Diversity of fungal pathogens infecting *Hordeum* L. in Macedonia, symptoms and morphology

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Abstract: During April, May and June 2006, 2007, 2008 and 2009 several plant pathogenic fungi are discovered on barley variety *Hordeum vulgare* L. Ement. Lam. in Macedonia. The monitoring confirms the presence of *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur, *Blumeria graminis* (*Erysiphe graminis* DC.), *Gaeumannomyces graminis* var. *tritici* (Sacc.) Arx & Oliver, *Puccinia hordei* Otth., and *Ustilago nuda* (Jensen) Rostr. The presence of anamorph *Septoria tritici* Rob ex Desm., which telemorph is *Mycosphaerella graminicola* (Fuckel.) Schroter and anamorph *Pseudocercosporella herpotrichoides* (Fron) Deighton which telemorph is *Tapesia yallundae* Wallwork & Spooner is discovered for the first time in Macedonia. The most spread fungus is *Cochliobolus sativus* (Ito & Kurib.) found in eight of nine monitoring areas.

Key Words: *Hordeum vulgare, Cochliobolus sativus, Blumeria graminis, Gaeumannomyces graminis* var. *tritici, Puccinia hordei, Ustilago nuda, Tapesia yallundae, Mycosphaerella graminicolla.*

Introduction

The most distributed barley variety in Macedonia is *Hordeum vulgare* L. Ement. Lam. It is breed on 55 000 ha with yield of around 3000 kg/ha (Statistical review: Agriculture, 2006, 2007 and 2008). Nine regions producing barley in Macedonia were monitored: Kumanovo, Skopje,

Bitola, Tetovo, Prilep, Stip, Kocani, Sveti Nikole and Probistip, during the period from January till June 2006, 2007, 2008 and January till April, 2009. The health condition of barley show that over 10% of entire yield lost is caused by several types of phytopathogenic fungi, parasiting barley in Macedonia. The presence of *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur, *Blumeria graminis* (Erysiphe graminis DC.), *Gaeumannomyces graminis* var. *tritici* (Sacc.) Arx & Oliver, *Puccinia hordei* Otth., and *Ustilago nuda* (Jensen) Rostr., is confirmed during this period. The presence of anamorph *Septoria tritici* Rob ex Desm., which telomorph is *Mycosphaerella graminicolla* (Fuckel.) Schroter, and anamorph *Pseudocercosporella herpotrichoides* (Fron) Deighton which teleomorph is *Tapesia yallundae* Wallwork & Spooner was confirmed for the first time in Macedonia. The aim of this article is to present the diversity of fungal pathogens, symptoms in the field and morphology of the microorganisms causing barley diseases in Macedonia.

Materials and methods

Plant material is collected during the field approbations of *Hordeum* L. in the regions of: Kumanovo, Skopje, Bitola, Tetovo, Prilep, Stip, Kocani, Sveti Nikole and Probistip in 2006, 2007, 2008 and 2009. Symptoms in the field are photographed and observed under binocular and microscope, mark OLYMPUS, model XS-402. Facultative fungal parasites are isolate on nutrient agar PDA (Nelson et al., 1983) and grown on 25℃, 7-10 days. For conidia induce, the pathogen was maintain on Czapeck's Solution Agar (Tuite, 1969), 10 days at temperature of 20-25℃. *Gauemanomuces graminis*, is isolated on selective medium SM-GGT3, (Mathre D.E., 2000);

The identity of fungi is confirmed by the morphology of the pathogen and the use of identification key (Agrios, third edition, 2007; Compenduum of baley diseases 2008; Compendium for Crop Production 2003, APS Press);

Pathogenicity is confirmed infecting health barley plants cv. "*Barun",* spraying the suspension with 10^7 CFU on the leaf surface and setting healthy seeds in infected soil;

