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Enzymatic antioxidant system in *Fraxinus* species and forms with varying susceptibility to black sawfly (*Tomostethus nigritus*)

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Degradation of European ash (*Fraxinus excelsior*) stands under the action of biotic stresses requires compensation for the loss of ash trees by searching for alternative local and introduced tree species. There is insufficient study on biochemical defense responses in the leaves of ash species and forms with various vulnerability to the damage caused by ash sawfly (*Tomostethus nigritus* F.). Our hypothesis is that there are differences in the resistance level of different ash tree species/forms to *T. nigritus* based on the study of their antioxidant enzyme system (AOS) activity and soluble protein (SP) content. The measurements of enzyme activity were carried out by spectrophotometric methods. Isoenzyme composition of benzidine peroxidase (BPx) was determined by isoelectric focusing. Based on visual observation, significant differences in susceptibility to black sawfly were found between three ash species and three ornamental forms of *F. excelsior*, the leaves of which were used for biochemical analyses. The results demonstrated a wide range of interspecific activity of enzymes and proteins, which indicates their involvement in plant adaptation to biotic stress. It has been shown that the high level of guaiacol-peroxidase (GPx) and catalase (CAT) activity in the leaves of ornamental forms may be related to their resistance to *T. nigritus*. The highest level of GPx activity and protein content in the Asian species *F. sogdiana* compared to other ash trees also indicates their important role and can give it benefits in protection against the leaf-chewing pest. Species-specificity of isoelectrofocusing (IEF) profiles of acidic isoperoxidases of BPx was recorded. The molecular forms of BPx with the highest level of activity in the species unaffected by *T. nigritus* were revealed. The highest inhibitory effect of the oligophage black sawfly on the antioxidant system and proteins was observed in the North American species *F. velutina*, which should be introduced with caution into urban green areas.

Keywords: *Fraxinus*; *Tomostethus nigritus*; soluble proteins; antioxidant enzymes; peroxidase isoenzyme composition.

Introduction

The surveys of recent years have shown a rapid dieback of ash trees in forests, green spaces of populated areas, as well as in field protection strips and roadside plantings. This picture is observed throughout Europe. The most important biotic factors found to be harmfully affecting the state of the *F. excelsior* population were the presence of the fungus *Hymenoscyphus fraxineus* (anamorph *Chalara fraxinea*) as a causative agent of ash dieback, and the emerald ash borer (*Agrilus planipennis*); their biology has been widely studied (Davydenko et al., 2019; Villari et al., 2016). Over the past 30 years, outbreaks of mass reproduction of *T. nigritus* (Fabricius, 1804) as a member of the family Tenthredinidae Latreille, 1802 (Hymenoptera) have been recorded in certain regions of Europe and Ukraine, complete defoliation of trees was sometimes noted in this period (Jess et al., 2017; Meshkova et al., 2017). One generation develops per year with a complete metamorphosis. Male larvae include 4 stages, and female larvae include 5 (Glavendekić & Mirić, 2011); intensive feeding on leaves occurs during these stages. *T. nigritus* is considered to be a narrow oligophagous species capable of feeding on a wide range of members of the genus *Fraxinus* L. Outbreaks can occur on non-endemic trees, such as *F. angustifolia* (Verheyde & Sioen, 2019). The insect is capable of spreading rapidly through urban infrastructure.

Introduction of plants is considered to be the most effective way to enrich and renew the plant complex in countries where novel plant species are used to benefit humans, as a way to maintain genetic diversity and to identify individual trees resistant to diseases and pests (Bačovsky et al., 2016; Nielsen et al., 2017; Kunakh et al., 2022; Miller & McMahan, 2022; Adamčíková et al., 2023). But, as noted by Lévesque et al. (2023), no native species alone or in combination can completely replace the ecological niche of European ash and related ecosystem services. This is further complicated by the fact that the productivity of non-native ash species in European forests and urban green space, their invasive potential and the risk of hybridization with native ash species are still unknown, and there is no scientifically based tolerance rating for most members of the genus *Fraxinus* (Percival et al., 2006; Lévesque et al., 2023).

The ash genus (*Fraxinus* L.) is composed of economically valuable woody plants; they include about 70 species and act as the major forest-forming component of the forests in temperate latitudes and mountain belts in the Northern Hemisphere as well as of Southeast Asian forests; they also widely used in green urban construction. A significant number of ash species are distributed in Europe, of which the common / European ash (*Fraxinus excelsior* L.) is the most common. It provides many ecosystem services, such as absorbing carbon and pollutants from air, soil and aquatic substrates, reducing flooding, minimizing the area of urban heat islands and maintaining wildlife in cities (Soldi et al., 2022).

In Ukraine, European ash (*F. excelsior* L.) is an autochthonous and forest-forming species of various native forests and a component of artificial plantations in agricultural landscapes and green infrastructure in cities. In urban green spaces, European ash is also represented by various varieties and decorative forms. Thus, according to Shlapak & Bayura (2014), in the cities of the Right Bank Forest Steppe of Ukraine, 95 decorative forms of *F. excelsior* are used in garden and park construction, among which are single-leaved 'Monofilla', multifolia 'Diversifolia' and the weeping 'Pendula'' (as the most commonly used). Besides the native ash species, non-native (introduced) species as Sogdian ash (*F. sogdiana* Bunge) (Tianshan ash) and velvet ash (*F. velutina*) (Arizona ash) grow only in the Botanical Garden of Oles Honchar Dnipro National University (DNU). Sogdian ash is distributed across Central Asia and China. This species is highly resistant to pest attacks (Nuer et al., 2014; Drenkhan et al., 2015). *F. velutina* is a fast-growing, light-demanding deciduous tree vulnerable to trunk burns; it tolerates alkaline, desert soils and is salt-resistant, but requires regular watering (Ma et al., 2022).

