

## Article

# Assessment of Resistance of Barley Varieties to Diseases in Polish Organic Field Trials

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**Abstract:** Leaf rust and net blotch are two important fungal diseases of barley. Leaf rust is the most important rust disease of barley, whereas net blotch can result in significant yield losses and cause the deterioration of crop quality. The best and the most environmentally friendly method to control diseases is to cultivate resistant varieties. The aim of the current study was to identify barley varieties with an improved resistance to leaf rust and net blotch in Polish organic post-registration trials conducted in the years 2020–2022. For this purpose, the cumulative link mixed model with several variance components was applied to model resistance to leaf rust and net blotch. It was found that the reference variety Radek was the most resistant to leaf rust, whereas variety Avatar outperformed the reference variety in terms of resistance to net blotch, although the difference between the two varieties was non-significant. In the present study, the use of the cumulative link mixed model framework made it possible to calculate cumulative probabilities or the probability of a given score for each variety and disease, which might be useful for plant breeders and crop experts. Both, the method of analysis and resistant varieties may be used in the breeding process to derive new resistant varieties suitable for the organic farming system.

**Keywords:** leaf rust; net blotch; barley organic trials; ordinal data; cumulative link mixed model



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## 1. Introduction

Barley is one of the world's staple crops. Leaf rust and net blotch, caused by *Puccinia hordei* and *Pyrenophora teres f. terres* [1,2], respectively, are the most important fungal disease of barley. The net blotch infection can result in significant yield losses amounting up to 40% [3], which may be of key importance for organic farmers. Moreover, this disease may lead to the deterioration of crop quality [4].

In 2020, the EU Commission approved a set of policy initiatives, the 'European Green Deal', the aim of which is to ensure sustainable development and climate neutrality in Europe by 2050. One way to achieve this goal is to increase the organic farming area to 25% by 2030. For this reason, the cultivation of resistant varieties is the best and the most environmentally friendly method for controlling diseases in all agricultural systems, including organic systems.

In Poland, new varieties of important crop species are tested first in value-for-cultivation-and-use (VCU) trials and only the best varieties are registered. Next, the newly registered varieties are assessed in post-registration trials. Based on the results of the post-registration trials, a recommendation for farmers is issued. Since 2022, organic varieties may be registered in Poland. Since 2014, the Research Centre for Cultivar Testing (COBORU) has conducted

organic post-registration trials on potatoes, cereals, and peas, first only at the Węgrzce experimental station and, since 2019, also at nine other locations. Since 2018, organic post-registration trials on cereals, peas, and soybeans have been performed in cooperation with the Institute of Soil Science and Plant Cultivation, State Research Institute (IUNG). As in conventional barley post-registration trials, resistance to leaf rust and net blotch is one of the observed traits, measured in ordinal scale.

Several methods of analyzing ordinal responses are reported in the literature [5–8]. Logistic regression is an approach commonly used in modeling disease incidence data. Piepho [9] described an application of the generalized linear mixed model in a single experiment for binary data. In a different study, Przystalski and Lenartowicz [10] applied the generalized linear mixed model framework to model overdispersed binomial data from a series of field trials. Przystalski et al. [11] used the cumulative link mixed model with two variance components to identify varieties resistant to oat crown rust. Recently, Zawieja et al. [12] applied a similar approach to analyze ordinal data from oil seed rape plant breeding trials. The aim of the current study was to identify barley varieties with improved resistance to leaf rust and net blotch in Polish organic post-registration trials conducted in the years 2020–2022. Furthermore, we present an application of the cumulative link mixed model to model ordinal data from a series of field trials. In contrast to Radzikowski et al. [13], in the current study, the recommendation of resistant varieties was based on results from eight locations across three years, and not from a single location across five years.

