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ЗА ПРИРОДНЕ НАУКЕ

MATICA SRPSKA
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BIPOLARIS SOROKINIANA (TELEOMORPH *COCHLIOBOLUS SATIVUS*) — CAUSER OF BARLEY LEAF LESIONS AND ROOT ROT IN MACEDONIA

ABSTRACT: Diseased barley plants (*Hordeum vulgare*), were noticed in the area of Kumanovo, Bitola, Probistip, Skopje and Kocani, at the beginning of March, 2006. Our investigations were carried out in the period from 2006 to 2009. The plants were highly diseased, probably in the stage of germination, dwarfed with necrotic leaves and with poorly developed root. A rotten root collar was noticed notice in some plants, which could be easily pulled out from the soil. Plants infected in a later developing stage became yellow from the top of the leaf, and many brown-olive, oval shape lesions were noticed. Conidia of *Bipolaris sorokiniana* (Sacc.) Shoen., were isolated from symptomatic lesions. Pseudothecia with asci and ascospores from teleomorph *Cochliobolus sativus*, were found on the barley straw in the same field the previous year.

KEY WORDS: ascuses, ascospores, conidia, *Hordeum vulgare*, *Cochliobolus sativus*, pseudothecia

INTRODUCTION

Barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) are the most important crops in the Republic of Macedonia, and are cultivated in over 88735,55 ha (wheat) and 32864,52 ha (barley) (National Statistics of Macedonia, 2007).

Barley and wheat, like all other crops, are host plants for many different pathogens, causal agents of diseases.

Cochliobolus sativus (Ito and kurib.) Drechsl. ex Dastur *Bipolaris sorokiniana* (Sacc in sorok.), the causal agent of spot blotch of barley (*Hordeum vulgare*) is a common foliar pathogen worldwide, but is particularly aggressive under conditions of high relative humidity and temperature associated with imbalanced soil fertility (Duveiller and Altamirano, 2000). It has a

wide host range and it is economically important for wheat (Mathre, 1982, Nutter et al., 1985).

Spot blotch of barley caused by *C. sativus* (Ito and Kurib.) Drechsl. ex Dastur *Bipolaris sorokiniana* (Sacc in sorok.) Shoem. *Helminthosporium sativum* Pamm, King and Bakke is responsible for low yield and poor quality in many parts of the world (Piening et al., 1976; Nutter et al., 1985). In susceptible barley cultivars, average yield losses of 16%—33% have been reported (Clark, 1979). The development of barley genotypes resistant, or tolerant, to spot blotch is considered to be the most economic way for controlling this disease.

Symptoms in barley

Spots (lesions) that are chocolate brown-to-black appear near the soil line or at the base of the sheaths that cover the seedling leaves. Infections may progress until the seedlings turn yellow and die, either before or after the emergence, thus reducing the stand. The latter case is more frequent. Affected seedlings may be dwarfed and have dark green leaves. Diseased barley seedlings commonly have weakened, dark brown rotted crowns and roots. When seedling infections are severe, plants may be dwarfed, the heads may not emerge completely, and the kernels are poorly filled (Ivanovic, 2001).

Barley plants that avoid serious seedling infection usually appear normal until about heading time, when the characteristic leaf lesions, of various sizes and shapes appear on the lower leaves after warm, moist weather. The center of each lesion is dark brown with gradual change into green colour at the edge of the leaf. Many spots are oblong or lens-shaped, with centers lighter brown than the edges. Where numerous, the lesions may merge, thus producing large irregular blotches. Heavily infected leaves dry out and die prematurely.

The centers of older lesions on both living and dead leaves have an olive-green cast caused by fungus growth and an abundant production of summer spores (conidia). Spot blotch starts on the older leaves and sheaths, spreading upward to the younger leaves. The lesions never have the netted appearance characteristic of net blotch.

A spot blotch is a very important disease in Macedonia but before this study no other studies conducted about the diversity of the *Cochliobolus sativus* populations in the barley fields have been carried out.

In this study the presence and distribution of *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) from different regions in Macedonia, and the possible ways of control and prevention were investigated.

MATERIAL AND METHODS

Host plants

Barley fields in Kumanovo, Bitola, Probistip, Skopje and Kocani areas, were used for this observation. Initial symptoms of common root rot in barley

on young seedlings from inoculum carried on the seed, or from infections originating from soilborne conidia near the seedling, were observed. Dark brown lesions appear on the outer coleoptile tissue and/or on the leaf base (Figure 1). Lesions may coalesce into long areas of necrotic brown tissue. In extreme cases, the entire seedlings may die. In most cases, however, the seedling will survive but the growth of the developing plant may be stunted (Figure 2).

