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ЧУВСТВИТЕЛЬНОСТЬ СЕВЕРОКАВКАЗСКОЙ И БЕЛОРУССКОЙ ПОПУЛЯЦИЙ *MICRODOCHIUM NIVALE* (FR.) SAMUELS & HALLET К ФУНГИЦИДАМ

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Розовая снежная плесень (возбудитель *Microdochium nivale*) – наиболее распространенный во всем мире низкотемпературный патоген. Изучена чувствительность двух географически отдаленных популяций возбудителя розовой снежной плесени (юга России и Республики Беларусь) к девяти современным фунгицидам. Для исследования отобраны фунгициды, включенные в Государственный каталог пестицидов и агрохимикатов, разрешенных к применению на территории Российской Федерации и рекомендуемые для обработки против снежной плесени. Материалом для изучения служила чистая культура гриба *M. nivale*. В исследовании использован метод агаровых блоков. Внесение растворов фунгицидов в питательную среду осуществляли двумя стандартными методами – вмешиванием в среду и растиранием препарата по поверхности среды шпателем. Выявлены препараты, обладающие 100%-м фунгицидным действием против обеих изучаемых популяций: Поларис, МЭ, Кинто Дуо, КС и Баритон Супер, КС. Препараты Оплот Трио, ВСК, Вайбранс Трио, ТКС, Максим Форте, КС показали 100%-ю эффективность только против белорусской популяции патогена. Определено, что применение двух методов внесения препарата в питательную среду (вмешивание и растирание по поверхности агара) имеет высокий коэффициент корреляции (для белорусской популяции – r_{xy}= 1,0, для северокавказской – r_{xy}= 0,99). Однако вмешивание меньше ингибирует рост колоний, поэтому является более предпочтительным в исследованиях по изучению чувствительности к препаратам чистой культуры гриба *M. nivale*. Выявлена статистически достоверная разница между чувствительностью к фунгицидам популяций географически отдаленных регионов (при использовании метода вмешивания F_t 5,32 < F_f 23,2, метода растирания – F_t 5,32 < F_f 37,7). Данные свидетельствуют о гетерогенности возбудителя снежной плесени по чувствительности к современному ассортименту протравителей семян.

Ключевые слова: снежная плесень, выпревание, *Microdochium nivale*, озимые зерновые культуры

SENSITIVITY OF THE NORTH CAUCASIAN AND BELARUSIAN POPULATIONS OF *MICRODOCHIUM NIVALE* (FR.) SAMUELS & HALLET TO FUNGICIDES

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Pink snow rot (pathogen *Microdochium nivale*) is the most common low-temperature pathogen worldwide. Sensitivity of two geographically distant populations of the pink snow rot pathogen (southern Russia and the Republic of Belarus) to nine modern fungicides was studied. The fungicides

included in the State Catalogue of pesticides and agrochemicals permitted for use in the Russian Federation and recommended for treatment against snow rot were selected for the study. The material for the study was a pure culture of the fungus *M. nivale*. The agar block method was used in this study. The fungicide solutions were introduced into the nutrient medium using two standard methods: by interfering with the medium and by rubbing the preparation on the medium surface with a spatula. The preparations with 100% fungicidal effect against both studied populations were identified: Polaris, OE, Quinto Duo, SC and Bariton Super, SC. Oplot Trio, WS, Vybrance Trio, FC, Maxim Forte, SC showed 100% efficacy only against the Belarusian population of the pathogen. It was determined that the use of two methods of introducing the preparation into the nutrient medium (intervention and rubbing on the agar surface) has a high correlation coefficient (for the Belarusian population - $r_{xy} = 1.0$, for the North Caucasian population - $r_{xy} = 0.99$). However, intervention is less likely to inhibit colony growth and is therefore preferable in drug sensitivity studies of pure culture of the fungus *M. nivale*. A statistically significant difference was found between the sensitivity to fungicides of populations from geographically distant regions (F_t *5.32* < F_f *23.2* for the intervention method, F_t *5.32* < F_f *37.7* for the rubbing method). The data indicate the heterogeneity of the snow rot pathogen in terms of sensitivity to the modern assortment of seed dressing agents.

