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ASSESSMENT OF *Parectopa robiniella* CLEMENS (LEPIDOPTERA: GRACILLARIIDAE) EFFECT ON BIOCHEMICAL PARAMETERS OF *Robinia pseudoacacia* UNDER CONDITIONS OF AN INDUSTRIAL CITY IN STEPPE UKRAINE

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Abstract

Shupranova L., Holoborodko K., Loza I., Zhukov O., Pakhomov O.: Assessment of *Parectopa robiniella* clemens (Lepidoptera: Gracillariidae) effect on biochemical parameters of *Robinia pseudoacacia* under conditions of an industrial city in steppe Ukraine. Ekológia (Bratislava), Vol. 41, No. 4, p. 340–350, 2022.

Antioxidant enzymatic activity and soluble protein content in *Robinia pseudoacacia* L. (black locust), invasive tree in Europe, plants were determined under feeding conditions of the caterpillars of *Parectopa robiniella* Clemens, 1863, invasive miner in Europe, in trees of three (I, II, III) age groups (5–10, 15–25, and 40 years). Biochemical analysis data showed significant differences between reference and experimental leaf samples in different urban ecosystems. There was no association between changes in catalase and peroxidase activity and protein content under the influence of leaf blotch miners depending on the tree age. Significant variability of proteins under *P. robiniella* influence was noted; the highest of its values were recorded in the leaves of III-aged trees. In most plantings (and especially in trees of age group III), an increase (1.2 to 2.7 times) in GPx activity or the absence of its changes was registered both in the ecologically favorable zone and in the locations affected by emissions from road transport and industrial enterprises. Plants of age groups I and II in the emission areas of metallurgical, pipe-rolling, and chemical industries experienced a significant decrease in GPx activity (by 20.2–44.6%) in the leaves damaged by the phytophage. In most of the plantings studied, catalase showed an increase/stability of its activity in all age groups of trees under condition of pest attack. The highest increase in CAT activity (2.1 times) was recorded in young *R. pseudoacacia* plants in an ecologically favorable area of the city.

Key words: invasive species, Gracillariidae, *Robinia pseudoacacia*, urban green plantings, technogenic load, protein content, antioxidant enzymes.

Introduction

Robinia pseudoacacia L. (black locust) is one of the most common and ecologically adaptable introduced tree species which has become an important (mostly negative) landscape element in such countries as Romania, Hungary, Italy, Slovenia, Croatia, Ukraine, etc. *R. pseudoacacia* L. is currently listed as an invasive alien species in Europe which has the highest negative environmental impact (Nentwig et al., 2018). Due to the active introduction into various types of ecosystems in many countries of the world, it is recognized as an invasive species having a high rate of adventivity (Rumlerová et al., 2016; Vítková et al., 2017; Wagner et al., 2017; Nicolescu et al., 2020; Vitková et al., 2020). The effect of *R. pseudoacacia* plantations on the decrease in the species diversity of forest and steppe vegetation has already been confirmed for a number of countries in Central Europe (Konaikova, Vakarenko, 2020; Montecchiari et al., 2020; Lukovičová et al., 2021). On the territory of Europe, a shift of its potential climatic niches towards the east was predicted under the conditions of climate change, with their reduction in Southern Europe and expansion in Central and North-Eastern Europe (Puchałka et al., 2021), where an increase in its invasive activity is possi-

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ble in the future. Taking into account the economic significance of black locust plantations in Ukraine, their bioproductivity, environmental, and energy potential have been widely studied (Sytnyk et al., 2017; Zverkovskyi et al., 2017). Previously, the absence of natural enemies outside the native area of the species was considered as one of the main reasons for the successful adventivity of *R. pseudoacacia* (Cierjacks et al., 2013). However, several large-scale invasions of phytophagous insects have been recorded in the European area of *R. pseudoacacia* in the last two decades.

Nowadays, the main phytosanitary risk of *R. pseudoacacia* existence within the European area is considered to be a spread of invasive species of North American origin, primarily from the family of the leaf blotch miner moths (Lepidoptera: Gracillariidae). Among the complex of invasive phytophages, *Parectopa robiniella* (Clemens, 1863) is characterized by the largest-scale invasion in Ukraine (Holoborodko et al., 2021). Since *P. robiniella* appearance, this species has become the object of numerous environmental and biological studies (Enescu, Dănescu, 2013; Guo et al., 2018; Kirichenko et al., 2019; Wilkaniec et al., 2021). In Eastern Europe, it is an insufficiently studied species. Some bioecological features of *P. robiniella* were studied in the conditions of the Steppe and Forest-steppe zone of Ukraine (Holoborodko et al., 2018; Shvydenko et al., 2021). This pest species was found on the leaves of *Robinia pseudoacacia* growing in artificial forest stands of different functional assignments throughout Ukraine. Caterpillars of *Parectopa robiniella* feed on leaf mesophyll and form whitish mines of complex shape about 1.4 cm^2 in size. Within its native area, *P. robiniella* acts as oligophage that damages the leaves of several woody species of Fabaceae: *Robinia pseudoacacia* L., *R. hispida* L., *R. viscosa* Vent., *Amorpha fruticosa* L., *Desmodium* sp., *Galactia volubilis* (L.) Britt., and Meibomia sp. (Davis, De Prins, 2011). The absence of natural enemies and diseases within the novel area, and an almost unlimited food resource contribute to the rapid and large-scale spread of this invasive insect.

