Modification in the pathogenicity of the *Pyrenophora teres* population under the influence of fungicides of the triazole and strobilurin classes

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Abstract. The research examined the effects of fungicides of chemical classes (triazole and strobilurine) (Amistar Gold, SC, 1 l/ha; Amistar Extra, SC, 1 l/ha; Delaro, CS, 1 l/ha) on the pathogenicity of the causative agent of net blotch of barley (ascomycetes Pyrenophora teres). A change in the aggressiveness of the fungal population was revealed after the introduction of fungicides in various concentrations into the nutrient medium according to the following indicators: the diameter of colonies of the fungus P. teres (Amistar Gold, SC – 33.1 mm, Amistar Extra, SC – 32.1 mm, Delaro, CS – 31.9 mm, control – 27.0 mm), a decrease in the latency period (Amistar Gold, SC – 5.1 days, Amistar Extra, SC – 5.6 days, Delaro, CS – 4.8 days, control – 6.2 days), and average virulence of populations (Amistar Gold, SC – 2.8 points, Amistar Extra, SC – 2.4 points, Delaro, CS – 1.9 points, control – 3.6 points) in comparison with the variant without fungicides treatments. The suppression of the physiological processes of the phytopathogenic fungus, a decrease in the virulence of the population and a pathological acceleration of the processes of mushroom maturation under the influence of fungicides based on triazoles and strobilurines were determined.

1 Introduction

Barley is one of the main grain crops grown in the world, the production and sales of which, according to FAOSTAT forecasts, have an annual upward trend (FAOSTAT, 2024). In the conditions of the Russian Federation, due to the predominance of a cold climate, the winter form of barley accounts for only 10-13%, but the expediency of cultivating winter barley in comparison with other forms and crops is due to a number of advantages, such as high stable yields, grain quality, optimal maturation periods, unpretentiousness of the crop, etc. [1, 2]. To build an effective system of protection of winter barley from diseases, methods of processing seed material and vegetative plants with fungicides based on various chemical classes are most widely used in the country. According to the State Catalog of Pesticides and Agrochemicals approved for use on the territory of the Russian Federation (dated

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05/14/2024), 188 fungicides have been approved and recommended for use on winter barley against the dominant pathogenic complex, net blotch of barley (the causative agent of the disease is *Pyrenophora teres* Drechsler ascomycete). Modern technological schemes for the protection of grain crops include mandatory processing of seed material and several treatments for vegetation [3]. Nevertheless, in industrial agrocenoses, the emergence and accumulation of pesticide-resistant strains of phytopathogens is everywhere observed, which leads to an increase in consumption rates, multiplicity, and a decrease in the effectiveness of both widely used and new fungicides due to the appearance of cross-resistance [4].

Preparations containing triazoles and strobilurines are most widely used in protective schemes of agricultural barley crops in the territory of the Russian Federation [5]. Despite the fact that the use of fungicides based on strobilurines and triazoles made it possible to ensure phytosanitary stability of crops at the end of the 20th century, in recent decades the accumulation of species with multiple resistance to fungicides in agrocenoses has led to a decrease in the effectiveness of standard protective measures, and also necessitated the withdrawal from industrial circulation of entire classes of fungicides and the introduction of chemicals with fundamentally new action mechanisms [6, 7]. Older fungicides (for example, based on copper or sulfur) affect many target sites of plant pathogens, being multisite inhibitors, which significantly slows down the appearance of resistant strains of pathogenic microorganisms [8]. Modern systemic fungicides act mainly on individual target sites, so just one gene mutation can cause a change in the target site, which leads to the rapid emergence and subsequent consolidation of resistance. To avoid destabilization of the phytosanitary condition of production crops, it becomes necessary to study the effect of different standards of application of fungicides based on triazoles and strobilurines recommended by manufacturers on the pathogenicity signs of the regional *P. teres population.* The data obtained will be used in the preparation of protection schemes for winter barley, considering the anti-resistant strategy of using fungicides with various mechanisms of action.

The purpose of the study was to determine the pathogenicity indicators (aggressiveness and average virulence) of *P. teres* fungi populations isolated from barley plants after treatment with fungicides based on triazoles and strobilurines in different application standards from those recommended by manufacturers.

