Saudi Journal of Biological Sciences 29 (2022) 103401

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Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Pyrenophora teres: Population structure, virulence and aggressiveness in Southern Russia

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ARTICLE INFO

Article history:
Received 4 March 2022
Revised 28 June 2022
Accepted 27 July 2022
Available online 1 August 2022

Keywords:
Drechslera teres
Spot
Barley
Sporulation
Pathogenicity

ABSTRACT

The barley net blotch agent *Pyrenophora teres* (Died) Drechs. is one of the dominant fungal pathogens in agricultural crops worldwide. Here we aim to study the aggressiveness and virulence of *P. teres* populations collected at different ontogenesis stages (BBCH 30 and BBCH 47) from winter barley cultivars of various resistance types: moderately resistant, moderately susceptible and highly susceptible. We observed a direct proportional relationship between cultivar resistance and the aggressiveness of *P. teres* populations collected in both growth phases of the host plant. The isolates collected at an early stage of host plant development have a large difference in aggressiveness criteria: colony growth rate, sporulation intensity, latency period, plant damage degree, and the number of identified races. At the BBCH 30 growth stage, the growth rate of fungus colonies selected from a resistant cultivar is 1.2 times higher than that of a susceptible cultivar. The growth rate of colonies selected from resistant and susceptible cultivars in the earlier BBCH 30 stage is 1.04 times higher than the growth rate of colonies selected from the later phase. The sporulation intensity of fungal populations selected from a resistant cultivar is higher than that of populations selected from a susceptible cultivar (for BBCH 30–5.4 times, for BBCH 47–4.0 times); and it is 1.3 times higher in an earlier phase of plant development. Correlation between colony growth rate and spore formation rate in the BBCH 30 is r = 0.4. A high correlation level (r = 0.9) and notable difference between the variants were revealed when studying the duration of the latent period. The average value of plant damage by the *P. teres* from resistant cultivar is 4 times higher than from the susceptible cultivar in the BBCH 30 stage; and 12 times – in the BBCH 47 stage. There is a moderate negative correlation between the plant damage degree and the number of races identified from the fungal population, r = −0.59 for the BBCH 30, r = −0.8 for the BBCH 47. The number of races identified from *P. teres* populations collected in the late phase of plant growth was one third less. Our study helped to acquire new knowledge about intrapopulation processes under the influence of various factors – plant growth stage and cultivar genotype. The results obtained are the basis for the development of adaptive-integrated techniques for managing populations of the hemibiotrophic pathogen, barley net blotch.

1. Introduction

The barley net blotch agent *Pyrenophora teres* (Died) Drechs. is one of the dominant fungal pathogens in agricultural crops in Russia and worldwide (Dontsova, 2015; Muria-Gonzalez et al., 2020). The resistance of new barley cultivars is overcome by new virulent isolates. Isolates produce several clonal generations during the season, survive wintering and, in the absence of natural competition, reproduce extensively (Oguz and Karakaya, 2015). This results in a significant yield loss over several years.

The willingness to consume organic food produced using only natural fertilizers has led to the production of high-quality organic grains (Cappelli and Cini, 2021). Currently, the technology of grow-

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103401
1319-562X/

ing several dominant pathogen-resistant cultivars is one of the main methods of integrated plant protection in production crops (Wallwork et al., 2016). Crop breeders around the world have been dealing with the challenge of developing long-term resistant barley cultivars for over 60 years (Abebe, 2021). This strategy provokes an increase in the virulent population of the pathogen in accordance with the cultivar genotype. It also leads to clonal reproduction and subsequent dominance of the most aggressive races, worsening the phytosanitary situation (Ronen et al., 2019). This results in crop losses and increased costs for various types of crop protection (Novakazi, 2020). Since new races of parasitic fungi may be more virulent, ongoing monitoring is needed to study the timing of overcoming resistance in new cultivars. The ratio between genetic diversity, the size of pathogen populations and host plants determines the severity of the disease (Afanasenko, 2010).

A certain genetic variation in patterns of pathogen infectivity and aggressiveness exists in the genetic diversity of a pathogen population. This criterion is measured by traits that determine the relationship of the "parasite-plant-host" pathosystem, such as colony growth rate, number of virulent races, sporulation intensity, etc. (Sultana et al., 2018). According to Dontsova (2015), the barley net blotch was found to infect wheat in recent years due to the arid climate of Southern Russia. Dontsova also notes an increase in the harmfulness of hemibiotrophic parasites on crops. Historically, the net blotch was first registered in 1928, and in just 20–30 years the disease became widespread and extremely harmful in all regions of barley cultivation (Afanasenko, 2010; Dontsova, 2015). This issue is particularly acute in Krasnodar Krai, as it occupies a leading position among Russian regions in the cultivation of winter barley with an average sown area of about 144 thousand hectares (Firsova et al., 2018). Net spotting of barley is the most dangerous and harmful disease in the south of Russia, as crop losses reach 45 % (Dontsova, 2015). Currently, the disease in the region is controlled using different approaches, chemical and biological preparations, as well as using resistant cultivars. The introduction of resistant cultivars is an actual and the best method of protecting barley crops. It is necessary to supplement the fundamental knowledge about the change in the structure of the pathogen population when growing resistant cultivars. Microevolutionary processes has also changed the population structure. Since the increase in the use of pesticides provoked a high frequency of mutations, identifying and fixing the new ones, as well as the most adapted strains and races. Their rapid spread led to the emergence of new sources of epidemics.

