JOURNAL OF PLANT PROTECTION RESEARCH Vol. 54, No. 1 (2014)

Blumeria graminis f. sp. *hordei* virulence frequency and the powdery mildew incidence on spring barley in the Wielkopolska province

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Received: September 17, 2013
Accepted: January 14, 2014

Abstract: Powdery mildew caused by fungi *Blumeria graminis* f. sp. *hordei* is one of the most common barley diseases in Polish meteorological conditions. The powdery mildew fungus is made up of different races and forms that are highly specialized. Barley cultivars might be resistant to a certain race of the mildew fungus, but susceptible to another race. Development of the disease is rapid in temperatures from 12 to 20°C, and in humid weather. The aim of the two-year experiment was to assess *B. graminis* f. sp. *hordei* virulence frequency and powdery mildew occurrence on five spring barley cultivars. Virulence frequency of the pathogen depended on place and term of exposition. The occurrence of powdery mildew on spring barley cultivars depended on virulence frequency of the pathogen and weather conditions.

Key words: *Blumeria graminis* f. sp. *hordei*, spring barley, virulence frequency

Introduction

After wheat, rye, and triticale, barley is one of the important cereal crops grown in Poland (about 12% of the total cereal growing area). Spring barley is more popular because it has advantages such as a short vegetation period as well as feeding and brewing values.

Aside from unfavourable meteorological or soil conditions, most yield losses are caused by fungal diseases and pests. Powdery mildew caused by *Blumeria graminis* f. sp. *hordei* is one of the most important diseases of barley in Poland. Usually spring barley is less seriously affected than winter barley. *Blumeria graminis* (de Candolla) Speer is a genetically diverse pathogen with different special forms and races. Many races can be distinguished within *B. graminis* f. sp. *hordei*. Some of them disappear as a consequence of the introduction of resistant barley cultivars and other races appear which are able to infect new barley cultivars (Czembor and Czembor 1998, 1999, 2001, 2005; Bocianowski *et al*. 2003; Gacek *et al*. 2004).

In the countries where powdery mildew is a problem, including Poland, yield losses may exceed 50%, although average losses are smaller and reach about 10–20%. Yield reduction is due to loss of functional green leaf area, reduced kernel weight, smaller numbers of kernels per ear, and tillers per plant. Reduction in quality characteristics is important for malting barley. There are several ways of controlling the disease. The primary one is the use of genetically mildew-resistant cultivars. This is a cheap and environmentally safe method. Powdery mildew can also be controlled with fungicides but these are ecologically undesirable and their frequent use may speed up the evolution towards resistance to fungicides. In agricultural practice, integrated control is often applied. This means cultivation is done of several more or less resistant cultivars, sometimes in mixtures, and supplemented by fungicides as required. The efficacy of using resistant cultivars depends on pathogen virulence (Helms Jørgensen 1994; Gacek *et al*. 2004; Czembor and Czembor 2005).

The aim of the research work was to carry out an assessment of *B. graminis* f. sp. *hordei* virulence frequency and powdery mildew occurrence on five spring barley cultivars.

Materials and Methods

In the 2011–2012 growing seasons, experiments were done on the evaluation of *B. graminis* f. sp. *hordei* virulence frequency, at one (2011) and two (2012) places: Bąków (50°57′58′′ N, 18°18′45′′ E) the site of the "Plant Breeding Smolice Ltd. – IHAR Group" (Opolskie province), and the Research Station for Variety Testing, Kościelna Wieś (51°47′08′′ N, 18°00′34′′ E) (Wielkopolskie province). In

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the experiment, 25 near-isogenic lines of the spring barley, cultivar Pallas (Table 1), were used as test plants for virulence frequency studies. One- to two- leaf seedlings of near-isogenic lines were exposed to infection for about one week in the field near plots where five spring barley cultivars (Antek, Basza, Blask, Rubinek, Skarb) were grown (Table 2). After incubation for 10 days, mildew colonies were counted on the seedlings. The number of colonies per plant was expressed as the per cent on the standard (Pallas). During every vegetation season, three expositions (terms 1–3) were done (end of April till the middle of June 2011 and 2012) – table 3. Powdery mildew incidence on five spring barley varieties was evaluated using a 1–9 scale (where 9 meant fully resistant, and 1 meant fully susceptible). These scores were transformed to percentage-of whole-plant-infection-data. Then the area under the disease progress curve (*AUDPC*) was calculated (Shaner and Finney 1977; Finckh and Wolfe 1997; Finckh *et al*. 1999; Woźniak-Strzembicka and Nadziak 2001):

$$AUDPC = \sum_{i=1}^{n} \left\{ \left(\frac{y_i + y_{i-1}}{2} \right) \left(x_i - x_{i-1} \right) \right\},$$

where:

y_i – percentage of visible infection (y_i/100) at the i-th observation

x_i – the day of the i-th observation

n – the total number of observations (modified from Shaner and Finney 1977).

