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Vertical distribution of foliar pathogens on wheat

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Abstract. *Wheat (bread and durum) is the most widely distributed crop in Bulgaria. Leaf spotting diseases of wheat are associated mainly with a complex of three fungal pathogens, which cause septoria leaf blotch. Lately, Pyrenophora tritici-repentis has been increasingly observed in Bulgaria. The purpose of the present investigation was to estimate the vertical distribution of these important leaf spotting pathogens during the post-anthesis period making a comparison between bread and durum wheat cultivars. P. tritici-repentis was the ubiquitous fungus in all studied sites with higher frequency of occurrence from lower to upper leaf layers. The inciters of septoria leaf blotch were predominant on bread wheat especially on cultivar Enola due to its susceptibility to Zymoseptoria tritici. In the lower and middle leaf layers they were more frequent than P. tritici-repentis. On durum wheat P. tritici-repentis was the most prevalent pathogen in all leaf layers especially on cultivar Saturn, while Z. tritici was found in 6 samples only in 2013. Stagonospora avenae f.sp. triticea was established on T. aestivum and T. durum cultivars with slightly prevalence on durum wheat. S. nodorum was recorded very seldom (on bread wheat) or missing at all (on durum wheat).*

Keywords: *Pyrenophora tritici-repentis, Drechslera tritici-repentis, Zymoseptoria tritici, Stagonospora avenae* f. sp. *triticea, Stagonospora nodorum, Phaeosphaeria avenaria* f. sp. *triticea*

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

Introduction

Wheat (bread and durum) is the crop with the highest production in European agriculture. It is the most widely distributed crop in Bulgaria, where occupies about 64% of cereals and 36% of arable land. Leaf spotting diseases of wheat are associated mainly with a complex of fungal pathogens known as septoria leaf blotch (SLB), which consists of *Mycosphaerella graminicola* (Fückel) J. Schröter in Cohn (anamorph *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous), *Phaeosphaeria nodorum* (E. Müller) Hedjarroude (anamorph *Stagonospora nodorum* (Berk.) E. Castellani & E.G. Germano) and *Phaeosphaeria avenaria* (G.F. Weber) O.E. Eriksson f. sp. *triticea* T. Johnson (anamorph *Stagonospora avenae* (A.B. Frank) Bisset f. sp. *triticea* T. Johnson) (Shipton et al., 1971; King et al., 1983; Eyal et al., 1987; Eyal, 1999; Rodeva, 2004). Lately, *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) has been observed with increasing frequency in Bulgaria (Todorova, 2006; Nedyalkova et al., 2013). The relative prevalence of leaf spotting fungi in the individual cultivars varied with year and location (Fernandez et al., 1996). These diseases cause significant damage and losses in yield and crop quality. The negative effect on wheat plants is mainly due to reduced photosynthetic area and accelerated leaf senescence. Vertical distribution of foliar pathogens must be taken in consideration since as higher is the position as greater is impact on crop productivity. The purpose of the present investigation was to estimate the vertical distribution of the main leaf spotting pathogens during the post-anthesis period making a comparison between bread and durum wheat cultivars.

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Material and methods

Two cultivars of bread wheat – *Triticum aestivum* L. (Sadovo 1 and Enola) and two cultivars of durum wheat – *T. durum* Desf. (Predel and Saturn) were included in the study. Field surveys were performed in 2012 and 2013. Samples were taken from three localities: the district units of Executive Agency for Variety and Seed Control in General Toshevo, Burgas and Radnevo. Leaf material was collected from three different leaf layers: lower (L), middle (M) and upper (U). The investigation was carried out in the phytopathological laboratory of the Institute of Plant Physiology and Genetics, Sofia. The disease symptoms were described. The fungal structures of taxonomic importance were studied microscopically. The isolations were made from leaf pieces or directly from fruiting bodies developed in the spots. The pathogen identification was based on a combination of morphological and cultural characteristics. Two main leaf spotting causal agents were estimated: 1) *P. tritici repentis* (*Ptr*) and 2) the causal agents of septoria leaf blotch (SLB) (*Z. tritici, S. avenae* f.sp. *triticea* and *S. nodorum*), which were considered as an assembly group. Meteorological data were collected on site at the three locations.

Results

Altogether 998 leaf samples were collected and examined, including 457 and 541 in 2012 and 2013, respectively (Table 1). The main tendency was *Ptr* to be the predominant pathogen in both years in all leaf layers except for the lower level in 2013.