Some researchers (Poudel et al., 2017; Dinglasan et al., 2019; Vasighzadeh et al., 2021), studying the genetic structure of *P. teres*, note the high genetic diversity of the pathogen. The principles of maintaining crop genetic diversity through cultivar diversity can significantly reduce the incidence. Differentiated adaptation of pathogen isolates to specific host cultivars complicates the strategy for disease-resistant cultivars (Afanasenko, 2010). Research on barley resistance to net blotch begins in the 1920s, when Geschele (1928) showed that it was quantitatively inherited. By the end of the 1950s, at least three genes proved to be effective against *P. teres* isolates. Then, in 1977, Bockelman et al. reported on the first resistance loci that could be localized in the genome (Bockelman et al., 1977; Abebe, 2021). In the 1990s, a number of studies were carried out to study plant resistance in the field (Steffenson et al., 1996). At present, due to recent advances in molecular marker techniques, resistance genes at quantitative trait loci (QTL) are known across all seven barley chromosomes, and many of them are specific to either *Ptt* or *Ptm*.

Genetic protection of grain crops involves comprehensive studies of the emergence of new species and races of pathogenic micromycetes. The breeding process involves the use of genetic diversity, search for resistance donors, the possibility of combining resistance to several pathogens at once. The main work on the study of pathogenesis is carried out in the seedling phase, since this phase is the most vulnerable for the plant (Wonneberger et al., 2017; Amezrou et al., 2018). At the same time, the German researcher Novakazi (2020) identifies a significant QTL number associated with resistance at both growth stages. According to Abebe (2021), only 75 % of resistance genes are effective in both seedling and adult stages. To our knowledge, the problem of changing the pathogenicity of the fungus and the physiological mechanisms of interaction in the ontogeny of the host plant have not yet been fully characterized. In research (Volkova et al., 2020) was indicated that net blotch resistant barley cultivars produce the most aggressive and rapidly reproducing pathogen populations. Studies of *P. teres* populations collected from cultivars with different resistance in different phases of host plant ontogeny have never been carried out in Southern Russia. There is a need to supplement studies of microevolutionary changes in the pathogen population under the influence of cultivar genotype and stages of host plant development.

Here we aim to study the aggressiveness and virulence of *P. teres* populations harvested from a host plant at different stages of ontogeny in winter barley cultivars of varying resistance. Two research objectives were set: firstly, to study the change in the structure of the pathogen population selected from cultivars of different resistance in different phases of ontogenesis according to the criteria of aggressiveness; secondly, to study the change in the structure of the pathogen population according to the virulence criterion.

The hypothesis of the study is that the degree of resistance and the phases of ontogenesis of the host plant affect the structure of the *P. teres* population.

2. Materials and methods

2.1. Collection of field samples

Experimental cultivars were sown on October 10, 2019. The study used the infrastructure and objects of the "State collection of entomoacariphages and microorganisms" (https://ckp-rf.ru/usu/585858/?sphrase_id=5369152) and "Phytotron for the isolation, identification, study and maintenance of races, strains, phenotypes of pathogens" (https://ckprf.ru/usu/671925/?sphrase_id=3926639). The predecessor is pure steam. Infectious material was selected against the natural infectious background in early April (growth stage BBCH 30). Then, trial field plants were selected from the soil and transferred to 3 flowerpots (5 plants per flowerpot) for further growth in a climate chamber KBWF 720 (temperature + 25. 0 °C, humidity 80 %, fluorescent lamps 13,000 lx, spectral distribution (250–785 nm)) up to the BBCH 47growth stage. The fungus was collected from the affected leaves of barley cultivars rated as: moderately resistant (MR) cultivar Versal, moderately susceptible (MS) cultivar Kubagro-1 and highly susceptible (HS) cultivar Romans, sown in the South of Russia. For the convenience of the study, each population was assigned a code name (Table 1).

Table 1
The codes for each *Pyrenophora teres* population.

Cultivar	Growth phase	Code name
Cultivar Versal	BBCH 30	MR-30
Cultivar Versal	BBCH 47	MR-47
Cultivar Kubagro-1	BBCH 30	MS-30
Cultivar Kubagro-1	BBCH 47	MS-47
Cultivar Romans	BBCH 30	HS-30
Cultivar Romans	BBCH 47	HS-47

2.2. Isolation of monoconidial isolates

One isolate was selected from one leaf. We used no more than two leaves per plant. Upon drying at 20 °C, spots (5 × 10 mm) were cut out from the leaves; the surface was disinfected with 1 % sodium hypochlorite solution for 2 min, then washed 3 times with sterile water for 2 min and dried on sterilized filter paper (Baturo-Ciesniewska et al., 2012). Disinfected plant sections were placed on carrot-beetroot agar (130 ml beets, 130 ml carrots, 20 g of agar per 1 L of water) and incubated for 5 days at 23 °C under UV lamps (30 W UVB 280–315 nm) (Liu et al., 2012). In 5 days, transfer of one conidia from one spot was made with a sterile needle into a dish with a beet-carrot nutrient medium. Isolates collected at an earlier growth phase were stored in a refrigerator at + 4 °C. Isolates taken from the second sample were used fresh.

2.3. Screening of aggressiveness parameters

Three parameters determined the pathogen aggressiveness: colonies growth rate, sporulation intensity, and the latent period between infection and the first symptoms of the disease (Dudka et al., 1982; Pakhratdinova et al., 2017). For the experiment, we used 10 isolates of the fungus from each cultivar. The growth rate of the pathogen was determined by transferring mycelial discs to carrot-beetroot agar and incubating for 10 days in the dark at 23 °C, then measuring the diameter of the colony. The intensity of isolate sporulation was studied by transfer and subsequent incubation in Petri dishes with carrot-beetroot agar for 14 days under near-UV light (Afanasenko et al., 2009). 10 ml of conidial suspension was prepared from each Petri dish, then the number of spores in 1 ml of suspension was counted using a Levenhuk MED 45 T microscope. Isolates with a concentration of 2×10^3 conidia per 1 ml were classified as weakly sporulating, 5×10^3 conidia per 1 ml – medium sporulating and 7×10^3 conidia per 1 ml – strongly sporulating, respectively.

