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The effect of *Tetraneura ulmi* L. galling process on the activity of amino acid decarboxylases and the content of biogenic amines in Siberian elm tissues

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Abstract

Tetraneura ulmi (L.), a member of Eriosomatinae subfamily, is one of the gall-forming aphids occurring on elms. Sap-sucking behaviour of founding mothers results in the formation of new plant organs. This study documents the changes in the content of plant biogenic amines (putrescine, cadaverine, spermidine, tryptamine, spermine and histamine) and key enzymes of their biosynthesis: lysine decarboxylase (LDC), tyrosine decarboxylase and ornithine decarboxylase (ODC) in galls and other parts of Siberian elm (*Ulmus pumila* L.) leaves during the galling process. The direction and intensity of these changes for particular amines and enzymes were dependent on the stage of gall development and part of the galling leaf. Generally, the amine content tended to increase in gall tissues during the 1st and 2nd period of the galling process and decreased in later phases. LDC and ODC activities were markedly enhanced, especially in gall tissues at the initial stage of the galling process.

Keywords: polyamines, *Ulmus pumila*, lysine decarboxylase, tyrosine decarboxylase, ornithine decarboxylase

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Introduction

Approximately 10% of the 4400 known aphid species worldwide are true gall formers. They are sap-sucking insects and have complex life cycles, with alternating sexual and parthenogenetic generations. Several types of true gall-inducing aphids, belonging to the subfamily Eriosomatinae, genus *Tetraneura*, have been described on elm trees. While most members of this genus have oriental and eastern Palearctic distribution, *Tetraneura ulmi* L. seems to be native to northwestern Europe. This species commonly occurs on various *Ulmus* species as the primary host in Europe, across Asia to eastern Siberia and has also been introduced to North America (Blackman & Eastop, 1994; Wool, 2004).

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In the spring, the first instar of *T. ulmi* fundatrix hatches from eggs laid in bark crevices of trees. After hatching, the larva migrates to young developing leaves, finds a suitable site and starts the galling process. Only that morph is capable of inducing a gall and each gall is induced by a single aphid. Within about 3–4 weeks, fundatrix develops inside the growing gall. The fully grown gall is stalked, approximately bean-shaped, green, smooth and shiny. The mature fundatrix gives birth to the offspring that develops into winged aphids during next 2–3 weeks. At the turn of May and June, the gall starts to turn yellow and is filled with migrant nymphs. The gall cracks open just above the stalk usually in the end of the first decade of June. After the gall opens, emigrants emerge and fly to the roots of numerous graminaceous plants – the secondary host, where they begin the development of new parthenogenetic colony (exules). In autumn, sexuparae (mothers) develop on the roots of secondary host, remigrate to the primary host and produce the sexual generation. Oviparous females lay

fertilized eggs (Urban, 2003; Wool, 2004; Kmieć & Kot, 2007; Suzuki *et al*., 2009). However, anholocyclic overwintering on grass roots or in ants' nests has been observed (Blackman & Eastop, 1994).

