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Symptoms, Etiology and Control of Sooty Blotch and Flyspeck in Bulgaria

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Abstract

Sooty blotch and flyspeck of apple (*Malus domestica* Borkh.) is a disease complex (SBFS) that causes superficial blemishes on fruits, leaves and stems. The cosmetic damages of the cuticle occur in humid temperate regions. This results in shortening the storage life of the fruits due to increased water loss. Fungi associated with SBFS have not been described in Bulgaria yet. We obtained a few isolates from discrete sooty blotch and flyspeck symptoms on apple fruits and leaves of the cultivars Melrose, Idared and Winter Banana. Two of them were identified as *Peltaster fruticola* Johnson and *Geastrumia polystigmatis* Batista & M. L. Farr, using morphological and cultural characteristics of in vitro and primary pathogenicity tests. The screening of the fungicides was carried out under laboratory conditions using Thornberry method. It showed that the most efficient against *Geastrumia polystigmatis* were Tiram 80% (as Tiram) – 97.8%, followed by Cyprodinil (as Chorus) – 71.81% and Folpet + Triadimenol (as Shavit) – 64.63% inhibition on mycelial growth. Copper hydroxide 87.7% (as Vitra) – 98.66%, Tiram 80% (as Tiram) – 98.54%, Cyprodinil (as Chorus) – 95.76% and Copper hydroxide 77% (as Funguran) – 95.69% highly inhibited the radial growth of *Peltaster fruticola*.

Keywords: sooty blotch and flyspeck, apple, etiology, *Peltaster fruticola, Geastrumia polystigmatis,* in vitro fungicide screening

Introduction

Sooty blotch and flyspeck of apple (*Malus domestica* Borkh.) is a disease complex (SBFS) that is favored by cool, rainy weather, densely planted gardens, not ventilated places, nitrogen fertilization, poor spraying. SBFS causes superficial blemishes on fruits, leaves and branches. This results in shortening the storage life of the fruits due to increased water loss. Fruits covered with SBFS fungi lose their relative weight faster, they are characterized by earlier shriveling and wrinkling than the healthy ones (Mirzwa-Mróz et al., 2012). Sooty blotch and flyspeck is a disease caused by a complex of 60 different species of fungi that colonize the epicuticular wax layer on apple). Sooty blotch was first described as being caused by *Dothidea pomigena* Schw. by Schweinitz, 1832 (Williamson et al., 2000). Groves (1933) categorized colonies into four groups: punctate, ramose, fuliginous and rimate, based on mycelial patterns and fruiting bodies (Williamson et al., 2004). SBFS is caused by a complex of epiphytic fungi, including *Peltaster fructicola, Geastrumia polystigmatis, Leptodontium elatius, Zygophiala iamaicens. Geastrumia polystigmatis* is the predominant species on apple, whereas *Peltaster* species are more common on reservoir hosts. Species distribution varied among sites (Madeiras, 2014). *Peltaster fructicola* Johnson, Sutton et Hodges (Johnson et al., 1996, 1997) is one of the few major components of the sooty blotch and flyspeck complex on apple fruits. *Geastrumia polystigmatis* was first described in 1960 by Batista et al. Like *Peltaster sp., Geastrumia polystigmatis* forms ramose colonies with pycnothyria (Johnson et al., 1996, 1997). A later investigations associated *Peltaster fructicola* with colonies showing the punctate mycelial type, whereas *Geastrumia polystigmatis* – with ramose colonies (Williamson et al., 2000, 2004; Mayfield, 2013; Madeiras, 2014). Johnson et al. (1996) observed that colonies of *Peltaster fructicola* were dark brown to black on WA and that some isolates produced an extracellular pigment visible in the agar. According to Williamson et al. (2004) colonies of *Peltaster fructicola* on WA were white to pale brown and the pigment was visible only on PDA, where colonies were compact, light dray to dark brown or black. Colonies of *Geastrumia polystigmatis* grew better on PDA than MEA and WA. The colony was dense

and tight to the surface of the agar in MEA and PDA plates and comparatively sparse in WA. Both *Geastrumia polystigmatis* and *Peltaster fructicola* were sensitive to thiophanate-methyl, mancozeb and cyprodinil. They were also recommended for use against apple scab (Madeiras, 2014).

