Distribution of Pathotypes with Regard to Host Cultivars in French Wheat Leaf Rust Populations

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ABSTRACT

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Isolates of wheat leaf rust collected from durum and bread wheat cultivars in France during 1999-2002 were analyzed for virulence on 18 Thatcher lines with single genes for leaf rust resistance (*Lr* genes). Sampling focused on the five most widely grown bread wheat cultivars (two susceptible and three resistant) to allow statistical comparison of diversity indexes between the cultivars. Leaf rust populations from durum and bread wheats were different. The diversity of the bread wheat leaf rust pathotypes, as measured by the Shannon index, ranged from 2.43 to 2.76 over the 4 years. Diversity for wheat leaf rust resistance was limited in the host since we postulated only seven seedling resistance genes in the 35

cultivars most widely grown during 1999-2002. Leaf rust populations were strongly differentiated for virulence within bread wheat cultivars, and diversity was higher on those that were resistant, mainly due to a more even distribution of virulence phenotypes than on susceptible cultivars. The pathogen population on the susceptible cv. Soissons was largely dominated by a single pathotype (073100), whereas all other pathotypes virulent on cv. Soissons either decreased in frequency or remained at a low frequency during the period studied. Several pathotypes including the most complex one were found only on resistant cultivars, even though most of them were virulent on the susceptible cv. Soissons. Specific interactions were necessary, but not always sufficient, to account for pathotype distribution and frequencies on the cultivars, suggesting that selection for virulence to host resistance genes is balanced by other selective forces including selection for aggressiveness.

Wheat leaf rust, caused by *Puccinia triticina* Eriks, was among the most damaging foliar diseases of wheat in Europe at the end of the 20th century. Damaging disease levels have been noted regularly in western Europe (except the UK), Italy, and eastern and southeastern Europe (namely Yugoslavia and Romania) (25). During the last 30 years, particularly in western European countries, fungicides have been used to control diseases including leaf rust in most years (25). Although selection for resistance to leaf rust has remained an issue for wheat breeders, it has not been a top priority. This probably explains the limited studies on this pathosystem in Europe as compared with other regions like North America (12,14,20) and Australia (27). Pathogen populations have been monitored and host resistance genes postulated from 1966 to 1993 in the Czech Republic (4). A more recent study by Mesterhazy et al. (23) that attempted to coordinate a 4-year survey of *P. triticina* in western, southern, and eastern Europe showed a great variation in the distribution of virulence phenotypes between years and countries. However, no interpretation of the observed diversity could be provided because of a lack of knowledge of the host resistance genes used in these countries. Park et al. (29) postulated leaf rust resistance genes in western European wheat cultivars grown in 1995, and related this to a survey of *P. triticina* populations in the same year. In this study, it was noted that western Europe comprised different epidemiological units, with different climates and different host populations, and that the role of host selection on the pathogen was diffi-

cult to estimate because virulence on the genes with the highest frequencies in the host population, *Lr13* and *Lr14a*, had not been monitored. Moreover, this study was limited to 1 year. The necessity to place the results of population studies in a perspective of several years was stressed by Brown (5).

In this paper, we describe a 4-year study of host and pathogen populations in which we placed special emphasis on selection by the host. To characterize host selection as accurately as possible, the sampling scheme was designed to collect leaf rust isolates from identified cultivars, providing more information than what would be gained from pooling results from many different cultivars (36). The pathogen population was characterized through two complementary approaches: (i) a classical survey of virulences on durum wheat cultivars and minor bread wheat cultivars to provide a broad view of virulences in *P. triticina* populations in France; and (ii) a detailed sampling on the five main bread wheat cultivars. We focused our sampling on the most widely grown cultivars, whether resistant or susceptible, to obtain sample sizes large enough to be considered representative of the population present on each of these cultivars. The study was limited at the spatial scale to one country, France, assuming that it could be regarded as a single epidemiological unit to overcome the above-mentioned difficulties of heterogeneity at the continental scale.

MATERIALS AND METHODS

Sampling. Because 98% of wheat fields in France are sprayed with fungicides (3), samples were collected from a network of unsprayed nurseries planted annually in approximately 50 different locations throughout the wheat-growing area by breeders (the French Wheat Breeders group 'Club des Cinq') or extension services (Arvalis-Institut du Végétal). For bread wheat, the sampling effort focused on the most widely grown cultivars (Table 1)

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planted as checks in all nurseries, i.e., Soissons, Isengrain, and Trémie from 1999 to 2002, and Apache and Orvantis from 2001 to 2002. On each site and for each cultivar, a few infected leaves were collected during May or June (a single collection date per location) from small plots (10 to 20 m²) from which one single-pustule isolate was selected and increased, giving one isolate per cultivar and location. In addition, in the same nurseries, we collected leaf rust samples from minor bread and durum wheat cultivars. Since the minor cultivars were not present in each location, the sample size for each minor cultivar was less than 10.

The different populations considered were (i) the population P_d growing on durum wheat cultivars; (ii) the population P_b growing on all bread wheat cultivars, including five major and several minor cultivars; and (iii) the five populations (P₁ to P₅) growing on the five major bread wheat cultivars, P₁ to P₅ being subpopulations of P_b. Selection by host could thus be evaluated for the influence of host species by comparing P_d and P_b and the influence of resistance genes within the bread wheat species by comparing no matter what given population (from P₁ to P₅), to another. Moreover, P_b allowed a comparison of our results with the published data on populations of *P. tritricina* in other countries, where the cultivar is not taken into account in the sampling scheme. The sample from P_d was collected in 1999, 2000, and 2001 from 8, 7, and 16 cultivars, respectively, yielding 19, 16, and 47 isolates, respectively, with a total of 82 isolates for the 3 years. A minimum sample size of 50 was achieved on cvs. Soissons (P₁) and Isengrain (P₂), whereas collections from resistant cultivars (Trémie, Apache, and Orvantis, P₃, P₄, and P₅, respectively) were more limited and yielded a sample size from 18 to 55 depending on the year (Table 2). The sample from P_b was obtained from 18 different bread wheat cultivars in 1999 and 2000, 13 cultivars in 2001, and 21 cultivars in 2002, yielding a sample size from 168 to 234 depending on the year (Table 2) with a total of 821 isolates for the 4 years.

Pathotype determination. Prior to inoculation, all healthy material was grown in air-filtered cabinets in a glasshouse at temperatures between 15 and 25°C and with a 14-h photoperiod (day-light supplemented by 400 W Na-lamps).

