RESULTS OF THE MONITORING OF THE POPULATION OF *BLUMERIA GRAMINIS* F. SP. *HORDEI* IN THE LATGALE REGION OF LATVIA IN 2007

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Abstract

In 2007, samples of the pathogen were collected both in sporulation and cleistothecia phases from commercial barley fields of susceptible varieties of spring barley near the town Daugavpils. For virulence detection the microinoculation technique was used. Frequencies of virulence genes and frequencies of pathotypes were calculated. Virulence genes *Va1*, *Va3* and *Va13* were presented with medium to medium-high frequencies, frequencies of *Va6*, *Va7 Va9*, *Va12*, *Vk* and *Vla* were medium-high. Virulence frequencies for all genes mentioned above increased in cleistothecia phase. Resistance factors from 'Steffi', 'Goldie' and 'Meltan' are still rather effective. Not any isolate with the virulence against *SI1* was detected in the population in 2007. Frequencies of all virulence genes involved in the monitoring were stable during last three years. A large number of pathotypes were detected. The dominant pathotype *a6 a7 a9 a12 k la* was present both in sporulation and cleistothecia phases.

Key words: powdery mildew, virulence frequencies, barley, resistance, pathotypes.

Introduction

Powdery mildew, caused by the fungus *Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei* Em. Marchal is one of the most devastating diseases of barley /Masterbroek, Balkema-Boomstra, 1995/. The fungus is an obligate pathogen of barley that is able to survive on green organs only, particularly on leaves /Dreiseitl, Jurečka, 2003; Dreiseitl, 2007/. Thus, powdery mildew can be very destructive to barley, reducing photosynthesis, growth and, finally, yield. At first, powdery mildew can be observed as small white patches of fluffy fungal mycelium (a phase of vegetative sporulation) on the surface of the lower leaves. Infections can also occur on the leaf sheaths and ears. During the growing season, the mycelium often becomes dotted with black cleistothecia, which are the sexual fruiting bodies of the fungus.

Host-pathogen interactions are based on the conception of "gene-for-gene", meaning interactions between host resistance genes and cognate pathogen avirulence genes /Halterman, Wise, 2004/.

There are two mainstays of powdery mildew control in the integrated crop protection system: i) selection for cropping the most resistant among available varieties, and ii) use of effective fungicides. Development of resistant varieties is the most economical and environmentally safe method reducing the application of fungicides to combat this disease /Naghavi et al., 2007/. The problem of barley powdery mildew control is the often-observed ability of the pathogen to evade measures directed against it. Thus, powdery mildew has a number of characteristics, which favour a rapid adaptation rate, such as its relatively short generation time, with sexual recombination throughout the year (formation of cleistothecia). Another important reason for the some-times-rapid adaptive response is the nature of spread of the pathogen, as newly adapted pathotypes can be carried relatively quickly by wind over a wide area. The plant-breeding history provides many examples of varieties, which at first had excellent mildew resistance but lost it within a few years because of adaptative changes of the pathogen /Felsenstein, Kuck, 1998/.

The most significant feature of *B. graminis* f. sp. *hordei* is the high level of pathogenic variability encountered in natural populations. This has repeatedly been demonstrated in different regions of the world where the disease is a problem. The amount of genetic variation found in a local population of *B. graminis* f. sp. *hordei* depends on the season due to the partly asexual mating system and the short generation cycles. It is proved that *B. graminis* f. sp. *hordei* is able to develop new races, which may rapidly spread across Europe and render the formerly resistant varieties susceptible /Limpert, 1987; Gacek, Czembor, 1988; Müller et al., 1996; Czembor, 2000/. That is why regular observations of regional populations are necessary for understanding the process of the evolution and dissemination of the pathogen. The population of *B. graminis* f. sp. *hordei* has been monitored in the Latgale region of Latvia since 1995 /Rashal et al., 1997; Rashal et al., 2000 a; Rashal et al., 2000 b; Kokina, Rashal, 2001; Kokina, Rashal, 2004, Rashal et al., 2004; Kokina, Rashal, 2005; Kokina, Rashal, 2006/. The objective of this paper is to characterize the pathogen population in the region in 2007.

Materials and Methods

In 2007, samples of *Blumeria graminis* f. sp. *hordei* were collected both in the sporulation and cleistothecia phases from commercial barley fields of susceptible varieties of spring barley (unknown resistance genes) in the Latgale region of Latvia, near the town Daugavpils. Samples of barley leaves with well-developed vegetative sporu-lation or cleistothecia were used. Sampling size and dates of the pathogen collecting are presented in Table 1.

Collected conidia spores were settled on detached healthy and fully-expanded primary leaves of the susceptible barley variety 'Otra'. Samples of cleistothecia were stored at the temperature of 4–8° C. First leaves of barley variety 'Otra' were used for isolation and multiplication of single colonies from samples both sporulation and clei-stothecia phases. For isolation of ascospores from cleistothecia host leaf segments with well-developed fruiting bodies were put on wet filter paper on the lid of a Petri plate and cultivated in the dark at the temperature of 18–20° C for 3–4 days. Then the lids with cleistothecia were put on a Petri plate with the 'Otra' leaf segments. When the filter paper dried out, the swollen cleistothecia contracted and "shot" the ascospores out, thus

infecting the host leaves. Infected leaf segments were incubated at the temperature 18–20° C under artificial light. After 3–4 days, well-developed conidia appeared which were used for isolation of single colonies.

