Virulence Analyses of the Powdery Mildew Population on Wheat in the Czech Republic in 1995–1998*

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Abstract

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In 1995–1998, the frequency of selected virulences in the powdery mildew (*Blumeria graminis* f. sp. *tritici*) population on wheat in the Czech Republic was investigated. Samples of conidia were collected by a mobile version of a spore-trap. A total of 1739 one-colony isolates were analysed on a set of five varieties as differentials. High frequencies (> 50%) were found for virulences *Vm2*, *Vm3a*, *Vm5* and *Vm17*, lower frequencies (< 50%) for combinations *Vm9,2,1* and *Vm2,6* and for *Vm4b*. There were no significant changes in virulence frequencies except for genes *Vm5* and *Vm9,2,1* that showed one-year variations. Differences between the eastern part (Moravia) and western part (Bohemia) of the Czech Republic were mostly insignificant for most examined genes. A significantly higher virulence frequency was found in Moravia for genes *Vm3a* (in 1995) and *Vm17* (in 1996), and in Bohemia for the combination *Vm9,2,1* (in 1995). A significant correlation (tested by Kendall's tau B values) existed between *Vm2* and *Vm5* in 1997 and 1998, and between *Vm4b* and *Vm5* in 1995 and 1998. Values of gametic disequilibrium were also studied. Changes in the complexity of isolate virulence were determined to be small and showed no definite trend.

Key words: *Blumeria graminis* f. sp. *tritici*, syn. *Erysiphe graminis* f. sp. *tritici*; virulence; wheat; population, Czech Republic

Powdery mildew (*Blumeria graminis* f. sp. *tritici*) occurs on wheat each year and causes yield loss up to 30 % (VERREET 1995). Average losses can be estimated to be about 5%. One of the alternatives in an integrated protection system is to grow resistant varieties. Since there is still a lack of effective sources of resistance, only few of the registered varieties are resistant. Moreover, virulent pathotypes that are able to overcome corresponding resistance genes will sooner or later appear in the pathogen population. The pace of this change depends on adaptation mechanisms, particularly on direct selection. The objective of the present study was to quantify selected virulences and characterise some other parameters in the pathogen population.

MATERIAL AND METHODS

A mobile spore-trap (SCHWARZBACH 1979) was used to collect random samples of conidia each year between 20 May and 10 June. The routes of collection were divided into Moravian and Bohemian parts and crossed the most important wheat-growing regions. The routes were 2072, 1841, 1804 and 1329 km long and cut into sections of 50 to 150 km. The single conidia were placed and cultivated on leaves of the susceptible variety Carsten V (planted under infection-free conditions) in Petri dishes with water agar (6 g agar per l distilled water, 30 ppm benzimidazole). After 10 to 11 days of cultivation under natural light conditions and 18–20 °C, the colonies were used for inoculation of leaf segments of differentials (Table 1) using a varipipette with replaceable tips (DREISEITL 1998). Seven days later, responses of individual test varieties possessing respective resistance genes were scored by reading

Table 1. A set of differential varieties of wheat

Pm resistance genes	Differential varieties
2	Ulka
3a	Asosan
4b	Weihenstephan M1
5	Hope
2,6	Kenya Civet
9,2,1	Normandie
17	Amigo

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infection types (IT) according to a 0–4 scale. Isolates exhibiting IT 4 and 3-4 were considered virulent.

Virulence frequencies were assessed for individual resistance genes in all sections analysed. If only a low number (< 10) of isolates was collected in one section, samples from adjacent sections were combined to ensure the validity of results (FELSENSTEIN 1991). Significance of differences in virulence frequency among years and between the Moravian and Bohemian parts of samples was calculated using the nonparametric Krustal-Wallis one-way analysis by ranks (WEBER 1992).

Associations between two genes or combinations of virulence genes were tested using Kendall's tau B criterion that shows a level of dependence of two ordinal variables (KOSCHIN *et al.* 1992).