The formation of the gall is a unique group of interactions between the insect and their host plants. Galling aphids have the ability to control and manipulate the growth of the host plant. They can programme plant development, resulting in the production of novel and neoplastic formations that do not exist in the normal development of trees (El-Akkad, 2004; Wool, 2004). In entire wall of *T. ulmi* galls both hyperplasia and hypertrophy were observed. The external part of the gall's wall is formed by large cells and the part closest to the gall's chamber has smaller cells. However, all of them are storage parenchymal cells with thickened cell walls. There is only one collateral vascular bundle situated in the gall's wall (Alvarez *et al*., 2013). The galling process was shown to decrease the photosynthesis rate and chlorophyll content in case of Brazilian peppertree infested by leaf-galling psyllid, *Colophyta latiforceps* (Bruckhardt) (Prade *et al*., 2016). In consequence, plant height and biomass was also reduced. In Siberian elm infested by *T. ulmi*, at the time of gall initiation, part of lamina near each gall stalk became discoloured and corrugated (Kmieć & Kot, 2007). Urban (2003) reported that this area covered about 6% of the leaf blade. Leaves with 1–3 galls situated on the distal part of the blade are the most frequent ones (Kmieć & Kot, 2007, 2010). However, in mass outbreaks, up to 21 galls can occur on one leaf (Urban, 2003). Plant tissues involved in gall formation are characterized by cytological and morphological changes, which are beneficial for the feeding and development of the gallers. Different insect species, inducing open and close galls, are able to correct the nutritive value in the feeding place through local increase of the content of amino acids, sugars and other plant nutrients (Giron *et al*., 2016). Moreover, manipulation in cell biochemistry of *Piptadenia gonoacantha* (Mart.) by gall inducers may involve accumulation of polyphenols and indole-3-acetic acid (IAA) (Bedetti *et al*., 2014). During such a response, oxidative stress was generated by ROS (reactive oxygen species) occurring in the site of polyphenol accumulation. Bailey *et al*. (2015) observed that galling Hymenoptera and Diptera were able to synthesize IAA from tryptophan, and such an insect-derived auxin was implicated in gall formation. The galls induced by aphid *Pemphigus populi* (Courchet) on *Populus nigra* (L.) leaves contained more auxins, gibberelins and zeatin than the ungalled leaf tissue (El-Akkad, 2004). According to Agarrwal *et al*. (2016), during hypersensitive response (HR) induced by avirulent biotype of the Asian rice gall midge within tissues of resistant cv. of *Oryza sativa* (L.), carbon and nitrogen metabolism was deregulated by C/N shift. In this case, genes involved in tetrapyrrole synthesis and lipid peroxidation were upregulated, and those responsible for chlorophyll synthesis and photosynthesis were down-regulated. In addition, the level of γ-butyric acid (GABA), which may play a role of insect neurotransmitter or plant defensive compound, was elevated at the feeding site. Nabity *et al*. (2013) showed that feeding of leaf-galling phylloxera induced stomata formation as well as assimilation and importation of carbon into the gall. The expression of genes associated with glycolysis and fermentation as well as water, nutrient and mineral transport were also elevated during this response. Hassian fly (*Mayetiola destructor* Say) also disturbed C/N metabolism in wheat tissues (Zhu *et al*., 2008). The plants infested by these insects showed a 36% decrease of free carbon-containing compounds and a 46% increase of free nitrogen-containing biomolecules. Such changes were achieved by deregulation of genes involved in some central metabolic pathways, i.e., glycolysis, tricarboxylic acid cycle and amino acid biosynthesis.

The presented data clearly suggest that the biochemical response of plants against galling insects is specific for particular systems of these interactions. The participation of different primary and secondary plant metabolites as well as some phytohormones in this phenomenon has been described. However, there is little information about the importance of polyamines (PAs) in the gall formation, in spite of their well-known involvement in the formation of green islands, which are caused by cereal response to fungal infections, analogous to the galling process (Walters, 2003). In this field, Subramanyam *et al*. (2015) showed that virulent larvae of *M. destructor* induced a strong increase of PAs in wheat correlated with increasing levels of these compounds in the body of insects. This response was typical only for the susceptible cultivar and was associated with an increase in the abundance of transcripts encoding ornithine decarboxylase (ODC) (*Ta-odc*), s-adenosylmethionine synthetase (*Ta-sams*) and s-adenosylmethionine decarboxylase (*Hfr-samdc*). However, the role of these biomolecules in other interaction systems between galling insects and their hosts is not clear.

The present study focused on the changes in the content of plant biogenic amines and key enzymes of its biosynthesis in galls, and other parts of Siberian elm (*Ulmus pumila* L.) leaves involved in the galling process induced by the aphid *T. ulmi* L.

Material and methods

Plant material

U. pumila L., known as Siberian elm, is native to central Asia, eastern Siberia, Mongolia, northern China, and Korea. Due to the resistance to Dutch elm disease and phloem necrosis, Siberian elm has been used to breed resistance into elm hybrids. Furthermore, it is cultivated throughout the Americas, Asia and Europe, because it tolerates a wide range of growing conditions (Mittempergher & Santini, 2004; Seneta & Dolatowski, 2006). The plant material was collected from trees, which are part of urban green areas of Lublin, Poland (51.24°N, 22.57°S) in 2015.

Sampling

The samples were collected in three stages of gall development: 1st stage – the initial period of galling. Galls were green, about 5 mm in height with one fundatrix larva inside. In the 2nd stage, the galls were green and fully grown. There were fundatrix and her young offspring inside. The 3rd stage involved the time period just before the gall opening. Galls were yellowish with nymphs in the fourth stadium and migrants inside. One sample constituted 50–100 leaves (depending on the size of the galls) with galls collected from trees within the reach of the hand. In each sampling point, intact leaves in a similar age (located at the same site on the shoots without galling leaves) were taken as a control. Galling and intact leaves were detached from the same trees with scissors, kept in plastic bags, and brought to the laboratory within 1 h after collection. In the laboratory, the leaves were prepared for biochemical analysis. Firstly, all galls were removed from the leaves by a scalpel. Subsequently the aphids were removed from galls by a soft brush. Parts of the leaf blade with visible

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damage were cut off. There were four combinations of the experiment: (1) control (intact) leaves, (2) undamaged part of lamina (without visible discoloration and corrugation) of galling leaves, (3) damaged part of galling leaves lamina, (4) galls.

