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Abstract – With standardised near isogenic line (NIL) differentials co-operators were able to present the first comprehensive virulence survey of the European wheat leaf rust population (1996–1999). The work included pathotype identification of 2608 isolates and field tests of NILs. *Lr9* and *Lr19* were very effective all over Europe. *Lr24*, *Lr25,* and *Lr28* were also effective, but in some countries and locations substantial virulence frequencies were observed. In addition, the genes *Lr12, Lr13, Lr22a, Lr34, Lr35* and *Lr37* were effective at the adult plant stage, but locally less so. In general, the indoor seedling tests and adult plant field tests showed good agreement. Virulence to *Lr1*, *Lr2a*, *Lr24, Lr25*, *Lr28* and *Lr29* tended to increase in the period, for the other *Lr*-genes the virulence frequency remained more or less stable. Among the 105 pathotypes identified none was clearly predominant in Europe.

leaf rust / wheat / virulence / pathotypes / breeding for resistance

Résumé – **La situation en Europe pour la virulence de la rouille brune chez le blé.** L'utilisation d'une gamme d'hôtes différentiels commune composée de lignées isogéniques (NIL) a permis aux auteurs de réaliser le premier inventaire exhaustif de la population européenne de rouille brune du blé (1996–1999). Deux mille six cent huit isolats ont été identifiés et les NIL ont été évaluées au champ. *Lr9* et *Lr19* se sont révélés efficaces dans toute l'Europe. *Lr24*, *Lr25* et *Lr28* ont également été efficaces, mais la fréquence des virulences correspondantes était non négligeable dans certains pays et certains lieux. Les gènes *Lr12, Lr13, Lr22a, Lr34, Lr35* et *Lr37* ont été efficaces au stade adulte, excepté dans quelques lieux. En général, les résultats des tests au stade plantule en conditions contrôlées ont été cohérents

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avec ceux obtenus au stade adulte au champ. Les fréquences de virulence sont demeurées stables au cours de la période étudiée, sauf pour *Lr1, Lr2a, Lr24, Lr25* et *Lr29*, dont les fréquences de virulence correspondantes tendaient à augmenter. Aucun des 105 pathotypes identifiés n'est apparu clairement dominant en Europe.

rouille brune / blé / virulence / pathotypes / sélection pour la résistance

1. Introduction

Wheat leaf rust is caused by the rust fungus *Puccinia triticina* [1]. Often used synonyms of this pathogen are *P. tritici, P. recondita* in short form, or *P. recondita* Rob. ex Desm. f. sp. *tritici* (Eriks.) Carl. Leaf rust is a major disease in most wheat growing areas [26]. More than 45 resistance genes to this disease have been identified up to now and to most of them the pathogen has developed virulence. Virulence surveys aim to detect new pathotypes and monitor shifts of pathotype frequencies, and to help breeders in proposing efficient resistance strategies to this disease. Such virulence survey work has a long tradition [12].

As stated by Zadoks and Bouwman [39], "race identification of wheat leaf rust has been messy for a long time" in Europe. This was caused by the lack of agreement on a standard differential set to discriminate between pathotypes. The situation was similar in America and Australia. Zadoks and Bouwman [39] noted that the international situation has much improved. McIntosh et al. [21] described the differential sets used in Australasia [24], North America [15, 18, 29], Middle America [2], South Africa [25], and India [22]. There are substantial differences between those sets. McIntosh et al. [21] did not mention a differential set used in Europe, although virulence surveys have been conducted in various countries for several years [3–5, 7, 11, 13, 20, 23, 33, 35]. Each country or laboratory has used and maintained its own differential set based mostly on those used in the traditional American surveys, but according to local needs they added or deleted certain lines in the set.

Recently various European laboratories set up differential sets based on the 'Thatcher' Near Isogenic Lines (NIL) series developed by Dyck in Canada (see Refs. in [21]). This was a first step towards standardisation of the virulence survey procedures.

The present paper reports (i) the implementation of a common procedure of virulence survey of wheat leaf rust and (ii) the determination of virulence and pathotype frequencies in Europe, by means of seedling and field tests.

2. Materials and methods

2.1. Differential set

Dr. Kolmer (Winnipeg, Canada) kindly provided the differential lines (Near-isogenic lines, NILs, in 'Thatcher' background) in 1994. At the Cereal Research Institute, Szeged, the lines were sown in 10 m long rows, 30 cm apart with 8 cm inter-plant spacing. All heads of 20 plants from each line were covered by paper bags before anthesis to prevent cross-pollination and only those were harvested of which the morphology and infection type (IT) were typical to the majority of plants in that line. These isolated and checked plants provided the basic seeds for distribution to the co-operators and further multiplication. In co-operation with Dr. Bartoš, some lines showing ambiguous IT were tested in the seedling stage to verify their identity.

Fifteen NILs were chosen to assemble a core differential set for use in all European countries. This set consisted of the 'Thatcher' NILs carrying the resistance genes *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr9, Lr11, Lr15, Lr17, Lr19, Lr21, Lr23, Lr24, Lr26,* and *Lr28*, respectively. These differentials are used also in other continents, their reaction is expressed in seedlings, and they were considered relevant to detect new virulences in the leaf rust

population. Some co-operators included more than these 15 differential lines, especially the differentials carrying genes *Lr25, Lr29* and *Lr30*.

