

EFFECT OF SURFACE AMINO ACIDS ON THE GROWTH OF *PELTASTER FRUCTICOLA* – FUNGUS ASSOCIATED WITH SOOTY BLOTCH COMPLEX

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Abstract: Studies undertaken in 2002–2004 on ‘Golden Delicious’ apple fruits showed the presence of amino acids on the surface of their skin. Amount of total free amino acids ranged from 2.5 to 3.0 mg/L. In *in vitro* bioassays it was found that amino acids as a basic source of nitrogen did not activate germination of conidia of *Peltaster fructicola* (Johnson). However amino acids stimulated elongation of germ tubes and early hyphal growth. It was confirmed that fruit washings also contained sugars that stimulated both conidial germination and germ tube elongation. We proved also that different carbon to nitrogen proportions significantly affected *P. fructicola* growth. Proportions of C:N were evaluated according to newly introduced amino acid sugar index (ASI).

Key words: *Peltaster fructicola*, sooty blotch, surface amino acids, sugars, apple skin, conidial germination, germ tubes, C:N ratio

INTRODUCTION

Sooty blotch of apple is caused by a complex of pathogens that develop only on the apple surface. The fungi do not penetrate cuticle or utilize any components of epicuticular waxes (Belding et. al 2000). Thus their growth should depend primarily on the presence of nutrients on the surface of the apple skin. It is commonly known that nutrients can not only trigger spore germination but also influence formation of germ tubes and further hyphal growth (Beckman and Payne 1983; Abdel and Arbab 1985; Xu and West 1992; Nelson and Hsu 1994; D’Enfert 1997). The presence of amino acids, sugars and organic acids on aerial plant surfaces was documented by several authors (Fiala et. al 1990; Mercier and Lindow 1998; Lindow and Brandl 2003; Wrona 2002). Our previous studies showed that sugars on the cuticle surface provide a source of carbon and play a major role in germination of conidia of

sooty blotch fungi (Wrona 2002). For subsequent developmental stages pathogens require assimilable nitrogen sources to form protein. The role of amino acids as nitrogen sources in the growth of sooty blotch mycelium has not been determined. The investigations carried out by Elson et al. (1998) and Chaky et. al (2001) on *Heminthosporium solani* and *Colleotrichum graminicola* indicated that fungal growth may depend also on different carbon to nitrogen ratio (C:N). Differences in carbon to nitrogen ratio in nutrient substrate may contribute to changes in pathogenicity level and can facilitate most processes that result in a successful infection.

The aim of this study was to characterize the concentration and composition of free amino acids present on the apple skin and gauge their effect on conidial germination and germ tube elongation of *P. fructicola*.

MATERIAL AND METHODS

In 2002–2004 ‘Golden Delicious’ apple fruits with no sooty blotch signs were sampled from unsprayed orchards of southern Poland at harvest, to determine the presence and concentration of amino acids and surface sugars on the apple skin. On the surface of 15 fruits taken annually from each of 25 selected trees, five 1 cm² areas were randomly designated. On each area 0.25 ml of distilled water was applied with micropipette for 30 min. (air temp 22°C). Collected solutions were analyzed chromatographically by HPLC method (column dimension 250 × 4.0 mm, eluent A: 20 mM CH₃COONa, pH 4.2, 5% THF, B:ACN gradient 20–36% B, 0–20 min., 36–38% B, 20–28 min., 38% B, 28–34 min., 38–59% B, 34–51 min., 57–67% B, 51–56 min. Flow 1.2 ml/min., detection fluorescence Ex. 263 nm, Em. 313 nm, temp. 30°C, injection 10 µl. As amino acids standard Sigma Co. Kit was used. Amino acids content in fruit washings was carried out spectrophotometrically. A coloration assay was performed by ninhydrin reaction (Yemm and Cocking 1955) and absorbance was measured with spectrophotometer (PC-spectro) at 350 nm in 1 cm² cell with asparagine and distilled water as reference. The concentration of surface sugars was assayed by Ferro-cyanide method (Dubois et. al 1956); absorbance was measured at 660 nm, with D-glucose and distilled water as reference. Concentration of surface sugars and amino acids was expressed in mg/L.

