

## The study of new fungus species causing apple sooty blotch

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### ABSTRACT

In southern Poland sooty blotch symptoms are caused by 8 fungus species and 3 fungus forms. In addition to *Triospermum myrti* (Lind.) Hughes, *Phialophora sessilis* de Hoog and *Peltaster fructicola* (John.) Sutton et Hodges described previously, the occurrence of 5 new fungus species and 3 fungus forms was found. They are the cause of the occurrence various sooty colored spots on the surface of apple fruit. The newly recognized species comprise the two belonging to *Triospermum* (*T. camelopardus* (Ing.) Dann et Mc Dougall, *T. acerinum* Syd.). The next group includes 3 fungi recognized as sooty molds, among them the most common *Aureobasidium pullulans* (de Bary) Arnoud and *Cladosporium cladosporioides* (Fres.) de Vries and the rare *Torula herbarum* (Pers.) Link. These fungi grow on fruits in orchards where no honeydew insects are present. Very interesting and lesser-known groups were fungus isolates from genera *Septoria*, *Ramularia* and *Pseudocercospora*. *P. sessilis* occurred rarely and *P. fructicola* only exceptionally.

## INTRODUCTION

Up to 1996, the fungus *Gloeodes pomigena* (Schw.) Colby was thought to cause apple sooty blotch. Johnson et al. (1996) when performing research studies in the USA proved that the disease symptoms are caused by at least three fungus species. For the first time they described the species *P. fructicola* produce a branched (ramosae) or point (punctate) thallus on the surface of apples. *Leptodontidium elatius* (Mang.) de Hoog was assigned to thallus of sooty mycelium (fuliginous). The third fungus species *Gaestrumia polystigmatis* (Bat.) Farr produced a branched thallus (ramosae). American scientists, however, found no fungus species corresponding to *G. pomigena* (Johnson et al. 1997). Also research studies performed in the years 1996 – 2002 in Germany did not confirm that *G. pomigena* was responsible for sooty blotch. However, it was found that sooty spots were produced by *T. myrti*, *T. camelopardus*, *P. sessilis*, *P. fructicola* and unrecognized fungi belonging to genus *Mycosphaerella* (Kern 2001, Noga et al. 2001, Feldmann et al. 2003). In Poland the occurrence of *T. myrti*, *P. sessilis* and *P. fructicola* was reported (Wrona 2003, Grabowski and Wrona 2004, Wrona and Grabowski 2004). However, in further research studies a fungus complex responsible for apple sooty blotch was identified.

## MATERIAL AND METHODS

This research study was carried out on the fruits of 10-year apple (cultivars ‘Golden Delicious’, ‘Idared’, ‘Jonagold’, ‘Rubinola’, ‘Topaz’) taken from selected orchards where no fungicide spraying was used. Chemical pest control was employed for honeydew producing insects (aphids, apple leaf suckers, scales). For this purpose 4 insecticide treatments were carried out in the period from May to October. For this reason no honeydew secretion was present on the leaves and fruits in the orchards under investigations, thus disabling development of sooty molds. The study was carried out in orchards located in the Kraków, Sandomierz and Nowy Sącz regions. In the years 2004 – 2006, 20 apples of each cultivar with clearly visible sooty blotch symptoms were taken from eight orchards within each region. At the laboratory of the Department of Plant Protection the fungi were isolated from sooty spots on the fruits by moving the fragments of mycelia and conidia onto a water agar with 2% apple juice added. The growing fungus colonies were successively inoculated on the PDA and MA agar. The resulting isolates were incubated at 20°C and relative humidity above 90% and then identified by using keys and mycological studies (Carmichael 1971, Ellis 1971, Ainsworth 1973, Ingold 1975, Kendrick and Nag Raj 1979, Gams et al. 1998, Williamson and Sutton 2000).

The next step was to determine the ability of isolated fungi to produce sooty spots on the surface of apples. The researches were carried out both in field and laboratory conditions. For this purpose water suspension of mycelium and spores at the concentration of  $2 \times 10^4$  infection units in 1 ml was prepared. In orchards the 10 fruits of cultivar 'Topaz' were sprayed with the suspension of every isolate at harvesting phase. The fruits were then tightly enclosed with a plastic sheeting to maintain high humidity after inoculation. Under laboratory conditions the 10 fruits of cultivar 'Topaz' were placed in tight glass containers with the humidity above 97%. Fruits in every container were sprayed with the suspension of every isolate. After 3 weeks it was assessed if typical sooty spots appeared on the apple fruits. *In vivo* and *in vitro* isolates causing sooty mycelium spots were considered as belonging to sooty blotch complex.

## RESULTS

In the years 2004 – 2006 3592 isolates containing 8 fungus species and 3 forms were isolated from lesions found on apples. New species and forms were discovered that so far had not been considered as responsible for apple sooty blotch. Research studies performed in southern Poland in previous years indicated that sooty spots on the surface of apples are caused by colonization the fruit skin by *T. myrti*, *P. sessilis* and *P. fructicola*.

