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Present status of *Zymoseptoria tritici* (*Mycospharella graminicola* /Fuckel/ Schroter) of the wheat cultures in the Republic of Macedonia

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(Manuscript received 6 July 2017; accepted for publication 31 August 2017)

Abstract. *In the Republic of Macedonia, wheat is a very important crop and it is grown on an area of around 250.000 ha. The most important regions for wheat growing are: Bitola, Kumanovo, Sveti Nikole, Skopje, Probistip, Kocani, Veles and Stip. The most important deseases on wheat are: Tapesia yallundae Wallwork and Spooner with its anamorphic stage Pseudocercosporella herpotrichoides (Fron) Deighton causer of the desease „eyespot" on barley and wheat; Puccinia graminis f. spp. tritici; Puccinia racondita f. spp. tritici; Gaeumannomyces graminis var. tritici; Bipolaris sorokiniana (Sacc.) Shoemaker; Blumeria graminis var. tritici and Zymoseptoria tritici (Mycospharella graminicola (Fuckel) Schroter). Many new diseases on wheat causing significant economic damage to producers are observed in Macedonia. The main aim of this article is to present the symptoms, morphology and protective measures of Zymoseptoria tritici (Mycospharella graminicola), the most widely spread fungal pathogens on wheat in the Republic of Macedonia. In the period between 2014 and 2016, the pathogen fungi on wheat with the highest intensity were: Zymoseptoria tritici, Tapesia yallundae, Puccinia graminis, Puccinia recondita, Gaeumannomyces graminis, Bipolaris sorokiniana, Blumeria graminis. The intensity of the diseases and the damages – yield losses of wheat, differed from year to year and between regions, depended on the sensitivity of the wheat varieties. The smallest yield loss was identified in wheat producers who treated the wheat with pesticides at least twice for vegetation season.*

Keywords: wheat, eyespot, diseases, symptoms, morphology

Introduction

Wheat is the most important small grain crop used in the daily diet of people with high business, agro-technical and economic importance for the Republic of Macedonia. The most common wheat varieties grown in the country are: *Mila, Radika, Pobeda, Orovchanka, Lepoklasa, Zvezdana, Symonida* and *Milenka*, with an average yield of around 3500-4000 kg/ha. Wheat production in Macedonia is intended only for domestic consumption.

Constant monitoring of the health conditions of wheat is performed in the area under cultivation during the vegetation period. Field control is performed mostly in the areas of Kochani, Stip, Sveti Nikole, Skopje, Bitola, Kumanovo and Prilep. The anamorphic stage of Septoria tritici was discovered as early as 1842 by Desmazierss (Arsenijevic, 1965). Many years later, the telemorphic stage was also discovered by Sanderson (1972, cited by Halama, 1996). This stage was discovered and discribed in the Republic of Macedonia for the fisrt time in 2007 (Karov et al., 2008b). *Mycosphaerella graminicola*, Synonym: *Septoria tritici*. Correct taxonomic name: *Zymoseptoria tritici*, is a species of filamentous fungus, an ascomycete in the family *Mycosphaerellaceae*. It is a wheat plant pathogen causing septoria leaf blotch that is difficult to control due to resistance to multiple fungicides. The pathogen today causes one of the most important diseases of wheat, some of which cause significant economic losses (Karov et al., 2008, 2009).

Septoriza of the wheat has been expanding in the last ten years. The damages from this fungus could amount to 60% (Jovicevic, 1960). Shipton et al. (1971) wrote that the damanges could go even beyond 60%. Through an experiment, Arsenijevic (1965) has confirmed that in the conditions of artificial innoculation the yield has decreased between 37-56%.

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The aim of the study is to present the symptoms, morphology and protective measures of *Zymoseptoria tritici* (*Mycospharella graminicola* /Fuckel/ Schroter), the most widely spread fungal pathogens on wheat in the Republic of Macedonia.

Material and methods

Sympthomatic plant material is collected during the field vegetations of wheat in the period 2007-2016. Symptoms in the field were photographed and observed under binocular and microscope of the brand Olimpus, model XS-402.

