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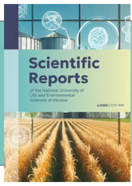

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Assessment of frost and drought resistance in species and cultivars of the genus *Ligustrum* L. for nursery stock production

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Abstract. The relevance of this study is driven by the need to improve the assessment of plants' adaptive potential for urban landscaping, particularly in Kyiv, where factors such as water deficiency and temperature fluctuations are critical in selecting species capable of withstanding environmental stress while maintaining ornamental value. This study aimed to investigate the frost and drought resistance of species and cultivars of the genus *Ligustrum* L., enabling an evaluation of their adaptive capacity for use in urban environments. Physiological methods were employed to assess indicators such as water retention capacity, water deficit, water content, heat resistance, and frost resistance. The findings indicated that *Ligustrum vulgare* L. and its cultivars exhibit high frost resistance, as evidenced by minimal tissue damage at -25°C, averaging 7.0-13.9 points. Other species demonstrated minor tissue damage at -20°C (on average, less than 2 points), suggesting that this temperature is not critical for them. At -25°C, the overall damage score increased significantly. In particular, *Ligustrum ovalifolium* Hassk. and its cultivars exhibited scores ranging from 14.2 to 24.8, while *Ligustrum japonicum* Green Century and *Ligustrum ibota* Musli recorded scores of 30.7 and 26.3, respectively. These values approach critical thresholds; however, during the growing season, cells and tissues demonstrated the ability to

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recover. *Ligustrum vulgare* L., *Ligustrum ovalifolium* Hassk., and certain cultivars displayed considerable drought resistance, as indicated by relatively minor fluctuations in leaf moisture content. The results also highlight the stable waterregulating function of these species, along with high heat resistance, particularly at 50°C. Notably, an increase in temperature to 60°C proved critical for all species, leading to partial browning or complete necrosis of leaf blades. The assessment of these parameters facilitates the selection of initial material for the production of resilient nursery stock suited to urban environmental conditions

Keywords: nursery stock; leaf blade; landscaping; water regime; resilience; frost damage

Introduction

One of the key aspects of developing plant compositions for urban landscaping is the assessment of their condition and resilience to stress factors, which enables the selection of an optimal plant assortment for greening large cities. Therefore, understanding plant sensitivity to stressors, considering the impact of both natural and anthropogenic factors on growth and development, is essential. As a highly urbanised environment, the city of Kyiv is characterised by challenging ecological conditions. As noted by I.O. Sviatohorov (2024), these include temperature fluctuations, limited summer precipitation, and a relatively high level of pollution. Thus, evaluating the adaptive potential of species within the genus *Ligustrum* L. under such conditions is a crucial task in urban landscaping.

S.I. Kuznetsov *et al.* (2020) described *L. vulgare* L. as a hardy plant, capable of withstanding both drought and frost. It is also adaptable to various soil types and tolerates urban conditions, including dust, smoke, and gas pollution. Additionally, this species is noted for its ability to be pruned and shaped, making it versatile for landscaping. *Ligustrum lucidum* W.T. Aiton, on the other hand, is considered moderately frost-resistant, able to withstand temperatures down to -20°C. It requires moderate moisture and is tolerant of smoke and gas pollution. T. Tkaczenko *et al.* (2023) highlight the potential of *L. vulgare* L. to partially purify air by trapping particulate matter, thus improving air quality. T. Pashayev (2024) emphasised the versatility of *L. vulgare* L. in urban landscaping. It is particularly well-suited for creating hedges and other structured plantings. This species' ability to withstand fluctuating temperatures and moisture levels makes it a valuable asset in urban green spaces. Its resilience and aesthetic appeal allow it to function as both a decorative feature and a functional barrier. Furthermore, *L. vulgare* L. exhibits strong recovery after winter, making it a reliable choice for long-term use in diverse climates.