Fungi associated with SBFS have not been described in Bulgaria yet.

Material and Methods

Symptom diversity

Symptoms of sooty blotch and flyspeck (SBFS) were described on fruits and leaves of the apple cultivars Winter Banana, Melrose and Idared, in orchards and private gardens in different regions of the southern slopes of the Balkan MountainS and Sredna Gora Mountain (Kalofer, Staro Zhelezare and Panagyurishte) in August-September and during storage production in 2009-2010. The mycelial types of SBFS fungal colonies on apple fruits were examined.

Isolation of fungi from the apple skin with sooty blotch and flyspeck symptoms

Apples with symptoms of sooty blotch and flyspeck were used as a test material. Fruits and leaves from the cultivars Winter Banana, Melrose and Idared were collected in the autumn of 2009 and 2010. Koch's postulates were applied to determine the etiology of the obtained isolates. Isolations were made as described by Johnson et al. (1996), Wrona et al. (2004) and Batzer et al. (2005). The fungal colonies were transferred with sterile scalpel into Petri dishes with Potato Dextrose agar (PDA). Streptomycin at the concentration of 40 ppm was added to inhibit the growth of saprophytic microflora. The plates were incubated at 24 ^{0}C in dark for 7 days.

Pathogenicity of the isolates from apple fruits

Suspension of 15-day culture of each isolate including mycelial fragments and conidia (determined by a microscopic method) was prepared for proving the pathogenicity of the obtained isolates. It was supplemented with 0.5% apple juice for accelerating the growth of the studied fungi. Fruits were disinfected with 90% ethyl alcohol and washed with sterile water. Each fruit was labeled with 5 circles with a diameter 25 mm to show the inoculated zones. Each zone was observed with a magnifying lens for establishing traces of natural infection with sooty blotch and flyspeck. The inoculation suspension was laid in each zone with a cotton bud and left to dry. Five zones were infected in each fruit and the spots were tamponaded with sterile distilled water, used as a control. Six fruits were infected by each isolate. The apples were slightly pulverized with sterile distilled water and put in an exicator at 80% humidity at a room temperature of 20-22^{0}C. The fruits were studied on the 7th, 14th and 21st day after the infection. The symptoms of sooty blotch and flyspeck and the types of the colonies found in the infected zones were reported (Wrona et al., 2004).

Cultural and morphological characterizations of the isolates

The cultural characteristics (mycelial colonies, density, presence of fruiting bodies and sclerotium-like bodies and diameter growth of the colonies) were determined on PDA, SNA (synthetic nutrient agar), MA (malt agar), CA (cherry agar), OA (oatmeal agar) and PA (peas agar). The morphological signs in the isolates were described on cultures sporulating on PDA. Mycelial plugs (10 mm diam.) from 15-day old cultures on PDA were placed upside down onto three plates (one plug per plate). All the plates were incubated for 7 days at 24^{0}C in dark. Three perpendicular measurements of the colony diameters were made and the diameter of the plug was subtracted to determine the diameter growth rate (Batzer et al., 2005).

Morphological characteristics (spore size, colour, shape) were determined using the microscopic method (Olympus Optical CO. LTD, Japan). Fifty measurements of spores from each putative pathogenic species were examined at x 400 magnification under a light microscope. The identification of the pathogenic isolates was carried out by using mycological paper (Johnson, 1994; Johnson et al., 1996, 1997).

