*Agronomy Research* **4**(Special issue), 231–236, 2006

# Monitoring the population of *Blumeria graminis* f. sp. *hordei* in the South-Eastern part of Latvia

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**Abstract.** The population of *Blumeria graminis* f.sp. *hordei* in the South-Eastern part of Latvia (Latgale region) has been monitored since 1995. In 2005, samples of the pathogen were collected both as conidia and cleistothecia from commercial barley fields. Frequencies of virulence genes, pathotypes and their complexity were calculated. Genes *Va1, Va3* and *Va13* were presented with medium-high frequencies and frequencies of *Va6, Va7, Va9, Va12, Vk* and *VLa* ranged from medium-high to high. A large number of pathotypes was detected, which reflects wide genetic diversity in the pathogen population.

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

## INTRODUCTION

The obligate plant pathogenic fungus *Blumeria graminis* (DC.) Golovin ex Speer f.sp. *hordei* Em. Marchal is the causal agent of powdery mildew on barley. During the infection process under favourable conditions, the fungus develops haustoria inside the epidermal host cell and utilizes nutrients from host cells. Powdery mildew is most frequently found on barley leaf surface as white patches (a phase of vegetative sporulation), which turn into dense mycelia with black fruiting bodies (cleistothecia) during the growing season. The pathogen can infect all aerial parts of the plant, reducing photosynthesis, growth and efficiency.

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). Accordingly, just one mutation may cause a pathogen to become virulent. Spores of the pathogen are spread by wind over large distances across Europe (Limpert et al., 1999; Hovmøller at al., 2000; Brown & Hovmøller, 2002). As a consequence new virulent races also could spread broadly if they advance, from the point of view of natural selection.

Regular observations of regional populations are necessary for understanding the process of the evolution and dissemination of the pathogen as well as for choosing the best strategy of breeding for resistance. In the Southeastern part of Latvia (the Latgale region), the population of *Blumeria graminis* f.sp. *hordei* has been monitored since 1995 (Rashal et al., 1997; Rashal et al., 2000a; Rashal et al., 2000b; Kokina & Rashal, 2001; Kokina & Rashal, 2004; Rashal et al., 2004; Kokina & Rashal, 2005).

The aim of this paper is to characterize the pathogen population in the Latgale region in 2005.

## MATERIALS AND METHODS

In 2005, samples of *Blumeria graminis* f.sp. *hordei* were collected both in the vegetative spore and cleistothecia phase from commercial fields of susceptible varieties of spring barley (unknown resistance genes) in the Southeastern part of Latvia (Latgale region). Sampling size and time of the pathogen are presented in Table 1. Only samples of barley leaves with well-developed colonies (phase of vegetative sporulation) or cleistothecia were used.

**Table 1.** Sampling time and number of isolates from asexual and sexual phase of *Blumeria graminis* f.sp. *hordei* collected in the South-Eastern part of Latvia in 2005.

| Date of sampling | Phase of the pathogen | Number of isolates |
|---|---|---|
| July 20 | conidia | 31 |
| August 11 | cleistothecia | 33 |
| | Total: | 64 |

The primary step for virulence testing was isolation of single colonies. The first leaves of universally susceptible barley variety 'Otra' were used for the isolation and multiplication of single colonies from samples of sporulation and cleistothecia. For isolation of ascospores from cleistothecia, the host leaf segments with well-developed fruiting bodies were put on wet filter paper on the lid of the Petri plate and cultivated in darkness at the temperature of 18–20° C for 3–4 days. Then the lids with cleistothecia were put on the 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 2.** Differentials used for detection of virulence gene in the population of *Blumeria graminis* f.sp. *hordei* in the South-Eastern part of Latvia in 2005.

| Differentials | Main resistance genes |
|---|---|
| *P01* | *Mla1* |
| *P02* | *Mla3* |
| *P03* | *Mla6* |
| *P04B* | *Mla7* |
| *P08B* | *Mla9* |
| *P10* | *Mla12* |
| *P11* | *Mla13* |
| *P17* | *Mlk* |
| *P23* | *MlLa* |
| *SI1* | *Ml(SI1)* |
| 'Steffi' | *Ml(St1), Ml(St2)* |
| 'Goldie' | *Mla12, MlLa, U* |
| 'Meltan' | *Mla13, Ml(Im9), Ml(Hu4)* |

For testing single colonies, a standard set of differentials was used (Table 2) (Kølster et al., 1986). Differentials were grown under laboratory conditions (18–20° C, photoperiod 10 h) until well-developed first leaves appeared. For inoculation of leaf segments of differentials, the microinoculation technique was used (Dreiseitl, 1998). Incubation of inoculated differentials was carried out under 18–20°C in light with a photoperiod of 10 h.

