incompatibility with Camp Rémy, CRR2178R, CRR2182R, and CRR2983R and the new isolate was incompatible with CRR2178R and CRR2083. Asterisks above bars indicate the level of the significant difference between isolates within line or cultivar: *, **, and *** indicate $P < 0.05$, 0.001, and 0.0001, respectively; NS = nonsignificant at $\alpha = 0.05$. Plus and minus below the graphs indicate the presence or absence, respectively, in individual lines or cultivars of identified genes for qualitative resistance (*Yr* genes) and of quantitative trait loci conferring resistance against *P. striiformis*.

were in accordance with the previous study, where the resistance genes of Camp Rémy were mapped (30).

None of the host genotypes carried seedling resistance effective against the isolate that represented the race which was first observed in Europe in 2011 (www.wheatrust.org). In both the field and the greenhouse, this isolate produced highly contrasting results on most host genotypes compared with the old isolate. The general picture was that RILs with a high number of previously defined QTL became significantly more diseased when exposed to or inoculated with the new isolate. In contrast, host genotypes that were susceptible or moderately susceptible to the old isolate had higher levels of resistance to the new isolate. The susceptibility of CRR2088 indicated that all four QTL for APR in this line had been fully overcome by this isolate. The disease severity on CRR2020 (six QTL) was similar to CRR2088 in the field but lower in the greenhouse. This may imply that the two additional QTL in CRR2020 are influenced by the environment. Both field and greenhouse results also indicated the existence of at least one QTL in the susceptible parent Récital. In a previous study of resistance in 'Renan', where Récital was included as susceptible parent, a QTL of limited effect was identified in Récital (16). In the greenhouse, we found that CRR2178R and CRR2083 were more resistant to the new isolate than both parents. An explanation could be that components of resistance derived from both parents are combined in these lines. The two lines share *QYr.inra.5BL1* from Camp Rémy and one hypothesis is that combining this QTL with a resistance from Récital may result in effective resistance. This hypothesis is supported by the moderate resistance in the greenhouse of the line CRR2178S which only carries *QYr.inra.5BL1*. In the field, however, both CRR2178R and CRR2083 were moderately susceptible to the new isolate whereas the parent Camp Rémy was fully resistant. The disease level on CRR2178S was also higher in the field compared with the greenhouse, an effect that could be due to a low infection rate conferred by QTL *QYr.inra.5BL1* in the greenhouse. These results indicate that the resistance present in these three lines and in Camp Rémy is highly influence by the environmental conditions or experimental settings. In the field, an initially low disease level may be amplified through successive infection cycles, thus masking the effect of resistance genes conferring low infection. The influence of environment on APR has often been reported where several studies have revealed an effect of light level and temperature

TABLE 5. Pearson's correlation coefficients between four disease variables measured in the greenhouse for two *Puccinia striiformis* isolates on adult plants of 11 wheat genotypes^a

Variables	Infection type	Latent period ^b	Lesion length	Disease area ^b
Infection type	...	-0.80	0.84	0.62
Latent period ^b	-0.82	...	-0.74	-0.61
Lesion length	0.89	-0.67	...	0.69
Disease area ^b	0.79*	-0.67	0.90***	...

^a Coefficients were calculated separately for the old isolate (below the diagonal and bold) and the new isolate (above the diagonal); * and *** indicate $P < 0.05$ and 0.001, respectively, for comparison of correlations coefficients between isolates.

^b Data for latent period and disease area were log transformed.

TABLE 6. Eigen vectors of principal component (PC) analysis for the four variables infection type, latent period, lesion length, and disease area

Variables	PC1	PC2
Infection type	0.512	-0.215
Latent period	-0.492	0.654
Lesion length	0.508	0.173
Disease area	0.488	0.704
Total variation (%)	89.7	5.8
Eigenvalue	3.587	0.231

(1,5). In a study on leaf (brown) rust, Renan was found to be resistant in the field despite being susceptible in the greenhouse (data not published). Furthermore, 'Soissons', which has been grown in large areas in France since 1989, is still resistant to stripe rust in the field despite showing moderate susceptibility in greenhouse tests before registration (4). There is also a risk that some of the interactions observed in the field could be due to natural infections or cross infection between experimental plots. However, based on control cultivars and assessments of surrounding fields, this was not a problem in the present study. The influence of environment and the effect of individual QTL could be studied further by the two pairs of near-isogenic lines, CRR2182 and CRR2178, which seemed to reveal an environmental effect on the expression of *QYr.inra.2DS* (CRR2182R) and *QYr.inra.2BS* (CRR2178R). *QYr.inra.2DS* seemed to result in elevated susceptibility in the greenhouse while *QYr.inra.2BS* appeared to enhance resistance to the new isolate under greenhouse conditions. A more complete elucidation of the effects of individual QTL and the influence of the environment on their expression will require additional genetic studies involving the new pathogen isolate.