Gametic disequilibrium in the pathogen population, which gives the difference between the assessed virulence for the combination of two resistance genes and theoretical virulence, was tested according to HOVMØLLER and ØSTERGÅRD (1991):

$$D(V_xV_y) = p(V_xV_y) - p(V_x) \, . \, p(V_y)$$

where: $D(V_xV_y)$ – gametic disequilibrium
$p(V_xV_y)$ – virulence frequency for the combination of resistance genes xy
$p(V_x)$ – virulence frequency for resistance gene x
$p(V_y)$ – virulence frequency for resistance gene y

Virulence complexity was assessed as a percentage of the sum of virulences studied in all years (Vm2, Vm4b, Vm5, Vm2,6, Vm9,2,1) and corresponding with the isolate frequency (DREISEITL 1997).

RESULTS

The virulence frequencies in individual years for the Moravian and Bohemian parts and for the whole Czech Republic are summarised in Table 2. The frequency of Vm2 and Vm2,6 virulences was stable in the period studied. In both cases, differences among years were not significant. The mean frequency over the years was 59.7% for Vm2 and 48.1% for Vm2,6. The virulence frequency of Vm4b varied from year to year and tended to decrease. However, the differences were not significant either. The mean frequency over the years of Vm4b virulence was 35.7%. Significant changes in frequency were found only for Vm5 and Vm9,2,1 virulences. The former showed a considerable decrease in the frequency in 1997, while Vm9,2,1 was more frequent in 1996 than in the other years. The mean frequency of Vm5 virulence is high (82.5%) in contrast to a low one of Vm9,2,1 (24%). The frequency of Vm3a virulence was examined in 1995 only and showed an average of 52% for the Czech Republic. The frequency of Pm17 virulence was only investigated in 1996 and was 67%.

Highly significant differences between the Moravian and Bohemian part of the population (using the nonparametric Kruskal-Wallis one-way analysis) were found only with Vm3a virulence (Table 3) which was more frequent in the Moravian part. A similar discrepancy of virulence frequency was found for Vm17. In this case, however, the difference was smaller and barely significant. A significant difference in virulence frequency was also determined for gene combination Vm9,2,1. However, these differences between the Moravian and Bohemian part were apparent only in 1995. In the following years, the differences were below the significance limit.

Parameters of Kendall's tau B expressing the closeness of dependence between pairs of resistance genes or gene combinations, including the dependence significance for each year, are given in Tables 4. Highly significant correlations were found between virulences with gene *Vm2*. The only exception is the year 1997 when the correlation

Table 2. Relative virulence frequency assessed in 1995–1998

Year	Region	Vm2	Vm3a	Vm4b	Vm5	Vm2,6	Vm9,2,1	Vm17
1995	Moravia	60.5	58.6	39.5	85.5	49.0	17.3	
	Bohemia	63.0	33.1	42.5	81.1	48.0	29.1	
	CR in total	**61.2**	**52.0**	**40.2**	**84.3**	**48.8**	**20.3**	
1996	Moravia	64.3		37.5	95.6	53.1	33.0	72.3
	Bohemia	58.0		37.0	92.8	47.8	30.8	60.9
	CR in total	**61.5**		**37.2**	**94.3**	**50.7**	**32.0**	**67.2**
1997	Moravia	59.2		39.5	67.3	50.3	17.0	
	Bohemia	58.2		26.1	65.5	41.8	20.6	
	CR in total	**58.7**		**32.4**	**66.3**	**45.8**	**18.9**	
1998	Moravia	56.7		27.7	83.9	46.0	24.6	
	Bohemia	59.4		45.8	87.5	87.5	49.0	
	CR in total	**57.5**		**33.1**	**85.0**	**46.9**	**24.7**	

Table 3. A level of significance of differences in virulence frequency between the Czech and Moravian parts using the nonparametric Kruskal-Wallis one-way analysis