In vitro screening of fungicides for control of causal fungus of sooty blotch and flyspeck on apple

A screening was carried out under laboratory conditions. The inhibiting effect of Cyprodinil (as Horus 50 WG), Tiram 80% (as Tiram 80 WG), 87,7% Copper hydroxide (as Funguran OH50 WP), Folpet + Triadimenol (as Shavit F72 WP), 80% Sulfur (as Acoidal WG) and Dithianon (as Delan 700 WG) on the mycelium growth of the pathogenic species was tested using Thornberry's method (Thornberry, 1950). Petri dishes (9 mm diameter) contained 9 ml PDA medium and 1 ml fungicide. Petri dishes containing 10 ml of fungicide-free PDA medium were used as control. Each Petri dish was inoculated with 1 cm disc of 15-day old fungal culture in the centre. Three dishes for each treatment were used as control. All the inoculated plates were incubated at 23-24^0 C in dark, until the mycelial growth reached the edge of

the control plate. Three perpendicular measurements of the colony diameters were made and the plug diameter was subtracted to determine the diameter growth rate (Batzer et al., 2005). The percentages of the linear mycelial growth reduction of the pathogenic fungi were calculated using the following formula:

$$I\ \% \ = \ \frac{C \ - \ T}{C} \ x \ 100$$

Where:

I% – index of fungal mycelial growth reduction

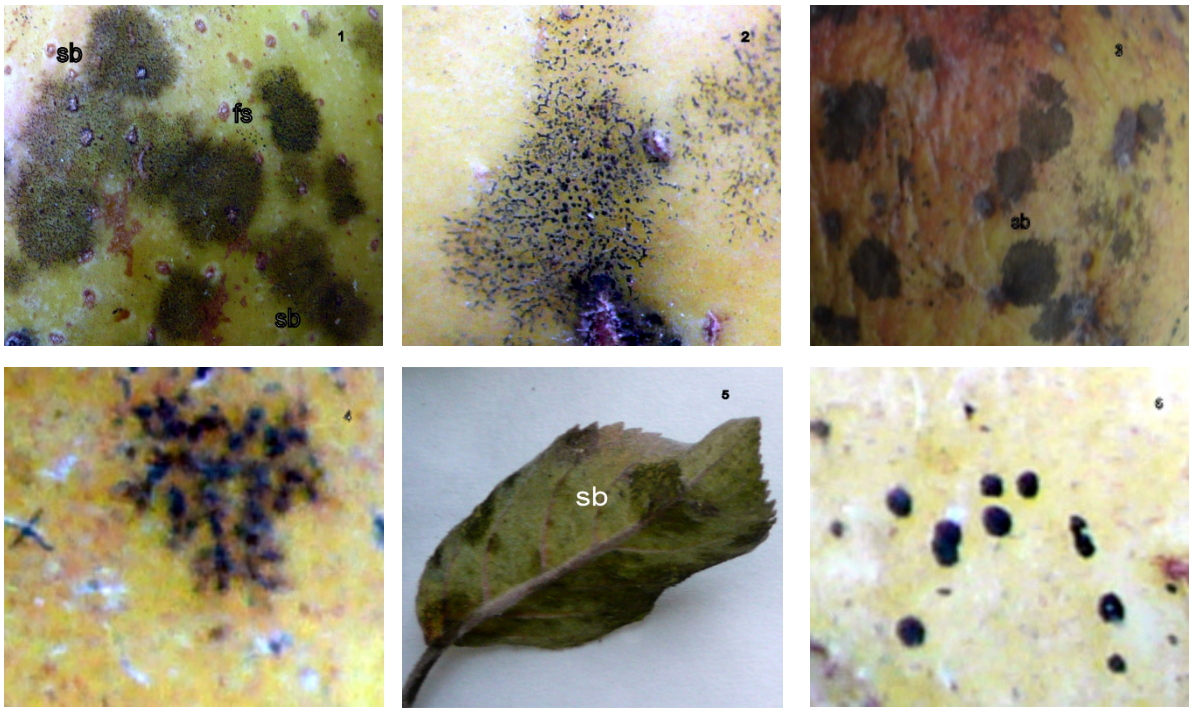
C – mycelial diameter in the control

T – mycelial growth in the treatment

Results

Characterization of the symptoms

Symptoms of sooty blotch and flyspeck (SBFS) were described on fruits, leaves and shoots of the apple cultivars Winter Banana, Melrose and Idared, collected from orchards and private yards in different regions of the southern slopes of the Balkan Mountains and Sredna Gora Mountain (Kalofer, Staro Zhelezare and Panagyurishte) in August-September and during storage production in 2009-2010. Sooty blotch symptoms are related to the occurrence of fungal colonies of a radiating structure and scattered small fruiting bodies (pycnidia) in them. They merge to cover most of the apple fruit surface. Symptoms may appear 3-4 days after petal fall but the more severe form of the disease that we observed, appeared from the end of summer till the beginning of autumn (cv. Winter Banana) and during fruit storage (cv. Melrose and Idared). The development of punctuate olivaceous to black colony types was observed on the fruits of the cultivars Idared and Winter Banana and on the fruits of cv. Merlose the colonies were initially diffuse but become blacker and denser of the ramose type. The symptoms on dark-coloured fruits were less obvious and more difficult to diagnose compared to those on pale-coloured yellow apples. A large number of mycelial colonies, olive green in colour, with single black fruit bodies were found on the leaves and leaf petioles of Winter Banana cultivar (Figs. 1-5).



Figures. 1- 6. Mycelial types of fungi in the sooty blotch and flyspeck complex on fruits and leaves. 1 and 3. Colonies of sooty blotch (SB). 2. Punctate type. 4. Ramose type. 5. Symptoms on the leaves. 6. Flyspeck (FS)

The observed symptoms of flyspeck were well-outlined black sclerotium-like bodies gathered in groups of different size. Most often the colonies found were about 1 to 3 cm in diameter, although sometimes they covered a larger part of the apple surface. The colonies comprised of several to 20 shiny black pycnothyria (Figs. 1-5). The symptoms of sooty blotch and flyspeck established in the present study usually appeared as mixed infections

and that is why the term sooty blotch and flyspeck complex fungi is used.

Fungal species determination

In result of the biological analysis of apple fruits having the symptoms of sooty blotch and flyspeck, six isolates were obtained and studied for pathogenicity. Symptoms appeared in two of the isolates. The colonies formed were of two mycelial types – ramose and punctuate types. Two putative isolates were delineated based on the mycelial type on the apple fruits, on the morphological and cultural characteristics of the fungal colonies in vitro. We suppose that the first isolate belongs to *Peltaster fructicola* Johnson. At the beginning the mycelium was white turning to grayish brown as getting older. The hyphae thickness was 1.5-4.0 µm. Conidiospores were egg-shaped, unicellular, transparent or crystal blue, their size being 3.6-7.2 x 0.7-1.1 µm. They were formed in spherical pycnothyria 81-113 µm in diameter. More often pycnothyria were randomly scattered and more rarely arranged in concentric rings. Our data were similar to those of Williamson et al. (2000, 2004) and Wrona et al. (2004). The colonies of *Peltaster fructicola* developed in the best way on PDA and the slowest growth was reported on SNA. On PDA, the colonies were loose at the beginning, getting denser after the 10th day. They were gray-white in young cultures and gray-green to gray-brown in mature cultures. On SNA the colonies were dense, olive green, pale brown to brown in colour (Tabl. 1).

Table 1. Cultural characteristics of *Peltaster fructicola* in vitro

Artificial culture media	colony diameter growth/mm			Cultural characteristics
	7th day	14th day	21st day	
Malt agar	7.8	35.1	63.3	Dense, dark brown
Pea agar	12.6	29.6	48.3	Dense, gray brown
Potato Dextrose agar	9.5	35.1	72.3	Dense, gray brown
Synthetic nutrient agar	7.3	24.5	41.5	Dense, olive green
Cherry agar	8.1	30.8	52.8	Dense, olive green
Oatmeal agar	7.1	29.1	43.4	Dense, gray brown

On the basis of the morphological and cultural characteristics,described on six culture media, we suppose that the second isolate belongs to *Geastrumia polystigmatis* Batista & M. L. Farr. (Johnson et al., 1996, 1997). The colonies developed in the best way on oatmeal agar (73.65 mm/d, dense, green-brown), on malt agar (73.58 mm/d, sleazy, gray-brown) and PDA (64.66 mm/d, sleazy, green-brown). The rest of the studied media with an exception of cherry agar (16.84 mm/d, dense, gray-brown) also provided good growth of *Geastrumia polystigmatis* in vitro. Microscopic analysis showed that a large number of pycnothyria 50-100 µm in diameter and fusiform conidial arms with 7-9 septa were formed in the dark diffused mycelium (Johnson, 1994; Williamson and Sutton, 2000). Conidiospores were not observed.