Changes in environmental factors, in particular, the attack of herbivorous insects, affect primary metabolism, such as the rate of photosynthesis, nitrogen uptake, protein content, antioxidant activity, and tree lifespan (Bond et al., 2007; Meinzer et al., 2011; Turfan et al., 2018; Shupranova et al., 2019; Holoborodko et al., 2022). The leaves are the main organ to produce organic matter, and they are the main source of carbohydrates for the plant (Liu et al., 2017). Leaf damage reduces the area of the photosynthetic surface and thus disrupts the entire production process in the plant.

Environmental factors, such as air pollution in cities inhabited by plants forage for leaf blotch miners, can affect leaf characteristics and thus interfere with the ability of insects to feed on mesophyll cells. The importance of leaf characteristics for plant resistance to miner attacks was investigated by comparing leaf strength and nutrient composition, surface wax, and secondary metabolites that induce the formation of chemical and tissue barriers and enhance cell repair processes in different plant species (Paterska et al., 2017). The long-term activity of phytophagous insects can negatively affect the production potential of the black locust (Csóka et al., 2017), whereas Holec et al. (2009) in their study found that the intensity of damage caused by *Parectopa robiniella* and *Macrosaccus robiniella* (Clemens, 1859) (Lepidoptera) activity was not critical for mature black locust trees. No significant differences were found in leaf damages caused by *M. robiniella*, *Parectopa robiniella,* and *Obolodiplosis robiniae* (Haldeman, 1847) (Diptera) on individual *Robinia pseudoacacia* trees of different ages growing in rural and urban environments.

Thus, a review of literature sources has shown that almost unexplored were found to be (1) biochemical mechanisms by which *R. pseudoacacia* withstands attacks of leaf blotch miner caterpillars; (2) which age groups of trees of this species will appear to be the most sensitive to the damaging effects of miner's caterpillars; and (3) the way in which different types of environmental pollution can affect the antioxidant protecting system of *R. pseudoacacia* against *Parectopa robiniella* influence. But the research on the features of *Robinia pseudoacacia* protecting responses against phytophage influence is of great importance, as it is expected that in the near future, the role of the black locust will increase due to its high ecological plasticity and biological characteristics that make it suitable for planting in drylands (Enescu, Dănescu, 2013). A study of the impact of disorders caused by the phytophage feeding occurred in *R. pseudoacacia*; both of different age groups and individual trees can contribute to the development of theoretical foundations for the selection and management of this species with the purpose of its restoration. The goal of this study was to investigate (1) the way in which protein-enzyme protective system of *R. pseudoacacia* leaves reacts to the damaging effect of *Parectopa robiniella* caterpillars under different conditions of air pollution, and (2) what age groups of *Robinia pseudoacacia* trees are the most sensitive to *Parectopa robiniella* attacks.

Material and methods

The study was conducted during the growing season of 2021 in parks of Dnipro city (Ukraine). For the study, six locations were selected in Dnipro city which is situated in the central part of the Dnipropetrovsk Oblast, on both banks of the Dnieper River, within the Northern steppe subzone, in the south-eastern part of Ukraine. The soil cover is represented by ordinary low humic chernozems. The territory has temperate-continental climate. Areas of the city have a complex relief structure. The left-bank part is represented by a floodplain flat relief with absolute surface marks of 51.0–72.6 m. The right-bank part is represented by a plain watershed highly dissected relief (0.8–0.9 km/km^2), with a height difference of 51.0–180.0 m. The average annual air temperature is about +8 °C; the average annual precipitation is 477 mm. Growing season period is 210 days.

The level of air pollution in the city is above average. The city's industrial complex is based on ferrous metallurgy enterprises, mechanical engineering, chemical industry, and energy.

Sampling was carried out in the first decade of September 2021, which corresponded to the period of maximum leaf damage by the invader, on the territory of Botanical Garden of Oles Honchar Dnipro National University (an environmentally suitable area, L1), and on six sites of different intensities of air pollution (L2–L6) (Fig. 1, Table 1).

The experiment was performed on 5 *Robinia pseudoacacia* specimens sampled from each site (a total of 30 individuals) of selected park areas within the city. Six groups of model trees were selected for the study of *R. pseudoacacia* of different ages: 5–10, 15–25, and 40 years of age (age groups I, II, and III, respectively)



Fig. 1. Map of the sampling units within the territory of Dnipro city (Ukraine).

Table 1. Description of the study locations.

Abbreviation	Location	GPS latitude, longitude
L1	Botanical Garden of Oles Honchar Dnipro National University (environmentally suitable area)	48°43‘08“ N, 35°03‘95“ E (height above sea level is 127 m)
L2	Metallurgists Square (western right-bank part of the city, near the Dnipro metallurgical plant)	48°28’26“ N 34°59’31“ E (height above sea level is 94 m)
L3	Lazaria Hloby Park (right next to the main road in the city center with heavy traffic)	48°47‘06“ N, 35°03‘20“ E (height above sea level is 56 m)
L4	Molodezhny Park (western right-bank part of the city, near the Dnipro Varnish-and-Paint Manufacturing Plant)	48°29‘08“ N, 34°56‘42“ E (height above sea level is 82 m)
L5	Manuylivsky Park (left-bank part of the city, near the main highway with heavy traffic, at a distance of 1.75 km from the Dnieper Pipe Rolling Plant)	48°29’13“ N, 35°03’40“ E (height above sea level is 56 m)
L6	Taras G. Shevchenko Park (central part of the city, area of intensive sedimentation of harmful impurities of plants of the left-bank south-eastern part: thermal power plant, pipe rolling plant)	48°46‘15“ N, 35°07 , 03 „ E (height above sea level is 82 m)

with presented leaf damage by *Parectopa robiniella* miner. For each site, leaves without miner damage and signs of ageing were sampled as a reference.

