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# Cultivar Mixtures as Part of Integrated Protection of Winter Barley from Leaf Diseases and Abiotic Stresses

Yana Yakhnik[1]*, Galina Volkova[1], Anastasia Danilova[1], Kirill Kutumov[2]

## Abstract

**Background:** Currently, cultivar mixtures are being extensively used in agriculture worldwide. The current research study aimed to identify the optimal proportion of cultivars with different resistance to pathogens to reduce the severity of leaf diseases in mixed cultivars against net blotch and leaf rust of barley.

**Methods**: Leaf rust severity was assessed using a modified Cobb scale, and net blotch severity with the E. E. Geshele scale. Disease severity was averaged from triplicate measurements on 25–30 plants. After harvest, 1000 seeds per plot were weighed. Differences were evaluated using the Fisher test (α = 0.05), and correlations via the Chaddock scale in Statistica 13.3.

**Results**: A high degree of inverse correlation was established between the proportion of resistant cultivars and the severity by both diseases r= – 0.93/– 0.99. The optimal mixture combination of a resistant and susceptible cultivar against net blotch was recorded as 1S:4R, and leaf rust as 1S:3R and 1S:4R.

**Conclusion**: In mixed barley crops, a strong inverse correlation was found between the proportion of resistant cultivars and disease severity (r = –0.93/–0.99). Disease severity correlated directly with humidity and temperature in spring, with an inverse correlation for net blotch in June. Using mixed cultivars enhances crop resistance to abiotic and biotic stresses, making it suitable for eco-friendly farming.

## Introduction

Globally, barley (*Hordeum vulgare* ssp. *vulgare* L.) ranks fourth in both yield and cultivation area of cereal crops just after wheat, rice and corn. It is grown in more than 100 countries [1]. A high adaptability to adverse environmental conditions (cold, drought, micronutrient-depleted soils) provides a wide range of agro-climatic growing zones. Barley remains a staple food for many people and is crucial for low-income families [2]. Barley is also widely used in brewing and as animal feed. Barley net blotch, caused by the fungus *Pyrenophora teres* Drechs., is one of the dominant pathogens in agricultural crops worldwide. It leads on average to about 40% yield loss, and in epidemic years - more than 70% [3]. Yield losses from leaf rust (the causative agent is the fungus *Puccinia hordei* Otth.) depend on the agro-climatic features of the region; on average, they are set at 30%, but in some cases can reach up to 62% [4].

An integrated pest management (IPM) is the best strategy to reduce the impact of plant diseases on crops. It includes crop rotation, fungicide treatment and the introduction of resistant cultivars. Fungicide treatment is the most common and effective way to control foliar diseases. However, it has a high pesticide load, which is complicated by an increase in pathogen resistance [5]. In regions with low soil fertility and insufficient moisture, the use of fungicidal treatments is not an economically justified method of control [6]. Currently, biologically safe pathogen control methods such as crop rotation, spatial isolation, cultivation of resistant cultivars, use of biological plant protection products and cultivation of genetically mixed cultivars are being actively developed and implemented [7].

Mixed crops are combinations of cultivars that are compatible in terms of biometric parameters, maturation time and quality indicators. Besides, they do not require additional costs to ensure phenotypic uniformity. The barrier effect provides an increase in the distance between plants of the same genotype. As a result, genetic diversity increases, which in turn leads to a slowdown in the co-evolution of the host plant and pathogen [8]. At the same time, it is necessary to regularly change the mixture composition, introducing new resistant and high-yielding cultivars into production to prevent the selection and reproduction of aggressive pathogen races [9]. One of the expected benefits of introducing mixed cultivars is both yield increase and stabilization [10]. Researchers noted that the likely mechanisms for achieving this effect for barley are a complex reduction in pathogenic pressure, differentiation in resource distribution, lodging reduction, as well as compensatory effect on the impact of abiotic stress factors.