2.2. Isolate sampling and assessment of virulence

The virulence survey was based on monopustule isolates. Each co-operator collected infected leaves from naturally infected plants of NILs and/or other wheat genotypes at several locations of their country. The urediospores from each leaf were transferred to a universally susceptible genotype and from the developing uredinia one or two monopustule-isolates were produced. In total 2608 monopustule isolates were tested, on average 655 per year (Tab. I). The samples represented populations from 12 European countries.

Spore suspensions or spore-talcum mixtures (1:3) of each isolate were applied onto the first leaf of seedlings of the differential NILs. After 24 or 48 h of 100% relative humidity under darkness, the seedlings were incubated at about 22/18 °C night/day. At some locations the temperatures were 3 °C higher or lower. Detached leaves were used only in Poland [9].

In Spain (1998) infected leaf samples were collected from several locations in the country and the spores of each sample were applied directly on the NILs in the greenhouse. In this case the virulence frequency is expressed relative to the total number of locations sampled (Tab. I). The data for other years and countries pertain to monopustule isolates as described above.

Seedling reactions were evaluated according to the Stakman scale (0–4) [34]. Infection types 2 and lower were interpreted as resistance/avirulence, IT 2+ and above susceptibility/virulence.

2.3. Adult plant tests

For the field evaluation the core differential set was mostly grown as unreplicated single rows or hill plots usually near wheat breeding germplasm. The trial consisted of the differential set of lines, including the lines with adult plant resistance genes, and was planted at 1 to 14 locations per country (five countries) per year. For evaluation of the disease severity and type of the natural infection, the modified Cobb's scale [2] was used. This code consisted of the percentage of total leaf area, covered with uredia or necrotic flecks, and the IT, e.g. 5R, 40MS, or 40MR-MS. Some authors used a scale 1–9 (1 resistant, 9 susceptible). The moment of the evaluation varied among the co-operators. One or more ratings were made and the latest was usually made at milk ripening when the upper leaves were still green. In the tables the latest scores are given. Since no fungicides were applied, other leaf pathogens could influence assessment of the severity of infection by leaf rust.

Table I. Number of monopustule isolates or bulk samples tested in the European virulence survey, 1996–1999.

Years	Countries												Sum
	F	D	I	CZ	SK	GB	SP	H	PL	BG	RO	CH	
1996	54	128	77	89	63	0	0	33	175	110	0	72	801
1997	92	92	61	44	30	4	7	100	205	52	0	0	687
1998	62	41	68	30	11	0	13*	80	330	62	14	0	698*
1999	69	58	0	33	35	45	16	0	152	0	14	0	422
Sum	277	319	206	196	139	49	16*	213	862	224	28	72	2608*

F = France, D = Germany, I = Italy, CZ = Czech Republic, SK = Slovakia, GB = Great Britain, SP = Spain, H = Hungary, PL = Poland, BG = Bulgaria, RO = Romania, CH = Switzerland.
0 = no data from that year.
*Bulk samples from different locations, not considered in Table III.

3. Results

3.1. Virulence frequencies

The virulence frequencies in each country for 1998 are presented in Table II. In that year the surveys covered 10 countries, so that the most comprehensive picture was obtained. In Table III the mean virulence frequencies over Europe are presented for each year.

Lr9 and *Lr19* were the only genes that were fully effective all over Europe. In most countries the respective NILs remained free of infection. The results obtained for other years indicate that a significant virulence to *Lr9* and *Lr19* still does not occur in Europe.

Virulence to *Lr24* was rare or absent in most countries, but relatively common in Bulgaria (30–50 %), and occurred also in Germany and Romania in 1998 (Tab. II). In 1999, the virulence frequency in Romania had increased to 86%. These results suggest a new pathotype spreading into Europe from Bulgaria, as the virulence frequency in that country was high before it rose in Romania.

Table II. Percentage of isolates of *Puccinia triticina* virulent at the seedling stage on single-gene differential lines in European countries in 1998.