Stress caused by herbivorous insect pests disrupts various metabolic processes involved in woody plant resistance mechanisms. Therefore, understanding the basic mechanisms of resistance is crucial for the identification and use of new plant species resistant to insect phytophages. Under the effect of pests, oxidative stress occurs in plant cells, related to escalated production of reactive oxygen species (ROS). As a result, key physiological processes such as photosynthesis, respiration and water exchange are disrupted, accompanied by secondary metabolism pathways aimed at protecting plant cells against herbivore invasion (Singh et al., 2013). The formation of ROS is prevented by the antioxidant system, which includes low molecular weight antioxidants (glutathione, tocopherols, ascorbic acid), enzymes that regenerate reduced forms of antioxidants, and enzymes that interact with ROS (Blokhina et al., 2003; Koch et al., 2016; Paterska et al., 2017; Materska et al., 2022). The intracellular level of hydrogen peroxide is regulated by a wide range of enzymes, the most important of which are catalase and peroxidases (Willekens et al., 1995; Foyer et al., 2017). Peroxidases have a high affinity for hydrogen peroxide and are involved in its utilization in plant cell organelles such as chloroplasts, mitochondria, cytoplasm, vacuole and cell wall (Anjum et al., 2016). This enzyme is also involved in various biochemical reactions, such as nitrogen metabolism, respiration, the formation of ethylene and auxin, lignin and suberin synthesis (in cell-wall formation), growth processes, tissues maturation and aging, IAA degradation, wound repair and protection from stresses occurred in the plant body (Takashima et al., 2013; Mittler, 2017; Petrova et al., 2022; Sahu et al., 2022).

Currently, there is insufficient study on biochemical defense responses in the leaves of both native and introduced ash species and forms with various degrees of vulnerability to the damage caused by the ash sawfly. The goal of the study was to research an activity of the defense enzymatic antioxidant system and soluble proteins content in the leaves of species and forms of the genus *Fraxinus* differently vulnerable to *T. nigritus* F. attacks.

Materials and methods

Plants of the genus *Fraxinus* from the collection of the Botanical Garden of the Oles Honchar Dnipro National University (Dnipro, Ukraine) (48°26'14'' N, 35°02'35'' E) were used as the object of study. *F. excelsior* L. (3 trees) and its three ornamental forms "Monofilla", "Diversifolia", "Pendula" (2 trees each), *F. velutina* Torr. (1 tree) and *F. sogdiana* (2 trees), growing in groups close to each other under the same light and temperature conditions, were used in the study. Sawfly damage to the trees was investigated in June 2022. The level of crown damage was assessed on a 5-point scale (Pap et al. 2014): 1 – no visible damage; 2 – 10–30% damage; 3 – up to 50% damage; 4 – severe damage (50–75%); 5 – very severe damage (>75%). Two species (*F. velutina*, *F. excelsior*) and 1 decorative form ('Monofilla') were damaged by ash sawfly. *F. sogdiana*, 'Diversifolia' and 'Pendula' were without visible damage by the phytophage.

For biochemical analysis, 5–7 leaves of medium formation from each tree (in tree replicates) with no signs of insect damage were selected on annual vegetative growth from the lower third of the southern-faced crown part in dry, clear weather, simultaneously with each ash species (Fig. 1).

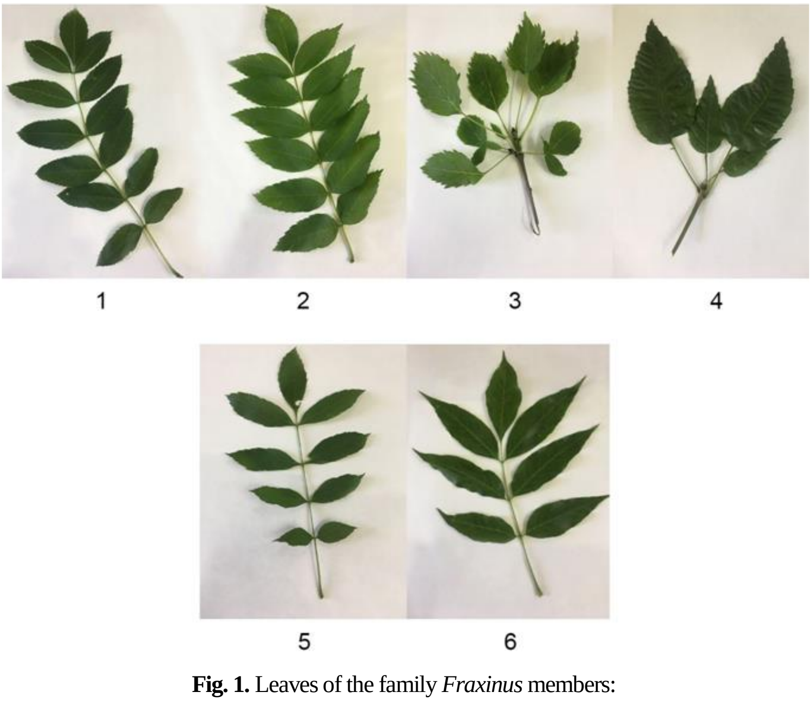

Fig. 1. Leaves of the family *Fraxinus* members:
1 – *F. excelsior* L.; *2* – 'Pendula'; *3* – 'Monofilla'; *4* – 'Diversifolia'; *5* – *F. velutina* Torr; *6* – *F. sogdiana* Bunge.

The leaves were washed with water and immediately used for enzyme extraction. To isolate the enzyme preparation, the ash 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 the extract was centrifugated for 20 minutes at 14,000 rpm. Supernatants were harvested to determine the content of lightly-soluble proteins (SP) and the activity and composition of benzidine-peroxidase, guaiacol-peroxidase, and catalase. The measurements of enzyme activity were carried out by spectrophotometric methods. Activity of benzidine-peroxidase (BPx, EC 1.11.1.7) [donor: hydrogen peroxide oxidoreductase] was determined by the Gregory method (1966). Changes in optical density were registered for 1 min at 490 nm after adding 1% H_2O_2 to the reaction mixture (Tris-HCL buffer, pH 7.4, 0.02 mM benzidine, and the sample volume 0.2 mL). The result was expressed in U/g FW. The protein content was determined with colorimetric analysis of the total coarse extract of the protein sample by the Bradford (1976) method and expressed in mg/g of fresh weight.

