

## Rare Virulences of Barley Powdery Mildew Found in Aerial Populations in the Czech Republic from 2009 to 2014

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### Abstract

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In breeding plants for resistance to disease pathogens major genes are often used. They mostly confer a resistance, which can be overcome by a corresponding pathogen virulence specific to the resistance conferred by that gene. Therefore, monitoring of new virulences in the pathogen population is an integral part of both the study of resistance and practical breeding. The aim of this paper is to present the detection of rare virulences that have been found in aerial populations of *Blumeria graminis* f.sp. *hordei* collected in the Czech Republic during the last six years. In total, 822 random isolates originating from cultivated spring and winter barley fields were evaluated on 37 differential varieties and 12 isolates carrying rare virulences were found. At least four virulences to resistances in Laverda, Psaknon, Venezia and SJ048311 were novel. In contrast, four rare isolates avirulent to the resistance *Ha* were also found. No isolate avirulent to resistances *Ra*, *HH* (*a8*) and *Lo* was detected. Monitoring of new virulences allows the selection of resistant donors for breeding new varieties and the identification of new resistances.

**Keywords:** *Blumeria graminis* f.sp. *hordei*; *Hordeum vulgare*; specific resistances

In breeding plants for resistance to disease pathogens major genes are often used. They generally confer race-specific resistance (JØRGENSEN 1994) when each resistance gene of the host is matched by a particular virulence of the pathogen (FLOR 1971). Therefore, monitoring the occurrence and frequency of virulence in the pathogen population is an integral part of the study of resistance and its practical application in breeding.

In the Czech Republic, barley is represented by spring and winter types. The former is processed for the domestic and overseas markets in the malting and brewing industry and as an animal feedstuff, whereas winter barley is used mostly for the latter. Powdery mildew, caused by the biotrophic fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*), is the most frequent disease of barley and is especially prevalent on spring-sown susceptible varieties (DREISEITL 2011a). During the last 50 years the severity of powdery mildew has been exacerbated by growing short-stawed

high-tillering barley varieties, increased fertilizer rates, especially nitrogen, and concurrent cultivation of spring and winter varieties with a substantial increase in the area of the latter.

Growing resistant varieties is an effective measure to suppress the incidence and damage caused by diseases, especially in widely-grown crops such as wheat and barley in the temperate zone. Since the beginning of domestic breeding barley for powdery mildew resistance (FADRHONS 1962), the study of the pathogen population (BRÜCKNER 1963) has been closely related to experiments focused on looking for effective resistance resources (BRÜCKNER 1964).

Investigations of populations became more widely researched first, when the spectrum of physiological races constituting the pathogen population (BRÜCKNER 1963; NOVER *et al.* 1968) was replaced by virulence analysis (WOLFE & SCHWARZBACH 1975) and second, conventional sampling the pathogen from plants (WOLFE 1968) was replaced by collecting spores

from the air using a specialized device (SCHWARZBACH 1979). This facilitated the fast gathering of many randomly sampled isolates (LIMPERT 1987; WOLFE *et al.* 1992). An alternative approach was the exposure of seedling leaves to spores present in the air (EYAL *et al.* 1973; BROWN *et al.* 1993).

The first virulence analysis of a Czech aerial population of the pathogen took place in 1988 by exposing young plants of differential varieties in the field (DREISEITL 1991a). It was followed by analysing the local aerial population using a stationary version of a spore sampler (DREISEITL & SCHWARZBACH 1994) and finally completed by surveying aerial populations with a mobile version of this device (DREISEITL 1997). The results of studies on the Czech population were part of a European survey (HOVMØLLER *et al.* 2000) and are now incorporated into the network of information relating to the structure of the world's population (DREISEITL & WANG 2007; DREISEITL & KOSMAN 2013; DREISEITL 2014a).

To gain more knowledge about pathogen populations, an appropriate differential set containing genotypes with selected genes of specific resistance is crucial. The differential set for the study of the domestic population was initially composed of only 9–14 mostly domestic genotypes (DREISEITL 1991a, b; DREISEITL & SCHWARZBACH 1994) that contained most of the first resistance genes used in Europe (BROWN & JØRGENSEN 1991). These domestic genotypes were soon replaced (DREISEITL 1998) by standard lines (KØLSTER *et al.* 1986). Later, these standard differentials, which still served to compare temporal changes in the population, were complemented by a set of selected mainly current varieties (DREISEITL 2004). From 2009 a third part was added to the set. This comprised barley genotypes containing specific resistances effective against all pathotypes maintained in a pathogen gene bank (DREISEITL 2011b, c) or against which only rarely occurring virulent pathotypes were detected.

