

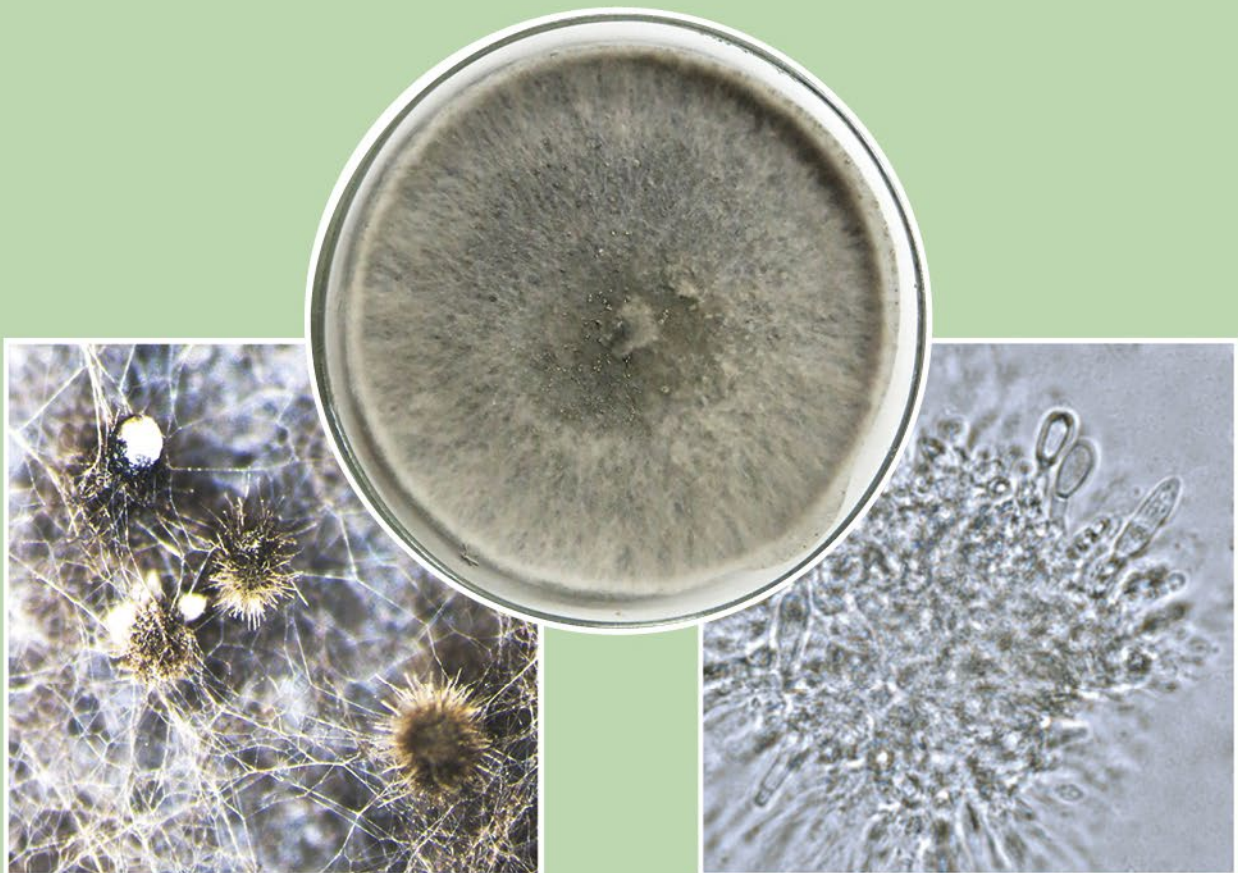


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CHARACTERIZATION OF *ZYMOSEPTORIA TRITICI* POPULATIONS IN BELARUS BY MORPHOLOGIC AND CULTURAL FEATURES

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Septoria leaf blotch caused by *Zymoseptoria tritici* is one of the most harmful diseases in Belarus. Isolates of the pathogen were obtained from northern, central and southern populations: in 2018–2019, which varied significantly among in the structure of colonies. The rate of fungal isolates forming yeast-like colonies decreased from 55.0% in the North of Belarus to 6.7% in the South, whereas incidence of filamentous isolates increased from 31.3% to 80.0%, respectively. In the northern population, phenotypic diversity was high, while in the southern population it was the lowest (Shannon's index was 1.53 and 1.14, respectively).