Table 1. Resistance characteristics of near-isogenic Pallas lines (Brown and Helms Jørgensen 1991; Jørgensen 1994)

No.	Near-isogenic line Pallas	Resistance genes	Resistance	Corresponding virulence of *B. graminis*
1	P01	*Mla1*	Al (Algierian)	Va1
2	P02	*Mla3*	Ri (Ricardo)	Va3
3	P03	*Mla6, Mla14*	Sp (Spontaneum)	Va6+Va14
4	P04A	*Mla7, Mlk, + ?*	Ly (Lyallpur), Kw (Kwan)	Va7+Vk
5	P04B	*Mla7, + ?*	Ly (Lyallpur)	Va7
6	P06	*Mla7, Ml(LG2)*	Ly (Lyallpur), LG (Long Glumes)/Iso 26R	Va7+VLG2
7	P07	*Mla9, Mlk*	MC (Monte Christo), Kw (Kwan)	Va9+Vk
8	P08A	*Mla9, Mlk*	MC (Monte Christo), Kw (Kwan)	Va9+Vk
9	P08B	*Mla9*	MC (Monte Christo)	Va9
10	P09	*Mla10, Ml(Du2)*	Du (Durani)	Va10+VDu2
11	P10	*Mla12*	Ar (Arabische)	Va12
12	P11	*Mla13, Ml(Ru3)*	Ru (Rupee)	Va13+VRu3
13	P12	*Mla22*	HOR 1657	Va22
14	P13	*Mla23*	HOR 1402	Va23
15	P14	*Mlra*	Ra (Ragusa)	Vra
16	P15	*Ml(Ru2)*	Ru (Rupee)	Va13
17	P17	*Mlk*	Kw (Kwan)	Vk
18	P18	*Mlnn*	Nigrinudum	Vnn
19	P19	*Mlp*	Psaknon	Vp
20	P20	*Mlat*	Atlas	Vat
21	P21	*Mlg, Ml(CP)*	Goldfoil, We (Weihenstephan)	Vg+VCP
22	P22	*mlo5*	Mlo	Vo
23	P23	*Ml(La)*	La (Laevigatum)	V(La)
24	P24	*Mlh*	Hanna	Vh
25	Pallas–standard	*Mla8*	Heils Hanna	Va8

Table 2. Characteristics of spring barley cultivars (Najewski 2012)

Cultivar	Resistance against *B. graminis*		
	level[1]	source of resistance	genes
Antek	6.4	Ly+un	*Mla7 + ?*
Basza	7.8	Mlo	*mlo*
Blask	7.4	Ly+un	*Mla7 + ?*
Rubinek	8.0	Mlo	*mlo*
Skarb	7.4	MC (Monte Christo)	Mla9+Ml (IM9)

[1]data from the Research Centre for Cultivar Testing Słupia Wielka using a 1–9 scale (1-fully susceptible, 9-fully resistant)

Table 3. Dates of near-isogenic lines Pallas line expositions

Place/Year	2011	2012
Bąków	18–25.05	–
	01–08.06	18–25.05
	14–21.06	25.05–01.06
Kościelna Wieś	–	10–18.05
	–	19–25.05
	–	06–15.06

The obtained results were statistically evaluated. Firstly, the normality of the distribution of the studied traits was tested using Shapiro-Wilk's normality test (Shapiro and Wilk 1965). To asses differences between near-isogenic lines of Pallas, places, and years, the analysis of variance (ANOVA) was performed. Then, to choose near-isogenic lines with the highest and lowest powdery mildew incidence, multiple comparisons of the means for lines using the test based on least significant difference (LSD), was done. Data analysis was performed using the statistical package GenStat 15th edition.