Table 1. Vertical distribution of the foliar pathogens according to the study year

Year	Number of samples	Ptr				SLB			
		L	M	U	Total	L	M	U	Total
2012	457	107	107	103	317	57	60	23	140
2013	541	51	114	153	318	92	94	37	223
Total	998	158	221	256	635	149	154	60	363

Table 2. Vertical distribution of the foliar pathogens according to the study site

Location	Number of samples	Ptr				SLB			
		L	M	U	Total	L	M	U	Total
General Toshevo	332	49	73	65	187	61	68	16	145
Burgas	422	93	104	101	298	43	56	25	124
Radnevo	244	16	44	90	150	45	30	19	94
Total	998	158	221	256	635	149	154	60	363

Ptr was the ubiquitous fungus in all studied locations with increasing occurrence from lower to upper leaf layers (Table 2). Species involved in the SLB group had quite equal distribution in the lower and middle layers and decreased in the upper one. The three sites were similar on a macro scale in frequency of foliar pathogen occurrence although there were distributional differences among the sites, as well as within an individual site.

The occurrence of studied fungi depended to a greater or lesser extent on susceptibility of the cultivar to which samples belong (Table 3). On durum wheat cultivars *Ptr* was more prevalent in comparison with SLB in all leaf layers. Cultivar Saturn expressed higher susceptibility to *Ptr*. SLB inciters were predominant on bread wheat especially on cultivar Enola due to its susceptibility to *Z. tritici*. In the lower and middle leaf layers they were more frequent than *Ptr*.

Because of similar field symptoms the various diseases belonging to SLB complex were recorded as a single entity in this study but the contribution of different species to the SLB was unequal (Table 4). *Z. tritici* was very frequent on bread wheat. On durum wheat this species was found for the first time in 2013. Only 6 samples were related to *Z. tritici:* 3 of General Toshevo, 1 of Bourgas and 2 of Radnevo. All were of the lower leaf layer with the exception of 1 sample from Radnevo of the upper layer. *S. avenae* f.sp. *triticea* were recorded on *T. aestivum* and *T. durum* cultivars with slightly prevalence on durum wheat. *S. nodorum* was established very seldom on bread wheat (5 of 589 samples) or missing at all on durum wheat (0 of 409 samples).

The disease caused by *Ptr* is known as tan spot or yellow leaf spots. Tan spot and SLB diseases incited similar leaf symptoms. Tan spot produced elongated lens-shaped tan-colored lesions, 6 – 12 mm in length that enlarged with age. Lesions were surrounded by a

Table 3. Vertical distribution of the foliar pathogens according to the host cultivar

Cultivar	Number of samples	Ptr				SLB			
		L	M	U	Total	L	M	U	Total
Sadovo 1	252	30	43	68	141	54	41	16	111
Enola	337	22	60	64	146	75	79	37	191
Predel	182	41	61	42	144	12	23	3	38
Saturn	227	65	57	82	204	8	11	4	23
Total	998	158	221	256	635	149	154	60	363

Table 4. Distribution of SLB samples according to the species affiliation of the causal agents

Site	Year	Number of samples	*Z. tritici*		S. *avenae* f.sp. *triticea*		S. *nodorum*	
			Ta	Td	Ta	Td	Ta	Td
General Toshevo	2012	51	37	0	6	4	4	0
	2013	94	58	3	1	32	0	0
Burgas	2012	50	39	0	4	7	0	0
	2013	74	68	1	1	4	0	0
Radnevo	2012	39	9	0	21	8	1	0
	2013	55	52	2	1	0	0	0
Total		363	263	6	34	55	5	0

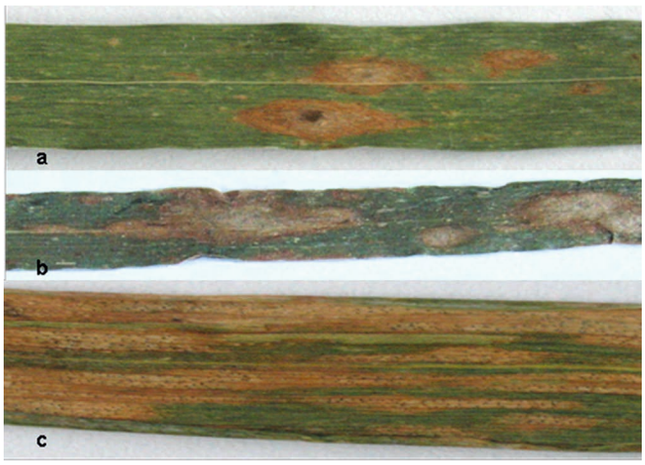

Figure 1. Symptoms of leaf spotting diseases of wheat caused by: a. *Pyrenophora tritici-repentis*; b. *Phaeosphaeria avenaria* f. sp. *triticea*; c. *Mycosphaerella graminicola*