	Vm2	Vm3a	Vm4b	Vm5	Vm2,6	Vm9,2,1	Vm17
1995	0.365	0.005^{**}	0.910	0.821	0.777	0.017^{*}	
1996	0.149		0.773	0.193	0.501	0.630	0.043^{*}
1997	1.000		0.100	0.855	0.361	0.361	
1998	0.171		0.121	0.558	0.329	0.696	

*(**) significance $P = 0.05$ (0.01)

between *Vm2* and *Vm2,6* was just below the significance limit. In 1995, significant correlations were assessed between virulences Vm3a–Vm4b, Vm3a-Vm5, and Vm4b–Vm5. In the following 2 years, associations between genes *Vm4b* and *Vm5* were not significant. In spite of that, values of Kendall's tau B for *Vm4b–Vm5* were higher than for other combinations, particularly in 1996. In 1997 and 1998, a significant correlation was assessed between *Vm2–Vm5* and in 1998 between virulences *Vm4b–Vm5* and *Vm2,6–Vm5*.

Genetic linkage of two or more virulence genes in the powdery mildew population was evaluated using values of so-called gametic disequilibria (Tables 5). The results are in accordance with correlations between virulence genes and express relative values of increased associations in comparison with expected values. The highest values were recorded in 1998 when deviations were +2.8% for genes *Vm2* and *Vm4b*, +2.7% for *Vm2*,6 and *Vm5*, and +1.2% for *Vm4b* and *Vm5*.

Virulence complexity was assessed for only those virulence genes studied during the whole four-year period so that inter-annual changes could be compared. For each year, the proportions of isolates were arranged according to the number of virulences carried (Table 6). In general, changes in the complexity are small. While the virulence complexity slightly increased in 1996, it declined in 1997. Although it increased again in the following year, it did not reach the level of 1995 and 1996.

DISCUSSION

The effectiveness of resistance genes depends on the frequency of corresponding virulence genes in the regional pathogen population and can be classified into four groups (FELSENSTEIN *et al.* 1992). Based on this classification, the resistance controlled by gene *Pm2* belongs to the group with low or insignificant protective effects; the resistance controlled by genes *Pm2*,6 is on the border between this group and the next which imparts reduced but significant protective effects. However, as reported by HANIŠOVÁ *et al.* (1997), there is a difference in reaction between juvenile and adult plants in varieties possessing resistance genes *Pm2*,6. They tend to have a higher resistance at later growth stages. Similarly, ECKER and LEIN (1994) confirmed that the combination with gene *Pm6* affects the so-called adult plant resistance, increasing its importance. Therefore, a direct correlation between Vm2,6 virulence and resistance under field conditions cannot be expected. Instead, it may be assumed that the assessed stability of virulence frequency for Vm2,6 in the population is a result of the relation between gene *Pm6* and adult plant resistance in spite of an increasing number of varieties with gene combination *Pm2,6*.

Based on the frequency of Vm4b virulence, the resistance controlled by gene *Pm4b* is classified in the group with reduced but significant effects. Gene *Pm4b* has been used in wheat breeding for a long time, but to a limited extent only (ECKER & LEIN 1994). Nevertheless, virulence for this gene still remains on a relatively low level even though the effectiveness of Pm4b is lower under field conditions. Good resistance is found only in varieties that combine gene *Pm4b* with genes *Pm5* or *Pm2*,6, i.e., with genes that are related to adult plant resistance (KLEM *et al.* 1995).

Despite of a high frequency of Vm5 virulence, which should rank varieties with the corresponding resistance gene *Pm5* in the group with low effects, these varieties exhibit low infection under field conditions, particularly at later growth stages (KLEM *et al.* 1995). This relates to the so-called adult plant resistance (ECKER & LEIN 1994) when gene *Pm5* is combined with other suitable resistance genes.

