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A mildew infection resistance study of winter barley varieties and their mixtures by the logistic model

Ewa Bakinowska[1]*, Anna Tratwal[2], Kamila Nowosad[3], Jan Bocianowski[4]

[1] Institute of Mathematics, Poznan University of Technology, Poznan, Poland

[2] Department of Pests Methods Forecasting and Plant Protection Economy, Institute of Plant Protection – National Research Institute, Poznan, Poland

[3] Department of Genetics, Plant Breeding and Seed Production, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

[4] Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, Poznan, Poland

Abstract

Biological diversity within a mixture field allows for better use of habitat and agro-technical conditions by the mixtures, which can be seen by higher and more stable yields than varieties sown separately. Our studies were conducted in the growing seasons 2011/2012–2014/2015 as field experiments with four winter barley varieties (Bombaj, Gil, Gregor, Bażant) and three, two- and three-component mixtures (Bombaj/Gil, Bombaj/Gregor, Gil/Gregor/Bażant). Seven different chemical treatments with fungicides were applied. The aim of this study was to compare the different varieties of winter barley with their mixtures for resistance to powdery mildew infection. To achieve this aim the logistic model for the analysis of data was used. Of the varieties under consideration, the best and the most resistant variety was Gregor, while the weakest and the most susceptible to diseases (powdery mildew) was Gil. This variety was also significantly weaker than any of the other mixtures taken into account. Moreover, it was so weak that when it was included in mixtures with other varieties, it weakened these mixtures as well.

Keywords: cumulative probabilities, powdery mildew, variety mixtures

Introduction

The existing rules and guidelines for integrated plant protection emphasize the application of all possible and available methods aimed at reducing the development of harmful organisms to a level of harmlessness. Protection systems for plant cultivation should include any available methods of pest control, but at the same time, they should take into consideration natural processes of self-regulation which occur in agro-systems and try to support those processes. One method of protecting plants from agrophages may be the introduction of an alternative form of growing plants that is currently being used in production practices,

i.e. sowing mixtures. Growing mixtures (mainly cereal and cereal-leguminous crops in Poland became widespread in the 1990s). In recent years, mixtures (cereal and cereal-legumes) have constituted 17–18% of the entire cereal cultivation acreage.

The most important advantage of growing cereal mixtures is the biodiversity they introduce, which thanks to the distinctive features of newly introduced plants allow for a better use of environmental resources, without disrupting the environment's biological balance (Finckh *et al.* 1999, 2000; Newton *et al.* 2009). Biological diversity within a mixture field offers better

use of habitat and agro-technical conditions by the mixtures, which is reflected in higher and more stable yields, than varieties sown separately (Newton *et al.* 2009; Tratwal and Walczak 2010).

In cultivar testing and plant breeding experiments, certain traits, such as frost resistance, lodging, breaking of straw or susceptibility to diseases, are assessed on an ordinal scale. Consequently, a methodological problem appears – how to compare particular varieties with mixtures for their resistance to diseases, e.g. powdery mildew. To analyze this kind of data, a logistic model can be used.

A fixed logistic model was applied by Bakinowska *et al.* (2012) in the statistical analysis of downy mildew infection data in field pea varieties. A more general mixed logistic model was applied by Bakinowska *et al.* (2016) also to downy mildew data in pea. Furthermore, a simple logistic model was used by Bakinowska and Kala (2007) to compare lodging in varieties of seed pea.

The aim of this study was to compare different varieties of winter barley with their mixtures for each one's resistance to powdery mildew infection. Additional objectives were to determine which of the varieties of barley was the most resistant to powdery mildew infection, and which strength of fungicides was the most effective. To achieve this aim, collected data was analyzed using the logistic model.

Materials and Methods

Experimental design

The studies reported here took place in the growing seasons 2011/2012–2014/2015. Field experiments with four winter barley varieties (Bombaj, Gil, Gregor, Bażant) and three, two- and three-component mixtures (Bombaj/Gil, Bombaj/Gregor, Gil/Gregor/Bażant) were conducted. Winter barley cultivars sown in pure stands and mixtures combined with different treatments of fungicides were tested at two sites, namely, the Experimental Station for Variety Testing Słupia Wielka (Wielkopolska District) and the Plant Breeding Station Bąków (Opole District). The experiment was carried out on 5 m² plots in four replicates.