Lr Genes	Reference stock	Countries									
		F	D	I	CZ	SK	SP*	H	PL	BG	RO
Lr1	Centenario/6*Thatcher R.L.6003	3.0	52.0	5.9	23.0	27.0	57.0	12.5	16.0	42.3	86.0
Lr2a	Webster/ 6*Thatcher , R.L.6016	0.0	58.6	1.5	23.0	9.0	36.0	15.0	11.0	9.6	79.0
Lr2b	Thatcher*6/Carina, R.L.6019	5.0	65.5	20.6	33.0	36.0	64.0	65.0	19.0	51.9	93.0
Lr2c	Thatcher*6/Loros, R.L.6025	97.0	82.8	95.6	100.0	100.0	79.0	97.5	42.0	96.2	100.0
Lr3a	Democrat/6* Thatcher, R.L.6002	69.0	31.0	45.6	100.0	91.0	64.0	65.0	97.0	100.0	93.0
Lr9	Thatcher*6/Transfer, R.L.6010	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–**
Lr11	Thatcher*6/Hussar, R.L.6053	56.0	100.0	80.9	100.0	100.0	100.0	90.0	100.0	100.0	100.0
Lr15	Thatcher*6/Kenya W1483, R.L.6052	11.0	51.7	13.2	77.8	73.0	21.0	55.0	95.0	25.6	100.0
Lr17	Thatcher*6/ K.Lucero, R.L.6008	13.0	100.0	8.8	100.0	91.0	36.0	67.5	96.0	100.0	100.0
Lr19	Thatcher*7/Tr.4 A.elong.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lr21	Thatcher*6//RL 5406=Tetra C., R.L. 6043	3.0	79.3	97.0	100.0	100.0	86.0	87.5	98.0	100.0	100.0
Lr23	Lee FL310/6*Thatcher, R.L. 6012	3.0	96.6	86.8	100.0	100.0	100.0	40.0	1.0	86.5	100.0
Lr24	Thatcher*6/Agent, R.L.6064	0.0	6.9	0.0	0.0	0.0	0.0	0.0	0.0	46.2	14.0
Lr25	Thatcher*7/Transec, R.L.6084	– *	3.4	0.0	–	–	0.0	–	0.0	–	–
Lr26	Thatcher*6/St-1.25, R.L.6078	18.0	48.3	38.2	90.0	73.0	7.0	80.0	98.0	–	100.0
Lr28	Thatcher*6/C77.1, R.L.6079	0.0	–	8.8	0.0	0.0	21.0	0.0	69.0	44.2	86.0
Lr29	Thatcher*6/CS7D/Ag#11, R.L.6080	–	0.0	1.5	–	–	0.0	–	61.0	73.1	–
Lr30	Thatcher*6/Terenzio, R.L.6049	–	69.0	92.6	–	–	64.0	–	96.0	100.0	–
Lr32	Thatcher*7/3Ae. sq, R.L.6086	–	96.6	–	–	–	50.0	–	98.0	–	–
Lr33	Thatcher*6/PI58548, R.L.6057	–	100.0	–	–	–	100.0	–	100.0	–	–
Lr34	Thatcher*6/PI58548, R.L.6058	–	–	–	–	–	100.0	–	100.0	–	–
Lr37	Thatcher*8/VPM1, R.L.6081	–	96.6	–	–	–	100.0	–	96.0	–	–
Lr38	Thatcher*6/T7, R.L.6097	–	13.8	–	–	–	14.0	–	6.0	–	–
Lr44	Thatcher*6/T. spelta 7831	–	75.9	–	–	–	100.0	–	94.0	–	–
LrB	Thatcher*6/Carina, R.L.6051	–	100.0	–	–	–	43.0	–	98.0	–	–
LrW	Thatcher*6/V336	–	6.9	–	–	–	0.0	–	30.0	–	–

*Bulk populations, ** Cases not tested.
F = France, D = Germany, I = Italy, CZ = Czech Republic, SK = Slovakia, SP = Spain, H =Hungary, PL = Poland, BG = Bulgaria, RO = Romania.

Virulence to *Lr25* was also rare, but there are insufficient data to conclude about its distribution and spread over Europe. Relatively low virulence frequencies were observed in Italy (1997 and no virulence in 1998) and in Germany (1998). Also the virulence frequencies to *Lr38* and *LrW* were low, and those genes, therefore, may also be of some interest for breeders.

Virulence frequencies to some *Lr*-genes were variable among countries. For example, the frequencies of virulence to *Lr17* and *Lr26* were relatively low in south-west and southern Europe, but high in central and eastern Europe. For other virulences, e.g. the virulence to *Lr28*, the differences in frequency between countries and years were not easy to interpret. Part of the variation may be due to the fact that some resistance genes (e.g. *Lr17* and *Lr23*) may cause intermediate infection types to avirulent isolates [21], leading to difficulties in interpretation between co-operators.

The virulence frequencies to *Lr2c* and *Lr11* were very high and often 100%. In none of the countries these genes were sufficiently effective.

The frequency of virulence to *Lr34* is hard to determine, since this gene does not cause a hypersensitive reaction [27]. In seedling tests the *Lr34* would merely cause a slightly lower IT [21] or only a longer latent period [27]. It is therefore not surprising that the high IT on the *Lr34* differential was interpreted as virulence of the isolates (Tab. II). Also *Lr37* is considered as effective adult plant resistance gene [21].

The virulence frequencies to *Lr1, Lr2a, Lr24, Lr25, Lr28* and *Lr29* showed a tendency to increase over the period 1996–1999 (Tab. III). The virulence frequencies to the other *Lr*-genes were more stable (e.g. *Lr2b*, *Lr3a*, *Lr17*) or fluctuated in time (e.g. *Lr30*).

Table III. Percentage of isolates of *Puccinia triticina* virulent at the seedling stage on 18 single-gene differential lines in European countries in 1996–1999.