To confirm the role of surface amino acids in the growth of *P. fructicola*, conidial germination and germ tube elongation in fruit washings, amino acids, D-glucose, mixture of D-glucose and amino acids, water (control) were estimated.

Evaluation of amino acids-sugars index (ASI)

To determine how different carbon to nitrogen proportions can affect *P. fructicola* growth, solutions containing various concentrations of amino acids and sugars were tested. Amino acid mixture consisted of: L-arginine, L-asparagine, L-glutamine, glycine, L-alanine, L-phenylalanine, L-lysine taken in equal weight proportions. Subsequently solutions of 0.4, 0.5, 0.8, 1.0, 1.2, 1.5, 2.0 and 3.1 mg/L were prepared. Solutions of D-glucose were added in proportions to obtain the C:N ratio about 10:1. Amino acids-sugars index (ASI) was evaluated as $ASI = [(Ac \times Sc) \div (1 + Ac + Sc)]$ (Wrona and Filipecki, data unpublished). Where Ac equals concentration of surface amino acids (mg/L), Sc equals the concentration of surface sugars (mg/L).

Germination of *P. fructicola* conidia and elongation of germ tubes

The germination of conidia and elongation of germ tubes was determined in:

1. distilled water (control),
2. D-glucose standard solution (10 mg/L),
3. amino acids standard solution (3 mg/L),
4. mixture of D-glucose(10mg/L) and amino acids (3 mg/L),
5. fruit washings obtained from the surface of 'Golden Delicious' apple fruits,
6. solutions of individual amino acids: L-arginine, L-asparagine, L-glutamine, glycine, L-alanine, L-phenylalanine, L-lysine, each of concentration 3 mg/L.

All tested solutions were standardized with phosphatic buffer to pH 6.5 (PerpHecT pH meter 310). Conidia of *P. fructicola* (5×10^5 /ml) were taken from 14-day old cultures growing on potato dextrose agar, in sterile distilled water and in solutions mentioned above were used. Conidial germination and germ tube elongation were observed on glass slides every 2 hr for 10 hr and percentage of conidia producing germ tubes was counted and germ tube length was measured. All observations were performed under magnification $\times 100$. Percentage of germinating spores and germ tube length were estimated for 80 spores per slide and 25 slides per treatment. Studies were repeated three times.

RESULTS AND DISCUSSION

HPLC revealed following amino acids on the fruit surface: L-arginine, L-asparagine, L-glutamine, glycine, L-alanine, L-phenylalanine, L-lysine. The total amount of free L-amino acids on 'Golden Delicious' apple skin ranged from 2.5 to 3.0 mg/L.

Initially amino acids solutions had no direct effect on the germination of conidia *P. fructicola* (Fig. 1A). After keeping for 10 hr in standard solutions of L-amino acids, percentage of germinated spores ranged from 12% (in L-phenylalanine) to 33% (in L-arginine), whereas 26% of spores germinated in distilled water. Similar results were reported by Elson et. al. (1998), Osharov and May (2000), Chaky et. al (2001) and Lapaire and Dukle (2003) for *H. solani*, *C. graminicola*, *Aspergillus nidulans* and *Cercospora zea-maydis*. They found that neither organic nor non-organic nitrogen sources can induce conidial germination, whereas some of amino acids like L-phenylalanine or L-methionine may even suppress hyphal growth and inhibit formation of conidia (Elson et. al 1998). Our studies also showed that incidence of germinated spores of *P. fructicola* in L-phenylalanine was much lower (12%), than in distilled water (26%). Conidial germination was highest (92%) in fruit washings obtained from the surface of 'Golden Delicious' apples (Fig. 1A). It was also observed that percentage of germinated spores (90%) in D-glucose solution of 10 mg/L was almost at the same high level as in the fruit washings which included both amino acids and sugars (data not shown).