During the growing season 2012/2013 the studies were carried out at one site, the Experimental Station for Variety Testing Słupia Wielka. The experiment at the Plant Breeding Station Bąków was completely destroyed by late frost in the spring (March). As a result of this natural occurrence, 25% of the plots were destroyed. Seven different chemical treatments with fungicides were applied:

– untreated plots (control), dose K;
– single treatment application with ¼ (dose A), ½ (dose B) and full (dose C) dosage of fungicides (at the beginning of shooting);
– treatments with ¼ (dose D), ½ (dose E) and full (dose F) dosages of fungicides but applied twice over the growing season (at the beginning of shooting and at the full/end of shooting).

At the beginning of shooting, a mixture of two fungicides was used – Amistar 250 SC + Tilt Plus 400 EC. At the end of shooting, Tilt Plus 400 EC was applied.

During the vegetation season powdery mildew infection was observed 3–5 times and measured using an 1–9 scale (where 9 – fully resistant, 1 – fully susceptible).

Statistical model

In plant breeding trials, one of the observed characteristics is resistance to powdery mildew, which is assessed on an ordinal scale. When observations on plant varieties are carried out in different localities (sites), in different subsequent years and different doses of fertilizing are applied, it is usually assumed that such data follow the multinomial distribution. This distribution is determined by probabilities π_{ijkpr}, where π_{ijkpr} is the probability that the jth variety (or component mixtures, $j = 1, …, 7$) belongs to the ith category ($i = 1, …, 9$) with respect to the test trait (disease intensity), for kth dose ($k = 1, …, 7$), at the pth site ($p = 1, 2$) in rth year ($r = 1, 2, 3$). It is obvious that for a given variety, site, year, and dose $\sum_{i=1}^{a} \pi_{ijkpr} = 1$, where: a is the number of categories. Various methods have been proposed to model such responses (e.g. Tutz 2012). The cumulative link mixed model can be written as (Mcculloch and Searle 2001; Chen and Kuo 2001):

$$\log \frac{\gamma_{ijkpr}}{1 - \gamma_{ijkpr}} = \alpha_i + \beta_j + \gamma_k + \delta_p + \eta_r + \phi_{jp} + \rho_{jr}, \quad (1)$$

where: $\gamma_{ijkpr} = \pi_{1jkpr} + \pi_{2jkpr} + ... + \pi_{ijkpr}$ denotes the ith cumulative probability, corresponding to the jth variety for the kth dose, at the pth site, in the rth year, α_i is the cut-off point of the ith category, and β_j is the effect of the jth variety γ_k the effect of fertilization dose. The cut-off point, variety effects and dose effects are assumed to be fixed. The site effect δ_p, the year effect η_r, variety × site effect φ_{jp}, and variety × year effect ρ_{jr} are assumed to be random. Moreover, it is assumed that:

$$\delta_p \sim N(0, \sigma_s^2), \quad \eta_r \sim N(0, \sigma_y^2),$$

$$\varphi_{jp} \sim N(0, \sigma_{vs}^0), \quad \rho_{jr} \sim N(0, \sigma_{v \times y}).$$

The main purpose of the present analysis was to estimate unknown values of the cumulative probabilities detailed in model 1, using the data collected.

The maximum likelihood method approximation, under the restriction $\beta_b = 0$, was used for the estimation of the unknown parameters in model 1.

To test the hypothesis:
$$H_0 : \beta_j = 0,$$
against $\qquad\qquad\qquad\qquad\qquad\qquad (2)$
$$H_1 : \beta_j \neq 0,$$

the *t*-test statistic $t = \hat{\beta}_j / \hat{\sigma}_j$ was used, where: $\hat{\beta}_j$ is the estimated effect of the *j*th variety and $\hat{\sigma}_j$ is the estimated standard error of $\hat{\beta}_j$. Under the null hypothesis (2), the test statistic *t* has a *t*-Student distribution.

Results

The same data set was analyzed in two ways. In the first analysis, each variety was compared with its mixture. In the analysis, the doses of fertilization, the localities and the consecutive years were taken into account. To obtain the probability of resistance to infection π_{ijkpr}, the parameters in model 1 were estimated. Due to the small number of observations and the large number of parameters, the interactions were omitted for varieties Gil and Bażant. Some of the calculations were performed in SAS (Statistical Analysis System, version 9.3) using "procedure glimmix" (for the cumulative link mixed model). The results of the estimation are presented in Table 1.