Isoenzyme composition of BPx was determined by isoelectric focusing (IEF) in a 5% horizontal polyacrylamide gel (PAAG) using Ultraphor (LKB, Bromma, Sweden), pH range 2.5–6.0. IEF was performed at +10 °C. The stained gels were scanned and analysed using 1D Phoretix software, by which the specific gravity (%) of each isoform in the total peroxidase spectrum was determined. For comparative analysis, the molecular forms of peroxidase were identified by Rf values (relative electrophoretic mobility of isoperoxidases).

Activity of guaiacol-dependent peroxidase (GPx, EC 1.11.1.7) was evaluated according to Ranieri et al. (2001) and expressed in mM TG/g FW * min. Catalase activity (CAT, EC 1.11.1.6) [hydrogenperoxide: hydrogen-peroxide oxidoreductase] was assessed according to Goth (1991), and the results were expressed in μM H_2O_2/mg protein.

The results of studies on enzyme activity and soluble protein content were presented as the average x ± SD (standard deviation). Tukey's honestly significant difference test was used to test differences between group means to assess the significance. The results obtained were subjected to univariate analysis of variance (ANOVA) using Statistica (StatSoftWare Past, 4.03, Norway) (Hammer et al., 2001).

Results

The resistance of plants was assessed by the degree of damage of the leaves during their invasion by the black sawfly (Table 1). Among the studied ash species/forms, the most affected by the black sawfly were *F. velutina* and *F. excelsior* (Table 1).

Minor damage to the crown was inherent in the ornamental form of European ash "Monofilla" (10%). Resistant to black sawfly were *F. sogdiana* and ornamental forms "Diversifolia" and "Pendula".

The analysis of the results revealed mainly the reliability of differences (P < 0.05) by enzyme activity and content of lightly-soluble proteins between the ash species and forms (Fig. 2).

Table 1
Degree of damage to species/forms of the genus *Fraxinus T. nigritus*

Species and forms of the genus *Fraxinus*	Tree age, yrs.	Level of tree damage by *Tomostethus nigritus* F., %	Geographic origin
European/common ash (*F. excelsior* Linnaeus, 1753)	75	40	Northern and Central Europe
'Monofilla'	15	10	
'Diversifolia'	75	0	
'Pendula'	75	5	
Tianshan ash (*F. sogdiana* Bunge)	73	<1	Central Asia and China
Velvet ash (*F. velutina* Torr)	75	94	South-Western North America

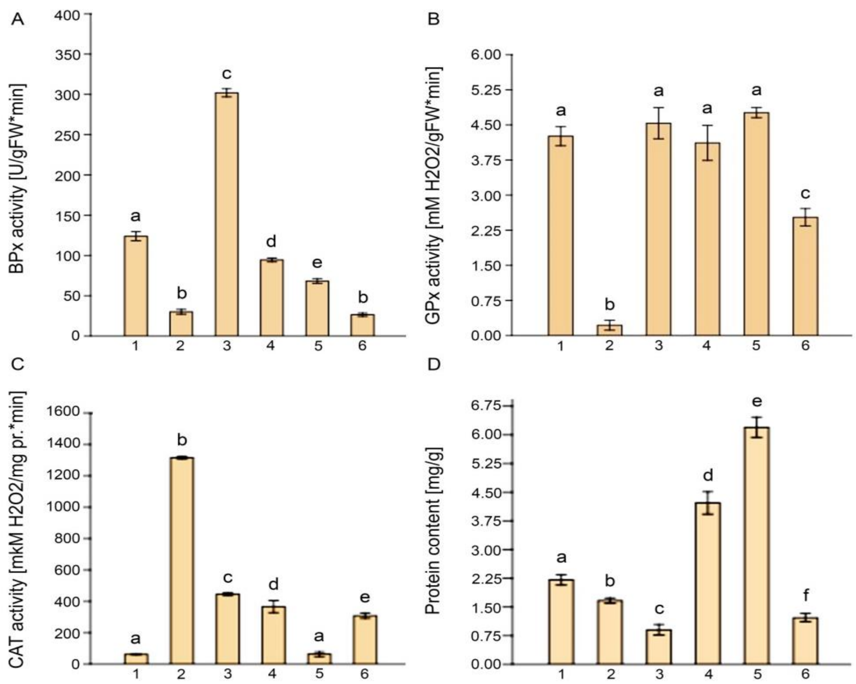

Fig. 2. Antioxidant enzyme activity and soluble protein content of *Fraxinus* species leaves: *A* – benzidine peroxidase, *B* – guaiacol peroxidase, *C* – catalase, *D* – proteins; means ± SD of five biological replicates; 1–6 – see Figure 1 different letters on the columns indicate significant differences according to Tukey-test, P < 0.05; n = 7

Among the studied species and forms, the value of benzidine-peroxidase activity in the leaves of the European ash form 'Diversifolia' was the greatest and exceeded that of native *F. excelsior* by 2.4 times, and of the forms 'Monofilla' and 'Pendula' by 10 and 3.2 times, respectively (P < 0.01). The introduced species Sogdian and velvet ash showed significantly less BPx activity compared to both *F. excelsior* (1.8 and 4.7 times, respectively) and its form 'Diversifolia' (4.4 and 11.4 times, respectively) (P < 0.01). The lowest activity was found in *F velutina* and in European ash form 'Monofilla' (Fig. 2A).