Currently, the use of mixed crops, as an environmentally friendly way to control foliar diseases, is very relevant and has become widely introduced into industrial agriculture [10,11]. A linear relationship was found between the decrease in disease incidence and the level of general resistance of the mixture in two-component mixtures containing cultivars resistant and susceptible to powdery mildew (the causative agent - *Blumeria graminis* (DC.) Speer f. sp. *hordei* Marchal.) in various proportions [11]. Ukrainian researchers established a direct correlation between the severity by dwarf rust of spring barley and the weight of 1000 seeds (r = 0.973, r = 0.980). They also noted a significant effect of an increase in the severity of leaf disease on a decrease in the seed quality [12]. It is necessary to supplement information on the severity correlations by net blotch and dwarf rust and the weight of 1000 seeds.

Understanding the interaction between host plant, pathogen, and environment provides the basis for managing crop diseases in a rapidly changing, uncontrolled environment of abiotic factors. The severity of plants by net blotch and leaf rust directly depends not only on the stage of development and cultivar, but also on the temperature and humidity of the environment [13]. Kosiada's studies revealed a direct dependence of *P. teres* barley disease intensity on temperature and humidity increases in the controlled laboratory [14]. Researchers from Tunisia studied the effect of temperature and light on the growth of a pure culture of *P. teres* under salt stress [15]. Climatic effects have been widely studied for the main types of rust diseases of wheat and *Puccinia striiformis* f.sp. *hordei*. Data on the effect of temperature and humidity on the disease are understudied for barley leaf rust [13,16]. Additionally, it is required to supplement data on the effect of temperature and humidity on the severity of net blotch and leaf rust in the field in ontogeny of plant.

The introduction of mixed barley into production directly depends on the agro-climatic characteristics of the region and the pathogenic pressure of dominant diseases. The optimal combination in a mixture of shares of resistant and susceptible cultivars and the impact of these combinations on economic efficiency is understudied. Also, the question of the influence of temperature and humidity of individual months of plant vegetation on the severity of rust and hemibiotrophic diseases has not been studied. The present research project was designed and executed with the aim to develop the optimal proportion of different pathogen resistance cultivars to reduce the severity of leaf diseases and increase economic efficiency in mixed crops against net blotch and leaf rust, as well as to determine the effect of regional climatic features (temperature and humidity) on the severity of leaf diseases and weight 1000 seeds of winter barley. We found the optimal mixture combination against leaf diseases and influence correlation was found between the average monthly humidity and temperature and the severity of both diseases.

## Methods

**Place of research, soil and climatic conditions**

In this research, we used the scientific equipment 'Phytotron for isolation, identification, study and maintenance of races, strains, phenotypes of pathogens' [17]. The objects of the bioresource collection of the Federal Research Center of Biological Plant Protection 'State Collection of Entomoacariphages and Microorganisms' were used in the research. Cultivars were sown in the field on plots of 6 $m^2$ in three repetitions. The predecessor is pure steam. An artificial infectious background was created for dwarf rust (a mixture of urediniospores with talc, load - 10 $kg^{-6}/1$ $м^2$, ratio 1:100). Additional inoculation is not required for net blotch experiments due to the presence of a high natural infection background.

The climate of the studied area is temperate continental; there is a sufficient amount of moisture and light with a long growing season. The soil is leached chernozem. The depth of the humus horizon is 80 - 150 $m^{-1}$. Humus content in the arable (0 – 20 $m^{-1}$) soil layer is 3.39%; mobile phosphorus is 18.2 $kg^{-6}/100$ $kg^{-3}$; mobile potassium compounds are 30.6 $kg^{-6}/100$ $kg^{-3}$; soil reaction is slightly acidic - 5.5 - 6.5 pH. Exchangeable acidity is absent. Hydrolytic acidity varies from 2 to 4 $kg^{-6}$-equiv/100 $kg^{-3}$. Base saturation is 85 - 95%. The climatic data of temperature and humidity of studied area are obtained from the open database Raspisaniye Pogodi Ltd [18].

The 2019–2020 growing season was characterized by a dry autumn. There was a significant lack of moisture in winter and spring, returned frosts in spring, atmospheric and soil drought. The weather in 2020 was favorable for the severity of net blotch and leaf rust. The 2020–2021 growing season was characterized by a lack of precipitation in autumn, snowy and cold winters, and a prolonged cold spring. Therefore, ripening time of grain crops was shifted by an average of 10 days. The weather was favorable for the severity of net blotch; the severity of leaf rust was low. The 2022 growing season was characterized by heavy snowfalls in January and frosts in March. Spring was cold and prolonged, but warmer than in 2021. The ripening time of grain crops has also been shifted, as in 2021. The weather was unfavorable for the severity of net blotch; quite intensive severity of leaf rust was observed.