2 Materials and Methods

The affected leaves with symptoms of net blotch of barley were selected from winter barley production crops in the Krasnodar Territory in 2023. The isolation, identification, and reproduction of the pathogen was carried out using the scientific equipment «Technological line for obtaining microbiological plant protection products of a new generation» (https://ckp-rf.ru/catalog/usu/671367/). No more than two leaves were used from the plant [9]. After drying at 20°C, stains (5×10 mm) were cut from the leaves, the surface was disinfected with 1% sodium hypochlorite solution for 2 minutes, then washed 3 times with sterile water for 2 minutes and dried on sterilized filter paper. The disinfected plant areas were placed on carrot-beet agar (120 ml of beetroot, 120 ml of carrot, 20 g of agar per 1 liter of water) and incubated for 5 days at a temperature of 23°C under ultraviolet lamps (30 W UVB 280-315 nm) [9]. After 5 days, one conidia from one spot was transferred with a sterile needle to a dish with beetroot-carrot nutrient medium for reproduction.

Plants of the Romans variety susceptible to the pathogen were sown in 0.5 l pots of 10-12 plants per pot and placed in the conditions of the Binder KBWF 720 climatic chamber (temperature +25.0°C, humidity 80%, fluorescent lamps 13000 lux, spectral distribution (250-785 nm)). Inoculation was carried out in the phase of 2-3 leaves, treatment with preparations was carried out after 3 days [7]. Widely used chemical fungicides – derivatives of triazoles and strobilurines were selected: Amistar Gold, SC, 1 l/ha (azoxystrobin 125 g/l

+ diphenoconazole 125 g/l); Amistar Extra, SC, 1 l/ha (azoxystrobin 200 g/l + ciproconazole 80 g/l); Delaro, CS, 1 l/ha (prothioconazole 175 g/l + trifloxystrobin 150 g/l). The study was carried out using five treatment options with preparations with different rates of use as a percentage of those allowed in agriculture: 0% (control without fungicide), 50%, 100% (option with a rate of use allowed in agriculture), 150%, 200%.

After treatment, a pure culture of the *P. teres* pathogen was isolated from barley plants and the parameters of aggressiveness (colony diameter, latency period, population sporulation) and virulence were studied. Repeated infection with isolated and propagated by inoculum *P. teres* was carried out to determine the development of barley seedling disease after inoculation by various populations and to identify the incubation period of each studied population of the fungus.

To determine the degree of plant damage and the incubation period of each of the experimental populations, 5 plants of the susceptible Romans variety were sown in 3 pots (0.25 ml). After inoculation, the plants were placed in a humid chamber in the dark for 24 hours, then transferred to the KBWF 720 climate chamber. The degree of plant damage was considered after 7 days, the incubation period was determined by the first symptoms of the disease on a standard scale [9].

In a pure culture of the *P. teres* fungus, the diameter was determined by transferring mycelial discs to carrot-beet agar, incubating for 7 days at a temperature of 23°C and measuring the colony diameter. The intensity of sporulation was studied after incubation of a pure culture of the pathogen for 10 days under ultraviolet lamps, then the sporulation of each experimental variant was calculated. Isolates with a concentration of 2×10^3 conidia in 1 ml were classified as weakly regulated, to medium-regulated - 5×10^3 conidia in 1 ml, to strongly regulated - 7×10^3 conidia in 1 ml.

The determination of the average virulence of *P. teres* populations isolated after treatments that differ from the recommended standards was carried out according to a generally accepted method using an international set of differentiating varieties [10]. The racial composition was characterized using the octal system. The reaction types, estimated in points in the range from 1 to 5, were attributed to resistance, from 5.1 to 10 – to susceptibility. Each of the three classes was assigned a value of 4, 2, and 1, respectively. If the value "virulence" is set, then the triplet data is summarized. They have three numbers on them, which are the name of the race.

Three repetitions were used in each experimental version. The statistical difference of the samples was evaluated using the Fisher criterion (α=0.05). The value of average virulence was determined as the average score of disease development in differentiator varieties. The calculation was performed using the Statistica software version 13.3 (http://statsoft.ru/products/trial/).

