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ORIGINAL PAPER

Accumulation of amino acids and phenolic compounds in biochemical plant responses to feeding of two different herbivorous arthropod pests

Katarzyna Golan[1] · Cezary Sempruch[2] · Edyta Górska-Drabik[1] · Paweł Czerniewicz[2] · Bożena Łagowska[1] · Izabela Kot[1] · Katarzyna Kmieć[1] · Klaudia Magierowicz[1] · Bogumił Leszczyński[2]

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Abstract The study aimed at comparing the changes in the content of free amino acids, phenolic compounds and the activity of PAL and TAL caused by two piercing-sucking arthropods: the grape mealybug (*Pseudococcus maritimus* Ehrh.) and the two-spotted spider mite (*Tetranychus urticae* Koch) in the leaves of orchid and strawberry, respectively. The obtained results show that the amino acid content and the ratio of amino acids to phenolic compounds increased in both plant species infested by the mealybug and the mite. However, such response was weakly dependent on changes in activity of the analysed enzymes. The pest feeding affected accumulation of the phenolic compounds, since the induction of the PAL activity in mealybug-infested orchid leaves during the first 5 h of the experiment preceded the increase in phenolic compounds during the first week of insect feeding. Instead, the increased activity of TAL was accompanied by elevated levels of phenolic compounds in the leaves of strawberry infested by mites. Mechanisms of biochemical plant responses induced by infestation of the studied herbivorous arthropods are discussed.

Keywords Amino acids · Grape mealybug · Phenolic compounds · Phenylalanine and tyrosine ammonia-lyases · Two-spotted spider mite

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✉ Katarzyna Golan
katarzyna.golan@up.lublin.pl

[1] Department of Entomology, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland

[2] Department of Biochemistry and Molecular Biology, Siedlce University of Natural Sciences and Humanities, Prusa 12, 08-110 Siedlce, Poland

Introduction

Amino acids and phenolic compounds play different roles in plant metabolism and biochemical defence against herbivorous arthropods. Amino acids are primary metabolites limiting the nutritive value of plant tissues for phytophagous insects. Generally, the level of these compounds is increased in plant tissues infested by piercing-sucking insects (Sempruch et al. 2011). This response results from the stimulation of nutrient flow to the feeding places of aphids in order to maintain the sustenance. Such tendency was observed in different plant species infested by *Elatobium abietinum* (Walk.), *Aphis fabae* (Scop.), *Diuraphis noxia* (Kord.), *Rhopalosiphum padi* (L.), *Sitobion avenae* (F.), *Shizaphis graminum* (Rond.) and *Bemisia tabaci* (Genn.)—Blackmer and Byrne (1999), Sandström et al. (2000), and Sempruch et al. (2011). Moreover, Sempruch et al. (2012, 2014) reported that the activity of some enzymes involved in different steps of amino acid biosynthesis and their further metabolism was also altered by aphid feeding. Proteomic analysis of biochemical changes in *Pisum sativum* (L.) infested by the pea aphid showed that the resistant pea cv. was characterised by: the reduction of proteins related to photosynthesis and amino acid biosynthesis, increased accumulation of wound signal molecules, such as lipoxygenases and leucine aminopeptidases, and activation of the ascorbate–glutathione cycle, while the susceptible cv. showed an increase in the primary metabolism pathway (particularly amino acid biosynthesis; Carillo et al. 2013).

On the other hand, secondary phenolic metabolites are generally regarded as defensive molecules. They can prevent insect oviposition on the host plant and disturb larval growth (Caretto et al. 2015). For example, flavonoids were shown to inhibit feeding, growth, development and

oviposition of various insect species (Simmond 2001, 2003; Lattanzio et al. 2006). The negative impact of tannins on insect growth stems from their ability to produce complex proteins and reduce digestibility. Thus, these compounds may act as enzyme inactivators. Moreover, tannin oxidation in the insect can also be considered as a defensive mechanism. According to Chrzanowski et al. (2012), phenolic acids extracted from black currant, sour cherry, walnuts and green husks prolonged the prereproductive period of *S. avenae* and reduced their daily fecundity. In addition, wounding and herbivory induced cell wall modifications, such as of cell wall-bound phenolics, lignins, suberin and cuticle-associated phenolics, which may act as physical barriers preventing infections and arthropod feeding (Bernards and Båstrup-Spohr 2008).

