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Effects of *Sitobion avenae* (Fabricius 1775) versus *Oulema melanopus* (Linnaeus 1758) and *Leptinotarsa decemlineata* (Say 1824) on selected amino acid decarboxylases activity within host plant tissues

Cezary Sempruch[1], Bogumił Leszczyński[1], Marcin Protasiuk[1], Krystyna Zarzecka[2]

[1]Department of Biochemistry and Molecular Biology, Siedlce University of Natural Sciences and Humanities
Prusa 12, 08-110 Siedlce, Poland
[2]Department of Plant Cultivation, Siedlce University of Natural Sciences and Humanities,
Prusa 14, 08-110 Siedlce, Poland
e-mail: cezar@uph.edu.pl

ABSTRACT

The work was a comparison of changes in the activity of two key enzymes of polyamine biosynthesis – ornithine decarboxylase (ODC) and lysine decarboxylase (LDC) caused by the feeding of *Sitobion avenae* (Fabricius 1775), *Oulema melanopus* (Linnaeus 1758) and *Leptinotarsa decemlineata* (Say 1824) within tissues of the triticale and potato, respectively. Obtained results showed that attacks by all tested insect species reduced ODC activity after two days of feeding and induced it later one. In case of LDC, the increase was observed during all study periods for *L. decemlineata* and after two days and one week for *S. avenae*. Feeding by *O. melanopus* resulted in a constant decrease of LDC activity during the performed observations.

KEY WORDS: *Sitobion avenae*, *Oulema melanopus*, *Leptinotarsa decemlineata*, amino acid decarboxylases, insect-plant interactions

INTRODUCTION

The pattern of plant responses towards attack by herbivorous insects is dependent on their feeding styles. Chewing insects damage plant tissues, inducing responses to wounding, but piercing-sucking insects (i. e. Hemiptera) cause

rather slight wounding and induce other defence mechanisms (Ferry *et al.,* 2004). According to Groppa & Benavides (2008), an increase in content of free polyamines and their hydroxycinnamic acid derivatives (HCAAs), as well as induction of key enzymes for their biosynthesis may result as an effect of wounding of the infested plant tissues. Mechanical wounding of *Brassica napus* (L.) leaves elevated arginine decarboxylase (ADC) activity and level of putrescine (Cowley & Walters, 2005). Tryptamine or tyramine and such HCAAs as *p*-coumaroyltyramine and feruloyltyramine accumulated within tissues of wounded transgenic tobacco lines expressing tryptophan decarboxylase (TDC) and tyrosine decarboxylase (TYDC), respectively (Guillet & de Luca, 2005). Tebayashi *et al.* (2007) stated that ovipositional deterrence of the leaf miner *Liriomyza trifolii* (Burgess in Comstock 1880) acquired by *Capsicum annuum* L. after jasmonic acid (JA) treatment resulted from caffeoylputrescine accumulation. In addition, synthetic *p*-coumaroylputrescine (CP) was characterized by similar activity. Silencing the NaMYB8 transcription factor that controls biosynthesis of CP and dicaffeoylspermidine in *Nicotiana attenuata* (Torr. ex Wats.) allowed better performance for *Manduca sexta* (Linnaeus 1763) and *Spodoptera littoralis* (Boisduval 1833) (Bassard *et al.,* 2010).

Our earlier studies showed that grain aphid feeding influenced the activity of key enzymes of polyamines and tyramine biosynthesis within triticale tissues depending on the plant genotype, aphid density and duration of infestation (Sempruch *et al.,* 2008; 2009; 2010b). Moreover, various patterns of changes in polyamines and tryptamine content in triticale cultivars (cvs.) with different susceptibility to aphids was observed in response to *Rhopalosiphum padi* (Linnaeus 1758) attack (Sempruch *et al.,* 2012). After two weeks of infestation, the putrescine level decreased and cadaverine, spermidine and tryptamine was enhanced within tissues of more resistant triticale cv., while in case of susceptible ones, the amine content was reduced. However, the mechanism of these interactions is still not clear. We hypothesise that the changes in amine content and activity of the key enzymes of their biosynthesis within triticale tissues may have at last partially resulted from wounding of plant tissues during puncturing of epidermis, mesophyll, and parenchyma cells. Thus, the presented work is a report on changes in the activity of two key enzymes of polyamine biosynthesis – ornithine decarboxylase (EC 4.1.1.17) and lysine decarboxylase (EC 4.1.1.18) caused by the sucking-piercing cereal aphid, versus such chewing species as the cereal leaf beetle and the Colorado potato beetle within tissues of the triticale and potato respectively.