Infected field-collected leaves were wiped gently on 7-day-old seedlings of the wheat cv. Michigan Amber that had been treated with 20 ml of a maleic hydrazide solution (0.25 g of maleic hydrazide per liter of H₂O) to prevent emergence of secondary leaves and increase the size of uredinia. Inoculated seedlings were placed for 24 h in a dew chamber at 15°C, then in the glasshouse. One week after inoculation, the seedlings were trimmed so that only one plant with one uredinium remained in each pot. Cellophane bags were then placed on the pots to prevent contamination between isolates. Spores from a single uredinium were collected with a cyclone collector into a 00 gelatin capsule. Spores were increased by adding 0.5 ml of light mineral oil to each capsule and spraying the resulting spore suspension onto 7-day-old Michigan Amber seedlings. The spores collected 10 days after inoculation were divided into two batches, one to inoculate a differential set and one for storage at -80°C. The differential sets were sown in pressed-peat pots (3 × 3 cm²) containing a commercial compost (peat substrate; Gebr. Brill Substrate, Georgsdorf, Germany) with four seedlings per pot and two pots per line (eight seedlings for each differential line). The differential set comprised 17 Thatcher differentials, *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr13*, *Lr14a*, *Lr15*, *Lr16*, *Lr17*, *Lr20*, *Lr23*, *Lr26*, *Lr37*, the Australian cv. Harrier carrying *Lr17b* (32) and the susceptible check cv. Morocco. After inoculation with a spore suspension, the sets were placed in a dew chamber for 24 h at 15°C and then for 9 days in a growth chamber maintained at 22°C with a 16-h photoperiod given by metal halide lamps (350 µE). Infection types on the differentials were read 10 days after inoculation according to Stakman et al. (35). Each isolate was assigned a six-digit code adapted from the system described by Gilmour (7): the 18 differential isolines were arranged in six sets of three to assign to each isolate an octal pathotype code.

Postulation of resistance genes. All cultivars belonging to the list of the 10 most widely grown cultivars in France, in any of the years 1990-2002, were analyzed to determine which leaf rust resistance genes were present. Nine of these cultivars were tested at the Plant Breeding Institute, The University of Sydney, Australia.

TABLE 1. Percentage of wheat area planted to five cultivars in France during 1999-2002^a

Cultivar	Registration year	Pedigree	1999	2000	2001	2002
Soissons (<i>Lr14a</i>)	1988	Hybride naturel dans 35/Iena	15	11	11	9
Isengrain (<i>Lr14a</i>)	1997	Apollo/Soissons	8	11	14	14
Trémie (<i>Lr10</i> , <i>Lr13</i>)	1992	SE32/Moulin	11	8	6	4
Apache (<i>Lr13</i> , <i>Lr37</i>)	1998	Axial/NRPB 84-4233	—	—	8	17
Orvantis (<i>Lr10</i> , <i>Lr13</i> , <i>Lr37</i>)	1999	Thésée/Disponent/Monopol ³ Torfrida	—	—	—	4

^a The percentage of the most commonly grown cultivar for each year is given in bold.

TABLE 2. Calculation of indices of richness, diversity, and evenness for populations of *Puccinia tritricina* collected from bread wheat cvs. Soissons (P₁), Isengrain (P₂), Trémie (P₃), Apache (P₄), and Orvantis (P₅) during 1999-2002

Statistic	1999 ^a				2000 ^a				2001						2002					
	P ₁	P ₂	P ₃	P _b ^b	P ₁	P ₂	P ₃	P _b	P ₁	P ₂	P ₃	P ₄	P ₅	P _b	P ₁	P ₂	P ₃	P ₄	P ₅	P _b
Sample size	53	48	32	168	57	50	36	187	63	61	55	19	18	234	47	52	20	31	24	231
Richness	16	17	9	35	12	20	16	45	13	11	14	12	10	34	11	13	11	14	12	42
H'	2.354	2.170	1.749	2.763	1.533	2.021	2.554	2.691	1.556	1.181	2.313	<u>2.351</u> ^c	2.033	2.430	1.561	1.627	2.151	2.434	2.200	2.677
Confidence interval ^d	2.25-2.60	2.03-2.51	1.58-2.07	2.70-2.94	1.30-1.90	1.81-2.67	2.55-2.65	2.60-2.90	1.30-1.97	0.91-1.59	2.24-2.47	<u>2.40-2.41</u>	1.95-2.22	2.31-2.67	1.32-1.93	1.35-2.11	2.11-2.32	2.43-2.51	2.15-2.37	2.60-2.90
N1	10.526	8.762	5.748	15.843	4.631	7.549	12.855	14.752	4.740	3.258	10.108	10.497	7.637	11.360	4.763	5.091	8.596	11.403	9.023	14.538
G	7.184	5.333	4.531	9.834	2.592	3.289	10.623	5.533	2.507	1.880	8.198	9.256	5.786	5.072	2.910	2.683	6.897	9.707	7.024	7.860
Evenness (E5)	0.649	0.558	0.744	0.595	0.438	<u>0.350</u> ^c	0.812	0.330	0.403	0.390	0.790	0.869	0.721	0.393	0.508	0.411	0.776	0.837	0.751	0.507
Confidence interval ^d	0.52-0.73	0.40-0.57	0.56-0.81	0.52-0.62	0.37-0.47	<u>0.33-0.34</u>	0.66-0.89	0.29-0.34	0.35-0.42	0.35-0.40	0.64-0.87	0.74-0.96	0.48-0.83	0.34-0.42	0.40-0.57	0.36-0.42	0.56-0.86	0.71-0.92	0.51-0.84	0.43-0.54

^a P₄ and P₅ could not be sampled in 1999 and 2000 because cvs. Apache and Orvantis, recently registered, were not present in the nurseries.

^b P_b indices were calculated from a sample including isolates from all bread wheat cultivars (minor cultivars and five major cultivars).

^c The underlined values, calculated from the observed sample, were not included in the confidence interval calculated with the BCa method.

^d Confidence intervals were calculated by the bootstrapping approach (BCa method) by Grünwald et al. (8).

lia, following the procedure described by Park et al. (29). For the remaining cultivars, multipathotype tests were conducted in a growth room under the conditions described above for pathotype determination. A selection of 15 pathotypes of *P. tritricina* was used (Table 3). Spore suspension (3 mg of spores per ml of mineral oil) was sprayed on the first leaves of 12 seedlings of each cultivar using 0.7 ml per tray of 240 seedlings. A set of differential cultivars was included in each test to check the identity and purity of the isolate as well as the infection type. Infection types were scored according to Stakman et al. (35).