To conduct biochemical analyses, compound leaves of medium formation without signs of ageing were sampled on the annual vegetative growth, from the lower third of the crown of the south-

Table 2. Estimation the effects of *Parectopa robiniella* ($F = 17.5$, $p < 0.001$), age ($F = 23.8$, $p < 0.001$), and their interaction on the enzyme activity with the use of GLM ($F = 4.8$, $p < 0.001$).

Parameter	R^2_{adj}	Model			Residual			*F*-ratio	*p*-level
		Sum of squares	df	Mean sum of squares	Sum of squares	df	Mean sum of squares		
GPx	0.43	3322.2	5	664.4	3217.5	30	107.25	6.20	<0.001
CAT	0.60	101877.8	5	20375.6	52853.0	30	1761.77	11.57	<0.001
Protein	0.41	15.5	5	3.1	15.8	30	0.53	5.87	<0.001

Table 3. Content of soluble proteins in *Robinia pseudoacacia* leaves of tree different ages under the effect of *Parectopa robiniella* (n = 5; mean ± SD).

Location	Protein content, mg/g					
	Age group I		Age group II		Age group III	
	reference	experiment	reference	experiment	reference	experiment
L1	0.98 ± 0.11[a]	1.86 ± 0.08[b]	1.01 ± 0.05[a]	0.97 ± 0.01[b]	1.04 ± 0.24[a]	1.16 ± 0.03[b]
L2	1.33 ± 0.02[a]	2.28 ± 0.04[b]	1.63 ± 0.04[a]	1.40 ± 0.02[a]	4.65 ± 0.09[a]	5.78 ± 0.05[b]
L3	2.88 ± 0.00[a]	1.52 ± 0.05[b]	2.64 ± 0.04[a]	2.55 ± 0.01[b]	4.11 ± 0.01[a]	1.57 ± 0.01[b]
L4	1.18 ± 0.07[a]	1.32 ± 0.09[b]	1.11 ± 0.11[a]	1.37 ± 0.04[b]	1.57 ± 0.02[a]	0.83 ± 0.08[b]
L5	0.77 ± 0.02[a]	1.92 ± 0.02[b]	1.27 ± 0.05[a]	1.39 ± 0.07[b]	3.35 ± 0.05[a]	3.78 ± 0.03[b]
L6	4.85 ± 0.68[a]	3.98 ± 0.45[a]	4.05 ± 0.63[a]	3.80 ± 0.66[a]	2.72 ± 0.49[a]	3.18 ± 0.47[a]

Note: the values in the columns marked with different letters (a, b) differed significantly according to Tukey test, $P < 0.05$.

Table 4. Guaiacol peroxidase activity in leaves of *Robinia pseudoacacia* trees of different ages under the influence of *Parectopa robiniella* (mean ± SD).

Location	Activity of GPx, μmol TG/min ·g FW					
	Age group I		Age group II		Age group III	
	reference	experiment	reference	experiment	reference	experiment
L1	17.48 ± 0.47[a]	33.93 ± 0.73[b]	26.60 ± 1.21[a]	42.47±1.21[b]	10.98 ± 0.24[a]	38.97 ± 0.55[b]
L2	37.85 ± 0.29[a]	20.98 ± 0.43[b]	30.59 ± 0.89[a]	60,55 ± 0.95[b]	21.35 ± 0.59[a]	26.81 ± 0.23[b]
L3	42.57 ± 0.92[a]	54.10 ± 1.14[b]	52.21 ± 2.70[a]	63.40 ± 1.84[b]	40.20 ± 2.06[a]	38.99 ± 1.35[a]
L4	1.04 ± 0.18[a]	11.29 ± 0.65[b]	28.75 ± 0.37[a]	22.95 ± 0.75[b]	26.56 ± 0,83[a]	27.81 ± 0.44[a]
L5	15.17 ± 1.02[a]	33.14 ± 1.10[b]	42.88 ± 0.85[a]	43.73 ± 0.83[b]	20.72 ± 1.06[a]	49.36 ± 0,80[b]
L6	22.55 ± 0.79[a]	55.24 ± 0.88[b]	22.28 ± 0.95[a]	23.58 ± 0.86[a]	15.39 ± 0.44[a]	40.79 ± 0.25[b]

Notes: the values in the columns marked with different letters (a, b) differed significantly according to Tukey-test, $P < 0.05$; GPx – guaiacol-peroxidase; TG – tetraguaiacol; FW – fresh weight.

ern exposure; the sampling was performed in dry, clear weather, 5 pieces from 5 black locust trees, at the same time in each experimental site. For biochemical analysis, the leaves were washed with water and immediately used for enzyme extraction. To isolate the enzyme preparation, the black locust leaves (0.3 g) were homogenized in 6 ml of 0.05 M Tris-HCL buffer, pH 7.4 with 0.5% polyvinylpyrrolidone (PVP). Extraction was performed at +4°C for 1 hour and centrifugated for 20 minutes at 14,000 rpm.

The measures of antioxidant enzymes guaiacol peroxidase and catalase activity were carried out using spectrophotometry. Guaiacol-dependent peroxidase (GPx, EC 1.11.1.7) activity was evaluated according to Ranieri et al. (2001) and expressed in μmol TG/min ·g FW. Catalase activity (CAT) was evaluated according to Goth (1991), and the results were expressed in μmol H_2O_2/min ·g FW. The concentration of soluble proteins (LP) was determined using the Bradford protein assay (1976).