Chemical analysis

The amine assay

The amines were analysed according to the method described by Flores & Galston (1982) with minor modifications (Horbowicz *et al.*, 2011). Fresh plant tissues were homogenized in 5% (v/v) perchloric acid in an ice-cooled mortar. Plant homogenates were centrifuged, and in order to amines in the supernatant, benzoyl chloride was used for 45 min at 35°C. Benzoyl derivatives were extracted by shaking the reaction mixture with ethyl acetate. The extraction was repeated twice, and pooled acetate layers were evaporated to dryness at 40°C. The residue was dissolved in high-performance liquid chromatography (HPLC) mobile phase (acetonitrile–water, 45:55) and filtered through 0.45 µm filter.

HPLC analysis was performed with an Agilent liquid chromatograph (1200 series). The mobile phase flow rate was 1.0 $cm^3 \times min^{-1}$. Benzoylated amines were eluted isocratically at 30°C using an Eclipse XDB-C18 RP analytical column (4.6×150 mm^2, 5 µm particle size) and a C18 guard column. The benzoyl amine derivatives were detected at 245 nm using a diode array detector, and amine contents was calculated from standard curves of commercially available standards.

Enzyme assays

Fresh plant material was homogenized with 0.2 M phosphate buffer (pH 8.2) containing β-mercaptoethanol and ethylenediaminetetraacetic acid (EDTA) for ODC extraction, 0.2 M Tris–HCl buffer pH 5.6 for LDC and 0.5 M acetate buffer (pH 5.6) for tyrosine decarboxylase (TyDC). The resulting extracts were purified by filtration through two layers of cheese-cloth and centrifugation at 18,000 *g* at 5°C.

ODC activity was assayed with the use of a spectrophotometric method described by Ngo *et al.* (1987). The enzyme extract was mixed with the substrate (ornithine in phosphate buffer pH 8.2, containing pirydoxal-5-phosphate). Enzymatic reaction was conducted at 30°C for 30 min, and then it was stopped by the addition of 10% trichloroacetic acid, 4 M NaOH and 1-penthanol. Organic layer was separated and transferred into glass tubes containing 10 mM trinitrobenzosulfonic acid (TNBS) in 1-pentanol, and dimethylsulfoxide (DMSO). After vigorous shaking and centrifugation, the absorbance of the organic layer, containing TNBS-putrescine, was measured at a wavelength of 426 nm.

Determination of LDC activity was conducted according to the method of Phan *et al.* (1982). Enzyme extract was added to a 20-µM lysine solution in extractive buffer (TRIS–HCl, pH 5.6) with the addition of 0.1 µM of pirydoxal-5-phosphate (PLP). After incubation at 30°C for 20 min, the enzymatic reaction was stopped by the addition of 1 M potassium carbonate and 10 mM aqueous solutions of TNBS. TNBS-cadaverine was extracted with toluene, and the absorbance of the toluene layer was measured at 340 nm.

The method of Phan *et al.* (1983) was applied to determine the TyDC activity. Tyrosine solution (8 mM) with 0.1 µM of PLP was used as a substrate for enzymatic reaction, the mixture was incubated at 30°C for 30 min, and the reaction was stopped by 1 M potassium carbonate and 10 mM TNBS. The obtained product (TNBS-tyramine) was extracted with toluene, and absorbance of the toluene layer was measured at 340 nm.

For the activity assays of all enzymes, a UV–VIS spectrophotometer (Hewlett Packard 8453) was used. The activity of enzymes was calculated as µM of appropriate amine, generated during 1 h of enzymatic reaction by 1 mg of enzymatic protein. Protein quantity in the enzymatic extracts was assayed according to the method of Lowry *et al.* (1951).

Statistics

The biochemical assays were conducted in three independent repeats. The data were log transformed before the analysis to correct for normality and to meet homoscedasticity assumptions. The results were statistically verified using one-way ANOVA. Significant differences between means were determined by Tukey's honestly significant difference (HSD) test at the significance level of $\alpha = 0.05$. The arithmetic means with SD are presented in the table and figures. All statistical analyses were conducted using Statistica for Windows, v. 9.1 (StatSoft Inc., 2010).