Resultes

***Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur** the casual agent of spot blotch and common root rot of barley and wheat, is discovered in the area of: Kumanovo, Skopje, Bitola, Tetovo, Prilep, Stip, Kocani and Sveti Nikole. This pathogen cause a disease called "Foot and root rot of cereals" or **"**Common root rot". Primary symptoms, occurred in the early stage of barley development on coleoptiles, subcrown, primary and secondary roots due to the presence of conidia inoculum in soil and seed (Agrios, 2005). At the end of the winter and early in the spring, underground plant parts appeared yellow and not developed enough. The pathogen from soil inoculum destroy the vascular system, thus the plant is unable to supply the nutrients and appear yellow and stunted as compared to healthy plants (Fig. 1). The second infections become from conidia produced on the wheat straw or diseased grass hosts (Ivanovic M., 1992). Second infections appear on leafs as black to brown flacks which expand into oval shaped black flack not exceeding 1cm in length (Fig. 2). Some of the plants recover from the infection (Fig. 3) and mature normally, but the infection progress every year due to the multiplication of the inoculum in the soil. Microorganism, survive as pseudothecia on wheat residues in the field (Fig. 4). Pseudothecia cracks and liberate asci and ascospores, which don't cause infection (Fig.5). Large number of conidia having 3-6 septa, dark green to brown colored, 21-47 μm in lenght and 16-18 μm width (Fig. 6) and olivaceous, septed mycelium with large number of conidiophores (Fig. 7) are observed microscopically. Microscopic observation of conidia grown on Czapeck's agar after one week showed occurrence of globular bodies (Fig. 6) and germination of conidia (Fig. 8).

Fig.1 Primary infection of *Cochliobolus sativus*

Fig.2 Secundary infection of *Cochliobolus sativus*

Fig. 3 Plant recovering from the infection of *Cochliobolus sativus*

Fig. 4 Pseudothecia on wheat residues

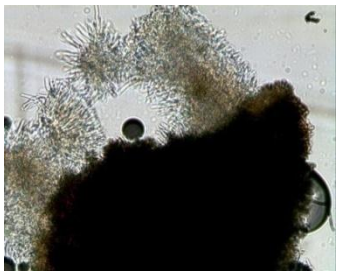

Fig. 5 Liberation of asci and ascospores from pseudothecia

Fig. 6 Conidia of C. sativus and presence of globular bodies

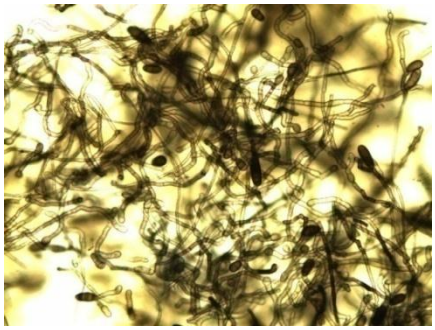

Fig. 7. Mycelia and conidiophores

Fig.8 Germination of conidia

Blumeria graminis **(Erysiphe graminis DC.)** cause disease called "Powdery mildew". The pathogen appears every year in every barley area. The intensity depends on weather conditions during the end of the winter and beginning of the spring. Most damaged was 2007, when the humidity during May and June was very high and the average temperature was 20℃. The damage is estimated on 5% of yield loss. Symptoms appear as whitish mycelium on the leaf surface and stem with many black cleistothecium in it (Fig. 9 and Fig. 10). Cleistothecium crack and liberates 25 mature and immature accuses (Fig. 11 and Fig. 12). Primary infections become from ascospores (Fig. 12) which produce mycelium. Mycelium, penetrate directly in the plant, after short time a surface colony and conidia are formed and these conidia cost second infections (Wiese, 1977).

Fig. 9 Black cleistothecium in grey mycelium on the surface of the leaf

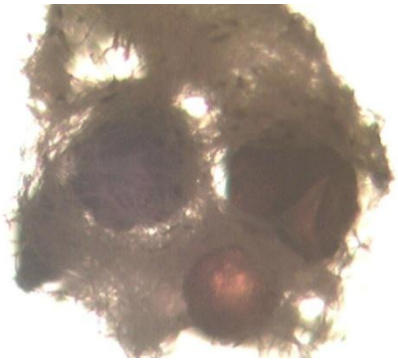

Fig. 10 Cleistothecium in mycelium

Fig. 11 Cracking of cleistothecium and liberation of asci

Fig. 12 Mature and immature asci and liberation of ascospores

Gaeumanomyces graminis* var. *tritici (Sacc.) Arx & Oliver, is more familiar as "Take-all" disease on cereals. Crowns and main roots of plants infected with take-all, generally are shiny blue-black in color and have more root rot than with "common root rot", resulting in stubbed off roots with take-all. Plants can usually be pulled from the soil much easier than healthy plants or ones with common root rot due to the week development of the root (Fig.14) The pathogen was discovered for the first time in 2005 in the area of Sveti Nikole, Stip and Kocani. In April 2009 it was discovered in the region of Bitola, too. The pathogen overwinters in infected barley and grass plant roots and stems, and in plant host debris, as perithecium (Mathre, 2000) (Fig. 15). Perithecium produces asci and eight ascospores (Fig.16 and fig. 17). Most infections are caused by mycelium coming in contact with the roots of the plants and penetrate directly trough pegs. The plants die because of the destroyed vascular system (Naiki et al., 1983) (Fig. 13). Ascospores liberated from asci developed on straw, cause the second infections on the leaf (Fig.14). Symptoms from the second infections appeared as dark brown to black lesions around 1cm in length. The infection on the leaves was over 10% of the leaf surface. In *in vitro* conditions perithecia developed on C'zapeck agar on 20˚C in a period of five weeks (Fig.18 and Fig. 19).