The data in Table 1 show that while variety Bombaj was not significantly different from its mixtures, both mixtures are better. Variety Gil is significantly different from its mixtures and both mixtures were better than Gil. Variety Gregor was not significantly different from its mixtures and both mixtures were worse than the primary variety (Gregor). Variety Bażant was not significantly different from its mixture. The mixture was worse.

The best effect on resistance to powdery mildew infection was achieved using doses F and E. All doses were compared with dose K (control). The ranking of varieties and doses are displayed in Table 2 (from the best to the worst).

Based on the estimates included in Table 1, the probabilities of disease resistance were determined. Figures 1–8 present the estimated probabilities of powdery mildew infection for each variety separately (Bombaj, Gil, Gregor, Bażant) in comparison with their varieties for dose F (the best effect) and for dose K (control dose). The most expected is probability π_1, indicating no infection (fully resistant). The least expected is probability π_9, indicating full susceptibility to the disease.

In the second analysis, all varieties and mixtures (jointly) were compared. Similar to the one presented in the previous paragraph, the doses of fertilization, the localities and the consecutive years were taken into account. All varieties were compared with mixture Gil/Gregor/Bażant. The findings of such an analysis are presented in Table 3. Only variety Gil differed significantly from the others, that is, it was much worse. Other varieties were not significantly different from Gil/Gregor/Bażant. The ranking of varieties and doses is displayed in Table 4 (from the best to the worst).

Based on the estimates shown in Table 3, the probabilities of disease resistance were determined, taking into account all varieties and mixtures jointly. Figures 9–10 present the estimated probabilities of powdery mildew infection for all varieties and mixtures for dose F and for control dose K, as in the previously discussed analysis.

Discussion

One of the cheapest and relatively easiest ways of diversifying and, at the same time, increasing the durability of genetic resistance of modern varieties under production conditions is cultivating them with different types of variety mixtures (Matyjaszczyk 2015). More recently, complex interbred populations have been created in line with the concept of evolution in plant breeding (Philips and Wolfe 2005).

Cultivation of cereal in mixtures restores biodiversity, which, thanks to the distinct features of varieties introduced, allows for better use of natural diversity of environment and the development of sustainable agricultural practices. In the 1990s, using a mixture of different varieties became more popular in agriculture. Studies were conducted on other varieties of spring barley to determine which mixtures are disease-resistant, inter alia, to powdery mildew. Four basic conclusions can be drawn: (1) mixtures are often more resistant to diseases and pathogens that cause them than pure varieties, (2) the yield of mixtures is generally higher than the yield of pure varieties, (3) mixture resistance to disease lowers the costs of applying fungicides, (4) mixtures can be grown as well as a pure variety (Tratwal *et al*. 2007).

Powdery mildew resistance is recorded on an ordinary scale. Several methods have been proposed for analyzing ordinal data. For such sets of data, it is adequate to use the logistic model (Mccullagh 1980; Mccullagh and Nelder 1989; Miller *et al*. 1993). To illustrate this, Mila *et al*. (2004) applied a fixed logistic model to develop explanatory models of Sclerotinia stem rot prevalence in four north central states (USA). Similarly, Hampel and Hartmann (2011) studied multi-location frost resistance data obtained over

Table 1. Estimated parameters and their significance for comparison of the pure varieties with their mixtures taking into account the doses (Analysis 1)