Weather conditions were more favourable for the occurrence of powdery mildew in Bąków than in Kościelna Wieś. Precipitation and temperatures were usually higher in Bąków than in Kościelna Wieś (Table 7).

Results and Discussion

The obtained results from the experiments with the *B. graminis* f. sp. *hordei* virulence frequency showed that in the *B. graminis* population there were genotypes able to affect all tested plants representing different sources of powdery mildew resistance. In all three terms of the expositions there were significant differences among near-isogenic lines and among years (Table 4). Differences among places were statistically significant in the first and second terms (Table 4). In other expositions, interactions were observed, namely in term 1 – interaction between Pallas lines and places, in all three terms – interactions between Pallas lines and years (Table 4). Pallas lines with the highest and lowest incidence of powdery mildew are shown in table 5.

In the first term at Bąków in 2011 (Fig. 1, Table 5), the highest virulence was noticed in relation to six lines (P01, P10, P11, P14, P15, P17) with resistance genes *Mla1, Mla12, Mla13+Ml*(*Ru3*)*, Mlra, Ml*(*Ru2*)*, Mlk* and corresponding frequency Va1, Va12, Va13+VRu3, Vra, Va13, and Vk. Low powdery mildew frequency was observed in relation to eight lines (P07, P08A, P08B, P13, P19, P20, P22, P24) with resistance sources: Monte Christo+Kwan, Monte Christo, HOR 1402, Psaknon, Atlas, Mlo, and Hanna.

In term 2 at Bąków in 2011 (Fig. 2, Table 5), the highest virulence was noticed in relation to four lines (P01, P03, P04B, and P15). Low powdery mildew frequency was observed in relation to seven lines (P07, P08A, P08B, P13, P19, P22, and P24) with resistance sources: Monte Christo+Kwan, Monte Christo, HOR 1402, Psaknon, Mlo, and Hanna.

In the last exposition at Bąków in 2011 (14–21.05.) (Fig. 3, Table 5), the highest virulence was noticed in relation to one line P15 with the resistance gene *Ml(Ru2)* (Rupee) and corresponding frequency Va13. Low powdery mildew frequency was observed in relation to fourteen lines with resistance sources: Monte Christo+Kwan, Monte Christo, Durani, Arabische, Rupee, HOR 1657, HOR 1402, Kwan, Psaknon, Atlas, Mlo, Laevigatum, and Hanna.

In the first spring exposition of 2012 (Kościelna Wieś 10–18.V) (Fig. 4, Table 5), in relation to these five: P03, P04A, P04B, P15, P17, high virulence frequency (70–100% compared to the standard – Pallas) was noticed. Low powdery mildew virulence frequency was noticed in relation to five lines with resistance genes *Mla3, Mln, Mlp, Mlat,* and *mlo5*.

In the second term exposition of 2012 (Bąków 19–25.05, Kościelna Wieś 18–25.05) (Fig. 5, Table 5), low virulence in relation to 19 (Bąków) and four (Kościelna Wieś – P08B, P18, P19, P22) lines was observed. High virulence frequency was observed in relation to one line – P15 with Rupee resistance (Bąków) and P03 with Spontaneum resistance (Kościelna Wieś).

In term 3 of 2012 (Bąków 06–15.06, Kościelna Wieś 25.05–01.06) (Fig. 6, Table 5), at Bąków, in relation to lines P15, P17, P18, P21, and P24, and at Kościelna Wieś in relation to five (80–100% of Pallas) lines (P02, P12, P14, P15, P17), high virulence frequency was noticed. In the same spring exposition in 2012 (Fig. 6, Table 5), we observed the lowest virulence in relation to 14 lines at Bąków and in relation to four lines at Kościelna Wieś.

Table 4. The results of ANOVA for near-isogenic Pallas line expositions (values of mean squares)

Term of exposition	Source of variation					
	location	year	line	location × year	year × line	residual
1	2,258.7**	94,752.7***	1,812.0***	532.6***	1,386.4***	222.7
2	6,168.6***	11,528.2***	6,144.6***	296.7	1,374.4***	548.7
3	2,355.0	84,372.0***	4,555.5***	679.0	2,559.2***	959.9

**significant at 0.01 level; ***significant at 0.001 level

Table 5. Highest and lowest powdery mildew incidence on Pallas lines in terms of expositions