The seedlings of the highly susceptible cultivar Romans were sown 5 plants in 3 pots (0.25 ml) to determine the degree of plant damage and the latent period of each of the experimental populations. Fungal populations selected from cultivars with different resistance to *P. teres* were used for inoculation. The spore suspension concentration (a mixture of 10 isolates selected from one cultivar) was 5000 conidia mL^{-1} (Afanasenko et al., 2009). After inoculation, the plants were placed for 24 h in a humid chamber in the dark, then transferred to a climatic chamber KBWF 720 (temperature + 25.0 °C, humidity 80 %, fluorescent lamps 13,000 lx, spectral distribution (250–785 nm). The degree of plant damage was measured after 10 days; the latent period was determined by the first symptoms of the disease using the standard Babayants scale (Babayants et al., 1988; Afanasenko et al., 2009).

2.4. Determination of the virulence composition of the population

We did an experiment to determine the racial structure of the pathogen population using an international set of differentiating cultivars (Afanasenko et al., 2009; Dinglasan et al., 2019). We used for the experiment 30*P. teres* isolates from each cultivar. One set of differentiator cultivars was inoculated with one isolate. To obtain the inoculum, a pure culture with one spore was grown under near-UV light at 25 °C for 14 days. A spore suspension was obtained by filling Petri dishes with sterile water and Tween 20 surfactant. The concentration of the inoculum suspension was adjusted to 40×10^3 conidia per ml (Afanasenko et al., 2009). Inoculation was carried out by spraying; the plants were then placed in a humid chamber in the dark. After 24 h, the pots were placed in a climatic chamber (temperature + 25.0 °C, humidity 80 %, fluorescent lamps 13.000 lx). In 7 days, the marker cultivars were evaluated according to Tekauz (1985) international scale. The racial structure was characterized using the octal system. Nine cultivars are arranged in strict order and divided into three triplets. Types of reactions, evaluated in points in the range from 1 to 5, were attributed to resistance, 5.1–10 – to susceptibility. Each of the three classes was assigned a value of 4, 2, and 1, respectively. If the value is "virulence", then the triplet data are summarized. They are marked with three numbers, which are the name of the race. The experiment was repeated three times.

2.5. Statistical analysis

The statistical difference between the samples was assessed using Fisher's exact test at a = 0.05 significance level. The Chaddock's scale was used to evaluate the relationship between the signs. The value of the average virulence was determined by D. Martens formula: M = ΣPg/n, where *Pg* is the number of isolates virulent to all differentiating cultivars; *n* is the total number of isolates. The Shannon diversity index described the level of *P. teres* genetic diversity according to the formula: Hw = −Σpi ln(pi), where *pi* is the frequency of the *i*-th phenotype in this population, *n* is the total number of isolates of the studied population. Statistica software (version 13.3) calculated the results (https://statsoft.ru/products/STATISTICA_Base/).

3. Results

3.1. The growth rate of colonies of P. Teres

We registered significant differences in the growth rate of colonies of *P. teres* isolates collected in the BBCH 30 growth phase from winter barley cultivars with different pathogen resistance (Table 2). The average value of the growth of MR-30 colonies was maximum and amounted to 9.0 mm per day. MS-30 colonies grew more slowly at a rate of 8.6 mm per day. The growth of HS-30 colonies was even slower, averaging 7.6 mm per day.

A statistically significant difference was found between the growth rate of colonies MR-30 and MS-30 ($F_t5.3 < F_f7.2$), MS-30 and HS-30 ($F_t5.3 < F_f56.3$), MR-30 and HS-30 ($F_t5.3 < F_f15.0$). Pathogen colony growth rate, selected from the medium susceptible cultivar MS-30, is higher than from the susceptible cultivar HS-30 by 1.0 mm per day. Fungus colonies growth rate from resistant cultivar MR-30 is higher than from HS-30 by 1.4 mm per day.

Statistical analysis revealed small but statistically significant differences between the growth rate of colonies of fungal isolates collected from the studied cultivars at the BBCH 47 growth stage (between MR-47 and MS-47 ($F_t5.3 < F_f23.2$), MS-47 and HS-47 ($F_t5.3 < F_f117.7$), MR-47 and HS-47 ($F_t5.3 < F_f37.8$)) (Table 3). The growth rates difference was insignificant. It averaged 8.4 mm per day for the MR-47 population; 8.0 mm per day for the MS-47 population and 7.8 mm per day for HS-47. Fungus colonies growth rate from the medium susceptible cultivar MS-47 is 0.2 mm higher than HS-47 per day. MR-47 colonies grew faster than HS-47 colonies by 0.4 mm per day.

3.2. Sporulation intensity of Pyrenophora teres populations

The MR-30 fungus population showed the highest sporulation capacity – $7.0 \pm 1.0 \times 10^3$ conidia per 1 ml (Fig. 1). The MS-30 population of *P. teres* with a sporulation intensity of $4.3 \pm 0.7 \times 10^3$ conidia per 1 ml can be attributed to medium sporulating colonies. The lowest level of sporulation was shown by the HS-30 population, the average value was $1.3 \pm 0.3 \times 10^3$ conidia per 1 ml. A statistically significant difference was found between the sporulation

Table 2
Growth rate of *Pyrenophora teres* isolates collected from barley in BBCH 30 growth stage.