| | Winter barley cultivars and their mixtures | | | | | | | | | | |
| | Bombaj | | | Gil | | | Gregor | | | Bażant | |
parameters	estimate[a]	t-statistic	parameters	estimate[a]	t-statistic	parameters	estimate[a]	t-statistic	parameters	estimate[a]	t-statistic
Intercept 4	-8.313	-3.71	Intercept 4	-6.262	-2.81	Intercept 4	-8.606	-4.01	Intercept 4	-7.648	-3.87
Intercept 5	-5.617	-2.77	Intercept 5	-3.987	-1.83	Intercept 5	-6.621	-3.41	Intercept 5	-6.008	-3.41
Intercept 6	-3.473	-1.73	Intercept 6	-2.317	-1.06	Intercept 6	-4.913	-2.57	Intercept 6	-3.918	-2.29
Intercept 7	-1.445	-0.72	Intercept 7	-0.289	-0.13	Intercept 7	-2.866	-1.51	Intercept 7	-2.212	-1.3
Intercept 8	1.145	0.57	Intercept 8	2.279	1.04	Intercept 8	0.113	0.06	Intercept 8	0.223	0.13
Bombaj/Gil	-0.039 (0.34)	-0.11	Bombaj/Gil	-0.966 (0.21)	-4.61*	Bombaj/Gregor	0.622 (0.48)	1.3	Gil/Gregor/Bażant	0.342 (0.21)	1.62
Bombaj/Gregor	-1.175 (0.34)	-3.45	Gil/Gregor/Bażant	-1.667 (0.22)	-7.58*	Gil/Gregor/Bażant	1.097 (0.48)	2.29	Bażant	0	–
Bombaj	0	–	Gil	0	–	Gregor	0	–	–	–	–
Dose A	0.364 (0.32)	1.16	Dose A	0.088 (0.32)	0.28	Dose A	-0.044 (0.32)	-0.14	Dose A	0.151 (0.39)	0.39
Dose B	-0.979 (0.31)	-3.11*	Dose B	-1.140 (0.31)	-3.68*	Dose B	-1.133 (0.32)	-3.52*	Dose B	-0.479 (0.38)	-1.28
Dose C	-0.077 (0.31)	-0.25	Dose C	-0.358 (0.30)	-1.18	Dose C	-0.510 (0.32)	-1.6	Dose C	-0.244 (0.38)	-0.64
Dose D	-0.534 (0.32)	-1.69	Dose D	-1.045 (0.32)	-3.3*	Dose D	-1.204 (0.33)	-3.69*	Dose D	-0.881 (0.38)	-2.33
Dose E	-1.538 (0.32)	-4.76*	Dose E	-1.963 (0.33)	-6.03*	Dose E	-2.155 (0.35)	-6.2*	Dose E	-1.670 (0.40)	-4.15*
Dose F	-1.945 (0.33)	-5.83*	Dose F	-2.652 (0.34)	-7.69*	Dose F	-2.487 (0.36)	-6.97*	Dose F	-2.003 (0.42)	-4.79*
Dose K	0	–	Dose K	0	–	Dose K	0	–	Dose K	0	–

[a] number in brackets denotes standard error of an estimate (of effects); Intercept i – cut-off point of the ith category; *$p < 0.01$

Table 2. Ranking of varieties and doses (Analysis 1)

Ranking of varieties	Winter barley cultivars and their mixtures			
	Bombaj	Gil	Gregor	Bażant
The best	1. Bombaj/Gregor	1. Gil/Gregor/Bażant*	1. Gregor	1. Bażant
	2. Bombaj/Gil	2. Bombaj/Gil*	2. Bombaj/Gregor	2. Gil/Gregor/Bażant
The worst	3. Bombaj	3. Gil	3. Gil/Gregor/Bażant	
Ranking of doses	Doses			
The best	Dose F*	Dose F*	Dose F*	Dose F*
	Dose E*	Dose E*	Dose E*	Dose E*
	Dose B*	Dose B*	Dose D*	Dose D
	Dose D	Dose D	Dose B*	Dose B
	Dose C	Dose C	Dose C	Dose C
	Dose K	Dose K	Dose A	Dose K
The worst	Dose A	Dose A	Dose K	Dose A

*$p < 0.01$

several years using a fixed logistic model, where cultivar and environmental effects were treated as fixed factors. In our experiment, it was more adequate to use the so-called mixed logistic model, in which the effects of location (site) and consecutive years were treated at random. This approach is an extension, because the conclusions to this study are important not only for the experimental sites, but can also be extrapolated to regions represented by these sites. Despite the evident advantages the logistic model offers for analyzing this
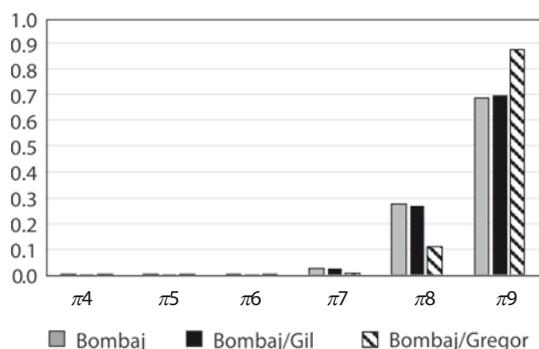

Fig. 1. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Bombaj and its mixtures, Dose F
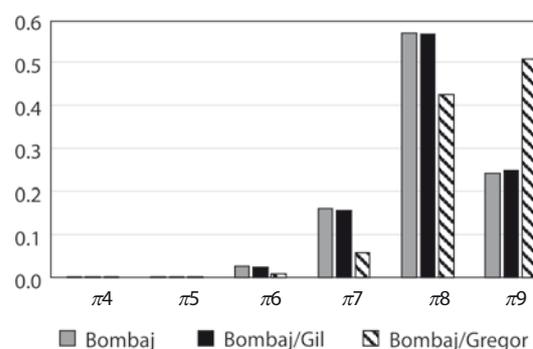