Evaluating how well plants withstand drought and heat is crucial for determining their suitability in urban environments. In cities, high temperatures and water shortages are significant challenges for plant growth. V. Bessonova *et al.* (2024) have emphasised the importance of studying the heat resistance of trees in the northern steppe region of Ukraine. These factors directly affect the survival and aesthetic appeal of plants exposed to intense sunlight and high temperatures. Similarly, research is being conducted to assess drought tolerance. D.S. Gordienko *et al.* (2021) examined the impact of climate on the resilience of English roses in the forest-steppe zone of Ukraine. L. Leotta *et al.* (2023) have focused on developing new strategies to enhance the resistance of ornamental trees to abiotic stresses (nonliving stresses such as drought or high temperatures) in Mediterranean climates. This research is essential for developing methods to help plants adapt to climate change in urban settings. The use of physiological methods to measure factors like water deficit, water content, water-holding capacity, and heat resistance provides valuable quantitative data. These measurements can reveal how well plants adapt to harsh temperatures (Demydov *et al.*, 2023; Yavas *et al.*, 2024).

J. Dong *et al.* (2022) have highlighted the critical role of aquaporins, located in cell membranes, in maintaining water balance under various stress conditions. Research has demonstrated that aquaporins directly contribute to a plant's cold tolerance. Specifically, the authors studied the aquaporin gene family in *Ligustrum* × *Vicaryi* Rehd. The use of species and cultivars of the genus *Ligustrum* L. in urban landscaping requires a thorough understanding of their adaptive potential, particularly their frost and drought resistance. This study aimed to evaluate the drought and frost resistance of *Ligustrum* L. species and cultivars within this genus.

Materials and Methods

The study on the potential drought and frost tolerance was conducted between 2023 and 2024. The research took place at the Plant Physiology and Microbiology Laboratory of the Institute of Horticulture, National Academy of Agrarian Sciences of Ukraine. The study involved species and cultivars of the genus *Ligustrum* L., specifically *Ligustrum vulgare* L., *L. vulgare* Atrovirens, *L. vulgare* Aurea, *Ligustrum ovalifolium* Hassk., *L. ovalifolium* Aureum, *L. ovalifolium* Green Diamond, *L. ovalifolium* Vicari, *Ligustrum ibota* Musli, and *Ligustrum japonicum* Green Century. The research adhered to ethical standards outlined in the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

To assess drought and frost tolerance, vegetative plant material was collected from mother plants aged 3-5 years. The selected plants were free from any visible damage caused by pests or diseases. These plants were grown in semi-shaded conditions on moderately fertile and moist soils. The frost tolerance of *Ligustrum* L. species and cultivars was evaluated using the methodology recommended by M.O. Bublyk *et al.* (2013). This involved directly freezing plant shoots in a laboratory setting. During the winter dormancy period, typically the second ten-day period of December to the first ten-day period of February, well-formed, one-year-old shoots (20-25 cm in length) were collected from the middle section of the plant crowns.

The process of determining frost tolerance involved lowering the temperature within a freezer chamber. A DW-40L92 refrigeration chamber (Qingdao Haier Biomedical Co. Ltd., China) was used, which allowed for the cooling of the experimental plant samples down to -40°C. The freezing was conducted using a gradual temperature reduction method, decreasing by approximately 5°C increments until reaching -20°C and -25°C. These specific temperature levels were selected to reflect the average winter temperatures observed in Kyiv between 2019 and 2024. The lowest recorded temperature during this period was -18°C (Meteoblue, n.d.). After freezing the oneyearold shoots of *Ligustrum* L. species and cultivars at -20°C and -25°C, a microscopic analysis was performed to assess tissue damage in comparison to the control samples (unfrozen). Once the desired temperature was reached, the experimental samples were held in the refrigeration chamber for 4 hours. This duration was necessary to allow for nucleation and ice formation processes to occur within the plant tissues. The next step involved determining the extent of frost damage to the shoot tissues. This evaluation was based on the intensity of browning observed in anatomical cross-sections. After freezing, a certain period of time is required for the signs of frost damage to become apparent. As noted by S. Maslovata (2016), it can take up to 7 days at room temperature for signs of frost damage to become visible. The next stage involved partially immersing the shoots in water, followed by the preparation of anatomical sections. Three primary zones were selected for analysis: the shoot apex, the middle section (the centre of the internode, between the buds), and the node itself, with the cut made directly through the bud. These sections were placed on microscope slides and examined under an MBS-10 microscope (TM MICROmed, Ukraine). During the anatomical-microscopic evaluation of the one-year-old growth, the extent of damage to individual tissues (bark, cambium, wood, and pith) was determined based on the visual browning observed in the cross-section. The degree of tissue damage was assessed using a six-point scale (0 to 5 points) for each anatomical section. The maximum total tissue damage score for the one-year-old growth was 65 points (Bublyk *et al*., 2013).