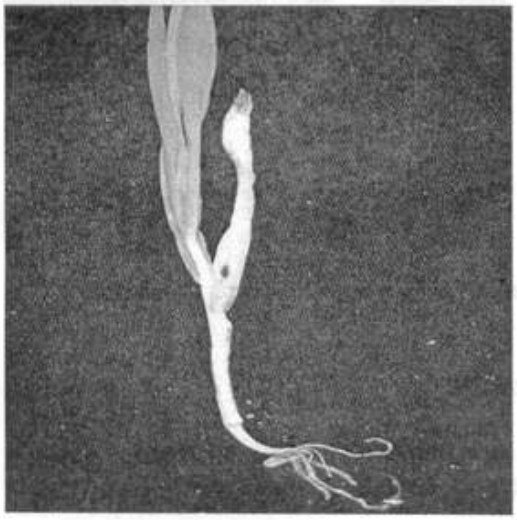

Fig. 1 — Dark lesions on the coleoptile

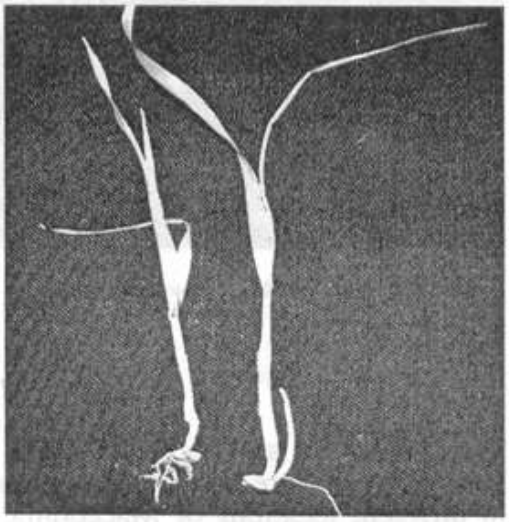

Fig. 2 — Stunting of seedlings infected and leaf bases caused by *C. sativus* by the common root rot organism

Dark brown-to-black spots may appear on the glumes and kernels. The black point at the germ (embryo) end of a kernel is a common symptom (Figure 3). Kernels infected early are shriveled and lightweight. Barley seeds, healthy and infected, were put on filter paper for observation (Figure 4).

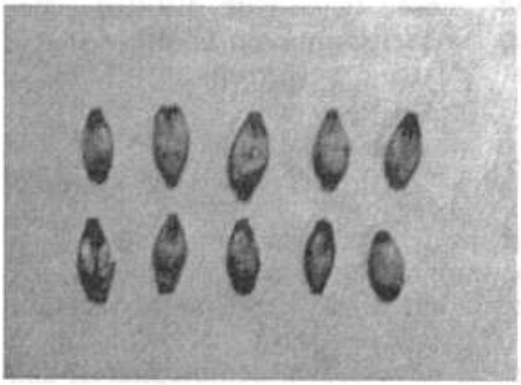

Fig. 3 — Black point of barley seeds

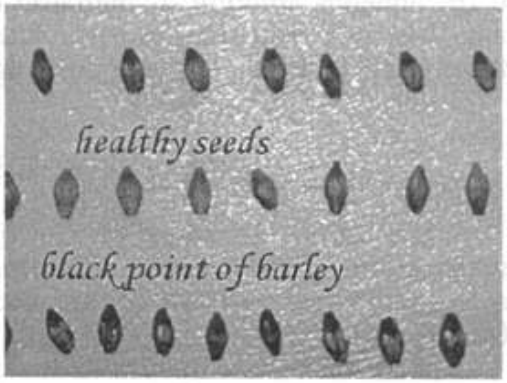

Fig. 4 — Barley seeds on filter paper

In this observation analysis of wheat straw from the previous year collected from the same field, was also performed (Figure 5).