The values of guaiacol-peroxidase activity ranged within 0.22–4.76 mM TG/g FW (Fig. 2B). The values of activity in European ash form 'Monofilla' were very low (11.5–21.6 times less compared to the other forms and species studied) (P < 0.05). In two ash species (*F. excelsior* and *F. sogdiana*) and two forms ('Diversifolia' and 'Pendula'), this indicator was at the level of 4.12–4.76 mM TG/g FW * min). There was no significant difference in GPx activity between European ash and its forms 'Diversifolia' and 'Pendula' (P = 0.014; P = 0.182, respectively). The values of GPx activity in the leaves of *F. angusifolia* were higher compared to European ash by 11.7%.

The velvet ash had a lower GPx activity by 68.4%, 79.4% and 62.8% compared to native *F. excelsior* and its decorative forms 'Diversifolia' and 'Pendula', respectively, and by 88.1% (P < 0.05) compared to Sogdian ash.

Catalase activity in the studied ash leaves ranged within a wide range: 63.0 to 1315.2 μM H_2O_2/mg pr. (Fig. 2C). The lowest activity was observed in the leaves of *F. excelsior* and *F. sogdiana*, and no significant difference has been established between these ash species (P = 0.96). The greatest activity was found in the decorative form 'Monofilla'. Compared to other samples, the range of exceeding this indicator was at the level of 2.9–20.9 times. The levels in catalase activity of the forms 'Diversifolia' and 'Pendula', as well as the species *F. velutina* were approximately the same (308.8–446.9 μM H_2O_2/mg pr.). Compared to the native European ash species, CAT activity in the leaves of the forms 'Diversifolia', 'Pendula' and the species *F. velutina* was 7.1, 5.8, and 4.9 times greater, respectively ($P < 0.01$).

The total number of benzidine-peroxidase isoenzymes found in all samples was 10 (Fig. 3A-D).

Fraxinus excelsior includes 6 isoperoxidases, while its decorative form 'Monofilla' includes 2, 'Diversifolia' and 'Pendula' includes 4 ones (Fig. 3A).

Fraxinus sogdiana and *F. velutina* include 3 and 1 isoforms, respectively. Only one component of Rf = 0.48 was consistently present in all ash species and forms studied, but its expression was significantly different (Fig. 3B, C). A greater proportion of this isoform was inherent in the decorative form 'Pendula', narrow-leaved and velvet ash, and the lowest proportion was found in European ash. Compared to *F. excelsior*, the activity of this zone was significantly greater in *F. sogdiana* by 96.4% ($F = 50.36$; $P = 2.1\ 10^{-3}$), in *F. velutina* by 71.7% ($F = 76.9$; $P = 9.0\ 10^{-4}$). In the leaves of decorative forms, the activity of the isoform of Rf = 0.48 was slightly greater in 'Monofilla' by 21% ($F = 1.94$; $P = 0.237$); in the forms 'Diversifolia' and 'Pendula' it was significantly increased compared to native European ash by 39.4% and 73.8%, respectively ($F = 22.72$; $P = 8.9\ 10^{-3}$; $F = 37.86$; $P = 3.5\ 10^{-3}$). The presence of isoperoxidase of Rf 0.73 was common in *F. excelsior* and its decorative forms. Activity of this isoform in the decorative ash forms was almost at the same level with native European ash ($P > 0.05$). Isoperoxidase of Rf 0.55 was found to be common in the ash form 'Pendula', and isoperoxidase of Rf = 0.61 was found to be common in the form 'Diversifolia' (the isoperoxidase proportion in this form was less by 37.8%), but no statistically significant difference between *F. angusifolia* and European ash form 'Diversifolia' was observed ($F = 4.34$; $P = 0.106$).

The protein content also ranged widely from 0.90 ('Diversifolia') up to 6.20 (*F. sogdiana*) respectively (Fig. 2D). A statistically significant difference in protein content between the native ash species and its decorative forms 'Monofilla' and 'Diversifolia' showed significantly lower SP content compared to *F. excelsior*: by 24.4 ($F = 238.8$; $P < 0.001$) and by 59.3% ($F = 838.8$; $P < 0.001$). In the decorative form 'Pendula' and the species *F. sogdiana* significantly increased SP content was found compared to *F. excelsior* by 90.9 ($F = 754.7$; $P < 0.001$) and by 180% ($F = 3403.2$; $P < 0.001$), respectively. A statistically significant difference in this parameter was observed between the native species and the introduced velvet ash: in the latter, the content of SP was up 44.8% less ($F = 628.0$; $P < 0.001$) compared to *F. excelsior*.

Discussion

Taking into account the importance of studying oxidoreductases and proteins as well as their variations between different genotypes in the genus *Fraxinus*, the response of activity and composition of enzymes and proteins in following 3 ash species and 3 decorative forms: *F. excelsior* L., its forms 'Monofilla', 'Diversifolia', 'Pendula', *F. sogdiana* Bunge (section *Fraxinus*); as well as North American species *F. velutina* Torr. (section *Fraxinus*) were investigated in relation to black sawfly (*T. nigritus*) attacks. Verheyde & Sioen (2019) found that defoliation mainly affected *Fraxinus angustifolia* Vahl, 1804 'Raywood', while neighboring trees of *F. excelsior* were mostly unaffected. On the contrary, our research in 2022 in the Botanical Garden of DNU (steppe zone of Ukraine) recorded an outbreak of ash sawfly (*T. nigritus*), which affected the European ash (by 40%) and its decorative form 'Monofilla' (by 10%) and, especially, the introduced species *F. velutina* (by 95%), while the Sogdian ash was almost unaffected (<1%). This is consistent with the data of Matošević et al. (2003), who observed severe defoliation of *F. excelsior*, but found that *F. angustifolia* at the same time was almost unaffected. Interestingly, two decorative forms *F. excelsior* 'Diversifolia' and 'Pendula' were not affected by *T. nigritus*. These data indicate a different vulnerability of the members of the genus *Fraxinus* to effects caused by *T. nigritus*. Therefore, the study of plant-phytophage relationships at the biochemical level is important for understanding the nature of adaptation of both native and introduced *Fraxinus* members to the action of the herbivorous insect.