Pathogens were isolated on nutrient PDA (Nelson et al., 1983) and grown at 25°C for 7-14 days. For candida induction, the pathogen was kept in Czapeck's agar solution (Tuite, 1969) for ten days at temperature of 20-25°C. The identity of fungi was confirmed by the morphology of the pathogen and the use of identification key (Bozidar et al., 1990; Agrios, 2007). Pathogenicity on wheat was confirmed by infecting healthy wheat plants of Mila variety.

Results and discussion

Symptom

Regarding the symptoms, the results obtained show that the disease appears in the course of the entire vegetation process on all parts of the plant that are above the ground. The first symptoms are observed on the lower leaves as chlorotic spots. The infection progresses on the upper leaves, while the lower leaves become chloritic with the presence of black pycnidia. Later on, the spots

Figure 1. Field symptoms on wheat variety „Pobeda"

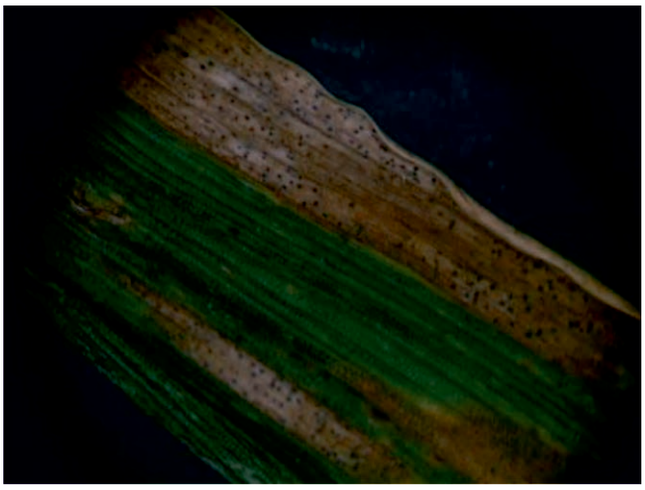

Figure 2. Pycnidia are oval and black with diameter of 17 – 96 µm

connect and get bigger. The most intensive period of the desease is in May and June. At that time, the spots reach the level of 1-5 x 4-15 mm.

Later on, the chlorotic spots become grey with dark edges (Figure 1). The pathogen remains in the necrotised tissue, but the necrosis enlarges outside the necrotised part of the wheat as well due to the influence of the toxin which is produced by the fungus.

Morphology

The blotches contain asexual (Pycnidia) and sexual (Pseudotecia) fructifications. The asexual, anamorph stage of the fungus is called *Septoria tritici* Rob. Ex Desm. Pycnidia are oval and black with diameter of 17 – 96 µm (Figures 2 and 3).

Pycnidia contain and produce a great number of hyaline micropicnospores (which measure 1-1.3 x 8-9.5 µm) and macropicnospores (which measure 1.5-3.5 x 35-98 µm). Germination of pycnidiospores can be lateral or terminal (Figure 4).

The sexual state – teleomorph - was observed for the first time at the end of May in 2008 in the area of Probishtip, Republic of Macedonia in the form of Peritecia on the lower part of the stem (Karov et al., 2008b).

Peritecia are subepidermal, globase, dark brown with diameter of 72-95 µm, carrying a lot of asci. Asci measure 11-13 x 30-38 µm. Asci are limpid with two layers containing eight ascospores. Ascospores are hyaline, eliptical and two-celled with dimension 2.5-4 x 9-16 µm (Figure 5).

Primary infection of plants is by airborne ascospores, i.e. by entry of ascospores into the leaves via stomata. Pycnidia develop in the lesions of wheat. A large number of micropycnospores and macropycnospores are formed in the pycnidia. Depending on the climate conditions, this pathogen fungus can survive the winter in the crop debris of the pycnidia and the mycelia. In such a case, pycnospores germinate and cause the primary infection. Pycnidia with micro- and macropycniodiospores are formed during spring and summer in the lesions of wheat. Secondary spread of pycnidiospores is performed by contact and rain dispersal. Pseudothecia and pycnidia develop within lesions.