Data analysis. Diversity for virulence phenotypes in the populations was estimated by the Shannon index

$$H' = -\sum p_i \ln(p_i)$$

in which p_i is the frequency of the i th phenotype. Since differences in an index of diversity can reflect variation in richness (i.e., the number of genotypes observed), evenness (i.e., the distribution of the genotypes), or both, we have calculated these indices and computed means and confidence intervals whenever possible. Richness was evaluated by the number of phenotypes, and evenness evaluated by the index E_5 proposed by Grünwald et al. (9):

$$E_5 = (G - 1)/(N_1 - 1)$$

in which G is the Stoddart and Taylor index, $G = 1/\sum p_i^2$, and $N_1 = e^{H'}$.

We used the bootstrapping approach recommended by Grünwald et al. (9) to obtain confidence intervals on the diversity index H' (Shannon index) and evenness index E_5 , using the BCa (accelerated bootstrap procedure) with the 'R' software (R Development Core Team (2004). R is a language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna, Austria; ISBN 3-900051-07-0, <http://www.R-project.org>). However, in our calculations, this method twice yielded a confidence interval that did not include the population mean. In these two cases (underlined in Table 2), the ratio of richness to sample size was very high. Because the theory is based on asymptotic approximation, our sample size might have been too small in these two cases.

Index $N_1 = e^{H'}$ represents the number of equally common genotypes that would produce the same diversity as H' . Because G weighs the number of abundant genotypes more strongly and N_1 weighs rarer genotypes more strongly (9), different rankings of the populations were obtained in some cases depending whether H' (and N_1), or G were considered.

Calculation of a diversity index was performed for the bread wheat populations P_1 to P_5 and P_b , but not for the durum wheat population P_d because of the small sample size.

RESULTS

Comparison of P_d and P_b . In the durum wheat population P_d , a total of 16 pathotypes was identified (Table 4), of which seven were found very rarely (only once a year in the samples) or not at all on bread wheat. The most frequent pathotype was 012020, virulent on three genes only (*Lr2c*, *Lr10*, and *Lr20*), whereas this pathotype was identified only three times in P_b (Table 5). In the bread wheat population P_b , a total 104 pathotypes was identified (Table 5), with 34 to 45 different pathotypes identified each year, of which 16 to 25 were found only once in the sample. The proportion of these pathotypes found only once in the year was roughly constant, from 47 to 60% across the 4 years. Virulence frequencies on *Lr13*, *Lr14a*, *Lr16*, and *Lr20* (Table 6) were significantly different between durum wheat and bread wheat.

Pathotype distribution on the five major wheat cultivars.

On the two susceptible cvs. Soissons and Isengrain, disease level was high (80 on the modified Cobb scale; 30) in the 4 years the day samples were collected. In contrast, there was at most, one pustule per flag leaf on cvs. Trémie (*Lr10* and *Lr13*) and Orvantis (*Lr10*, *Lr13*, and *Lr37*) in some years, and the maximum disease level on these cultivars was between 1 and 5. Cv. Apache was more variable in its leaf rust response with a disease level usually between 1 and 10, but reaching 50 at one location in 2002.

The distribution of the pathotypes in the populations P_1 to P_5 is plotted in Figure 1. Pathotypes found only once at most in each year were pooled in a group named "rare". As soon as a pathotype was found at least twice in 1 year, it was plotted individually. Because the distributions on cvs. Soissons and Isengrain were rather similar, the first nine pathotypes on the x axis are the same for these two cultivars. There were two dominant pathotypes in 1999: (i) pathotype 073100 (virulent on *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr10*, and *Lr14a*; frequency 28 and 33%); and (ii) pathotype 014103 (virulent on *Lr2c*, *Lr13*, *Lr14a*, *Lr26*, and *Lr17b*; frequency 19 and 25%) for cvs. Soissons and Isengrain, respectively. The frequency of pathotype 014103 decreased over the following years, dropping to 0 in 2002 that resulted in a population dominated by pathotype 073100 that represented 54 to 72% of the isolates. The population on these two cultivars was also composed of five to nine pathotypes found at low frequencies, between 2 and 13%, and rare pathotypes found only once, representing 2 to 11 pathotypes each year, i.e., between 5 and 26% of the population.

All pathotypes found on cvs. Soissons (except pathotype 016404; Fig. 1) and Isengrain (except for four belonging to the group of rare pathotypes) possessed virulence for the gene *Lr14a* postulated in these two cultivars.

TABLE 3. Standard isolates of *Puccinia tritricina* used in multipathotype tests for postulation of resistance genes

Isolate	Pathotype ^a	Virulence at the seedling stage ^b
B9405-2B	075337	<i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr3ka</i> , <i>Lr13</i> , <i>Lr14a</i> , <i>Lr15</i> , <i>Lr17</i> , <i>Lr17b</i> , <i>Lr20</i> , <i>Lr26</i> , <i>Lr37</i>
B9407-1CA3	050124	<i>Lr2c</i> , <i>Lr3bg</i> , <i>Lr14a</i> , <i>Lr20</i> , <i>Lr37</i>
B9201-2C3	014103	<i>Lr2c</i> , <i>Lr13</i> , <i>Lr14a</i> , <i>Lr17b</i> , <i>Lr26</i>
B9387-1A1	073100	<i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr3ka</i> , <i>Lr10</i> , <i>Lr14a</i>
B9506-2B	014103	<i>Lr2c</i> , <i>Lr13</i> , <i>Lr14a</i> , <i>Lr17b</i> , <i>Lr26</i>
B950365	073100	<i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr3ka</i> , <i>Lr10</i> , <i>Lr14a</i>
B9384-1C1	075337	<i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr3ka</i> , <i>Lr13</i> , <i>Lr14a</i> , <i>Lr15</i> , <i>Lr17</i> , <i>Lr17b</i> , <i>Lr20</i> , <i>Lr26</i> , <i>Lr37</i>
B950019-A	012026	<i>Lr2c</i> , <i>Lr10</i> , <i>Lr17b</i> , <i>Lr20</i> , <i>Lr37</i>
B9414-1CA2	170106	<i>Lr1</i> , <i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr14a</i> , <i>Lr17b</i> , <i>Lr37</i>
B8907-3B4	050124	<i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr14a</i> , <i>Lr20</i> , <i>Lr37</i>
B950506A	106232	<i>Lr1</i> , <i>Lr10</i> , <i>Lr13</i> , <i>Lr15</i> , <i>Lr17</i> , <i>Lr17b</i> , <i>Lr20</i>
B77SaBa	775237	<i>Lr1</i> , <i>Lr2a</i> , <i>Lr2b</i> , <i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr3ka</i> , <i>Lr13</i> , <i>Lr15</i> , <i>Lr17</i> , <i>Lr17b</i> , <i>Lr20</i> , <i>Lr26</i> , <i>Lr37</i>
B347	777234	<i>Lr1</i> , <i>Lr2a</i> , <i>Lr2b</i> , <i>Lr2c</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr3ka</i> , <i>Lr10</i> , <i>Lr13</i> , <i>Lr15</i> , <i>Lr17</i> , <i>Lr20</i> , <i>Lr37</i>
B9834-E	016504	<i>Lr2c</i> , <i>Lr10</i> , <i>Lr13</i> , <i>Lr14a</i> , <i>Lr16</i> , <i>Lr37</i>
B00M057-A	166336	<i>Lr1</i> , <i>Lr3</i> , <i>Lr3bg</i> , <i>Lr10</i> , <i>Lr13</i> , <i>Lr14a</i> , <i>Lr15</i> , <i>Lr17</i> , <i>Lr17b</i> , <i>Lr20</i> , <i>Lr37</i> , <i>Lr27</i> + <i>Lr31</i>