The results of enzyme activity and soluble protein content analyses were presented as the average x value ± SD (standard deviation). Tukey's honestly significant difference test was used to test differences between group means for significance. The differences were found to be statistically significant at $p < 0.05$. The data obtained were analyzed using Statistica software package (version 8, StatSoft, USA). A multiple generalized linear model (GLM) (Statistica software package) was used to test the significance of the plant biochemical characteristics dependence on environmental predictors (*P. robiniella*, tree age). The adjusted coefficient of multiple determinations (R^2_{adj}) was calculated as part of the GLM.

Table 5. Changes in catalase activity in *Robinia pseudoacacia* tree leaves of different ages under the effect of *Parectopa robiniella* (n = 5, mean ± SD).

Location	Activity of CAT, μmol H_2O_2/min ·g FW					
	Age group I		Age group II		Age group III	
	Reference	Experiment	Reference	Experiment	Reference	Experiment
L1	186.18 ± 1.28 [a]	390.58 ± 1.38 [b]	218.61 ± 0.57[a]	199.88 ± 6.93[b]	199.47 ± 5.78 [a]	257.54 ± 1.92[b]
L2	185.69 ± 6.18[a]	227.11 ± 4.99[b]	255.64 ± 44.46[a]	482.36 ± 31.88[b]	243.81 ± 46.61[a]	242.48 ± 32.43[a]
L3	231.43 ± 3.02[a]	300.83 ± 1.83[b]	289.49 ± 4.33[a]	342.34 ± 4.33 [b]	246.25 ± 8.41 [a]	126.13 ± 6.01[b]
L4	269.64 ± 4.93[a]	254.98 ± 1.11[b]	242.27 ± 3.84[a]	288.30 ± 1.94 [b]	389.51 ± 4.84[a]	197.96 ± 3.85[b]
L5	179.42 ± 1.28 [a]	231.74 ± 1.28[b]	–	–	–	–
L6	360.35 ± 30.43[a]	305.46 ± 32.05[b]	329.32 ± 32.39[a]	342.12 ± 22.08[a]	331.89 ±24.11[a]	315.94 ± 8.99[a]

Notes: the values in the columns marked with different letters (a, b) differed significantly according to Tukey-test, P < 0.05; CAT – catalase; FW – fresh weight.

The one-way analysis of variance (ANOVA) was used to determine the dependence of enzyme activity on protein accumulation. Each panel shows the correlation coefficient value (d). Mine-free leaves were used a reference for each monitoring location. We tested the following null hypotheses: 1) the enzymatic activity and soluble protein content are independent of plant age and effects of *P. robiniella*; 2) the correlation between the activity of various enzymes and soluble protein content is independent of age and effects of *P. robiniella*.

Results

The application of the generalized linear model (GLM) showed statistically significant ($p < 0.001$) effects of phytophage, tree age, and their interaction on guaiacol peroxidase, catalase activity, and soluble protein concentration (Table 2).

The study of changes in LP content showed that all age groups in different urbocoenoses revealed multidirectional changes in the black locust leaves under the damaging effect of leaf blotch miners (Table 3).

In the vast majority of experimental samples, an increase in this parameter was recorded in trees of age group I (and especially sampled from L1 location) by 92% ($p = 4{,}15 \cdot 10^{-4}$), L2 by 71% ($p = 6{,}36 \cdot 10^{-4}$), and L5 by 2.5 times ($p = 3{,}40 \cdot 10^{-10}$). A significant decrease in this parameter was recorded in the location of intensive vehicle emissions (L3): by 47.2 ($p = 1.01^{-6}$), 3.5 ($p = 0.025$), and 61.8% ($p = 2.73^{-10}$) in the age groups I, II, and III, respectively. Statistically insignificant inhibition of the parameter was detected in planting of L6 by 17.9% ($p = 0.137$) in age group I, by 6.2% in age group II ($p = 0.668$), and by 11.6% at L1 (environmentally suitable area) ($p = 0.492$) in age group III. In age group II of the trees, an increase in this parameter was registered in two following cases: on the emission area L5 of the pipe rolling plant by 9.5 ($p = 0.04$) and on chemical production area (L4) by 23.8% ($p = 0.018$). Age group III of the black locust plants also showed an increase in protein content due to the damaging effects of *P. robiniella* in two cases: on L2 location (metallurgical production) by 24.3 ($p = 4.60 \cdot 10^{-5}$) and on L5 by 12.9% ($p = 0.004$).

In moth-affected leaves sampled from the groups of plants in different plantings, intra-individual variability of highly soluble protein content had a low variation coefficient (0.14–18.0%). Affected plants in most cases showed significantly higher GPx activity in all age groups of plants from the experimental sites compared to the intact plants (Table 4).