## 2. Materials and Methods

### 2.1. Data

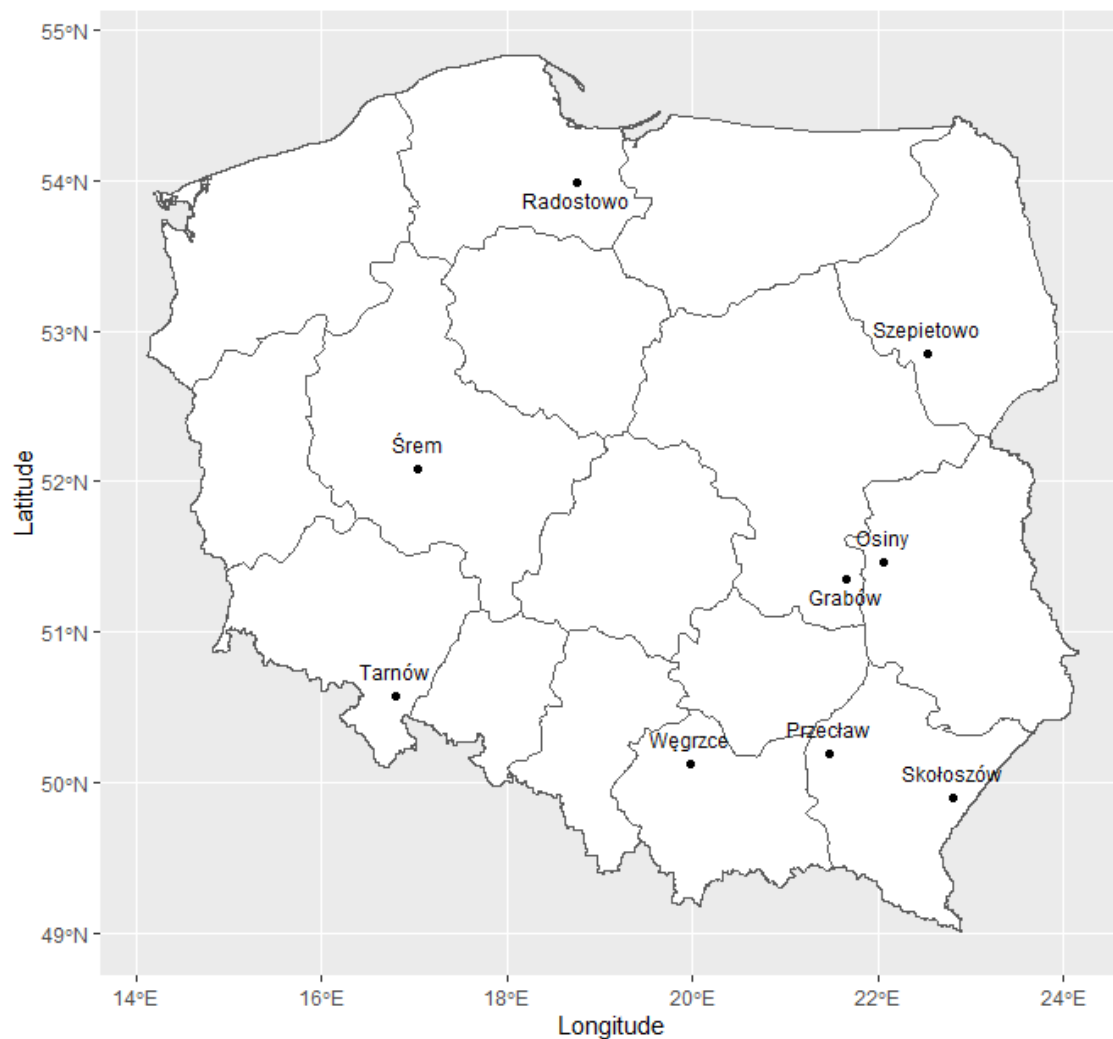
The data sets consisted of spring barley organic trials performed in the years 2020–2022. Each trial was laid out in a randomized complete block design with four replicates. The trials were performed in cooperation with IUNG at the experimental stations (sites) belonging to COBORU and IUNG (see Figure 1). The names of the sites used in the present study are given in Table 1.

**Table 1.** Sites used in 3-year organic variety trials conducted in 2020–2022.

Site	Year			Geographical Coordinates		
	2020	2021	2022	Latitude	Longitude	m a.s.l.
Grabów (Gr)	x	x	x	51°36' N	21°39' E	156
Osiny(Os)	x	x	x	51°47' N	22°05' E	143
Przeclaw (Prz)	-	-	x	50°11' N	21°44' E	230
Radostowo (Ra)	-	-	x	53°59' N	18°45' E	40
Skołoszów (Sko)	x	x	-	49°53' N	22°44' E	230
Szepietowo (Sze)	x	x	x	52°50' N	22°53' E	149
Śrem (Sr)	-	-	x	52°05' N	17°02' E	76
Tarnów (Tar)	x	x	x	50°34' N	16°47' E	300
Węgrzce(We)	x	x	x	50°07' N	19°59' E	385

Of all the experimental sites, only Węgrzce, Osiny, and Grabów have certificated organic fields. The field certification is ongoing in Przeclaw, Radostowo, Skołoszów, Śrem, and Tarnów. In Szepietowo, the field was organically managed, but not certified.

During the three years of the study, there were 12 varieties tested in total. Since we were interested in comparing the resistance of these varieties in the organic system, we focused on the varieties tested for three years. A list of varieties used in the present study with their country of origin and registration year is given in Table 2.



**Figure 1.** Map of Poland showing locations of experimental sites.

**Table 2.** Varieties used in organic trials conducted from 2020 to 2022.

i	Variety	Country	Registration Year
1	Radek	Poland	2015
2	Avatar	Poland	2019
3	Bente	Germany	2017
4	Etoile	Germany	2018
5	Farmer	Poland	2018
6	KWS Vermont	Germany	2016
7	Mecenas	Poland	2019
8	MHR Fajter	Poland	2018
9	Pilote	Switzerland	2018
10	Rubaszek	Poland	2014

Disease severity was assessed by crop experts at different stages of plant growth, according to the BBCH code [14]. In the case of leaf rust, disease severity was assessed at the BBCH68-73 growth stage (end of flowering–early milk) on the two upper leaves. For net blotch, disease severity was assessed at the BBCH52-59 growth stage (heading; 20% of the inflorescence emerged–end of heading; inflorescence fully emerged) on the upper three leaves. It should be emphasized that in the trials within the present study, disease severity was caused by natural field infection. For each disease, the disease severity was scored in the canopy, distributed in several places along the plot. The final assessment was carried

out at the maximum degree of plant infection, shortly before the physiological phase of leaf drying, but not too late, as dried plant parts would make the assessment difficult.

For both diseases, severity was scored on an ordinal scale from 1 to 9, where 9 means no infection [15]. Only one measurement per plot was made for each disease and each variety. Since we were interested in a comparison of varieties, environments (the combination of year and place) with a low intensity of a given disease were removed from further analysis.

## 2.2. Statistical Analysis

In the present study, a cumulative link mixed model was used to assess the susceptibility to leaf rust and net blotch of ten varieties in a series of organic field trials. The cumulative type model was first introduced by McCullagh [6] (see also [5–7]). For clarity, throughout the current study ‘environment’ (Env) refers to a combination of year and location.

