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INFLUENCE OF FRUCTOSE AND GLUCOSE OCCURRING ON FRUIT SURFACE ON THE GROWTH OF FUNGI THAT CAUSE SOOTY BLOTCH OF APPLE

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Abstract: The fungi that cause sooty blotch grow only on the apple skin, so they use appropriate nutrients which are present on the fruit surface. It has been shown that when the first symptoms of sooty blotch occur a noticeable increase of glucose and fructose content both on the apple skin and in juice is observed. Such increase occurs at pH 4.4. An effect of surface glucose and fructose on the growth of pathogens responsible for the disease was also confirmed by evaluating the germination of conidia of *Phialophora sessilis* de Hoog and *Peltaster fructicola* Jonhson in solution of above mentioned sugars, distilled water and standard d-glucose solutions.

Key words: sooty blotch, plant cuticle, cuticle permeability, surface sugars

INTRODUCTION

Sooty blotch is a superficial disease caused in Poland (Wrona 2003) by the three fungi: *Tripospermum myrti* (Lind) Hughes, *Phialophora sessilis* de Hoog and *Peltaster fructicola* Jonhson. These fungi develop only on the apple skin, while making no damage to cuticle. Thus, their growth should depend on the presence of nutrients in an assimilable form on the apple skin. The research studies carried out by Belding et al. (2000) have proved that pathogens causing sooty blotch are not capable to utilize any components of epicuticular waxes or aminoacids as a basic source of carbon. Thus one may conclude that sugars present on the apple skin are of utmost importance to their growth. Schreiber and Skrabs (2001) and Schreiber (2001) demonstrated that the sugar concentration can depend on such factors as pH on the fruit skin and environmental factors (relative humidity, temperature). They showed that higher relative humidity and temperature, result in higher degree of cuticle permeability, thus also higher sugar concentration on the apple skin. The permeability can be modified by microorganisms. They produce ionized carboxyl groups that reduce pH on the apple skin and increase in this way cuticle permeability to water and dissolved nutrients in it (Knoll and Schreiber 1998).

The aim of this paper is to determine eventual role of glucose and fructose concentration on the apple skin on the germination of conidia of two fungi (*P. sessilis, P. fructicola*) that cause sooty blotch and their further growth. Also the relation among surface sugar, sugar in apple juice and pH of juice was determined.

MATERIAL AND METHODS

The studies were carried out on 'Novamac' apple fruits in the years 2000–2002. The fruits were taken from the orchard in Garlica near Cracow, every two weeks in the period between July and October to determine: sugar content in fruit juice, pH of juice as well as glucose and fructose concentration on the apple skin. Simultaneously, in the same intervals the incidence and severity of disease was evaluated, and then the germination of conidia of *P. sessilis* and *P. fructicola* in water and solutions of fructose and glucose was estimated.

Sugar content in fruit juice, pH of juice and reducing sugar (fructose and glucose together) concentration on the apple skin were determined.

To determine the concentration of reducing sugars on the apple skin every two weeks 5 fruits were taken from 25 selected trees. On a surface of each fruit 4 areas of 1 cm^2 were set up. On each area 0.25 ml of distilled water was applied with micropipette and the collected solution was analyzed spectrophotometrically (Kosuge and Hewitt 1964). The color reaction was carried out by ferrocyanide method. The absorbance was measured with a spectrophotometer at wavelength of 660 nm in 1 cm^2 cell with water as a reference. At the same dates the sugar content and pH of fruit juice were also measured. The fruit juice was obtained with juice extractor and then centrifuged to remove remains of parenchyma and pectin. For such prepared juice the average percentage sugar content was measured with a refractometer. The juice acidity was determined with an electronic pH meter.

Evaluation of sooty blotch severity on the fruits

The incidence and severity of sooty blotch on Novamac fruits were estimated at the same dates as analysis of sugars and pH. The percentage of affected fruits and mean degree of infection (Tab. 1) were calculated (every two weeks) on the base of 25 fruits that were taken in random from various parts of 50 selected trees.

Germination of conidia of *P. sessilis* and *P. fructicola*

The germination of conidial spores of both pathogens was determined in distilled water, d-glucose solutions of 0.5 mg/dl and 1.0 mg/dl and reducing sugar solution obtained from the surface of 'Novamac' fruits.