The aim of this paper is to present the detection of rare virulences to resistances possessed by genotypes within the third part of the differential set that have been found in the Czech Republic during the last six years.

## MATERIAL AND METHODS

**Collection of isolates.** Random population samples of pathogen spores originating from cultivated spring and winter barley fields collected from the

air were obtained by means of a jet spore sampler (SCHWARZBACH 1979) mounted on the roof of a car. Spores were sampled by driving across the Czech Republic in six years (2009–2014) at the end of May to the beginning of June, when tillering of spring barley had usually ended and winter barley was at the ear emergence stage. Freshly detached healthy and fully-expanded primary leaves of the susceptible barley variety Stirling (DREISEITL & PLATZ 2012) were placed in 120 mm Petri dishes on water agar (0.8%) modified with benzimidazole (40 mg/l) (BWA) – a leaf senescence inhibitor – and inserted in the bottom of the spore sampler. Collected spores settled on leaves while travelling and dishes with detached leaves were replaced for each of the 14 sections of the sampling route, which was about 1000 km (Table 1). During sampling, dishes with fresh as well as with exposed leaves were kept in a refrigerator at about 8°C. After sampling, exposed leaves were transferred to Petri dishes of 150 mm diameter with fresh BWA.

**Multiplication of isolates.** To multiply inoculum, dishes with leaves and collected spores were incubated for 11 to 13 days at  $19.0 \pm 1^\circ\text{C}$  under 12 h artificial light at  $30 \pm 5 \mu\text{mol/m}^2/\text{s}$ . Conidia from each single-spore colony were sucked into a replaceable tip of a varipipette AW 1000 and then blown off the tip into a micro-settling tower using a 10 ml syringe. Further details of the method used are described in DREISEITL (2008). By this way dishes with leaf seg-

Table 1. Sampling routes for collecting barley powdery mildew isolates from the air across the Czech Republic

Designation	Sampling route section	Distance (km)
A	Brno–Kroměříž	65
B	Brno–Znojmo	60
C	Brno–Břeclav	50
X	Brno–motorway 1 (90 <sup>th</sup> km)	95
D	Přáslavice–Vyškov	60
E	Přáslavice–Ostrava	65
F	Olomouc–Šumperk	50
I	Praha–motorway 1 (90 <sup>th</sup> km)	90
K	Praha–Plzeň	80
L	Praha–Petrohrad	75
M	Praha–Lovosice	75
N	Praha–Turnov	80
O	Praha–Hradec Králové	85
Y	Praha–circle	70

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Table 2. Virulence frequency of 822 barley powdery mildew isolates sampled from the air in the Czech Republic from 2009 to 2014 and recorded on 37 differential varieties

Differential	Growth habit <sup>a</sup>	Resistance <sup>b</sup>	Virulence frequency (%)					
			2009	2010	2011	2012	2013	2014
Xanadu	S	U (Mlo)	0					
IN0614	S	U (Mlo)	0					
LP1506.1.96	S	U	0	0				
Pallidum 107	S	U	0	0				
SI-1	S	SI-1	0	0				
HM-407	W	U	0	0	0	0		
Bonita	W	U	0	0	0	0	0	0
Camilla	S	U (SI-1)	0	0	0	0	0	0
Venezia	W	WI-1	0	0	<b>0.7</b>	2.1		
Br.4190a1	W	U	0	0	<b>0.7</b>			
Wendy	W	U (Lv U)	0	10.8				
NORD02610/24	W	U (Lv)	<b>0.7</b>					
NORD03025/6	W	U (Lv)	<b>0.7</b>					
Souleyka	W	U (Lv)	<b>0.7</b>					
Laurena	W	U (Lv)	<b>0.7</b>					
Laverda	W	U (Lv)	<b>0.7</b>	11.5				
Spilka	S	U	<b>1.4</b>	3.8				
KM-2929	S	U		0				
KM-12/2010	S	U		0	0	0	0	
KM-14/2010	S	U		0	0	0	0	0
Sara	S	Ri Tu2		0	0	0	0	0
Psaknon	S	Ps		0	0	<b>0.7</b>	<b>0.9</b>	<b>1.7</b>
NORD07017/69	W	U			0	0	0	0
SJ048311	W	U			0	0	0	<b>0.8</b>
SK-4770	S	U			0	<b>0.7</b>	<b>0.9</b>	0
AC07/624/34	S	U				0	0	0
NORD12/1122	S	U (Mlo?)					0	0
E-388/01	S	U					0	0
KWS11/251	S	U					0	0
Nigrate	W	Ni					0	0
Octavia	S	U (Mlo)						0
SY412-329	S	U						0
SJ123063	S	U						<b>0.8</b>
W. 37/136	W	Ha	<b>98.6</b>	100.0	100.0	100.0	<b>98.1</b>	100.0
Dura	W	Ra	100.0	100.0	100.0	100.0	100.0	100.0
Pallas	S	HH	100.0	100.0	100.0	100.0	100.0	100.0
Lomerit	W	None (Lo)		100.0				
No. isolates			144	157	150	144	107	120
No. differentials			21	21	15	15	17	19