Keywords: morphological features, population, Septoria leaf blotch, sporulation, *Zymoseptoria tritici*

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Introduction

The fungus *Zymoseptoria tritici* (Desm.) Quaedvl. & Crous (synonym *Septoria tritici* Desm.) is among the main causative agents of Septoria leaf blotch in many regions of the world (Fones, Gurr, 2015; Torriani et al., 2015; Zeleneva, 2019), including Belarus (Krupenko et al., 2017). The disease harmfulness is displayed as yield decrease up to 50% (Torriani et al., 2015). Under *in vitro* conditions *Z. tritici* forms colonies which differ in color, shape, colony type and other characters (Cordo et al., 1997; Sudnikova et al., 2010; Vechet, Vydrova, 2011; Zeleneva, 2019).

In Belarus, studies of morpho-cultural features of *Z. tritici* have been initiated in 2011 (Sklimenok et al., 2011; Sklimenok, Buga, 2012). It has been established that isolates from different populations formed predominately yeast-like black corrugated colonies with or without pink margin at frequency rates of 64 and 43%, respectively (Sklimenok, 2015). Further studies of morpho-cultural properties of *Z. tritici* are of interest for consequent monitoring of fungicide sensitivity and other important biological features.

Materials and Methods

The studies have been performed in laboratory of phytopathology of Institute of Plant Protection in Priluki, Minsk region, Belarus. The Septoria leaf blotch-affected leaves of winter wheat have been sampled in 2018 and 2019 during surveys across northern, central and southern agroclimatic zones of the Republic, which provided for the designation of the respective populations of the fungus.

The leaves have been taken from each field in diagonal direction at the distance of at least 5 m. The sampled specimens have been air-dried and stored at +4°C. Potato sugar agar (PSA) was used to study morpho-cultural properties of the fungus (Sklimenok et al., 2011). A pycnidium has been excised

from the leave with a needle and placed into a 100 µl drop of sterile water on the surface of PSA medium supplemented with 5% of streptomycin then the drop has been smeared over the medium. The plates were incubated for 7–10 days at 20°C. Then one of the newly emerged colonies was sown to obtain one monoonidial isolate per leaf (Sklimenok et al., 2011). Each isolate has been grown in four replicates and grown at 20°C for 30 days to define morphotype, size and spore production intensity of the colonies according to Sanin et al. (2008) and Sanina (1991). Morphological diversity of the populations has been accessed using Shannon's index (Kolmer et al., 2003).

Results and Discussion

Zymoseptoria tritici isolates formed yeast-like, mycelial and mixed types of colonies with varied frequencies. Yeast-like colonies were predominant in northern populations (55%) while in southern ones, mycelial colonies were prevailing (80%). In the central agroclimatic zone, the quotes of yeast-like and mixed types were about the same (36–37%).

The analyzed populations of *Z. tritici* were variable in morphology (Table 1). As many as eight morphotypes of the fungus were found with black corrugated yeast-like colonies (morphotype I b) as the most abundant (43.0%) in the northern population. In central population, there were six morphotypes,

where Ib colonies were the most frequent (31.0%) and the frequencies of mixed and (II a) and mycelial colonies (III a) were also relatively high – 25.6 and 27.8%, respectively. In southern agroclimatic zone, only four morphotypes were present, and white or grey mycelial colonies (III a) were prevailing at the rate of 53%. Thus, the highest phenotypic diversity of the fungal isolates is detected in the northern population. This can be explained by hydrothermal condition, as this part of the Republic is distinguished by lower average daily temperature values and high precipitation rates.