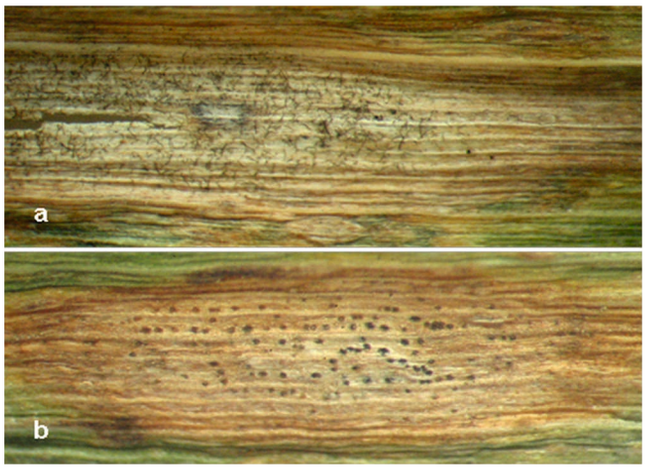

Figure 2. Sporulation of leaf spotting fungi on wheat leaf: a. conidiophores and conidia of *Pyrenophora tritici-repentis*; b. pycnidia of *Phaeosphaeria avenaria* f. sp. *triticea*

yellow halo and a dark spot occurred in the center, where the leaf tissues were initially infected. The symptoms observed on bread and durum wheat were quite similar (Figure 1a). *S. avenae* f.sp. *triticea* provoked development of irregularly-shaped, oval to elongated necrotic spots or lesions (Figure 1b). Stagonospora nodorum blotch was indistinguishable from stagonospora avenae blotch. Identification of both species in the field was difficult or practically impossible. Microscopic examination and isolations were necessary. The damages incited by *Z. tritici* tended to be linear, restricted laterally, elongated blotches, which enlarged longitudinally along the leaves appearing parallel-sided (Figure 1c). Lesions caused by all studied foliar pathogens could coalesce to form large blotches, predisposing leaves to premature senescence.

The initial symptoms of tan spot and stagonospora leaf blotch were partly similar and difficult to distinguish. Lesions of both diseases were light tan in color, irregularly oval to pointedly elongate, and often with distinct chlorotic zone extending in a longitudinal direction. Diseases could be distinguished mainly by fungal reproductive structures. The lesions incited by *Ptr* contained a small dark brown spot near the center and became darker with aging due to the production of black, erect, simple (not branched), single or in small groups conidiophores, supporting single cylindrical conidia

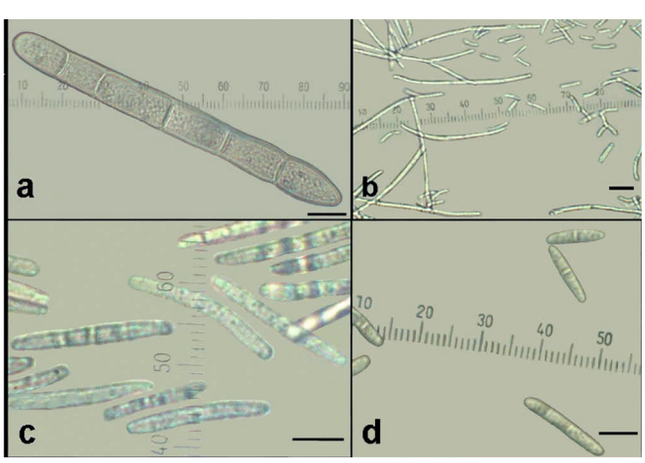

Figure 3. Conidia of leaf spotting fungi: a. *Pyrenophora tritici-repentis*; b. macro- and microconidia of *Mycosphaerella graminicola*; *Phaeosphaeria avenaria* f. sp. *triticea*; c. d. *Phaeosphaeria nodorum*
Scale bars: a = 20 µm; b, c, d = 10 µm

(Figure 2a). As the lesions of stagonospora leaf blotch matured, the centers became pale, gray-brown with numerous small black dots (pycnidia) (Figure 2b).

Confirmation of the foliar pathogens was made by a light microscope observation. Conidia of *Ptr* were hyaline (non-pigmented) to light brown, cylindrical, with one to nine transverse pseudosepta (commonly 5 –7), 50 – 200 x 12 – 20 µm. The conical tapering of the basal cell of the conidium, giving the appearance of a snake head, was very diagnostic of this fungus (Figure 3a). *Z. tritici* produced two types of pycnidia: normal size (90 – 170 µm in diameter) bearing only macroconidia or mixed macro- and microconidia (Figure 3b) and smaller one (65 – 120 µm in diameter) that contained only microconidia. The latter were found more frequently on sheath. Macroconidia were hyaline, thin-walled, filiform, straight or slightly curved, gradually tapered from the obtuse base to an acute apex, with 3 – 5 indistinct septa, 30 – 70 x 1 – 3 µm. Microconidia were hyaline, thin-walled, filiform, straight or slightly curved, unicellular, 8 – 10 x 0.8 – 1.0 µm. Conidia of *S. avenae* f.sp. *triticea* were hyaline, thin-walled, guttulate, cylindrical, straight or slightly curved, with obtuse apices and 1 to 5 transverse septa (commonly 3 – 4), 17 – 48 x 2.5 – 4.5 µm (Figure 3c). Conidia of *S. nodorum* were hyaline, thin-walled, cylindrical, straight or slightly curved, with obtuse apices and 0 to 3 transverse septa, 13 –28 x 2 – 4 µm (Figure 3d).