MATERIAL AND METHODS

The field experiments were carried out at the Agricultural Experimental Station in Zawady near Siedlce (Central – Eastern Poland) with the use of the winter triticale cv. Witon and the potato cv. Żagiel.

Fifteen ears of the aphid-free triticale plants were infested with at last three wingless females of *S. avenae* each in the third decade of June on an experimental field (2.0 x 9.0 m) and marked with red ribbon. The same number of control plants (without aphids) was selected simultaneously and marked with white ribbon. The aphid number on infested plants was counted after two days, one week and two weeks from the beginning of the experiment. Five infested and five control ears were collected for chemical analysis at every observation term. The tests using the cereal leaf beetle were conducted on the same experimental fields, however, 30 flag leaves were infested by one larva each and 30 control plants were selected. The infested and control plants were also marked with red and white ribbon respectively. The pest number was counted on 10 leaves, which were next collected at particular terms of the experiment (two days, one week and two weeks). In the case of the Colorado potato beetle, three potato plants cultivated on the 5.55 x 16.88 m experimental fields were infested by at last five *L. decemlineata* individuals, both larvae and adults, each in the second decade of June. The same number of control plants (without the beetles) was simultaneously selected and both the control as well as tested plants were marked with a ribbon. The insects on infested plants were counted and the collection of plant material (infested leaves from one tested and one control plant at each term of the experiment) were conduced similarly to tests on the aphids and the cereal leaf beetle.

ODC and LDC were extracted from fresh plant material (Sempruch *et al.,* 2008; 2010b) and their activities were assayed spectrophotometrically according to Ngo *et al*. (1987) and Phan *et al.* (1982), respectively. Enzyme activity was expressed in mm of putrescine (in case of ODC) or cadaverine (LDC) generated during 1 hour by 1 mg of enzymatic protein, estimated according to Lowry *et al.* (1951).

All analyses were conducted in three independent replications. The obtained results were subjected to Kruskal-Wallis' test as a non-parametric alternative to ANOVA after rejection of data normality with the chi-square test. The U-test was used to analyze differences in the enzyme activities within plants infested by aphids and the control ones. Spearman's correlation on rank (r) was calculated for determining the interactions between the insect density and duration of infestation, and changes in enzyme activities. The acceptance level of statistical significance was $p \leq 0.05$. The presented results are an arithmetic means with standard errors. All statistical analyses were conducted using Statistica Software for Windows version 9.0 (StatSoft Inc. 2010).

RESULTS

The density of *S. avenae* and *L. decemlineata* on their host plants during collection of plants for chemical analysis is presented in Table 1. The aphid number was not statistically different at particular terms of the experiment, instead, the

Colorado potato beetle density was characterised by a decreasing tendency. The number of *O. melanopus* was constant during the experiment at the level of one individual per flag leaf.

Table 1. Density of pest population on the host plants during the experiment.

Insect per place of infestation	Duration of infestation			Statistics
	Two days $\bar{X} \pm SE$	One week $\bar{X} \pm SE$	Two weeks $\bar{X} \pm SE$	
	Insect den sity			
S. avenae per ear	4.02 ± 0.38	5.00 ± 0.32	4.20 ± 0.38	$H_{(2, N = 15)} = 2.94$ $p = 0.23$
O. melanopus per flag leaf	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	$H_{(2, N = 15)} = 0.00$ $p = 1.00$
L. decemlineata per potato plant	10.00 ± 0.58	7.00 ± 0.58	6.50 ± 0.29	$H_{(2, N = 9)} = 5.80$ $p = 5.80 \times 10^{-2}$

Kruskal-Wallis' test: comparison of the insect density at particular terms of plant material collection.

Statistical analysis showed significant differences in ODC ($H_{(17, N=54)} = 48{,}17$; $p = 1.00 \times 10^{-4}$) and LDC ($H_{(17, N=54)} = 51{,}05$; $p < 1.00 \times 10^{-14}$) activity within tissues of plants infested by tested insects and control ones. It was shown that ODC activity decreased during the early phase (after two days) of the experiment and increased during the next terms (one and two weeks) within tissues of all analyzed host plants infested by the insects (Tab. 2). However, these changes were statistically confirmed at one and two weeks for *S. avenae* and at all studied terms for *O. melanopus* and *L. decemlineata*.

Table 2. Effect of insect feeding on ornithine decarboxylase activity within tissues of host plants during the experiment.