Fig. 13 Primary infection of G. graminis

Fig.14 Secondary infection of G. graminis

Fig. 15 Perithecium of G. graminis on straw

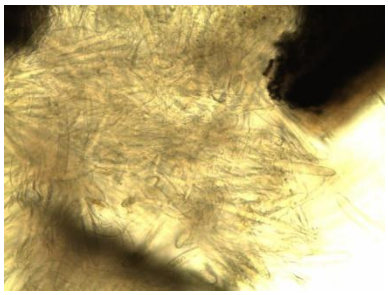

Fig. 16 Liberation of asci from perithecium of G. graminis

Fig. 17 Asci and ascospores of G. graminis

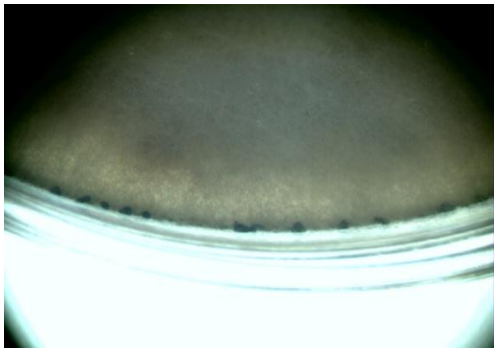

Fig. 18 Perithecia on C'zapeck agar

Fig. 19 Microscopic view of perithecia in *in vitro* conditions

***Puccinia hordei* f.sp. *tritici* Otth** cause the disease called **"**Stem rust". Symptoms are observed in the area of Bitola, Skopje, Probistip and Prilep, every year since 2006. Yellow to orange uredosoruses, elliptical and parallel with the axis, appear on stem and leafs. Over 65% of the plant surface was covered with these uredosoruses (Fig. 20). The fungus forms five different types of spores (Kurt et al., 2005). Microscopical observations showed the presence of uredospores and teleutospores (Fig. 21). Uredospores are one celled, orange and oval with diameter of 17- 21μm. Teleutospores are two-celled, elliptical and slightly sagged between the cells. The carion of the teleutospores is well diferenced and clearly seen (Fig. 19).

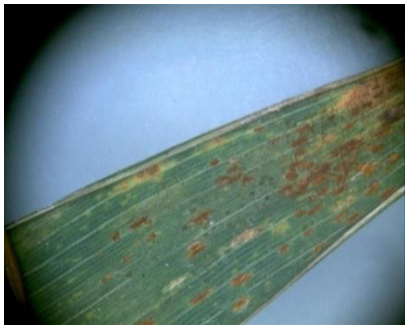

Fig. 20 Uredosoruses of *Puccinia hordei* f.sp. *tritici* on the leaf surface

Fig. 21 Uredospores and teleutospores of *Puccinia hordei* f.sp. *tritici*

***Ustilago nuda* (Jensen) Rostr.** or "Loose smut" is discovered in the area of Kocani and Bitola in several small parcels where growers used untreated seed from their own production. This pathogen usually is successfully controlled with seed disinfection (karboksin), otherwice it can be very destructive. The pathogen completely destroyed the germs, the seed is full with black powdery smut (Fig. 22 and Fig. 24), consists of teleutospores from the fungus. Teleutospores are distributed with the wind and spread the infection on large distances (Cummins et al., 2003). Microscopic observations showed the presence of brown teleutospores (Fig. 23). Teleutospores are formed from the cells of the mycelium. They are spread with the wind and infect the healthy flowers and later the seed. The pathogen survive in form of inactivate mycelium in the seed, until it becomes to germinate, and the mycelium

become active (Ivanovic, 1992). Teleutospores observed under microscope have oval shape and smooth walls (Fig 21).