Keywords: snow rot, rotting, *Microdochium nivale*, winter cereals

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Conflict of interest
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INTRODUCTION

Microdochium nivale (Fr.) Samuels & Hallet is the most common low-temperature phytopathogen affecting cereals and grasses in the areas with stable snow cover [1]. In the Republic of Belarus, the disease is one of the most common harmful ones [2]. According to V. Gorshkov et al. [3], in Russia as early as the 1970s, snow rot was considered a disease affecting cereals only in the regions where plants were covered with snow for at least 100 days. In the southern regions of Russia, the pathogen occurred sporadically until 1995; since 2000 the dynamics of its occurrence and development have been observed. At present, the pathogen is widespread throughout the country where winter grain crops are cultivated on an industrial scale.

Effective protection of winter cereal crops against pink snow rot is possible by treating the seeds with fungicidal preparations based on contact active substances, such as fludioxonil and prochlorazole [2]. European researchers also indicate that the only available mechanism of chemical control of the disease in Northern Europe is pre-sowing seed treatment with fungicides [4]. Currently, a number of specialized fungicide seed dressing agents have been developed and approved for use, which can significantly reduce the lesion of plants by the causative agent of pink snow rot [5]. At the same time, there is no effective strategy for protecting crops against pink snow rot due to a number of restrictive measures, such as reducing the list of approved drugs and peculiarities of winter treatment. In our opinion, foliar application of

fungicides to protect winter grain crops from snow rot is difficult to carry out in the autumn period due to the fact that the air temperature during fungicide application should not be below 12 °C, and 10-14 days should pass from the moment of treatment until winter grain crops go into dormancy (autumn cessation of vegetation).

At the same time, the problem of resistance is acute and has great negative consequences in plant protection in the farms of almost all categories [6]. One of the first published data on the loss of *M. nivale* sensitivity to benzimidazoles is the research of the problem of pink snow rot lesion of golf courses. Ten years later, the problem of snow rot resistance to this active ingredient was already widespread [7]. Resistance to strobilurins was first revealed by French researchers when analyzing the causes of epiphytoty in 2007, 2008 [8]. Field tests of strobilurin-based fungicides efficacy were conducted and the formation of several resistance mechanisms as well as positive cross-resistance was found at once. Currently, the dominant position in the market of chemical fungicides is occupied by triazoles, widespread introduction of which has improved the phytosanitary situation in the grain fields. However, the studies of O.P. Gavrilova et al. [9] indicated that 45% of *M. nivale* strains are resistant to triazole-containing preparations. It is worth noting that an increase in the rate of drug consumption leads to the acceleration of the formation of resistant forms of pathogens and to the destruction of useful soil microflora.

In multi-year studies of Belarusian scientists who screened the most common disinfectants, high biological efficacy of Bariton, SC (86.9%), Quinto Duo, SC (92.4%), Maxim Forte, SC (93.4%), and 100% efficiency of Quinto Plus, SC were noted [2]. A sensitivity study of *M. nivale* isolates sampled on wheat crops in the Central Black Earth region of the Russian Federation showed complete inhibition of the colony growth by Maxim, SC and Quinto Duo, SC [5].

The data on the sensitivity of *M. nivale* population to the basic assortment of fungicides in the south of Russia are insufficient and need to be supplemented.

The purpose of the study was to investigate the sensitivity of two geographically distant populations of the pink snow rot pathogen to nine modern fungicides.