Lr genes	Years				Mean
	1996	1997	1998*	1999	
Lr1	17.2	18.9	29.7	33.7	24.9
Lr2a	19.8	18.9	23.0	27.2	22.2
Lr2b	40.9	47.1	43.2	42.4	43.4
Lr2c	85.5	87.4	90.5	82.5	86.54
Lr3a	68.9	75.8	76.8	69.6	72.8
Lr9	1.0	0.0	0.0	0.0	0.3
Lr11	84.1	79.3	91.9	93.3	87.1
Lr15	60.1	53.4	55.8	68.8	59.5
Lr17	69.9	62.7	75.1	67.9	68.9
Lr19	0.0	0.0	0.0	0.7	0.2
Lr21	76.7	58.8	85.0	72.0	73.1
Lr23	53.0	38.3	71.5	44.9	51.9
Lr24	5.3	4.1	7.4	12.0	7.2
Lr25	1.9	0.0	1.1	9.8	3.2
Lr26	53.7	67.7	68.2	64.2	63.4
Lr28	18.4	6.6	36.0	29.8	25.2
Lr29	33.0	0.0	33.4	60.9	31.8
Lr30	83.7	60.7	89.4	93.0	75.7
Mean	**43.0**	**37.8**	**48.8**	**48.5**	**44.5**
No. of isolates	801	687	698	422	2608

* The bulk populations collected and tested in Spain are not included in this column.

3.2. Effectiveness of the Lr-genes in the field

In the field test under natural infection, the disease severity on the differentials differed highly between countries and between locations within countries (Tab. IV). The data from 1996 and 1997 are not shown, as they were similar to those obtained in 1998 and 1999.

The leaf rust severity on Thatcher was in some cases lower than on some of the NILs.

Lr9 and *Lr19* remained free of infection, although traces of infection were sometimes observed on the *Lr19* differential. These genes were the most effective resistance genes in Europe.

Other seedling resistance genes that appeared to be widely effective in the field tests, were *Lr24, Lr25,* and *Lr38*. The effectiveness of *Lr28* and *Lr29* fluctuated strongly between locations and years.

The field evaluation also allowed evaluation of the effectiveness of resistance genes that are

Table IV. Leaf rust severity in the field on single-gene differential lines in some European countries in 1998 and 1999.

Lr Gene	Romania 1998	Hungary 1998				CH ** 1998	GB 1999	PL 1998	PL 1999
	Fundulea	Szeged	Marton.	Táplán	Budapest	Rkholz	Aberyst.	Kr. **	Kr
Lr1	70MS-S	40MS-S	60S	50R-MR	0	2	18MS	3	40MR
Lr2a	80MS-S	40R-MR-MS	70S	80MS	0	4	18S	3	30MS
Lr2b	80MS-S	–[#]	50S	80MR-MS	30 S	5.5	25S	6	60MS
Lr2c	80S	60MS-S	40S	80MS	40-50MS	6	35S	6	50MS
Lr3	80S	80MR-MS-S	50 S	30R	20 MS	6	45S	7	60MS
Lr3bg	40MS	40MR, S	80S	80MS-S	30-40MS	6	45S	5	70MS
Lr3ka	80S	20R-MR	70S	tR-MR	30-40MS	5	45S	6	40MS
Lr9	–	0	0	0	0	1	0	1	–
Lr10	80S	30MR-MS	20S	60MR-MS	10MR-MS	5	–	6	30MR
Lr11	60MS	30MR	30S	–	5MR-MS	4.5	45S	6	50MS
Lr12-A[†]	70MS-S	20MR-MS	1R-40MS	30R-MR	0	3	–	3	30MR
Lr13-A	80S	40R-MR	0	30R-MR	t R-MR	3	30S	4	50MS
Lr14a	80S	40MR-MS-S	–	40MS	50S	5.5	50S	6	60MS
Lr14b	70MS	10MR	15MS	40MR-MS	10MR-MS	3.5	–	5	70MS
Lr15	80S	20MR-MS	1R-40 S	80MS	40-50MS-S	6	50S	5	70S
Lr16	80S	40MR-MS	50S	60MS	30MS-S	4.5	-	5	50MS
Lr17	–	10MR	1 R	20R-MR	t MS	3	35MR	6	60MS
Lr18	30MS	10R	0-40MR	10R-MR	t MR-MS	2.5	–	3	10R
Lr19	0R	tMR	t R	0	0	1	tR	1	0
Lr20	30MR-MS	10R-MR	0-10MR	40MR	t MR-MS	2	35S	5	30MS
Lr21	70MS-S	10MR	0	60MR-MS	0	2.5	30X	6	50S
Lr22a-A	50MS	tMR	0-30 MR	10R-MR	0	3.5	–	2	10MR
Lr23	80S	2R-MR	1 R	40MS	20MR-MS	3	40X	3	2R
Lr24	0R	t MR	0	t MR	t MR	1	0	1	0
Lr25	–	t MR	0	0	10-30MR-MS	1	–	4	0
Lr26	80S	5R-MR	80S	60MS	30 MS	4.5	45S	7	60MS
Lr28	20MR	5R	0-5 R	80MS	0	1	15R	5	10S
Lr29	90MS-S	30MR-MS-S	0	t MR	0	1	–	3	0
Lr30	90S	5MR	60S	80MS	30MS	4.5	–	7	20MR
Lr32	80MS-S	10R-MR	0 (50 S)	30R-MR	t MR	3	–	5	20MR
Lr33	80MS-S	40MS	30 MS	80MS	0	5	–	7	60MS
Lr34-A	80MS-S	20MR-MS	0 (5 MR)	60MS	t MR	4.5	–	3	10MR
Lr35-A	tMS	0	0-50 R	–	t MR	1	–	3	5R
Lr37-A	50MR-MS	5MR	0	10R-MR	t MR	1	35MR	2	0
Lr38	0R	0	0	–	0	1	–	3	10MR
Lr44	30MR-MS	5 MR	3 R	60MR	20MS	1	–	5	30S
LrB	–	60MS-S	1 R	40MS	30MS	8	–	2	40MS
LrW	–	40MS	0	-	0	2.5	–	3.5	20MR
Thatcher	50S	70S	60S	80S	80-100S	5.5	–	5	80-100S