**Research materials**

For the study, Russian commercial cultivars of winter barley were selected based on phenotypic compatibility and type of use. Two cultivars were selected with different resistance to leaf rust - resistant cultivar Gordey (hereinafter R) and susceptible cultivar Mykhailo (hereinafter S). Two cultivars were selected with different resistance to net blotch - resistant cultivar Joseph (hereinafter R) and susceptible cultivar Romans (hereinafter S). The cultivars used in the leaf rust infestation trial are resistant to net blotch. Before sowing, the seeds of the cultivars were thoroughly mixed in the ratio 1S:1R, 1S:2R, 1S:3R, 1S:4R; pure cultivars were also sown. There were no fungicidal treatments on the experimental plots.

**Assessment and measurement methods**

Diseases were recorded during the period of maximum severity on plants inside the plots to avoid the edge effect. We determined the intensity of leaf rust the severity using a Modified Cobb Scale. We determined the severity to net blotch leaves using the E. E. Geshele Scale [19]. We determined the average severity of leaf diseases from the total number of examined plants in triplicate (at least 25–30 plants in each replication). After harvest, 1000 seeds were randomly selected from each plot (Automatic Seed Counter MSC-C) and weighed (ViBRA ALE 223).

**Statistical analysis**

The statistical difference between the samples was assessed using the Fisher test at a significance level of $\alpha = 0.05$. The Chaddock scale was used to interpret the degree of correlation. We obtained the average value for the calendar month from 8 daily measurements to analyze the indicators of humidity and temperature. Monthly averages were calculated for the main growing season of the studied region, that is, for March, April, May and June. The calculation was performed using the Statistica software version 13.3.

## Results

The severity of net blotch in barley in 2020 and 2021 was average for the annual rates in this region (Table 1). The share of plot location influence did not exceed 2–3% (at $F_{3.12} = 7.68$, $p = 0.0009$ for 2020, $F_{3.12} = 3.43$, $p = 0.03$ for 2021). In 2022, the weather did not strongly contribute to the severity of leaf diseases; there was no inter-sampling effect (at $F_{3.12} = 0.3$, $p = 0.7$). The weather in 2020 and 2022 contributed to the severity of leaf rust on leaves, in 2021 the severity was minimal (at $F_{3.12} = 5.79$, $p = 0.004$ (2% influence share) for 2020, F3 $F_{3.12} = 0.3$, $p = 0.7$ for 2021, $F_{3.12} = 0.27$, $p = 0.7$ for 2022). The data indicates sample representativeness for each pathogen over the three years of the study. The degree of disease severity consistently decreased with an increase in the proportion of resistant cultivar in the mixed crops in both the net blotch and leaf rust disease severity studies. Data suggests a beneficial effect of mixed cultivars in reducing disease severity when using susceptible cultivars. The weight of 1000 seeds in the net blotch experimental plots were found to be maximum in 2021 - 45.80 $kg^{-3}$, in 2022 the weight was 40.46 $kg^{-3}$; in 2020, the minimum seed yield of 31.62 $kg^{-3}$ was noted (Figure 1). In the leaf rust infected plots, the maximum yield was found in 2022 - 44.80 $kg^{-3}$. In 2021, the yield was 41.25 $kg^{-3}$, the minimum indicators for the weight of 1000 seeds - 32.34 $kg^{-3}$ on average for all plots were noted in 2020.

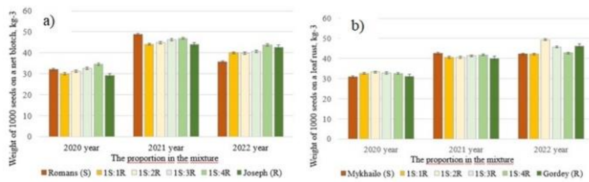


**Figure 1:** Weight of 1000 seeds in mixtures of resistant and susceptible cultivars against net blotch (a) and leaf rust (b) in 2020-2022, $kg^{-3}$.

The mixture efficiency for each of the infectious plots varies depending on the proportions of resistant and susceptible cultivars. In 2020 and 2022, we observed a trend towards an increase in the mass of 1000 seeds in mixed crops compared to mono-varietal susceptible and resistant crops (Figures 2 and 3).