A statistically significant increase in peroxidase activity on all locations studied was observed in the leaves sampled from *Robinia pseudoacacia* trees of age group I. On the location of chemical industry emission (L4), trees of age group III showed only a trend of increasing GPx activity by 4.7% ($p = 0.083$). The most significant increase in peroxidase activity on almost all the locations studied was noted in the leaves of trees of the age group III, which ranged from 21.4 to 254.9%. Group II-aged trees on two locations (L1 and L2) showed a significant increase in enzyme activity by 59.7 and 97.9% ($p = 8{,}75 \cdot 10^{-5}$; $p = 2{,}33 \cdot 10^{-6}$), and only a trend of increasing activity ($p = 0.283$; 0.153, respectively) was observed in two others (L5 and L6). In the age group I, a significant increase in GPx activity by 94.1, 145.0, and 118.5%, respectively, was recorded on three monitoring locations (L1, L5, and L6) ($p < 0.001$). A statistically significant decrease in guaiacol peroxidase activity was found in two plantings on the emission area of metallurgical production (L2; age group I) and chemical industry area (L4; age groups I and II; $p < 0.001$). Variability in GPx activity of damaged leaves in groups of plants growing in different contaminated areas had a low variation coefficient (0.6–6.7%).

In the analyzed groups of trees from different urbocenoses, catalase activity values were also different (Table 5).

Leaf samples with moth mines showed an increase in enzyme activity (17.8 to 110.0%; $p < 0.001$) in age group I on 4 locations.

In age group II, an increase in enzyme activity in different urbocenoses (L2, L3, L4, and L6) also prevailed due to insect attacks and, especially, in the area of metallurgical production influence (L2), namely, by 88.7% ($p = 1{,}99\ 10^{-3}$). The most significant inhibition of catalase activity was recorded in insect-damaged leaves sampled from areas affected by vehicle (L3) and chemical production (L4) emissions by an average of 49% ($p < 0.001$). Intra-individual variability of catalase activity in damaged leaves sampled from groups of plants in different park stands had low- and medium-variation coefficients (0.3–19.1%).

Generalized response of biochemical parameters (Fig. 2) on the phytophage damaging effect as a function of tree age showed that GPx activity increased significantly only in the leaves sampled from trees of age group III, namely, by 64.8% ($p = 2.01\ 10^{-5}$). In other age groups, a trend of increasing guaiacol-dependent peroxidase activity was noted (by 36.0%, $p = 0.059$, and by 26.2%, $p = 0.062$, respectively). At the same time, the level of variability

Table 6. GLM test of the effect of different predictors on the slope of GPx and CAT dependence on protein content.

Effect	GPx		CAT	
	F-ratio	*p*-level	*F*-ratio	*p*-level
Age group	1.13	0.34	0.23	0.80
P. robiniella	0.43	0.52	0.34	0.56
Protein	63.00	0.00	14.59	<0.001
Age group × *P. robiniella*	0.28	0.75	1.17	0.33
Age group × Protein	0.95	0.40	1.33	0.28
P. robiniella × Protein	0.48	0.50	2.93	0.10
Age group × *P. robiniella* × Protein	0.89	0.42	0.61	0.55

in GPx activity of phytophage-damaged leaves increases by 9.4 and 20.6% in trees of age groups I and II, respectively, compared to the reference, and a decrease in this parameter by 49.3% was observed in age group III.

Fig. 2 shows a significant increase in CAT activity in trees of age group I by 21.1% ($p = 0.023$), and in age groups II and III only the trends (by 23.7%, $p = 0.240$) and by 4.5% ($p = 0.852$), respectively, were observed. An increase in variability of enzyme activity in miner damaged leaves sampled from trees of age groups II and III by 1.9 and 2.0 times, respectively, was recorded compared with the reference. In age group I, variability of catalase activity decreased by 29.8%. In all the studied age groups of trees, changes in the content of soluble proteins (Fig. 2) under the influence of *P. robiniella* were insignificant ($p > 0.05$). Protein accumulation was characterized by the highest level of variability among the studied parameters: 44.7–69.9% in the reference leaves and 48.5–79.5% in the experimental ones.

Our results indicated the relationships between the studied parameters under the influence of predictors. The activity of GPx and CAT enzymes increased with an increase in protein concentrations both in reference leaves and in leaves under the influence of leaf blotch miners (Fig. 3). The maximum correlation coefficient (r = 0.95) between guaiacol-peroxidase, catalase, and protein was typical for reference leaves and age group of trees, and for CAT–LP relationship in trees of age group II. Under conditions of phytophage influence, a significant decrease in the intensity of the relationship between GPx activity and protein concentration in age groups I and III was observed. A slight increase in GPx–LP relationship was recorded in the age group II of trees (Fig. 3A). A significant increase in the intensity of CAT–Protein relationship in the leaves sampled from trees of III age group was found (Fig. 3B).

The analysis of the results showed that positive correlation did not depend on the influence of black locust miners and the tree age (Table 6).

The main effect of each factor without taking into account the protein concentration was insignificant ($p > 0,05$).