Cultivar	Growth rate of colonies of pathogen isolates, mm day									
	1	2	3	4	5	6	7	8	9	10
MR Versal	8.3 ± 0.5	8.5 ± 0.2	8.7 ± 0.7	8.8 ± 0.6	8.9 ± 0.2	9.0 ± 0.8	9.0 ± 0.6	9.0 ± 0.8	9.3 ± 0.8	10.6 ± 0.8
MS Kubagro-1	7.3 ± 0.6	7.5 ± 0.4	8.1 ± 0.3	8.7 ± 0.6	8.7 ± 0.5	8.8 ± 0.5	9.1 ± 0.7	9.1 ± 0.4	9.2 ± 0.6	9.2 ± 0.5
HS Romans	6.3 ± 0.4	6.3 ± 0.5	6.6 ± 0.4	7.3 ± 0.4	7.5 ± 0.4	7.6 ± 0.4	8.3 ± 0.7	8.4 ± 0.7	8.7 ± 0.7	9.0 ± 0.7

Table 3
Growth rate of *Pyrenophora teres* isolates collected from barley in BBCH 47 growth stage.

Cultivar	Growth rate of colonies of pathogen isolates, mm day									
	1	2	3	4	5	6	7	8	9	10
MR Versal	7.5 ± 0.4	8.0 ± 0.7	8.1 ± 0.3	8.1 ± 0.6	8.1 ± 0.7	8.3 ± 0.3	8.5 ± 0.6	8.8 ± 0.7	9.1 ± 0.5	9.1 ± 0.8
MS Kubagro-1	7.4 ± 0.6	7.5 ± 0.5	7.6 ± 0.4	7.8 ± 0.6	8.1 ± 0.7	8.3 ± 0.7	8.4 ± 0.5	8.4 ± 0.4	8.4 ± 0.5	8.5 ± 0.4
HS Romans	7.4 ± 0.5	7.5 ± 0.5	7.6 ± 0.6	7.7 ± 0.3	7.9 ± 0.6	7.9 ± 0.5	7.9 ± 0.6	8.1 ± 0.7	8.1 ± 0.7	8.1 ± 0.5

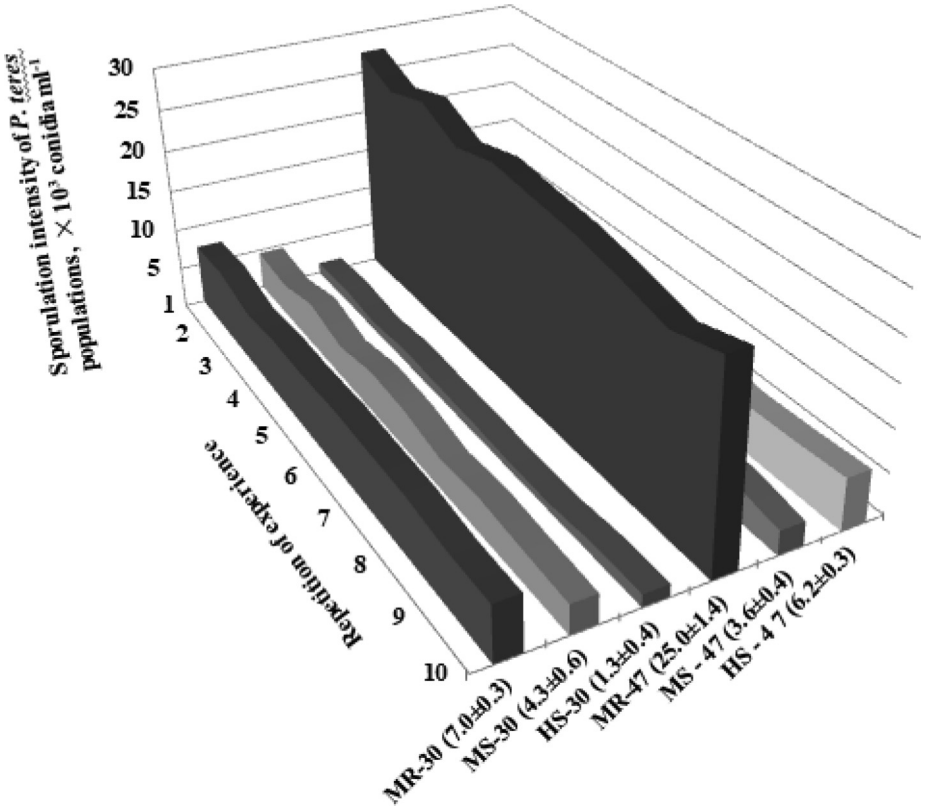

Fig. 1. Sporulation intensity of *Pyrenophora teres* populations collected from different development phases, $\times 10^3$ conidia ml^{-1}.

intensity of colonies MR-30 and MS-30 ($F_t5.3 < F_f58.6$), MS-30 and HS-30 ($F_t5.3 < F_f29.3$), MR-30 and HS-30 ($F_t5.3 < F_f51.1$).

The highest sporulating capacity was observed in the *P. teres* MR-47 population – 25.0 ± 1.5 × 10^3conidia per 1 ml. The lowest value was found in the MS-47 fungus – 3.6 ± 0.8 × 10^3conidia per 1 ml. The HS-47 pathogen population isolated from a highly susceptible cultivar demonstrated the ability to sporulate 6.2 ± 0. 9 × 10^3 conidia per ml.