Line	Term 1		Term 2			Term 3		
	Bąków 2011	Kościelna Wieś 2012	Bąków 2011	Bąków 2012	Kościelna Wieś 2012	Bąków 2011	Bąków 2012	Kościelna Wieś 2012
P01	67 b	10	107 b	75	50	45	85 a	30
P02	55	5	100	20 a	30	45	33 a	80
P03	62	100	138 b	60	80	68	73 a	40
P04A	56	70	93	35 a	40	62	65 a	40
P04B	56	80	107 b	48 a	40	68	77 a	40
P06	48	40	82	45 a	30	62	77 a	50
P07	13 a	30	13 a	27 a	20	23 a	28 a	30
P08A	17 a	10	12 a	18 a	20	19 a	50 a	40
P08B	10 a	10	19 a	15 a	10	12 a	17 a	40
P09	46	30	36	32 a	40	12 a	50 a	60
P10	85 b	40	88	60	50	19 a	70 a	50
P11	86 b	20	77	97	40	23 a	103	60
P12	46	40	44	53 a	40	19 a	108	100
P13	0 a	10	7 a	32 a	20	12 a	77 a	40
P14	96 b	40	100	47 a	50	45	100	80
P15	101 b	70	120 b	128 b	60	100 b	160 b	100
P17	73 b	80	73	57 a	50	23 a	133 b	80
P18	50	5	56	37 a	10	45	127 b	40
P19	14 a	5	21 a	18 a	10	24 a	92	40
P20	31 a	5	33	15 a	20	19 a	102	30
P21	56.94	30	63	28 a	40	48	128 b	40
P22	2.78 a	5	0 a	23 a	10	12 a	33 a	30
P23	23.06 a	10	27 a	20 a	30	32 a	73 a	20
P24	60.00	40	69	55 a	50	32 a	193 b	40

Letter 'a' – lowest incidence of powdery mildew without significant differences
Letter 'b' – highest incidence of powdery mildew without significant differences

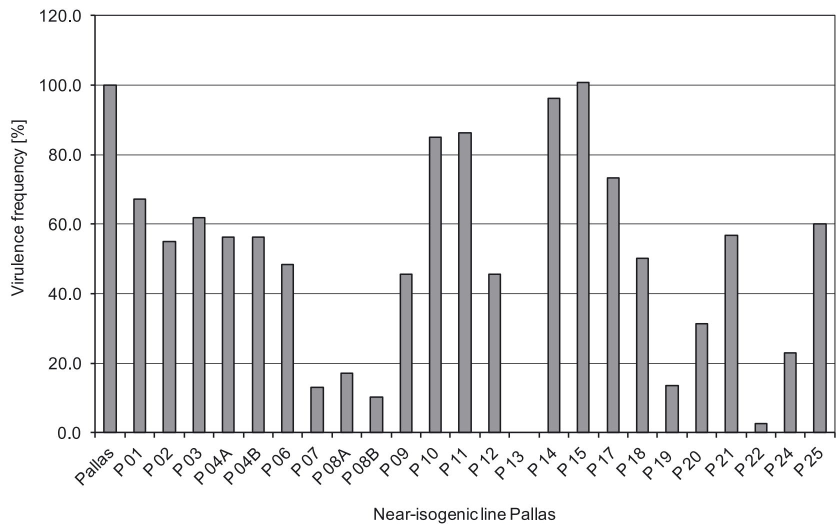

Fig. 1. Powdery mildew (*B. graminis* f. sp. *hordei*) virulence frequency at Bąków – 18–25.05.2011

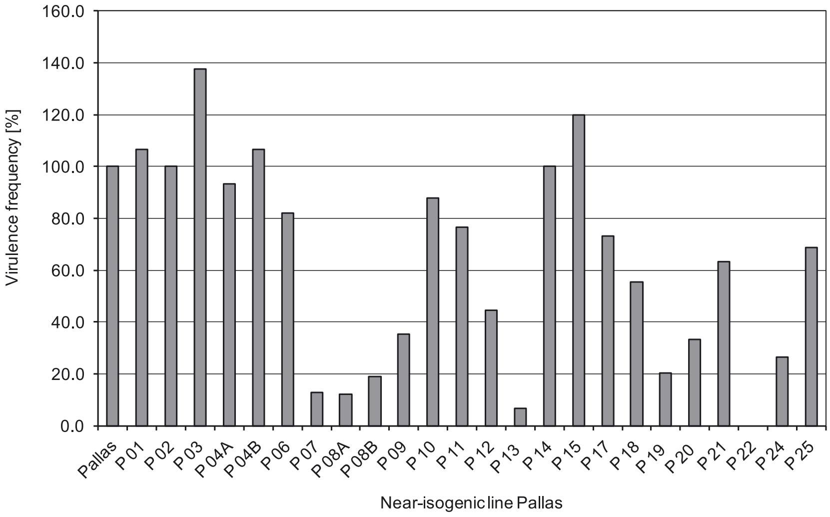

Fig. 2. Powdery mildew (*B. graminis* f. sp. *hordei*) virulence frequency at Bąków – 01–08.06.2011