* Scoring was made using the modified Cobb's scale, S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant. X intermediate IT, t = traces.

** Switzerland, Zürich-Reckenholz, and Poland, Krakow, scoring was made on a 1-9 scale, 1 indicating no symptoms.

–: no data available, 0: no infection recorded.

[†]: –A: resistance genes that are predominantly effective in the adult plant stage [21].

known to be expressed primarily or only in the adult plant stage. Of these adult plant resistance genes, *Lr35* was the most widely and strongly effective. The effectiveness of the other adult plant resistance genes (*Lr12, Lr13, Lr22a, Lr34* and *Lr37*) appeared to vary among locations. Most of these genes normally do not cause complete resistance, but intermediate types of infection and less protection at high temperatures [21].

3.3. Pathotype composition

Pathotype composition was not determined in all countries and all years. The most comprehensive data were obtained in 1998 (Tab. V). The total number of pathotypes identified over five countries in that year was 105. The countries had very few pathotypes in common. A predominant pathotype, accounting for 16 to 30% of the population, could be identified in each country, except in Bulgaria where the population was composed of many pathotypes, each with a frequency lower than 6%. The predominant pathotypes were rather simple in France and Italy (1 to 4 virulence genes), whereas they were complex (up to 13 virulence genes) in Poland, Bulgaria and Hungary. There were hardly any or no predominant pathotypes in common between countries. The data indicate great genetic diversity in the European population of the wheat leaf rust fungus, especially in south-eastern Europe.

Table V. Frequency (as percentage) of the dominant, and several other, pathotypes of *Puccinia triticina* among the 105 pathotypes identified in five European countries in 1998.

Virulent on lines with *Lr* genes	Countries*				
	F	H	I	BG	PL
2c	12.0	–	–	–	–
2c,21,23	–	–	16.2	–	–
2c,11	12.0	–	–	–	–
2c,11,21,23	–	12.5	29.4	–	–
3,11,15,17,21,26,	–	–	–	2.0	17.1
3,11,15,17,21,26,28	–	–	–	–	25.4
2c,3	16.0	–	–	–	–
2c,3,11	16.0	–	–	–	–
2c,3,11,21,23,26	–	2.5	11.8	–	–
2c,3,11,15,17,21,26,28	–	–	–	2.0	14.6
1,2c,3,11,17,21,23,26	–	–	–	5.9	–
1,2c,3,11,17,21,23,28	–	–	–	5.9	–
1,2c,3,9,11,15,17,21,23,24,26,28				2.0	
2b,2c,3,11,17,21,23	–	–	–	5.9	–
2b,2c,3,11,17,21,23,26	–	–	–	5.9	–
2b,2c,3,11,15,17,21,26	–	22.5	–	–	1.5
2a,2b,2c,11,15,17,21,26		5.0			3.7
1,2a,2b,2c,3,11,15,17,21,26	–	10.0	–	–	–
1,2a,2b,2c,3,11,15,17,21,23,26,28				2.0	
1,2a,2b,2c,3,11,15,17,21,23,24,26,28				2.0	
Total	56.0	52.5	57.4	33.6	62.3

*F= France, H= Hungary, I= Italy, BG= Bulgaria, PL = Poland.

4. Discussion

This paper reports on the first attempt to set up a comprehensive virulence survey of the wheat leaf rust pathogen in Europe. Major improvements were the agreement of a standardized differential set and a common seed stock of NILs. Nevertheless, differential lines that were not true to type were still used locally. The standardisation and the international co-operation enabled detection of such erroneous materials, and the rectification of them.