Fig. 22 Destroyed germs of *Ustilago nuda*

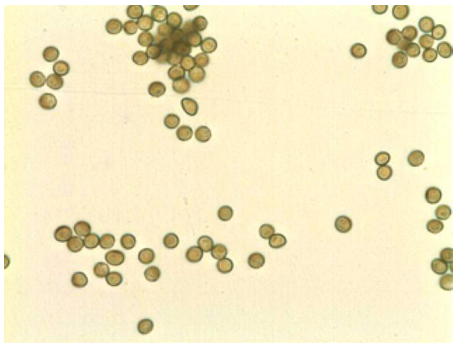

Fig. 23 Teleutospores of *Ustilago nuda*

Fig. 24 Symptoms of "Loose smut"
in the field

***Mycosphaerella graminicola* (Fuckel.) Schroter** or "Septoria leaf blotch". During the period of June 2007 and 2008, small yellow areas are observed in the field of barley area in Tetovo, Bitola and Prilep. First symptoms appear on lower leaves as chlorotic spots. The infection progress on upper leaves, while lower leaves become chlorotic with the presence of black piknidia (Fig. 25) representing the anamorphic stadium of the fugus called ***Septoria tritici* Rob ex Desm.** Picnidia are oval and black with diameter of 71-96 μm. Pycnidium cracks and liberates a great number of micro (8-9,5μm) and macropycnospores (Fig 25 and Fig.26)

which cause the infection. Teleomorphic stadium of the fungus, *Mycosphaerella graminicola* is observed in 2008 in the area of Tetovo and Bitola. Perithecia are dark brown to black in color with diameter of 72-95μm (Fig. 27). Asci are limpid and have two layers, ascospores are elliptical and two-celled (Fig. 28).

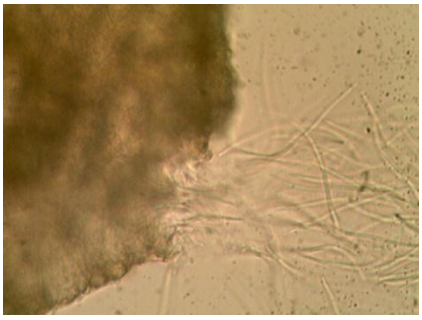

Fig. 25 Cracking of pycnidia and Liberation of pycnospores

Fig. 26 Macro and micropicnospores of *Septoria tritici*

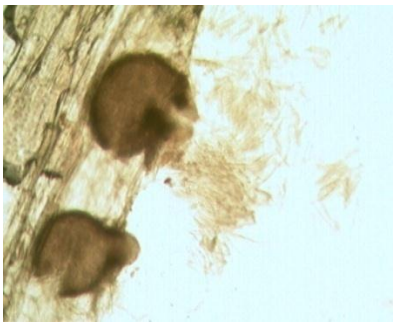

Fig. 27 Perithecium of *Mycosphaerella graminicola* and liberation of asci

Fig. 28 Asci and ascospores

The pathogen overwinters as mycelium and conidia within pycnidia, in infected seed and diseased plant refuse, left in the field. Seedling infections result in damping off, and provide inoculums for subsequent infections (Halama, 1996). The infection in the diseased area in Macedonia is spread from the diseased plant straw left in the field (Fig.27).

***Tapesia yallundae* Wallwork & Spooner** cause disease called "Eyespot". It is a teleomorphic stage of the fungus which anamorph is ***Pseudocercosporella herpotrichoides* (Fron) Deighton**. Symptoms appeared as elliptical or "eye" shaped lesions at the stem basis (Fig. 29 and Fig 30). The eyespot lesions are elliptical, greyish to olivaceous inside with dark

brown margins (Fig. 30). Later the inside of the lesions become sunken, and develop fungus-darkened centers. In sever development of the disease, the stem may break through the lesion where it is the most weakened. In the beginning, mycelium is white to gray, latter becoming light brown to olivaceous. It produces conidiophores and long, tight conidia (Fig. 31), which cause the infection in spring. These infections cause eyespot symptoms. Development of the disease can cause lodging or whiteheads of the grain head. Sexual stage occurs on straw debris (Colbach et al., 1998). Microscopic observation of field material show the presence of apothecia, asci (Fig. 32) and ascospores (Fig. 33) which spread the infection on long distance (Lucas et al., 2000).