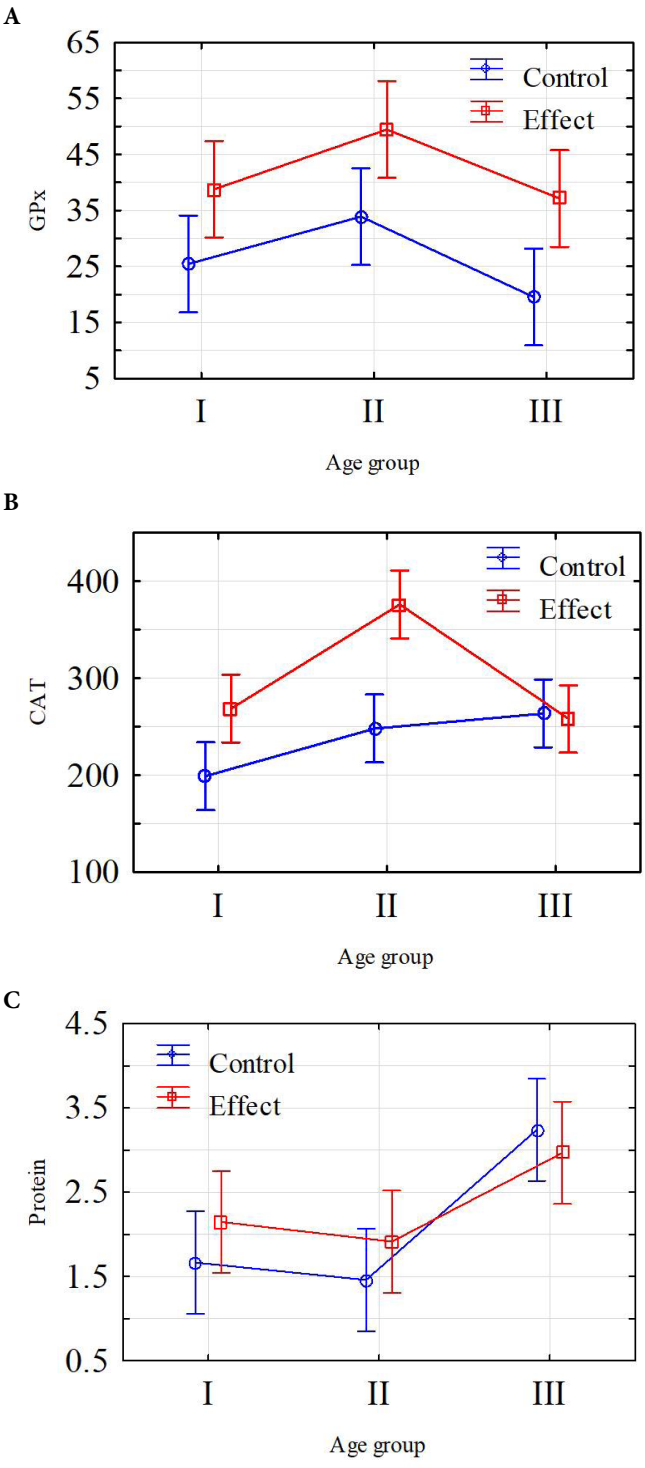


Fig. 2. Response of biochemical parameters to experimental exposure (*Parecota robiniella*) as a function of age.

Discussion

Robinia pseudoacacia is a tree species widely spread in parks, squares, gardens, and alleys of Dnipro city; it refers to a species resistant to unfavorable conditions of industrial cities in the Steppe zone (Korshykov et al., 2018). Currently, black locust is suffering from damage by *Parecota robiniella*; this raises the question of the stability of *Robinia pseudoacacia* plantings. In Ukraine, this invasive leaf blotch miner appeared relatively recently and is insufficiently studied with regard to physiological and biochemical mechanisms of *R. pseudoacacia* protection