<sup>a</sup>W – winter barley, S – spring barley; <sup>b</sup>U – unknown when the differential was used first, some of these resistances have been identified and are shown in parenthesis; frequencies of virulences/avirulences considered rare are in bold

ments of the first part of the differential set were inoculated. After evaluation (results are not shown here) the conidia of isolates were used for inoculation of the third part of differential set (results are the topic of the present contribution).

**Differentials.** A set of 37 barley varieties represented the third part of differential set (Table 2). This set was continuously supplemented with selected genotypes against which no virulence had been found in corresponding resistance tests and which did not contain the non-specific resistance *Mlo*. If the virulence frequency exceeded 2%, it was not further considered as rare and the corresponding genotype was incorporated in another part of the differential set. Eight standard genotypes with known resistances were used. The resistance of the other genotypes was unknown at the time of their addition to the differential set. Four of the 37 differentials were included to detect rare avirulences. In any one year 15–20 differentials were used.

**Production of plant material.** About 25 untreated seeds of each susceptible or differential variety were sown in a pot (80 mm diameter) containing a peat-based potting mix. Seedlings were kept in a mildew-proof greenhouse under natural daylight. Leaf segments of differential varieties 20 mm long were cut from the central part of healthy fully-expanded primary leaves when second leaves were emerging. Three leaf segments of each differential were placed adjacently in a 150 mm Petri dish containing BWA with the adaxial surface upward.

**Inoculation.** Inoculation of the differential set used here was done in a circular metal settling tower of 150 mm diameter and 415 mm in height. For each isolate, a Petri dish with leaf segments from the differential varieties was placed at the bottom of the tower. Conidia of each isolate from a leaf segment of a variety with fully developed pathogen colonies were shaken onto a square piece (40 × 40 mm) of black paper to visually estimate the amount of inoculum deposited. This was then gently rolled to form a blowpipe and conidia of an isolate were blown through a side hole of 13 mm diameter in the upper part of the settling tower over the Petri dish. The inoculum density was about 5–8 conidia/mm<sup>2</sup>. The dishes with inoculated leaf segments were kept under described incubation conditions.

**Virulence determination.** Reaction type (RT) according to the response of each differential to a corresponding isolate was scored eight days after inoculation on a 0 to 4 scale (TORP *et al.* 1978) where

RT 4 or 3–4 were considered virulent to the corresponding resistance gene(s). Each virulent isolate was tested on at least two replications.

**Numerical designations of isolates.** Numerical designations of isolates were based on their virulence patterns on the set of the 12 differentials (Table 3), which were divided into 4 triplets. Each of the digits indicates virulence or avirulence on the three differentials of the respective triplet. If virulence to a corresponding resistance gene was detected, the first differential line is given the value 1 (2<sup>0</sup>), the second line has the value 2 (2<sup>1</sup>), and the third line has the value 4 (2<sup>2</sup>). Therefore, each digit can have a value from 0 (no virulence on any of the three differential lines) up to 7 (= 1 + 2 + 4) denoting virulence on each of the three differential lines (GILMOUR 1973; LIMPET & MÜLLER 1994). The resulting number (reverse-octal notation) defines the virulences of the isolates and consequently their classification as pathotypes.

## RESULTS

**2009.** In total 144 isolates using 20 differential varieties were examined. One isolate was virulent to the resistance of five differentials, including Laverda, two isolates were virulent on Spilka and two isolates were avirulent to the resistance *Ha*. No isolate was virulent to the resistance of 11 differentials and no avirulence to the resistance of Dura and Pallas was detected (Table 2).