Results

Changes in the activity of amino acid decarboxylases

Statistical analysis showed significant differences in the activity of the amino acid decarboxylases in Siberian elm tissues during the galling process (figs 1–3). The results of the ANOVA test in the initial period of gall formation were as follows: $F_{3,8} = 10.68$ at $P = 3.59 \times 10^{-4}$ for LDC, $F_{3,8} = 94.60$ at $P = 10^{-6}$ for TyDC, $F_{3,8} = 213.24$ at $P < 10^{-7}$ for ODC. The highest increase of the activity in this period was recorded for ODC. In the tissue of young galls and healthy parts of galling leaves, the activity of this decarboxylase was about 15-fold higher as in the control leaves. Over a fourfold increase of its activity in damaged parts of galling leaves was observed (fig. 3). The activity of TyDC in the analysed elm tissues remained at a similar level with the exception of damaged parts of galling leaves, in which the activity of this decarboxylase was significantly reduced. LDC activity was significantly increased only in the gall tissue compared with the control leaves. In healthy and damaged parts of galling leaves, only a slight decrease and increase of its activity, respectively, was observed. The results of the ANOVA test for the activity of amino acid decarboxylases in elm tissues during the period of fully developed galls were as follows: $F_{3,8} = 267.67$ at $P < 10^{-7}$ for LDC, $F_{3,8} = 100.56$ at $P = 10^{-6}$ for TyDC, $F_{3,8} = 226.15$ at $P < 10^{-7}$ for ODC. In the second stage of the galling process, when galls were green, fully grown, with fundatrix and larvae inside, a similar pattern of all the analysed decarboxylase activities was observed. A significant decrease was found of their activity in galling leaves and gall tissues compared with the control leaves. In the case of TyDC, the highest suppression of its activity (almost 22-fold) was observed in the damaged part of leaves and galls (fig. 2). In the third stage of gall development, when galls were mature just before the opening, significant differences in decarboxylase activities were detected: $F_{3,8} = 256.74$ at $P < 10^{-7}$ for LDC, $F_{3,8} = 12.75$ at $P = 2.05 \times 10^{-3}$ for TyDC, $F_{3,8} = 56.067$ at $P = 10^{-5}$ for ODC. The activity of LDC was significantly lower in galling leaves and especially in gall tissues compared

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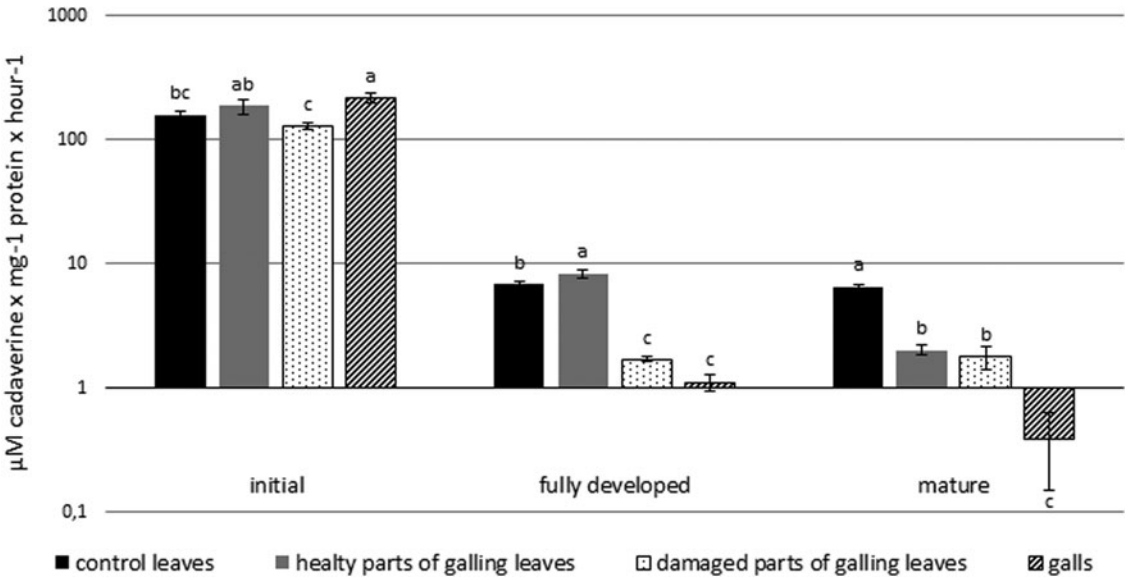

Fig. 1. The lysine decarboxylase (LDC) activity in Siberian elm tissues during galling process of *Tetraneura ulmi* L. ANOVA for: initial period of galling $F_{3,8} = 10.68$ at $P = 3.59 \times 10^{-4}$, stage of fully developed galls $F_{3,8} = 267.67$ at $P < 10^{-7}$ and stage of mature galls $F_{3,8} = 256.74$ at $P < 10^{-7}$. In each stage of gall development the bars signed by different letters indicate significant differences at $P \leq 0.05$ (Tukey's HSD test).

