

## Assessment of the vertical spread of main fungal pathogens causing leaf spot

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### Abstract

Nedyalkova, S. & Chavdarov, P. (2024). Assessment of the vertical spread of main fungal pathogens causing leaf spot. *Bulg. J. Agric. Sci.*, 30(6), 1033–1039

During the period 2020–2021, we studied the occurrence and distribution of leaf spot fungal pathogens on durum wheat in Bulgaria. The study was conducted in three regions of the country, namely, Field Crop Institute – Chirpan, Sadovo and Tatarevo, on the vertical distribution of *P. tritici-repentis* and septoria leaf blotch (SLB) in two varieties of common wheat (Sadovo 1 and Andino) and two varieties of durum wheat (Helix and President). The vertical spread of foliar pathogens is of crucial importance, as the higher position the pathogens reach, the greater their negative impact on crop productivity is. In the conducted study, an assessment was made of the vertical spread of the most important foliar pathogens in the post-flowering period, comparing common and durum wheat varieties. The relative prevalence of foliar pathogens in individual cultivars varied by year and habitat. Differences in the vertical distribution of the studied foliar pathogens were probably due to the intensity of sporulation. The increasing frequency of occurrence of Ptr in the middle and upper levels compared to the causative agents of septoria can be explained by the ability of this species to form reproductive structures in a shorter period of time than *Z. tritici* and *Pa. avenae* f.sp. *triticea*, although they are also present in the lower levels. The frequency of occurrence varied depending on the leaf position (lower, middle, upper) and on the susceptibility of the variety from which the sample was taken. Pathogen *P. tritici-repentis* was spread in all investigated regions with an increasing frequency from the lower to the upper leaf positions.

**Keywords:** fungal pathogens; leaf spots; *Triticum durum*; *Triticum aestivum*; *Pyrenophora tritici-repentis*

### Introduction

Durum wheat is a traditional staple crop in Bulgaria. The main purpose of durum wheat is its use as an indispensable raw material in the production of pasta and other types of food products. A significant problem concerning its production is the occurrence of disease pathogens leading to decrease in yield and crop quality. The distribution and relative significance of foliar pathogens in some growing areas has changed drastically as a result of new market tendencies, which cause changes in the farming practices and the introduction of new varieties. The monitoring of these changes is important in order to undertake appropriate and timely mea-

asures to prevent the spread of diseases. Information on the most common fungi causing leaf spotting on durum wheat will help order the priorities in breeding for disease resistance.

Fungal pathogens on wheat considerably limit the obtained yields and deteriorate the quality of production. The caused diseases can lead to 15–20% annual loss, with main contributors being rusts, leaf spotting and wheat blast (Figuroa et al., 2018).

Wheat septoria are important diseases with substantial impact on wheat production in numerous countries in the world (Figuroa et al., 2018). Their increased economic importance is due to the introduction of high-yielding, low-

stemmed, sensitive wheat varieties; changes in the crop-cultivation technology; increased use of nitrogen fertilizers and falling behind in the use of genetic resistance compared to increased resistance to other leaf pathogens such as rusts and powdery mildew (Shipton et al., 1971; King et al., 1983; Eyal et al., 1987; Eyal, 1999; Cunfer & Ueng, 1999; Rodeva, 2004; Lucas et al., 1999).

A major component in the integrated septoria management is genetic resistance. No complete wheat resistance has been established to *Zimoseptoria tritici*, *Parastagonospora nodorum* and *Parastagonospora avenae* f. sp. *triticea*, but the varieties significantly differ in their response to each of these pathogens (necrosis and/or pycnidia). This variability can be used in breeding for resistance. Reports were delivered on wheat resistance to *Z. tritici* (Rosielle, 1972; Gough & Smith, 1976; Krupinsky et al., 1977; Rosielle & Brown, 1979; Eyal et al., 1983; Brown et al., 2001; Ruzgas et al., 2002; Adhikari et al., 2003; 2004a; 2004b; Chartrain et al., 2004; Simón, 2010; Francki, 2013; Arraiano & Brown, 2017) and to *Pa. nodorum* (Krupinsky et al., 1977; Scharen & Eyal, 1980; Rosielle & Brown, 1980; Rufty et al., 1981; Ruzgas et al., 2002; Abeyssekara et al., 2009; Friesen et al., 2009; Friesen & Farris, 2010; Phan et al., 2018). Sources of resistance to both pathogens were established among varieties of genera *Aegilops* and *Agropyron*, and in some case also successfully transferred into the wheat genome (Gough & Tuleen, 1979; Trotter et al., 1983; Krupinsky & Berdahl, 1984; Ecker et al., 1990a; 1990b; Assefa & Fehrmann, 1998; Murphy et al., 2000; Loughman et al., 2001).