Table 2. Cultural characteristics of *Geastrumia polys tigmates*

Artificial culture media	colony diameter growth/mm			Cultural characteristics
	7th day	14th day	21st day	
Malt agar	8.83	42.17	73.58	Sleazy, gray brown
Pea agar	12.83	36.16	62.25	Sleazy, gray brown
Potato Dextrose agar	10.5	37.51	64.66	Sleazy, green brown
Synthetic nutrient agar	7.33	20.18	58.36	Dense, dark gray
Cherry agar	8.5	10.4	16.84	Dense, gray brown
Oatmeal agar	8.51	41.52	73.65	Dense, green brown

In vitro screening of fungicides for control of causal fungus of SBFS on apple

Copper hydroxide 87.7% (as Vitra) – 98.66%. Tiram 80% (as Tiram) – 98.54%, Cyprodinil (as Chorus) – 95.76% and Copper hydroxide 77% (as Funguran) – 95.69% showed the strongest inhibiting effect on *Peltaster fruticola* mycelial growth, which was established after carrying out the in vitro screening. The growth of *Geastrumia polystigmatis* colonies was most strongly suppressed by Tiram 80% (as Tiram) – 97.8%, followed by Cyprodinil (as Chorus) – 71.81% and Folpet + Triadimenol (as Shavit) – 64.63%. Taking into account the joint action of both pathogens as causative agents of sooty blotch and flyspeck on apple, it is possible to use those of them, which show the best effect after in vivo experiments. Some fungicides, such as Cyprodini, Dithianon and Folpet + Triadimenol that are efficient against apple scab (*Venturia inaequalis*), could be used for simultaneous control of both diseases.

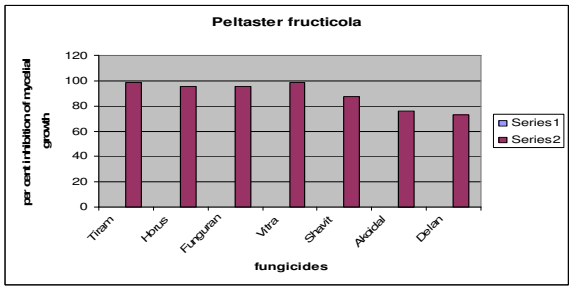


Figure 7. Inhibibition effect of the fungicides on the growth of *Peltaster fructicola* in vitro

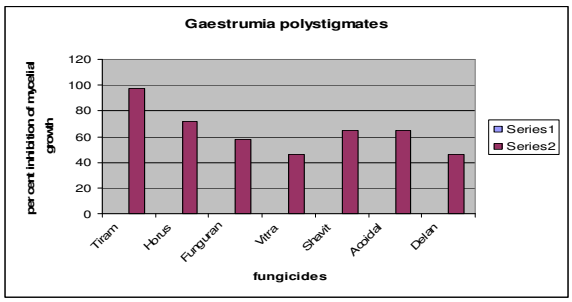


Figure 8. Inhibibition effect of the fungicides on the growth of *Geastrumia polysigmatis* in vitro

Conclusion

Sooty blotch and flyspeck disease appears sporadically in Bulgaria, most often in the mountainous areas, where humidity levels are higher and for a longer period. Until now it has not been a problem either during vegetation or during apple storage. But the climatic changes in the recent years should strengthen the attention to sooty blotch and flyspeck fungi complex, taking into consideration the disease spread not only in the USA but also in many European countries, some of them neighbouring Bulgaria, such as Poland, Spain, Germany, Turkey, Serbia (Wrona et al., 2004; Mirzwa-Mróz, 2012; Ivanovic, 2010; Mayfield, 2013). The results obtained in the present investigation set the beginning of the study on sooty blotch and flyspeck fungi complex in Bulgaria.

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