The pathogen spores were taken from 14-day cultures growing on the PDA. From each fungal isolate the suspensions containing 5×10^5/ml fungal spores in water and solutions mentioned above were prepared. The germinating spores were observed for 5 hours, and every hour the percentage of those that produced hyphal growth was counted. The number of conidia for *T. myrti* was too small to evaluate any germination.

Table 1. Degrees of apple fruits infection

Degrees	Affected surface [%]
1	> 20
2	21–40
3	41–60
4	above 60

RESULTS AND DISCUSSION

The tests carried out in the years 2000–2002 on 'Novamac' apple fruits showed the presence of reducing sugars on the apple skin and their direct effect on the growth of fungi causing sooty blotch.

During the first year i.e. between June 30 and July 30 very low concentration of surface glucose and fructose was observed (from 0.13 to 0.18 mg/dl). During this period no disease symptoms were found on the fruits. A significant (more than twice) increase in glucose and fructose content occurred only between July 30 and August 15, when its concentration rose up to 0.37 mg/dl, while pH of apple juice increased up to 4.4. Simultaneously, it was found that sugar concentration in fruit juice increased up to 10.1% (Tab. 2). During this period the first symptoms of sooty blotch appeared on the fruits. Such considerable increase of sugar concentration both in the fruit juice and on the skin could be referred to pH changes of the apple juice. At pH about 4.0 a significant increase in β-amylase activity lead to apple starch breakdown into glucose and fructose (Tetlow and Matthew 2004). That is why in the period between the end of July and the mid August when the first disease symptoms occur, the glucose and fructose concentration increases not only in

Table 2. Relationship between occurrence of sooty blotch of apple on 'Novamac' fruits and sugar concentration in juice and reducing sugars on fruit surface

Sampling date	Sugars in apple juice [%]	Fructose and glucose occurring on fruit surface [mg/dl]	pH of apple juice	Percentage of infected fruits	Mean degree of infection (1–4)
		2000			
30.06	5.0	0.13	2.9	0	–
16.07	7.2	0.14	3.3	0	–
30.07	8.2	0.18	3.6	**0**	–
15.08	**10.1**	**0.37**	**4.4**	**7**	**1.5**
01.09	11.6	0.45	4.5	16	1.9
17.09	13.4	0.61	4.8	28	2.6
01.10	14.9	0.81	4.8	30	3.7
		2001			
30.06	5.0	0.12	2.7	0	–
16.07	6.7	0.15	3.2	0	–
30.07	8.2	0.18	3.5	**0**	–
16.08	**9.9**	**0.34**	**4.3**	**5**	**1.2**
30.08	11.2	0.49	4.4	15	1.7
15.09	12.4	0.65	4.7	25	2.4
30.09	14.9	0.82	4.9	30	3.5
		2002			
01.07	5.1	0.10	2.9	0	–
17.07	7.0	0.15	3.0	0	–
04.08	8.3	0.19	3.4	**0**	–
17.08	**10.0**	**0.39**	**4.4**	**5**	**1.1**
02.09	11.3	0.44	4.6	17	1.8
16.09	12.9	0.63	4.7	25	2.5
01.10	14.6	0.80	4.9	33	3.3

the fruit juice but also on the apple skin. Afterwards, i.e. between August 15 and October 1 it was found that further increase in concentration of reducing sugars on the apple skin was accompanied by gradual increase of sugar content in the juice. At the crop maturity phase of the Novamac fruits, the concentration of surface sugars exceed 0.8 mg/dl, and sugars in juice amounted to 14.9%. High concentration of glucose and fructose in this period was accompanied by extreme incidence and severity of sooty blotch. The percentage of affected fruits for this cultivar reached 30%, and recorded severity was 15.7. In the years 2001 and 2002 similar relationships were observed (Tab. 2).

An effect of the presence of reducing sugars on the fruit skin on the growth of fungi that cause sooty blotch was also confirmed by evaluation of germination of conidial spores. The tests indicated that the 0.8 mg/dl solution of reducing sugars obtained from the fruit skin considerably stimulated germination of spores both for *P. sessilis* and *P. fructicola*. Already after keeping for 4 hours in the reducing sugar solution, above 77% of the *P. sessilis* spores germinated. In d-glucose solution of 1.0 mg/dl this percentage was a little higher, while in distilled water only 28% of spores germinated (Fig. 1). Similar dynamic of germination was observed for *P. fructicola*. After 4 hours in the reducing sugar solution 83% of conidia germinated, in 1.0 mg/dl d-glucose solution this figure was higher. Concurrently, in distilled water less than 35% of spores germinated (Fig. 2).