^a Six-digit code based on a 18-*Lr* genes differential set (Thatcher lines *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr13*, *Lr14a*, *Lr15*, *Lr16*, *Lr17*, *Lr20*, *Lr23*, *Lr26*, the Australian cv. Harrier (*Lr17b*), and *Lr37*).

^b Tested for pathogenicity on the 18 lines listed above in addition to the Thatcher lines with the resistance genes *Lr9*, *Lr19*, *Lr24*, line CS2A/2M (*Lr28*), and the Australian cv. Gatcher (*Lr10*, *Lr27* + *Lr31*).

The population P₃ from cv. Trémie was composed of three to four pathotypes with intermediate frequencies of 9 to 31%, then three to seven pathotypes with low frequencies (2 to 7%), and some rare pathotypes representing 7 to 25% of the population (Fig. 1). Pathotypes 016404, 016504, and 016206, combining virulences for *Lr10* and *Lr13* present in Trémie, were the main components of P₃ in 1999 and 2000. The population then changed with the rapid emergence of a new group of pathotypes. This group included three closely related pathotypes also virulent on *Lr10* and *Lr13*, pathotype 166337 that was found only twice in 2000 and increased to 13% of P₃ in 2001 and pathotypes 166336 (differing from 166337 by avirulence on *Lr26*) and 166376 (differing from 166336 by virulence on *Lr23*) representing 16% and 9%, respectively, of P₃ in 2001. This group of three pathotypes accounted for 38% of P₃ in 2001. Pathotypes 166337 and 166376 were not detected in 2002, but pathotype 166336 remained at a frequency of 25%. Pathotype 073100, although avirulent on *Lr13* (one of the resistance genes of cv. Trémie), was found each year in P₃ at frequencies between 3 and 14%. Of the 32 pathotypes found during the 4 years on cv. Trémie, 13 were avirulent either on *Lr10* (four pathotypes) or *Lr13* (eight pathotypes) or on both genes (one pathotype).

The distribution of pathotypes was more balanced on cv. Apache (*Lr13* and *Lr37*), with the population comprising eight pathotypes at frequencies between 3 and 16% and a group of rare pathotypes representing 19 to 26% of the population. The population on cv. Orvantis (*Lr10*, *Lr13*, and *Lr37*) comprised the lowest number of pathotypes, with only four pathotypes at frequencies between 6 and 33% and rare pathotypes representing 33% of the population. All four pathotypes found on cv. Orvantis were present on Apache. In addition, one found on Apache (pathotype 166336) was also found on Trémie and a second (pathotype 075317 virulent on *Lr2c*, *Lr3a*, *Lr3bg*, *Lr3ka*, *Lr13*, *Lr14a*, *Lr15*, *Lr17*, *Lr26*, *Lr17b*, and *Lr37* at the seedling stage) was not found on any other cultivar. Again, as for Trémie, although pathotype 073100 was avirulent on *Lr13*, it was present on Apache and Orvantis at a frequency as much as 17%.

Diversity indices. The highest richness index, calculated as the number of phenotypes in the sample, was always obtained on the population P_b (Table 2). In 1999, the lowest richness was observed on cv. Trémie (P₃), but in 2000 it was observed on cv. Soissons (P₁). In 2001 and 2002, richness was almost constant across the cultivars, ranging between 10 and 14.

Diversity and evenness for each year across cultivars. The diversity index *H'* was higher in population P_b than in populations P₁ to P₅ for the 4 years and this difference was significant in most cases (12 of 16). For the 4 years, diversity on cv. Soissons was the same as on cv. Isengrain. In 1999, diversity on cv. Trémie was significantly lower than on cvs. Soissons and Isengrain, whereas in 2000, it was higher than on cv. Soissons and the same as on cv. Isengrain. In 2001 and 2002, diversity on the two susceptible cultivars was significantly lower than on the three resistant cvs. Trémie, Apache, and Orvantis.

In 1999, evenness was the same across the cultivars. In 2000, evenness on Trémie was not only higher than on other cultivars, but also on the global population. In 2001, evenness on Soissons, Isengrain, and the global population was significantly lower than on the resistant cvs. Trémie, Apache, and Orvantis. In 2002, the situation was not so clear, differences being significant in only one-half of the cases. Evenness on Isengrain was lower than on any other cultivar except Soissons.

Diversity and evenness for each cultivar across years. In 1999, diversity and evenness on cv. Soissons were higher than in the three other years. Diversity on cv. Isengrain was rather constant across the years, but was lowest in 2001. Some variations in evenness were observed, which was highest in 1999 and lowest in 2000. For cv. Trémie, diversity was lowest in 1999, reached a maximum in 2000, and then decreased in 2001 and 2002, whereas evenness was constant across the 4 years. Diversity and evenness were stable for cvs. Apache and Orvantis between 2001 and 2002. For the population P_b, diversity was very stable across the years, varying between 2.43 and 2.76, whereas evenness varied, with a maximum (0.595) in 1999, a minimum (0.350) in 2000, and then increased to reach in 2002 the same level (0.507) as in 1999.