Table 1. Sampling date and number of isolates of *Blumeria graminis* f. sp. *hordei* collected in the Latgale region of Latvia in 2007

Sampling date	Phase of the pathogen	Number of isolates
July 15	conidia	40
August 9	cleistothecia	44
	Total number of isolates:	84

For testing single colonies the standard set of differentials was used (Table 2). Plants were grown in laboratory conditions at 18–20° C, photoperiod 10 h. Primary leaf segments of each differential, approximately 20 mm long, were placed on the 0.7% water agar with 35 ppm benzimidazole /Dreiseitl, 2004/. For inoculation of the leaf segments the microinoculation technique /Dreiseitl, 1998/ was used. The conidia of one colony were sucked into a tip of micropipette, and then blown into a micro-settling tower. After inoculation with conidia of single colony, the microsettling tower was sterilized by 98% ethanol. Incubation of inoculated segments of differentials was carried out under 18–20° C in light with a photoperiod of 10 h.

Table 2. A set of differentials used for detection of virulence genes in *Blumeria graminis* f. sp. *hordei* samples collected in the Latgale region of Latvia in 2007

Differentials	Main resistance genes
P01	*Mla1*
P02	*Mla3*
P03	*Mla6*
P04B	*Mla7*
P08B	*Mla9*
P10	*Mla12*
P11	*Mla13*
P17	*Mlk*
P23	*MlLa*
SI1	*Ml(SI)*
ʻSteffiʼ	*Ml(St1), Ml(St2)*
ʻGoldieʼ	*Mla12, MlLa, U*
ʻMeltanʼ	*Mla13, Ml(Im9), Ml(Hu4)*

After 7–9 days of incubation, reaction types produced by the response of each differential to a corresponding *B. graminis* f. sp. *hordei* isolate were scored according to the 0–4 scale /Torp et al., 1978/. Leaf segments with infection type 0–3 were classified as resistant, segments with infection type 4 – as susceptible. Frequencies of virulence

genes and frequencies of pathotypes were calculated. The mean complexity was calculated as a weighted mean of complexities of all presented isolates.

Results and Discussion

Frequencies of virulence genes Va1, Va3 and Va13 in the Latgale population in 2007 varied from 29 to 33% in samples of conidia and from 47 to 59% in cleistothecia (Figure 1). A clear tendency to increase frequencies of these virulence genes was observed in conidia samples in comparison with cleistothecia. Such tendency was detected also in previous years /Kokina, Rashal, 2004; Kokina, Rashal, 2005; Kokina, Rashal, 2006/. The most significant difference between conidia and cleistothecia samples was detected for Va13.
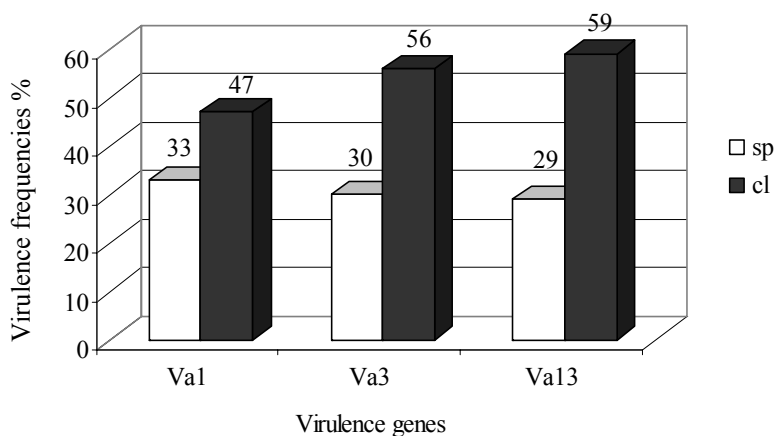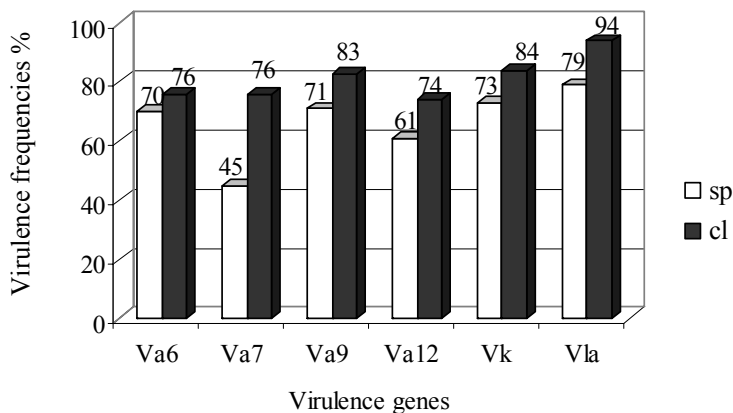

Figure 1. Virulence frequencies (%) of *Va1, Va3* and *Va13* detected in *Blumeria graminis* f. sp. *hordei* samples collected in the Latgale region of Latvia in 2007. Difference between virulence frequencies in sporulation and cleistothecia phases is significant for *Va13* (P > 0.95)

Frequencies of virulence genes Va6, Va7, Va9, Va12, Vk and Vla in the pathogen population in 2007 are presented in Figure 2. In phase of conidia virulence frequencies varied from 45 to 79%; in the phase of cleistothecia frequencies were higher: from 61 to 94%. Significant difference between samples was detected for Va7 (45 and 76%, respectively). Earlier very considerable differences between conidia and cleistothecia phases were detected for the gene Va7 as well.