Fig. 2. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Bombaj and its mixtures, Dose K
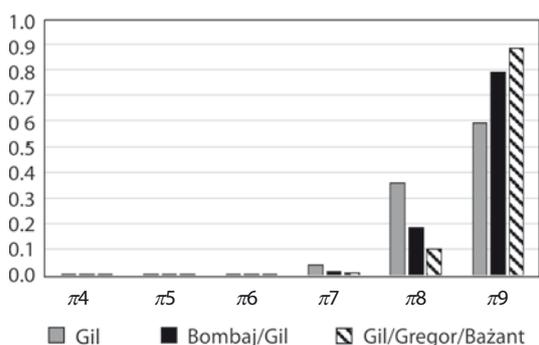

Fig. 3. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Gil and its mixtures, Dose F
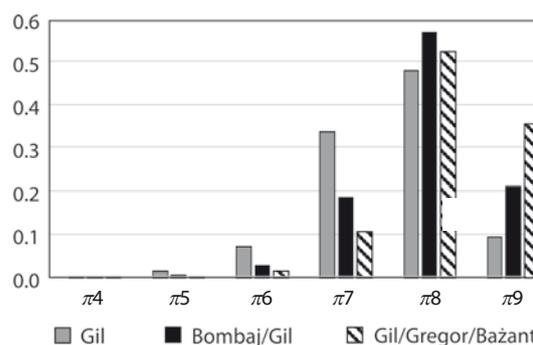

Fig. 4. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Gil and its mixtures, Dose K
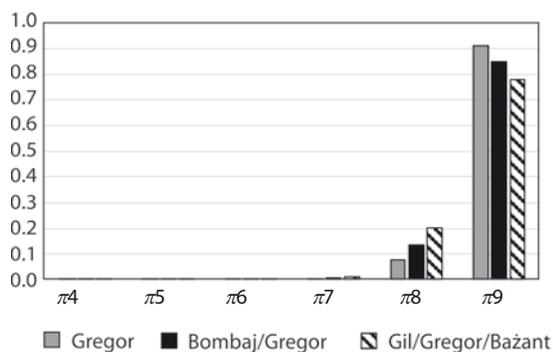
Fig. 5. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Gregor and its mixtures, Dose F
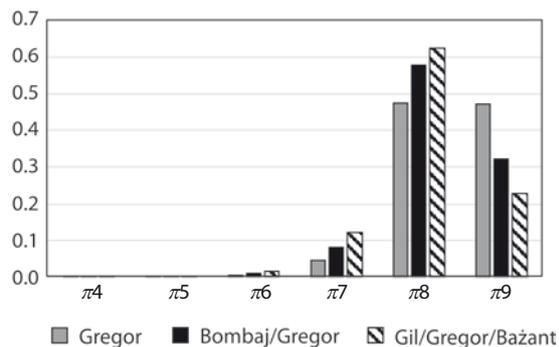

Fig. 6. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Gregor and its mixtures, Dose K
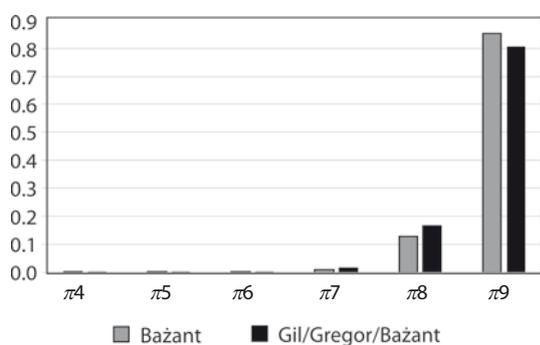

Fig. 7. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Bażant and its mixture, Dose F
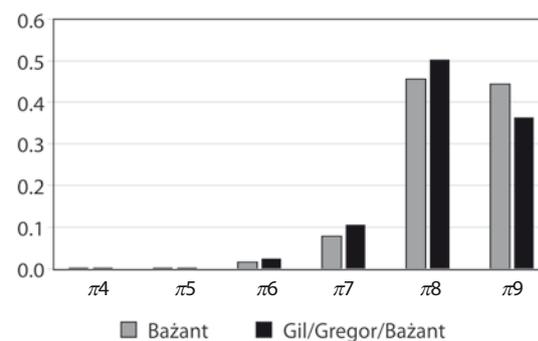