Race specificity for quantitative resistance against stripe rust as shown here has previously been demonstrated for leaf rust (*P. hordei*) on barley in a study where pathogen isolates were selected to optimize identification of race specificity (32). In a different study on stripe rust, a QTL for APR was found to be effective against only one of three isolates (39). However, it is possible that this QTL against stripe rust represented a gene for qualitative resistance because some genes of this type are mainly expressed in adult plants (7). Our results are in agreement with the hypothe-

sis that all resistance genes interact in a gene-for-gene manner of either major or minor effects (41). In addition, our results are supported by the fact that quantitative resistance in cultivars related to Camp Rémy apparently has been partly overcome under field conditions in England (1).

The virulence phenotypes of the new isolate was detected for the first time in Europe in 2011 (www.wheatrust.org). The isolate used here shows incompatible interaction with seedlings of French Renan (*Yr3* and *Yr17*) despite the fact that it carries virulence for both *Yr3* and *Yr17* (C. de Vallavieille-Pope, unpublished data). Identification of undetected resistance by an uncommon isolate was also observed in a study where an isolate from Lebanon identified *Yr25* in the three European differential lines 'Heines VII', 'Heines Peko', and 'Strubes Dickkopf' (11). The unusual character of the isolate representing the new race from 2011 was supported by a ready production of telia on both seedlings and adult plants (data not shown). This is in contrast to previously common European isolates and may suggest a sexual origin in the near past (3).

Breeding for durable resistance based on genes for quantitative resistance has been a main focus for CIMMYT for >25 years (46). Experimental studies and field observations have reported stability of quantitative resistance across years and locations when artificially inoculated with a particular isolate or during natural infection (8,10). The results we present here should encourage the identification and maintenance of a diverse array of QTL and genes for quantitative resistance to *P. striiformis* in the wheat germplasm. Our study also suggests that cultivars should be tested in the epidemiological environments where use is intended. Using the same cultivar across different regions might lead to un-