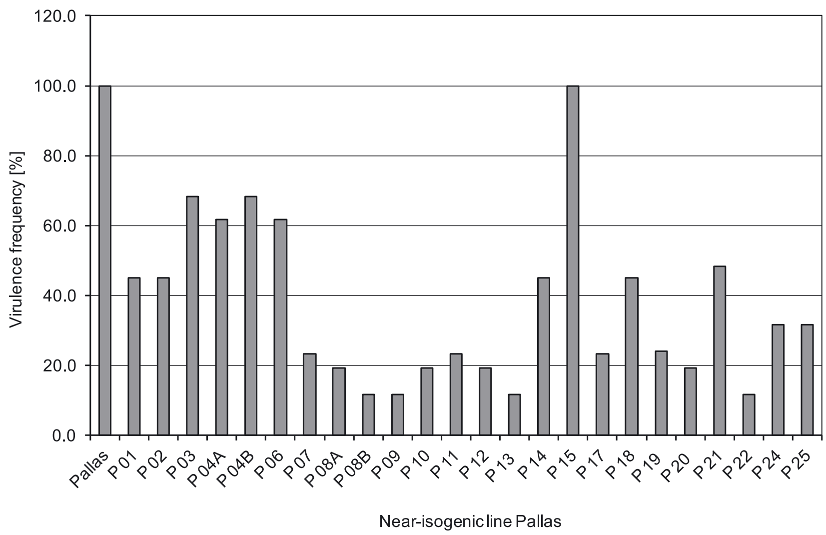

Fig. 3. Powdery mildew (*B. graminis* f. sp. *hordei*) virulence frequency at Bąków – 14–21.06.2011

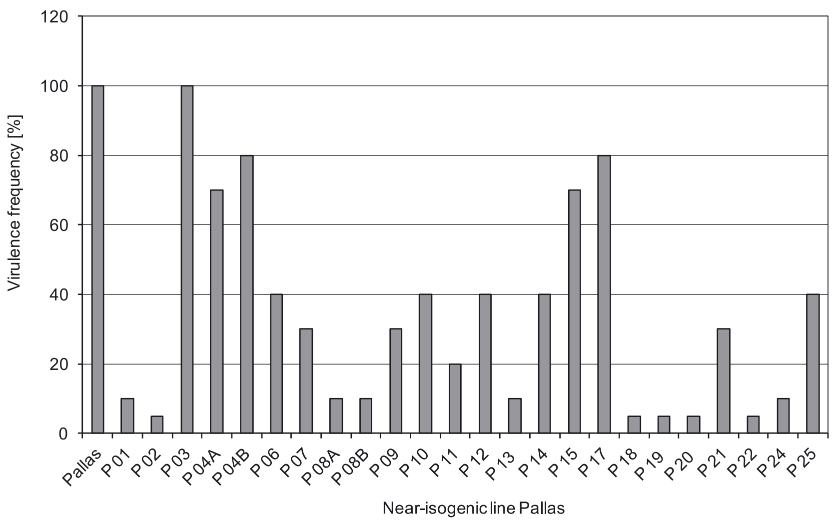

Fig. 4. Powdery mildew (*B. graminis* f. sp. *hordei*) virulence frequency at Kościelna Wieś – 10–18.05.2012

