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Effect of *Sitobion avenae* (Fabricius, 1775) feeding on the free amino acid content within selected parts of triticale plants

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ABSTRACT

The influence of grain aphid feeding on the content of free amino acids within ear, shoot and root tissues of winter triticale was studied. Obtained results showed that during the peak of density of *Sitobion avenae* (Fabricius, 1775) the population level of the amino acids was elevated within infested ears. In shoots of the aphid-infested plants the amino acid content was reduced, while in the case of root tissues only the level of nonessential amino acids was decreased and the level of the essential ones was not statistically different from that of the control group. Obtained results are discussed below with reference to biochemical mechanisms of triticale response to the aphid infestation.

KEY WORDS: grain aphid, *Sitobion avenae*, triticale, free amino acid

INTRODUCTION

Phloem sap as a main source of nutrients for many of hemipterous species (i.e. aphids) is characterised by 0.8 – 4.5% amino acid content (Sändstrom & Moran, 1999). It is not an efficient amount, thus sucking-piercing insects collect high quantities of phloem sap and induce plant metabolism to more intensive biosynthesis of biomolecules necessary to their growth and development (Doorsher, 1988). Therefore, the content of free amino acids increases within tissues of different plant species infested by *Elatobium abietinum* (Walker, 1849), *Aphis fabae* (Scopoli, 1763) and *Bemisia tabaci* (Gennadius, 1889) (Blackmer & Byrne, 1999).

Changes in amino acids content may be systemic because of a possible redirection of nutrients flow to the spot of aphid feeding from other plant tissues (Blackmer & Byrne, 1999). On the other hand, plants activate defence mechanisms consistently working on blocking the phloem sap flow towards the spot of aphid attack through triggering sieve tubes by the calose or lectins connected with the local increase of Ca^{2+} concentration (De Wet & Botha, 2007). According to Sändstrom *et al*. (2000) cereal aphids *Schizaphis graminum* (Rondani, 1852) and *Diuraphis noxia* (Kurdjumov, 1913) caused strong increase of amino acids (especially essential) within phloem sap of wheat and barley and these changes affected various parts of infested leaves. However, there has been no available information so far on how aphids influence the amino acid content within cereal tissues distant from the spot of attack, such as vegetative aerial parts and roots, following the infestation of ears. The present paper focuses on the influence of grain aphid - *Sitobion avenae* (Fabricius, 1775) feeding on the ears of winter triticale on free amino acid content within ear, shoot and root tissues.

MATERIAL AND METHODS

Winter triticale (*Triticosecale*, Wittm. ex A.Camus) Lamberto cv. was utilized in the experiments. The abundance of *S. avenae* on the triticale and its influence on amino acid content were estimated under field conditions at the Agricultural Experimental Station in Zawady near Siedlce (central-eastern Poland).

Estimation of the abundance of the grain aphid

The grain aphid population density on the studied triticale was estimated according to the method described by Wratten *et al.* (1979) and Lykouressis (1984). The observations were carried out since the arrival of aphids on the triticale, until their disappearance (G.S.52 – 88; Tottman & Broad, 1987), at one-week intervals. The number of aphids on 25 blades and the percentage of infested plants were noted. The values of estimated parameters were calculated as a means of three replicates in three various experimental fields (1.5 x 3m).

Influence of the grain aphid feeding on amino acid content within triticale tissues

Ears of 25 plants were artificially infested by five wingless females of *S. avenae* at early milk-ripestage (G.S.73) and isolated with cheese cloth isolators. Control ears (without aphids) were prepared in a similar way. The aphid number was maintained at the same level on the infested ears during the experiment. Infested plants and control plants were collected after one week, divided into ears, shoots and roots and the amino acid assay was immediately performed on them.

Amino acid assay

Free amino acids were extracted from plant material freeze-dried with 80% ethanol and purified on the ion-exchange column (Amberlit IR - 120 [H^+]) according to Lasheen *et al.* (1970) method. The samples were analyzed with the aid of the amino acids analyser T-339 (Microtechna, Praha).