Based on the study of the activity of peroxidases (BPx and GPx) in the leaves of ash species and forms, it can be stated that this parameter reflects the degree of plant adaptation to the environment. According to BPx activity in European ash and its forms, the following rating was established (from less to more vulnerable to the insect affect): 'Diversifolia' → *F. excelsior* → 'Pendula' → 'Monofilla'. It should be noted that *Fraxinus excelsior* maintained a moderate level of peroxidase activity despite a fairly high level of pest damage (40%). By the research of Petrova et al. (2022), *Fraxinus excelsior* is a species very suitable for urban landscaping due to its significant tolerance to stressful environmental conditions, and it has a significant adaptive reserve which has not yet been realized. If one compares the vulnerability of introduced ash species to ash sawfly with a native species by this indicator, then the lowest response of BPx was noted in *F. velutina*.

Native and introduced ash species differ in both activity and component composition of benzidine-peroxidase. Isoenzyme spectra of peroxidases are used to detect interspecies differences, plant ontogenetic age, and stress effects (Prisedsky et al., 2017). Analysis of peroxidase isoenzyme composition revealed a clear distinction between the ash species studied, as well as the distinction based on the relative proportions of isoperoxidases that were common to all species studied. A study of the isoenzyme profiles of benzidine-peroxidase in various ash species and forms revealed a total of 10 isoperoxidases within the acidic pH range (2.5–6.0). The peroxidase profiles of the studied ash species and forms were similar, representing their close phylogenetic relationship. One component of Rf 0.48 was consistently present in all ash species and forms studied, but its expression was significantly different. The greatest proportion of this isoform (20.5%, 20.8% and 23.5%) was found in Sogdian ash, the decorative form 'Pendula' and velvet ash, respectively, and the smallest was found in native European ash (12%). In native European ash, this fact was compensated by the high activity of isoperoxidase of Rf 0.73 (17.7%). It should be noted that the presence of isoperoxidase of Rf 0.73 was the common in *F. excelsior* and its decorative forms, but it was not present in the introduced species. Activity of this isoform in decorative ash forms was almost at the level same with native European ash. Thus, the results showed a clear species specificity of the IEF profiles of BPx ash trees, which differed in both the number and activity of isoperoxidases. Similar results have been found in other woody plant species. Lehner & Cardemil (2003) determined changes in cell wall peroxidase activity and isoform profiles of seedlings of the legumes *Prosopis tamarugo* Phil. and *Prosopis chilensis* Mol. to assess their resistance to wounding. According to their data, a significant increase in the activity of acidic isoforms in *P. tamarugo* was observed, while in *P. chilensis* the activity level of the main isoforms of peroxidase increased. It has been shown that the invasion of the pest *P. robiniella* enhanced the defence system of *R. pseudoacacia* leaves by stimulating the activity of antioxidant enzymes and changing the expression of isoperoxidases (Seliutina et al., 2020; Shupranova et al., 2023).

The study of guaiacol-peroxidase activity in ash trees of various species and forms showed that the greatest activity was recorded in the leaves of Sogdian ash, European ash and its forms 'Diversifolia' and 'Pendula', and the smallest activity was observed in *F. velutina* and, especially, in the decorative form 'Monophylla' (19.3 times less compared to *F. excelsior*). High values of GPx activity may be associated with its participation in the processes of stress adaptation to unfavorable environmental conditions and, especially, with the enhancement of the lignification process (physical defense of cells) under the action of biotic stresses (Miller & McMahan, 2022). Poland et al. (2015) reported that in most resistant *Fraxinus* taxa, the majority of emerald ash borer (*Agrilus planipennis*) larvae died because of the physical defense response of the host plant. Therefore, in the leaves of Sogdian ash, a high level of guaiacol-peroxidase activity may be associated with an increase in the lignification process under the action of biotic stresses.