Over 20 major STB resistance genes were mapped (Brown et al., 2001; McCartney et al., 2002; Brown et al., 2015). Sources of quantitative resistance, which is longer lasting under field conditions and often provides protection against various pathogen genotypes, have also been identified. Reports were made regarding 167 genomic regions with QTLs associated with STB resistance (Brown et al., 2015). Phenotyping these QTLs proved their part in various stages of the disease development – fructification, necrosis and latent period. Laboratory methods were developed to test young plant resistance (Arraiano et al., 2001). According to Chartrain et al. (2004) pyramiding several resistance genes is an efficient long-term strategy for STB resistance breeding. To achieve satisfactory long-term resistance, it is necessary to use genetically diverse material.

In some variety-isolate combinations, the response of young wheat plants to *Z. tritici* can predict the response of mature plants (Danon & Eyal, 1990; Kema & Silfhout, 1997).

Progress in improving STB resistance was achieved by crossing lines from several European breeding programmes (Brown et al., 2001).

A comparative study on the response of common and durum wheat to foliar pathogens revealed significant variation between varieties, but not when grouped by species. Among both types of wheat, there are varieties with both low and high levels of attack by the leaf spot complex. *Ptr* was isolated more often from durum than common wheat, whereas *Pa. nodorum* – more often from common wheat (Fernandez et al., 1996). Types of wheat genotypes with complex resistance to STB, SNB and *Ptr* were established (Šíp et al., 2005; Ali et al., 2008).

Breeding for combined resistance to diseases is a perspective strategy in creating new cultivars. The goal of modern resistance breeding is adequate resistance to major diseases, and not just high resistance to one disease. An important step is the elimination of highly susceptible lines, which are not only heavily attacked, but also provide inoculum for other varieties. In practice, resistance breeding is based on breeding under field conditions and depends on the natural occurrence of the studied diseases.

The aim of this study was to assess the vertical distribution of major fungal pathogens causing leaf spot.

## Material and Methods

The study focuses on two cultivars of common wheat, Sadovo-1 and Andino, and two cultivars of durum wheat, Helix and President. Samples were taken from three locations: FCI – Chirpan, Sadovo and vill. Tatarevo (Haskovo). Leaf material was collected from three different leaf positions: lower (L), middle (M) and upper (U). All collected materials were placed in paper bags, and after one week of air drying at room temperature they were gathered in polyethylene bags and stored in a fridge at 4–5°C.

Microscopic observations were made on fungal structures, formed on infected plant tissue and grown in culture media. The main method to determine the species composition of the fungal pathogens that participated in the leaf spotting complex was the comparative-morphological one. The symptoms were described as found on the naturally infected durum wheat plants and were compared to the ones on common wheat caused by the respective pathogens. Identification was performed by means of direct observation of the pathogen structures formed on plant tissue and grown in culture media and it was based on a combination of morphological and cultural traits. The inoculum for testing the fungal growth and fructification was obtained by culturing the source isolates on PDA at 22°C in the dark. Discs with diameter of 4 mm were cut from the periphery of actively growing colonies and placed in the center of Petri dishes (d = 90 mm), each containing 25 ml of the respective grow-

ing medium. The inoculated Petri dishes were incubated at 22°C. Two mutually perpendicular colony diameters were measured to assess the mycelial growth.