Statistics

Kruskal-Wallis analysis of variance by ranks was applied after the rejection of data normality with chi-square test. Differences in the amino acid concentrations within plants infested by the aphids and control ones were confirmed by Mann-Whitney's U-test. The acceptance level of statistical significance was $P \leq 0.05$. The presented data are an arithmetic means with standard errors. All statistical analyses were conducted with Statistica software for Windows 9.0 (StatSoft Inc., 2010).

RESULTS

Dynamics of a grain aphid population

Obtained results showed differences in density of *S. avenae* population during particular growth stages of the triticale ($H_{(5, N = 18)} = 15.74$ at $p = 7.60 \times 10^{-3}$ for aphid number and $H_{(5, N = 18)} = 13.49$ at $p = 1.92 \times 10^{-2}$ for the percentage of infested plants). The first appearance of the grain aphid occurred during heading (G.S.55; Tottman & Broad, 1987) (Fig. 1). The aphid density increased at later growth stages reaching peak density at early milk-ripe stage (G.S.73) in the case of a number of individuals per blade and at the late milk-ripe stage (G.S.78) for the percentage of infested plants. Then the aphid population was strongly reduced at the early dough-ripe stage (G.S.82).

Changes in amino acid content within triticale infested with a grain aphid

The obtained results proved that grain aphid feeding caused statistically significant changes in the free amino acid content within the analyzed parts of triticale plants ($H_{(17, N = 54)} = 49.90$ at $p < 10^{-14}$). The levels of essential, nonessential and total free amino acids (the sum of essential and nonessential ones) increased within ears infested by aphids, while within shoots and root tissues they decreased

(Fig. 2). Essential amino acids within root tissues were excluded, since their content in the control plants and aphid-infested ones was not significantly different.

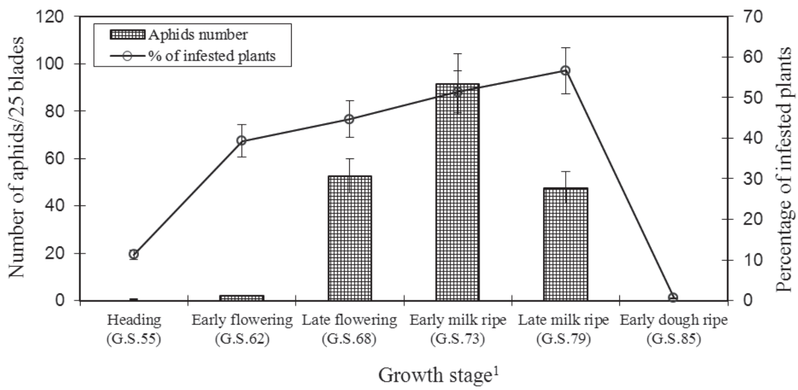

Figure 1. Population dynamics of a grain aphid on the studied winter triticale
[1]Growth stage of triticale given according to Tottman & Broad (1987) scale.

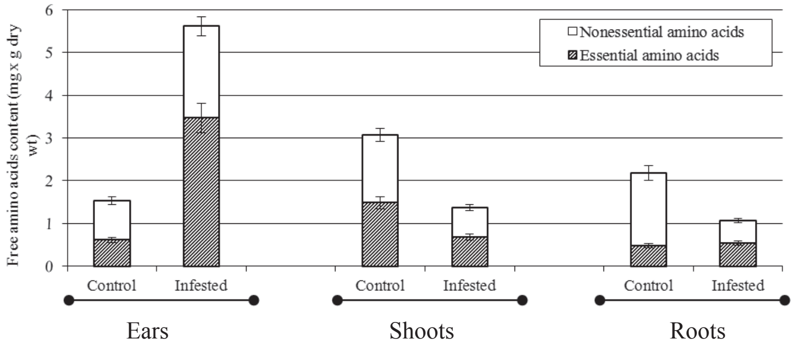

Figure 2. Influence of grain aphid feeding on the total content of free amino acids within analysed triticale organs

Differences between amino acid content within the control-group plants and aphid-infested plants were statistically confirmed ($U = 0.00$ at $p = 0.05$) with the exception of essential amino acids in root tissues ($U = 3.00$ at $p = 0.51$).