Relative importance of the different resistance genes in the host population. Resistance gene *Lr13* was the most commonly detected gene, found in 25 of 35 cultivars analyzed (Table 7) and was also the most widely deployed gene present in average on 40.1% of the wheat area during 1999-2002. *Lr14a*, although found in only seven of the cultivars, was similar to *Lr13* by the importance of cultivation (35% of the wheat area) because of the dominance of cv. Soissons (*Lr14a*). Fourteen of 35 cultivars examined had either *Lr13* or *Lr14a* alone. In addition, resistance genes *Lr3*, *Lr10*, *Lr20*, *Lr26*, and *Lr37* were postulated in the cultivars (Table 7). Three cultivars had no detectable resistance

TABLE 4. Number of isolates of *Puccinia triticina* collected from durum wheat (population P_d) in France in 1999-2001, identified for virulence phenotype on 18 differential hosts

Virulence phenotype ^a	Virulences (<i>Lr</i> genes)	Year			Total
		1999	2000	2001	
012020	2c, 10, 20	5	6	28	39
073100	2c, 3a, 3bg, 3ka, 10, 14a		6	7	13
012062	2c, 10, 20, 23, 17b	1		8	9
075337	2c, 3a, 3bg, 3ka, 13, 14a, 15, 17, 20, 26, 17b, 37	3			3
077317	2c, 3a, 3bg, 3ka, 10, 13, 14a, 15, 17, 26, 17b, 37	2			2
012120	2c, 10, 14a, 20			2	2
771237	1, 2a, 2b, 2c, 3a, 3bg, 3ka, 15, 17, 20, 26, 17b, 37	2			2
010120	2c, 14a, 20	2			2
012400	2c, 10, 16		2		2
014103	2c, 13, 14a, 26, 17b	2			2
775237	1, 2a, 2b, 2c, 3a, 3bg, 3ka, 13, 15, 17, 20, 26, 17b, 37	1			1
071615	2c, 3a, 3bg, 3ka, 15, 16, 17, 26, 37	1			1
016404	2c, 10, 13, 16, 37		1		1
075317	2c, 3a, 3bg, 3ka, 13, 14a, 15, 17, 26, 17b, 37			1	1
012126	2c, 10, 14a, 20, 17b, 37		1		1
012122	2c, 10, 14a, 20, 17b			1	1
Total number of isolates		19	16	47	82
Total number of pathotypes		9	5	6	16

^a Six-digit code based on a 18 *Lr* genes differential set (Thatcher lines *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr13*, *Lr14a*, *Lr15*, *Lr16*, *Lr17*, *Lr20*, *Lr23*, *Lr26*, the Australian cv. Harrier (*Lr17b*), and *Lr37*).

gene. Combinations of two resistance genes were postulated for 13 cultivars, and combinations of three resistance genes were postulated for only three cultivars, of which two were registered recently (Orvantis in 2000 and Caphorn in 2001).

DISCUSSION

Since virtually all commercial wheat fields in France are sprayed with fungicide we had to collect isolates from unsprayed

blocks in experimental nurseries. As evaluated with the Shannon index, nursery collections were more diverse than field collections in the prairie area in Canada in 1990 (15), however, based on the Rogers index and contingency table analysis, they were regarded as similar. Although it was stated that nurseries could not accurately reflect the level of diversity occurring in commercial fields (18), it was concluded in a later study (19) that in most areas there were no consistent differences in pathotype diversity between field and nursery collections.

TABLE 5. Number of isolates of *Puccinia triticina* collected from bread wheat (population P_b) in France during 1999-2002 and identified by virulence phenotype on 18 differential hosts

Virulence phenotype ^a	Virulences (Lr genes)	Year				Total
		1999	2000	2001	2002	
073100	2c, 3a, 3bg, 3ka, 10, 14a	36	76	98	67	27
016404	2c, 10, 13, 16, 37	17	10	17	21	65
016206	2c, 10, 13, 15, 17b, 37	10	6	12	28	56
016504	2c, 10, 13, 14a, 16, 37	14	14	11	16	55
014103	2c, 13, 14a, 26, 17b	27	9	6		42
077317	2c, 3a, 3bg, 3ka, 10, 13, 14a, 15, 17, 26, 17b, 37	6	4	12	15	37
075317	2c, 3a, 3bg, 3ka, 13, 14a, 15, 17, 26, 17b, 37	9	1	8	14	32
166336	1, 3a, 3bg, 10, 13, 14a, 15, 17, 20, 17b, 37			11	17	28
166337	1, 3a, 3bg, 10, 13, 14a, 15, 17, 20, 26, 17b, 37		2	10		12
016505	2c, 10, 13, 14a, 16, 26, 37			7	3	10
010103	2c, 14a, 26, 17b	6		4		10
073120	2c, 3a, 3bg, 3ka, 10, 14a, 20	3	3		3	9
016501	2c, 10, 13, 14a, 16, 26	1	6		1	8
075717	2c, 3a, 3bg, 3ka, 13, 14a, 15, 16, 17, 26, 17b, 37		5	2		7
071100	2c, 3a, 3bg, 3ka, 14a	5	1		1	7
166376	1, 3a, 3bg, 10, 13, 14a, 15, 17, 20, 23, 17b, 37			6		6
014503	2c, 13, 14a, 16, 26, 17b			6		6
016500	2c, 10, 13, 14a, 16		4	1		5
016100	2c, 10, 13, 14a	1	3		1	5
014100	2c, 13, 14a	5				5
116206	1, 2c, 10, 13, 15, 17b, 37			1	3	4
077337	2c, 3a, 3bg, 3ka, 10, 13, 14a, 15, 17, 20, 26, 17b, 37				4	4
075337	2c, 3a, 3bg, 3ka, 13, 14a, 15, 17, 20, 26, 17b, 37	3		1		4
012002	2c, 10, 17b		2	2		4
016503	2c, 10, 13, 14a, 16, 26, 17b		4			4
012004	2c, 10, 37				4	4
016104	2c, 10, 13, 14a, 37			2	2	4
777634	1, 2a, 2b, 2c, 3a, 3bg, 3ka, 10, 13, 15, 16, 17, 20, 37	3				3
077500	2c, 3a, 3bg, 3ka, 10, 13, 14a, 16		3			3
014206	2c, 13, 15, 17b, 37		1	2		3
012020	2c, 10, 20	1	1		1	3
071501	2c, 3a, 3bg, 3ka, 14a, 16, 26	3				3
071303	2c, 3a, 3bg, 3ka, 14a, 15, 26, 17b		3			3
012006	2c, 10, 17b, 37	1			2	3
012206	2c, 10, 15, 17b, 37				3	3
016405	2c, 10, 13, 16, 26, 37		1	1	1	3
016306	2c, 10, 13, 14a, 15, 17b, 37		3			3
006506	10, 13, 14a, 16, 17b, 37				2	2
016202	2c, 10, 13, 15, 17b		2			2
012404	2c, 10, 16, 37				2	2
010502	2c, 14a, 16, 17b	1			1	2
016216	2c, 10, 13, 15, 17, 17b, 37			1	1	2
077310	2c, 3a, 3bg, 3ka, 10, 13, 14a, 15, 17			2		2
074114	2c, 3a, 3bg, 13, 14a, 17, 37		1	1		2
073501	2c, 3a, 3bg, 3ka, 10, 14a, 16, 26	1	1			2
073301	2c, 3a, 3bg, 3ka, 10, 14a, 15, 26		2			2
073101	2c, 3a, 3bg, 3ka, 10, 14a, 26		2			2
071337	2c, 3a, 3bg, 3ka, 14a, 15, 17, 20, 26, 17b, 37	1	1			2
016407	2c, 10, 13, 16, 26, 17b, 37	1	1			2
012226	2c, 10, 15, 20, 17b, 37		1		1	2
014102	2c, 13, 14a, 17b			1	1	2
Number of isolates		168	188	234	231	821
Number of pathotypes		35	45	34	42	104
Number of pathotypes found once in four years ^b		13	15	9	16	53
Unique pathotypes ^c		21	25	16	25	53