In Polish conventional and organic post-registration trials, the disease intensity is observed in ordinal scale. Usually, it is assumed that, conditionally on the linear predictor, the observed data have a multinomial distribution, which depends on probabilities  $\pi_{ijkl}$ , where  $\pi_{ijkl}$  denotes the probability that the  $j$ -th variety ( $j = 1, \dots, J$ ) belongs to the  $i$ -th category ( $i = m, \dots, I$ , and  $m$  is the lowest category in the series of field trials) at the  $k$ -th environment ( $k = 1, \dots, K$ ) and in the  $l$ -th replicate ( $l = 1, \dots, L$ ). Let  $\gamma_{ijkl}$  denote the  $i$ -th ( $i = m, \dots, I - 1$ , and  $m$  is the lowest category in the series of field trials) cumulative probability corresponding to the  $j$ -th variety ( $j = 1, \dots, J$ ) at the  $k$ -th environment ( $k = 1, \dots, K$ ) and in the  $l$ -th replicate ( $l = 1, \dots, L$ ). Then, the cumulative link mixed model can be written as

$$\eta_{ijkl} = \text{logit}(\gamma_{ijkl}) = \log\left(\frac{\gamma_{ijkl}}{1 - \gamma_{ijkl}}\right) = \theta_i - \alpha_j - u_k - w_{jk} - t_{kl}, \quad (1)$$

where  $\theta_i$  is the fixed cutpoint of the  $i$ -th category, and  $\alpha_j$  is the fixed effect of the  $j$ -th variety. Further,  $u_k$ ,  $w_{jk}$ , and  $t_{kl}$  denote, in model (1), the random effects of environments, of variety  $\times$  environment interactions, and of replicates nested within the environments, respectively. It is assumed that  $u_k \sim N(0, \sigma_u^2)$ ,  $w_{jk} \sim N(0, \sigma_w^2)$ , and  $t_{kl} \sim N(0, \sigma_t^2)$ .

The main objective of the analysis was to estimate the unknown probabilities and cumulative probabilities in model (1) based on the experimental data. The estimates of unknown parameters in model (1) were obtained by applying the maximum likelihood method with the Laplace approximation, under restriction  $\alpha_1 = 0$  [16,17]. In Polish organic trials, all varieties are treated as standard, but in model (1), only one standard can be used. In conventional trials, the Radek variety was the most resistant to both diseases among the varieties used in the present study [18]. For this reason, this variety was treated as a reference (standard) variety.

Because, the restriction  $\alpha_1 = 0$  was used to estimate unknown parameters, the variety effect  $\alpha_j$  ( $j = 2, \dots, J$ ) can be interpreted as a comparison with the reference variety. To test the significance of each comparison, we tested the following null hypotheses:

$$H_0 : \alpha_j = 0, \quad j = 2, \dots, J. \quad (2)$$

For each comparison, statistic

$$z_j = \frac{\hat{\alpha}_j}{\hat{\sigma}_j} \quad (3)$$

was used as the test statistic [17], where  $\hat{\alpha}_j$  is the estimated effect of the  $j$ -th variety, and  $\hat{\sigma}_j$  is the estimated standard error of  $\hat{\alpha}_j$ . Under the null hypothesis, the test statistic  $z_j$  has an approximate standard normal distribution.

The calculations were performed in R [19] using the `clmm` function from the ‘ordinal’ package [17].

### 3. Results

The median (med), minimum (min), maximum (max), and the most frequently observed scores of leaf rust and net blotch in the three studied years are reported in Table 3. Graphical summaries of both data sets are given in Supplementary Figure S1.

**Table 3.** Summary statistics for leaf rust and net blotch: median (med), minimum (min), maximum (max), and the most frequently observed score (mfv).

Data Set	Year <sup>†</sup>	med	min	max	mfv
Leaf rust	20 (3)	7	5	9	7
	21 (5)	8	5	9	8
	22 (5)	6	1	9	6
Net blotch	20 (6)	7	2	9	7
	21 (6)	7	2	9	7
	22 (4)	7	1	9	7

<sup>†</sup> The numbers in brackets denote the number of sites included in the analysis.

It can be noticed that in 2020 and 2021, the observed intensities of leaf rust ranged from 5 to 9, while for net blotch, the observed intensities varied from 2 to 9. In 2022, for both diseases, the observed intensities varied from 1 to 9. Moreover, the most frequently observed scores for leaf rust were 7, 8, and 6 in 2020, 2021 and 2022, respectively. For net blotch, the most frequent score was 7 in all studied years.

Both data sets were analyzed using model (1). For net blotch, the estimate of the variance component for replicates nested within environments was equal to zero. For this reason, this term was removed from the model, and the data set was re-analyzed. The estimates of the variance components for both data sets are given in Table 4. It can be noticed that for both diseases, the variance component for the environments was higher than the variance component for the variety × environment interaction (Table 4).

**Table 4.** Estimated variance components in the cumulative link mixed model for both data sets.

Data Set	Variance Component		
	Env	Variety × Env	Env × Rep
Leaf rust	22.20	4.01	0.26
Net blotch	5.85	1.52	–

For both data sets the analyses provided estimates of unknown parameters (Table 5). The second and fourth columns of Table 5 provide estimates of cutpoints and variety effects with standard errors (in brackets) for leaf rust and net blotch, respectively. The third and fifth columns of Table 5 provide values of the z test statistic for leaf rust and net blotch, respectively.