L-Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) is a key phenolic biosynthesis enzyme (Chaman et al. 2003). In plant tissues, it catalyses bioconversion of L-phenylalanine to *trans*-cinnamic acid, which is a key step in the shikimic pathway (Dixon et al. 2002). Some authors reported that L-tyrosine ammonia-lyase (TAL; EC 4.3.1.) was also involved in the synthesis of phenolic compounds via the shikimic pathway (Watts et al. 2006). However, the data obtained for maize suggested that the same polypeptide chain showed PAL and TAL activity (Rösler et al. 1997). It was demonstrated that PAL activity was correlated with polyphenol and *o*-dihydroxyphenol contents in developing olive under different watering treatments (Tovar et al. 2002). Overexpression of PAL in transgenic tobacco plants elevated the level of chlorogenic and *p*-coumaric acids (Howles et al. 1996). PAL activity in apple was correlated with simple phenols, particularly with phenolic acids (Ju et al. 1995). In addition, PAL and TAL are known to be involved in plant responses against aphid infestation. PAL activity was significantly increased in cotton leaves infested by the cotton aphid (*Aphis gossypi* Glov.) and in wheat infested by *S. avenae*; moreover, resistant cultivars (cvs.) were characterised by a significantly higher PAL activity than susceptible ones (Li et al. 2008; Han et al. 2009). Ciepiela and Niraz (1993) observed an increase of TAL activity in wheat leaves under *S. avenae* infestation. During this response, PAL activity was also stimulated, but only in case of less susceptible cv. According to Chaman et al. (2003), barley cv. less susceptible to *Shizaphis graminum* (Rond.) showed a significantly higher specific activity of PAL than the more susceptible cv., and these differences were connected with increased accumulation of free salicylic acid (SA) in more resistant plants and conjugated SA in both barley cvs. Different expression of PAL in transgenic tobacco cvs. resulted in a highly increased content of phenolic compounds, such as chlorogenic acid, total flavonoids and rutine. However, these changes were not correlated with the survival of larvae of the specialist herbivore, *Manduca sexta* (L.), and generalist species, *Heliothis virescens* (F.). On the other hand, the participation of PAL in acquired systemic resistance is possible, because SA was shown to induce the accumulation of PAL mRNA, synthesis of new PAL proteins, as well as an increase of its activity and phenolic content in the grape berry (Wen et al. 2005, 2008). However, in tobacco cell cultures, methyl jasmonate (MeJA) induced PAL mRNA accumulation, the activity of this enzyme and synthesis of coumarin: scopoletin and scopolin (Manisha et al. 1998).

Eleftherianos et al. (2005) showed that *R. padi* induced increase in total amino acids in maize and barley tissues without affecting the phenol concentration, while *S. avenae* feeding caused decrease in phenol levels but did not change the total amino acids in these cereal species. Moreover, other data suggest that the mechanisms of biochemical plant responses to herbivory associated with the accumulation of amino acids and phenolic compounds, as well as changes in the activity of PAL and TAL, may vary in different plant species. In addition, it is possible that these responses are dependent on other factors, such as the species and number of herbivores and duration of infestation. Therefore, the current work is the first report that focuses on changes in the content of free amino acids and phenols and the activity of PAL and TAL caused by two systematically distant piercing-sucking arthropods, i.e. the grape mealybug (*Pseudococcus maritimus* Ehrh.) and the two-spotted spider mite (*Tetranychus urticae* Koch) in the leaves of orchid and strawberry, respectively.

Materials and methods

Plant and insect materials

The experiment was conducted in the laboratory of the Department of Entomology, University of Life Sciences in Lublin. Plant materials were the orchids (*Phalaenopsis* × hybridum 'Innocence') without inflorescence shoots and strawberry (Fragaria ssp.) frigo runner plants. Orchid plants were grown in plastic transparent pots of a 12-cm diameter, filled with coarse pine bark bedding. Young strawberry plants with four compound leaves were grown in plastic green pots of an approx. 15-cm diameter, filled with universal base. The plants were located in a cultivation chamber on textile subirrigation mats (Polprotex) covered with black agrofabric. Plants were placed at a distance of 20 cm from each other. Flooding orchid plants with tap water once a week was the only care treatment applied. Strawberry plants were watered twice a week and extra liquid fertilizer blend with a combination of nitrogen, phosphorus and potassium (NPK; 50 ml/10 l of water) was added once a week. The experiment included orchids (after

a 4-week adaptation period) in the phase of seven fully developed leaves, without inflorescence shoots, colonized with *P. maritimus* individuals, derived from laboratory breeding performed on Phalaenopsis × hybridum 'Innocence' for 6 months preceding the experiment. Strawberry plants were fertilized until four compound leaves (a "trifoliate") were formed. The plants were colonized with *T. urticae* individuals derived from laboratory cultures conducted on bean (*Phaseolus* L.) for 2 weeks preceding the experiment. Five young females or third instar larvae of *P. maritimus* and five nymphs of *T. urticae* were transferred onto each plant of Phalaenopsis × hybridum 'Innocence' and strawberry, respectively, using a bristle brush. Herbivorous arthropods could move freely on the plant.

The sample consisted of 3 plants, on which the insects were feeding for 1, 2, 5 and 24 h as well as 7 and 14 days, respectively. Plants without pests served as controls. The experiment was conducted in three replications for each combination. The same experimental conditions were applied in the cultivation chambers for all the plants used during this study (temperature 27 ± 1 °C; humidity 50 ± 5%, photoperiod L:D = 16:8).