^a Six-digit code based on an 18-Lr genes differential set (Thatcher lines Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr3ka, Lr10, Lr13, Lr14a, Lr15, Lr16, Lr17, Lr20, Lr23, Lr26, the Australian cv. Harrier (Lr17b), and Lr37).

^b Pathotypes found only once in 4 years have been pooled in a group and their virulence formula is not given here.

^c Number of pathotypes found only once for each year.

Diversity and evenness in P_b were stable across the 4 years. The diversity in P_b , as measured by the Shannon index, ranged between 2.43 and 2.76. These values were very similar to those observed in some epidemiological areas of the United States (18,19). In the eastern and prairie areas of Canada in the period 1931-1987, the Shannon index was mostly between 1 and 2 and it dropped below 1 in the prairies after the introduction of resistant cultivars (14), but the use of only four differential lines resulted in lower diversity as compared with other studies using more differential lines. In Israel, with isolates derived from durum wheat and bread wheat and in an area where a high diversity could be expected because of the presence of indigenous wild *Triticum* and *Aegilops* spp. relatives, the Shannon index values were very close to those calculated in our study, ranging between 2.147 and 2.969 in 1994-1997 (21).

The Shannon index calculated for France in a previous study in 1995 (29) was lower than the values obtained here, ranging between 0.75 and 1.67. In the 1995 study, sample sizes were smaller than in our study. The Shannon index underestimates diversity in small samples as compared with big samples (19). Although Park et al. (29) used 25 differentials while we used only 18, they did not include *Lr13* and *Lr14a*, the most frequent resistance genes (Table 7) in the French wheat host population. Using these resistance genes in our differential set may have permitted discrimination of more pathotypes. Although the differential set we used differed from that used by Park and Felsenstein (28) for a few lines, it is likely that the two predominant pathotypes 17 and 39 (28) found in Europe in 1995 were the same as pathotypes 014103 and 073100 in our study, respectively. On the other hand, pathotypes 3 and 6, common in France in the 1995 study, were not found in our study. Aerial spore trapping as used in the 1995 study has been shown to reflect mainly pathogen populations from fields bordering the sampling route (10). The spores trapped from pathotypes 3 and 6 might therefore, have come mainly from Sideral (*Lr13* and *Lr14a*), the second most widely grown cultivar in these areas in 1995. The sharp decline in the cultivation of Sideral from 1998 and lack of a sample from it in the current study may explain why pathotypes 3 and 6 were not detected during the 1999-2002 period.

Comparison between durum wheat (P_d) and bread wheat (P_b). The first level of differentiation made in French wheat leaf rust populations was between isolates originating from durum and bread wheat. Although virulence frequencies (Table 5) could be

biased because of the smaller size of the sample derived from P_d , the frequency of virulence on *Lr20* was much higher on P_d than on P_b , and the frequency of virulence on *Lr13*, *Lr14a*, and *Lr16* was lower on P_d than on P_b . Resistance gene *Lr20* is infrequent in bread wheat cultivars (Table 7). Information about *Lr* resistance genes in the durum wheat cultivars is missing so we do not know if the high virulence frequency for *Lr20* in P_d results from selection by host. The most frequent pathotype on durum wheat, 012020, represented 47% of all pathotypes collected in 3 years for durum wheat, whereas it was only 0.3% of pathotypes collected in 4 years from bread wheat, which is not surprising given that 012020 did not have the virulences matching the resistance genes carried by the main bread wheat cultivars. Almost one-half of the pathotypes found on durum wheat were rare or not found at all on bread wheat. Thus, we concluded that leaf rust populations from durum and bread wheat were different as was shown in Morocco (6), Mexico (33), and international collections (11,26). Pathotype 012062, found in 2001 in France (Table 5), appears to be the same as the new race BBG/BN detected in 2001 on durum wheat in Mexico (34).

With the differential set used, we were able to detect differences between P_d and P_b , thus, to conclude to a differential selection exerted by host species. A study of selection by host within P_d would require the characterization of the resistance genes in the durum wheat cultivars, and the definition of a corresponding differential set. Moreover, it is possible that *P. trititica* and *P. recondita* occur on durum wheat in France since both species occur in the Mediterranean area, (Italy, Morocco, Spain, and Portugal) (2).

Diversity and distribution of pathotypes in the bread wheat leaf rust populations (P_1 to P_5). Differences between the years could be found in diversity or evenness for some cultivars, but no year was characterized by a general increase or decrease in these two indexes. Diversity was similar in the two populations from susceptible cvs. Soissons (P_1) and Isengrain (P_2) in the 4 years, but it was lower than in the three populations from resistant cvs. Trémie (P_3), Apache (P_4), and Orvantis (P_5) in all years except 1999 where it was lower in P_3 than in P_1 and P_2 . Since evenness was the same across the cultivars in 1999, the difference between P_3 on one hand and P_1 and P_2 on the other hand in that year was due to a lower richness in P_3 . In the two following years, 2000 and 2001, the significant differences in diversity between susceptible and resistant cultivars were attributed to differences in

TABLE 6. Virulence frequencies on *Lr* genes in wheat leaf rust populations collected from durum wheat (P_d) and bread wheat (P_b) cultivars in France in 1999-2002