3 Results and Discussion

After inoculation of winter susceptible barley seedlings with populations isolated from plants after fungicide treatments, it was found that the development of the disease was statistically lower in experiments using the drugs Amistar Gold, SC (F_f 36.2>F_t 10.1) and Amistar Extra, SC (F_f 121.0>F_t 10.1) (Table 1, Fig. 1). The development of the disease after inoculation by populations isolated after treatment with Delaro, SC, on average did not significantly differ from the control variant (F_f 0.23>F_t 9.3). Correlation analysis revealed an inverse linear relationship between experimental variants of isolated pathogen populations after treatment with preparations with azoxystrobin and the norm of fungicide use (r= -0.95 (Amistar Gold, SC); r= -0.66 (Amistar Extra, SC)). Despite its widespread use, this active substance remains highly effective against the fungus *P. teres* [11, 12]. A moderate positive correlation was found between populations isolated after plant treatment with different standards of the

preparation Delaro, CS from those recommended by manufacturers, the development of plant disease (r= 0.43). Delaro, CS fungicide is a two-component based on active substances from the chemical classes triazoles (protioconazole) and strobilurines (trifloxystrobin). According to the literature, mutations occurring under the action of a protioconazole-based preparation do not reduce the sensitivity of the *P. teres* fungus population to fungicides, which implies an increase in the heterogeneity of the population without the action of stabilizing selection [13]. Thus, it was determined that treatment with fungicides Amistar Gold, SC (average disease development score of 2.8 points), Amistar Extra, SC (average disease development score of 2.0 points) suppresses the physiological processes of the phytopathogenic fungus *P. teres*. A decrease in the aggressiveness of the pathogen was revealed in comparison with the control variant, the development of the disease after inoculation of which was 5.0 points.

Table 1. The development of the disease after inoculation with populations of *Pyrenophora teres* isolated from plants after treatment with fungicides of various standards of use, the Romans variety (laboratory complex of the FSBSI FRCBPP, 2024).

Fungicide	Disease development, score					
	0 %*	50 %	100 %	150 %	200 %	Average value
Amistar Gold, SC	5.0±0	3.8±0.47	3.0±0	2.3±0.47	2.3±0.47	2.8±0.62
To control, %	-	75.0	60.0	45.0	45.0	56.3
Amistar Extra, SC	5.0±0	1.5±0.24	2.4±0.47	2.5±0.47	1.5±0	2.0±0.48
To control, %	-	30.0	48.0	50.0	30.0	39.5
Delaro, SC	5.0±0	3.0±0.47	6.8±.94	6.6±0.82	5.3±0.47	5.4±1.5
To control, %	-	60.0	135.0	132.0	105.0	108.0

* – the rate of application from the permitted in agriculture, %

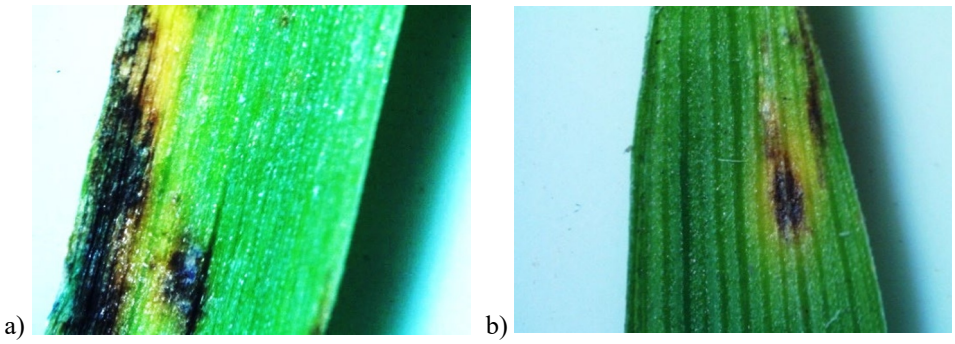

Fig. 1. The development of the disease after inoculation with populations of *Pyrenophora teres* isolated from plants after treatment with fungicides in the norm recommended by manufacturers: a) Delaro, SC; b) Amistar Extra, CS (7 days after inoculation, mag. * 4, orig. Yakhnik, FSBSI FRCBPP, 2024).