3.3. The latent period of Pyrenophora teres populations

We registered significant differences between isolates of populations from various barley cultivars when studying the duration of the latent period of *P. teres* development (Fig. 2). Minimal latency and greater leaf damage were observed after inoculation with the MR-30 population. Already on the third day, the first manifestations were detected on 0.5 % of the leaf surface. Also on day 3, isolated manifestations of the disease (0.2 %) were observed on plants inoculated with the MS-30 population. The HS-30 pathogen population showed the least aggressiveness; the first symptoms on individual plants were observed only on the fourth day (0.1 %). A high level of correlation was determined between the degree of plant damage by various types of inoculums from the first manifestations of the disease (r = 0.9) and a substantial difference was found between the variants MR-30 and MS-30 ($F_t6.6 < F_f558.0$), MS-30 and HS-30 ($F_t5.9 < F_f1102.4$), MR-30 and HS-30 ($F_t6.6 < F_f200.4$).

We did an experiment to study the duration of the latent period of disease manifestation after inoculation with a population collected from cultivars with different resistance in the BBCH 47 growth stage. This experiment proved that the pathogen popula-

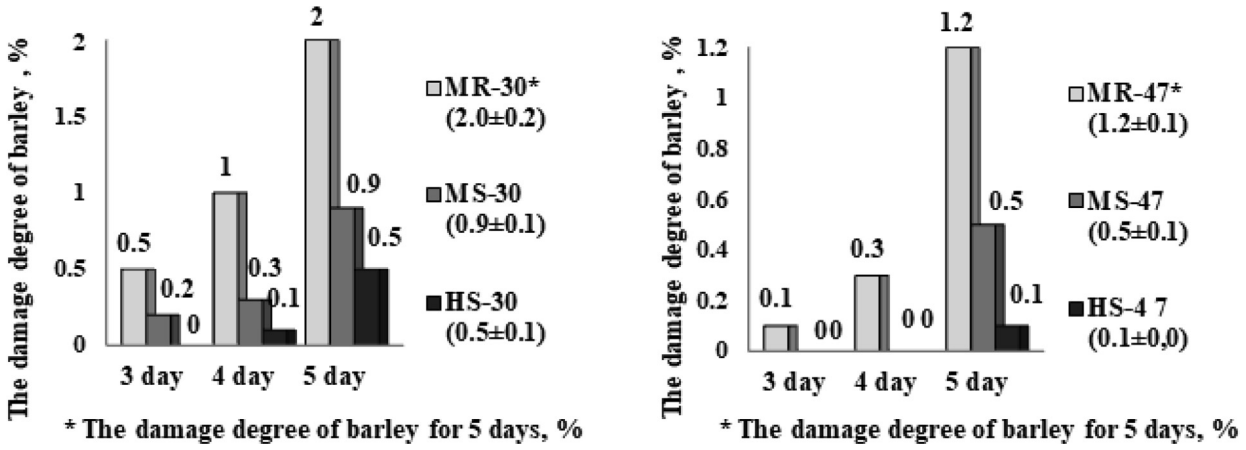

Fig. 2. Duration of the latent period and the damage degree of isolates collected from various barley cultivars, %.

tion from a moderately resistant cultivar was the most aggressive. The first manifestations of the disease were noted on the 3rd day (0.1 %). The first symptoms of the disease after the inoculation of MS-47 (0.5 %) and HS-47 (0.1 %) were observed on the 5th day. A high level of correlation (r = 0.9) and a significant difference between the variants was also revealed when studying the duration of the BBCH 47 latent period between the populations MR-47 and MS-47 (F_t5.9 < F_f79.7), MS-47 and HS-47 (F_t5.9 < F_f399.1), MR-47 and HS-47 (F_t6.6 < F_f93.1).

3.4. The virulence composition of Pyrenophora teres populations

Fig. 3 illustrates barley damage indicators at the corresponding stages BBCH 30 ((MR-30 (10 ± 1.4), MS-30 (25 ± 1.2), HS-30 (65 ± 3.0)) and BBCH 47 (MR-47 (30 ± 2.8), MS-47 (17.6 ± 1.2), HS-47 (70 ± 5.4)), mean virulence and number of identified races in *P. teres* populations collected from barley cultivars with different resistance.

For the *P. teres* MR-30 population with 10 % plant damage, the number of identified races was 18; for the MS-30 population, 25 % and 12 races; for the HS-30 population, 65 % and 13 races, respectively.

P. teres MR-47 population caused an average plant infection rate of 30 %, the lowest average virulence of 15.8 %, and the highest race diversity of 12 races. The MS-47 population caused the lowest plant damage among the experimental samples – 17.6 %, had the highest average virulence value of 20.8 % and the average number of identified races – 10. The HS-47 population led to the greatest plant damage – 70 %, high average virulence – 20.0 % and the smallest number of identified races – 7.

Comparative analysis of isolates of the BBCH 30 stage showed significant differences in the number of identified races of the fungus (Fig. 4). The highest occurrence was noted for race №. 651 (7.7 %) and race №. 551 (6.6 %), which were found in all populations of *P. teres* of the BBCH 30 stage. The MR-30 and HS-30 populations, selected from moderately resistant and highly susceptible cultivars, had 6 coinciding races. The MR-30 and MS-30 populations contained 4 identical races, and a pairwise comparison of the MS-30 HS-30 populations revealed 3 matching races.