Chemical analysis

Free amino acids were extracted from 4 g of fresh plant material through homogenization with 80% ethanol solution. Obtained suspension was centrifuged 12,000×*g* for 20 min at 4 °C. Supernatant was used for further analysis. Amino acids content was assayed with the ninhydrin method (Robyt and White 1987). The analysis was performed by the addition of ninhidrin reagent (0.2 mg × cm^{-3} of ninhidrin in 20 mM acetic acid-acetate buffer, pH 5.0) to amino acid extract. The mixture was heated 10 min at 100 °C and then cooled. Absorbance of reaction product was measured at 570 nm with an UV–Vis spectrophotometer (Hewlett Packard 8453). The level of amino acids in plant extracts was read from the calibration curve drawn for 10–200 μM × dm^{-3} concentrations of phenylalanine.

Fresh plant material (4 g) was homogenized with 80% methanol in the aim of the total phenol extraction. Crude extract was next centrifuged at 12,000×*g* for 20 min at 4 °C. Total phenol content was assayed in the supernatant with the Folin–Ciocalteau method according to Stratil et al. (2006). Absorbance of products of the phenol reaction with Folin–Ciocalteau reagent was measured spectrophotometrically with an UV–Vis spectrophotometer (Hewlett Packard 8453) at 750 nm, and phenol content was appointed with a calibration curve prepared for gallic acid at a concentration range from 10 to 100 μg × 0.2 cm^{-2}.

The method described by Ciepiela and Niraz (1993) was applied for determination of PAL and TAL activity. Plant material (10 g) was cut and homogenised with cold 0.1 M borate buffer of pH 8.8. The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C and supernatant was used as a crude enzymatic extract. The reaction mixture consisted of 2 cm^3 of borate buffer, 1 cm^3 of 0.1 M L-phenylalanine solution in borate buffer, 0.2 cm^3 of enzymatic extract and 3 cm^3 of water. The mixture was incubated at 30 °C for 30 min. In case of TAL, 0.1% solution of L-tyrosine in borate buffer was used as a substrate, and other conditions of the assay was the same than for PAL. The concentrations of products of enzymatic reactions were assayed spectrophotometrically at 290 nm for PAL and 333 nm for TAL and read out from calibration curves made for different concentration of *trans*-cinnamic acid (for PAL) and *p*-coumaric acid (for TAL). Activity of the enzymes was calculated as a μM of the appropriate product generated by one mg of enzymatic protein during 1 h of the reaction. Protein content in enzymatic extract was assayed with the Lowry et al. (1951) method.

Statistics

All chemical analyses were assayed in three independent replicates. The distribution of the obtained data was determined with the *Chi* square (χ^2) test. One-way analysis of variance (ANOVA) was performed for data with a normal distribution (protein content in enzymatic extracts, PAL activity in orchid leaves, amino acids to phenols ratio in strawberry leaves). The Kruskal–Wallis' test, as a non-parametric alternative for ANOVA, was applied for data with failed normality (content of amino acids and total phenols; TAL activity; PAL activity in strawberry and amino acids to phenols ratio in orchid leaves).

Differences in content of studied compounds and the enzyme activities within analysed plants were analysed by the Student's *t* test (for data with normal distribution) or the Mann–Whitney U test (in other cases).

The acceptance level of statistical significance was $p \leq 0.05$. The results presented in tables and figures are arithmetic means with standard deviations (SDs). All statistical analyses were conducted with Statistica for Windows version 12.0 (StatSoft Inc. 2015).

Results

Changes in content of the amino acids

Statistical analysis showed significant differences in amino acid content in orchid ($H_{5,\,N\,=\,18} = 15.13; p = 9.8 \times 10^{-3}$) and strawberry leaves ($H_{5,\,N\,=\,18} = 15.13; p = 9.8 \times 10^{-3}$) in particular variants of the experiment. Generally, amino acid levels increased about fivefold in orchid leaves infested

by *P. maritimus* already after the first hour of insect feeding (Table 1). This response persisted during the following time points of the infestation and was strongest after 1 week. An exception was the variant 24 h., during which the increase of the amino acids was the weakest and amounted to only slightly more than twofold multiple of the control values. A similar response was recorded in strawberry leaves infested by *T. urticae* (Table 1). However, in this case, amino acid level was increased about twofold after the first hour of the infestation, but the initial level of these compounds was more than tenfold higher compared to orchid leaves. The response was the strongest after 1 day (24 h) and 1 week of the two-spotted spider mite feeding.

Changes in content of the total phenols

The content of total phenols at different periods of herbivory was significantly different in orchids ($H_{5,\ N\ =\ 18} = 16.46$; $p = 5.7 \times 10^{-3}$) and in strawberries ($H_{5,\ N\ =\ 18} = 16.01$; $p = 6.8 \times 10^{-3}$). It was increased after 1 day and 1 week, while it was decreased during the first 5 h; after 2 weeks, it was induced in orchid leaves infested by the grape mealybug (Table 2). As regards the strawberry infested by the mite, an increase of these compounds was observed at all time points with the exception of the 2-week period, when the changes were not statistically significant. This response was the highest after 1 week of the infestation and the lowest after 1 day.

