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# Virulence Frequency to Powdery Mildew Resistances in Winter Barley Cultivars

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**Abstract**: Virulence frequencies to powdery mildew resistances in winter barley cultivars mostly registered in the Czech Republic were studied in 2007 and 2008. Random samples of the air populations originating from winter and spring barley fields were obtained by means of a mobile version of a jet spore sampler. Conidia were sampled by driving across the Czech Republic. In total 349 isolates were studied and 17 differentials were used. The virulence frequencies to specific resistances of given cultivars showed wide range from 0% to 100%. Nine differentials were used to distinguish 134 pathotypes, of which 32 representing 63.9% of isolates were detected in both years. Pathotype 773, which broke down the resistance of eight differentials, was the most abundant. In 2008, lower virulence frequencies to all differentials, and thus lower population complexity, were determined, which may be caused by different regional origins of the isolates examined. Importance of the study of the given pathogen population is discussed in terms of successful breeding of resistant barley cultivars.

**Keywords**: barley; *Blumeria graminis* f.sp. *hordei*; *Hordeum vulgare*; powdery mildew resistances; pathogen populations; virulence frequencies

Winter barley (*Hordeum vulgare* L.) is a widely grown crop in the Czech Republic and powdery mildew caused by the fungus *Blumeria graminis* f.sp. *hordei* = *Bgh* is the most common disease on it (Dreiseitl 2007a). *Bgh* is an obligate pathogen that is able to survive on host green organs only. Growing winter barley gives the pathogen a greater chance of surviving by coincidence of the vegetation period of later tillers of spring barley and the period of emergence of first plants from volunteer winter barley as well as by a multiple number of host plants, particularly in winter. Growing susceptible cultivars facilitates a mass pathogen reproduction during the long vegetation period of this crop.

To limit the damage to barley, genetic resistance is an effective, economically sound and safe alternative to fungicide application. At least since 1970, there has been no cultivar in the registered assortment of winter barley that would miss a specific-resistance gene to powdery mildew (Dreiseitl 2007b). In spite of that, under conditions favourable for the pathogen development, many cultivars of winter barley are heavily infected by powdery mildew (Dreiseitl 2007c). That would not be possible without high frequencies of corresponding virulences in the pathogen population. It means that the practical effectiveness of each resistance is limited by the adaptable population of the pathogen (Dreiseitl 2003). Therefore, the objective of this study was to determine the virulence frequency to resistances possessed by the currently registered cultivars of winter barley and to analyze some other simple characteristics of the given pathogen population.

## MATERIALS AND METHODS

Two *Bgh* populations (2007 and 2008) were used for the assessment of virulence frequencies to powdery mildew resistances in winter barley cultivars and for determination of virulence associations (= pathotypes).

**Sampling of isolates**. Random samples of *Bgh* conidia originating from cultivated winter and spring barley fields were obtained from the air by means of the mobile version of a jet spore sampler (Schwarzbach 1979). The sampling of conidia was done by driving across the Czech Republic on 25 and 26 May 2007 (about 1600 km) and on 30 and 31 May 2008 (about 1900 km). The collected conidia settled on detached healthy and fully-expanded primary leaves of the susceptible barley cultivar Pallas in 2007 (Kølster *et al*. 1986) or Stirling in 2008 (Dreiseitl unpublished) that were placed in glass Petri dishes 120 mm in diameter with 0.8% water agar and 40 ppm benzimidazole and inserted in the bottom part of the spore sampler. Dishes with fresh detached leaves of a susceptible barley cultivar were exchanged after approximately 100 km, taking into account agroecological conditions in the region. During the sampling, dishes with fresh as well as with exposed leaves were kept in a car refrigerator at 10°C.

**Incubation**. After sampling the exposed leaves were transferred into glass Petri dishes 150 mm in diameter with fresh agar. To reproduce the collected conidia, dishes with leaves were kept in an incubation room for 11 days at the temperature of 19.0°C ± 0.5°C and under 12 h artificial light at 30 ± 5 μmol/m$^2$ per s.

**Differential sets**. The set of differential cultivars (differentials) in 2007 was comprised of nine cultivars and in 2008 of 17 cultivars. Based on the postulation method, it is known that all these cultivars carry different specific resistance genes to powdery mildew. However, the knowledge of the presence of these genes in several differentials is still preliminary or incomplete. Mostly registered cultivars were used except Vanessa and line LP 6-552 (Table 1). For the given purposes seeds of differentials from the Czech official variety trials were used.

**Growing of cultivars**. About 25 seeds of each susceptible or differential cultivar were sown in a pot (80 mm in diameter) filled with a mixture 2:1 of peat (Peat moss substrate B, produced by Rašelina Soběslav, Czech Republic) and soil. Plants were grown in a greenhouse at 25°C ± 5°C for 10–11 days. Of each differential, leaf segments 20 mm in length were removed from the central part of healthy, fully-expanded primary leaves and three of them were placed on the above-mentioned agar in plastic Petri dishes 90 mm in diameter.