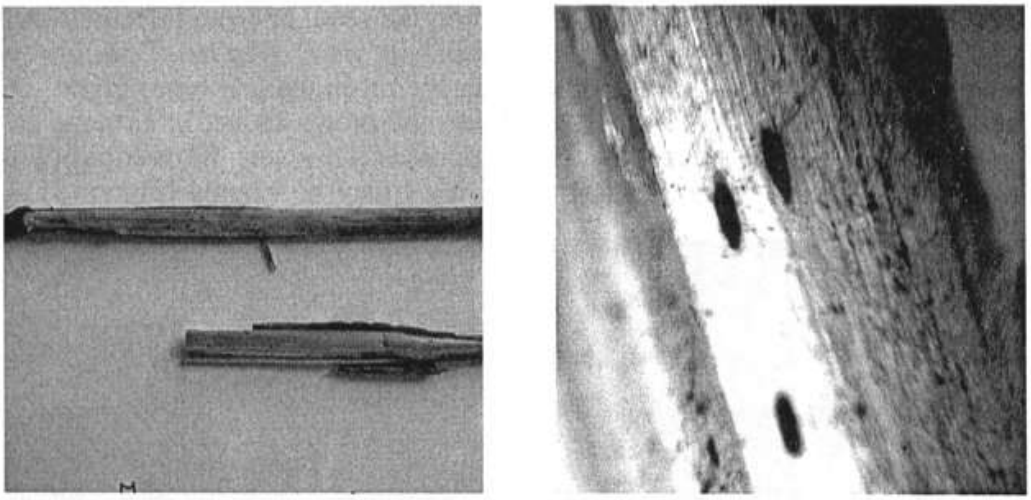

Fig. 5 — Barley straw with presence of pseudothecia from teleomorph *C. sativus*

Fungal isolates

During the seasons 2006, 2007, 2008 and 2009, the isolates from *Cochliobolus sativus* were obtained from barley leaves showing spot blotch symptoms in different locations of Macedonia.

Isolates from plant tissue. In order to isolate the pathogen from infected tissues on various media, it is best to wash the tissue well with running water for several minutes and up to 1 or 2 hours. After that, leaf surfaces were sterilized in 0.1% aqueous solution of mercuric chloride, rinsed twice with distilled water and dried between filter papers. The sterilized leaves were cut into small pieces 5 mm long and transferred to Petri dishes containing potato dextrose agar medium (PDA).

Isolates from infected barley seeds. The seeds were sterilized in the 0.1% aqueous solution of mercuric chloride, rinsed twice with distilled water and dried between filter papers. After that, the seeds were cut lengthwise and transferred to Petri dishes containing potato dextrose agar medium (PDA).

RESULTS

The anamorph stage of *Cochiobolus sativus* was easily isolated from symptomatic tissue placed under moist conditions. The mycelium of *Bipolaris sorokiniana* had an olive colour, and when older, it had dark or black colour (Figure 6).

More often, we found individual or group olive-brown conidiophores, and large brown phragmoid (cross walls only in one direction) conidia borne laterally and terminally on the conidiophore. Conidia were formed quickly and were quite evident by their characteristic dark brown oval shape with thick cross walls (Figure 7). They usually appeared within a few days at a room temperature.

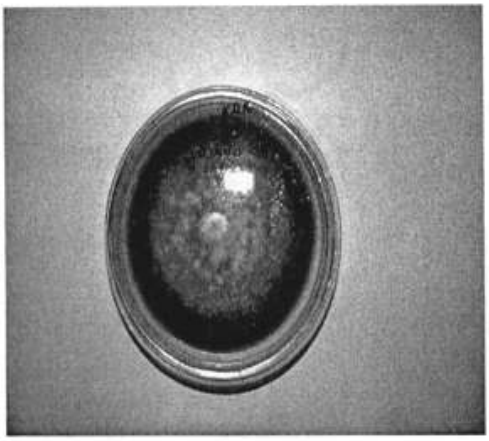

Fig. 6 — Olive micelia on nutrient agar

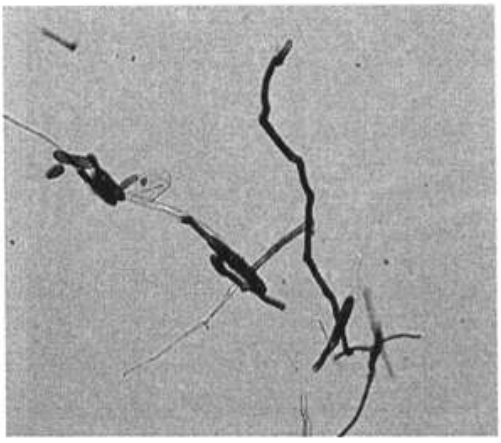

Fig. 7 — Conidiophores and conidia of common root rot pathogen, *C. sativus*

In microscopic observations, 3—7 septata conida, from conidialen stadium of *Cochiobolus sativus, Drechslera sorokiniana* (Syn. Bipolaris sonokiniana) were found. In more cases, conidia were with 5 to 6 septa, cylindrical with oval point, with dimensions of 5—6 mm in width and 120—140 mm in length, and had olivaceous colour (Figure 8).