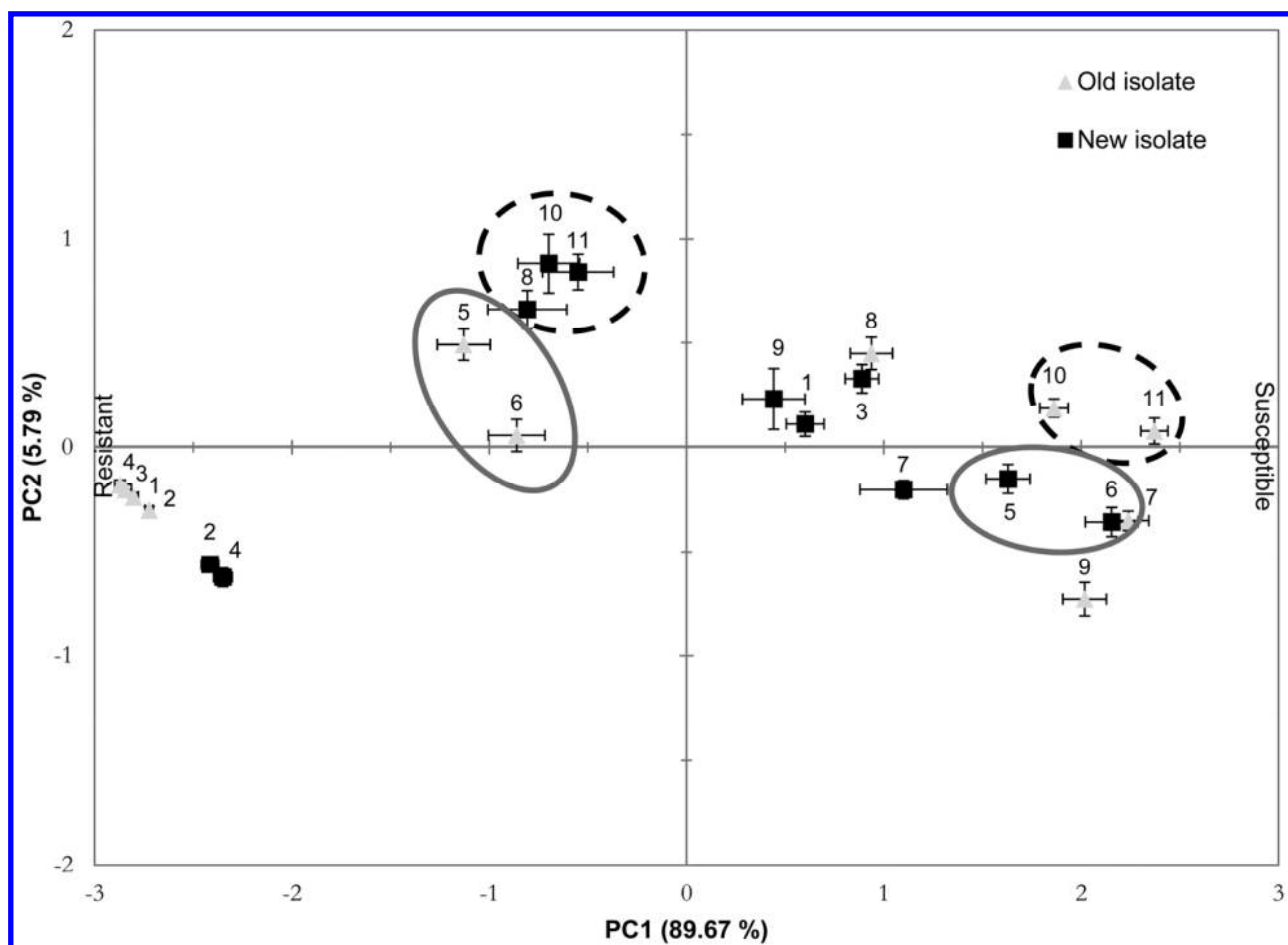


Fig. 3. Plot of the first (PC1) and second (PC2) principal component means ($\pm$ standard error) from an analysis of the four variables (infection type, latent period, lesion length, and disease area) for two *Puccinia striiformis* isolates on adult plants of 11 different wheat genotypes (Table 3): 1 = Camp Rémy; 2 = CRR2178R; 3 = CRR2182R; 4 = CRR2083; 5 = CRR2020; 6 = CRR2088; 7 = CRR2019; 8 = CRR2182S; 9 = CRR2178S; 10 = CRR2283; and 11 = Récital.

expected results as genetically different pathogen populations are encountered. The previously acclaimed race-nonspecific nature of quantitative resistance is most likely an effect of combining several genes with different effects in the same cultivar, which makes it unlikely that the pathogen can accumulate the required virulence within a short timespan (14,31). However, the nature of quantitative resistance is still not fully understood (43).

The high correlation between the epidemiological parameters found for both the old and the new isolate indicates that all parameters are measures of the overall ability of the pathogen to grow on a particular host genotype. In two cases, the correlation coefficient was significantly higher for the old isolate than for the new isolate. This may reflect that the interaction between the host genotypes and the new isolate involved some unidentified resistance genes or QTL. The high correlation between parameters allowed us to perform a PCA which integrated results for all parameters and gave an informative illustration of the interaction between host genotype and isolate. Most of the variation in data was explained by the first principal component and all parameters contributed almost equally to this component. This indicates that all parameters are reliable estimates of the interaction between the pathogen and the host. Therefore, in future studies, focus could be directed to specific parameters, depending on the purpose of the study. The second component in the PCA only explained a small amount of the variation in data but showed some divergence of results for LP and disease area. For some interactions, a relatively long LP was associated with a relatively large disease area and vice versa. This might be explained by some QTL having a more specific effect on a certain phase of the infection process. Our discovery of significant isolate specificity (or race specificity) of quantitatively inherited APR resistance to *P. striiformis* stresses the difficulties in predicting the durability of disease control based on the nature of the inheritance or the phenotypic expression of resistance to *P. striiformis*. These results also point to the necessity of using pathogen isolates of diverse origin in breeding programs. Increasing knowledge of resistance in European wheat, together with the previously unidentified resistance in Réctal found here, has made it clear that intensive and long-term selection by European cereal breeders has led to accumulation of diverse resistance genes, not all identified in commercial cultivars. Epidemics caused by emerging strains in other parts of the world in recent years might be a result of a generally lower selection for resistance against *P. striiformis*.

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