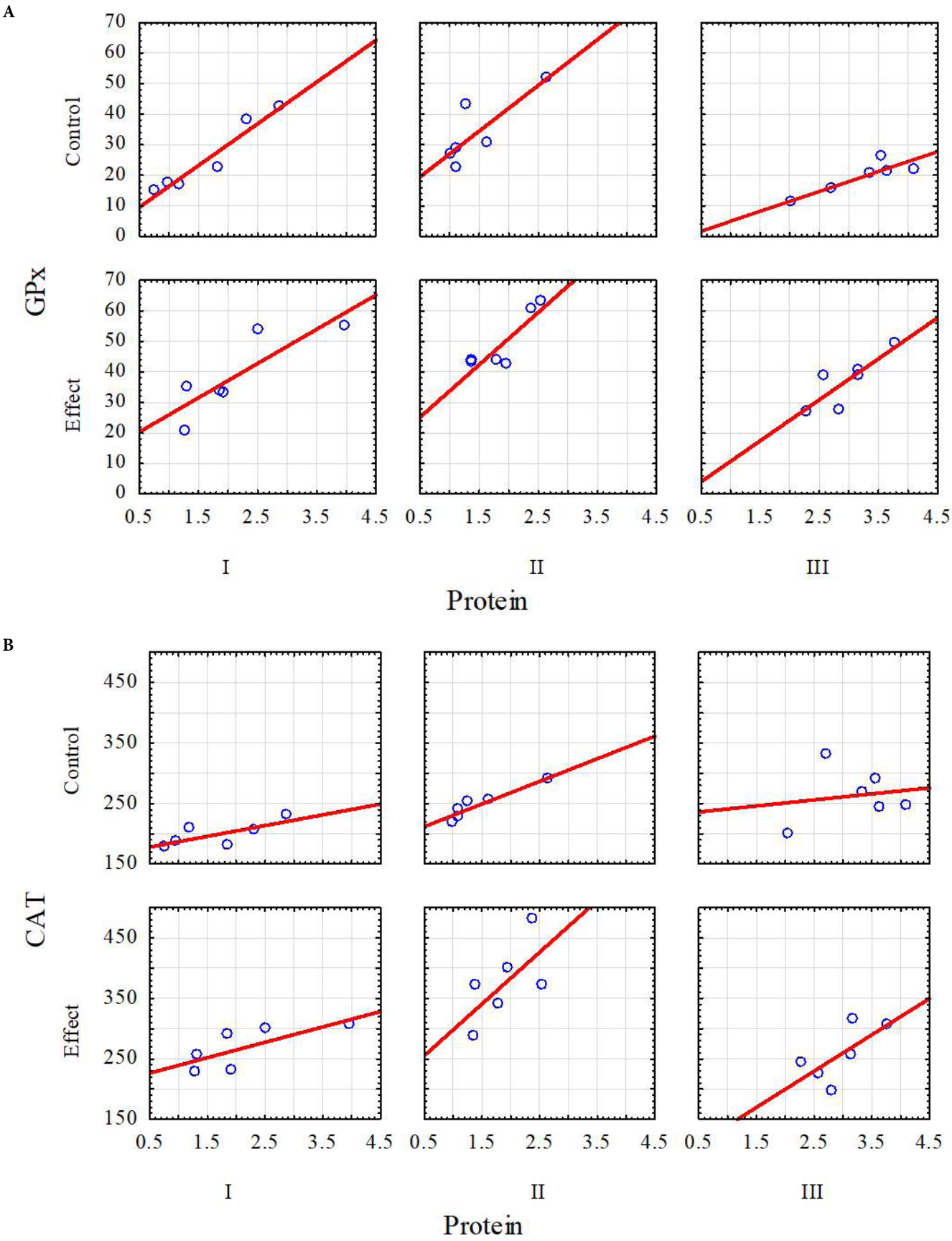


Fig. 3. GPx (A) and CAT (B) scatter plot depending on protein content.

against its influence. But the complex effect of biotic and abiotic factors can lead to the fact that plants can lose their resistance to severe urban conditions (Polle, Rennenberg, 2019; Wilkaniec et al., 2021). Such negative consequences would have an even greater impact on the functional state of young trees planted for landscaping of industrial cities. According to Korshykov et al. (2018), the highest viability of street tree plantings is typical in the age category of 21–30 and 31–40 years. The uncertainty of "plant-phytophage" interaction in the natural conditions of their existence requires the study of protection mechanisms for different-aged *R. pseudoacacia* plants against attacks of invader insects in urban environments. As a number of authors have shown, tree size and age influence various changes in their structure and function, affecting the processes of growth, germination, reproduction, or carbon accumulation (Meinzer et al., 2011; Duan et al., 2017).

Protein and carbohydrates are particularly important nutrients for herbivorous insects because of stimulating growth and reproduction (Canbolat, 2012). But their content in plants is highly variable. Although these macromolecules have the same caloric content, they are functionally very different for insects. The results evidenced that insects prefer protein with low total macronutrient content in the available form (Le Gall, Behmer, 2014). Proteins provide the animal body with the amino acids needed to build new tissues, while carbohydrates are commonly used as a key source of energy needed to support protein biosynthesis. In plants, protein content varies both at intra-species, inter-species level, and in individual plants, depending on the type (leaves, seeds, stems) and age of tissue (young, old leaves) (Mattson, 1980).

As our studies have shown, the content of soluble proteins was varied significantly between the age groups of *R. pseudoacacia* trees; the range of values was 0.77–4.85 mg/g in 5–10-year-old trees; 1.01–4.05 mg/g in 15–25-year-old trees; and 1.04–4.65 mg/g in 40–year-old trees. Mean protein content in the total sample was significantly higher in the older age group (III) in both the reference (by 26.5 and 45.5%, respectively, for groups I and II) and experimental samples (by an average of 45.8%) compared to young trees. The higher content of soluble proteins in old trees was also noted in other works (Turfan et al., 2018). The analysis determined that protein amount decreased after an insect attack on the leaves of trees of all age groups in the location where motor vehicle emissions were the main pollutants (L3), but this parameter increased in the location of pipe rolling plant emissions (L5). In age group II, this pattern was also found in the ecologically suitable location (L1) and in the location of metallurgical production influence (L2), and in age group III, a decrease in the content of soluble proteins was also observed in the location L4 (area of chemical production emissions). But the vast majority of leaves damaged by the pest in *R. pseudoacacia* of age groups I and III showed an increase in protein concentration of the studied plantings. In age group II, only two of five locations (L4 and L5) showed increased protein synthesis. The results of the variance analysis showed a positive correlation between the activity of enzymes and protein; this correlation did not depend on tree age and the influence of environmental factors. Thus, protein system of the plant reacts differently to leaf infestations with insects.

Based on a number of studies (Bilgin et al., 2010; Singh et al., 2013), there is usually a decrease in the intensity of metabolic processes (including a decrease in the rate of protein synthesis) under conditions of biotic stress; it has a protective nature, thus keeping a vital potential of cells necessary for subsequent repair. In addition, it has been reported (Zogli et al., 2020) that *Schizaphis graminum* (Rondani, 1852) (Hemiptera) feeding on the leaves of *Panicum virgatum* L. inhibits the accumulation of proteins involved in photosynthesis. In our study, it was shown that accumulation of soluble protein content was observed in most plantings and, especially, in age group I. As was noted (Zogli et al., 2020), such a plant response to insect damage may be essential for the involvement of protein enzymes in the biosynthesis of protective secondary metabolites. In addition, it has been established induction of direct protection factors, such as protease inhibitors, protective peptides in response to leaf feeding by chewing insects, and other toxic proteins that reduce the process of leaf digestion by phytophages (McHale et al., 2006; Acevedo et al., 2015; Emebiri et al., 2016; Meriño-Cabrera et al., 2018), as well as secondary metabolites (alkaloids, phenolic compounds).