Changes in amino acids to phenols ratio

The ratio of amino acids to phenols was significantly changed in strawberry ($F_{5,\ 12} = 14.11$; $p = 1.13 \times 10^{-4}$) and orchid ($H_{5,\ N\ =\ 18} = 15.83$; $p = 7.3 \times 10^{-3}$) infested by the studied arthropod species (Table 3). The changes were especially distinct in orchid infested by *P. maritimus*, as this coefficient was 5.8- to 8.8-fold elevated with the exception of the 24-h time point (1.3-fold increase). The ratio was also increased 1.4- to 2.2-fold in strawberry leaves in response to infestation with *T. urticae*.

Table 1 Changes in amino acid content in leaves of studied plant species infested by *P. maritimus* and *T. urticae*

Duration of infestation	Species of plant and herbivore	
	Orchid *P. maritimus*	Strawberry *T. urticae*
	Amino acid content (μM × g fresh wt.)	
0 h (control)	0.36 ± 0.03	10.41 ± 0.52
1 h	1.88 ± 0.22*	18.97 ± 1.57*
5 h	2.19 ± 0.03*	17.84 ± 0.88*
24 h	0.71 ± 0.01*	23.54 ± 2.90*
1 week	2.56 ± 0.08*	23.09 ± 3.64*
2 weeks	2.28 ± 0.37*	14.44 ± 0.70*

* Values significantly different than control at $p \leq 0.05$ (Mann–Whitney' U test)

Table 2 Changes in total phenol content in leaves of studied plant species infested by *P. maritimus* and *T. urticae*

Duration of infestation	Species of plant and herbivore	
	Orchid *P. maritimus*	Strawberry *T. urticae*
	Total phenol content (μM × g fresh wt.)	
0 h (control)	1.47 ± 0.18	35.16 ± 1.00
1 h	1.18 ± 0.18*	41.77 ± 0.83*
5 h	1.18 ± 0.18*	42.42 ± 0.35*
24 h	2.12 ± 0.18*	37.70 ± 0.47*
1 week	1.77 ± 0.18*	54.93 ± 0.30*
2 weeks	1.06 ± 0.18*	36.17 ± 0.24

* Values significantly different than control at $p \leq 0.05$ (Mann–Whitney' *U*-test)

Table 3 Changes in value of amino acids/total phenols ratio in leaves of studied plant species infested by *P. maritimus* and *T. urticae*

Duration of infestation	Species of plant and herbivore	
	Orchid *P. maritimus*	Strawberry *T. urticae*
	Amino acids/total phenols	
0 h (control)	0.25 ± 0.03	0.29 ± 0.01
1 h	1.57 ± 0.19*	0.46 ± 0.05**
5 h	1.89 ± 0.06*	0.42 ± 0.02**
24 h	0.33 ± 0.03*	0.63 ± 0.08**
1 week	1.45 ± 0.08*	0.41 ± 0.07*
2 weeks	2.20 ± 0.03*	0.40 ± 0.02**

* Values significantly different than control at $p \leq 0.05$ (Mann–Whitney' U test for orchid infested by *P. maritimus* and Student's *t* test for strawberry infested by *T. urticae*)

** Values significantly different than control at $p \leq 0.01$ (Student's *t* test)

Changes in PAL and TAL activities

PAL activity was significantly changed at various periods of infestation in orchid ($F_{5,\ 12} = 22.03$; $p = 1.2 \times 10^{-5}$) and strawberry ($H_{5,\ N\ =\ 18} = 12.01$; $p = 3.46 \times 10^{-2}$) leaves. It was induced during the first 5 h and reduced after 1 day in mealybug-infested orchid leaves (Table 4). However, in subsequent time points, the response was compensated and enzyme activity was similar to the control. A decrease in PAL activity was detected in strawberry leaves, and this change was statistically confirmed during the first 5-h and 2-week time points of the mite infestation.

Table 4 Changes in activity of L-phenylalanine ammonia lyase (PAL) and L-tyrosine ammonia lyase (TAL) in leaves of studied plant species infested by *P. maritimus* and *T. urticae*

Duration of infestation	Species of plant and herbivore			
	Orchid		Strawberry	
	P. maritimus		*T. urticae*	
	Activity of analysed enzymes			
	PAL (μM *trans*-cinnamic acid/mg of protein/h; $\bar{x} \pm$ SD)	TAL (μM *p*-coumaric acid/mg of protein/h; $\bar{x} \pm$ SD)	PAL (μM *trans*-cinnamic acid/mg of protein/h; $\bar{x} \pm$ SD)	TAL (μM *p*-coumaric acid/mg of protein/h; $\bar{x} \pm$ SD)
0 h (control)	3.34 ± 0.20	12.06 ± 1.85	0.88 ± 0.06	0.35 ± 0.01
1 h	6.33 ± 1.03**	7.68 ± 1.16*	0.79 ± 0.01*	0.36 ± 0.01
5 h	5.73 ± 0.57**	5.52 ± 0.58*	0.73 ± 0.01*	0.40 ± 0.03*
24 h	1.99 ± 0.30**	3.53 ± 0.01*	0.80 ± 0.03	0.49 ± 0.03*
1 week	3.23 ± 0.70	3.74 ± 0.99*	0.80 ± 0.02	0.38 ± 0.03
2 weeks	3.23 ± 0.55	4.46 ± 0.97*	0.76 ± 0.03*	0.42 ± 0.01*