Our data therefore suggest that the influence of fruit washings on conidial germination of *P. fructicola* was due primarily to the effect of sugars that can be considered as an activating signal during the first phases of sooty blotch colonization. Amino acids as a main nitrogen source appeared to be essential to hyphal development (Fig. 2A). Germ tubes grew most rapidly in fruit washings and mixture of

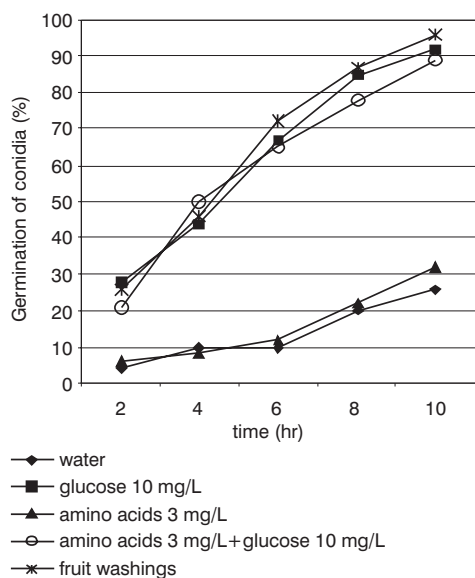


Fig. 1A. Germination of conidia of *Peltaster fructicola* in fruit washings and tested solutions, at PH 6.5

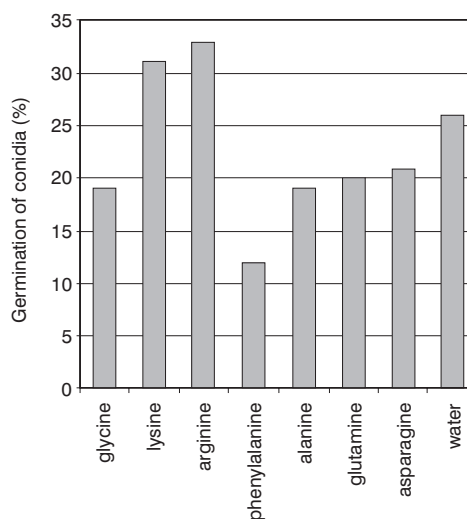


Fig. 1B. Germination of conidia of *Peltaster fructicola* in solutions of tested amino acids (3 mg/L) after 10 hr, at PH 6.5

amino acids (3.0 mg/L) and D-glucose (10 mg/L). The length of germ tubes in both tested solutions was similar and after 10 hr of incubation reached 50 and 48 μm re-

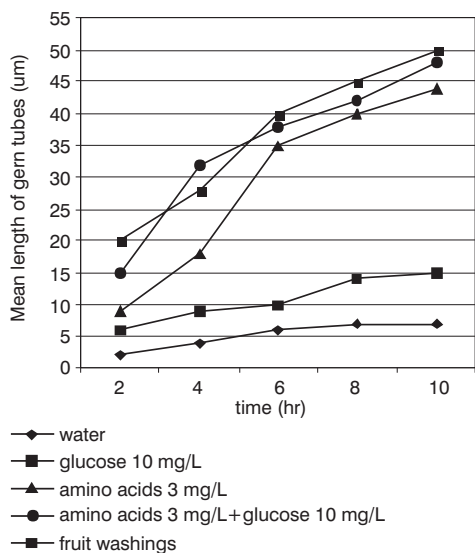


Fig. 2A. Elongation of germ tubes of *Peltaster fructicola* in fruit washings and tested solutions, at PH 6.5

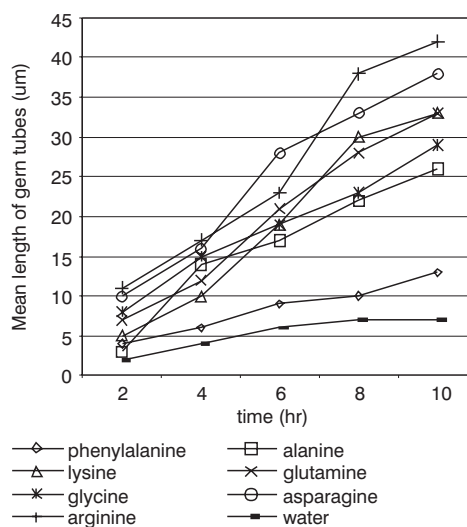


Fig. 2B. Elongation of germ tubes of *Peltaster fructicola* in tested solutions of amino acids (3 mg/L) after 10 hr, at PH 6.5