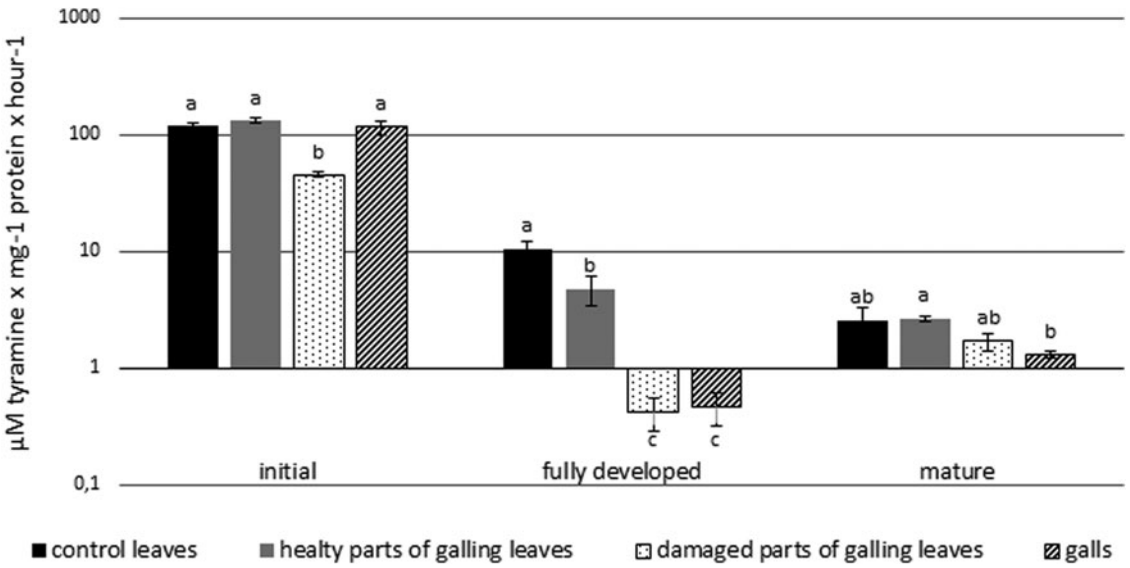

Fig. 2. The tyrosine decarboxylase (TyDC) activity in Siberian elm tissues during galling process of *Tetraneura ulmi* L. ANOVA for: initial period of galling $F_{3,8} = 94.60$ at $P = 10^{-6}$, stage of fully developed galls $F_{3,8} = 100.56$ at $P = 10^{-6}$ and stage of mature galls $F_{3,8} = 12.75$ at $P = 2.05 \times 10^{-3}$. In each stage of gall development the bars signed by different letters indicate significant differences at $P \leq 0.05$ (Tukey's HSD test).

with the control. The activity of TyDC in galling leaves and galls was similar to its activity in the control leaves. However, ODC activity in healthy part of galling leaves was higher, but it was lower in galls compared with the control leaves.

Changes in the content of biogenic amines

In the initial period of gall formation, the content of six amines was measured in Siberian elm tissues (table 1). In young galls, the level of cadaverine, spermidine and histamine was the highest; spermine was the lowest; while putrescine and tryptamine concentrations were similar to the level in other analysed tissues. The content of all amines in damaged and undamaged parts of galling leaves was similar to the control leaves, except for putrescine. In the stage of fully developed galls, putrescine was not detected in the analysed elm tissues. The highest content of the amines remained in gall tissues. In the damaged parts of galling leaves, the level of