Virulence genes Vst, Vgo and Vme were presented in the Latgale population with medium frequencies (Figure 3) which means that corresponding resistance factors from 'Steffi', 'Goldie' and 'Meltan' are still rather effective in the region.

Barley line SI1 was considered as a new resistance source for the control of powdery mildew /Hovmøller et al., 2000/. Not any isolate with the virulence to SI1 was detected in the Latgale pathogen population in 2007, as well as in previous years. Further observation of this virulence is necessary in Latvia and elsewhere in Europe.

Frequencies of all virulence genes, included in the monitoring, were approximately in the same level as in the previous years (2005–2006). Virulences of some of them increased gradually in previous years, for example genes Va1, Va3 and Va13. Frequencies of other virulence genes, such as Va6, Va7, Va9, Va12, Vk and Vla were stable high during several last years. This indicates stabilization of the pathogen population, at least regarding investigated genes.

Figure 2. Virulence frequencies (%) of Va6, Va7, Va9, Va12, Vk and Vla detected in Blumeria graminis f. sp. hordei samples collected in the Latgale region of Latvia in 2007. Difference between virulence frequencies in sporulation and cleistothecia phases is significant for Va7 (P > 0.95)
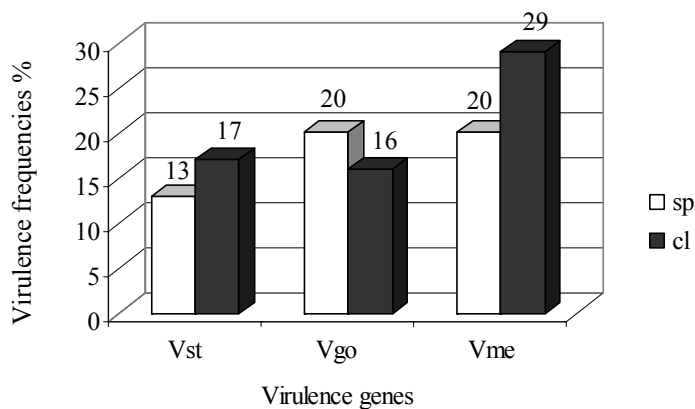
Figure 3. Virulence frequencies (%) of *Vst, Vgo* and *Vme* detected in *Blumeria graminis* f. sp. *hordei* samples collected in the Latgale region of Latvia in 2007

A large number of pathotypes were detected in the Latgale region in 2007. Similarly to previous years, the dominant was the pathotype a6 a7 a9 a12 k la, which was present both in sporulation and cleistothecia phases with frequencies 18 and 23%,

respectively. The pathotype included virulence genes with high frequencies in the population and was described as the most frequent pathotype during previous years /Kokina, Rashal, 2006/. The pathotype a1 a3 a13, defined earlier as new and dangerous under Latvian conditions /Kokina, Rashal, 2004/, was not detected in the pathogen population in 2007.

Complexity (number of virulence genes per isolate) varied from 2 (in conidia) to 12 (in cleistothecia). The pathotypes with all virulences except VSI were present in phase of cleistothecia. Obviously, increasing the frequency on individual virulence genes leads to an increase in the complexity in the pathogen population: it was found that the mean complexity in sporulation and cleistothecia was statistically significant (5.9 ± 0.3 and 8.2 ± 0.3, respectively).

Conclusions

Results of the investigation show that the population of *B. graminis* f. sp. *hordei* in the Latgale region was stable for the last 2–3 years regarding virulence genes involved in the monitoring. Occurrence and frequency of matching virulences in the pathogen population define the efficiency of a resistance gene /Dreiseitl, Pařízek 2003/. According this, resistance genes *Mla6*, *Mla7*, *Mla9*, *Mla12*, *Mlk* and *Mlla* are not effective under Latvian conditions, which can be explained by extensive and long-term use of genes mentioned above in commercial varieties in several European countries. Resistance genes *Mla1*, *Mla3* and *Mla13* lost their effectiveness during the last years. Although virulence genes *Vst*, *Vgo* and *Vme* are present in the Latgale population with medium frequencies at the moment, it could not be excluded, that corresponding resistance will also lose its effectiveness in forthcoming years.

That is why regular observations of local populations are critical for choosing the best strategy of breeding for resistance. Including in the monitoring additional pathogen virulence genes, matching the new resistance genes, involved in the barley breeding in other regions of Europe in recent years, is also necessary.

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