Insect	Plant	Duration of infestation		
		Two days $\bar{X} \pm SE$	One week $\bar{X} \pm SE$	Two weeks $\bar{X} \pm SE$
		ODC activity (μM putrescine × mg-1 protein × hour-1)		
S. avenae	control	75.35 ± 1.58	73.64 ± 3.49	70.22 ± 0.81
	infested	70.63 ± 8.78	92.92 ± 4.46	86.36 ± 2.29
	U3; p	1.00; 0.13	$0.00; 4.95 \times 10^{-2}$	$0.00; 4.95 \times 10^{-2}$
O. melanopus	control	68.87 ± 2.02	39.14 ± 2.60	49.23 ± 1.68
	infested	57.03 ± 3.99	49.58 ± 1.76	63.08 ± 1.84
	U3; p	$0.00; 4.95 \times 10^{-2}$	$0.00; 4.95 \times 10^{-2}$	$0.00; 4.95 \times 10^{-2}$

Insect	Plant	Duration of infestation		
		Two days $\bar{X} \pm SE$	One week $\bar{X} \pm SE$	Two weeks $\bar{X} \pm SE$
		ODC activity (µM putrescine × mg-1 protein × hour-1)		
L. decemlineata	control	93.54 ± 1.59	91.35 ± 3.79	80.61 ± 7.66
	infested	64.09 ± 5.96	109.72 ± 2.42	109.75 ± 4.25
	U3; p	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}

Mann-Whitney's U-test; comparison of ODC activity within tissues of plants infested by insects and control ones.

The aphid infestation caused an increase of LDC activity within triticale tissues after two days and one week and a decrease after two weeks; instead, the cereal leaf beetle feeding caused a reduction in enzyme activity during the whole period of infestation (Tab. 3). Potato leaves infested by the Colorado potato beetle were characterized by the opposite, a constant increase of LDC. All changes in LDC activity within host plants infested by the insects were statistically confirmed.

An analysis of correlations proved significant positive interaction between the number of Colorado potato beetles and LDC activity within potato leaves ($r_9 = 0.77$, $p = 1.46 \times 10^{-2}$). Moreover, duration of infestation was negatively correlated with LDC activity within triticale leaves infested by *O. melanopus* ($r_9 = -0.94$, $p = 9.60 \times 10^{-5}$) and positively with ODC activity within potato leaves attacked by *L. decemlineata* ($r_9 = 0.74$, $p = 2.32 \times 10^{-2}$).

Table 3. Effect of the insect feeding on lysine decarboxylase activity during the experiment.

Insect	Plant	Duration of infestation		
		Two days $\bar{X} \pm SE$	One week $\bar{X} \pm SE$	Two weeks $\bar{X} \pm SE$
		LDC activity (µM cadaverine × mg-1 protein × hour-1)		
S. avenae	control	48.12 ± 2.04	61.90 ± 3.00	41.14 ± 1.34
	infested	63.36 ± 2.58	74.47 ± 3.66	33.85 ± 0.98
	U3; p	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}
O. melanopus	control	51.14 ± 1.54	38.30 ± 3.01	32.77 ± 3.35
	infested	37.56 ± 2.69	24.74 ± 1.47	18.15 ± 2.04
	U3; p	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}
L. decemlineata	control	15.44±1.36	15.58±1.19	10.68±2.05
	infested	29.24±2.56	23.72±2.11	24.25±2.16
	U3; p	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}	0.00; 4.95×10^{-2}

Mann-Whitney's U-test; comparison of LDC activity within tissues of plants infested by insects and control ones.

DISCUSSION

In the case of all tested insect species, the population density did not show an increasing tendency during the experimental period. This is particularly evident in the case of *S. avenae,* since summer parthenogenetic generations of the aphids reveal high reproductive potential. However, the rate of fecundity of cereal aphids may be reduced by unfavourable climatic factors (Leszczyński, 1990). It is possible that this phenomenon may be important in the case of our studies, since the experimental period (late spring-early summer of 2011) was characterised by high rainfall and relatively low air temperature (frequently made observations). On the other hand, the reduction in the development of the insect population may be an effect of plant chemical responses.