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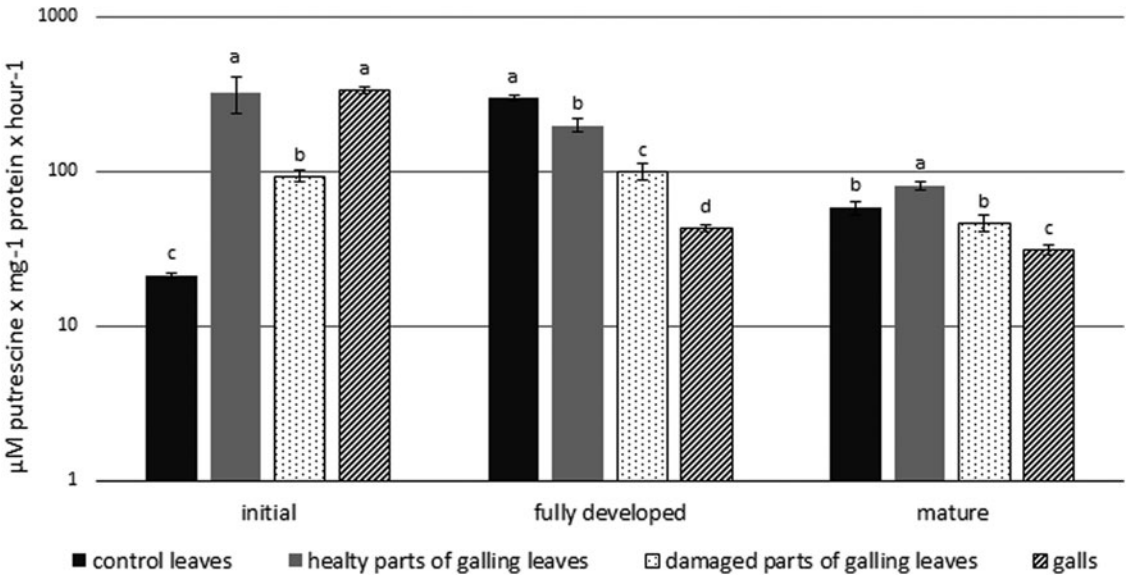

Fig. 3. The ornithine decarboxylase (ODC) activity in Siberian elm tissues during the galling process of *Tetraneura ulmi* L. ANOVA for: initial period of galling $F_{3,8} = 213.24$ at $P < 10^{-7}$, stage of fully developed galls $F_{3,8} = 226.15$ at $P < 10^{-7}$ and stage of mature galls $F_{3,8} = 56.067$ at $P = 10^{-5}$. In each stage of gall development the bars signed by different letters indicate significant differences at $P \leq 0.05$ (Tukey's HSD test).

Table 1. The content of polyamines (µg × g^{-1} FW) in Siberian elm tissues during the galling process of *Tetraneura ulmi* L.

Stage of gall development	Tissue	Putrescine	Cadaverine	Spermidine	Tryptamine	Spermine	Histamine
	Control leaves	19.88 ± 0.32 a	0.78 ± 0.15 b	7.70 ± 0.79 b	46.88 ± 3.19 a	3.99 ± 0.51 ab	7.46 ± 1.64 b
Initial	Leaves UP	19.16 ± 1.52 a	0.54 ± 0.02 b	7.11 ± 0.16 b	48.99 ± 2.52 a	4.92 ± 0.49 a	6.99 ± 0.83 b
	Leaves DP	12.17 ± 0.79 b	0.83 ± 0.05 b	7.06 ± 0.52 b	55.18 ± 3.87 a	2.54 ± 0.27 b	7.65 ± 0.59 b
	Gall	18.38 ± 0.86 a	1.57 ± 0.27 a	16.70 ± 2.01 a	56.20 ± 4.86 a	0.87 ± 0.15 c	12.57 ± 0.72 a
ANOVA		**$F_{3,8} = 50.13$**	**$F_{3,8} = 24.62$**	**$F_{3,8} = 53.64$**	**$F_{3,8} = 4.57$**	**$F_{3,8} = 63.01$**	**$F_{3,8} = 12.28$**
P		**1.60×10^{-5}**	**2.16×10^{-4}**	**1.20×10^{-5}**	**3.81×10^{-2}**	**7.00×10^{-6}**	**5.10×10^{-4}**
	Control leaves	0.0	0.45 ± 0.30 b	2.54 ± 0.06 b	13.47 ± 0.37 b	0.47 ± 0.04 b	8.23 ± 0.55 b
Fully	Leaves UP	0.0	0.29 ± 0.02 c	2.52 ± 0.17 b	11.15 ± 0.68 bc	0.0 d	5.33 ± 0.37 c
Developed	Leaves DP	0.0	0.0 d	2.39 ± 0.03 b	10.39 ± 0.47 c	0.31 ± 0.03 c	8.50 ± 0.72 b
	Gall	0.0	0.74 ± 0.05 a	6.88 ± 0.46 a	24.48 ± 1.68 a	1.63 ± 0.05 a	16.20 ± 0.54 a
ANOVA		–	**$F_{3,8} = 249.18$**	**$F_{3,8} = 335.9$**	**$F_{3,8} = 140.42$**	**$F_{3,8} = 1085.09$**	**$F_{3,8} = 208.38$**
P		–	**$<10^{-7}$**	**$<10^{-7}$**	**$<10^{-7}$**	**$<10^{-7}$**	**$<10^{-7}$**
Mature	Control leaves	0.0	0.0	1.26 ± 0.11 b	4.50 ± 0.25 c	0.0	0.0 c
	Leaves UP	0.0	0.0	3.54 ± 0.10 a	19.38 ± 1.33 a	0.0	11.09 ± 0.29 a
	Leaves DP	0.0	0.0	3.36 ± 0.08 a	16.91 ± 0.67 b	0.0	8.54 ± 0.72 b
	Gall	0.0	0.0	0.0 c	0.0 d	0.0	0.0 c
ANOVA		–	–	**$F_{3,8} = 1254.66$**	**$F_{3,8} = 466.37$**	–	**$F_{3,8} = 634.92$**
P		–	–	**$<10^{-7}$**	**$<10^{-7}$**	–	**$<10^{-7}$**