One of the main criteria for the aggressiveness of populations is the duration of the incubation period, that is, the length of time from the moment of infection to the manifestation of the disease symptoms. Statistically significantly more time passed before the development of the first symptoms of the disease after inoculation by the population isolated from the plant after treatment with the fungicide Amistar Gold, SC. The average value was 4.7 days with the development of disease symptoms in the control variant 4.2 days after inoculation (F_f 49.9>F_t 10.1) (Table 2). The incubation period after inoculation with populations isolated after treatment with Amistar Extra, SC (F_f 0.52>F_t 9.3) and Delaro, CS (F_f 0.6>F_t 9.3) did not reveal statistically significant differences. A correlation was found between an increase in the consumption rate of the fungicide and the incubation period of *P.*

teres populations in experiments with Amistar Gold, SC (r=0.37) and Amistar Extra, CS (r=0.78), no correlation was found for Delaro, SC (r= -0.02).

Table 2. The incubation period of the *Pyrenophora teres* population isolated from plants after treatment with fungicides of different concentrations, the Romance cultivar (laboratory complex of the FSBSI FRCBPP, 2024).

Fungicide	Incubation period, day					
	0 %*	50 %	100 %	150 %	200 %	Average value
Amistar Gold, CS	4.2±0.24	4.8±0.24	4.7±0.24	4.8±0.47	4.5±0.41	4.7±0.12
To control, %	-	115.1	111.1	115.1	107.1	112.1
Amistar Extra, SC	4.2±0.24	4.0±0	4.3±0.24	4.3±0.47	4.5±0.62	4.3±0.18
To control, %	-	95.2	103.2	103.2	107.1	102.2
Delaro, CS	4.2±0.24	4.8±0.24	3.3±0.33	3.5±0.41	4.8±0.47	4.1±0.7
To control, %	-	115.1	79.4	83.3	115.1	98.2

* – the rate of application from the permitted in agriculture, %

It was found that fungicide treatments had a statistically significant effect on the growth of isolated colonies of the fungus *P. teres.* The population isolated after treatment with Amistar Gold, SC, on average exceeded the diameter of the control variant colonies (isolated from plants without treatment) by 24.4% (F_f 703.2>F_t 7.7), with Amistar Extra, SC – by 20.6% (F_f 57.4>F_t 7.7), with Delaro, CS – by 20.0% (F_f 31.3>F_t 7.7) (Table 3). The maximum growth rates of fungal colonies were revealed in the experiment with the drug Amistar Gold, SC, the average value of all variants was 33.1 mm. An increase in the rate of preparation consumption correlated with the growth of colonies of isolated populations in all cases (Amistar Gold, SK r= 0.6; Amistar Extra, SC r=0.48; Delaro, CS r=0.57). According to the literature, the effect of various stress factors triggers the protective mechanisms of living organisms, as a result of which such phenotypic changes as accelerated maturation and increased toxin formation are recorded, which probably caused the acceleration of mycelium growth of a phytopathogenic fungus in comparison with the variant without the introduction of toxicants [13, 14].

Table 3. The diameter of colonies of *Pyrenophora teres* populations isolated from plants after treatment with fungicides of various application standards (laboratory complex of the FSBSI FRCBPP, 2024).

Fungicide	Diameter of colonies, mm					
	0 %*	50 %	100 %	150 %	200 %	Average value
Amistar Gold, SC	26.6±1.02	33.7±0.47	33.3±0.94	32.7±0.47	32.7±0.94	33.1±0.42
To control, %	-	126.6	125.3	122.8	122.8	124.4
Amistar Extra, SC	26.6±1.02	32.7±0.94	31.7±0.94	33.7±0.47	30.3±0.47	32.1±1.26
To control, %	-	122.8	119.0	126.6	114.0	120.6
Delaro, CS	26.6±1.02	33.3±0.94	31.0±0.82	29.7±0.94	33.7±0.94	31.9±1.6
To control, %	-	125.3	116.5	111.5	126.6	120.0
Average to control, %		124.7	117.8	119.4	121.2	-

* – the rate of application from the permitted in agriculture, %

P. teres populations isolated after fungicide treatments were significantly different from the control variant in terms of sporulation intensity in pure culture (Table 4). After treatment with Amistar Gold, SC (F_f 17.9>F_t 10.1), the average sporulation value was $4.0*10^3$ conidia in 1 ml of solution. After treatment with Delaro, CS (F_f 18.2>F_t 10.1), the average sporulation

value was detected at the level of $3.1*10^3$ conidia in 1 ml. There were no significant differences in comparison with the control variant in the cups with populations isolated after plant treatments with fungicides Amistar Extra, SC (F_f 3.03>F_t 10.1). The average sporulation value was 5.4 $*10^3$ conidia in 1 ml. All experimental variants, including control, were classified as medium-sporulating. With an increase in the consumption rate of preparations in the experimental variants, an inverse correlation was observed with the intensity of sporulation of *P. teres* fungus colonies isolated from plants (Amistar Gold, SC r= -0.48; Amistar Extra, SC r= -0.85; Delaro, CS r= -0.79).