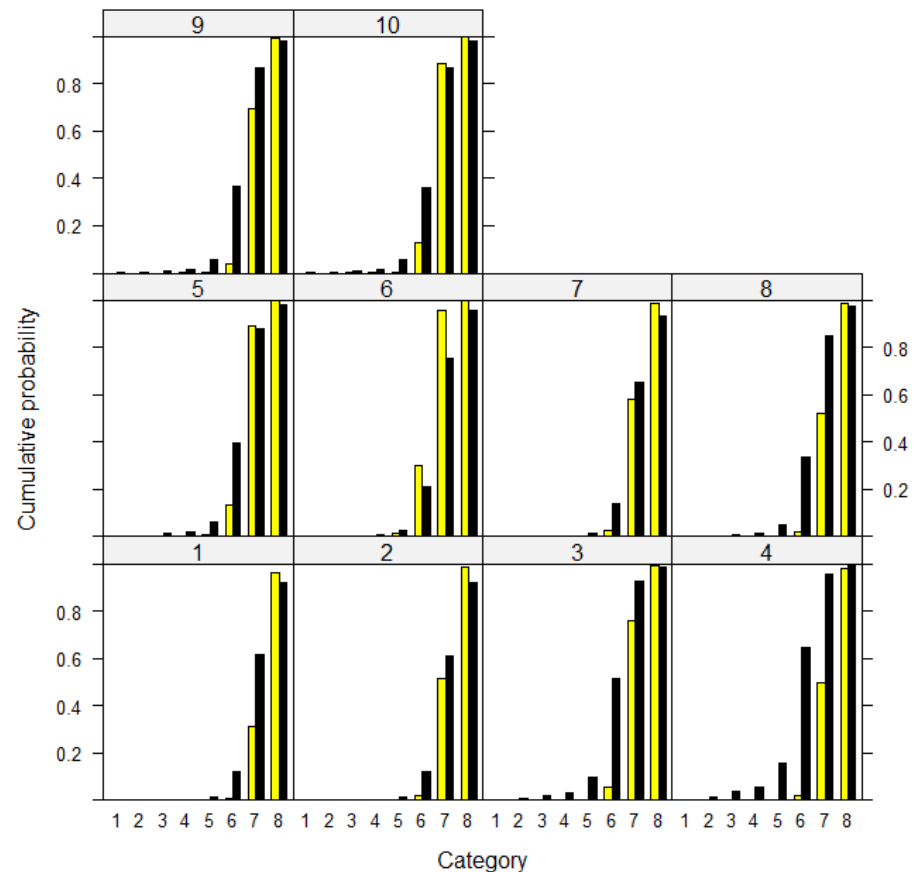
It can be noticed that all the varieties were less resistant to leaf rust than the standard variety Radek (1). However, only the differences between varieties Bente (3), Farmer (5), KWS Vermont (6), Rubaszek (10), and the standard variety Radek were significant. A different pattern can be observed for net blotch. In this case, variety Avatar (2) turned out to be slightly more resistant than the reference variety Radek, but the difference was non-significant. Furthermore, varieties KWS Vermont (6) and Mecenas were slightly less resistant than the reference variety, but the differences were non-significant. The other varieties were significantly worse than the standard variety in terms of resistance to net blotch.

Next, for both data sets, the values of cumulative probabilities (given that there are no random effects as presented in Table 5) were calculated and are plotted in Figure 2. The cumulative probabilities for leaf rust and net blotch are given in yellow and black, respectively (Figure 2).

**Table 5.** Estimates of cutpoints, variety effects, and values of the test statistic *z* for leaf rust and net blotch.

Parameter	Leaf Rust		Net Blotch		
	Estimate <sup>a</sup>	Test Statistic <i>z</i> <sup>b</sup>	Estimate <sup>a</sup>	Test Statistic <i>z</i> <sup>b</sup>	
Cutpoint1	$\theta_1$	−15.63 (1.82)	−8.57	−9.30 (0.91)	−10.21
Cutpoint2	$\theta_2$	−13.26 (1.68)	−.87	6.94 (0.80)	−8.72
Cutpoint3	$\theta_3$	−10.97 (1.61)	−6.81	5.81 (0.76)	−7.61
Cutpoint4	$\theta_4$	−9.24 (1.57)	−5.90	−5.42 (0.75)	−7.18
Cutpoint5	$\theta_5$	−8.00 (1.54)	−5.19	−4.22 (0.73)	−5.74
Cutpoint6	$\theta_6$	−4.74 (1.48)	−3.20	−1.95 (0.72)	−2.72
Cutpoint7	$\theta_7$	−0.79 (1.45)	−0.54	0.48 (0.71)	0.68
Cutpoint8	$\theta_8$	3.23 (1.46)	2.22	2.47 (0.72)	3.44
Radek	$\alpha_1$	0	0	0	0
Avatar	$\alpha_2$	−0.86 (0.92)	−0.93 <sup>ns</sup>	0.04 (0.55)	0.07 <sup>ns</sup>
Bente	$\alpha_3$	−1.95 (0.93)	−2.11 *	−2.02 (0.55)	−3.68 ***
Etoile	$\alpha_4$	−0.79 (0.92)	−0.85 <sup>ns</sup>	−2.55 (0.55)	−4.59 ***
Faremer	$\alpha_5$	−2.88 (0.93)	−3.08 **	−1.52 (0.55)	−2.78 **
KWS Vermont	$\alpha_6$	−3.91 (0.94)	−4.17 ***	−0.64 (0.54)	−1.18 <sup>ns</sup>
Mecenas	$\alpha_7$	−1.11 (0.92)	−1.22 <sup>ns</sup>	−0.14 (0.55)	−0.26 <sup>ns</sup>
MHR Fajter	$\alpha_8$	−0.88 (0.92)	−0.96 <sup>ns</sup>	−1.27 (0.55)	−2.31 *
Pilote	$\alpha_9$	−1.61 (0.92)	−1.76 <sup>ns</sup>	−1.41 (0.55)	−2.57 *
Rubaszek	$\alpha_{10}$	−2.83 (0.92)	−3.06 **	−1.37 (0.54)	−2.52 *

<sup>a</sup> The numbers in brackets denote the standard error of an estimate. <sup>b</sup> \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; <sup>ns</sup> non-significant.