Leaves DP – damaged parts of galling leaves; leaves UP – undamaged parts (without visible changes in appearance) of galling leaves; means marked with the same letter in the column (in each stage of the galling process) do not differ significantly at $P = 0.05$ (Tukey's HSD test).

spermidine and histamine was similar to the control leaves; however, the content of tryptamine and spermine was statistically lower. The content of cadaverine in damaged parts of galling leaves was not detected. The level of spermidine and tryptamine in undamaged parts of galling leaves was similar to the control leaves and damaged parts, but cadaverine and histamine were statistically lower compared with the control. Spermine was not detected (table 1). In the stage of mature galls, only spermidine, tryptamine and histamine were detected, but not in all analysed elm tissues. None of PAs was found in gall tissues. In galling leaves (both parts), the level of amines was higher than in the control ones.

Discussion

The results clearly proved that the galling process induced by the aphid *T. ulmi* in Siberian elm leaves was connected with changes in the content of biogenic amines and the activity of key enzymes of their biosynthesis. Direction and intensity of these changes for particular amines were dependent on the

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stage of gall development and part of galling leaf. Generally, the amine content tended to increase in gall tissues during the 1st and 2nd period of the galling process and decreased later on. Different modes of changes were observed in case of spermine and putrescine at the initial stage of gall development, when these amines were characterized by a lower content or lack of significant changes, respectively. The content of spermidine, tryptamine and histamine was also increased in damaged and undamaged parts of leaves in the last stage of gall development. The present study partly confirmed the results of Subramanyam *et al.* (2015), who reported an increase of PA contents in wheat infested by *M. destructor*. Such response was first observed for putrescine, and then spermidine and spermine, and it was typical only for the interaction between susceptible wheat cv. and virulent larvae. Moreover, PA contents in infested plants were correlated with the increasing levels of these biomolecules in insect tissues (Subramanyam *et al.*, 2015). The authors supposed that PAs were the terminal metabolic products utilized by the insects, as the transcripts of wheat PA oxidase were not increased in the infested plants. In their opinion, these compounds could protect the midgut of virulent larvae from basal defences. It may suggest that *T. ulmi* is able to force the host plant to induce the synthesis of compounds it requires, similarly to Hassian fly larvae. Such a conclusion is supported by the reports on the beneficial effect of exogenous PAs and tyramine on the physiology of herbivorous insects (Carter *et al.*, 1998; Zhang *et al.*, 2008; Wu *et al.*, 2010). However, our earlier studies showed that PAs and tyramine at mM concentrations had rather a tendency to disturb the feeding and survival of cereal aphid species, such as *Sitobion avenae* (Fabr.) and *Rhopalosiphum padi* (L.) (Sempruch *et al.*, 2010, 2016*a*). In addition, the level of cadaverine, spermidine and tryptamine was elevated in triticale infested by bird cherry–oat aphid but only in case of more resistant cv. (Sempruch *et al.*, 2012). Thus, we conclude that the role of these biomolecules in aphid–plant interactions is still not clear and it needs further studies. Various directions of changes in the biogenic amine contents in Siberian elm induced by *T. ulmi* (particularly in parts of the leaves involved in the galling process and the same leaf parts at the following stages of gall formation) also suggest that the role of these compounds might change in galls and neighbouring plant tissues during the galling process. The changes in gall tissue appear to be beneficial for the insects during the 1st and especially the 2nd period of the galling process, therefore, the direction of these transformations had an opposing character in damaged and undamaged parts of the leaves. The remarkably high concentrations of amino acids in galls of different *Tetraneura* species on *Ulmus davidiana* var. *japonica* have also been found by Suzuki *et al.* (2009).