Table 4. Sporulation of the *Pyrenophora teres* population isolated from plants after treatment with fungicides of different concentrations (laboratory complex of the FSBSI FRCBPP, 2024).

Fungicide	Conidium in 1 ml $*10^3$, pcs.					
	0 %*	50 %	100 %	150 %	200 %	Average value
Amistar Gold, SC	5.8±0.82	4.7±0.47	3.0±0	3.5±0.47	4.7±0.47	4.0±0.75
To control, %	-	80.5	52.3	60.3	80.5	68.4
Amistar Extra, SC	5.8±0.82	5.8±0.47	5.8±0.47	5.4±0.41	4.7±0.43	5.4±0.45
To control, %	-	100.6	100.6	92.5	80.5	93.5
Delaro, CS	5.8±0.82	4.7±0.94	1.9±0.24	3.5±0.47	2.3±0.09	3.1±1.1
To control, %	-	80.5	52.3	60.3	40.2	53.3

* – the rate of application from the permitted in agriculture, %

The latency period required by the pathogenic organism to reinfect the host was observed on average in all experimental variants below the control, which ranged from 79.9% to 86.7% of the control variant. The data indicate an acceleration of physiological processes during the microevolutionary process caused by the toxic effects of fungicides (Table 5, Fig. 2). The variance analysis revealed differences in experimental variants when culturing populations isolated after treatment with fungicides Amistar Gold, SC (F_f 12.1>F_t 10.1), Amistar Extra, SC (F_f 10.4>F_t 10.1) and Delaro, CS (F_f 27.3>F_t 10.1) on nutrient medium. An inverse correlation between the consumption rate of the fungicide and the latency period was revealed in experimental versions with the drugs Amistar Gold, SC (r= -0.69) and Delaro, CS (r= -0.73). There was no correlation between the latency period of populations isolated after treatment with Amistar Extra, SC, and the rate of preparation consumption (r= -0.03).

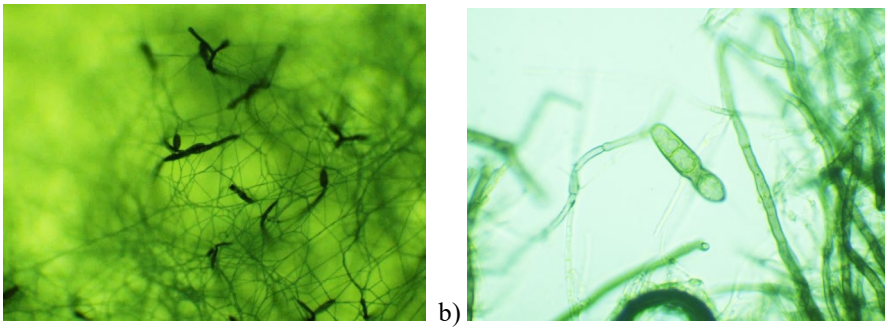

Fig. 2. The onset of sporulation (a, mag. 4/0.10) and sporulation (b, mag. 10/0.25) of colonies of *Pyrenophora teres* fungus isolated from plants after treatment with Amistar Extra, SC fungicide, was 150% of the norm recommended by manufacturers (orig. Yakhnik, FSBSI FRCBPP, 2024).

Table 5. The latent period of *Pyrenophora teres* populations isolated from plants after treatment with fungicides of various application standards (laboratory complex of the FSBSI FRCBPP, 2024).

