JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 44, No. 4 (2004)

ETIOLOGY OF APPLE SOOTY BLOTCH IN POLAND

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Accepted: November 18, 2004

Abstract: Investigations have been undertaken to determine which fungi species are responsible for occurrence of sooty blotch disease in Poland. It was found that disease complex is caused by *Tripospermum myrti* (Lind) Hughes, *Phialophora sessilis* de Hoog and *Peltaster fructicola* Jonhson. There was no evidence of the presence fungus described as *Gloedes pomigena* which was previosly considered as a casual agent of apple sooty blotch disease in Poland.

Key words: sooty blotch, etiology, *Peltaster fructicola*, *Phialophora sessilis*, *Tripospermum myrti*, *Gloedes pomigena*

INTRODUCTION

Although sooty blotch is one of a relatively common disease occurring in many apple growing regions in the world, there was no information on its cause until 1830. According to Jonhson (1994), Schweintz was the first who described disease symptoms as dark spots on fruits and named the pathogen *Dothidea pomigena*. Futher investigations on etiology of sooty blotch were incoherent. Jonhson et al. (1997) quotes that Spraque considered *Asteroma pomigena* the fungus responsible for the disease, while according to Saccardo the causal agent of sooty blotch was the fungus called *Phyllachora pomigena*, and this term was used in the scientific literature till 1900. Then Shelby (1900) found that both sooty blotch and flyspeck are caused by the same pathogen – *Leptothyrium pomi*. However, Colby (1920) proved that sooty blotch and flyspeck are two different diseases caused by two different pathogens. Colby (1920) attributed symptoms called sooty blotch to the fungus *G. pomigena* (Schw.) Colby. According to this author the fungus formed a dark, septate, partially branched thallus growing only on the apple skin. Colby (1920) observed also pycnidia and spores of this pathogen. Pycnidia could be black, jellied inside, while spores were conidial and hyaline (unicellular) of 10–20 μ × 4–7 μ in size. The fungus *G. pomigena* was regarded to be the only cause of apple sooty blotch until 1994, when Jonhson (1994) and Jonhson et al. (1996; 1997) showed that this disease in the US territory was caused by three fungal species: *Leptodontium elatius*

(Mangenot) de Hoog, *Geastrumia polystygmatis* Batista et Farr and *Peltaster fructicola* Jonhson. They found no fungus that could correspond to the description of *G. pomigena*. Also the studies carried out in Germany and the Netherlands did not confirm existence of this fungus.

It was concluded that sooty blotch in Poland can be caused by fungi that have not been described yet as causal agents. The aim of this study was to identify fungal species responsible for apple sooty blotch in Poland

MATERIAL AND METHODS

Isolation of fungi from the apple skin with sooty blotch symptoms

Apples with symptoms of sooty blotch were used as a test material. Fruits were collected every year from the region of southern Poland in the years 2000–2002, at the amount of 180 specimens (ca 22 per cultivar), when disease symptoms were well developed, i.e. at the end of September/early October. Fruits came from the following apple cultivars: Jonagold, Golden Delicious, Champion, Cox Orange, Novamac, Witos, Florina and Freedom. Due to the fact that fungi causing sooty blotch grow only on apple skin, no chemical desinfection was used and specimens were not washed in water, either. The mycelium was removed with a scalpel and placed onto water agar (15 g agar/1000 ml of distilled water). Water agar was used as an initial substrate, as it favoured the elimination of various saprophytic organisms present on apple skin. To inhibit the growth of bacteria and species of Penicillium, streptomycin at the concentration of 40 ppm and 40% lactic acid at the amount of 15 ml in 1000 ml of water agar were added. Plates were incubated for 7 days at 22–24°C, and then consecutive transfers onto water agar (without streptomycin and lactic acid) were made. Contaminating microorganisms, mainly bacteria and fungi from genera *Penicillium, Alternaria, Cladosporium* and yeast-like fungi were eliminated. To obtain pure isolates of other fungal colonies numerous transfers to PDA medium were made. In further research studies on pathogenicity and identification of obtained isolates only PDA medium was used.