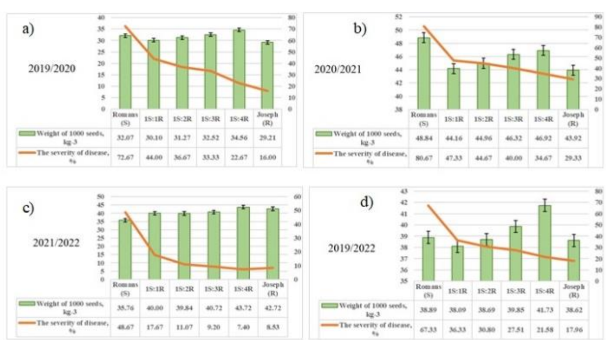


**Figure 2:** The severity of diseases (%) and weight of 1000 seeds ($kg^{-3}$) in different proportions of resistant/susceptible cultivars at the infectious site with net blotch of barley.

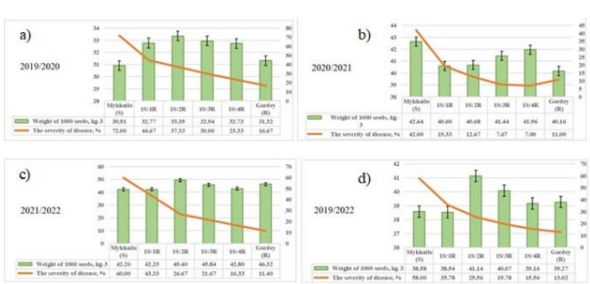


**Figure 3:** The severity of diseases (%) and weight of 1000 seeds ($kg^{-3}$) in different proportions of resistant/susceptible cultivars at the infectious site with leaf rust of barley.

**The influence of temperature and humidity on the severity of plant diseases and the weight of 1000 seeds**

During the study period, a low level of average monthly humidity indicator was observed in April 2020 (Table 2). The difference was 35.7%; in other months of the study, the difference in indicators did not exceed 11.1%. March 2022 was the coldest at the beginning of the growing season. A high degree of inverse correlation between the proportion of resistant cultivars and the severity by both diseases was revealed. (r= – 0.93/– 0.99). No correlation was found over the years between the proportion of resistant cultivar in the mixture and the weight of 1000 seeds in the net blotch test (r= – 0.12 (2020); r= – 0.67 (2021); r= 0.93 (2022). We note moderate correlation in 2020 and 2022 in a study of the effect of resistant cultivar proportions on the weight of 1000 seeds in the leaf rust test (r= 0.38 (2020); r= 0.49 (2022)) and inverse noticeable correlation in 2021 (r= – 0.66).

A weak dependence of the degree of net blotch severity on the average monthly humidity was found for a resistant cultivar in March (r= 0.22), April (r= 0.11) and June (r= 0.06), and a high dependence in May (r= 0.82). The humidity effect on the severity of the susceptible cultivar was moderate in March (r= 0.43), May (r= 0.46), and June (r= 0.37), but weak in April (r= 0.11).

Correlation analysis between the average monthly indicators of humidity and the severity to barley by leaf rust showed a high degree of dependence for both studied cultivars in March, April, and May (r from 0.79 to 0.99; in March, a noticeable degree of dependence (r = 0.67)), as well as a high degree inverse correlation in June (r from -0.74 to -0.98). Correlation analysis of average monthly temperature and the severity of net blotch showed an increase in influence during the spring months for resistant (March (r= 0.25), April (r= 0.50), May (r= 0.99)) and susceptible cultivars (March (r= 0.45), April (r= 0.86), and May (r= 0.92)). A noticeable (for R) and high correlation (for S) in April and a very high dependence of net blotch severity on temperature in May were noted. In June, a high degree of inverse correlation was found for both resistant (r= – 0.95) and susceptible cultivars (r= – 0.74).