4.1. Sampling

The host genotypes on which the isolates are collected influence the survey results. If the isolates are collected from plants with effective genes the survey will of course lead to higher frequencies of the corresponding virulent pathotypes. Since a major purpose of virulence surveys is to provide information on new virulences to breeders, it is justified to sample on cultivars with certain commercially interesting *Lr*-genes. A more representative picture of the structure of the leaf rust population is obtained when samples are collected from universally susceptible wheat lines, preferably collected by mobile spore traps [32]. By such a sampling new pathotypes important for the breeding have a lower chance of being identified. The present survey had not been standardised with respect to the sampling strategy, which may partly explain differences in virulence frequencies among countries. To serve breeders, future surveys should be carried out on isolates collected on cultivars or breeding lines for which new pathotype development is particularly relevant (viz. *Lr9, Lr19, Lr24, Lr25* and *Lr28*).

4.2. Virulence frequencies

The 2608 isolates analysed give a comprehensive picture of the virulence frequencies in the European wheat leaf rust fungus population. The results of the field test agreed quite well with those of the seedling tests. Both tests indicated that the frequencies of virulence to genes *Lr9, Lr19, Lr24, Lr25* and *Lr28* are low in large parts of Europe, or even in the whole of Europe. The same is probably true for virulence to the adult plant resistance gene *Lr35*. It is relevant to note that, as far it is known, none of these genes is currently deployed on a large scale in European wheat cultivars [38].

The genes to which virulence is rare in Europe are more or less the same as reported in other continents [6, 8, 14, 21, 26, 28, 31, 36, and 37]. Among the exceptions is *Lr24*, which is widely ineffective in North and South America and South Africa, but effective in Australia and the Indian subcontinent [22] and Europe. Maybe the most striking difference is the apparently frequent occurrence of virulence against *Lr21*. Virulence to this gene has been reported to occur locally at low frequencies in Europe [23] and elsewhere (Huerta-Espino, cited in [21]). Our data suggest that virulence to this gene is widespread and common now (Tabs. II and III). For this gene, it is, however, possible that in some situations avirulent isolates are interpreted as virulent in seedling tests [21]. Our results also suggest that virulences to *Lr24* and *Lr25* are not as rare as in a previous survey [23].

The resistance genes that were postulated to be most common in the modern European winter wheat germplasm, were *Lr3a*, *Lr10*, *Lr13*, *Lr14a*, *Lr20*, *Lr26* and *Lr37* [38]. To most of these genes the virulence frequencies appeared to be high (*Lr3a, Lr26*, Tab. II) and/or the level of protection in the field was low (*Lr10, Lr14a*, Tab. IV). In the case of *Lr26* the virulence frequency is especially high in eastern Europe, where cultivars carrying *Lr26* were very popular. The gene *Lr37* is the most effective of the presently deployed resistance genes in Europe.

The average frequency of virulences appeared to increase slightly for the *Lr*-genes included in the surveys (43% in 1996 to 49% in 1999). However, the period of the study is too short, and the sampling methods not sufficiently standardised to draw firm conclusions on an overall virulence increase in Europe.

4.3. Value of field tests

The field tests provide another approach to survey the occurrence of virulences. Most survey papers report on virulence frequencies obtained in seedling tests. Only a few attempts have been made before to use field tests in order to evaluate virulences in rust populations [21].

The NILs were sown mostly in breeding nurseries where the number of pathotypes is expected to be much higher than in commercial fields, because of the much larger genetic variation in resistance genes present in the nurseries. As the differential set is exposed to spores of all different local pathotypes in different parts of the countries, the infection severity indicates the general protective ability of the respective *Lr*-genes under field conditions. However, the observation of a lower severity on the susceptible check Thatcher, as compared to some NILs with *Lr* genes, suggests that this field testing does not permit a precise quantitative ranking of the efficiency of the genes. The good general agreement between the field tests (Tab. IV) and the virulence surveys on seedlings (Tab. III), the possibility of the field test to assess the effectiveness of genes for adult plant resistance, and the low labour input required for field tests, imply that field tests are an efficient alternative to seedling tests.

4.4. Adult plant resistance

In all four years of field tests, *Lr35* and *Lr37* gave a better protection than the other adult plant resistance genes. Most adult plant resistance genes cause incomplete types of resistance. Avirulent pathotypes would merely cause lower infection levels than virulent pathotypes, and such differences are, in this type of test, much harder to detect than for (a)virulences to qualitative types of resistance. Winzeler et al. [38] reported that among the winter wheat cultivars with the highest susceptibility to wheat leaf rust, some were probable carriers of *Lr13*, indicating low effectiveness of this gene. It is unclear whether the variation in performance of *Lr13*-carrying cultivars is due to variation for corresponding virulence in the local pathogen populations, or due to interactions between *Lr13* with genes in the genetic background. It has been reported, for example, that combinations between *Lr13* and *Lr34* result in very high levels of resistance, due to synergism between both genes [10, 30].

4.5. Pathotype composition

The 1998 data from five countries indicate a great diversity in pathotype composition between countries. Altogether 105 pathotypes were identified on a total sample of 592 isolates. This implies that on average each pathotype was represented by only 5 to 6 isolates. Especially in eastern Europe the variation in the pathogen population and the average number of virulence factors per pathotype were greater than in central, south and western Europe. In our survey, the pathotype composition varied greatly between years in the same country, as has been reported for France, Italy and Hungary [7, 11, 20]. Such diversity between years and regions has been reported also for the US [19].