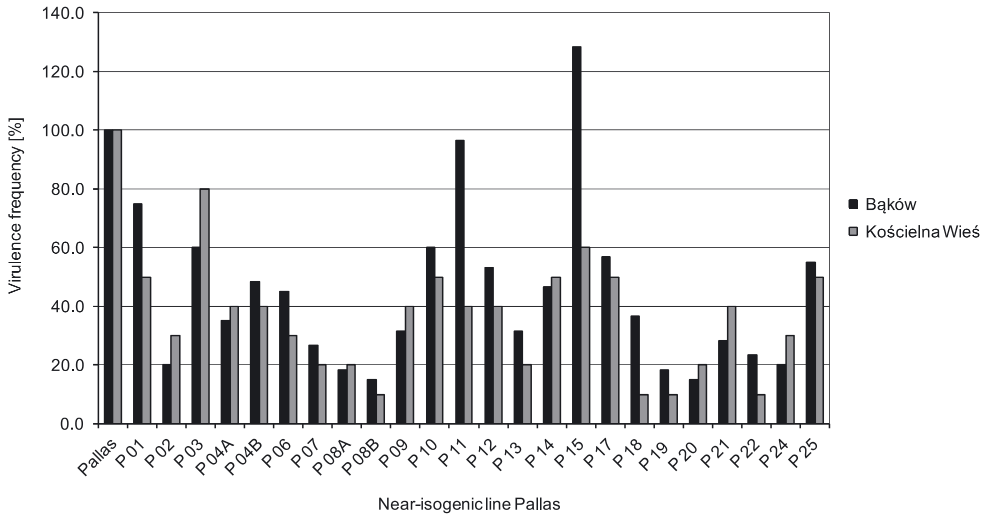

Fig. 5. Powdery mildew (*B. graminis* f. sp. *hordei*) virulence frequency at Bąków (19–25.05.2012) and Kościelna Wieś (18–25.05.2012)

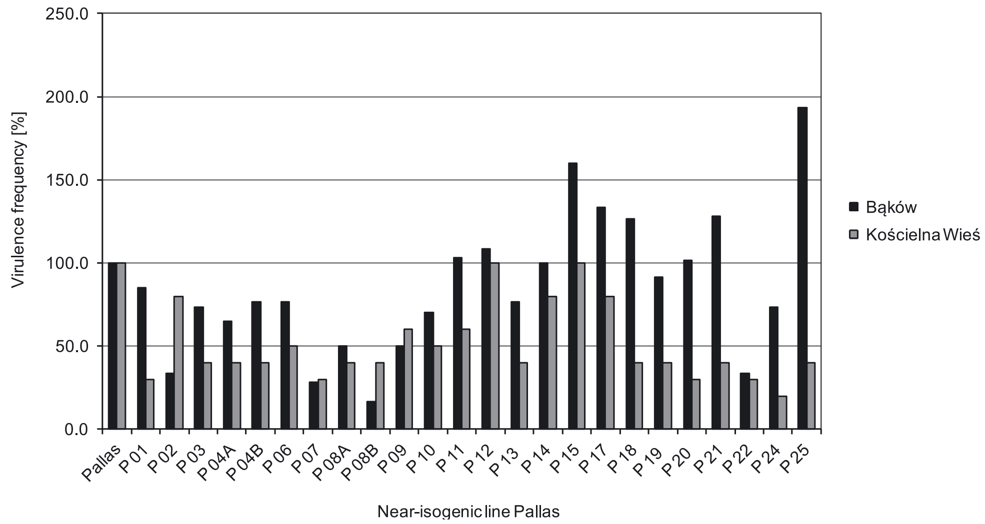

Fig. 6. Powdery mildew (*B. graminis* f. sp. *hordei*) virulence frequency at Bąków (06–15.06.2012) and Kościelna Wieś (25.05.–01.06.2012)

In conclusion, during three spring expositions at Bąków 2011 and 2012, high powdery mildew virulence frequency was noticed in relation to four lines with resistance genes *Mla3, Mla7+Mlk, Mla7*, and *Ml(Ru2)*. Low virulence frequency was noticed in relation to Monte Christo+Kwan, Monte Christo, HOR 1402, Psaknon, Mlo, and Laevigatum resistance.

In Kościelna Wieś, during three spring expositions in 2012 (Fig. 4–6, Table 5), high virulence was noticed in relation to four lines (P03, P04B, P15, P17) with Spontaneum, Lyallpur, and Kwan resistance – corresponding frequency Va6+Va14, Va7, Va13, and Vk. Low powdery mildew frequency was observed in relation to lines with resistance sources Nigrinudum, Psaknon, Mlo, and Laevigatum – corresponding virulence Vnn, Vp, Vo and V(La).