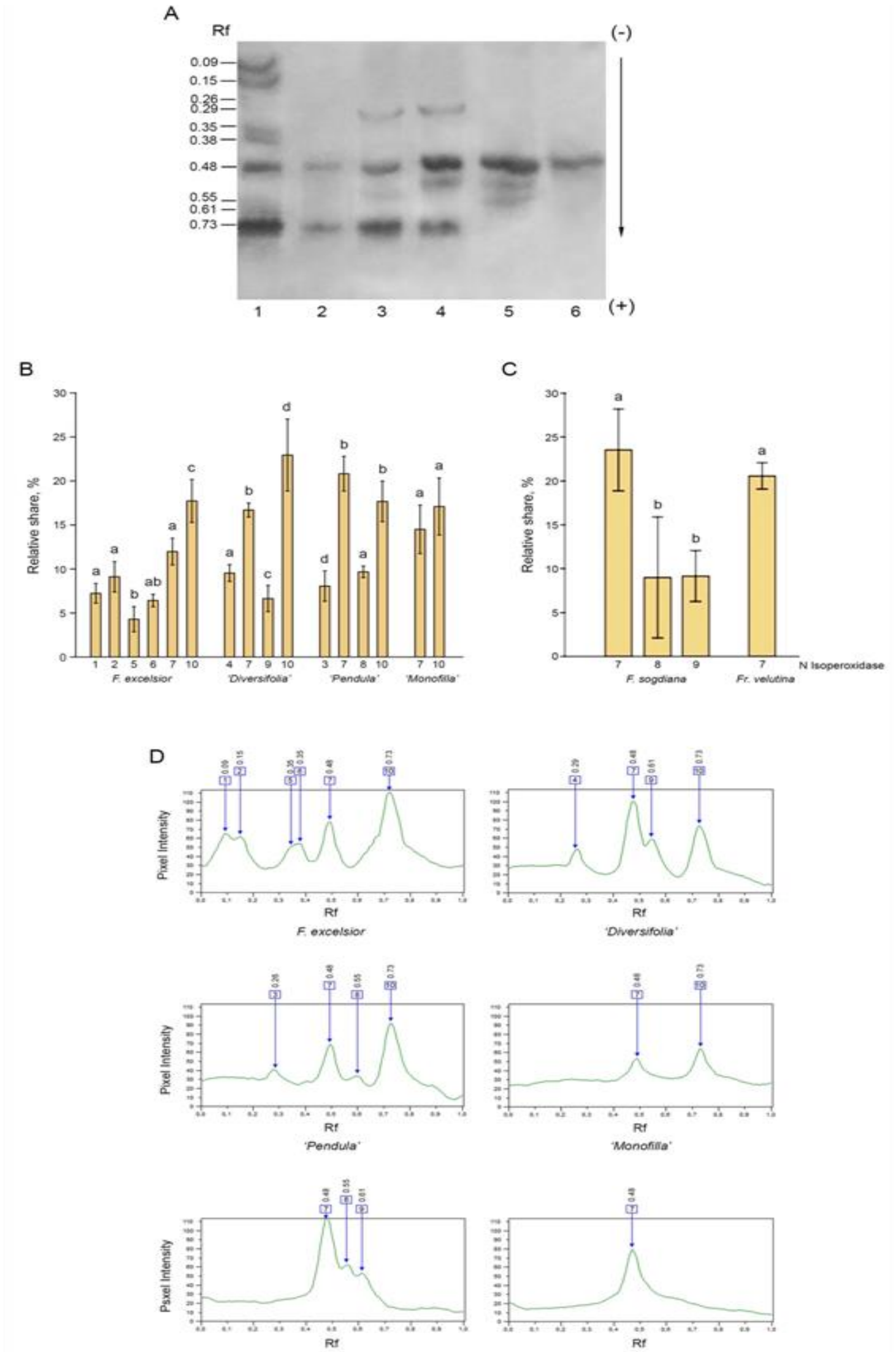

Fig. 3. IEF analysis of BPx leaves of genus *Fraxinus*: *A* – IEF profiles of peroxidase; Rf – values of electrophoretic mobility of isoperoxidases; *B*, *C* – the relative share of isoforms (%, n = 7); *D* – densitometric analysis

Catalase is actively involved in the utilization of hydrogen peroxide in plants, and in such a way that it contributes to the maintaining of reduced equivalents and energy in cells (Sahu et al., 2022). As our studies have shown, the highest catalase activity was found in the leaves of European ash, single-leaf form 'Monofilla', and the lowest was found in native European ash; it can be arranged as follows (from larger to smaller values): 'Monofilla' → 'Diversifolia' → 'Pendula' → *F. excelsior*. The ash species showed the following rating regarding CAT activity: *F. velutina* → *F. sogdiana* = *F excelsior*. The obtained data showed the important role of catalase in antioxidant processes in leaves of decorative forms of *F. excelsior* due to the elimination of hydrogen peroxide during biotic stress. Our results suggest that increased CAT proportion in the total antioxidant activity in leaves of *F. excelsior* 'Monofilla' indicates compensation of the reduced BPx and GPx activity and increased participation of the enzyme in the mechanism of photosynthetic defense process (Shupranova et al., 2022). The high constitutive level of CAT activity in the leaves of the decorative form 'Monofilla' in view of very low peroxidase activity was probably sufficient to provide defence response against the black sawfly attacks.

Whilst the ecological importance of plant soluble proteins and available carbohydrates in the development of plant-insect interactions is known, there are relatively few studies on their concentration in the plant leaves (Deans et al., 2016). To date, there is little information available to support variations in plant protein profiles for native and introduced species of *Fraxinus* genus, and especially on differences related to the effects of herbivorous insects on the trees. Analysis of the soluble protein content in leaves of 3 tree species (*F. excelsior*, *F. velutina*, *F. sogdiana*) and 3 decorative forms of *F. excelsior* ('Monofilla', 'Diversifolia', 'Pendula') showed high variability of this parameter. According to the SP content, the studied ash trees can be divided into three groups: I – leaves with the lowest content ('Diversifolia'); II –leaves with an average SP content: *F. velutina*, 'Monofilla' and *F. excelsior*); III – leaves with the greatest protein content (*F. sogdiana* and the forms 'Pendula'). In general, according to this indicator, the rating of ash trees can be arranged as follows: *F. sogdiana* → 'Pendula' → *F. excelsior* → 'Monofilla' → *F. velutina* → 'Diversifolia'. Under conditions of various stress types, a decrease in metabolic processes were usually observed, including a decrease in the rate of protein synthesis, which has a protective nature maintaining the vital potential of cells for their further recovery (Zogli et al., 2020). In the case studied, protein content was observed to be the greatest in insect-unaffected ash trees (*F. sogdiana* and 'Pendula'). A similar result was obtained for the species *A. parviflora*, which exhibits high resistance to the horse chestnut leaf miner *Cameraria ohridella* (Shupranova et al., 2024). SPs concentration was observed to be the lowest in the form 'Diversifolia' resistant to black sawfly attacks; these patterns require additional research.

The results of this study confirmed the hypothesis that antioxidant enzymes and leaf proteins are important components of the defense system in the interaction between *Fraxinus* plants and the herbivorous pest *T. nigritus*.

Conclusions

The obtained results showed a wide-range interspecies variability in the enzyme activity of antioxidant defense system and protein content in the leaves of European ash (as a native species), its decorative forms and of Sogdian and velvet ash (as introduced species). Different vulnerability of the studied ash trees to the impact of *Tomostethus nigritus* F. was found. Antioxidant enzymes in decorative European ash forms 'Diversifolia' and 'Pendula', as well as the species *F. sogdiana* were revealed to be more effective in the development of resistance to the attacks of black sawfly. Among other ash species, the North American species *F. velutina* was the most vulnerable to the insect impact; it was characterized by the lowest peroxidase and catalase activity. The results of the study can be beneficial for appreciation of the adaptive capacity of ash species in the conditions of the Northern steppe of Ukraine when introduced in green urban planning.

The authors declare no conflict of interest.