Commonly the predominant pathotypes in one country were not found in any other country. This result seems in contrast with the main conclusion of Park and Felsenstein [23] who found 4 predominant and widespread pathotypes across western Europe. These pathotypes were very similar to those present in former Czechoslovakia in the past 20 years. Their results suggested that migration across Europe resulted in some common features in pathotype composition between European countries. However, their survey [23] concentrated on western Europe and did not cover the whole continent. A survey in the USA [17] demonstrated that on a continent, several subpopulations can be distinguished, each with their own pathotype composition, but similarities can be found [16].

4.6. Breeding aspects

It is of interest that none of the widely effective *Lr*-genes are exploited so far in commercially grown wheat cultivars in the countries covered by

this study. Some of these genes, however, are reputed to be associated with inferior agronomic performance. *Lr19* is associated with undesirable yellow-pigmented flour [21], *Lr25* with poor performance [21] and also *Lr*9 may have adverse side-effects. A derivative of the Swiss cultivar 'Arina' in which the *Lr9* gene had been introduced, yielded significantly less than the original version of 'Arina' [Winzeler, pers. comm. 1999]. Despite the possible risks, alien genes are now widely used in crossing programs [21, 36].

Pyramiding resistance genes has been suggested already a long time ago (see citations in [21] and [26]). In fact, many cultivars already contain more than one resistance gene to leaf rust [21, 38]. However, the strategy would only be effective in cases where the virulence frequencies to each of the *Lr* genes are negligible. Our study indicates some candidate genes that could be used for such a pyramiding, possibly in combination with genes like *Lr34* that tends to enhance the effects of genes that would result in incomplete resistance, if applied alone.

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References

[1] Anikster Y., Bushnell W.R., Eilam T., Manisterski J., Roelfs A.P., *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats and rye, Can. J. Bot. 75 (1997) 2082–2090.

[2] Anonymous, CIMMYT Report on Wheat Improvement, Mexico D.F., p. 115, 1976.

[3] Bartos P., Huszar J., Virulence of the wheat leaf rust population in Slovakia in 1996, Biológia (Bratislava) 53 (1998) 99–105.

[4] Bartos P., Stuchlikova E., Wheat leaf rust races/pathotypes in the Czech Republic in 1997–1998, Plant Prot. Sci. 35 (1999) 51–56.

[5] Bartos P., Stuchlikova E., Hanusova R., Physiologic specialization of wheat leaf rust (*Puccinia persistens* Plow. var. *triticina* (Eriks.) Urban et Markova) and stem rust (*Puccinia graminis* Pers. subsp. *graminis*) in the Chech and Slovak Republics in the years 1991–1993), Genetika a Slechteni 30 (1994) 253–267.

[6] Boskovic M.M., Boskovic V.J., Jerkovic J., Evaluation of wheat genotypes for differentiating pathotypes of *Puccinia recondita tritici*, Proc. of the 9th Cereal Rust & Powdery Mildews Conference, 2–6 Sept. Lunteren, The Netherlands, 1996, p. 134 (Abstr.).

[7] Casulli F., Pasquini M., Pathogenicity of *Puccinia recondita* f. sp. *tritici* in Italy from 1993 to 1996, Phytopathol. Mediterranea 27 (1998) 51–57.

[8] Chen W.Q., Qin Q.M., Chen Y.L., Yan S.B., Virulence dynamics of *Puccinia recondita* f. sp. *tritici* in China during 1992–1996, Acta Phytopathol. Sinica 28 (1998) 101–106.

[9] Felsenstein F.G., Hsam S.L.K., Zeller F.J., Distribution of virulence of *Erysiphe graminis* f. sp. *avenae* in western Europe, Proc. of the 9th Cereal Rust and Powdery Mildews Conference, 2–6 Sept. Lunteren, The Netherlands, 1996, p. 136 (Abstr.).

[10] German S.E., Kolmer J.A., Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat, Theor. Appl. Genet. 84 (1992) 97–105.

[11 Goyeau H., de Vallavieille-Pope C., Population structure and sampling of *Puccinia recondita* f. sp. *tritici* in France during 1993–1995, Proc. of the 9th Cereal Rust and Powdery Mildews Conference, 2–6 Sept. Lunteren, The Netherlands, 1996, p. 136 (Abstr.).

[12] Johnston C.O., Maines E.B., Studies on physiologic specialisation *in Puccinia triticina*. U.S. Dept. Agric., Tech. Bull. 313 (1932) 1–22.

[13] Jones E.R.L., Brown rust of wheat. UK Cereal Pathogen Virulence Survey 1998, Ann. Rep., 1999, pp. 34–42.

[14] Kolmer J.A., Physiologic specialization of *Puccinia triticina* in Canada, 1997, Plant Dis. 83 (1999) 194–197.

[15] Kolmer J.A., Liu J.Q., Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1995, Can. J. Plant Pathol. 19 (1997) 166–170.

[16] Kolmer J.A., Liu J.Q., Virulence and molecular polymorphism in international collections of the wheat leaf rust fungus *Puccinia triticina*, Phytopathology 90 (2000) 427–436.