Fungicide	Incubation period, day					
	0 %*	50 %	100 %	150 %	200 %	Average value
Amistar Gold, SC	6.2±0.24	5.2±0.24	5.8±0.47	4.2±0.47	5.0±0.82	5.0±0.57
To control, %	-	83.3	94.1	67.2	80.6	81.3
Amistar Extra, SC	6.2±0.24	5.0±0.41	5.8±0.47	5.7±0.71	5.8±0.47	5.6±0.33
To control, %	-	80.6	94.1	91.4	94.1	90.1
Delaro, CS	6.2±0.24	5.5±0.62	4.2±0.24	4.7±0.24	4.8±0.82	4.8±0.46
To control, %	-	88.7	67.2	75.3	78.0	77.3

* – the rate of application from the permitted in agriculture, %

The average virulence of *P. teres* fungi populations isolated from plants after treatment with fungicides with different application standards from those recommended by manufacturers in the control variant was 3.6 points (Fig. 3). Also, at the control level, the virulence of the population isolated after treatment with Amistar Gold, SC was revealed, with a norm recommended by manufacturers of 3.6 points, which may be characterized by the appearance of strains resistant to difeconazole in the pathogen population [15]. In the remaining experimental variants, the average virulence of populations isolated after treatment with preparations in different from the recommended standards of use by manufacturers was found to be lower than the control variant. In the experimental version with the preparation Amistar Gold, SC the average virulence was 2.8 points (77.9% of the control variant), Amistar Extra, SC – 3.0 points (84.1% of the control variant), Delaro, CS – 1.9 points (52.5% of the control variant). A negative correlation was found between the rate of preparation consumption and average virulence in all experimental variants (Amistar Gold, SC r= -0.72; Amistar Extra, SC r= -0.38; Delaro, CS r= -0.66). Low doses of toxicants are one of the factors of the emergence and consolidation of resistant strains in local agrocenoses, since genetic variability in the population includes differences in sensitivity [16]. Despite the fact that individual mutations provide only partial resistance, which is phenotypically reflected in the low virulence of the population, an increase in heterogeneity leads to the risk of more resistant to fungicides and aggressive pathogens.

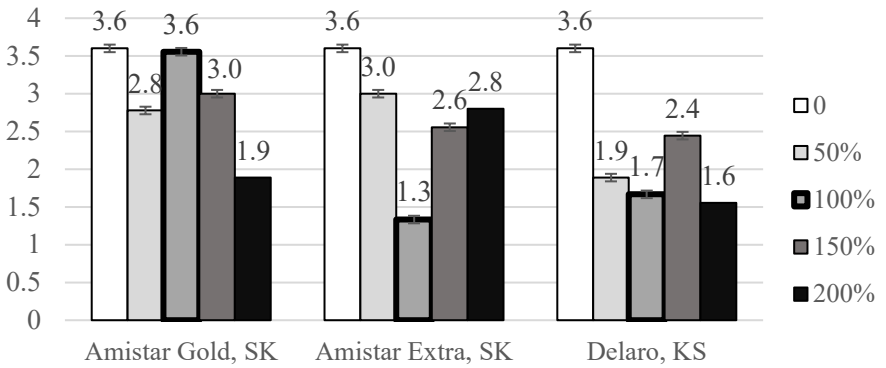

Fig. 3. The value of the average virulence of *Pyrenophora teres* populations isolated after plant treatment with fungicides (laboratory complex of the FSBSI FRCBPP, 2024).

4 Conclusion

As a result of the research conducted to study the effect of triazole and strobilurine class fungicides on the pathogenicity of the *P. teres* fungus, it was revealed that after treating plants with fungicides different from the recommended norms, the diameter of colonies isolated from *P. teres* plants significantly increases (Amistar Gold, SC – 33.1 mm; Amistar Extra, SC – 32.1 mm; Delaro, CS – 31.9 mm; control – 27.0 mm) and the latency period decreases (Amistar Gold, SC – 5.1 days; Amistar Extra, SC – 5.6 days; Delaro, CS – 4.8 days; control – 6.2 days). *P. teres* populations isolated from plants after pesticide treatments have a lower average virulence (Amistar Gold, SC – 2.8 points; Amistar Extra, SC – 2.4 points; Delaro, CS – 1.9 points) compared with the control (3.6 points).

A direct correlation was found between the growth of colonies of the *P. teres* fungus and an increase in the rate of use of drugs (Amistar Gold, SC r=0.6; Amistar Extra, SC r=0.48; Delaro, CS r=0.57). Sporulation intensity (Amistar Gold, SC r= -0.48; Amistar Extra, SC r= -0.85; Delaro, CS r= -0.79) and average virulence (Amistar Gold, SC r= -0.72; Amistar Extra, SC r= -0.38; Delaro, CS r= -0.66) isolated from plants after fungicide treatments of populations with an increase in the rate of use, it decreased.

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