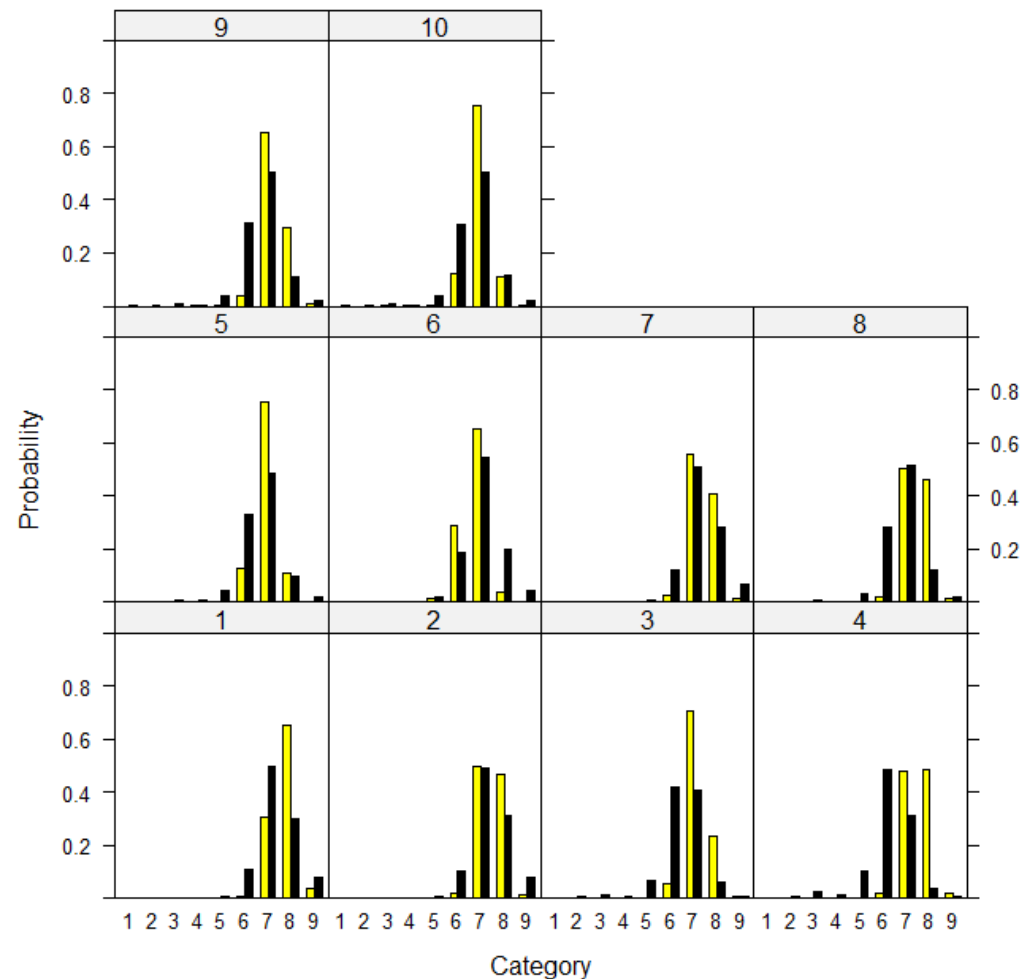


**Figure 2.** Cumulative probabilities for leaf rust (yellow) and net blotch (black). Bars over the numbers denote probability of obtaining score not larger than given number. Notation: 1—Radek, 2—Avatar, 3—Bente, 4—Etoile, 5—Farmer, 6—KWS Vermont, 7—Mecenas, 8—MHR Fajter, 9—Pilote, 10—Rubaszek.

For example, for variety Avatar (2) and leaf rust (yellow bars), a value of 0.52, calculated from the values given in Table 5, means that this variety will receive a score no larger than 7 with the probability of 0.52 (Figure 2). Furthermore, in the case of leaf rust, the probabilities of receiving a score of 8 or higher by varieties Radek (1) and Etoile (4) were

0.68 and 0.50, respectively. For other varieties, the probability was less than 0.5. On the other hand, for net blotch, the probability of receiving a score of 8 or higher by the most resistant varieties (Radek and Avatar) was approximately equal to 0.39.

Finally, for both data sets, in using the cumulative probabilities (Figure 2), the probabilities of obtaining a given score were calculated (Figure 3). The probabilities for leaf rust and net blotch are given in yellow and black, respectively (Figure 3).



**Figure 3.** Probabilities for leaf rust (yellow) and net blotch (black). Bars over the numbers denote probability of obtaining score equal to given number. Notation: 1—Radek, 2—Avatar, 3—Bente, 4—Etoile, 5—Farmer, 6—KWS Vermont, 7—Mecenas, 8—MHR Fajter, 9—Pilote, 10—Rubaszek.

In the case of leaf rust (yellow bars), one can observe that for the reference variety Radek (1), the most probable score was 8 (Figure 3). A different pattern was observed for varieties Avatar (2) and Etoile (4). For these two varieties, scores 7 and 8 were equally probable. For the other varieties, the most probable score was 7. On the other hand, for net blotch (black bars), the most probable score for a majority of the tested varieties was 7. For the most susceptible variety, Etoile (4), the most probable score was 6. A different pattern was observed for variety Bente (3). For this variety, scores of 6 and 7 were equally probable (Figure 3). Furthermore, for the most resistant varieties in terms of net blotch (Radek and Avatar), the probability of obtaining a score of 8 was equal to 0.3 (Figure 3).