**2010.** A total of 157 isolates using 20 differential varieties were examined. Two differentials, which had subsequently been found to carry the non-specific resistance *Mlo*, and four other differentials with an identical resistance to Laverda were excluded. Five differentials were added on which no virulent isolate had been found. No new virulence was detected, but the virulence frequency on Laverda considerably increased.

**2011.** One hundred and fifty isolates using 15 differential varieties were examined. Laverda, Wendy and Spilka were removed because the corresponding virulence frequencies increased considerably and respective virulent isolates were, therefore, not considered rare. Furthermore, the resistance *Lv* had been detected in Wendy accompanied by another minor (unknown) resistance. SI-1 was omitted because it was found to carry an identical resistance to Camilla, and KM-2929 because of problems with seed homogeneity. LP 1506.1.96 and Pallidum 107

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were also removed even though no isolate virulent to their resistances had been detected in the two preceding years, and Lomerit because no avirulence was found. Three differentials were new additions and their resistances, as well as resistances of seven other differentials were fully effective. The first virulent isolate was recorded on Venezia, which was also virulent on Br.4190a1.

**2012.** In total 144 isolates using 15 differential varieties were examined; Br.4190a1, which probably carries an identical resistance to Venezia was excluded whereas AC 07/624/34 was added. The first isolate virulent on Psaknon and another isolate virulent to the resistance of SK-4770 were detected. No virulence against the resistance of nine differentials was found.

**2013.** The number of isolates examined was 107 using 17 differential varieties. Two differentials were excluded: Venezia because the corresponding virulent isolates had become more common, and HM-407 because of poor seed germination. Four new differentials were added. Isolates virulent on Psaknon and SK-4770 were once again detected. In addition, two isolates avirulent to *Ha* were found. No new

virulences were detected and no virulence to the resistance of 12 differentials was found.

**2014.** One hundred and twenty isolates using 19 differential varieties were examined. KM-12/2010 was omitted because its resistance is probably identical to KM-14/2010. Three new differentials were added since an isolate virulent on SJ 123063 was detected. An isolate with a new virulence to the resistance of SJ 048311 and two isolates virulent on Psaknon were found. No virulence to the resistance of 13 differentials was detected.

**Rare virulence / avirulence.** For this study 822 isolates were used, 12 of which carried rare virulences to seven differentials (Venezia, Laverda, Spilka, Psaknon, SJ048311, SK-4770 and SJ123063) and four isolates possessed a rare avirulence to the resistance *Ha* (Table 3). The isolates differed in the spectra of virulences to 12 standard barley genotypes and thus each of them typifies different pathotypes. The virulence complexity of isolates to resistance genes in standard genotypes ranged from 5 (isolates I-8/2009 and B2-1/2014) to 10 (K-25/2009 and L-9/2009). No isolate avirulent to resistances *Ra*, *HH* and *Lo* was detected.

Table 3. Twelve isolates of barley powdery mildew carrying rare virulences (*V*) to resistances in particular varieties and four isolates carrying rare avirulence *AvHa* found among 822 isolates sampled from the air in the Czech Republic from 2009 to 2014 and showing their virulence to the resistance of standard lines and pathotype designation

Year	Isolate	Rare virulence/avirulence	Standard line and its main <i>Ml</i> resistance gene												Pathotype
			P01 <i>a1</i>	P02 <i>a3</i>	P03 <i>a6</i>	P04B <i>a7</i>	P08B <i>a9</i>	P10 <i>a12</i>	P11 <i>a13</i>	P17 <i>k1</i>	P23 <i>La</i>	P21 <i>g</i>	P20 <i>at</i>	P15 <i>(Ru2)</i>	
2009	I-8	VLaverda			+	+			+		+			+	4154
2009	K-25	VSpilka	+	+		+	+	+	+	+	+	+		+	3775
2009	L-9	VSpilka	+		+	+	+	+	+	+	+	+		+	5775
2011	K-2	VVenezia		+	+	+		+			+	+		+	6545
2012	I-5	VSK-4770	+	+	+	+		+			+	+			7541
2012	X-30	VPsaknon	+	+	+	+		+	+			+		+	7515
2013	L-1	VPsaknon			+	+		+	+	+		+			4531
2013	L-17	VSK-4770	+				+	+	+	+		+	+	+	1637
2014	B2-1	VSJ048311			+	+		+				+		+	4505
2014	C-2	VSJ123063	+		+	+		+	+			+		+	5515
2014	I-6	VPsaknon	+		+	+		+		+	+	+		+	5565
2014	M-3	VPsaknon	+		+	+		+	+		+	+			5551
2009	I-12	<i>AvHa</i>	+			+	+	+		+		+		+	1725
2009	O-8	<i>AvHa</i>			+	+		+	+		+	+	+	+	4557
2013	C-2	<i>AvHa</i>		+	+	+		+		+		+		+	6525
2013	L-24	<i>AvHa</i>	+		+	+	+	+			+	+		+	5745