According to the Rogers distance, the differences between populations in the BBCH 30 phase are low and insignificant (R = 0.22 (HS-30/MS-30), R = 0.19 (HS-30/MR-30), R = 0.20 (MR-30/MS-30). The greatest difference was found between populations of susceptible and moderately susceptible cultivars. On average, differences between populations in the later development phase of the BBCH 47 host plant are also low, but the Rogers distance increases when compared with the most resistant cultivar (R = 0.16 (HS-47/MS-47), R = 0.31 (HS-47/MR-47), R = 0.28 (MR-47/MS-47)). The data obtained are consistent with the above results on the significant racial diversity of the resistant cultivar. Modern breeding takes place by pyramiding several resistant genes, the interaction of which leads to a general resistance of the plant to disease (Afanasenko, 2010). Thus, studies by other scientists confirm that the genetic diversity of the pathogen evolutionarily depends on

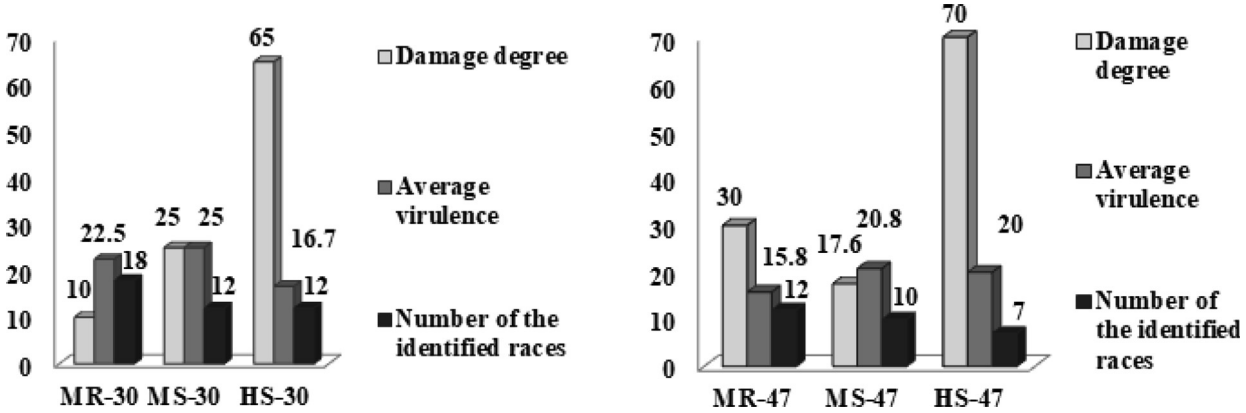

Fig. 3. Damage degree, average virulence and number of races in *Pyrenophora teres* populations collected from barley cultivars of different resistance at different stages of host plant development.

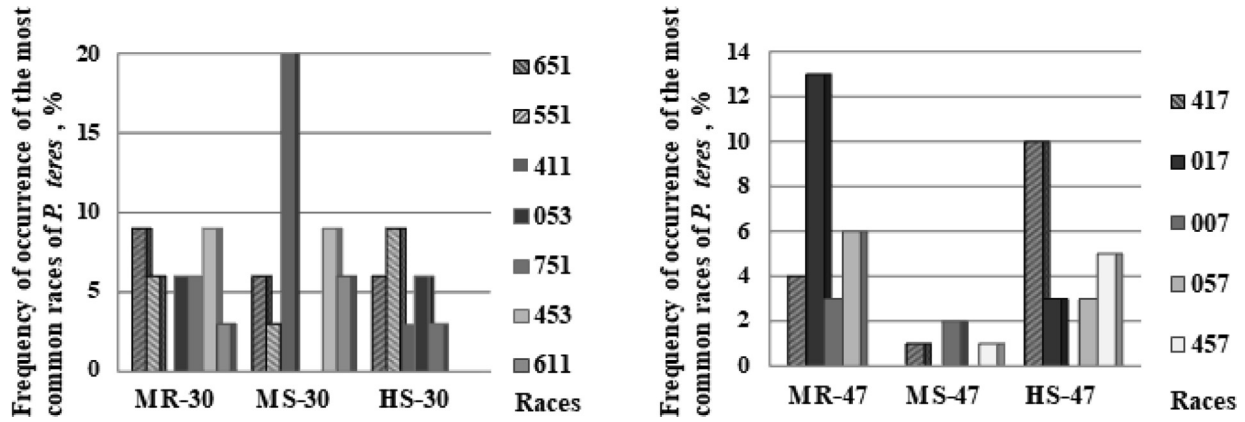

Fig. 4. Frequency of occurrence of the most common races in *Pyrenophora teres* populations collected from barley cultivars of different resistance in BBCH 30 and BBCH 47 growth phases.Note. Races found in populations collected from all barley cultivars are highlighted in black.

the genetic diversity of the host plant (Linde and Smith, 2019). The greatest difference was found between moderately resistant and susceptible cultivars. The Rogers distance indicates moderate changes in the structure of the pathogen in the ontogenesis of resistant and susceptible cultivars (R = 0.43 (HS), R = 0.46 (MR)) and minor changes in the structure of a moderately susceptible cultivar(R = 0.28 (MS)).

4. Discussion

We found the following pattern in pathogen populations collected from cultivars in both phases of plant development: an increase in the growth rate of pathogen colonies with an increase in plant resistance. Fungus colonies growth rate selected from resistant cultivar MR-30 is 1.2 times higher than that of HS-30. The growth rate of MR-47 fungus colonies selected from a resistant cultivar is 1.07 times higher than that of HS-47 colonies. It stands to mention that in the earlier phase of the BBCH 30 growth stage, the difference in the growth rate of *P. teres* MR-30 and HS-30 colonies was 1.1 times higher compared to that of the BBCH 47. At the same time, the difference between the average growth rate of fungal colonies in both phases was small (8.4 mm per day for BBCH 30; 8.1 mm per day for BBCH 47) ($F_t4.2 < F_f67.1$). The two-way analysis of variance determined differences between populations in growth phases ($F_t4.1 < F_f14.3$) and varietal resistance ($F_t3.2 < F_f44.9$). Thus, we concluded that isolates selected from less pathogen resistant cultivars and isolates selected in a later phase of host plant development had a lower colony growth rate in pure culture. Presumably, this derives from the fact that coevolution of the parasite and the host plant ensures synchronization of the biological rhythm of organisms, determining the maximum aggressiveness of the parasite during the most susceptible stage of the host (Westwood et al., 2019).