Moreover, in the present study, the level of the majority of the analysed amines was reduced in damaged and undamaged parts of galling leaves during the initial period of the galling process and at the time when the galls were fully developed. Similar tendency was found for spermidine and tryptamine in tissues of mature galls. This may mean that in some tissues of Siberian elm leaves, the rate of catabolic amine transformation was increased and/or their biosynthesis was reduced during the studied stages of gall development. Degradation of PAs, catalysed by diamine oxidase and PA oxidase, generate considerable quantities of hydrogen peroxide. H_2O_2 may subsequently induce programmed cell death (PCD). Such transformations were characteristic of a HR developed by tobacco against tobacco mosaic virus (Yoda *et al.*, 2009). ROS, including hydrogen peroxide, were also involved in biochemical plant defence against infestation by aphids (Moloi & van der Westhuizen, 2006; Łukasik *et al.*, 2012; Sytykiewicz, 2014). Oxidative stress was generated by ROS occurring in place of polyphenol accumulation in cells of *P. gonoacantha* (Mart.) infested by gall inducers (Bedetti *et al.*, 2014). Moreover, HR was developed as a result of incompatible interactions between avirulent biotype of Asian rice gall midge and their host *O. sativa* (Agarrwal *et al.*, 2016). The response was associated with extensive reprogramming of the transcriptome and metabolome of rice. It was characterized by generation and release of ROS, i.e., singlet oxygen and resulted in PCD mediated by lipid peroxidation.

Our results also proved that the induction of ODC and LDC, but not TyDC, was at least partly involved in the regulation of the amine balance during the galling process induced by *T. ulmi* on Siberian elm leaves. However, in spite of the ODC induction in all analysed parts of *U. pumila* leaves at the 1st stage of gall development, the content of putrescine was not elevated. It may be important information since putrescine was the first PA that was increased in a susceptible wheat cv. under infestation by virulent larvae of *M. destructor* (Subramanyam *et al.*, 2015). Putrescine participates in plant responses against abiotic and biotic stresses through regulation of osmotic balance, membrane functioning, gene expression, modulation of metabolic pathways and detoxification of ROS (Fariduddin *et al.*, 2013). For example, application of exogenous putrescine reduced generation of protein oxidation in *Salvinia natans* L. under hydrogen peroxide treatment (Mandal *et al.*, 2014). However, it is possible that the induction of ODC indirectly altered the biosynthesis of other PAs (especially spermidine) during the first step of gall formation induced by *T. ulmi* in Siberian elm leaves. In addition, an increase of cadaverine content, correlated with the induction of LDC activity in gall tissues, might also be connected with a reduced level of putrescine. According to Tomar *et al.* (2013), the decreased level of putrescine in stress-tolerant plants may be compensated by spermine and cadaverine. Cadaverine, similarly to other Pas, is capable of binding A- or B-DNA forms, and such binding may stabilize the structure of DNA. On the other hand, the induction of LDC activity in response to *R. padi* infestation was characteristic of less susceptible cvs of triticale, wheat and maize (Sempruch *et al.*, 2013, 2015, 2016*b*). Since such a phenomenon was not observed in case of susceptible plants, it is possible that this enzyme and its products constitute part of plant defensive responses against the aphid feeding. Thus, it can be concluded that the mode of amino acid decarboxylase participations in aphid–plants interactions is strictly dependent on the genotype of the herbivore and its host.

Finally, it can be said that biogenic amines were involved in the gall formation in Siberian elm leaves infested by *T. ulmi.* However, their role was changing depending on the stage of gall development and part of the galling leaf. At this research stage, it is not possible to explicitly state, whether these changes are caused by a flow of the analysed compounds to gall tissues from the neighbouring parts of the leaf, or the modification in the rate of their metabolic transformations. However, the results of enzymatic assays suggest that the changes in amine levels in the studied interactions might be at least partly caused by the regulation of the activity of ODC and LDC, but not TyDC. Full explanation of the participation of such enzymatic mechanisms in interactions between plants and galling insects needs further studies focused on

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other enzymes from the amine biosynthesis and degradation pathway as well as on other products of these reactions. For example, ODC, LDC and TyDC were also shown to be involved in biosynthesis of hydroxycinnamic acid amides that may participate as neurotoxins in plant defence against herbivorous arthropods (Fixon-Owoo *et al*., 2003).

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