One of the main plant responses to herbivorous insect attacks is the induction of redox enzymes such as peroxidases and catalases. A number of authors have shown that the higher level of their activity caused the higher plant resistance to insect attacks (Gill et al., 2010; Taggar et al., 2012; Duan et al., 2014; Polle, Rennenberg, 2019; Toledo et al., 2021). The results obtained show that an increase or absence of changes in guaiacol peroxidase activity in pest-damaged leaves was the main pattern in most of the studied plantings of almost all age groups, except for the monitoring site located in the area of influence of chemical production emissions (L4), where a decrease in GPx activity of age groups I and II was recorded ($p < 0{,}001$). In the total sample, a tendency to increasing GPx activity was observed in age groups I and II, and no differences in enzyme activity were found between the reference and the experiment samples in age group III. Peroxidase activation in cells of the black locust plant under the pest influence can be either the result of its involving in antioxidant protection or a non-specific response to stress associated with strengthening cell walls via the formation of lignins and oxidative phenols (Takashima et al., 2013; Zhao et al., 2016; Khromykh et al., 2018). The results obtained on the high activity of GPx in places of leaf damages by pest insects were confirmed with a number of studies (Singh et al., 2013; Seliutina et al., 2020).

Catalase activity was also associated with the plant response to the attack of herbivorous insects. Heng-Moss et al. (2004) found a decrease in catalase activity for a sensitive buffalograss variety in response to insect *Blissus occiduus* Barber, 1918 (Hemiptera) feeding, while the resistant variety retained the activity of this enzyme. Rangasamy et al. (2009) reported no changes in catalase activity of wheat varieties in response to *Eurygaster integriceps* Puton, 1881(Hemiptera) feeding. A decrease in catalase activity was found during feeding of *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera) on *Aesculus hippocastanum* leaves (Shupranova et al., 2019).

A study of catalase activity in leaves of *Robinia psedoacacia* trees growing in locations of the industrial city differing in their ecological conditions showed significant differences in the enzyme activity; it depended on the tree age and the type of technogenic pollution of the environment. Three types of CAT response to the damage of black locust leaf cells were estab-

lished in all the locations studied: increase, decrease, and lack of response. In the total sample, as in the case of GPx, the leaves also showed a tendency to increase catalase activity by 20.7, 23.7, and 4.5%, respectively, for age groups I, II, and III after infection with chewing insects.

Based on the results obtained, simultaneously multidirectional changes in the activity of oxidative metabolism enzymes were established in individual experiments. In *R. pseudoacacia* trees growing in the ecologically favorable location (L1), an increase in the activity of both enzymes was recorded by 1.9 and 2.1 times (age group I), by 3.5 times and 29.1% (age group III), respectively, to GPx and CAT. In L2 location (metallurgical production emissions), a similar pattern was found in the leaves of trees of age group II, where the increase in GPx and CAT activity was at the level of 98.0 and 88.7%, respectively. A decrease in the activity of both GPx (by 33.7%) and CAT (by 5.4%) was observed only in L1 location. The third pattern concerns a decrease in GPx activity (by 44.6 and 20.2%) and an increase in CAT (by 22.3 and 19.0%), which was recorded in trees growing in the location of emission of metallurgical (L2, age group I) and chemical (L4, age group II) industries, respectively. In contrast, an increase in GPx activity (by 59.7%) and a decrease in CAT (by 8.6%) were found in age group II sampled from L1 location. Two more multidirectional changes were registered in the age group III of trees from two locations: L2 (increased GPx levels by 25.6% and no changes in CAT activity) and L4 (no changes in GPx and inhibition of CAT activity by 49.2%). Analysis of significant correlations between biochemical parameters showed that the degree of intensity of GPx-Protein and CAT-Protein relationships decreases in the leaves damaged by the miner in most trees, which may be a consequence of a violation in the work of the protein-synthesizing system as a result of *P. robiniella* influence as well as excessive intake of ingredients from vehicle and industrial emissions into the cells.

The studied patterns of changes in the activity of antioxidant defense enzymes may indicate changes in the resistance of *R. pseudoacacia* trees to the conditions of complex action of biotic and technogenic factors in the urban environment. Based on the literature data, it can be assumed that the black locust trees resistant to the complex influence of pest insects and pollutants were characterized by an increase or stability of guaiacol-peroxidase and catalase activity, while the black locust plants unresistant to such influence were characterized by a decrease in the activity of the enzymes. The presented results on changes in catalase and peroxidase activity indicate their essential role in detoxification of reactive oxygen species and maintaining plant homeostasis under unfavorable conditions.

Conclusion

The results obtained showed the multidirectional activity of enzymatic antioxidant protection system in *R. pseudoacacia* trees of different ages responding to the damaging effect of *Parecota robiniella* caterpillars, which indicates the high plasticity of this species to a complex of unfavorable conditions of the urbanized environment in the Steppe Dnieper region. It was shown that most of *Robinia pseudoacacia* trees responded to the miner caterpillar attack by inducing catalase and guaiacol peroxidase activity and total soluble protein content in their leaves. But metabolic disorders due to technogenic and biotic stresses were also reflected in a decrease in both the activity of antioxidant enzymes and the content of soluble proteins. In general, under the influence of the miner caterpillar feeding, the protective system of *R. pseudoacacia* leaves was strengthened by stimulating the activity of antioxidant enzymes. For effective use of *R. pseudoacacia*, it is necessary to take into account the interindividual plant variability to the complex effects of biotic and technogenic stresses. Determining adaptive mechanisms of *R.* p*seudoacacia* in response to various stressful environmental conditions can be important to managing the maintenance and restoration of its plantings.

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