Pathogenicity of fungal isolates

To examine pathogenicty of the isolated fungi a test was carried out on Golden Delicious fruits. For this purpose the fruits with no disease symptoms were collected in the mid-September from unsprayed trees. To eliminate an accidental infection by fungi responsible for sooty blotch, the apple skin was disinfected by immersing for 20 seconds in 70% ethyl alcohol, and then rinsed with distilled water. From each obtained isolate inoculum in the form of suspension containing 5×10^5 infection units per 1 ml of sterile distilled water was prepared. For this purpose 14-day old cultures growing on the PDA at 22–24°C were used. Apple juice was added to make the concentration of 0.5% in the prepared inoculum suspensions in order to enhance initial growth of the tested fungi. Each of 15 fruits were inoculated with each isolate on previously marked 5 zones (25 mm in diameter). Sixth area was a control treatment where distilled water with apple juice was used. The inoculation was performed by careful application of inoculum suspension using sterile gauze. The fruits were then sprayed with distilled water and placed separately in moist chamber at 20–22°C. Af-

ter 14–24 days from inoculation date the fruits showed spots which were formed by dark mycelium. Sooty blotch symptoms in the form of dark-olive blotches of 3–4 mm in diameter were observed and the corresponding isolate suspension was regarded as responsible for the disease. Isolates subsequently were divided in 4 groups according to their decreasing pathogenicity.

Identification of individual pathogenic fungal isolates

The isolates that were capable of causing sooty blotch symptoms on the inoculated fruits were identified.

In macroscopic description the color of mycelium, texture and growth rate on PDA at 22–24°C were estimated. In macroscopic description spore size, shape, structure, color, formation of hyphae and hyphal characteristics (presence of septa, width and color) were taken into account. The identification of pathogenic fungal isolates was carried out by using the mycological keys (Arx 1974; Ellis 1971; Kendrick 1992; Kendrick and Carmichael 1973; Kendrick and Nag Raj 1979) and mycological papers (Johnson 1994; Johnson et al. 1996; 1997).

RESULTS AND DISCUSSION

In the years 2000–2002 602 fungal isolates were isolated from apple showing sooty blotch symptoms. Pathogenicity was confirmed for 510 of them. There were significantly differences in severity and radial growth among fungal isolates. Pathogenic fungi were identified as: *Tripospermum myrti*, *Phialophora sessilis* and *Peltaster fructicola*. The most pathogenic appeared to be *P. fructicola* isolates, with average pathogenicity 88.7% and radial growth of 28.5 mm. In a number of 164 they were representative for first group (Tab. 1) Second group consisted of 249 isolates with average pathogenicity 72.4% and average radial growth of 19.3 mm. Those isolates were identified as *P. sessilis*. Third group covered *T. myrti* isolates, that were characterized by lowest pathogenicity of 53.9% and avarege radial growth of 15.1 mm. They were obtained in a number of 97 (Tab. 1).

Table 1. Pathogenicity of fungal isolates obtaine from apple showing sooty blotch symptoms

Group	Species	Severity [%]	Radial growth [mm]	Number of isolates in group	Av. severity [%]	Av. radial growth [mm]
I	*Peltaster fructicola*	> 80	> 25	164	88.7 a	28.5 a
II	*Phialophora sessilis*	79–60	24–17	249	72.4 b	19.3 b
III	*Tripospermum myrti*	< 60	< 17	97	53.9 c	15.1 c
IV	–	no pathogenicity	0.0	92	0.0	0.0

Means within the same column followed by the same letter are not significantly different from one another at p= 0.05, according to Duncan's test

The colonies of *P. fructicola* were olive-green or sometimes ashen, surface of colonies was slightly wrinkled, and the reverse dwas dark-olive. The linear growth was slow of about 45 mm/30 days. Conidial spores were unicellular, hyaline of 4.1 × 1.0 μ in size (Tab. 2). They were formed on nonseptated vegetative hyphae or in oval pycnidia of 60 × 30 μ in size.