#### 4. Discussion

The use of resistant barley varieties is the best and the most environmentally friendly method for controlling diseases in all agricultural systems, including organic farming. In the literature, disease severity is measured as the percentage of infected plants [10]

as a proportion of plot area infected by the disease or on a disease rating scale (see, e.g., [20–24]). The rating scales are typically from 0 or 1 to 9, where 0 and 1 mean no infection. However, depending on the study, different scales may also be used (see, e.g., [22,23]). In Bundessortenamt (the German agency in charge of variety testing), disease resistance is assessed on an ordinal scale from 1 to 9, where 1 is the desired situation. In the present study, disease severity was measured on an ordinal scale from 1 to 9, where 9 means no infection. It is known that diseases observed on the ordinal scale follow a multinomial distribution and should be modeled using either generalized linear models or generalized linear mixed models [24]. However, in practice, the ordinal scores on a 1-to-9 scale are treated as if they were normally distributed and analyzed using linear mixed models (see, e.g., [20,21]). The main issue with this approach is that the normality assumption of the errors is often violated, because this is only an approximation. For this reason, in the present study, the cumulative link mixed model approach was used to model ordinal data and to identify varieties resistant to leaf rust and net blotch. Przystalski et al. [11] and Zawieja et al. [12] applied a similar approach to find varieties resistant to oat crown rust and sclerotinia, respectively. The main advantage of the approach described in the current study, or cumulative-type models in general, is that the obtained results have a simple interpretation in terms of probability, which can be easily obtained, in contrast to the common approach (see, e.g., [8,16]). Furthermore, the model described in the current study can also be used to analyze the reversed ordinal scale. The interpretation of the results from such an analysis can be found in [22,23]. On the other hand, in many studies, the ordinal scores are analyzed using linear or generalized linear models with sites or environments treated as fixed effects (see, e.g., [22,23] and the references therein). This approach is often questioned because the results cannot be extended to the region represented by those sites. The results of such analyses are only valid for these experimental sites or environments. To solve this problem, Yates and Cochran [25] proposed to treat environments or sites as random. In a different study, Bakinowska et al. [23] compared a fixed logistic model and a cumulative link mixed model with several variance components. They showed that in all cases, the cumulative linear mixed model outperformed the fixed model in terms of goodness-of-fit criteria. Furthermore, they showed that the variety recommendations from the two models differ. For this reason, it is recommended to use cumulative link mixed models to model ordinal data from a series of field trials.

Our findings reveal that variety Radek was the most resistant to leaf rust, whereas variety Avatar was the most resistant to net blotch. In turn, Bente, Farmer, and Rubaszek were the most susceptible to both diseases. In comparison to the reference variety, varieties Avatar and Mecenas were inferior in terms of leaf rust resistance, whereas in the case of net blotch, only variety Avatar proved to be more resistant than variety Radek. However, the differences were non-significant. A different behavior was observed for variety KWS Vermont. The difference between this variety and the reference variety was significant in the case of leaf rust, while for net blotch, it was non-significant. The opposite was observed for varieties Etoile, MHR Fajter, and Pilote. In view of the barley genetic studies, the varieties susceptible to leaf rust either possess a single *Rph* gene (*Rph 1-22*; see, e.g., [26,27]); and the references therein) or do not possess any *Rph* gene. Park [28] pointed out that resistance provided by single *Rph* genes has often failed to provide long-term control of the disease because new virulent pathotypes of the pathogen can arise via mutation, introduction, selection, and/or recombination. In a different study, Yeo et al. [29] showed that the partial resistance of barley to leaf rust is polygenically inherited and is supposed to act on a minor-gene-for-minor-gene model [30–35]. This may suggest that the resistant varieties possess one of at least 20 partial resistance QTLs against barley leaf rust. Furthermore, the newly released resistant varieties (see Table 2) may possess a recently found *Rph28* gene at chromosome 5H [36]. In the case of net blotch, the resistant varieties may carry the resistance genes located on chromosome 3H [1] or QTLs associated with barley net blotch resistance [37–39]. To confirm all these hypotheses, further molecular studies on varieties used in the barley organic series of trials are needed. For this purpose, one could

apply the KASP and allele-specific PCR markers associated with net blotch resistance on chromosome 3H [1] or single-nucleotide polymorphism (SNP) markers described by Rozanova et al. [40] or Wonneberger et al. [41]. To date, no modern variety developed under organic farming conditions has been registered on the Polish National List. Thus, after successful verification, the resistant varieties may be used to breed new varieties with improved resistance to both diseases, which is suitable for the organic farming system.

Finally, it should be emphasized that the use of resistant varieties is the most environmentally friendly method of controlling cereal diseases, including barley. However, as in conventional agriculture, increasing the organic cereal cultivation area leads to a greater risk of disease intensification as a result of monoculture. For this reason, many scientists have proposed to use interspecies mixtures or variety mixtures instead of pure stand varieties [42–45]. Finckh et al. [42] argued that using variety mixtures increases the genetic distance between plants with the same genetic resistance to diseases. Furthermore, they concluded that variety mixtures limit the spread of pathogens by resistant plants that create a natural barrier. In a different study, Tratwal and Bocianowski [45] reported that spring barley variety mixtures were more resistant to powdery mildew than pure stand varieties. A similar effect was observed in Polish organic post-registration trials in terms of resistance to net blotch. In the years 2018–2019, the variety mixture Radek+Rubaszek+Soldo was slightly more resistant than the reference variety Radek, although the difference was non-significant. A different way of controlling plant diseases in all agricultural systems, including organic system, was proposed by Moya et al. [46]. In that study, the authors proposed the use of secondary metabolites of *Trichoderma*, such as volatile organic compounds (VOCs), which reduce plant diseases, including net blotch. In the same study, they concluded that VOCs emitted by *T. harzianum* and *T. longibrachiatum* have an antagonistic effect toward the pathogen *P. teres* and can be used to control the net blotch disease of barley. However, further research is needed to evaluate the effects of barley variety mixtures and/or the use of *Trichoderma* VOCs on the control of net blotch and other important barley diseases.