Discussion

The data presented in this paper illustrated only the incidence, not the severity of the studied pathogens. The frequency of occurrence of fungi differed depending on the leaf layer from which the samples were collected. The differences in the vertical distribution of the studied foliar pathogens probably were an expression of the rate at which these fungi can sporulate. The increasing frequency of *Ptr* in the middle and upper leaf layers in comparison with SLB inciters could be explained by its capacity to produce reproductive structures more speedily than *Z. tritici* and *S. avenae* f.sp. *triticea* even though they were also present in the lower layer. *Ptr* is a diurnal sporulator and conidia are disseminated by wind. High number of conidia occurred during afternoon hours after prolonged wetness periods (Francl, 1997). SLB are splash-dispersed diseases, which progress from the base to the top of

canopy. Conidia are incorporated in droplets resulting from the impact of rain drops on sporulating tissues of the lower infected leaves. *Z. tritici* has a latent period of 3 – 4 weeks (Shaw, 1990) while *Ptr* has a much shorter latent period of 5 – 8 days (Riaz et al., 1991), hence it seems likely that tan spot out-competed septoria tritici blotch.

The primary disease infection by *Ptr* is initiated by ascospores developed in pseudothecia on overwintered wheat straw. The release of ascospores starts in spring and can continue all the season contributing to the infection pressure (Ciuffetti and Tuori, 1999; Bankina and Priekule, 2011). Pseudothecia of *Ptr* were observed under the climate conditions of Bulgaria (Todorova, 2006; Nedyalkova et al., 2013). During the period of rainfall and high humidity multiple cycles of conidial production and release occurred, leading to rapid development of tan spot pathogen (Ronis and Semaškienė, 2006). It was found that a longer post-inoculation wet period and optimal temperature increased conidial germination, the number of germ tubes per conidium, the length of germ tubes and appressoria production (Hosford et al., 1987).

The infection by *M. graminicola* is initiated by air-borne ascospores and splash-dispersed conidia produced on residues of the previous season's crop. In some countries the pathogen is able to complete several sexual cycles per season (Kema et al., 1996) and ascospores are important sources of primary inoculum (Sanderson and Hampton, 1978; Shaw and Royle, 1989). The teleomorph *M. graminicola* has not been established in Bulgaria, yet. Moisture is required for all stages of SLB infection: germination, penetration, mycelial development and formation of pycnidia (Shaw, 1990).

Differences have been observed in the species composition and frequency of occurrence of fungi involved in SLB complex, where the causal agent of septoria tritici blotch *M. graminicola* was the dominant species. It is one of the most economically important wheat-infecting fungi in Europe (Eyal, 1981, 1999; Kema et al., 2008).

Symptoms produced by *Z. tritici* were distinct from those of *Ptr* and other two SLB inciters. It is possible that familiarity with symptoms would permit field identification of septoria tritici blotch. It is unlikely, however, that field symptoms of tan spot and stagonospora leaf blotch could be reliably distinguished. Although the occurrence of pycnidia in tan-colored lesions would indicate infection by *Stagonospora*, pycnidia were not always present.

Conclusion

The present investigation provided information on the frequency of the occurrence of the main leaf spotting pathogens on wheat (bread and durum) and their vertical distribution. *Ptr* was the ubiquitous fungus in all studied sites with increasing occurrence from lower to upper leaf layers. SLB inciters were predominant on bread wheat especially on cultivar Enola due to its susceptibility to *Z. tritici*. In the lower and middle leaf layers they were more frequent than *Ptr*. On durum wheat *Ptr* was the most prevalent pathogen in all leaf layers especially on cultivar Saturn, while *Z. tritici* was found in 6 samples only in 2013. *S. avenae* f.sp. *triticea* were recorded on *T. aestivum* and *T. durum* cultivars with slightly prevalence on durum wheat. *S. nodorum* was recorded very seldom (on bread wheat) or missing at all (on durum wheat).

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Studies performed on experimental animals should be carried out according to internationally recognized guidelines for animal welfare. That should be clearly described in the respective section “Material and methods”.

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