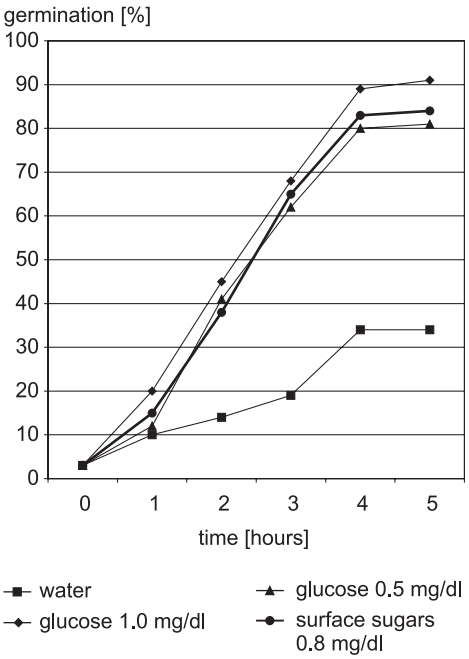

Fig. 1. Percentage germinated conidia of *Peltaster fructicola* in water, glucose, as well as in fructose and glucose occurring on fruit surface

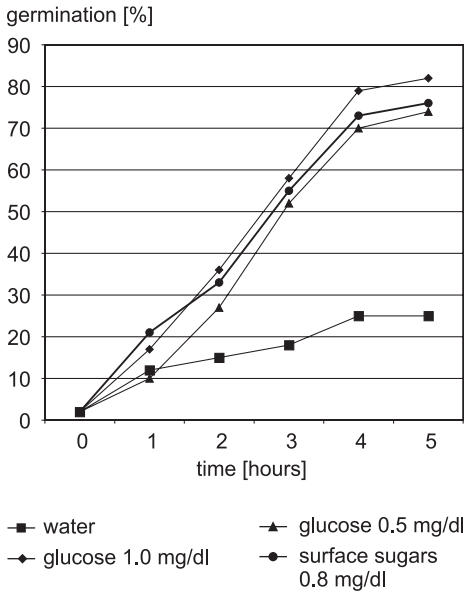

Fig. 2. Percentage germination of conidia of *Phialophora sessilis* in water, glucose, as well as in fructose and glucose occurring on fruit surface

Similar results were reported by Kosuge and Hewitt (1964) and Schreiber (2001), who found similar relationship for *Botrytis cinerea* responsible for grey mould of strawberry and grape berries. Due to the fact that substances such as aminoacids and hydrocarbons have no direct effect on germination and further growth of fungi causing sooty blotch (Belding et al. 2000), one may conclude that sugars present on the apple skin are their primary source of nutrients.

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POLISH SUMMARY

WPŁYW GLUKOZY I FRUKTOZY OBECNYCH NA POWIERZCHNI SKÓRKI JABŁEK NA ROZWÓJ SPRAWCÓW BRUDNEJ PLAMISTOŚCI

Grzyby wywołujące brudną plamistość jabłek *(Peltaste fructicola, Phialophora sessilis, Tripospermum myrti)* rozwijają się jedynie na powierzchni skórki jabłek, w związku z tym do ich wzrostu konieczna jest obecność substancji odżywczych na powierzchni owoców. Badania wykazały, że w okresie w którym pojawiły się pierwsze objawy brudnej plamistości stwierdzono wyraźny wzrost zawartości zarówno cukrów na powierzchni jak i cukrów w soku jabłek. Wzrost ten nastąpił przy pH 4.4. Wpływ cukrów obecnych na powierzchni skórki jabłek na rozwój sprawców choroby potwierdzono dodatkowo oceniając zdolność kiełkowania konidiów *P. sessilis* i *P. fructicola* w roztworze tych właśnie cukrów w odniesieniu do wody destylowanej oraz wzorcowych roztworów d-glukozy.