MATERIAL AND METHODS

The studies were carried out at the Republican Unitary Enterprise "Plant Protection Institute" (The agrotown of Priluki, Republic of Belarus) and the Federal Scientific Center for Biological Plant Protection (Krasnodar, Russia) in 2021, 2022 using the unique scientific installation (USI) base "Phytotron for isolation, identification, study and maintenance of races, strains, phenotypes of pathogens" (http://ckp-rf.ru/usu/ No. 671925) and BRC (bioresource collection) facilities of the FSBSI FSCBPP (Federal Scientific Center for Biological Protection of Plants) "State Collection of Entomoacariphages and Microorganisms" (USU registration No.: 671925). Climatic conditions were simulated using a Binder KBWF 720 climatic chamber.

Nine fungicides included in the State Catalogue of Pesticides were selected for the study: Maxim Forte, SC (azoxystrobin 10 g/l + tebuconazole 15 g/l + fludioxonil 25 g/l), Vybrance Trio, FC (sedaxane 25 g/l + tebuconazole 10 g/l + fludioxonil 25 g/l), Polaris, ME (imazalil 25 g/l + prochlorazole 100 g/l + tebuconazole 15 g/l), Quinto Duo, SC (prochloraz 60 g/l + triticonazole 20 g/l), Quinto Plus, SC (triticonazole 33, 3 g/l + fludioxonil 33.3 g/l + fluxapiroxad 33.3 g/l), Bariton Super, SC (protioconazole 50 g/l + tebuconazole 10 g/l + fludioxonil 37.5 g/l), Scarlet, ME (imazalil 100 g/l + tebuconazole 60 g/l), Oplot Trio, WSC (azoxystrobin 40 g/l + difenoconazole 90 g/l + tebuconazole 45 g/l), Credo, SC (carbendazim 500 g/l)[1].

The material was a pure culture of the fungus *M. nivale*. Monospore cultures were obtained, followed by a mixture of isolates from each region. The species identity of the isolates was

[1]State Catalogue of pesticides and agrochemicals permitted for use in the Russian Federation. Ministry of Agriculture of the Russian Federation. Pesticides. Official publication. M., 2021. vol. 1. 795 p.

determined using molecular methods. The belonging of the strains to *M. nivale* species was established using real-time PCR with species-specific primers [10, 11]. PCR was performed using a CFX96 Real-Time System thermal cycler (BioRad, USA). Pure culture of pink snow rot pathogen was screened on potato-glucose agar. The fungus was cultured at 10-15 °C (12 h) under 30W UVB lamps (280-315 nm). When screening fungicides, we used a modified agar block method. Calculation of drug amounts and preparation of their working solutions was performed using the Chekmarev calculation method according to the recommended norms of application for seed material treatment with standard working fluid consumption [12]. Fungicide solutions were introduced into the nutrient medium using two standard methods: by kneading in the medium and by rubbing the preparation on the medium surface with a spatula.

Statistical processing was performed using Statistica 13.3 software; the differences between the samples were evaluated using Fisher's criterion (with $a = 0.05$); the relationship between the features was calculated using the Cheddock scale. Biological efficacy was calculated using the generally accepted Abbott's formula on day 7 for inhibition of pathogen mycelial growth on solid nutrient medium[2].

RESULTS AND DISCUSSION

Complete inhibition of the growth of the Belarusian pathogen population colonies when the preparation was applied to the medium by interference was found in most fungicides (see Table 1); 100% suppression by the fungicide containing the active substance fludioxonil was also observed in the studies of *Microdochium* fungi strains isolated from cereals and grasses of different geographical origin [9]. Scarlet, ME caused incomplete inhibition of colony growth with a biological efficacy of 97.6%. The drug contains imazalil, a substance of imidazole class. The active ingredient is highly dangerous for aquatic biocenoses and toxic to humans, but it is distinguished by high activity against helminthosporiosis and fusarium rot of grain crops, as well as high activity against pathogens resistant to benzimidazole [13]. Imazalil-containing preparations have a synergistic effect against difficult to control diseases that are transmitted both through seeds and soil, but their presence in a preparation with other active substances with different mechanism of action reduces the risk of resistant strains of phytopathogens [14]. Tebuconazole, a third-generation triazole fungicide, is an effective systemic fungicide for seed pre-sowing treatment of cereal crops. It is important to note that tebuconazole-based products slow down the rate of resistance development to the whole group of triazoles. Biological efficacy of the preparation Credo, SC was 75.4%. The active ingredient carbendazim is one of the first systemic fungicides of benzimidazole class. In spite of wide manufacturing implementation and effective use, it has a number of drawbacks, such as slow movement through the host plant and rapid formation of resistant populations.