In 2021, in both infectious plots, the 1000-seed weight was higher in the crops of the cultivar susceptible to the pathogen (Table 3). Then there was a tendency to increase yields with an increase in the proportion of resistant cultivars in crops; and the minimum value was observed in crops with one resistant cultivar. Favorable weather in 2021 contributed to high yields, subject to the tolerance of the susceptible cultivar to net blotch, as a significant the severity to the leaves of the susceptible cultivar was noted.

| The proportion R in the mixture | The severity of net blotch (%) | | | The severity of leaf rust (%) | | |
|---|---|---|---|---|---|---|
| | 2020 year | 2021 year | 2022 year | 2020 year | 2021 year | 2022 year |
| 0.00 | 72.6±8.8 | 80.6±9.6 | 48.6±9.1 | 72.0±10.8 | 42.0±10.1 | 60.0±9.2 |
| 0.50 | 44.0±11.8 | 47.3±12.2 | 17.6±8.6 | 44.6±11.2 | 19.3±8.8 | 43.3±9.0 |
| 0.66 | 36.6±14.9 | 44.6±14.5 | 11.0±3.6 | 37.3±14.3 | 12.6±4.9 | 26.6±8.1 |
| 0.75 | 33.3±11.7 | 40.0±10.6 | 9.2±3.0 | 30.0±8.4 | 7.6±2.5 | 21.6±8.8 |
| 0.80 | 22.6±9.6 | 34.6±6.4 | 7.4±1.8 | 23.3±8.3 | 7.0±2.5 | 16.3±7.1 |
| 1.00 | 16.0±5.0 | 29.3±8.8 | 8.5±3.3 | 16.6±4.8 | 11.0±5.7 | 11.4±4.6 |

Values are means ± standard deviation, means with different letters within columns differ from each other ($\alpha = 0.05$): for the net blotch of barley $F_t 2.3 < F_f 58.4$ (2020), $F_t 2.3 < F_f 44.5$ (2021), $F_t 2.3 < F_f 107.9$ (2022); for the leaf rust of barley $F_t 2.3 < F_f 62.6$ (2020), $F_t 2.3 < F_f 55.4$ (2021), $F_t 2.3 < F_f 82.5$ (2022).

**Table 1:** The severity of net blotch and leaf rust in barley in the field, %.

| Month | The severity of diseases, % | 2020 | 2021 | 2022 | Rr/Sr | |
|---|---|---|---|---|---|---|
| | *P. teres* | R16.0/S72.7 | R29.3/S80.7 | R8.5/S48.7 | *P. teres* | *P. hordei* |
| | *P. hordei* | R16.7/S72.0 | R11.0/S42.0 | R11.4/S60.0 | | |
| March | Humidity, % | 69.5 | 75.0 | 76.6 | 0.22/0.43 | 0.97/0.67 |
| April | | 43.5 | 79.2 | 74.4 | 0.11/0.11 | 0.99/0.85 |
| May | | 64.4 | 53.3 | 61.1 | 0.82/0.46 | 0.79/0.99 |
| June | | 51.7 | 58.3 | 59.1 | 0.06/0.37 | −0.98/−0.74 |
| March | Temperature, °C | 9.3 | 4.5 | 2.9 | 0.25/0.45 | 0.96/0.65 |
| April | | 10.4 | 11.1 | 13.4 | 0.5/0.86 | 0.64/0.04 |
| May | | 16.5 | 18.0 | 15.2 | 0.99/0.92 | 0.03/0.57 |
| June | | 22.9 | 21.7 | 23.0 | −0.95/−0.74 | 0.49/0.88 |

Values R/S are meaning the severity of diseases resistant and susceptible cultivars for each pathogen. Values Rr/Sr are meaning the degree of correlation between the severity of diseases and humidity, the severity of diseases and temperature respectively.

**Table 2:** Correlation between monthly average humidity/temperature and the severity of net blotch and leaf rust in barley.