Pathogen overwinters as mycelium, pycnidia and pseudothecia on crop debris, autumn sown crops and volunteer host (Ponomarenko et al., 2011). Nakov et al. (1994) found that this pathogen fungus in Bulgaria overwinters in the mycelium of the infected leaves of the wheat.

Septoria tritici infects through stomata rather than by direct penetration and there is a long latent period of up to two weeks following infection before symptoms develop. Symptoms appear 14-21 days after penetration. The length of the incubation period depends on the sensitivity of the variety, the air temperature and the relative humidity of the air. *S. tritici* is a policyclic pathogen and there

Figure 3. Microscopic view of picnidia

Figure 4. Picnid with micro and macro picnospores

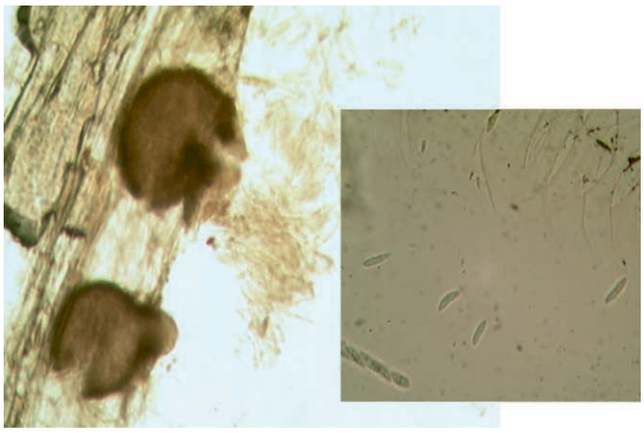

Figure 5. Ascospores are hyaline, eliptical and two-celled with dimension 2,5- 4 x 9-16 µm

can be up to nine generations of it in the course of one vegetation period of wheat (Arsenijevic, 1965).

In 2011, Quaedvlieg et al. (2011) introduced a new genus *Zymoseptoria* and proposed a new scientific classification: Kingdom: fungi, Phylum: *Ascomycota*, Class: *Dothideomycetes*, Subclass: *Dothydeomycetidae*, Order: *Capnodiales*, Family: *Mycosphaerellaceae*, Genus: *Zymoseptoria*, Species: *Zymoseptoria tritici,* Binomial name: *Myconosphaerella graminicola* (Fuckel.) J. Schroter.

Many authors and researchers accepted the new classification and the newly proposed genus *Zymoseptoria*. In the Republic of Macedonia, the scienties have also accepted and agreed with the proposal that the pathogen fungus *Zymoseptoria tritici* is causing leaf blotch of wheat.

Five different combinations of fungicides were examined in the period between 2014-2016 on wheat variety „*Pobeda*". Those were:

1. Propiconazole 250g/L
2. Epoksikonazol + Tiofanat metil
3. Propiconazol + difenokonazol
4. Epoksikonazol + Karbendazim
5. Ciprokonazol + Karbendazim
6. Kontrola (not treated, without fungicides).

Field examinations were conducted in Kochani and Kumanovo regions. First treatment with fungicides was performed in the phase when plants have three leaves, immediately after the first symptoms appear. The second treatment with fungicides was performed 90 days after the first one.

Conclusion

In the period between 2007 and 2016, the authors were monitoring regularly the health status of the crops, and in particular wheat and barley, in the biggest areas of the Republic of Macedonia where these crops are grown. In this period, several new pathogen fungi were detected on wheat and in Macedonia. The following pathogen fungi on wheat were identified (2014-2016) with the highest intensity: *Zymoseptoria tritici, Tapesia yallundae, Puccinia graminis, Puccinia recondita, Gaeumannomyces graminis, Bipolaris sorokiniana, Blumeria graminis*. The intensity of the diseases and the damages – yield losses of wheat – differed from year to year and between regions. This depended on the sensitivity of the wheat varieties. The smallest yield loss was identified in wheat producers who treated the wheat with pesticides at least twice. In the field examinations the best bilogical efficiency of wheat protection from a great number of fungal diseases was achieved with two treatments, with one of the following combinations of fungicides: Epoksikonazol + Tiofanat metil; Propiconazol + difenokonazol; Epoksikonazol + Karbendazim and Ciprokonazol + Karbendazim.

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The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods,Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

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