Obtained results showed similar patterns of changes in ODC activity not dependent on the insect mouth apparatus as well as host plant species. Thus, it is possible that the mechanism of interaction was also characterised by some similarities. Earlier studies proved that mechanical wounding induced an increase in polyamine content and the activity of key enzymes for their biosynthesis. For example, within wounded pulp tissue of green banana fruit, putrescine accumulated and ADC activity was induced, and the response was connected with ethylene evolution (Yoza *et al*., 1996). According to Cowley & Walters (2005), herbivorous insects induced plant defence response mainly *via* wounding of host tissues. Such a mechanism particularly characterised chewing insects, however, during probing behaviour, the aphid stylets transiently puncture epidermis, mesophyll, and parenchyma, inducing plant response to the infestation (Goggin, 2007). Thus, it is possible that the non-specific response based on the changes in ODC activity observed here was caused by wounding of plant tissues during insect feeding.

On the other hand, LDC activity was modified differentially depending on the insect and host plant. Aphid feeding resulted in the induction of the enzyme during the first week of infestation and in later reduction after two weeks. In the case of both chewing species, constant tendencies of changes were observed, although in opposite directions. Aphids usually penetrate plant tissues *via* intercellular route, and their influence on hosts result from assimilate uptake and injection of salivary secretions into phloem and xylem (Saheed *et al.,* 2007). The compounds secreted by aphids may translocate long distances within plant tissues, inducing systemic responses. Zhao *et al.* (2009) observed that grain aphid feeding on wheat increased activities of key enzymes regulated by both jasmonic acid (JA) and salicylic acid (SA) signalling pathways as well as relative transcript levels of their defense genes. According to Ferry *et al.* (2004), plant responses caused by sap-feeding insects (i.e. Hemiptera) have been shown to be similar to pathogen attacks connected with changes in polyamines accumulation and metabolism (Walters, 2003). On the other hand, transgenic tomato lines were characterized by an increase in glutamine, glutamate, asparagine, citrate, malate and fumarate

levels and a decrease in aspartate, threonine, valine, glucose and sucrose content developed by transformation from the yeast SAMDC gene (Mattoo *et al.,* 2010). This gene is related to the expression of S-adenosylmethionine decarboxylase (key enzyme of spermidine and spermine biosynthesis). Thus, it is suggested that polyamines may modify the nutritive value of plants for herbivorous insects.

However, there is little data on participation of the amines in interactions between herbivorous insects and their host plants. According to Sempruch *et al.* (2008; 2009; 2010b; 2012), the feeding of cereal aphids caused changes in accumulation of the polyamines and tryptamine within triticale tissues, and patterns of these changes are at least partially connected with regulation of some amino acid decarboxylases activity. Sempruch *et al.* (2010a) observed that placing of triticale seedlings in 0.01% solution of agmatine and cadaverine, and in 0.1% solutions of agmatine, cadaverine, putrescine, spermidine and spermine disturbed food assimilation and survival of *S. avenae* wingless females. Moreover, 1 mM and 10 mM concentrations of putrescine, cadaverine, spermidine, spermine, agmatine and tyramine influenced the feeding behaviour of *R. padi*, especially reducing activities connected with active and passive food ingestion (Sempruch *et al.*, unpublished data).

In conclusion, we can state that feeding of all tested insects affected ODC and LDC activity within tissues of the triticale and the potato and that the response was dependent on pest density in the case of the Colorado potato beetle as well as the duration of infestation for both chewing species. Similar patterns of changes in ODC activity suggest non specific response induced by wounding of plant tissues, while the LDC activity varied specifically in relation to both insect and plant species.

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Porównanie wpływu żerowania *Sitobion avenae* (Fabricius 1775), *Oulema melanopus* (Linnaeus 1758) i *Leptinotarsa decemlineata* (Say 1824) na aktywność wybranych dekarboksylaz w tkankach roślin żywicielskich

STRESZCZENIE

Celem podjętych badań było porównanie wpływu żerowania *Sitobion avenae* (Fabricius 1775) oraz *Oulema melanopus* (Linnaeus 1758) i *Leptinotarsa decemlineata* (Say, 1824) na aktywność dwóch kluczowych enzymów biosyntezy poliamin – dekarboksylazy ornityny (ODC) i dekarboksylazy lizyny (LDC) w tkankach pszenżyta ozimego i ziemniaka. Otrzymane wyniki wykazały, że wszystkie badane owady wywoływały spadek aktywności ODC po dwóch dniach żerowania oraz wzrost po jednym i dwóch tygodniach trwania eksperymentu. W przypadku LDC wzrost aktywności stwierdzono dla *L. decemlineata* we wszystkich rozpatrywanych terminach oraz dla *S. avenae* po dwóch dniach i jednym tygodniu żerowania. Zmiany w aktywności LDC wywołane żerowaniem *O. melanopus* wykazywały natomiast tendencje spadkowe.