* Values significantly different than control at $p \leq 0.05$ (Mann–Whitney' U test for orchid infested by *P. maritimus* and Student's *t* test for strawberry infested by *T. urticae*)

** Values significantly different than control at $p \leq 0.01$ (Student's *t* test)

TAL activity decreased in mealybug-infested orchid leaves during all observation periods ($H_{5,\ N\ =\ 18} = 14.75$; $p = 1.5 \times 10^{-2}$; Table 4). However, in strawberry infested by the studied mite species, the activity of this enzyme was elevated ($H_{5,\ N\ =\ 18} = 12.60$; $p = 2.74 \times 10^{-2}$), especially after 5 h, 1 day and 2 weeks of the infestation, and these changes were statistically significant.

Moreover, the protein concentration in enzymatic extracts obtained from the orchid leaves was increased under the mealybug infestation ($F_{5,\ 12} = 8.11$; $p = 1.3 \times 10^{-3}$), and this response was statistically confirmed after 5 h, 1 day and 2 weeks from the beginning of insect feeding (Table 5). This response was not significant with respect to strawberries infested by mites ($F_{5,\ 12} = 3.05$; $p = 5.25 \times 10^{-2}$).

Discussion

Although *P. maritimus* and *T. urticae* belong to different groups of arthropod species, both of these herbivores feed by piercing and sucking the plant tissue. The grape mealybug has a typical phloem-feeding behaviour with an exclusively extracellular route to phloem and periodic intracellular punctures (Calatayud et al. 1994, 2006; Cid and Fereres 2010), whereas *T. urticae* penetrates the leaf either between epidermal pavement cells or through a stomatal opening, without damaging the epidermal cellular layer (Egas et al. 2003; Bensoussan et al. 2016). The mites feed from mesophyll cells without preference for cell type and their feeding pattern was not affected by the plant host preference. Schmidt et al. (2009) documented changes in plant primary metabolism at early plant responses to infestation by different herbivores: *T. urticae* and *Spodoptera littoralis* Boisduval. These authors observed that the differences in signalling pathways and defence responses depended on the type of damage to the plants resulting from the direct feeding. An increase in the content of amino acids occurs in typical biochemical responses of plants infested by piercing-sucking insects. For example, such changes were induced in different host plants infested by the following species: *E. abietinum*, *A. fabae*, *D. noxia*, *S. graminum, R. padi*, *S. avenae*, and in *Cucumis melo* L. infested by the silverleaf whitefly (Blackmer and Byrne 1999; Sandström et al. 2000; Sempruch et al. 2011). Cell content-feeding spider mites can dehydrate the spongy mesophyll causing stomatal closure, which, in turn, can decrease photosynthesis and alter primary metabolism (Bondada et al. 1995). As reported by Schmidt et al. (2009), *T. urticae* feeding on cotton caused different increases in total nitrogen concentrations and local concentrations of free amino acid, such as glutamine, phenylalanine, lysine or tryptophan. A similar increase in the amino acid content was demonstrated in the present work in orchid and strawberry leaves infested by *P. maritimus* and *T. urticae*, respectively. This response was induced already after the first hour of the infestation and in case of orchid, it was approx. two times stronger than in strawberry. The peak of this reaction was recorded after the first day in strawberry or after 1 week in orchid. According to Zhou et al. (2015), herbivores enrich the amino acid content in their food by increasing free amino acid biosynthesis, inducing proteolysis to release free amino acids into

Table 5 Changes in protein concentration in enzymatic extracts obtained from leaves of studied plant species infested by *P. maritimus* and *T. urticae*

Duration of infestation	Species of plant and herbivore	
	Orchid *P. maritimus*	Strawberry *T. urticae*
	Protein concentration (mg × cm^{-3}) ekstraktu	
0 h (control)	0.86 ± 0.16	1.18 ± 0.08
1 h	1.13 ± 0.09	1.28 ± 0.05
5 h	1.38 ± 0.06**	1.27 ± 0.02
24 h	1.40 ± 0.12**	1.28 ± 0.03
1 week	1.14 ± 0.14	1.21 ± 0.03
2 weeks	1.27 ± 0.13*	1.22 ± 0.03

* Values significantly different than control at $p \leq 0.05$ (Student's t test)