Table 1. Elongation of germ tubes of *Peltaster fructicola* in relation to carbon and nitrogen proportions and amino acids-sugars index (ASI)

Amino acids mixture* (mg/L)	D-glucose (mg/L)	C:N ratio	Amino acids-sugars index (ASI)	Mean germ tube length (μm)
0.4	4.0	10:1	0.29	12.9
0.5	5.3	10.6:1	0.38	13.5
0.8	8.2	10.2:1	0.65	15.1
1.0	11.0	11:1	0.84	22.1
1.2	13.0	10.8:1	1.02	30.5
1.5	14.0	9.3:1	1.27	39.4
2.0	21.0	10.5:1	1.75	46.2
3.1	32.0	10.3:1	2.75	49.5

*Amino acid mixture consisted of: L-arginine, L-asparagine, L-glutamine, glycine, L-alanine, L-phenylalanine, L-lysine, taken in equal weight proportions

spectively. Less growth was found for individual or combined amino acids. In solution of amino acids mixture (3.0 mg/L) germ tubes reached 44 μm , but in the case of individual amino acids the longest tubes (42 μm) were noted for L-arginine and the shortest for L-phenylalanine (12 μm). The growth of germ tubes was least affected by D-glucose solution (15 μm) and sterile distilled water (7 μm).

Taken together our results suggest that conidial germination is triggered mainly by sugars components, whereas the elongation of germ tubes and further hyphal growth require nitrogen sources. However simultaneous presence of carbon compounds may also support fungal growth.

Our studies also indicated that *P. fructicola* growth depends not only on sugars and amino acids concentration in substrate but also on their mutual proportions. Proportion expressed as the C:N ratio appeared unrelated to germ tube growth (Table 1), whereas ASI values distinctly reflected germ tube elongation. Carbon to nitrogen proportions were optimal for fungal growth when ASI reached values above 0.84, further increase in ASI values from 0.84 to 2.75 contributed to extension of germ tubes from 22.1 μm to 49.2 μm .

It seems that also different amount of sugars (C) and amino acids (N) on the surface of the apple skin may significantly influence first critical phases of *P. fructicola* development. We therefore conclude that determination of carbon to nitrogen proportions as ASI for different apple cultivars could explain some of the still poorly understood aspects of different susceptibility to sooty blotch disease among apple cultivars.

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POLISH SUMMARY

WPLYW POWIERZCHNIOWYCH AMINOKWASÓW NA WZROST *PELTASTER FRUCTICOLA* – GRZYBA ZWIĄZANEGO Z KOMPLEKSEM BRUDNEJ PLAMISTOŚCI

Badania przeprowadzone na owocach odmiany 'Golden Delicious' wykazały na powierzchni skórki jabłek obecność aminokwasów. Koncentracja tych wolnych aminokwasów wahała się od 2.5 do 3.0 mg/L. Doświadczenia prowadzone w warunkach *in vitro*, udowodniły, że aminokwasy jako podstawowe źródło azotu nie aktywowały kiełkowania zarodników konidialnych *Peltaster fructicola*. Obecność aminokwasów stymulowała natomiast wydłużanie się strzępek rostkowych i początkowy wzrost strzępki. Badania potwierdziły również, że przesącz owoców odmiany 'Golden Delicious' zawierał także cukry, które stymulowały zarówno kiełkowanie konidii jak i wydłużanie strzępki rostkowej grzyba. Udowodniono także, że zmienne proporcje źródła węgla (cukry) do azotu (aminokwasy) znacząco oddziaływały na wzrost *P. fructicola*. Proporcje węgla do azotu wyliczono wg nowo wprowadzonego wskaźnika aminokwasowo-cukrowego (ASI).