The presence of *T. camelopardus* and *T. acerinum* was also observed in this study. It was found that without honeydew the fungi *A. pullulans*, *C. cladosporioides* and *T. herbarum* produced a compact sooty thallus on the fruit skin. So far these fungus species had been classified among sooty molds that grow on surface of leaves covered with honeydew produced by aphids.

In the present study, the author found that ripening fruits of sugar content above 9% are sites for developing mycelium of *A. pullulans*, *C. cladosporioides* and *T. herbarum* (data not shown). The occurrence of sooty spots in the form of sooty thallium on apples was recorded only during the period of prolonged precipitations and moderate temperatures (12-18°C). The colonization tests carried out on ripening fruits confirmed the obtained results. From lesions on the fruits the fungi belonging to the three genera: *Pseudocercospora*, *Septoria* and *Ramularia* were also isolated. Applied in the form of water suspension of mycelium and conidia, the fungi developed on the surface of apples at high humidity producing dark spots typical of sooty blotch. Probably, the obtained isolates are anomorphs of the fungus genus *Mycosphaerella*. To determine its species and to confirm this conclusion the PCR method should be used.

In addition, variable occurrence frequency was observed for individual isolates. The most common species was *T. myrti* that comprised from 20.10% to 30.07% of

all the obtained fungi in particular regions. Slightly lower occurrence (8.36-18.43%) in lesions was observed for the fungus *T. camelopardus*, hitherto not reported on fruits in Poland. *T. acerinum* was the rarest species observed among the fungus genus *Tripospermum*. This fungus was most frequently reported in the Kraków region (7.96%).

Another most common fungus group was *A. pullulans* and *C. cladosporioides*. They colonized ripping apples mainly in the Sandomierz Orchard Region. Under favorable conditions (high humidity) they covered a considerable area of the fruit skin. These two fungus species were common on individual apples. When considering the percentage of fungi among those responsible for apple sooty blotch in all the regions under investigation it was found that *T. myrti* (25.97%) predominates. The less common species are *A. pullulans* (16.45%), *C. cladosporioides* (16.45%) and *T. camelopardus* (14.98%). In the Kraków and Sandomierz regions the common species was *T. herbarum*. In the case of remaining fungus species the occurrence did not exceed 5% and depended on the region (Fig. 1).

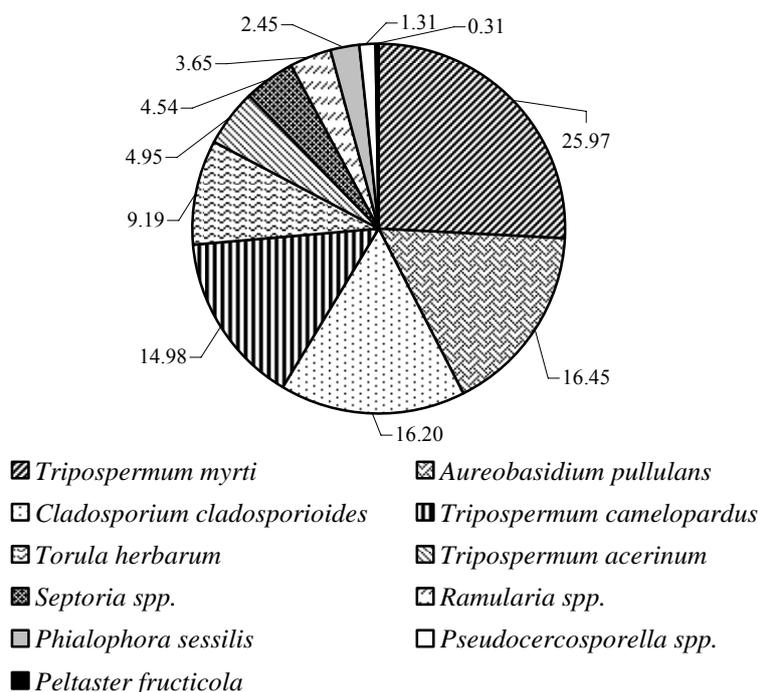


Fig. 1. Percentage share of fungi species in apple sooty blotch complex

All isolated fungi were able to form sooty spots on apple skin (Table 1). After applying inoculum onto ripening fruits, lesion occurrence was observed for *T. myrti* and *T. camelopardus* (incubation period of 12 days). The fungi caused the most intensive covering with blotches of the fruit surface. The produced spots were of irregular shape. The longest incubation period (14 days) was recorded for *P. fructicola*, *A. pullulans*, *C. cladosporioides*, *T. herbarum* and *Septoria* spp.

## DISCUSSION

The results of the study confirmed the presence of some fungus species causing apple sooty blotch in Poland. This concerns *T. myrti*, *P. sessilis* and *P. fructicola* (Grabowski and Wrona 2004, Wrona and Grabowski 2004). It was proved, however, that not only the above mentioned species but also other fungus species and forms can cause sooty blotch. *T. camelopardus* was relatively common in orchards under consideration. The presence of nits mycelium on apples was confirmed in southern Germany in 1998 (Kern 2001, Noga et al. 2001). In Poland, the occurrence of this species in fish pond waters is reported by Orłowska et al. (2004). Like other species belonging to genus *Tripospermum* they are connected with water environment and in high humidity conditions they can occur also on above ground plant parts (Ingold 1975, Betancourt et al. 1987, Bärlocher 1992, Czczuga and Orłowska 1995, Pascoal and Cássio 2004). For the first time it was confirmed in Poland that *T. acerinum* is also able to cause apple sooty blotch. The fungi belonging to genus *Tripospermum* (also telemorph *Capnodium*) cause blotches on various plant parts. Such fungi belong to the so-called sooty molds.