Screening of the fungicides inhibiting the growth of North Caucasian population of *M. nivale* also revealed differences in the biological effectiveness of the preparations. When the fungicide solution was added to the nutrient medium, complete inhibition of the growth of North Caucasian population of *M. nivale* was revealed in the experiment with the fungicides Polaris, ME, Quinto Duo, SC and Bariton Super, SC. It should be noted that Polaris, ME and Quinto Duo, SC contain the active ingredient prochloraz from the imidazole class. Due to high efficiency for inhibition of colony growth of all the studied populations, it can be determined that the substance is effective against *M. nivale* population. The high effectiveness of this active ingredient against bacterioses is also worth noting. Bariton Super, SC fungicide besides widespread tebuconazole includes the active ingredient of relatively recent introduction into production, protioconazole (triazole class), whose action is also aimed at increasing the habitus and power of the host plant. The

[2]Dospekhov B.A. Methodology of field experience. Moscow: Agropromizdat, 1985. 351 p.

Табл. 1. Скрининг эффективности фунгицидов против белорусской и северокавказской популяций *M. nivale* методом вмешивания (ФГБНУ ФНЦБЗР, 2022 г.)

Table 1. Screening of the effectiveness of fungicides against the Belarusian and North Caucasian populations of *M. nivale* by an intervention method (FSBSI FSCBPP, 2022)

Preparation	Belarussian population		North Caucasus population	
	Colony diameter, mm	Biological effectiveness, %	Colony diameter, mm	Biological effectiveness, %
Maxim Forte, SC	0	100	18,7 ± 1,25	57,3
Vybrance Trio, FSC	0	100	5,7 ± 1,2	87,0
Polaris, ME	0	100	0	100
Quinto Duo, SC	0	100	0	100
Quinto Plus, SC	0	100	10,0 ± 1,6	77,1
Bariton Super, SC	0	100	0	100
Scarlet, ME	1,3 ± 0,5	97,6	1,3 ± 0,3	96,9
Oplot Trio, WSC	0	100	3,0 ± 0,8	93,1
Credo, SC	13,7 ± 2,6	75,4	2,7 ± 0,6	93,9
Control	55,7 ± 1,2	–	43,7 ± 2,3	–

active ingredient fludioxonil (chemical class of phenylpyrroles) is one of the most popular and most successful classes of fungicides, since in 30 years of intensive use in agricultural plant protection almost no cases of field resistance were registered [15]. The mechanisms of its action have not yet been thoroughly studied. It has been revealed that the substance inhibits mainly conidia germination, embryonic tube and mycelial growth.

A high level of biological efficacy was observed in Scarlet, ME (96.9%), Credo, SC (93.9%) and Oplot Trio, WSC (93.1%). Oplot Trio, WSC fungicide contains azoxystrobin (strobilurine chemical class) and difenoconazole, tebuconazole (triazole chemical class). Azoxystrobin, being a synthetic analogue of natural toxins, positively affects photosynthetic activity and habitus of a host plant, but repeated use of the drug leads to rapid accumulation of resistant races of pathogens [14]. Diphenoconazole, in addition to fungicide, has a growth-stimulating effect on the plant; tebuconazole is widely used as an effective fungicide with a weak retardant effect.