## 5. Conclusions

The findings of the present study revealed that reference variety Radek was the most resistant to leaf rust, whereas variety Avatar outperformed the reference variety in terms of resistance to net blotch. In the case of leaf rust, the probability of receiving a score of 8 or higher by variety Radek was equal to 0.68. For net blotch, the probability of the same event was equal to 0.39. Further, variety Mecenias was slightly worse than the reference variety in terms of resistance to both diseases, but the differences were non-significant. Finally, varieties Bente, Farmer, and Rubaszek were significantly inferior to reference variety Radek in terms of resistance to both diseases. Thus, it may be concluded that varieties Avatar, Mecenias, and Radek should be recommended for organic farming. Finally, the resistant varieties may be used to breed new varieties with improved resistance to both diseases, which is suitable for the organic farming system.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture14050789/s1>, Figure S1: Graphical summaries of the two data sets: histograms. Figure S2: Graphical summaries of the two data sets: boxplots for environments. Figure S3: Graphical summaries of the two data sets: boxplots for varieties.

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## References

- Afanasenkov, O.; Rozanova, I.; Gofman, A.; Lashina, N.; Novakazi, F.; Mironenko, N.; Baranova, O.; Zubkovich, A. Validation of molecular markers of barley net blotch resistance loci on chromosome 3H for marker-assisted selection. *Agriculture* **2022**, *12*, 439.
- Backes, A.; Guerriero, G.; Barka, E.A.; Jacquard, C. *Pyrenophora teres*: Taxonomy, morphology, interaction with barley, and mode of control. *Front. Plant Sci.* **2021**, *12*, 614951.
- Steffenson, B.; Hayes, P.; Kleinhofs, A. Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. *Theor. Appl. Genet.* **1996**, *92*, 552–558.
- Burleigh, J.R.; Tajani, M.; Seck, M. Effects of *Pyrenophora teres* and weeds on barley yield and yield components. *Ecol. Epidemiol.* **1988**, *78*, 295–299.
- Agresti, A. *Analysis of Ordinal Categorical Data*; Wiley & Sons: New York, NY, USA, 1984.
- McCullagh, P. Regression model for ordinal data (with discussion). *J. Roy. Statist. Soc. Ser. B* **1980**, *42*, 109–127.
- Simko, I.; Piepho, H.P. Combining phenotypic data from ordinal rating scales. *Trends Plant Sci.* **2011**, *16*, 235–237.
- Tutz, G. *Regression for Categorical Data*; Cambridge University Press: Cambridge, UK, 2012.
- Piepho H.P. Analysing disease incidence data from designed experiments by generalized linear mixed models. *Plant Pathol.* **1999**, *48*, 668–674.
- Przystalski, M.; Lenartowicz, T. Comparing the resistance of mid-maturing maize varieties to European corn borer (*Ostrinia nubilalis* Hbn.)—Results from the Polish VCU registration trials. *Plant Breed.* **2017**, *136*, 498–508.
- Przystalski, M.; Tokarski, P.; Pilarczyk, W. A method for identifying oat varieties with improved resistance to oat crown rust from a series field trials. *Field Crops Res.* **2013**, *149*, 49–55.
- Zawieja, B.; Slebiada, L.; Mikulski, T. Progress in plant tolerance to the fungal disease Sclerotinia in breeding experiments on winter oilseed rape. *Biom. Lett.* **2023**, *60*, 23–35.
- Radzikowski, P.; Jończyk, K.; Feledyn-Szewczyk, B.; Jóźwicki, T. Assessment of resistance of different varieties of winter wheat to leaf fungal diseases in organic farming. *Agriculture* **2023**, *13*, 875.
- Hack, H.; Bleiholder, H.; Buhr, L.; Meier, U.; Schnock-Fricke, U.; Witzemberger, W.E. A uniform code for phenological growth stages of mono- and dicotyledonous plants—Extended BBCH scale, general. *Nachr. Deut. Pflanzenschutz.* **1992**, *44*, 265–270.
- Drażkiewicz, K.; Skrzypek, A.; Szarzyńska, J. *Cereals. Methodology for Value-for-Cultivation-and-Use (VCU) Testing in Ecological Conditions*; WGO-R/S/2/2020: Słupia Wielka, Poland, 2020. (In Polish)
- Tutz, G.; Hennevogel, W. Random effects in ordinal regression models. *Computat. Stat Data Anal.* **1996**, *22*, 537–557.
- Christensen, R.H.B. Ordinal—Regression Models for Ordinal Data. R Package Version 2022.11-16. Available online: <https://CRAN.R-project.org/package=ordinal> (accessed on 28 October 2023).
- Najewski, A.; Madajska, K.; Skrzypek, A.; Szarzyńska, J. *Descriptive List of Agricultural Plant Varieties: Cereals*; COBORU Publishing Office: Słupia Wielka, Poland, 2023. (In Polish)
- R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2023. Available online: <https://www.R-project.org/> (accessed 24 October 2023)
- Mikó, P.; Löschenberger, F.; Hiltbrunner, J.; Aebi, R.; Megyeri, M.; Kovács, G.; Molnár-Láng, M.; Vida, G.; Rakszegi, M. Comparison of bread wheat varieties with different breeding origin under organic and low input management. *Euphytica* **2014**, *199*, 68–80.
- Laidig, F.; Feike, T.; Klocke, B.; Macholdt, J.; Miedaner, T.; Rentel, D.; Piepho, H.P. Long-term breeding progress of yield, yield-related, and disease resistance traits in five cereal crops of German variety trials. *Theor. Appl. Genet.* **2021**, *134*, 3805–3827.
- Bakinowska, E.; Pilarczyk, W.; Osiecka, A.; Wiatr, K. Analysis of the downy mildew infection of field pea varieties using logistic model. *J. Plant Prot. Res.* **2012**, *52*, 264–270.
- Bakinowska, E.; Pilarczyk, W.; Zawieja, B. Analysis of downy mildew data on field pea: An empirical comparison of two logistic models. *Acta Agric. Scand. B Soil Plant Sci.* **2016**, *66*, 107–116.
- Stroup, W.W. Rethinking the analysis of non-normal data in plant and soil science. *Agron. J.* **2015**, *107*, 811–827.
- Yates, F.; Cochran, W.G. The analysis of groups of experiments. *J. Agric. Sci.* **1938**, *28*, 556–580.
- Derevnina, L.; Singh, D.; Park R.F. The genetic relationship between barley leaf rust resistance genes located on chromosome 2HS. *Euphytica* **2015**, *203*, 211–220.
- Hickey, L.T.; Lawson, W.; Platz, G.J.; Dieters, M.; Arief, V.N.; Germán, S.; Fletcher, S.; Park, R.F.; Singh, D.; Pereyra, S.; Franckowiak, J. Mapping *Rph20*: A gene conferring adult plant resistance to *Puccinia hordei* in barley. *Theor. Appl. Genet.* **2011**, *123*, 55–68.