[17] Leonard K.J., Roelfs A.P., Long D.L., Diversity of virulence within and among populations of *Puccinia recondita* f. sp. *tritici* in different areas of the United States, Plant Dis. 76 (1992) 500–504.

[18] Long D.L., Kolmer J.A., A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*, Phytopathol. 79 (1989) 525–529.

[19] Long D.L., Leonard K.J., Roberts J.J., Virulence and diversity of wheat leaf rust in the United States in 1993 to 1995, Plant Dis. 82 (1998) 1391–1400.

[20] Manninger K., Physiologic specialization of *Puccinia triticina* in Hungary during 1994–1998. Workshop on Disease Resistance and Cereal Leaf Pathogens beyond the Year 2000, COST Action 817, 11–12 November, Martina Franca, Italy, 1999, p. 45 (Abstr.).

[21] McIntosh R.A., Wellings C.R., Park R.F., Wheat Rusts – an Atlas of Resistance Genes, Kluwer Acad. Publ., Dordrecht, 1995.

[22] Nagarajan S., Nayar S.K., Bahadur P., Kumar J., Wheat Pathology and Wheat Improvement. Azad Hind Stores: Chandrigarh, India, 1986.

[23] Park R.F., Felsenstein F.G., Physiological specialization and pathotype distribution of leaf rust of wheat in Western Europe, 1995, Plant Pathol. 47 (1998) 157–164.

[24] Park R.F., Wellings C.R., Pathogenic specialization of wheat rusts in Australia and New Zealand in 1988 and 1989, Australas. Plant Pathol. 21 (1992) 61–69.

[25] Pretorius Z.A., Le Roux J., Occurrence and pathogenicity of *Puccinia recondita* f. sp. *tritici* on wheat in South Africa during 1986 and 1987, Phytolactica 20 (1988) 349–352.

[26] Roelfs A.P., Sing R.P., Saari E.E., Boers L.H.M., Rust diseases of wheat: Concepts and methods of disease management. Mexico, D.F. CIMMYT, 81 p., 1992.

[27] Rubiales D., Niks R.E., Characterization of *Lr34*, a major gene conferring nonhypersensitive resistance to wheat leaf rust, Plant Dis. 79 (1995) 1208–1212.

[28] Saini R.G., Livinder K., Mandeep K., Adult plant leaf rust *(Puccinia recondita tritici*) resistance on known *Lr* genes against three virulence variants of pathotype 77 from Indian sub-continent, Indian J. Agric. Sci. 68 (1998) 776–779.

[29] Samborski D.J., Wheat leaf rust, in: Roelfs A.P., Bushnell W.R. (Eds.), The cereal rusts. Vol. II, Academic Press, Orlando, 1985, pp. 39–59.

[30] Sawhney R.N., The role of *Lr34* in imparting durable resistance to wheat leaf rust through gene interaction, Euphytica 61 (1992) 9–12.

[31] Sawhney R.N., Sharma I.B., Rajesh K., Assessment and exploitation of genetic variation for resistance to *Puccinia recondita* for stabilizing wheat production, Indian J. Genet. Plant Breed. 58 (1998) 251–262.

[32] Schwarzbach E., A high throughput jet trap for collecting mildew spores on living leaves, Phytopathol. Z. 94 (1979) 165–171.

[33] Strzembicka A., Virulence of *Puccinia recondita* f. sp. *tritici* in Poland, J. Appl. Genet. 38B (1997) 101–105.

[34] Stakman E.C., Stewart D.M., Loegering W.Q., Identification of physiologic races of *Puccinia graminis* var. *tritici*, Agr. Re. Service E 617 (USDA), Washington D.C., ARS 617, 53 p., 1962.

[35] Todorova M., Pathotype specialization of *Puccinia recondita* f. sp. *tritici* in Bulgaria during 1973–1993, Cereal Rusts and Powdery Mildews Bull. 24 (1996) 54–59.

[36] Tomar S.M.S., Menon M.K., Introgression of alien genes for leaf rust (*Puccinia recondita*) resistance into bread wheat (*Triticum aestivum*) cultivars, Indian J. Agric. Sci. 68 (1998) 675–681.

[37] Watkins J.E., Rutledge S.S., Baenziger P.S., Youngquist W., Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Nebraska during 1995 and 1996, Plant Dis. 82 (1998) 679–682.

[38] Winzeler M., Mesterházy A., Park R.F., Bartos P., Csõsz M., Goyeau H., Ittu M., Jones E., Löschenberger F., Manninger K., Pasquini M., Richter K., Rubiales D., Schachermayr G., Strzembicka A., Trottet M., Unger O., Vida G., Walther U., Resistance of European winter wheat germplasm to leaf rust, Agronomie 20 (2000) 783–792.

[39] Zadoks J.C., Bouwman J.J., Epidemiology in Europe, in: Roelfs A.P., Bushnell W.R. (Eds.), The Cereal Rusts, Vol. II. Academic Press: Orlando, Florida, USA, 1985, pp. 329–369.