The biological efficacy of Vybrance Trio, FC was found to be 87.0%, and that of Quinto Plus, SC was 77.1% (see Fig. 1). These fungicides consist of a combination of active substances of triazole and phenylpyrrole chemical classes. Also, the fungicides Vybrance Trio, FC included a relatively new drug sedaxane, which has not only fungicidal action, but also, in combination with fludioxonil, provides prevention of pathogens with high potential for resistance development [15]. Minimum value of biological efficacy was found in Maxim Forte, SC - 57.3%. The drug contains a combination of active ingredients based on strobilurines (azoxystrobin), triazoles (tebuconazole) and phenylpyrroles (fludioxonil).

The use of the method of introducing the preparation into the nutrient medium with uniform distribution on the surface of agar plate with a spatula revealed the increase of the preparation efficiency in all experimental variants (see Table 2). When the substance is distributed over the agar surface, the area of this surface is similar to that of wheat seeds during dressing [12]. However, when the substance is introduced into the medium by the interference method (kneading in), the concentration of the preparation decreases, which provokes the growth of fungus colonies. When comparing the correlation between the results obtained using the two methods, the coefficient was found to be very high for both populations (for the Belarusian population r_{xy} = 1.0, for the North

Caucasian population r_{xy} = 0.99). Although the results had a direct correlation between the two methods of fungicide application in the medium, the samples were statistically different (for the Belarusian population F_t 5.12 < F_f 10713, for the North Caucasus - *Ft* 5.32 < *Ff* 430.4).

Screening of fungicides inhibiting the development of the Belarusian population of *M. nivale* on agar plates revealed 100% inhibition of mycelial growth when applying Maxim Forte, SC, Vybrance Trio, FC, Polaris, ME, Quinto Duo, SC, Quinto Plus, SC, Bariton Super, SC, Scarlet, ME, Oplot Trio, WSC (see Fig. 2). Mycelium growth was not completely inhibited by the application of Credo, SC, and the biological efficiency was 77.4%.

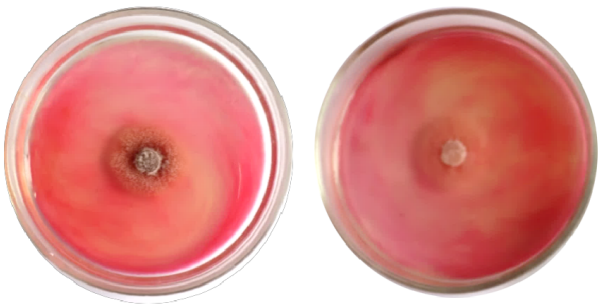

Рис. 1. Ингибирование роста колоний северокавказской популяции *M. nivale* при внесении препарата Кинто плюс, КС в среду методом вмешивания (слева) и растирания (справа)

Fig. 1. Inhibition of the growth of colonies of the North Caucasian population of *M. nivale* when introducing drug Kinto Plus, CS into the medium by the method of intervention (left) and rubbing (right)

Polaris, ME, Quinto Duo, SC, Bariton Super, SC, Scarlet, ME, Oplot Trio, WSC completely inhibited colony growth of the North Caucasian population of *M. nivale* on agar plates. When Credo, SC was applied, insignificant growth of colonies was observed; the biological efficiency was 97.7%. Supplementation of fungicide Vybrance Trio, FC also did not fully inhibit the colony growth, biological efficiency was 95,0%. The drug Quinto Plus, SC had this indicator at 88.5%. The lowest value of biological efficacy was observed in Maxim Forte, SC - 65.7%.

The comparative analysis between the fungicide sensitivity of pathogen populations from geographically distant regions revealed a statistically reliable difference between the results obtained (when using the interference method, F_t 5.32 < F_f 23.2, when using the rubbing method, F_t 5.32 < F_f 37.7). Thus, the results obtained indicate the heterogeneity of the pink snow rot pathogen in terms of sensitivity to fungicides.