**Inoculation**. Inoculation was performed in a metal micro-settling tower with the circular basis 90 mm in diameter and 200 mm in height, with a gap of 10 mm diameter in the central part of the upper cap. During the incubation, each *Bgh* colony usually developed 20 ± 5 thousand conidia of a new "generation" from the collected conidia. They were used to inoculate a set of leaf segments of differentials A (not mentioned here) placed in a Petri dish with the agar. The conidia of one colony were sucked into a replaceable tip of a varipipette AW 1000 set to 0.4 ml volume and then blown off the tip into a micro-settling tower using a syringe (10 ml) with a 180 mm long tube. Inoculum density was ca. 3 conidia/mm$^2$. The dishes with a set of leaf segments of differentials were exchanged one by one at the bottom of the tower and after inoculation they were kept under described incubation conditions. Before the inoculation of each dish with conidia of another colony, the micro-settling tower, surface of a laboratory desk and the tube were sterilized with 98% ethyl alcohol spread with cotton. After the evaluation of differential set A (results are not shown here) the conidia of one isolate were used in the same way for the inoculation of differential set B (results are the topic of the present contribution).

**Evaluation**. Reaction types (RTs) produced by the response of each differential to a corresponding *Bgh* isolate were scored nine days after inoculation according to the nine-point 0–4 scale including intertypes (Torp *et al*. 1978). The isolates that produced RT 4 or 3–4 on a differential were considered virulent on the corresponding resistant cultivars. In cases where virulence was manifested towards a differential with more resistance genes, the virulent isolate has more corresponding virulence genes. But when an isolate was avirulent, it could be due to the presence of one or more of these avirulence genes. Some or all of those isolates that are avirulent on this differential possessing more resistance genes may possess corresponding virulence gene(s) which cannot be expressed.

**Pathotype designation**. The isolates were assigned numerical designations based on their virulence to matching resistances in nine differentials used in both years (Tables 1 and 2). The differential set was divided into three triplets and each of the three digits indicates virulence or avirulence on the three differentials of the respective triplet (Limpert *et al*. 1994). If a virulence to the corresponding differential is detected, the first differential has the value 1 ($2^0$), the second differential has the value 2 ($2^1$), and the third dif-

ferential has the value 4 ($2^2$). Therefore, each digit can have a value from 0 (no virulence on any of the three differentials) up to 7 (= 1+2+4, virulent on each of the three differentials). The resulting number based on nine differentials defines the virulences of the isolates and consequently their classification as pathotypes (Tables 2 and 3).

**Data analysis**. Parameters for a comparison of both populations were calculated on the basis of virulence patterns of isolates on the set of the nine differentials in the given order (Table 3). Descriptive parameters of populations (virulence frequency, virulence complexity, number of pathotypes, abundance and diversity parameters shown in Table 4) were calculated for the isolates by the HaGiS program (Hermann *et al*. 1999).

## RESULTS

### Virulence frequency

In 2007, 189 *Bgh* isolates were examined (Table 1). The virulence frequencies to nine differentials varied from 43.4% (Vilna) to 93.6% (Merlot). In 2008, 160 pathogen isolates were examined and 17 differentials were used (all nine from 2007 and newly eight others). The virulence frequencies showed a substantially wider range (from 0% to 100%) due to newly included differentials. All examined isolates were characteristic of virulence to Lomerit possessing the resistance gene *Mla8* (the corresponding virulence frequency = 100%). On the contrary, none of the isolates was virulent to Laverda and only two isolates were virulent to Florian (the corresponding virulence frequency = 1.3%). Virulence frequencies to resistances of all nine differentials used in both years were lower in 2008 than in 2007 by 0.3% (Tiffany) to 29.0% (Gilberta) although virulence frequencies on Tiffany cannot be statistically distinguished and a difference on Vanessa was less significant than on the other differentials.

### Pathotype distribution

In 2007, 73 pathotypes, of which 25 showed frequencies higher than 2, were found on nine differentials used in both years (Table 4). Mean