Fig. 8. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. Bażant and its mixture, Dose K
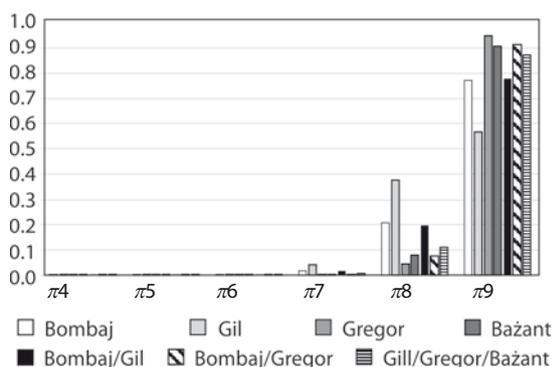

Fig. 9. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. All varieties and mixtures, Dose F
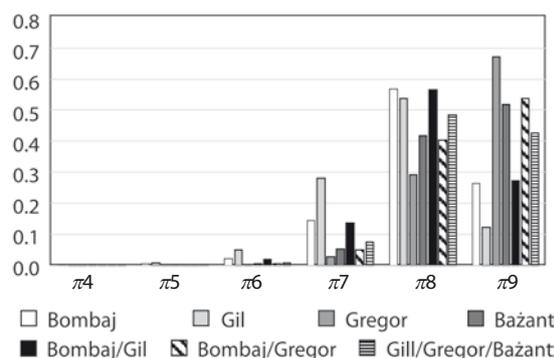

Fig. 10. The probability of resistance to powdery mildew, scores 9, 8, 7, 6 , 5, 4. All varieties and mixtures, Dose K

type of data, it is still not very popular in agricultural studies.

In addition, this work is important from a practical point of view. To the best of our knowledge, no studies have been devoted to the topic of the influence of winter barley variety mixtures combined with different fungicide treatments on disease reduction. Other

authors (e.g. Newton *et al.* 2002) in an experiment with spring barley variety mixtures combined with standard fungicide treatments showed 30–60% of powdery mildew reduction in mixtures.

Although standard mixtures of varieties of winter barley are better (with respect to yield and disease resistance), our experiment demonstrated that pure

Table 3. Estimated parameters and their significance for comparison of all varieties taking into account the doses (Analysis 2)

Parameters	Estimate[a]	t-statistic
Intercept 4	−8.338	−3.62
Intercept 5	−6.227	−2.74
Intercept 6	−4.411	−1.95
Intercept 7	−2.337	−1.03
Intercept 8	0.294	0.13
Bombaj	0.737 (0.52)	1.42
Gil	1.674 (0.52)	3.22*
Gregor	−1.020 (0.52)	−1.96
Bażant	−0.367 (0.52)	−0.7
Bombaj/Gil	0.691 (0.52)	1.33
Bombaj/Gregor	−0.443 (0.52)	−0.85
Gil/Gregor/Bażant	0	–
Dose A	0.124 (0.21)	0.6
Dose B	−1.004 (0.21)	−4.86**
Dose C	−0.341 (0.20)	−1.67
Dose D	−0.895 (0.21)	−4.32**
Dose E	−1.880 (0.22)	−8.68**
Dose F	−2.247 (0.22)	−10.01**
Dose K	0	–

[a]number in brackets denotes standard error of an estimate (of effects)
*$p < 0.05$; **$p < 0.01$

Table 4. Ranking of varieties and doses (Analysis 2)

Ranking of varieties	Variety
The best	1. Gregor
	2. Bombaj/Gregor
	3. Bażant
	4. Gil/Gregor/Bażant
	5. Bombaj/Gil
	6. Bombaj
The worst	7. Gil*
	Doses
The best	Dose F*
	Dose E*
	Dose B*
	Dose D*
	Dose C
	Dose K
The worst	Dose A

*$p < 0.01$

varieties may be more resistant than cultivars to powdery mildew. It is important to ascertain how efficient and strong the pure variety is. In the considered set of varieties, the best and the most resistant variety was Gregor, while the weakest and the most susceptible to diseases (powdery mildew) was Gil. This variety is also significantly weaker than any of the other mixtures taken into account. Moreover, it is so weak that when it was included in mixtures with other varieties, it weakened these mixtures as well (in comparison with pure varieties).

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