** Values significantly different than control at $p \leq 0.01$ (Student's t test)

the phloem and/or manipulating sink-source relationships to allow more transport of nitrogen into infested leaves. It was suggested that the main mechanism of local elevation of amino acids level by the insects with phloem-feeding behaviour, which include *P. maritimus*, is based on redirection of nutrient flow to the spot of feeding from other plant tissues (Blackmer and Byrne 1999; Sempruch et al. 2011). However, in case of *T. urtice,* which feeds from mesophyll cells, such manipulation appears to be rather less important. Earlier studies also showed that infestation of some species of host plants by the aphids affected activity of the enzymes involved in amino acids metabolism such as: glutamine synthetase (GS), glutamate synthase (GOGAT), aspartate aminotransferase (AspAT), alanine aminotransferase (AlAT), arginase, ornithine decarboxylase (ODC), lysine decarboxylase (LDC), tyrosine decarboxylase (TyDC) and proteases (Sempruch 2009; Zhou et al. 2015). Thus, it is possible that despite a similar direction of changes in the amino acid content, the mechanism of this interaction in both studied systems may be at last partly different. In addition, various severities of changes in the amino acid level could be associated with differences in the amount of these compounds in uninfested leaves, as their constitutive concentration in strawberry was almost 30-fold higher than in orchid. Nevertheless, an increasing ratio of the content of amino acids to phenols was observed in tissues of the studied plants, as a result of infestation by both arthropod species and this response was visibly stronger in orchid. This may suggest that these herbivores are well-adapted to overcome the constitutive chemical barriers associated with a deficiency of amino acids and an excess of phenolic compounds.

Excessive accumulation of amino acids induced by herbivores in the tissues of both studied plant species was rather poorly dependent on changes in the activity of the analysed enzymes. PAL was significantly induced in orchid infested by *P. maritimus* during the first 5 h of the infestation and suppressed at other time points. However, TAL activity was elevated only in strawberry, and the change was significant after 5 h, 1 day and 2 weeks of mite feeding. Our earlier studies (Sempruch et al. 2014) proved that some amino acid decarboxylases are also involved in orchid responses towards mealybugs infestation. It may suggest that regulation of amino acid accumulation in insect-infested plant tissues has a complex character and different biochemical agents may participate in this response. Alterations in PAL or/and TAL activity seem, however, to affect the accumulation of total phenols, because the induction of PAL activity in mealybug-infested orchid leaves during the first 5 h preceded the increase of total phenolics after 1 day and 1 week of insect feeding. In turn, higher TAL activity accompanied the elevated level of phenolic compounds in the leaves of strawberry infested by mites. Quantitative and qualitative alternations in phenols in response to insect attack are a known phenomenon, since these biomolecules play a major role in host plant resistance against herbivores (War et al. 2012). This thesis fully explains our results obtained for strawberry and *T. urticae*, because in this interaction, constant increase of total phenols in tissues of infested plants was stated, and this response was at least partly connected with induction of TAL activity. War et al. (2012) referred that an important role in plant defence against insects plays lignin, reducing insect feeding by decreasing nutritional value of leaf tissue. The increase in the rate of synthesis of this component in hybrid *Populus* (*P. deltoids* × *P. nigra*) was associated with over-expression of lignin-associated genes (*CAD/CAD*-like genes). However, in case of orchid and *P. maritimus*, such response was observed only after 24 h of feeding and disappeared after 2 weeks, which may be indicative of minor importance of this mechanism in orchid response to the feeding of the grape mealybug. A food source for this insect is phloem sap that is characterised by a low concentration of phenolic compounds. Moreover,

decrease of the total phenols in orchid leaves during the first 5 h and after 2 weeks of infestation by the grape mealybug may results from other transformation of these compounds, not studied in this work. For example, oxidation of phenols catalysed by polyphenol oxidase (PPO) and peroxidise (POD) is a known mechanism of plant defense against herbivorous insects (Chrzanowski et al. 2003). Products of these transformation, named quinones, are able to inhibit the protein digestion in hesrbivores. Quinones also exhibits direct toxity to insects (War et al. 2012). In addition, various patterns of the changes in the activity of the enzymes in the tissues of strawberry attacked by mites and orchid infested by scale insects may suggest potential differences in changes in the content of individual phenolic compounds. Chrzanowski and Leszczyński (2008) showed that cereal leaf beetle brought highest changes in the gallic, chlorogenic, syringic and ferulic acids in winter triticale; therefore, grain aphid feeding resulted in the increase of gallic, chlorogenic, vanilic, caffeic, syringic and salicylic acids in the same host plant species. However, this phenomenon, in the case of interactions described here, requires further studies. The present data confirm the results of earlier studies, showing that changes in the rate of aromatic amino acid deamination affect the content of simple phenols, polyphenols, *o*-dihydroxyphenols and phenolic acids in plant tissues (Ju et al. 1995; Howles et al. 1996; Tovar et al. 2002) and that aphid feeding modifies TAL and PAL activities (Ciepiela and Niraz 1993; Li et al. 2008; Han et al. 2009). PAL was also found to be activated in cucumber seedlings infested by *B. tabaci* and an increase of its activity was dependent on the duration of infestation (Zhang et al. 2008). The current study proved that other piercing-sucking arthropods, such as the grape mealybug and the two-spotted spider mite also affected the activity of these enzymes. It is possible that the mechanism of the regulation has a systemic character, because the increase in protein concentration was observed in the mealybug-infested orchid, but not in the mite-infested strawberry. However, such a phenomenon needs further research focused on the molecular regulation of biosynthesis of pure PAL protein. In the grape berry, the accumulation of PAL mRNA, de novo synthesis of enzymatic protein, an increase of its activity and a higher phenolic content were shown to be induced by SA (Wen et al. 2005, 2008). On the other hand, PAL was shown to be involved in SA biosynthesis. For example, the high specific activity of this enzyme in barley increased the content of free and conjugated forms of SA, and these changes, in turn, enhanced plant resistance against *S. graminum.* In addition, MeJA induced the accumulation of PAL mRNA and the activity of this enzyme (Manisha et al. 1998).