## Results and Discussion

Assessment was made on the vertical spread of the two most significant components in the leaf spot complex: *P. tritici-repentis* (*Ptr*) and septoria causal agents (*Z. tritici*, *Pa. avenae* f. sp. *triticea* and *Pa. nodorum*), which were considered as a collective group (septoria leaf blotch – SLB). The analysis was based on the comparison between two varieties of durum wheat, Helix and President, and two varieties of common wheat, Sadovo 1 and Andino.

A total of 782 leaf samples were collected and tested, including 349 in 2020 and 433 in 2021 (Table 1). The main tendency was that *Ptr* was the predominant pathogen in both years in all leaf positions, except the lower position in 2021.

*Ptr* was a widely spread pathogen in all the regions of the study with an increasing frequency from the lower to the higher positioned leaves (Table 2). The species included in the SLB group have almost the same distribution in the lower and middle positions and decrease in the upper one. The

three regions are similar in frequency of occurrence of the foliar pathogens, even though there were certain differences in the regions and between themselves.

The occurrence of the studied fungi also depended on the susceptibility of the given variety, from which the sample was taken (Table 3). In durum wheat varieties, *Ptr* was the predominant pathogen compared to SLB for all leaf positions. Variety President showed higher susceptibility to *Ptr*. The causative agents of SLB predominated in common wheat, especially in variety Sadovo-1, due to its susceptibility to *Z. tritici*. In the lower and middle positions, they were more frequently found than *Ptr*.

Due to the similar symptoms of different diseases related to the SLB-complex, they were considered as one subject of study, but the contribution of the individual species to SLB was not the same (Table 4). *Z. tritici* was the most frequently occurring pathogen in common wheat. In durum wheat, this species was first found in 2021 in 3 samples: 1 from FCI – Chirpan, 1 from Sadovo, and 1 from vill. Tatarevo. They were all from the lower position except 1 sample from vill. Tatarevo from an upper leaf position.

*Pa. avenae* f. sp. *triticea* was found on varieties *T. aestivum* and *T. durum* with a slight predominance in durum

**Table 1. Vertical distribution of foliar pathogens according to studied year**

Year	Number of samples	<i>Ptr</i>				SLB			
		L	M	U	Total	L	M	U	Total
2020	349	89	89	85	263	39	42	5	86
2021	433	33	96	135	264	74	76	19	169
Total	782	122	185	220	527	113	118	24	255

Legend: *Pyrenophora tritici-repentis* – *Ptr* ; septoria leaf blotch – SLB

**Table 2. Vertical distribution of foliar pathogens according to the studied region**

Region	Number of samples	<i>Ptr</i>				SLB			
		L	M	U	Total	L	M	U	Total
FCI – Chirpan	260	37	61	53	151	49	56	4	109
Sadovo	350	81	92	89	262	31	44	13	88
Tatarevo	172	4	32	78	114	33	18	7	58
Total	782	122	185	220	527	113	118	24	255

Legend: *Pyrenophora tritici-repentis* – *Ptr* ; septoria leaf blotch – SLB

**Table 3. Vertical distribution of foliar pathogens according to the studied variety**

Variety	Number of samples	<i>Ptr</i>				SLB			
		L	M	U	Total	L	M	U	Total
Andino	198	22	35	59	116	45	32	5	82
Sadovo 1	283	16	55	59	130	67	72	14	153
Helix	128	28	49	30	107	5	16	0	21
President	173	54	45	72	171	0	2	0	2
Total	782	120	184	220	524	117	122	19	258

Legend: *Pyrenophora tritici-repentis* – *Ptr* ; septoria leaf blotch – SLB

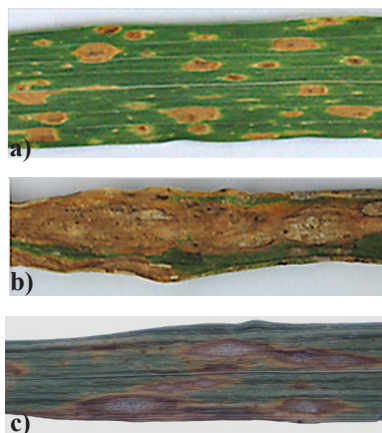
**Table 4. Distribution of SLB samples according to the species of causative agents**