Gene	1999		2000		2001		2002 ^a
	Durum	Bread	Durum	Bread	Durum	Bread	Bread
<i>Lr1</i>	0.16	0.02	0.00	0.03	0.00	0.13	0.10
<i>Lr2a</i>	0.16	0.02	0.00	0.01	0.00	0.00	0.00
<i>Lr2b</i>	0.16	0.02	0.00	0.01	0.00	0.00	0.00
<i>Lr2c</i>	1.00	0.99	1.00	0.98	1.00	0.88	0.90
<i>Lr3a</i>	0.47	0.44	0.38	0.57	0.17	0.66	0.54
<i>Lr3bg</i>	0.47	0.44	0.38	0.57	0.17	0.66	0.54
<i>Lr3ka</i>	0.47	0.44	0.38	0.56	0.17	0.53	0.46
<i>Lr10</i>	0.42	0.61	1.00	0.86	0.98	0.85	0.90
<i>Lr13^b</i>	0.42	0.62	0.06	0.46	0.02	0.53	0.61
<i>Lr14a^b</i>	0.47	0.79	0.44	0.84	0.23	0.83	0.69
<i>Lr15</i>	0.47	0.22	0.00	0.19	0.02	0.31	0.40
<i>Lr16^b</i>	0.05	0.26	0.19	0.29	0.00	0.20	0.21
<i>Lr17</i>	0.47	0.16	0.00	0.08	0.02	0.24	0.25
<i>Lr17b</i>	0.58	0.39	0.06	0.27	0.21	0.39	0.44
<i>Lr20^b</i>	0.74	0.08	0.44	0.05	0.83	0.13	0.12
<i>Lr23</i>	0.05	0.00	0.00	0.00	0.17	0.03	0.00
<i>Lr26</i>	0.58	0.36	0.00	0.26	0.02	0.25	0.19
<i>Lr37</i>	0.47	0.42	0.13	0.30	0.02	0.47	0.65

^a Durum leaf rust population was not studied in 2002.

^b The virulence frequencies were significantly different at $P < 0.05$ between durum wheat and bread wheat (one-way analysis of variance with the years considered as replicates).

evenness because evenness on the resistant cultivars was always higher than on the susceptible cultivars. The situation was similar in 2002, although differences in evenness between cultivars were not always significant. However, it is difficult to attribute the significant differences in diversity to the significant differences in richness because richness values for the different cultivars were very close, being between 11 and 14. The small sample sizes of isolates collected from resistant cultivars could have been responsible for the lack of significance in some cases. Despite this, the general trend from these results was that diversity was higher on resistant cultivars than on susceptible cultivars, and this difference was due to evenness as it is shown clearly on Figure 1. Evenness on the susceptible cultivars was low because of the dominance of two pathotypes, 014103 and 073100 in 1999, and a single pathotype, 073100 in 2000-2002, the frequency of which varied between 54 and 72%. In contrast, in the Canadian prairie area in 1990, the Shannon index for resistant cultivars (pooled population sampled from 13 cultivars) was significantly lower than for susceptible cultivars (pooled population sampled from five cultivars), although the Rogers index and contingency table analysis showed that there was no significant difference between populations from resistant and susceptible cultivars (15).

The distribution of pathotypes was very similar on the two susceptible cvs. Soissons and Isengrain that carried the largely ineffective resistance gene *Lr14a*, both of which also have a very similar genetic background. The populations P₁ (Soissons) and P₂ (Isengrain) were dominated by two pathotypes in 1999, after which one (014103) decreased in frequency and disappeared in 2002, while the other (073100) represented more than 70% of the population. The pathotype distribution was more balanced on the resistant cultivars as reflected by the evenness indexes being higher than on susceptible cultivars. A strong and rapid change in P₃ was detected when two of the most frequent pathotypes, 016504 and 016206, dropped in frequency in 2001 and were replaced by a group of three very similar pathotypes designated as 'group 166'. This group, absent in 1999, represented 6% of P₃ in 2000 and then 38% in 2001. Group 166 comprised the most virulent pathotypes (virulent on 12 of 18 differential lines) and differed greatly from the other pathotypes common in France by avirulence on *Lr2c*, virulence on Gatcher (results not presented here), combined virulence on *Lr1*, *Lr10*, and *Lr13*, combined virulence on *Lr3a* and *Lr3bg*, and avirulence on *Lr3ka*. The most similar pathotypes described in the literature were pathotypes MBD/SM and MCD/SM, dominant in CIMMYT nurseries in

(continued on next page)

Fig. 1. Frequencies of *Puccinia triticina* pathotypes on five major bread wheat cultivars (populations P₁ to P₅) in France during 1999-2002. Black bars = pathotype carrying the virulence gene(s) matching all the resistance gene(s) of the cultivar. Shaded bars and corresponding pathotype name in bold = pathotype lacking at least one of the virulence gene(s) matching resistance gene(s) of the cultivar. The pathotypes found only once at most for each year have been pooled in the category 'rare'. Susceptible cultivars are Soissons (P₁) and Isengrain (P₂). Resistant cultivars are Trémie (P₃), Apache (P₄), and Orvantis (P₅).

1990 (33), but with the difference that these latter pathotypes were virulent on *Lr28*, whereas group 166 pathotypes were avirulent on *Lr28* (results not presented here). Virulence for *Lr28* is a characteristic of North American populations of *P. triticina* because the frequency of virulence for this gene is very high in these populations (33). Races with the same virulence as 'group 166' were found in North America starting in the mid-1990s. These races were found to have very distinct amplified fragment length polymorphism (AFLP) patterns compared with other races (17).

The isolation of pathotype 073100, avirulent on *Lr13*, from three cultivars carrying this gene (Trémie, Apache, and Orvantis) was not unexpected because it was known that this resistance gene does not completely prevent sporulation by avirulent pathotypes. Although 98% of the wheat fields in France are sprayed with fungicide, inoculum is present every year as shown by epidemics occurring on any untreated plot. On a cultivar as susceptible as Soissons, infections can still be observed after treatments when environmental conditions are conducive. Infections can also be easily observed in newly planted commercial fields in the fall and it is likely that some inoculum survive on volunteers during the summer. The stability of the pathotype distribution on cv. Soissons over 4 years also suggested survival of local inoculum. The differences between pathotype distributions observed in

populations from cvs. Soissons and Trémie suggested that a strong selection was exerted on the inoculum by the cultivar.