The colonies of *P. sessilis* had a black leather velvety surface and were white in the central part. The reverse of these colonies was black or dark-olive. The linear growth was slow of about 40 mm/30 days. This fungus formed unicellular, hyaline, oval conidia of 3.0 × 1.8 μ in size, which were formed on short sessile phialides of 2.0 × 1.5 μ in size and with easily distinguishable dark collar. Mycelial hyphae were septate, septa spaced every 18.0 μ in average.

The colonies of *T. myrti* were dark brown and velvety, slightly lightened and bulged in the central part. The reverse of these colonies was black and linear growth was slowabout 40 mm/30 days. This fungus formed dark multicellular conidial spores of characteristic star-like shape (Tab. 2). They arose on conidophores of 75 μ in length and 6,5 μ in width. Mycelial hyphae were dark and contained septa spaced every 14 μ in average.

No fungus that could comply with the description of *G. pomigena* was found. Also the research work performed in Germany, the Netherlands and the United States (Jonhson 1994; Jonhson et al. 1996; 1997) did not confirm the presence of this pathogen. In Germany and the Netherlands the same fungal species as those of Poland, except for *Tripospermum cameloparculus*, were regarded to cause sooty blotch. In the US apart from *P. fructicola* also other fungal species such as *L. elatius* and *G. polystigmatis* are listed among fungi as causing the disease. So far the research work performed indicates that there are considerable differences in etiology of apple sooty blotch between Europe and America.

Table 2. Microscopic characteristics of spores and hyphae of pathogenic isolates

Spore characteristics			Hyphae characteristics			species
Shape and color	spore size (length × width)	formation	distance between septa	width	colour	
Oval, hyaline	av. 4.1 μ × 1.0 μ (3.6–7.2 × 0.7–1.1 μ)	on vegetative hyphae and oval pycnidia of average size of 60 μ × 30 μ (57–100 μ × 20–40 μ)	no septa	2–4 μ	hyaline	*Peltaster fructicola* Jonhson
	av. 3.0 μ × 1.8 μ (3.0–3.3 μ × 1.5–1.8 μ)	phialides, short sessile 2 μ in length, and 1.5 μ in width, dark collar, clearly visible	regular, spaced every 18 μ in average 10–30 μ	2.0–2.5 μ	hyaline	*Phialophora sessilis* de Hoog
Star-shaped staurospores, dark	cell base, in average 8.0 μ × 6.0 μ (7–9 μ × 5–7 μ). Straight arms of 25 μ in length, average base width of 5 μ In top section (1.0–2.0 μ). The arms have 1 to 4 walls.	on conidiphores, average length of 75 μ (65–82 μ) average width of 6.5 μ (4.0–5.7 μ)	regular, spaced every 14 μ in average 12–20 μ	4–8 μ	dark	*Tripospermum myrti* (Lind) Hughes

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POLISH SUMMARY

ETIOLOGIA BRUDNEJ PLAMISTOŚCI JABŁEK NA TERENIE POLSKI

Grzyb o nazwie *Gloedes pomigena* jak dotąd powszechnie uznawany jest w Polsce za sprawcę brudnej plamistości jabłek. Przeprowadzone w ostatnich latach na terenie Niemiec, Holandii i Stanów Zjednoczonych badania nie stwierdziły jednak obecności grzyba, który odpowiadałby opisowi tego patogena. Za przyczynę brudnej plamistości jabłek uznano tam natomiast kilka innych gatunków grzybów. Podjęto więc badania, których celem było określenie jakie gatunki grzybów odpowiedzialne są za występowanie brudnej plamistości jabłek w Polsce. Wykazano, że sprawcami choroby są *Tripospermum. myrti*, *Phialophora sessilis* oraz *Peltaster fructicola*. Nie stwierdzono natomiast obecności grzyba opisywanego jako *G. pomigena.*