Region	Year	Number of samples	<i>Z. tritici</i>		<i>Pat</i>		<i>Pn</i>	
			Ta	Td	Ta	Td	Ta	Td
FCI – Chirpan	2020	45	35	0	4	3	3	0
	2021	64	44	1	1	18	0	0
Sadovo	2020	24	19	0	2	3	0	0
	2021	64	60	1	1	2	0	0
Tatarevo	2020	19	5	0	9	4	1	0
	2021	39	37	1	1	0	0	0
Total		255	200	3	18	30	4	0

Legend: *Zymoseptoria tritici* – *Z. Tritici*; *Pyrenophora tritici-repentis* – *Ptr*; *Parastagonospora nodorum* – *Pn*

wheat. Species *Pa. nodorum* was found far more rarely in common wheat (4 out of 481 samples) and was missing as a whole in durum wheat (0 out of 301 samples).

The disease caused by *Ptr* is known as yellow leaf spot and has similar symptoms as the SLB-complex. The yellow leaf spots are initially lenticular, grow larger with age, become irregularly elliptical with pointed ends and reach 6-15 mm in size. The spots are surrounded by a yellow halo, and a dark spot remains in the center, where the infection has started (Figure 1a). The symptoms on common and durum wheat are similar. *Pa. avenae* f. sp. *triticea* causes the development of irregular, oval to oblong-oval shaped necrotic spots (Figure 1b). The *Pa. nodorum* spotting appears as relatively small, oval to lenticular, initially chlorotic, and later in the growing season – as confluent reddish-brown spots with pale center, where pycnidia are formed (Figure 1c).

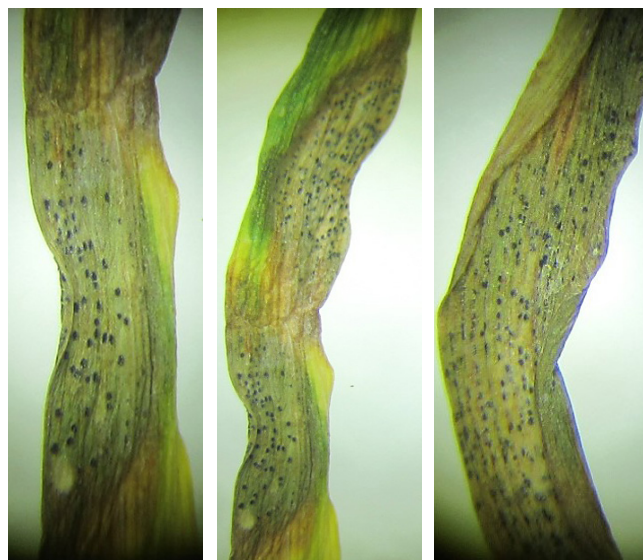


**Fig. 1. Symptoms caused by**  
**a) *Pyrenophora tritici-repentis*;**  
**b) *Parastagonospora avenae* f. sp. *triticea*;**  
**c) *Parastagonospora nodorum*;** on durum wheat leaves

The damages caused by *Z. tritici* appear on the lower leaves, especially on those in contact with soil. They form extensive, merging spots with a large number of pycnidia (Figure 2, Figure 3). On the leaves of adult wheat plants, the spots are oblong, linear, elongated, laterally limited by the nervature. They expand by the length of the leaf and are situated parallel.



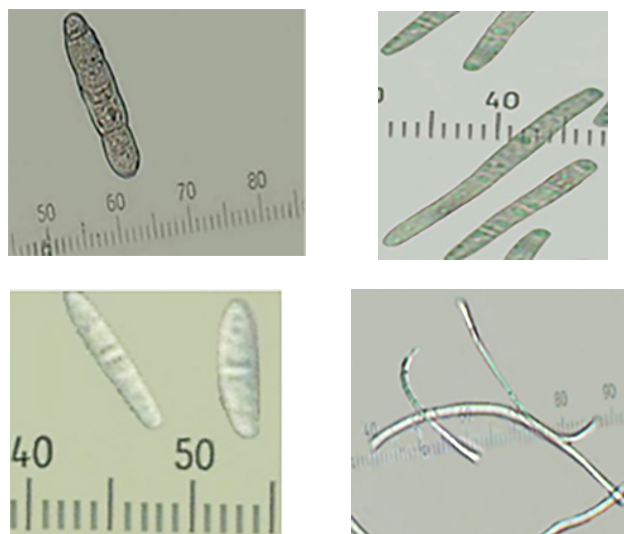
**Fig. 2. Leaf samples with found pycnidium of *Zymoseptoria tritici*, observed on durum wheat, (Helix) – observation on 31.03.2020**