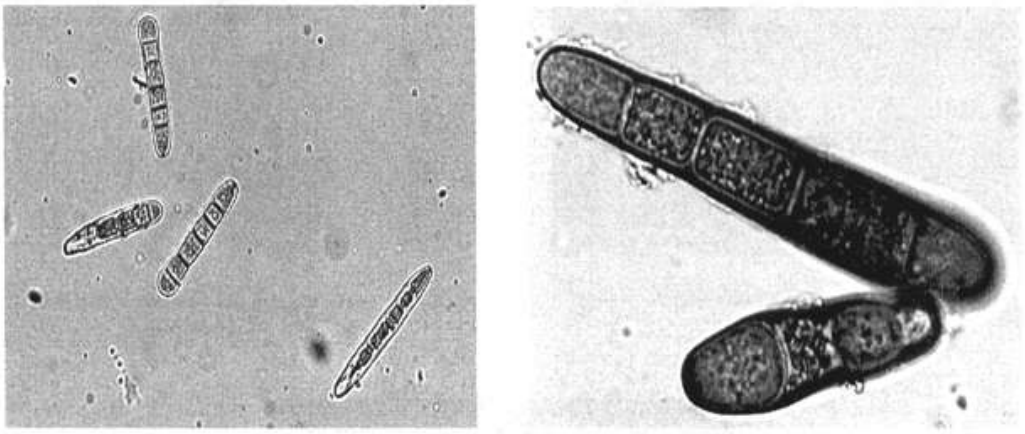

Fig. 8 — Conidii from *Drechslera sorokiniana*

Leaves from filter paper, at temperature of 25°C, after 5 days showed the presence of septic, granulated mycelia with olive color (Figure 9). On the plant tissue the presence of conidiophores and conidia of *Bipolaris sorokiniana* was also observed (Figure 10).

Pseudothecia with asci and ascospores from teleomorph *Cochliobolus sativus*, Pammel; King & Bakke, were found on barley straw from the previous year, on some fields (Figure 11 and 12).

Ascospores are usually slight, septic and discoloured with the dimensions of 6x320 mm and with 6 or 7 septate (Figure 13).