References

Adamcikova, K., Pažitný, J., & Pastirčáková, K. (2018). Individual resistance of *Fraxinus angustifolia* and *F. excelsior* clones to *Hymenoscyphus fraxineus*. Journal of Plant Protection Research, 58, 227–232.

Aldibekova, A., Kurmanbayeva, M., Aksoy, A., Permitina, V., Dimeyeva, L., & Zverev, N. (2023). Anatomical structure and phytochemical composition of a rare species *Fraxinus sogdiana* Bunge (Oleaceae) growing in different soils in Kazakhstan. Diversity, 15(6), 769.

Anjum, N. A., Sharma, P., Gill, S. S., Hasanuzzaman, M., Khan, E. A., Kachhap, K., Mohamed, A. A., Thangavel, P., Devi, G. D., Vasudhevan, P., Sofo, A., Khan, N. A., Misra, A. N., Lukatkin, A. S., Singh, H. P., Pereira, E., & Tuteja, N. (2016). Catalase and ascorbate peroxidase – representative H_2O_2-detoxifying heme enzymes in plants. Environmental Science and Pollution Research, 23(19), 19002–19029.

Bačovský, V., Vyhnánek, T., Hanáček, P., Mertelík, J., & Šafránková, I. (2017). Genetic diversity of chestnut tree in relation to susceptibility to leaf miner (*Cameraria ohridella* Deschka & Dimić). Trees, 31, 753–763.

Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxygen damage and oxygen deprivation stress: A review. Annals of Botany, 91, 179–194.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72, 248–254.

Bussotti, F., Pollastrini, M., Holland, V., & Bruggeman, W. (2015). Functional traits and adaptive capacity of European forests to climate change. Environmental and Experimental Botany, 111, 91–113.

Chen, J., Shen, Z. J., Lu, W. Z., Liu, X., Wu, F. H., Gao, G. F., Liu, G. L., Wu, C. S., Yan, C. L., Fan, H. Q., Zhang, Y. H., Zheng, H. L., & Tsai, C. L. (2017). Leaf miner-induced morphological, physiological and molecular changes in mangrove plant *Avicennia marina* (Forsk.) Vierh. Tree Physiology, 37, 82–97.

Davydenko, K., Borysova, V., Shcherbak, O., Kryshtop, Y., & Meshkova, V. (2019). Situation and perspectives of ash (*Fraxinus* spp.) in Ukraine: Focus on eastern border. Baltic Forestry, 25, 193–202.

Deans, C. A., Spencer, T., Behmer, S. T., Fiene, J., & Sword, G. A. (2016). Spatio-temporal, genotypic, and environmental effects on plant soluble protein and digestible carbohydrate content: Implications for insect herbivores with cotton as an exemplar. Journal of Chemical Ecology, 42, 1151–1163.

Drenkhan, R., Adamson, K., & Hanso, M. (2015). *Fraxinus sogdiana*, a Central Asian ash species, is susceptible to *Hymenoscyphus fraxineus*. Plant Protection Science, 51, 150–152.

Foyer, C. H., Ruban, A. V., & Noctor, G. (2017). Viewing oxidative stress through the lens of oxidative signalling rather than damage. Biochemical Journal, 474, 877–883.

Glavendekić, M., & Mirić, M. (2011). Štetočina jasena *Tomostethus nigritus* F. (Hymenoptera: Tenthredinidae). Biljni Lekar, 39, 639–644.

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta, 196, 143–152.

Gregory, R. P. F. (1966). A rapid assay for peroxidase activity. Biochemical Journal, 101, 582–583. https://doi.org/10.1042/bj1010582

Hammer, Ø., Harper, D. A. I., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4, 4.

Jess, S., Murchie, A., & Allen, D. (2017). First observation of *Tomostethus nigritus* (Fabricius) (Hymenoptera: Tenthredinidae) on urban ash trees in Ireland. Irish Naturalists' Journal, 35, 134–136.

Karban, R., & Baldwin, I. (1997). Induced responses to herbivory. University of Chicago Press, Chicago.

Koch, K. G., Chapman, K., Louis, J., Heng-Moss, T., & Sarath, G. (2016). Plant tolerance: A unique approach to control hemipteran pests. Frontiers in Plant Science, 7, 1363.

Kunakh, O. M., Ivanko, I. A., Holoborodko, K. K., Lisovets, O. I., Volkova, A. M., Nikolaieva, V. V., & Zhukov, O. V. (2022). Modeling the spatial variation of urban park ecological properties using remote sensing data. Biosystems Diversity, 30(3), 213– 225.

Lehner, G., & Cardemil, L. (2003). Differences in wound-induced changes in cell-wall peroxidase activities and isoform patterns between seedlings of *Prosopis tamarugo* and *Prosopis chilensis*. Tree Physiology, 23, 443–452.

Levesque, M., Eduardo, J. I. B., & Queloz, V. (2023). Potential alternative tree species to *Fraxinus excelsior* in European forests. Frontiers in Forests and Global Change, 6, 1048971.

Ma, X., Liu, J. N., Yan, L., Liang, Q., Fang, H., Wang, C., et al. (2022). Comparative transcriptome analysis unravels defense pathways of *Fraxinus velutina* Torr. against salt stress. Frontiers in Plant Science, 13, 842726.

Materska, M., Pabich, M., Sachadyn-Król, M., Konarska, A., Weryszko-Chmielewska, E., Chilczuk, B., Staszowska-Karkut, M., Jackowska, I., & Dmitruk, M. (2022). The secondary metabolites profile in horse chestnut leaves infested with horse-chestnut leaf miner. Molecules, 27, 5471.

Meshkova, V., Kukina, O., Zinchenko, O., & Davydenko, K. (2017). Three-year dynamics of common ash defoliation and crown condition in the focus of black sawfly *Tomostethus nigritus* F. (Hymenoptera: Tenthredinidae). Baltic Forestry, 23, 303–308.