| Month | Weight of 1000 seeds, $kg^{-3}$ | 2020 | 2021 | 2022 | Rr/Sr | |
|---|---|---|---|---|---|---|
| | *P. teres* | R29.2/S32.1 | R43.9/S48.8 | R35.8/S42.7 | *P. teres* | *P. hordei* |
| | *P. hordei* | R31.3/S30.9 | R42.0/S42.6 | R46.3/S42.2 | | |
| March | Humidity, % | 69.5 | 75.0 | 76.6 | 0.67/0.81 | 0.99/0.96 |
| April | | 43.5 | 79.2 | 74.4 | 0.90/0.97 | 0.98/0.99 |
| May | | 64.4 | 53.3 | 61.1 | 0.97/0.98 | 0.47/0.72 |
| June | | 51.7 | 58.3 | 59.1 | 0.77/0.89 | 0.98/0.99 |
| March | Temperature, °C | 9.3 | 4.5 | 2.9 | 0.65/0.76 | 1.0/0.95 |
| April | | 10.4 | 11.1 | 13.4 | 0.11/0.33 | 0.84/0.63 |
| May | | 16.5 | 18.0 | 15.2 | 0.60/0.40 | 0.23/0.01 |
| June | | 22.9 | 21.7 | 23.0 | −0.86/−0.7 | −0.17/−0.46 |

Values R/S are meaning the weight of 1000 seeds resistant and susceptible cultivars for each pathogen. Values Rr/Sr are meaning the degree of correlation between the weight of 1000 seeds and humidity, the weight of 1000 seeds and temperature respectively.

**Table 3:** Correlation between monthly average humidity/temperature and 1000 seed weight on the net blotch and leaf rust infected plots.

Analysis of average monthly humidity indicators and their effect on the weight of 1000 seeds showed a correlation in both cultivars of net blotch infected spot in March (r= 0.67 for R; r= 0.81 for S) and in June (r= 0.77 for R; r= 0.89 for S). In April and May, the yield weight indicators are in a very high dependence on humidity for both cultivars (r from 0.90 to 0.98). On the leaf rust infected spot, a very high correlation was noted in March, April, and June (r from 0.96 to 0.99); moderate for resistant cultivar (r= 0.47) and noticeable for susceptible cultivar (r= 0.72) correlation in May.

We noticed a significant correlation between the weight of 1000 seeds and the temperature in the net blotch infected spot in March (r= 0.65 for R; r= 0.76 for S) and in May (r= 0.60 for R; r= 0.40 for S (noticeable dependence)). In April, the correlation between the indicators was weak (r= 0.11 for R; r= 0.33 for S), and in June there was already a high inverse correlation for both cultivars (r= – 0.86 for R; r= – 0.70 for S).

## Discussion

Plant tolerance, as the ability to limit the negative impact on productivity and yield, is an important trait in breeding process [20]. In the long term, in the foliar diseases management, tolerant cultivars do not have a significant impact on the selection pressure of pathogen populations, unlike cultivars with significant resistance [21]. However, the advancement of this direction requires a deep understanding of plant phenotypic traits and protection mechanisms against abiotic stresses, as well as the mechanism of action of various parasitic fungal species on the host plant [22]. P. *hordei*, being a biotrophic parasite, has a significant negative effect on plant tissues, reducing photosynthesis and the amount of sucrose available for translocation and other uses by the plant. The mechanisms of influence of the biotrophic parasite on the plant are consistent with the results of our field studies. This indicates a low tolerance of barley cultivars to rust diseases, in particular to *P. hordei* (Figure 4a). The net blotch pathogen has two forms - *P.*

*teres f. maculata* and *P. teres f. teres*. Being genetically one species, these forms differ in the mechanisms of plant infection and toxic effects [23]. The net blotch pathogen generally has only local adverse effects such as tissue death and reduction of photosynthetic surface (Figure 4b). The adaptive mechanisms of various barley cultivars make it possible to maintain relative tolerance to the net blotch pathogen, which is consistent with the above results of the study.

We conducted a correlation analysis of field data on the plant severity by diseases, 1000 seeds weight and such abiotic factors as average monthly humidity/temperature of the main growing season (March, April, May, and June). The influence of these factors on the performance of two different resistance cultivars shown in monocultures was studied.

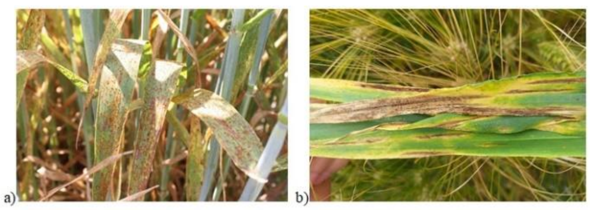


**Figure 4:** Disease symptoms caused by: a) *Puccinia hordei* (leaf rust of barley); b) *Pyrenophora teres* (net blotch of barley) (orig.)