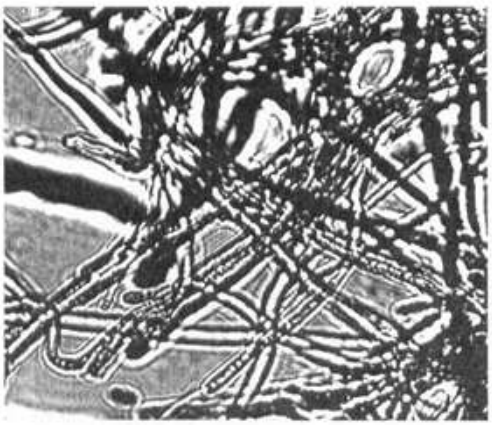

Fig. 9 — Mycelium on PDA

Fig. 10 — Conidiophores and conidia from *Cochliobolus sativus*

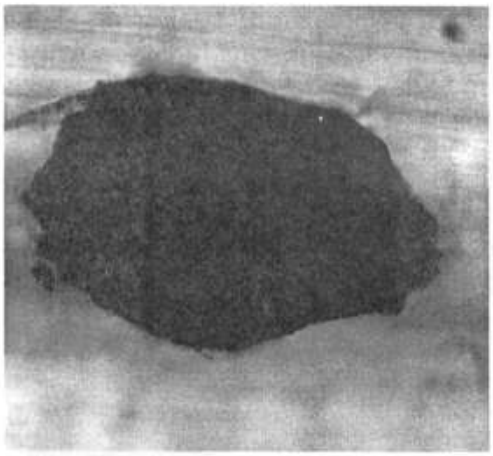

Fig. 11 — Pseudothecia

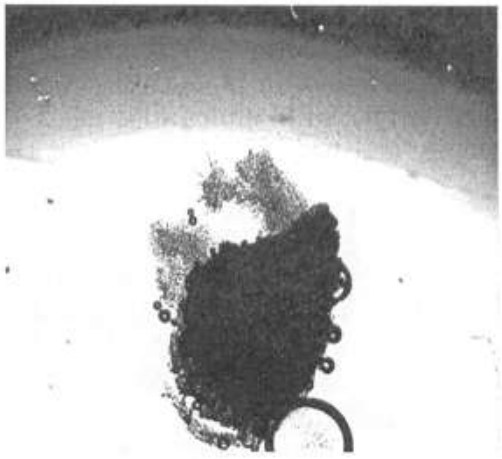

Fig. 12 — Cracking of pseudothecia and releasing of ascospores

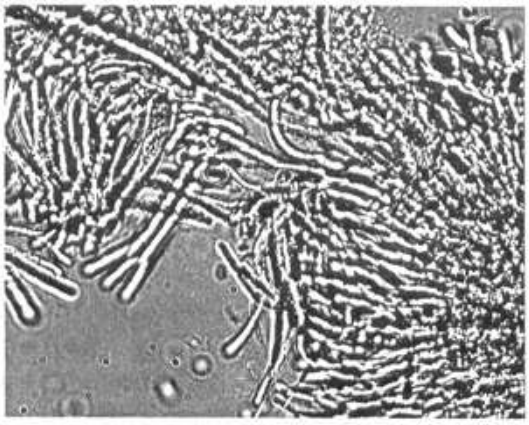

Fig. 13 — Ascospores

DISCUSSION

Cochliobolus sativus (Ito and kurib.) Drechsl. ex Dastur *Bipolaris sorokiniana* (Sacc in sorok.), the causal agent of spot blotch of barley (*Hordeum vulgare*) is a common foliar pathogen worldwide. It has a wide host range and it is economically important for wheat (Marthre, 1982, Nutter et al., 1985).

Diseased barley plants (*Hordeum vulgare*), were noticed in the areas of Kumanovo, Bitola, Probistip, Skopje and Kochani at the beginning of March, 2006. The plants were highly infected, probably in the stage of germination, dwarfed with necrotic leaves and with poorly developed root. A root rot collar was noticed in some plants, which could be easily pulled out from the soil. Plants infected in a latter developing stage became yellow from the top of the leaf and many brown-olive oval shape lesions were noticed. Conidia from *B. sonokiniana* were isolated from symptomatic lesions.

Pseudothecia with asci and ascospores from teleomorph Pammel; King & Bakke were found on the barley straw from the previous year, in some fields.

Our results indicate the recesseity of monitoring and controlling the barley plants in order to prevent the infections of *Cochiobolus sativus*, which can occur in all stages, from early spring, before germination, untill the end of the vegetation.

In all observed barley fields, the symptoms of spot blotch of barley, were present in the earliest phase of development, thus leading to a conclusion that the disease came from the seed material or soil in which pathogen had spent the winter.

Damages in the barley production during the mentioned years (from 2006 to 2009) were estimated to range from 30% tp 70% of yield losses.

Control and prevention

To reduce the infection in the barley fields, the most important practice is to use healthy and certified seed material. Before use, seeds must be treated with fungicide. If the infection already exists, as it was the case with our investigation in 2007, then it is necessary to treat the seeds with fungicide "propiconazol" in dosage of 0,5 l/ha, twice per season. With constant monitoring of plants, along with treated seeds of barley, the reduction in percentage of the infected grains could be detected next year.

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BIPOLARIS SOROKINIANA (TELEOMORPH *COCHIOBOLUS SATIVUS*) — УЗРОК ОШТЕЋЕЊА И ПЕГАВОСТИ ЛИСТА И ТРУЛЕЖИ КОРЕНА ЈЕЧМА У МАКЕДОНИЈИ

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Резиме

У првој недељи месеца марта 2006. године регистрована је болест јечма у околини Куманова, Битоља, Пробиштипа, Скопља и Кочана. Теренска и лабораторијска испитивања зараженог материјала су урађена у периоду од 2006. до 2009. године. Анализом је утврђено да су биљке највероватније биле заражене још у фази клијања. Констатоване су њихова закржљалост, пожутелост листова и слаба развијеност корена. Код неких биљака је забележена појава трулежи приземног дела стабла као и њихова слаба веза са подлогом. Код биљака које су касније заражене некроза се појавила прво на листовима и манифестована је преко многобројних кафено маслинастих пега неправилног елипсоидног облика. Из симтоматичних пега су издвојене конидије фитопатогене гљиве *Bipolaris sorokiniana* (Sacc.) Shoen.

Псеудотеци је са аскусима и аскоспорима телеморфног стадијума *Cochliobolus sativus* Pammel; King & Bakke, пронађене су у остацима сламе јечма од претходне године са исте парцеле у близини парцеле са зараженим биљкама.