It was also proved that there were statistically relevant differences in the content of particular free amino acids within analysed parts of control-group plants and aphid-infested plants ($H_{(89, N = 270)} = 241.70$ at $p < 10^{-14}$). The feeding of grain aphids significantly raised the level of amino acids within ear tissues of Lamberto cv. with the exception of tyrosine, phenylalanine and histidine (non-significant changes) (Tab. 1). The amino acid content within the shoots of aphid-infested plants was reduced, with the exception of aspartic and glutamic acids. However, such changes were not statistically confirmed in the case of threonine, isoleucine, phenylalanine and histidine. In the case of roots of the infested triticale, the level of such amino acids as aspartic acid, serine, proline, alanine, tyrosine and lysine was lowered, while the level methionine was raised, and in the case of threonine, glutamic acid, glycine, valine, isoleucine, leucine, phenylalanine and histidine their levels were the same as in the control group, even though statistical analysis confirmed these changes only for the aspartic acid and lysine.

Table 1. Influence of grain aphid feeding on the content of particular free amino acids within analysed organs of a winter triticale

Amino acid	Plant organs					
	Ears		Shoots		Roots	
	Control	Infested	Control	Infested	Control	Infested
Asp	0.02±0.01	0.18±0.03	0.07±0.02	0.07±0.01	0.11±0.04	0.03±0.02
	[1]U = 0.00, $p = 4.95x10^{-2}$		U = 4.50, p = 1.00		U = 0.00, $p = 4.95x10^{-2}$	
Thr*	0.13±0.03	1.31±0.34	0.15±0.05	0.11±0.02	0.12±0.02	0.12±0.03
	U = 0.00, $p = 4.95x10^{-2}$		U = 2.00, p = 0.28		U = 4.50, p = 1.00	
Ser	0.11±0.05	0.60±0.10	0.37±0.06	0.18±0.08	0.25±0.05	0.20±0.04
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 1.50, p = 0.19	
Glu	0.04±0.02	0.17±0.08	0.09±0.03	0.16±0.15	0.02±0.02	0.02±0.02
	U = 0.00, $p = 4.95x10^{-2}$		U = 3.50, p = 0.66		U = 4.00, p = 0.83	
Pro	0.05±0.04	0.25±0.08	0.05±0.01	0.02±0.01	0.08±0.04	0.07±0.03
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 3.50, p = 0.66	
Gly	0.04±0.03	0.38±0.10	0.32±0.08	0.02±0.02	0.03±0.02	0.03±0.03
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 4.50, p = 1.00	
Ala	0.43±0.03	0.56±0.07	0.63±0.11	0.23±0.04	0.33±0.13	0.15±0.06
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 3.50, p = 0.66	
Val*	0.05±0.02	0.46±0.11	0.24±0.04	0.05±0.02	0.05±0.03	0.05±0.01
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 4.50, p = 1.00	
Met*	0.02±0.02	0.24±0.05	0.12±0.02	0.05±0.03	0.01±0.01	0.02±0.02
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 3.50, p = 0.66	
Ileu*	0.08±0.03	0.30±0.05	0.12±0.03	0.10±0.04	0.01±0.01	0.01±0.01
	U = 0.00, $p = 4.95x10^{-2}$		U = 2.50, p = 0.38		U = 3.50, p = 0.66	
Leu*	0.05±0.02	0.78±0.11	0.44±0.11	0.19±0.08	0.04±0.03	0.04±0.01
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 4.50, p = 1.00	
Tyr	0.01±0.01	0.01±0.01	0.06±0.01	0.01±0.01	0.05±0.03	0.03±0.02
	U = 3.50, p = 0.66		U = 0.00, $p = 4.95x10^{-2}$		U = 2.00, p = 0.28	
Phe*	0.10±0.04	0.08±0.06	0.07±0.04	0.05±0.02	0.12±0.06	0.12±0.03
	U = 3.50, p = 0.66		U = 3.00, p = 0.51		U = 4.50, p = 1.00	
His*	0.05±0.05	0.17±0.09	0.11±0.02	0.07±0.05	0.05±0.03	0.05±0.01
	U = 1.00, p = 0.13		U = 1.50, p = 0.19		U = 4.00, p = 0.83	
Lys*	0.06±0.01	0.13±0.04	0.24±0.04	0.06±0.02	0.23±0.03	0.13±0.04
	U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$		U = 0.00, $p = 4.95x10^{-2}$	