After 7–9 days of incubation reaction types on the differentials were scored according to a 0–4 scale (Torp et al., 1978). Leaf segments of differentials with infection types 0–3 were classified as resistant, segments with infection type 4 – as susceptible. Frequencies of virulence genes, their distribution per isolate and frequency of pathotypes were calculated. The mean complexity was calculated as a weighted mean of complexities of all presented isolates.

## RESULTS AND DISCUSSION

Frequencies of the powdery mildew virulence genes *Va1, Va3* and *Va13* are presented in Fig. 1. Medium frequencies (from 26% to 39%) of the virulence genes were detected in samples of conidia. A clear tendency of increase in frequency of these virulence genes was observed in the phase of cleistothecia, as virulence frequencies varied from 45% to 54%. A similar tendency of virulence increase during the growing season was detected earlier, as well. In 2003–2004, considerable increase in frequencies of *Va1, Va3* and *Va13,* especially in cleistothecia (Kokina & Rashal, 2005) were detected in the Southeastern part of Latvia for the first time.

Frequencies of virulence genes *Va6, Va7, Va9, Va12, Vk* and *Vla* were medium-high to high in 2005, varying from 48% to 97% (Fig. 2). In samples of cleistothecia an increase in frequencies of several virulence genes was observed. The most considerable difference in virulence frequencies between conidia (48%) and cleistothecia (82%) phases was detected for *Va7*.

Resistance factors from 'Steffi', 'Goldie' and 'Meltan' are still effective; corresponding virulence genes are presented with low-medium frequencies (Fig. 3). During the same period virulences are presented more frequently than in previous years. No isolates with the correspondent virulence for *SI1* were detected in the pathogen population in 2005.

A large number of pathotypes was detected in the pathogen population in Southeastern Latvia in 2005 (Table 3). The dominant pathotype *a6 a7 a9 a12 k la* was present both in vegetative sporulation and cleistothecia phases with frequencies of 16.1% and 21.2%, respectively. This pathotype was designated as the most frequent pathotype in the population earlier, as well (Kokina & Rashal, 2005a). The pathotype *a1 a3 a13*, which was defined as new and dangerous under Latvian conditions in 2004 (Kokina & Rashal, 2004), was not detected in Southeastern Latvia in 2005.

The number of virulence genes per isolate of detected pathotypes varied from 1 to 12 (data not shown). The mean complexity in sporulation and cleistothecia phases was 5.97 ± 0.39 and 7.09 ± 0.36, respectively. Data proved increasing aggressiveness in the pathogen population during the growing season. A pathotype with one virulence gene *Va12* was detected in samples in the phase of sporulation. The most virulent pathotype with 12 virulences (all virulence genes except *VSI*) was present in phase of cleistothecia in 2005.

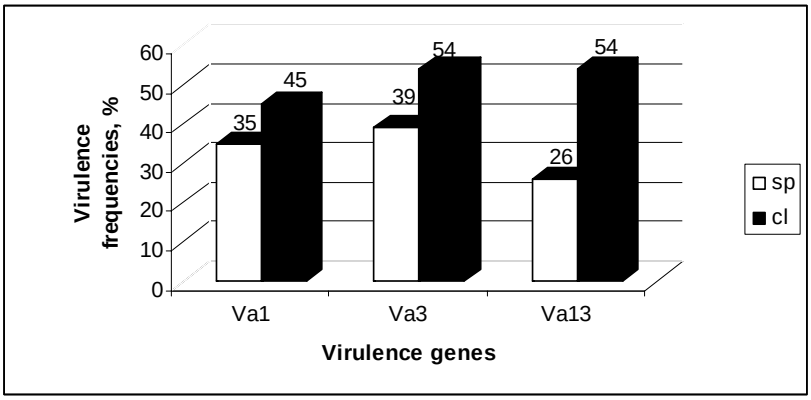


**Fig. 1.** Frequencies of virulences genes *Va1, Va3* and *Va13* in the South-Eastern part of Latvia in 2005. Difference between virulence frequencies in sporulation and cleistothecia phases is significant for *Va13* ($P > 0.95$).