28. Park, R.F. Pathogenic specialization and pathotype distribution of *Puccinia hordei* in Australia, 1992 to 2001. *Plant Dis.* **2003**, *87*, 1311–1316.
29. Yeo, F.K.S.; Bouchon, R.; Kuijken, R.; Loriaux, A.; Boyd, C.; Niks, R.E.; Marcel, T.C. High-resolution mapping of genes involved in plant stage-specific partial resistance of barley to leaf rust. *Mol. Breed.* **2017**, *37*, 45.
30. González, A.M.; Marcel, T.C.; Niks, R.E. Evidence for a minor-gene-for-minor-gene interaction explaining nonhypersensitive polygenic partial disease resistance. *Phytopathology* **2012**, *102*, 1086–1093.
31. Marcel, T.C.; Gorguet, B.; Ta, M.T.; Kohutova, Z.; Vels, A.; Niks, R.E. Isolate specificity of quantitative trait loci for partial resistance of barley to *Puccinia hordei* confirmed in mapping populations and near-isogenic lines. *New Phytol.* **2008**, *177*, 743–755.
32. Parlevliet, J.E.; Zadoks, J.C. The integrated concept of disease resistance: A new view including horizontal and vertical resistance in plants. *Euphytica* **1977**, *26*, 5–21.
33. Niks, R.E.; Fernandez, E.; van Haperen, B.; Bekele Aleye, B.; Martinez, F. Specificity of QTLs for partial and non-host resistance of barley to leaf rust fungi. *Acta Phytopathol. Entomol. Hung.* **2000**, *35*, 13–21.
34. Niks, R.E.; Qi, X.; Marcel, T.C. Quantitative resistance to biotrophic filamentous plant pathogens: Concepts, misconceptions and mechanisms. *Ann. Rev. Phytopathol.* **2015**, *53*, 445–470.
35. Qi, X.; Jiang, G.; Chen, W.; Niks, R.E.; Stam, P.; Lindhout, P. Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. *Theor. Appl. Genet.* **1999**, *99*, 877–884.
36. Mehnaz, M.; Dracatos, P.; Pham, A.; March, T.; Maurer, A.; Pillen, K.; Forrest, K.; Kulkarni, T.; Pourkheirandish, M.; Park, R.F.; et al. Discovery and fine mapping of *Rph28*: A new gene conferring resistance to *Puccinia hordei* from wild barley. *Theor. Appl. Genet.* **2021**, *134*, 2167–2179.
37. König, J.; Perovic, D.; Kopanhnke, D.; Ordon, F. Mapping seedling resistance to net form of net blotch (*Pyrenophora teres f. teres*) in barley using detached leaf assay. *Plant Breed.* **2014**, *133*, 356–365.
38. Grewal, T.S.; Rossnagel, B.G.; Pozniak, C.J.; Scoles, G.J. Mapping quantitative trait loci associated with barley net blotch resistance. *Theor. Appl. Genet.* **2008**, *116*, 529–539.
39. Grewal, T.S.; Rossnagel, B.G.; Scoles, G.J. Validation of molecular markers associated with net blotch resistance and their utilization in barley breeding. *Crop Sci.* **2010**, *50*, 177–184.
40. Rozanova, I.V.; Lashina, N.M.; Mustafin, Z.S.; Gorobets, S.A.; Efimov, V.M.; Afanasenko, O.S.; Khlestkina, E.K. SNPs associated with barley resistance to isolates of *Pyrenophora teres f. teres*. *Bmc Genom.* **2019**, *20* (Suppl 3), 292.
41. Wonneberger, R.; Ficke, A.; Lillemo, M. Identification of quantitative trait loci associated with resistance to net form net blotch in a collection of Nordic barley germplasm. *Theor. Appl. Genet.* **2017**, *130*, 2025–2043.
42. Finckh, M.R.; Gacek, E.S.; Goyeau, H.; Lannou, C.; Merz, U.; Mundt, C.C.; Munk, L.; Nadziak, J.; Newton, A.C.; de Vallavieille-Poppe, C.; et al. Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie* **2000**, *20*, 813–837.
43. Newton, A.C.; Begg, G.S.; Swanston, J.S. Deployment of diversity for enhanced crop function. *Ann. Appl. Biol.* **2009**, *154*, 309–322.
44. Kiær, L.P.; Skovgaard, I.M.; Østergård, H. Effects of inter-varietal diversity, biotic stresses and environmental productivity on grain yield of spring barley variety mixtures. *Euphytica* **2012**, *185*, 123–138.
45. Tratwal, A.; Bocianowski, J. Cultivar mixtures as part of integrated protection of spring barley. *J. Plant. Prot. Res.* **2018**, *125*, 41–50.
46. Moya, P.; Girotti, J.R.; Toledo, A.V.; Sisterna, M.N. Antifungal activity of *Trichoderma* VOCs against *Pyrenophora teres*, the causal agent of barley net blotch. *J. Plant. Prot. Res.* **2018**, *58*, 45–53.

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