Miller, F., & McMahan, E. (2022). Examining resistance of Asian, European, and North American ash and elm species to the emerald ash borer (*Agrilus planipennis*) (Coleoptera: Buprestidae). Great Lakes Entomologist, 55, 21–44.

Mittler, R. (2017). ROS are good. Trends in Plant Science, 22, 11–19.

Nielsen, L. R., McKinney, L. V., Hietala, A. M., & Kjær, E. D. (2017). The susceptibility of Asian, European and North American *Fraxinus* species to the ash dieback pathogen *Hymenoscyphus fraxineus* reflects their phylogenetic history. European Journal of Forest Research, 136, 59–73.

Nuer, M. A., Zhang, W., Yang, X. R., & Jia, N. (2014). Phenotypic plasticity on component size of compound leaves of *Fraxinus sogdiana* in Yili, Xinjiang. Journal of Northeast Forestry University, 42, 31–34 (in Chinese).

Pandey, V. P., Awasthi, A., Singh, S., Tiwari, S., & Dwivedi, U. N. (2017). A comprehensive review on function and application of plant peroxidases. Biochemistry and Analytical Biochemistry, 6, 308.

Pap, P., Stojnić, S., Nikolić, N., Orlović, S., Marković, M., Vasić, V., & Stevanov, M. (2014). Impact of *Erysiphe alphitoides* (Griffon & Maubl.) on leaf physiological parameters in pedunculate oak (*Quercus robur* L.) saplings. Baltic Forestry, 20, 2–9.

Paterska, M., Bandurska, H., Wysłouch, J., Molińska-Glura, M., & Moliński, K. (2017). Chemical composition of horse-chestnut (*Aesculus*) leaves and their susceptibility to chestnut leaf miner *Cameraria ohridella* Deschka & Dimić. Acta Physiologiae Plantarum, 39, 105.

Percival, G. C., Keary, I. P., & Al-Habsi, S. (2006). An assessment of the drought tolerance of *Fraxinus* genotypes for urban landscape plantings. Urban Forestry and Urban Greening, 5, 17–27.

Seliutina, O. V., Shupranova, L. V., Holoborodko, K. K., Shulman, M. V., & Bobylev, Y. P. (2020). Effect of *Cameraria ohridella* on accumulation of proteins, peroxidase activity and composition in *Aesculus hippocastanum* leaves. Regulatory Mechanisms in Biosystems, 11(2), 299–304.

Shlapak, V., & Bajura, O. (2014). Yasen zvychainyi (*Fraxinus excelsior* L.) ta yoho dekoratyvni formy v Pravoberezhnomu Lisostepu Ukrainy [Common ash (*Fraxinus excelsior* L.) and its ornamental forms in the right-bank Forest-Steppe of Ukraine]. Proceedings of the Forest Academy of Sciences of Ukraine, 12, 86–90 (in Ukrainian).

Shupranova, L. V., Holoborodko, K. K., Zhukov, O. V., Loza, I. I., Pakhomov, O. E., & Domnich, V. I. (2023). Effects of *Parectopa robiniella* on the antioxidant defense system in *Robinia pseudoacacia* trees of different ages. Taiwan Journal of Forest Science, 38, 249–263.

Shupranova, L., Holoborodko, K., Loza, I., Zhukov, O., & Pakhomov, O. (2022). Assessment of *Parectopa robiniella* Clemens (Gracillariidae Stainton, 1854) effect on biochemical parameters of *Robinia pseudoacacia* under conditions of an industrial city in Steppe Ukraine. Ekológia (Bratislava), 41(4), 340–350.

Shupranova, L., Holoborodko, K., Zhukov, O., Shulman, M., Loza, I., Seliutina, O., & Ivanko, I. (2024). Antioxidant activity and resistance traits of *Aesculus* species against *Cameraria ohridella* Deschka & Dimić infestation in the Ukraine steppe. Dendrobiology, 92, 89–100.

Singh, H., Dixit, S., Verma, P. C., & Singh, P. K. (2013). Differential peroxidase activities in three different crops upon insect feeding. Plant Signaling and Behavior, 8, e25615.

Soldi, E., Fuller, E., Tiley, A., Murphy, B. R., & Hodkinson, T. R. (2022). First report of the ash sawfly, *Tomostethus nigritus*, established on *Fraxinus excelsior* in the Republic of Ireland. Insects, 13(1), 6.

Verheyde, F., & Sioen, G. (2019). Outbreaks of *Tomostethus nigritus* on *Fraxinus angustifolia* ‘Raywood’ in Belgium. Journal of Hymenoptera Research, 72, 67–81.

Villari, C., Herms, D. A., Whitehill, J. G. A., Cipollini, D., & Bonello, P. (2016). Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer. New Phytologist, 209, 63–79.

War, A. R., Taggar, G. K., Hussain, B., Taggar, M. S., Nair, R. M., & Sharma, H. C. (2018). Plant defence against herbivory and insect adaptations. AoB Plants, 10, ply037.

Wilkaniec, A., Borowiak-Sobkowiak, B., Irzykowska, L., Breś, W., Świerk, D., Pardela, L., Durak, R., Środulska-Wielgus, J., & Wielgus, K. (2021). Biotic and abiotic factors causing the collapse of *Robinia pseudoacacia* veteran trees in urban environments. PLoS One, 16, e0245398.

Willekens, G. L., Inzé, D., Van Montagu, M., & Van Camp, W. (1995). Catalase in plants. Molecular Breeding, 1, 207–228.

Zhu-Salzman, K., Bi, J. L., & Liu, T. X. (2005). Molecular strategies of plant defense and insect counter-defense. Insect Science, 12, 3–15.

Zogli, P., Alvarez, S., Naldrett, M. J., Palmer, N. A., & Koch, K. G. (2020). Greenbug (*Schizaphis graminum*) herbivory significantly impacts protein and phosphorylation abundance in switchgrass (*Panicum virgatum*). Scientific Reports, 10, 14842.