Table 1. A differential set of 17 winter barley cultivars and corresponding virulence frequencies (%) of the pathogen found in the Czech Republic in 2007 and 2008

| Differential cultivar | Year of registration | 2007 (%) | 2008 (%) |
|---|---|---|---|
| Merlot | 2002 | 93.6 ± 1.8[a] | 75.6 ± 3.4 |
| Luran | 1998 | 92.1 ± 2.0 | 85.0 ± 2.8 |
| Tiffany | 1999 | 81.5 ± 2.8 | 81.2 ± 3.1 |
| Gilberta | 2006 | 74.6 ± 3.2 | 45.6 ± 3.9 |
| Carola | 2001 | 66.7 ± 3.4 | 55.0 ± 3.9 |
| Vanessa | – | 66.1 ± 3.4 | 61.9 ± 3.8 |
| Traminer | 2003 | 64.6 ± 3.5 | 54.4 ± 3.9 |
| Nelly | 2001 | 63.5 ± 3.5 | 53.8 ± 3.9 |
| Vilna | 2001 | 43.4 ± 3.6 | 31.9 ± 3.7 |
| Lomerit | 2002 | – | 100.0 |
| Babette | 2007 | – | 76.3 ± 3.4 |
| LP 6-552 | – | – | 71.9 ± 3.6 |
| Alinghi | 2007 | – | 48.8 ± 4.0 |
| Duet | 1999 | – | 15.6 ± 2.9 |
| Finesse | 2007 | – | 10.6 ± 2.4 |
| Florian | 2008 | – | 1.3 ± 0.9 |
| Laverda | 2007 | – | 0.0 |
| No. of isolates | | 189 | 160 |

[a]Standard error of binomial distribution

Table 2. Virulence spectra of abundant pathotypes of the powdery mildew pathogen found in the Czech Republic in 2007 and 2008

| Differential cultivar | Virulence spectra of abundant pathotypes of the powdery mildew pathogen | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 773 | 771 | 763 | 716 | 777 | 371 | 746 | 761 | 712 |
| Merlot | + | + | + | + | + | + | + | + | + |
| Luran | + | + | + | + | + | + | + | + | + |
| Tiffany | + | + | + | + | + | | + | + | + |
| Gilberta | + | + | | + | + | + | | | + |
| Carola | + | + | + | | + | + | | + | |
| Vanessa | + | + | + | | + | + | + | + | |
| Traminer | + | + | + | | + | + | | + | |
| Nelly | + | | + | + | + | | + | | + |
| Vilna | | | | + | + | | + | | |

Designation of the pathotypes according to Limpert *et al.* 1994; + virulence

virulence complexity of isolates in the examined population sample was 6.46 and mean pathotype complexity was 5.56. In 2008, 93 pathotypes were found, of which 33 exhibited frequencies higher than 2. Mean isolate complexity was 5.43 and mean pathotype complexity 5.04. In both years, a total of 349 isolates were studied that belonged to 134 pathotypes. Thirty-two pathotypes (23.9%) represented 223 isolates (63.9%) were found in both years. Out of them, 137 isolates (72.5% of the given population) were detected in 2007 and 86 isolates (53.8% of the given population) in 2008. One hundred and two pathotypes (76.1%) represented 126 isolates (36.1%) were found only in one of the two years. In 2007, pathotype 773 was the most abundant (Table 3), comprising 23 isolates (= 12.2%) and showing virulence complexity 8 (overcoming resistances of 8 out of 9 differentials). In 2008, pathotype 763 was the most abundant, comprising 9 isolates (= 5.0%) and showing virulence complexity 7.

Table 3. Frequency of abundant pathotypes of the powdery mildew pathogen found in the Czech Republic in 2007 and 2008

| Abundant pathotypes | 2007 (%) | 2008 (%) |
|---|---|---|
| 773 | 12.2 | 5.0 |
| 771 | 6.9 | 2.5 |
| 763 | 3.2 | 5.6 |
| 716 | 6.3 | 1.2 |
| 777 | 6.3 | 1.2 |
| 371 | 3.7 | 1.9 |
| 746 | 3.1 | 1.6 |
| 761 | 2.6 | 1.9 |
| 712 | 1.6 | 2.5 |

Designation of the pathotypes according to Limpert *et al.* 1994

Table 4. Population parameters of the powdery mildew pathogen in the Czech Republic in 2007 and 2008

| Population parameter | 2007 | 2008 |
|---|---|---|
| No. of isolates | 189 | 160 |
| No. of pathotypes | 73 | 93 |
| No. of pathotypes with frequency >1 | 25 | 33 |
| Mean of isolate complexity | 6.46 | 5.43 |
| Mean of pathotype complexity | 5.56 | 5.04 |
| Diversity – Simple | 0.39 | 0.58 |
| Diversity – Gleason | 13.74 | 18.13 |
| Diversity – Shannon | 3.74 | 4.31 |
| Diversity – Simpson | 0.97 | 0.99 |

## DISCUSSION

The pathogen of powdery mildew on barley is very adaptable to both resistance genes contained in grown cultivars and fungicidal active ingredients. The adaptability is reflected in the wide diversity of its population. It is also documented by the present results, by both high values of all four coefficients of diversity given in Table 4 and the high number of detected pathotypes and their relatively low frequencies. The adaptability of the given pathogen is a result of rapid and/or frequent operation of evolutionary forces in its population (Dreiseitl 2008). In contrast with most of the other host-pathogen systems, in the barley-powdery mildew pathogen interaction there are a lot of resistance genes, many others have been more or less described (Dreiseitl *et al.* 2007; Dreiseitl & Yang 2007; Řepková *et al.* 2006, 2008) and a large number of new genes, particularly in wild barleys (*Hordeum vulgare* ssp. *spontaneum* and *Hordeum bulbosum*), can be anticipated (Dreiseitl & Bockelman 2003; Dreiseitl & Dinoor 2004; Shtaya *et al.* 2007).