[1]Mann-Whitney U-test: comparison of amino acid content within control-group triticale plants and aphid-infested triticale plants, *amino acids essential for aphids.

DISCUSSION

It is well known that plant infestation by piercing-sucking insects causes changes in the amino acid composition within plant tissues. According to Fisher (1987), the feeding of green spruce aphid on spruce foliage caused the increase of histidine, isoleucine, leucine, phenylalanine, threonine and valine, and the decrease of aspartic acid and asparagine. Large colonies of *A. fabae* on *Vicia fabae* L. also raised amino acid levels, especially in the case of asparagine, aspartic acid, alanine, proline, phenylalanine, valine, leucine, isoleucine and methionine (Poehling, 1985). Gall-forming aphids strongly increased the asparagine content and slightly influenced lysine, glycine and serine level (Forrest, 1987). Blackmer & Byrne (1999) showed that an aggregation of *B. tabaci* nymphs on *Cucumis melo* L. significantly raised the total amino acid concentration as well as the content of 10 out of the 22 identified amino acids. Moreover, changes in amino acid content in response to aphid attack were dependent on the duration of infestation, aphid number and the cultivar of host plant (Sempruch & Ciepiela, 1998).

Data presented here fully confirmed the results of earlier experiments, since it was proved that the level of amino acids within triticale ears infested by a grain aphid at peak population density was generally raised. The accumulation of the analysed compounds in the spot of the aphid feeding was connected with a decrease in their levels within aerial vegetative parts and root tissues. It is possible that aphid influence on amino acid content within plant organs not affected directly by the attack is at least partially dependent on the redirection of nutrients flow to the spot of feeding. On the other hand, lack of significant changes in the content of amino acids within the roots of aphid-infested triticale plants may suggest other mechanisms of the analyzed interactions. For example, Sempruch *et al*. (2007) observed the induction of glutamine synthetase (GS) activity in seedlings of triticale after one week of infestation with *S. avenae*. Since GS is a key enzyme in glutamine biosynthesis, it is possible that changes in its activity may alter glutamine, glutamic acid and other free amino acids accumulation. However, this phenomenon needs more extensive studies focused on changes in amino acid content within phloem tissue and on the influence of aphid feeding at other stages of the amino acid metabolism.

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Wpływ żerowania mszycy zbożowej - *Sitobion avenae* (Fabricius, 1775) na zawartość wolnych aminokwasów w wybranych częściach roślin pszenżyta ozimego

STRESZCZENIE

Celem podjętych badań było określenie wpływu żerowania mszycy zbożowej – *Sitobion avenae* (Fabricius, 1775) w kłosach pszenżyta ozimego odmiany Lamberto na zawartość wolnych aminokwasów w kłosach, pędach i korzeniach. Otrzymane wyniki wykazały, że w okresie najwyższej liczebności populacji *S. avenae* w kłosach zaatakowanych przez mszyce następował wzrost zawartości aminokwasów. W źdźbłach i korzeniach odnotowano natomiast tendencje spadkowe w odniesieniu do większości analizowanych związków. Zaobserwowane zmiany sugerują indukcję biochemicznych mechanizmów reakcji pszenżyta na żerowanie mszycy zbożowej.