Fig. 29 Eyespot symptoms

Fig. 30 Eyespot lession on the steam base

Fig. 31 Conidia of T. yallundae

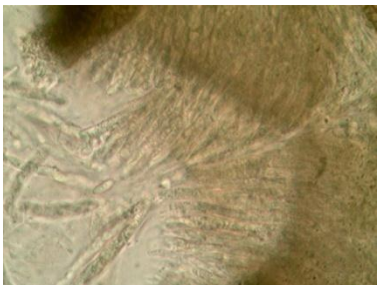

Fig. 32 Apothecia and asci

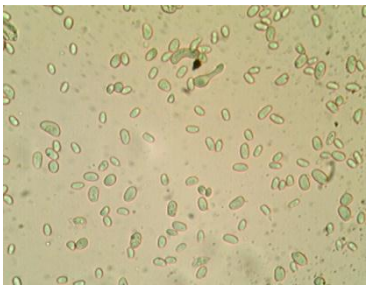

Fig. 33 Ascospores

Discussion

The investigation of barley health during last four years 2006, 2007, 2008 and 2009 showed the presence of great diversity of plant pathogenic fungi in Macedonia: *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur, *Blumeria graminis* (Erysiphe graminis DC.), *Gaeumanomyces graminis* var. *tritici* (Sacc.) Arx & Oliver, *Puccinia hordei* Otth., *Ustilago nuda* (Jensen) Rostr., *Mycosphaerella graminicolla* (Fuckel.) Schroter and *Tapesia yallundae*

Wallwork & Spooner. Favourable weather conditions at the end of the winter and beginning of spring, during these years, induce around 10% of yield loss, in barley production in Macedonia. Pathogens occurred mostly in areas where barley is produced every year without crop rotation. The most destructive and intensive was the pathogen *Cochliobolus sativus* in the production year 2007/2008 and 2008/2009 on barley, sawed in autumn (October and November) showing primary infections in December and January of investigated period. Secondary infections were observed in March and April 2008 and 2009. The most yield loss, around 20-45% was observed in the region of Kumanovo and Skopje in 2008.

The aim of this study was to make evidence of mycoses appeared on barley in Macedonia, estimation of yield loss, symptoms and morphology of the pathogen which cause the disease. During the research, the presence of three different varieties of fungi, their telemorphic and anamorphic stage, are discovered for the first time in Macedonia: Telemorph ***Tapesia yallundae*** **Wallwork & Spooner** and anamorph ***Pseudocercosporella herpotrichoides*** **(Fron) Deighton**, belonging to the class ***Ascomycetes***, order ***Leotiales***, family ***Dermataceae***, genus ***Tapesia***; Telemorph ***Mycosphaerella graminicola*** **(Fuckel.) Schroter,** anamorph ***Septoria tritici*** **Rob ex Desm.,** belonging to the class ***Loculoascomycetes,*** order **Dothideales,** family **Mycosphaerellaceae,** genus **Mycosphaerella** and Telemorph ***Cochliobolus sativus*** **(Ito & Kurib.) Drechsler ex Dastur,** which anamorph is ***Drechslera sorokiniana*** **(Sacc.) Subram. & Jain.,** belonging to the class ***Loculoascomucetes***, order ***Pleosporales***, family ***Pleosporaseae***, genus ***Cochliobolus***.

Republic of Macedonia doesn't export barley, most of these diseases are introduced in the country by import of seed material. Considering that most of these diseases are residue born diseases, crop rotation, on time seedling, soil dreaning and establishment of residue management is recommended to suppress the infection.

References

Agrios G.N., (2005). Plant Pathology. Fifth edition. USA: Elsevier Academic Press.

Colbach N., Saur L. (1998). Influence of crop management on eyespot development and infection cycles of winter wheat. European Journal of Plant Pathology, 104 (1): 37-48.

Cummins G.B., Hiratsuka, Y. (2003). Ilustrated genera of rust fungi. Third ed. Minesota, APS Press.

Halama P. 1996. The occurrence of *Mycosphaerella graminicola*, telemorph of Septoria tritici in France. Plant Pathology. 45, 135-138.

Ivanovic M., (1992). Miokoze biljaka. Beograd: Nauka.

Kurt J. Leonard and Les J. Szabo (2005). Stem rust of small grains and grasses caused by Puccinia graminis. Molecular Plant Pathology 6(2), 99-111.

Lucas J.A., Dyer, P. S. and Murray, T.D. (2000). Pathogenicity, host-specificity, and population biology of Tapesia spp., causal agents of eyespot disease in cereals. Advances in Botanical research 33: 226-258.

Mathre D.E. (2000). Take-all Disease on wheat, barley and oats. Plant Health Progress 10.1094/PHP-2000-0623-01-DG.

Naiki T and Cook, R.J. (1983). Factors in loss of pathogenicity in Gaeumannomyces graminis var. tritici. Phytopathology 73: 1652-1656.

Nelson P.E., Toussoun, T.A., and Marasas, W.F.O., 1983. Fusarium species: An illustrated Mannual for Identification. Pensylvania State University Press, University Park.

Tuite J. 1969. Plant Pathological Methods. Burgess Publishing Co., Mpls.

Wiese M.V. (1977). Compendium of Wheat Diseases. Minnesota, USA. APS.