The identified high frequency of virulence for gene *Pm17* in 1996 suggests only a low or insignificant effect of this gene for resistance. A high frequency of virulence for this gene was also reported by BARTOŠ *et al.* (1998). Therefore, virulence for this gene was not studied in subsequent years.

Any differences between the Moravian and Bohemian populations (Table 2) may be caused by the prevailing wind directions, so that changes in the population could be expected from west to east (FELSENSTEIN 1991). Highly significant differences in the regional frequency distribution of Vm3a virulence, which was more frequent in the Moravian population, are confirmed by analyses of the population of the whole Czech Republic carried out in 1994 (KLEM & TVARŮŽEK 1995) as well as by data on the distribution of the frequency of this virulence gene in Europe (FELSENSTEIN 1991). Analyses of the assortment of varieties which had been grown in former Czechoslo-

Table 4. Values of Kendall's tau B showing a level of dependence of individual virulence genes in 1995–1998

	1995					1996					1997				1998			
	Vm9,2,1	*Vm2,6*	*Vm5*	*Vm4b*	*Vm3a*	*Vm17*	*Vm9,2,1*	*Vm2,6*	*Vm5*	*Vm4b*	*Vm9,2,1*	*Vm2,6*	*Vm5*	*Vm4b*	*Vm9,2,1*	*Vm2,6*	*Vm5*	*Vm4b*
Vm2	0.351**	0.435**	0.001	–0.018	–0.030	0.022	0.408**	0.416**	0.036	0.002	0.239**	0.106	0.146*	-0.017	0.279**	0.326**	0.117*	0.121*
Vm3a	–0.061	0.025	0.090*	0.108*														
Vm4b	0.059	0.028	0.091*			0.059	–0.017	0.019	0.073		-0.054	0.023	0.043		-0.001	0.084	0.073	
Vm5	0.079	-0.005				–0.052	0.018	0.025			0.084	0.056			0.094	0.149**		
Vm2,6	0.356**					0.003	0.579**				0.230**				0.338**			
Vm9,2,1						-0.002												

*(**) significance $P = 0.05$ (0.01)

Table 5. Gametic disequilibria in the powdery mildew population on wheat in 1995–1998

	1995					1996					1997				1998			
	Vm2	*Vm3a*	*Vm4b*	*Vm5*	*Vm2,6*	*Vm2*	*Vm4b*	*Vm5*	*Vm2,6*	*Vm9,2,1*	*Vm2*	*Vm4b*	*Vm5*	*Vm2,6*	*Vm2*	*Vm4b*	*Vm5*	*Vm2,6*
Vm17						0.005	0.013	–0.006	0.001	0.000								
Vm9,2,1	0.069	–0.012	0.012	0.011	0.072	0.093	–0.004	0.002	0.135		0.046	–0.010	0.016	0.058	0.055	–0.001	0.009	0.069
Vm2,6	0.106	0.006	0.007	–0.001		0.101	0.005	0.003			0.026	0.005	0.013		0.080	0.020	0.027	
Vm5	0.000	0.016	0.016			0.004	0.008				0.034	0.010			0.021	0.012		
Vm4b	–0.004	0.026				0.000					–0.004				0.028			

Table 6. Virulence complexity in the powdery mildew population on wheat in 1995–1998

Number of virulences	1995			1996			1997			1998		
	n	Σ virulence	%	*n*	Σ virulence	%	*n*	Σ virulence	%	*n*	Σ virulence	%
0	17	0	3.5	8	0	1.3	20	0	6.4	17	0	5.3
1	93	93	18.9	111	111	18.1	72	72	23.1	64	64	20.0
2	140	280	28.5	169	338	27.5	102	204	32.7	87	174	27.2
3	120	360	24.4	122	366	19.8	63	189	20.2	76	228	23.8
4	89	356	18.1	144	576	23.4	47	188	15.1	55	220	17.2
5	33	165	6.7	61	305	9.9	8	40	2.6	21	105	6.6
Σ	492	1254		615	1696		312	693		320	791	
Complexity	**2.55**			**2.76**			**2.22**			**2.47**		

vakia in previous years did not reveal any variety possessing resistance to Vm3a (LUTZ *et al.* 1992). This regional distribution can be influenced by the flow of pathotypes virulent for gene *Pm3a* from Asia, or by earlier growing landraces carrying this gene (FELSENSTEIN 1991).