Table 1. Differentiation of *Zymoseptoria tritici* populations based on their morphotypes

Population	Morphotype frequency, %								Shannon's Index
	Yeast-like			Mixed			Filamentous		
	I a	I b	I c	II a	II d	II e	III a	III b	
Northern	1.6	43.0	10.6	7.4	4.7	1.6	28.0	3.1	1.53
Central	0.0	31.0	5.6	25.6	1.5	0.0	27.8	8.5	1.23
Southern	0.0	6.7	0.0	13.3	0.0	0.0	53.3	26.7	1.14

Таблица 1. Дифференциация популяций гриба *Zymoseptoria tritici* по морфотипам

Популяция	Частота встречаемости морфотипа, %								Индекс Шеннона
	Дрожжеподобный			Смешанный			Мицелиальный		
	I a	I b	I a	II a	I a	II e	I a	III b	
Северная	1.6	43.0	10.6	7.4	4.7	1.6	28.0	3.1	1.53
Центральная	0.0	31.0	5.6	25.6	1.5	0.0	27.8	8.5	1.23
Южная	0.0	6.7	0.0	13.3	0.0	0.0	53.3	26.7	1.14

Variability of *Z. tritici* isolates is confirmed by studies performed in Russia (Sudnikova et al., 2010; Zeleneva, 2019), Czech Republic (Vechet, Vydrova, 2011), Argentina (Cordo et al., 1997) and Alger (Harrat, Bouznad, 2018). Meanwhile, the connections between colony morphology and virulence of the fungal isolates remain unclear. For instance, Cordo et al. (1997)

reported that mycelial forms are more virulent while Zeleneva (2019) attributed this feature to the yeast-like ones.

The fungal isolates also displayed variation in colony diameter from 1.2 cm in the northern population to 1.6 cm in the central one and in sporulation intensity which was maximal (23.0 mln spores/cm²) in the northern population and minimal (7.8 mln spores/cm²) in the southern one (Table 2).

Table 2. Differentiation of *Zymoseptoria tritici* populations based on their growth rate and sporulation intensity

Population	Colony diameter, cm	Sporulation intensity, million spores/cm ²
Northern	1.2±0.0	23.0±2.3
Central	1.6±0.0	12.2±1.4
Southern	1.5±0.1	7.8±1.6

NB. Mean and standard error values are given.

Таблица 2. Дифференциация популяций гриба *Zymoseptoria tritici* по скорости роста и интенсивности спороношения

Популяция	Диаметр колоний, см	Интенсивность спороношения, млн спор/см ²
Северная	1.2±0.0	23.0±2.3
Центральная	1.6±0.0	12.2±1.4
Южная	1.5±0.1	7.8±1.6

Примечание. Представлены средние значения показателя ± стандартная ошибка.

The obtained data reveal remarkable phenotypic diversity of the fungus *Z. tritici* in Belarus. The variation can be found between the isolates originating from different populations, but also from the same field and even from a single host plant leaf (McDonald, Martinez, 1990; Linde et al., 2002).

The outcomes of this direction of research may have

practical implications concerning sensitivity to active antifungal substances. In particular, strobilurins are known to be more active against the fungal spores (Balba et al., 2007) largely present in the yeast-like colonies, while azoles primarily act on the mycelium (Mueller et al., 2013) and may be thus more effective against the mycelial form of *Z. tritici*.

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Краткое сообщение

ДИФФЕРЕНЦИАЦИЯ ПОПУЛЯЦИЙ ГРИБА *ZYMOSEPTORIA TRITICI* В БЕЛАРУСИ ПО МОРФОЛОГО-КУЛЬТУРАЛЬНЫМ ПРИЗНАКАМ

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Септориоз листьев озимой пшеницы, вызываемый грибом *Zymoseptoria tritici*, – одна из наиболее распространенных и вредоносных болезней в Беларуси. Проведено изучение морфолого-культуральных признаков изолятов гриба, выделенных из трех агроклиматических зон (популяций): северной, центральной и южной в 2018–2019 гг. Выявлена значительная вариабельность изолятов гриба *Z. tritici* по структуре колоний. Установлено, что доля изолятов, образующих дрожжеподобные колонии, снижается с 55.0% в северной части республики до 6.7% в южной, тогда как частота встречаемости мицелиальных колоний возрастает с 31.3% до 80.0%, соответственно. Большое разнообразие морфологических типов колоний отмечено в северной популяции, наименьшее – в южной (индекс Шеннона составил соответственно 1.53 и 1.14).

Ключевые слова: морфологические признаки, популяция, септориоз, спороношение, *Zymoseptoria tritici*

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