Similar results, especially with low frequency virulence in relation to Mlo resistance (corresponding virulence Vo) were obtained by other researchers (Gacek *et al.* 2004; Czembor and Czembor 2005).

In the experiment, due to diverse meteorological conditions, some differences in powdery mildew incidence on spring barley cultivars were observed (Tables 6, 7).

In the 2011 and 2012 vegetation seasons, at both Bąków and Kościelna Wieś, Antek was most severely infected by powdery mildew (Table 6). Cultivars Basza and Rubinek with Mlo resistance, were least infected by *B. graminis* f. sp. *hordei*.

According to the "gen for gen" Flor theory (Flor 1956), cultivars Antek and Skarb can be infected by powdery mildew races corresponding to Lyallpur and Monte Christo resistance. Based on the two-year experiment, it can be said that the results obtained from field observations are similar to the results obtained from near-isogenic Pallas lines expositions.

Differences between seasons, locations, and terms of observations of the powdery mildew, were presented in the form of a boxplot (Fig. 7). The largest range of observations was observed in term 3 in Bąków, 2012.

Table 6. Powdery mildew incidence according to the area under the disease progress curve (*AUDPC*) on spring barley cultivars in the 2011 and 2012 vegetation season

Year	Place	Cultivars/*AUDPC*				
		Antek	Basza	Blask	Rubinek	Skarb
2011	Bąków	514.54	38.80	190.22	50.52	224.87
	Kościelna Wieś	367.26	37.25	183.97	37.25	172.63
2012	Bąków	781.34	57.71	123.53	99.53	168.78
	Kościelna Wieś	162.44	31.91	94.53	23.76	56.49

Table 7. Weather data from the 2011 and 2012 vegetation seasons

Month (decades)	Bąków				Kościelna Wieś	
	temperature [°C] (average)		rainfall [mm] (sum)		temperature [°C] (average)	rainfall [mm] (sum)
	2011	2012	2011	2012	2012	2012
1st of March	1.7	2.3	7.5	10.2	1.3	2.0
2nd of March	6.8	7.3	8.5	6.5	7.2	0.5
3rd of March	12.3	9.5	7.3	14.4	8.3	7.5
1st of April	5.3	6.2	6.2	8.6	4.7	0.8
2nd of April	9.2	8.5	5.8	11.4	8.2	9.4
3rd of April	16.5	18.2	10.5	4.9	15.4	1.0
1st of May	15.5	16.4	15.9	12.5	15.9	9.8
2nd of May	14.2	13.8	22.5	19.2	13.1	5.3
3rd of May	16.9	18.5	10.7	9.9	17.4	10.5
1st of June	15.5	14.6	37.5	45.6	14.3	28.5

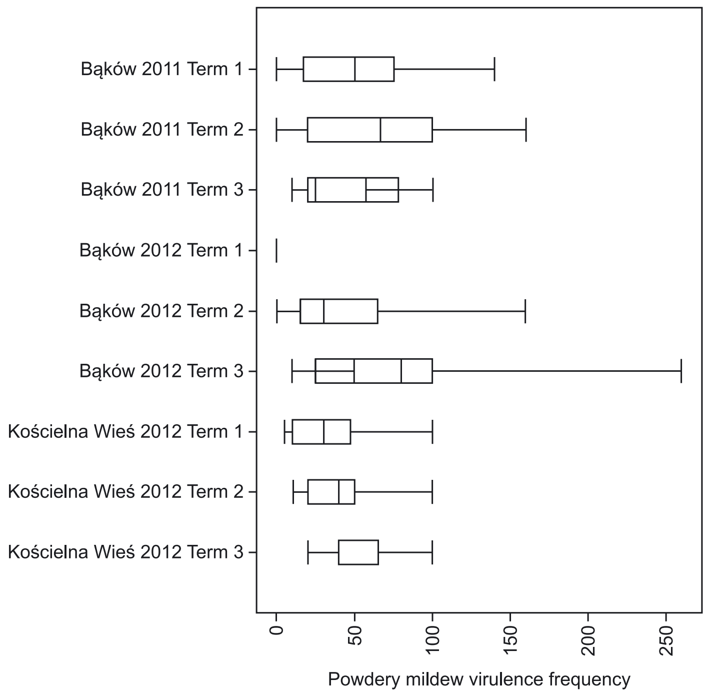

Fig. 7. Boxplot of the powdery mildew in both seasons, both locations, and for three terms

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