Distribution of pathotypes and resistance factors in the cultivars. In several cases, the distribution of some pathotypes on the different cultivars could not be explained by the selection exerted by specific host resistance genes only. Ten to eighteen pathotypes, although virulent on *Lr14a*, and therefore, virulent on cvs. Soissons and Isengrain carrying only *Lr14a* and no adult plant resistance gene, occurred in P_1 and P_2 but remained at frequencies much lower than the dominant pathotype 073100. Several virulence gene combinations, including the most complex one, were found only on resistant cultivars even though these multivirulent pathotypes were primarily virulent on the susceptible cultivars and despite the fact that our samples were collected from small plot nurseries, where exchange of inoculum between plots is expected to be frequent. The two cultivars from which populations were similar (Soissons and Isengrain) had the same resistance gene and a similar genetic background. It is particularly surprising that pathotype 073100 remained at a high and stable frequency during the 4 years, whereas all other virulent pathotypes either decreased in frequency (014103) or never increased in frequency in the population. Given that Soissons was a largely dominant cultivar in the period 1991-1999 and considering that

Fig. 1. (continued from preceding page)

pathotype 073100 was not frequent on the other dominant cultivars (except on Isengrain, which has the same resistance gene and a genetic background close to that of Soissons), it is unlikely that the dominance of 073100 in the global population could be accounted for by another reason than a strong aggressiveness on cvs. Soissons and Isengrain. This hypothesis is reinforced by considering the transition between 1999, where 073100 coexisted with 014103, and year 2000, where 073100 remained the only dominant pathotype. Although this has not been tested, this high frequency of 014103, virulent on *Lr13* in 1999, might result from the development of epidemics on cv Thésée (*Lr13*) that was largely cultivated from 1988 to 1997 and on which 073100 is avirulent.

It appears then that leaf rust epidemics on the susceptible cultivars were mainly caused by a single pathotype (073100) despite the presence of a fairly large diversity of other virulent pathotypes in the global population, and this for several years. In this situation, the knowledge of the frequency and distribution of resistance genes in the host population and of the virulence combinations in the pathogen population is not sufficient to predict or explain the distribution and frequency of the pathotypes. When isolates of *P. graminis* f. sp. *tritici*, virulent on commonly grown cultivars, were found at very low frequencies (31), it was suggested by Alexander et al. (1) that negative associations between virulence and traits affecting spore survival and reproduction may be responsible. In our case, a converse hypothesis may be proposed, in which a higher aggressiveness of pathotype 073100 could lead to a strong dominance of this pathotype over other pathotypes virulent on *Lr14a*. A few population studies show that the dominance of a subpopulation (pathotype, clonal lineage) in a global population can be accounted for by differences in aggressiveness (24). This can sometimes be explained by a better adaptation to specific climatic conditions (13). This latter explanation is unlikely here, given the high frequency of 073100 over several consecutive years. Other studies have concluded that rust population structure is likely to be influenced by characters other than the interaction of host resistance genes and corresponding genes in the pathogen (22,29). High levels of diversity maintained in North American wheat leaf rust populations indicate that selection for virulence to

host resistance genes is effectively balanced by other selective forces (16).

Group 166, virulent on *Lr10* and *Lr13*, occurred on cvs. Trémie (*Lr10* and *Lr13*) and Apache (*Lr13* and *Lr37*) but not on cv. Orvantis (*Lr10*, *Lr13*, and *Lr37*). Thus again, interactions with a specific resistance gene(s) cannot explain the absence of this group on cv. Orvantis, unless cvs. Apache and Orvantis have unknown and different adult-plant resistance gene(s). Pathotype 077317, although virulent on *Lr10* and *Lr13*, was absent or rare on cv. Trémie. For these populations, derived from resistant cultivars and where the structure observed could not be fully explained by interaction of known genes for resistance and avirulence, possible differences in aggressiveness may contribute to pathogen population composition, as for the dominance of 073100 on cvs. Soissons and Isengrain. However, unlike for the susceptible cultivars, it is not known here whether specific adult-plant resistance is present in the resistant cultivars. For example, Bartos et al. (4) concluded that race frequency in the former Czechoslovakia could not be explained on the basis of host selection, but the study of Park et al. (29) showed that many cultivars registered there carried *Lr13*, a gene that had not been considered in their study. In France, unexplained differences between leaf rust populations from cvs. Soissons and Thésée in 1993-1995 (8) may well have been due to the presence of the resistance genes *Lr14a* in Soissons and *Lr13* in Thésée, as virulence for these genes was not monitored in these years.

In summary, *P. triticina* populations in France were diverse for virulence despite the presence of only a few specific resistance genes in host bread wheat cultivars. The potential occurrence of recombination, which could explain the observed diversity, still has to be tested. Populations were strongly differentiated between durum wheat and bread wheat cultivars and within bread wheat cultivars. Diversity was high on those that were resistant, mainly due to an evenness found to be higher for resistance as compared with susceptible cultivars. The population structure described in our study stresses the importance for sampling procedures to take into account the host cultivar for measuring the influence of selective factors on wheat leaf rust populations, as completely different virulence or pathotype frequencies could be obtained, depending on the host cultivars represented in the sample.

Last, we showed with a large-scale study that specific interactions in the leaf rust–wheat pathosystem were necessary but not sufficient to account for pathotype distribution and frequency on the cultivars. Other factors like aggressiveness, could account for the dominance of one or a few pathotypes on some cultivars over several years.

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TABLE 7. Mean percentage of wheat area planted during 1999-2002 with the different *Lr* genes postulated in the 35 most commonly grown cultivars

<i>Lr</i> gene combination	Mean percentage of wheat area	No. of cultivars
No gene	4.3	3
<i>Lr10</i> , <i>Lr13</i>	10.0	3
<i>Lr10</i> , <i>Lr13</i> , <i>Lr26</i>	0.7	1
<i>Lr10</i> , <i>Lr13</i> , <i>Lr37</i>	0.7	2
<i>Lr13</i>	16.2	10
<i>Lr13</i> , 20	1.0	1
<i>Lr13</i> , <i>Lr14a</i>	5.3	2
<i>Lr13</i> , <i>Lr26</i>	2.5	1
<i>Lr13</i> , <i>Lr37</i>	2.9	3
<i>Lr14a</i>	29.4	4
<i>Lr14a</i> , <i>Lr37</i>	0.3	1
<i>Lr26</i>	0.6	1
<i>Lr3a</i>	0.6	1
<i>Lr3a</i> , <i>Lr13</i>	0.8	2
Total	75.3 ^a	35
Cumulative percentage of wheat area for each <i>Lr</i> gene		
<i>Lr</i> gene		
<i>Lr13</i>	40.1	
<i>Lr14a</i>	35.1	
<i>Lr10</i>	11.3	
No gene	4.3	
<i>Lr37</i>	4.0	
<i>Lr26</i>	3.2	
<i>Lr3a</i>	1.4	
<i>Lr20</i>	1.0	

^a The total is not 100% because the minor cultivars are not included in this table.

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