+Virulence on resistance genes of standard lines



## DISCUSSION

In annual resistance testing of spring barley many fully resistant varieties are found. They mostly carry the resistance *Mlo* that is widely distributed in both grown varieties and breeding lines (DREISEITL 2012). Such varieties are characterized by the occurrence of rare single colonies (JENSEN *et al.* 1992). However, almost all varieties containing *Mlo* also possess one or more specific resistances that prevent the expression of the typical phenotype of the resistance *Mlo*. Therefore, some varieties carrying *Mlo* are inadvertently included in differential sets. The resistance *Mlo* was subsequently identified in Xanadu and IN0614, which were then excluded from the differential set. NORD 12/1122, however, did not exhibit the '*Mlo*' phenotype nor necroses indicative of specific resistances in tests for resistance and inoculation with 227 isolates in two consecutive years.

A new virulence can arise in the pathogen population caused by mutation in an avirulent pathotype. Such mutants usually quickly disappear because of their lower viability. However, if spores of the mutant alight on a fully resistant variety, they can reproduce through their newly acquired virulence. Without intraspecific competition, the progeny of the mutant can successfully colonize so far a fully resistant variety even though the mutated progeny would succumb to intraspecific competition. Progeny carrying the new virulence are gradually selected on the variety that was originally fully resistant, and through a selective advantage will gain increased general viability. This also allows it to survive under conditions of intraspecific competition on other host varieties and, depending on the intensity and time of selection, to increase the virulence frequency in the population. Under conditions of intensive selection lasting several decades, the virulence frequency can approach to 100% as seen in, for example, *VHa*, or even to reach this level and fix the corresponding virulences in the given metapopulation, as has happened in for example, *VRa*, *VHH* (= *Va8*) and *VLo*.

A new virulence can occur in a local population of *Bgh* first, through mutation in that region, second by genetic transfer from another part of the metapopulation, or third from an accidental introduction from another metapopulation. Seven rare virulences were recorded of which at least four (to resistances in Venezia, Laverda, Psaknon and SJ048311) were novel. In the Czech Republic commercial varieties had been grown that possessed only one correspond-

ing resistance (*Lv*). It is, therefore, likely that most of these rare virulences originated outside the country.

Monitoring the occurrence of new virulences is important for several reasons. First, it indicates that a given population contains pathotypes that can overcome the corresponding resistance. Hence, neither breeding nor registration of new varieties with this resistance is acceptable because when widely grown, these varieties encourage pathogen reproduction and, therefore, resistance breakdown and loss of economic significance. Monitoring virulences also allows more effective identification of potential genetic resources intended for breeding new varieties (DREISEITL 2014b).

Detection of new virulences additionally allows the adoption of new resistances. Virulence to the resistance of Kangoo can be used as an example. Our gene bank of barley powdery mildew standards comprises all genotypes that were fully resistant, i.e. no virulent pathotype had been detected on them. Up to 2009 there were 29 such genotypes in the spring barley collection. Their resistance was fully effective and probably different from *Mlo*, although it was not known whether all the genotypes carried one common resistance or whether each contained a different resistance. In 2008 the first virulent isolates on Kangoo were obtained (DREISEITL 2008). Some of these isolates with new virulences were used in tests of 22 out of the 29 fully resistant spring barley genotypes. Eighteen of them, including Kangoo, contained identical, novel, and as yet undescribed resistance. These could be characterized based on their compatible reaction to pathotypes with new virulences, and were designated according to the first registered variety Roxana (resistance code *Ro*) (DREISEITL 2011b).

Similarly, after detecting the first isolate with a new virulence to the resistance of Laverda in 2009 (DREISEITL 2008, 2011d) 15 fully resistant genotypes of winter barley were tested using this new pathotype. Eleven of the 15 genotypes (including some varieties used in this report) possessed identical, novel resistances that were characterized according to their compatible reaction to pathotypes with new virulences. They were newly designated according to Laverda, resistance code *Lv* (DREISEITL 2011c). Therefore, monitoring the occurrence of new virulences facilitates not only more effective use of varietal resistance but also the identification of new resistances.

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