On average, we observe a rather high direct correlation between the average monthly humidity and the degree of net blotch severity in May and a weak correlation in April. This is consistent with the literature data on the direct influence of humidity indicators on pathogen development during clonal reproduction [24]. Martin and Clough [25] note that in the field, sporulation is directly related to both temperature and diurnal variation in moisture levels, as a 16-hour period is needed, followed by a dry leaf surface. The severity of barley leaf rust in the spring months is directly related to the average monthly humidity and inversely related to the average monthly humidity in June. The data are consistent with the studies of other researchers describing the conditions for the germination of *Puccinia hordei* uredospores in the presence of sufficient humidity in a wide temperature range [26]. According to our research, on average, for a susceptible cultivar to net blotch, there is a large dependence of the severity on the average monthly temperature indicators. *P. teres* requires temperatures above 2°C for germination and inoculation with conidia. As a result, the factor limiting the severity of the pathogen in March in this region is the temperature at sufficiently high humidity [27]. In 2022, we observed low temperatures in March. This may have contributed to the reduction in the number of infection cycles and, as a result, the overall decrease in the severity of plants compared to other years of the study. In the subsequent spring months, the average temperature was quite high. This contributed to multiple generations and extensive spread of the pathogen. The phase of milky-wax ripeness in the region falls in June, when rather high temperatures are observed. Our data indicates a high inverse correlation between pathogen severity and average temperatures. This, in turn, may suggest that high temperatures are a limiting factor in the summer months. We established the dependence of the leaf rust severity on the average monthly temperature in March from high (r= 0.96 for R) to noticeable (r= 0.65 for S). A high correlation was found for the resistant cultivar (r=0.96) in April, but not for the susceptible one (r=0.04). In May, the opposite dynamics were observed - the absence of correlation between the indicators on the resistant cultivar (r= 0.03) and the noticeable correlation on the susceptible cultivar (r= 0.57). In June, a significant correlation for the resistant cultivar (r= 0.49) and a very high correlation for the susceptible cultivar (r= 0.88) was revealed. We, therefore, assume that the increased average monthly temperatures in March (resumption of vegetation) and the stage of milky-wax ripeness (June) are of particular importance for barley leaf rust management. On average, the indicators are consistent with the generally recognized known data on the direct dependence of the mass of the obtained crop on the presence of sufficient moisture during the growing season. However, it should be taken into account that leaf rust severity in May and net blotch severity in March and June may affect the crop quality. Greatly infected net blotch crops cause an increase in the weight of 1000 seeds at high average monthly temperatures in March and May. However, these figures may decrease at high temperatures in June. High average monthly temperatures in March directly affect the weight of 1000 seeds on the leaf rust infected spots (r= 1.0 for R; r= 0.95 for S). The effect of high average monthly temperatures on the leaf rust infected spot in June has a negative correlation similar to that of the net blotch infected spot. For a resistant cultivar, the inverse correlation is weak (r= – 0.17); for the susceptible one it's moderate (r = - 0.46). Thus, in case of severe leaf rusting, it is necessary to consider high average monthly temperatures in March and April, which can significantly affect the yield. The results obtained are also consistent with previous studies on the direct effect of increasing humidity and temperature on the growth of phytopathogenic fungi due to the activation of metabolic processes [3,5,24]. When sowing mixed barley crops, a high degree of inverse correlation was established between the proportion of resistant cultivars and the severity by both diseases (r= – 0.93/– 0.99). The optimal mixture combination against net blotch was recorded as 1S:4R, and against leaf rust as 1S:3R and 1S:4R. A direct correlation was found between the average monthly humidity and the severity by both diseases in the spring months. A direct correlation was found between the average monthly temperature the severity by both diseases in the spring months and the inverse correlation for net blotch in June. The use of mixed cultivars can be applied in environmentally safe farming, since this method increases the resistance of crops to abiotic and biotic stresses.

## Acknowledgment

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## Author Contributions

Yana Yakhnik: Conceptualization, Methodology, Data Analysis, Writing – Review & Editing. Galina Volkova: Investigation, Data Curation, Writing – Original Draft. Anastasia Danilova: Investigation, Resources, Writing – Review & Editing. Kirill Kutumov: Data Curation, Investigation, Writing – Original Draft.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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