The sporulation intensity of *P. teres* colonies isolated from the medium susceptible cultivar MS-30 is 3.3 times higher than that from the susceptible cultivar HS-30; and from the resistant cultivar MR-30 it is 5.4 times higher than that from from the susceptible HS-30. The sporulation intensity of the HS-47 fungus colonies from the susceptible cultivar is 1.7 times higher than that of MS-47 colonies; and the sporulation of MR-47 colonies is 4.0 times higher than that of HS-47 colonies. The two-way analysis of variance revealed differences between populations both in growth phases ($F_t4.1 < F_f1811.0$) and varietal resistance ($F_t3.2 < F_f2145.0$). Pairwise comparison of the sporulation intensity of colonies collected at different growth phases of the host plant also showed significant differences. A consistently high ability to spore formation was noted in *P. teres* populations from a moderately resistant cultivar in both phases of host plant development (Fig. 5).

The correlation between the colonies growth rate and *P. teres* sporulation intensity in the BBCH 30 growth phase is moderate (r = 0.4). We register no such correlation (r = 0.06) in the fungus population collected from barley plants in the BBCH 47 growth phase. Kazakhstan researchers did not find a dependence of the colonies growth rate and sporulation intensity of isolates on the regional location. Instead, a direct negative correlation was found between the diameter of colony growth and the intensity of sporulation (Pakhratdinova et al., 2017). Other researchers do not note any correlation between *P. teres* colonies growth rate and sporulation capability of the isolates collected from zones differing in agroclimatic conditions (Baturo-Ciesniewska et al., 2012). In these studies, the growth phase of the host plant was not taken into account. Some researchers at the end of the 20th century suggested that the morphological and cultural characteristics of fungi are of little importance in plant pathology and plant breeding because they very rarely correlate with pathogenicity or virulence (Kiraj et al., 1970). An analysis of modern published data shows that the morphological and cultural characteristics of fungi, especially sporulation in *in vitro* culture, are an important indicator used as a criterion for assessing the harmfulness of the disease, resistance of cultivars and predicting pathogen development (Baturo-Ciesniewska et al., 2012; Pakhratdinova et al., 2017). Consequently, we assume that the resistant cultivar is a survival strategy potential activator for a phytopathogen, provoking sporulation intensification.

When studying the duration of the latent period of *P. teres* development, we also note that a pathogen population harvested at the BBCH 30 growth stage, after inoculated, results in a greater area of leaf surface damage than a population harvested at a later stage of plant development. The two-way analysis of variance revealed differences between populations both in growth phases ($F_t4.1 < F_f4.9$) and varietal resistance ($F_t3.3 < F_f7.5$). For the BBCH 30 growth stage, the average value of plant damage on day 5 by the MR-30 population is 4 times greater than HS-30; for the BBCH 47 growth stage, the average damage by the MR-47 population is 12 times greater than HS-47.

Thus, the correlation analysis of *P. teres* populations collected from barley cultivars with different degrees of resistance and at different stages of plant ontogeny growth revealed a direct proportional relationship between the resistance of the cultivar and the aggressiveness of the pathogen population. The greatest inverse relationship between the latent period and plant damage is observed in the early stages of plant ontogenesis. This poses an

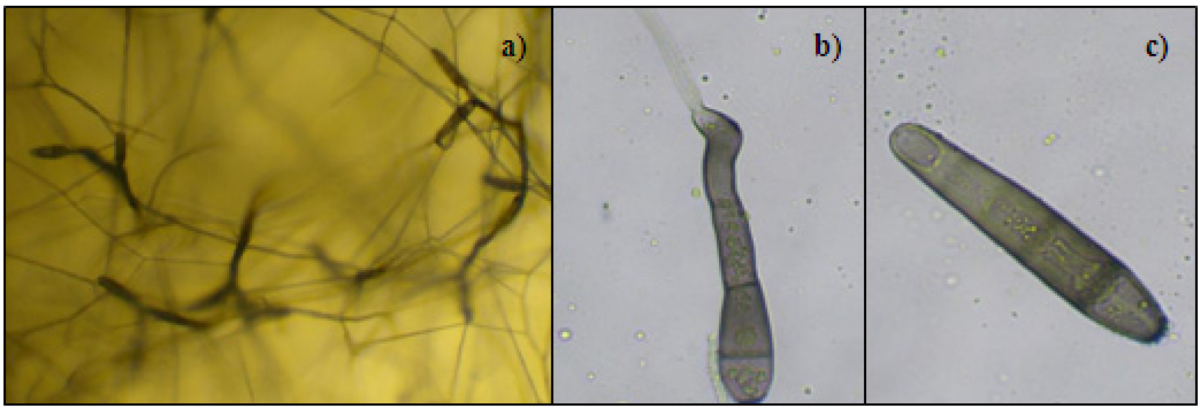

Fig. 5. a) *Pyrenophora teres* start of sporulation; b) growth of *Pyrenophora teres* conidia on a conidiophore c) 4-septate *Pyrenophora teres* conidia.

important problem of using integrated plant protection to avoid the accumulation of aggressive *P. teres* clones. Some other Russian researchers (Sheshegova and Shchennikova, 2020) came to similar conclusions, finding that there was a weak negative relationship between the length of the latent period and harmfulness of the disease (r = −0.33).