By growing on leaves often covered with sticky secretion made by aphids, they block access to light, thus inhibiting photosynthesis (Hughes 1976, Schouties 1980, Mańka 2005). Other fungus groups commonly considered as sooty molds include *A. pullulans* and *C. cladosporioides*. It was found in this study that these fungi often occur on the surface of the fruits producing large black spots. Aphid secretion on the fruits (or leaves) is not necessary for their development. Both *A. pullulans*, *C. cladosporioides* as *T. herbarum* appeared on ripening apples after prolonged rain period.

Another fungus group of the sooty blotch complex includes fungi belonging to genera: *Septoria*, *Ramularia* i *Pseudocercospora*, that so far, also in Poland was not included among those causing sooty blotch. Probably they are anomorphs of the fungi belonging to *Mycosphaerella*. The presence of similar fungi in the sooty and dot blotch was recorded by American researchers (Batzer et al. 2005). Previously, the same was reported for not identified anomorphs of the genus *Mycosphaerella* by German scientists (Noga et al. 2001). This fact confirms

Table 1. Fungi isolated from dark spots on the apples having sooty blotch symptoms

Fungi	Region						Ability to produce sooty spots on the fruits			
	Kraków			Sandomierz				Nowy Sącz		
	Number of isolates	Occurrence frequency	Number of isolates	Occurrence frequency	Number of isolates	Occurrence frequency		Number of isolates	Occurrence frequency	
<i>Tripaspermum myrti</i> (Lind.) Hughes	202	20.10	320	26.23	411	30.07	***			
<i>Tripaspermum camelopardus</i> (Ing.) Dann et Mc Dougall	184	18.31	102	8.36	252	18.43	+++			
<i>Tripaspermum acerinum</i> Syd.	80	7.96	24	1.97	74	5.41	++			
<i>Phialophora sessilis</i> de Hoog	42	4.18	17	1.39	29	2.12	++			
<i>Peltaster fructicola</i> (John.) Sutton et Hodges	3	0.30	0	0	8	0.59	+			
<i>Aureobasidium pullulans</i> (de Bary) Arnoud	140	13.93	281	23.03	170	12.44	+			
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	118	11.74	265	21.72	199	14.56	+			
<i>Torula herbarum</i> (Pers.) Link	122	12.14	144	11.81	64	4.68	+			
<i>Pseudocercospora</i> spp.	28	2.78	11	0.90	8	0.58	++			
<i>Septoria</i> spp.	30	2.99	25	2.05	108	7.90	+			
<i>Ramularia</i> spp.	56	5.57	31	2.54	44	3.22	++			
Total	1005		1220		1367					

\* + low, ++ medium, +++ high

the obtained results for anamorphs belonging to genera *Septoria*, *Ramularia* and *Pseudocercospora*. The fungi of genus *Cladosporium* can be an anamorph of *Mycosphaerella*. Compared to the previous studies a minute share of *P. sessilis* and *P. fructicola* in causing sooty blotch should be noted. The latter was not recorded during the 3-year field tests carried out in the Sandomierz orchard region. It was reported sporadically only in the remaining regions.

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**BADANIA NAD NOWYMI GATUNKAMI GRZYBÓW POWODUJĄCYMI  
BRUDNĄ PLAMISTOŚĆ JABŁEK**

Streszczenie: Objawy chorobowe brudnej plamistości jabłek w południowej Polsce są powodowane przez 8 gatunków i 3 formy grzybów. Oprócz opisanych wcześniej *Tripaspermum myrti*, *Phialophora sessilis* i *Peltaster fructicola* stwierdzono występowanie 5 nowych gatunków i 3 form grzybów. Powodują one powstawanie na powierzchni jabłek ciemnych plam o różnym charakterze. Wśród nowopoznanych gatunków dwa należą do rodzaju *Tripaspermum* (*T. camelopardus*, *T. acerinum*). Kolejną grupę stanowią 3 grzyby uznawane za tzw. sadzakowate, spośród których najczęściej występowały *Aureobasidium pullulans* i *Cladosporium cladosporioides*, rzadziej *Torula herbarum*. Rozwijały się one na owocach w sadach, gdzie nie żerowały owady wydzielające spadź. Mało rozpoznaną grupę stanowią izolaty grzybów z rodzajów: *Septoria*, *Ramularia* i *Pseudocercospora*. Zaobserwowano rzadkie występowanie *Phialophora sessilis*, sporadyczne *Peltaster fructicola*. Objawy brudnej plamistości jabłek są wywoływane przez kompleks różnych grzybów.

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