The population study sets a number of various aims in relation to resistance genes, from relatively simple assessment of virulence frequencies (Dreiseitl 2004) or analysis of detected pathotypes (Zhu *et al.* 2007) to complex survey of a continental population (Hovmøller *et al.* 2000) or characteristics of selected representatives of a global population (Dreiseitl *et al.* 2006; Dreiseitl & Wang 2007). This study is focused on the determination of virulence frequencies to specific resistances in selected cultivars disregarding whether these resistances have been identified.

A significant finding is a general decrease in virulence frequencies in 2008 that is not related to the size of the area planted with corresponding cultivars. The virulence frequency to the resistance of cv. Gilberta decreased to the greatest extent. The changes in plant pathogen populations are induced by operating evolutionary forces. If populations produced by cultivated crops are studied, the most frequent evolutionary force is usually directional selection on cultivars with corresponding resistance genes. However, the planting area of cv. Gilberta has been increasing slowly and is still small. Likewise, changes in the area under the other cultivars (that were not large in the given period) do not allow us to clarify the differences found. They might be hypothetically influenced by different output of collecting the two population samples in the given years because 86.9% of isolates were obtained from sampling routes of 520 km across the region of south and central Moravia in 2008, whereas in 2007 only 22.8% of the examined isolates originated from this area. To test potential regional differences in this typical air-borne pathogen is a subject of further investigations.

The virulence frequency to the three studied cultivars was studied already in 2004 (Dreiseitl 2004). In 2007, it increased in cv. Vanessa from 40.1% to 66.1% and in cv. Carola even from 14.1% to 66.7%. It is a big change, particularly in the latter cultivar. Such a change may be substantiated by the fact that the two cultivars possess at least one common resistance gene (*MlSt*) that is also present in some current cultivars of spring barley. In contrast, in cv. Duet some decline was found in virulence frequency ranging from 21.8% (2004) to 15.6% (2008). However, it cannot be in direct connection with the area planted with this cultivar or other cultivars because the most effective gene of resistance genes in cv. Duet (*MlDt*) is not carried by any other currently grown cultivar.

In general, the lower virulence frequencies in 2008 are expressed by a decrease in the isolate complexity from 6.46 to 5.43, whereas the pathotype complexity declined "only" from 5.56 to 5.04. It demonstrates that in both years, but particularly in 2007, pathotypes with higher complexity were much more abundant (the most abundant pathotype 773 has complexity 8). Using nine differentials, the theoretically highest accessible complexity is 9.0. Thus, the assessed decrease in the complexity of the 2008 population in comparison with that in the 2007 population means leaving the maximum and approaching the optimum of this parameter, which is a half of the theoretical range (4.5) considering the population diversity. The 2008 population is indeed markedly more diverse, which is supported not only by all four coefficients of diversity but also by a considerably higher number of detected pathotypes, even at a lower number of the isolates examined.

The pathogen population is the main limitation in breeding cultivars of each crop for resistance to a given disease. So, if programmes of breeding resistant cultivars, and particularly of growing these cultivars, are to be an adequate alternative to the protection against a certain disease, then the observation of a corresponding pathogen popu-

lation is necessary. Furthermore, there is a need of developing the methods for population studies themselves in order to provide the objective characterization of resistance in current cultivars, predict its effectiveness, and thus considerably contribute to the rational use of resistance in future cultivars.

Substantial progress has been made in breeding spring barley cultivars resistant to powdery mildew, in particular by using the gene *mlo*. In spite of that, a potential of this disease harmfulness is even higher than, for instance, 30 years ago (Dreiseitl unpublished). In the Czech Republic, it is due to the present area of winter barley (ca. 22% of total barley area), and it is particularly due to the fact that cultivars of this crop with effective resistance to the given disease have not been grown yet. Such more or less susceptible cultivars provide the pathogen with a large space for its reproduction because host plants of winter barley are present in the field for a much longer time than spring barley plants.

Our results, however, suggest a possible beginning of change in the resistance of winter barley cultivars because the effectiveness of unknown resistance in new cvs. Florian and especially Laverda is high. Under conditions of Central Europe the pathogen causing barley powdery mildew has no other possibility of survival on other host species than on cultivated barley. Therefore, wide growing resistant cultivars of not only spring barley but also winter barley would be a very efficient measure in reducing the harmfulness potential of this most frequent disease of barley.

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