In summary, we can conclude that the studied herbivore species influenced the free amino acid accumulation, total phenol content, PAL and TAL activities as well as protein concentration in enzymatic extracts within their host plants. Differences in direction, intensity and/or duration of these responses observed between both studied variants suggest that the mode of biochemical plant defence is dependent on the species of plant and herbivore. It is possible that specific plant responses based on primary and secondary metabolism and arthropod feeding behaviour are important factors regulating these mechanisms.

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References

Bensoussan N, Santamaria ME, Zhurov V, Diaz I, Grbić M, Grbić V (2016) Plant-herbivore interaction: dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. Fron Plant Sci 7. Article 1105

Bernards MA, Båstrup-Spohr L (2008) Phenylpropanoid metabolism induced by wounding and insect herbivory. In: Schaller A (ed) Induced plant resistance to herbivory. Springer, New York, pp 189–211

Blackmer JL, Byrne DN (1999) The effect of *Bemisia tabaci* on amino acid balance in *Cucumis melo*. Ent Exp Appl 93:315–319

Bondada BR, Oosterhuis DM, Tugwell NP, Kim KS (1995) Physiological and cytological studies of two-spotted spider mite, *Tetranychus urticae* K (Acari, Tetranychidae) injury in cotton. Southwest Entomol 20:171–180

Calatayud PA, Le Rü B (2006) Cassava-Mealybug Interaction. Institut de Recherché Pour le Development

Calatayud PA, Rahbe Y, Tjallingii WF, Tertuliano M, Le Rü B (1994) Electrically recorded feeding behaviour of cassava mealybug on host and non-host plants. Entomol Exp Appl 72:219–232

Caretto S, Linsalata V, Colella G, Mita G, Lattanzio V (2015) Carbon fluxes between primary metabolism phenolic pathway in plant tissues under stress. Int J Mol Sci 16:26378–26394. doi:10.3390/ijms161125967

Carillo E, Rubials D, Castillejo MA (2013) Proteomic analysis of pea (*Pisum sativum* L.) response during compatible and incompatible interactions with the pea aphid (*Acyrthosiphon pisum* H.). Plant Mol Biol. doi:10.1007/s11105-013-0677-x

Chaman ME, Copaja SV, Argandoña VH (2003) Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) activity, and resistance of barley to aphid infestation. J Agric Food Chem 51:2227–2231

Chrzanowski G, Leszczyński B (2008) Induced accumulation of phenolic acids in winter triticale (*Triticosecale* Wittm.) under insects feeding. Herba Pol 54:33–39

Chrzanowski G, Ciepiela AP, Sprawka I, Sempruch C, Sytykiewicz H, Czerniewicz P (2003) Activity of polyphenoloxidase in the ears of spring wheat and triticale infested by grain aphid (*Sitobion avenae*/F./). EJPAU, Ser Biol 6(2)

Chrzanowski G, Leszczyński B, Czerniewicz P, Sytykiewicz H, Matok H, Krzyżanowski R, Sempruch C (2012) Effect of phenolic acids from black currant, sour cherry and walnut on grain aphid (*Sitobion avenae* F.) development. Crop Prot 35:71–77

Cid M, Fereres A (2010) Characterization of the probing and feeding behavior of *Planococcus citri* (Hemiptera: Pseudococcidae) on grapevine source. Ann Entomol Soc Am 103(3):404–417. doi:10.1603/AN09079

Ciepiela AP, Niraz S (1993) Changes in activity of L-phenylalanine ammonia-lyase (PAL) and L-tyrosine ammonia-lyase (TAL) in flag leaves of winter wheat induced by feeding of grain aphid. Zesz Nauk WSRP w Sidlcach, ser Nauki Przyr 34:131–141 **(in Polish)**

Dixon RA, Achnine L, Kota P, Liu CJ, Reddy MSS, Wang LJ (2002) The phenylpropanoid pathway and plant defence a genomics perspective. Mol Plant Pathol 3:371–390

Egas M, Norde DJ, Sabelis MW (2003) Adaptive learning in arthropods: spider mites learn to distinguish food quality. Exp Appl Acarol 30:233–247

Eleftherianos I, Vamvatsicos P, Ward D, Gravanis F (2005) Changes in the levels of plant total phenols and free amino acids induced by two cereal aphids and effects on aphid fecundity. J Appl Entomol 130:15–19

Han Y, Wang Y, Bi JL, Yang XQ, Huang Y, Zhao X, Hu Y, Cai QN (2009) Constitutive and induced activities of defense-related enzymes in aphid-resistant and aphid-susceptible cultivars of wheat. J Chem Ecol 35:176–182