Табл. 2. Скрининг эффективности фунгицидов против популяций *M. nivale* методом растирания (ФГБНУ ФНЦБЗР, 2022 г.)

Table 2. Screening of the effectiveness of fungicides against *M. nivale* populations by a rubbing method (FSBSI FSCBPP, 2022)

Preparation	Belarussian population		North Caucasus population	
	Colony diameter, mm	Biological effectiveness, %	Colony diameter, mm	Biological effectiveness, %
Maxim Forte, SC	0	100	17,8 ± 3,0	65,7
Vybrance Trio, FSC	0	100	2,6 ± 0,8	95,0
Polaris, ME	0	100	0	100
Quinto Duo, SC	0	100	0	100
Quinto Plus, SC	0	100	6 ± 1,1	88,5
Bariton Super, SC	0	100	0	100
Scarlet, ME	0	100	0	100
Oplot Trio, WSC	0	100	0	100
Credo, SC	14,7 ± 2,6	77,4	1,2 ± 0,7	97,7
Control	65,0 ± 0,6	–	52 ± 4,3	–

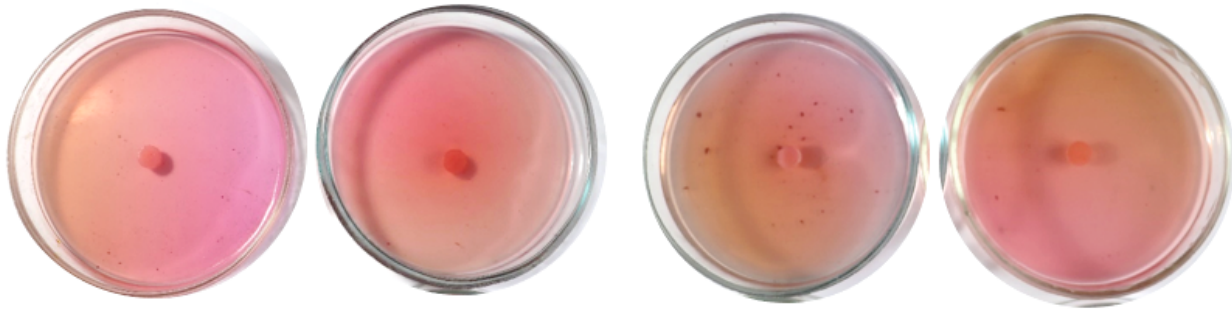

Рис. 2. Полное ингибирование роста колоний белорусской (слева) и северокавказской (справа) популяций *M. nivale* при внесении препаратов Скарлет, МЭ (слева) и Поларис, МЭ (справа) методом растирания

Fig. 2. Complete inhibition of colony growth of the Belarusian (left) and North Caucasian (right) populations of *M. nivale* when applying Scarlet, ME (left) and Polaris, ME (right) preparations by rubbing

CONCLUSIONS

1. Screening of nine chemical fungicides against *M. nivale* population in a pure culture revealed preparations with 100% fungicidal effect against both studied populations: Polaris, ME, Quinto Duo, SC and Bariton Super, SC. Oplot Trio, WSC, Vybrance Trio, FC, Maxim Forte, SC showed 100% efficacy only against the Belarusian population of the pathogen.

2. The use of two methods of introducing the preparation into the nutrient medium (kneading in and rubbing on the surface) has a high correlation coefficient (for the Belarusian population r_{xy} = 1.0, for the North Caucasian population r_{xy} = 0.99). The interference method inhibits the colony growth to a lesser extent; therefore, it is more preferable in the study of sensitivity to preparations of pure culture of fungus *M. nivale*.

3. A statistically significant difference was found between the sensitivity to fungicides of the populations from geographically distant regions (F_t 5.32 < F_f23.2 when using the interference method, F_t 5.32 < F_f 37.7 when using the rubbing method), indicating the heterogeneity of the pink snow rot pathogen in sensitivity to the modern assortment of seed dressing agents.

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