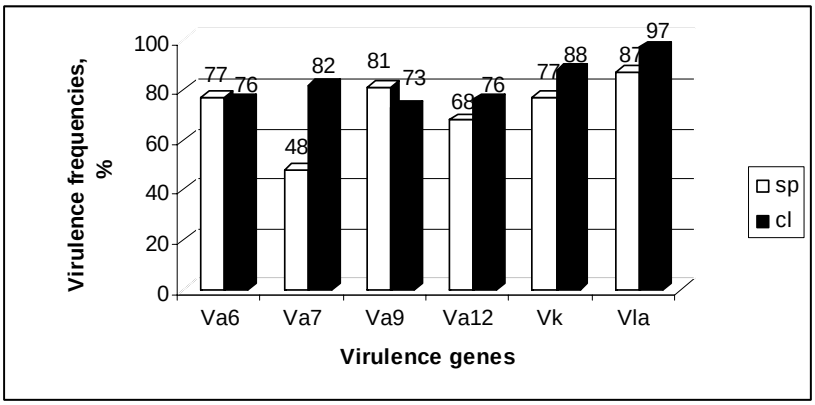


**Fig. 2.** Frequencies of virulences genes *Va6, Va7, Va9, Va12, Vk* and *Vla* in the South-Eastern part of Latvia in 2005. Difference between virulence frequencies in sporulation and cleistothecia phases is significant for *Va7* ($P > 0.99$).

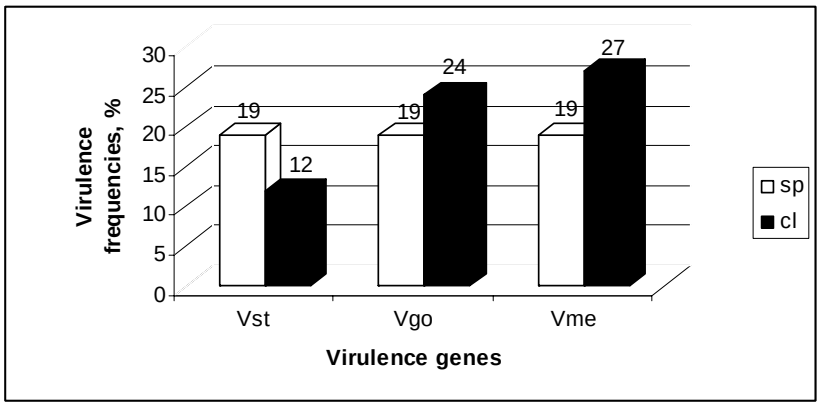


**Fig. 3.** Frequencies of virulences genes *Vst, Vgo* and *Vme* in the South-Eastern part of Latvia in 2005.

**Table 3.** The most frequent pathotypes in the pathogen population in the South-Eastern part of Latvia in 2005.

| Phase of the life cycle of the pathogen | Number of pathotypes | Pathotype | Frequency of the pathotype, % | Mean complexity |
|---|---|---|---|---|
| sporulation | 19 | *a6 a7 a9 a12 k la* | 16.1 | 5.97 ± 0.39 |
| | | *a1 a3 a6 a9 a12 k la* | 9.7 | |
| | | *a1 a3 a6 a9 a12 k la go* | 9.7 | |
| | | *a1 a3 a7 a9 a12 a13 k la* | 6.5 | |
| | | *a3 a6 a7 a9 a12 a13 la* | 6.5 | |
| | | *a6 a7 a9 k la st go me* | 6.5 | |
| | | *a6 a9 a13 k la st me* | 6.5 | |
| cleistothecia | 18 | *a6 a7 a9 a12 k la* | 21.2 | 7.09 ± 0.36 |
| | | *a1 a3 a6 a7 a9 a12 a13 k la go* | 12.1 | |
| | | *a6 a7 a9 a12 a13 la* | 9.1 | |

Presented data should be taken in consideration for elaboration of recommendations concerning the use of resistance genes in barley breeding programmes for the region.

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