Howles PA, Sewalt VJH, Paiva NL, Elkind Y, Bate NJ, Lamb C, Dixon RA (1996) Overexpression of L-phenylalanine ammonia-lyase in transgenic tobacco plants reveals control points for flux into phenypropanoid biosynthesis. Plant Physiol 112: 1617–1624

Ju ZG, Yuan YB, Lieu CL, Xin SH (1995) Relationships among phenylalanine ammonia-lyase activity, simple phenol concentrations and anthocyanin accumulation in apple. Sci Hortic 61:215–226

Lattanzio V, Lattanzio VMT, Cardinali A (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperto F (ed) Phytochemistry: advances in research 2006. Research Signpost, Trivandrum, pp 23–67

Li JB, Fang LP, Zhang YN, Yang WJ, Guo Q, Li L, Bi CL, Yang RZ (2008) The relationship between the resistance of cotton against cotton aphid, *Aphis gossypii*, and the activity of phenylalanine ammonia-lyase. Chin Bull Entomol 2008-03

Lowry JOH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:256–277

Manisha S, Goro T, Keiichi G, Takashi J, Makoto S, Nobuaki H, Mitsuo O (1998) Effects of methyl jasmonate and elicitor on the activation of phenylalanine ammonia-lyase and the accumulation of scopoletin and scopolin in tobacco cell cultures. Plant Sci 132:13–19

Robyt JF, White JW (1987) Qualitative and quantitative methods for determining biological molecules. In: Biological techniques theory and practice. Brook/Cole Publishing Company, Monterey, pp 213–252

Rösler J, Krekel F, Amrhein N, Schmidt J (1997) Maize phenylalanine ammonia-lyase has tyrosine ammonia-lyase activity. Plant Physiol 113:175–179

Sandström JP, Telang A, Moran NA (2000) Nutritional enhancement of host plant by aphids: a comparison of three aphid species on grasses. J Insect Physiol 46:33–40

Schmidt L, Schurr U, Röse USR (2009) Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves. Plant, Cell Environ 32:893–903. doi:10.1111/j.1365-3040.2009.01969.x

Sempruch C (2009) Participation of the amino acids in host plants-aphids interactions. Post Nauk Rol 338:89–101 **(in Polish)**

Sempruch C, Michalak A, Leszczyński B (2011) Effect of grain aphid (*Sitobion avenae* Fabricius, 1775) feeding on content of free amino acids within selected parts of triticale plants. Aphids Other Hemipterous Insects 17:139–145

Sempruch C, Leszczyński B, Chrzanowski G, Filipczuk A, Czerniewicz P, Wolska K (2012) Activity of aspartate aminotransferase and alanine aminotransferase within winter triticale seedlings infested by grain aphid (*Sitobion avenae* F.). J Plant Prot Res 52:364–367

Sempruch C, Golan K, Górska-Drabik E, Kmieć K, Kot I, Łagowska B (2014) Effect of mealybugs infestation on activity of amino acid decarboxylases in orchid leaves. J Plant Int 9:825–831. doi:10.1080/17429145.2014.954014

Simmonds MSJ (2001) Importance of flavonoids in insect–plant interactions: feeding and oviposition. Phytochem 56:245–252

Simmonds MSJ (2003) Flavonoid–insect interactions: recent advances in our knowledge. Pchytochem 64:21–30

StatSoft Inc (2015) Data Analysis Software System version 12. http://www.statsoft.com

Stratil P, Klejdus B, Kuban V (2006) Determination of total content of phenolic compounds and their antioxidant activity in vegetables—evaluation of spectrophotometric methods. J Agric Food Chem 54:607–616

Tovar MJ, Romero MP, Girona J, Motilv MJ (2002) L-phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. cv. Arbequina) fruit grown under different irygation regimes. J Sci Food Agric. doi:10.1002/jsfa.1122

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of plant defense against insect herbivores. Plan Signal Behav 7:1306–1320

Watts KT, Mijts BN, Lee PC, Manning AJ, Schmidt-Dannert C (2006) Discovery of a substrate selectivity switch in tyrosine ammonia-lyase, a member of the aromatic amino acid lyase family. Chem Biol 13:1317–1326

Wen PF, Chen JY, Kong WF, Pan QH, Wan SB, Huang WD (2005) Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. Plant Sci 169:928–934

Wen PF, Chen JY, Wan CB, Kong WF, Zhang P, Wang W, Zhan JC, Pan QH, Huang WD (2008) Salicylic acid activates phenylalanine ammonia-lyase in grape berry in response to high temperature stress. Plant Growth Regul 55:1–10

Zhang SZ, Zhang F, Hua BZ (2008) Enhancement of phenylalanine ammonia lyase, polyphenoloxidase, and peroxidase in cucumber seedlings by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) infestation. Agric Sci China 7:82–87

Zhou S, Lou YR, Tzin V, Jander G (2015) Alternation of plant primary metabolism in response to insect herbivory. Plant Physiol 169:1488–1498