**Fig. 3. Leaf samples with found pycnidium of *Zymoseptoria tritici*, observed on common winter wheat (Sadovo-1) – observation on 04.14.2020**

The spots caused by all the studied foliar pathogens can coalesce and form large necrotic damage, resulting in premature leaf death. These fungal species often infect wheat at the same time, causing similar symptoms and can be easily misidentified. Microscopic observations and isolations are needed.

The foliar pathogens were confirmed by means of observation with a light microscope. Conidia of *Pyrenophora tritici-repentis* were multicellular, hyaline to light brown, cylindrical, straight or slightly curved, with rounded ends, with 5 to 13 (most often 4 to 7) thin, colourless pseudosepta, with dimensions  $75\text{--}200 \times 13\text{--}19 \mu\text{m}$  (Figure 4a). Fruit bodies of the anamorph of *Parastagonospora avenae* f. sp. *triticea* – pycnidia, contained a large number of conidia, which were hyaline, thin-walled, smooth, cylindrical, straight or slightly curved, with oil droplets, rounded edges, 1 to 5 septa (most often 3–4),  $17\text{--}48 \times 2.5\text{--}4.5 \mu\text{m}$  (Figure 4b). *Parastagonospora nodorum* can be distinguished from *Pa. avenae* f. sp. *triticea* to a certain extent by the conidia morphology. For *Pa. nodorum* they are shorter (very rarely more than  $25 \mu\text{m}$ ) and have from 0 to 3 relatively well-noticeable septa (Figure 4c). Observation of isolates from *Zymoseptoria tritici* identified pycnidia of normal size (diameter  $85\text{--}190 \mu\text{m}$ ), which only contained macroconidia, which were hyaline, thin-walled, filamentous, gradually narrowing from rounded bases to a pointed apex, smooth, straight or curved, containing drops, with 3–5 indistinct septa,  $30\text{--}70 \times 1.0\text{--}2.5 \mu\text{m}$  (Figure 4d).



**Fig. 4. Conidial sporulation of a) *Pyrenophora tritici-repentis*; b) *Parastagonospora avenae* f. sp. *triticea*; c) *Parastagonospora nodorum*; d) *Zymoseptoria tritici***

Differences in the vertical distribution of the studied foliar pathogens were due to the sporulation intensity. The increasing frequency of *Ptr* in middle and upper positions compared to the septorioses agents can be explained with the ability of this species to form reproductive structures for a shorter period of time compared to *Z. tritici* and *Pa. avenae* f.sp. *triticea*, even though they can be also found in the low-

er positions. *Ptr* sporulates daily and conidia are spread by wind. A large number of conidia appear in the afternoons after prolonged wet spells. The Septorioses spores are spread by raindrops and the infection moves from the bottom to the top part of the crops. *Z. tritici* has a latent period of 3–4 weeks, whereas *Ptr* has a much shorter latent period of 5–8 days, which shows the greater competitiveness of *Ptr*.

## Conclusions

The assessment of the vertical distribution of *P. tritici-repentis* and septoria leaf spot as a collective group (septoria leaf blotch – SLB) in durum and common wheat varieties proved that the frequency of occurrence varies depending on the leaf position (lower, middle, upper) and the susceptibility of the variety from which the sample was taken. Pathogen *P. tritici-repentis* was found in all the studied regions with an increasing frequency from the lower to upper leaf positions.

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Received: June, 10, 2023; Approved: February, 02, 2024; Published: December, 2024