The high diversity of the racial composition of the *P. teres* population collected from a moderately resistant cultivar indicates a high evolutionary adaptability of the pathogen to overcome host plant resistance. The maximum virulence was observed in the population of the pathogen isolated from a moderately susceptible cultivar (25.0 %), and the lowest one (16.7 %) – in the fungus population from a highly susceptible cultivar in the BBCH 30 growth stage. Statistical analysis showed a high linear negative correlation between the damage degree of the host plant and the average virulence of the pathogen (r = −0.84). Plant damage degree and average virulence correlate weakly (r = 0.14) in the BBCH 47 growth stage.

The authors found a moderate negative correlation between the plant damage degree and the number of *P. teres* races isolated from populations in the BBCH 30 stage (r = −0.59), a high negative correlation in the BBCH 47 (r = −0.8). We did not find races identical to the BBCH 30 growth stage in the BBCH 47*P. teres* populations. Race №. 417 was noted in all populations of *P. teres* collected from barley cultivars at the BBCH 47growth stage, which amounted to 5 %. In populations MR-47 and HS-47, MR-47 and MS-47, two matching races were identified. In the MS-47 and HS-47populations, identical races were identified in 3 cases. According to Shannon's diversity index, the highest heterogeneity was found in the populations selected for the BBCH 30 growth stage (for MR-30 Sh, 2.77; for MS-30 Sh, 2.28; for HS-30 Sh, 2.24). It is noted that the highest indicators of genetic diversity were found in the most resistant cultivar. With a decrease in the resistance of the host plant, the index of genetic diversity of the pathogen decreases. A similar dynamics of increasing population heterogeneity with increasing cultivar stability is observed in the BBCH 47phase (MR-47 Sh – 2.14; for MS-47 Sh – 1.99; for HS-47 Sh – 1.59). On average, in the later phase of the host plant ontogenesis, there is less genetic diversity of the pathogen.

Comparison of the number of *P. teres* races in the two phases of host plant ontogeny revealed a 31 % decrease in the number of races in the population collected in the BBCH 47 stage compared to the earlier growth stage of BBCH 30. Pathogen populations HS-30 and HS-47, isolated from a highly susceptible cultivar, had a lower racial diversity, which may indicate both a decrease in the aggressiveness of the pathogen population in the ontogeny of the host plant and the elimination of the least physiologically adapted races. We determined that the populations selected from a moderately resistant cultivar coincided in racial composition with races of less resistant cultivars in 89 % of cases for the growth stage of BBCH 30 and in 40 % of cases at the growth stage of BBCH 47.

This current study confirms the need for the widespread introduction of polygenic cultivars in barley production crops, and also supplement the data on the need for extensive use of not only highly resistant, but also more susceptible cultivars to diseases. Analyzing the obtained data, we conclude that it is necessary to introduce an extended range of resistance genes into new cultivars at both juvenile and adult stages.

5. Conclusion

1. We found that the growth rate of colonies of isolates selected from a cultivar more resistant to *P. teres* is statistically higher than the growth rate of colonies from a susceptible cultivar. At the BBCH 30 stage, the growth rate of the population selected from the resistant cultivar was 1.2 times higher than that of the susceptible cultivar (by 1.4 mm per day). At the BBCH 47 stage, the difference between the growth rates was not high, but still statistically significant and amounted to 1.07 times (by 0.4 mm per day). In the earlier BBCH 30 stage, the difference in the growth rate of *P. teres* MR-30 and HS-30 colonies was 1.1 times higher compared to the BBCH 47 stage.
2. We discovered that sporulation intensity of *P. teres* colonies selected from a more resistant cultivar is statistically higher than that of fungus colonies selected from a susceptible cultivar. At the BBCH 30 stage, the sporulation intensity of the P. teres population selected from the resistant cultivar was 5.4 times higher than that of the population from the susceptible cultivar. It is 1.3 times higher than the difference in sporulation of the colonies selected at the BBCH 47 growth stage (4.0 times).
3. A moderate correlation was noted between colony growth rate and sporulation intensity of the *P. teres* population at the BBCH 30 growth stage (r = 0.4). Fungus population of the BBCH47 growth stage demonstrated no such correlation (r = 0.06).
4. A high correlation level (r = 0.9) and notable difference between the variants were revealed when studying the duration of the latent period. The difference between the inoculum selected from resistant and susceptible cultivars was 1 day for the BBCH 30, and 3 days for the BBCH 47. The average value of plant damage by the *P. teres* from resistant cultivar is 4 times higher than from the susceptible cultivar in the BBCH 30 stage; and 12 times – in the BBCH 47 stage.

5. There is a moderate negative correlation between the plant damage degree and the number of races identified from the fungal population, r = −0.59 for the BBCH 30, r = −0.8 for the BBCH 47.
6. *P. teres* isolates selected from barley plants in different phases of ontogeny growth belonged to different mismatched races. The highest population heterogeneity was observed at an earlier phase of plant development. With an increase in the resistance of a cultivar, an increase in heterogeneity occurs. Later growth stages mark a decrease by one third in the number of pathogen races.
7. Structure study of *P. teres* populations at various development phases helped to acquire new knowledge about intrapopulation processes under the influence of various factors – plant growth stage and cultivar genotype. The results obtained are the basis for the development of adaptive-integrated techniques for managing populations of the hemibiotrophic pathogen, barley net blotch.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the State Assignment of the Ministry of Science and Higher Education of the Russian Federation within the framework of research on the topic No FGRN-2022-0004.

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