Regarding the fact that gene *Pm3a* is not present in the European variety assortment, the assessed correlations among virulence genes suggest a critical importance of the association between genes *Vm4b* and *Vm5* in 1995. It can be assumed that this is a result of an increasing area under varieties which carry genes *Pm4b*,5. Among winter wheat varieties this combination was present in the variety Zdar (LUTZ *et al.* 1992) that was widely grown. Therefore, its effect on the population formation could be considerable. In the following years, the correlation was not significant, which can be explained by the declined area under Zdar.

A significant correlation between virulences Vm2 and Vm5 in 1997 and 1998, and Vm2,6 and Vm5 in 1998 may have been caused by the introduction of new varieties carrying a combination of genes *Pm2,6,4b,5* (in cv.s Siria and Contra) and of *Pm2,6,5* (in cv. Ritmo) (HANIŠOVÁ *et al.* 1997).

Gametic disequilibrium may be a result of selection, recombinations, random changes and mutations (HOVMØLLER & ØSTERGÅRD 1991). A parallel selection for two resistance genes has a crucial effect. The assessed values of gametic disequilibria correspond with the above-mentioned correlations between virulence genes. They are significant for genes *Vm4b–Vm5*, *Vm2–Vm5*, and *Vm2,6–Vm5*. Their positive values express the effect of direct selection of two virulences on the occurrence of a respective association.

The changes with time of the virulence complexity of isolates were low and no definite trend was observed. In the given period, there was no proof that the resistance genes present in the Czech assortment of wheat varieties had effected an increase in the complexity of corresponding virulences in the pathogen population.

For each year, there are some collections without tested virulence genes. This can be explained by growing commercial varieties that have no gene for resistance against prowdery mildew.

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Souhrn

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V letech 1995–1998 byla zjišťována četnost vybraných virulencí v populaci padlí travního na pšenici (*Blumeria graminis* f. sp. *tritici*) na území České republiky. Vzorky konidií byly získány odchytem pomocí mobilní verze lapače spor. Bylo analyzováno celkem 1 739 jednokupkových izolátů. Soubor diferenciačních odrůd se ve všech sledovaných letech shodoval v pěti odrůdách. Vysoká četnost (>50 %) byla zjištěna u *Vm2*, *Vm3a*, *Vm5* a *Vm17*, nižší (<50 %) u kombinací *Vm9,2,1*, *Vm2,6* a genu *Vm4b*. Nebyly zjištěny průkazné změny v četnostech virulence s výjimkou genů *Vm5* a *Vm9,2,1*, u nichž byly zaznamenány jednoleté výkyvy. Regionální rozdíly mezi moravskou a českou částí populace byly pro většinu sledovaných genů neprůkazné. Průkazně vyšší četnost virulence v moravské části byla zjištěna u genů *Vm3a* a *Vm17*. Průkaznost regionálních rozdílů v případě kombinace *Vm9,2,1* byla zjištěna pouze v roce 1995. V letech 1997 a 1998 byla mezi virulencemi Vm2 a Vm5 a v letech 1995 a 1998 mezi virulencemi Vm4b a Vm5 zjištěna průkazná závislost, testovaná hodnotami Kendallova tau B. Byly zjišťovány hodnoty gametických nerovnováh. Změny v komplexnosti virulence izolátů byly nízké, přičemž nebyl zjištěn jejich jednoznačný směr.

Klíčová slova: *Blumeria graminis* f. sp. *tritici*, syn. *Erysiphe graminis* f. sp. *tritici*; virulence; pšenice; populace; Česká republika

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