The damage assessment followed this scale: 0 – no damage observed; 1 – slight discolouration, damage to up to 20% of the tissue; 2 – moderate tissue damage (40%); 3 – moderate damage with clear browning at the interface with adjacent tissues (60%); 4 – severe damage (complete browning of the affected area, blackened borders with adjacent tissues, 80%); 5 – complete tissue necrosis (100%) (Maslovata, 2016).

To determine the potential drought tolerance, a series of laboratory experiments were conducted. These experiments included measuring water deficit, water content, water-holding capacity, and heat resistance, following the Scientific and Methodological Recommendations for the Study and Conservation of Genetic Resources of Fruit, Berry, Nut-Bearing, and Underutilized Crops (Trokhymchuk & Makarova, 2022). For the drought tolerance assessments, fully grown leaf blades were collected, primarily from the central part of the mother plants' crowns. The leaves were harvested early in the morning, before 8:00, to minimise rapid turgor loss. They were then placed in sealed bags to facilitate long-term transport. The collection period occurred during the first ten-day period of August.

The water deficit was determined as follows: initially, the leaf blades were weighed using precision scales. They were then immersed in a water-filled container, which was covered with glass to create an air chamber. The leaves remained submerged for 24 hours. After this period, they were removed from the water, gently blotted with paper towels to remove excess moisture, and immediately weighed again. The difference between the pre-saturation and post-saturation weights was then calculated.

To assess the water content, a method involving complete drying of leaf blades was employed. This allowed for the calculation of the total water content within the plant material. For this, previously prepared leaf blades from species and cultivars of *Ligustrum* L. were placed in metal weighing bottles, which had been weighed beforehand. The weighing bottles with the leaf blades were then weighed again. The weighing bottles with the leaf blades were then placed in an oven at 40°C for 24 hours or until the leaves were fully dried. After drying, the weighing bottles with the plant material were weighed again, and the corresponding calculations for the moisture content in the leaves were performed. The total water content (W) as a percentage of the fresh weight was determined using the following formula (1):

$$W = \frac{(b-c)}{(b-a)} * 100\%, \tag{1}$$

where a is the mass of the empty weighing bottle, g; b is the mass of the weighing bottle with the fresh leaf sample, g; c is the mass of the weighing bottle with the dry leaf sample, g. The water retention capacity was determined by measuring the gradual loss of moisture at room temperature. The experiment was conducted in a laboratory setting with a constant temperature of 23.8°C and a relative humidity of 44.5%. At the start of the experiment, the leaf blades were weighed, and subsequent measurements were taken after 2, 4, 6, and 24 hours. The water retention capacity (WRC) is considered higher if the moisture loss from the leaf blades is lower over a specific time period, and it is calculated using the formula (2):

$$WRC = \frac{B}{A} * 100\%, \tag{2}$$

where A is the moisture content at the start of the experiment, g; B is the moisture loss over a specific time period, g. The determination of heat resistance involved subjecting the leaf blades of the experimental plants to exposure to heat in an oven for 10 minutes at different temperatures: 40, 50, and 60°C. After roasting, the leaf blades were immersed in a 0.2% hydrochloric acid (HCl) solution, which primarily facilitated the faster manifestation of damaged areas on the leaves. The immersion times in the acid were 1, 3, and 5 minutes. Afterwards, the damage was assessed according to the following scale in percentages: 0 – leaves without damage; 0-15 – slight damage; 16-50 – moderate damage; 51-100 – severe damage or complete leaf blade death. The processing and analysis of the results were carried out in Microsoft Excel (Mezhenskyi, 2017). The measurement results are presented as the mean ± standard error (x ± SE).

Results and Discussion

The analysis of damage data on shoots revealed that the severity of tissue damage caused by low negative temperatures is influenced by both the species or cultivar and the degree of temperature reduction. Specifically, the control samples, which were not subjected to freezing, showed no damage in any of the tested plants, as illustrated in Figure 1 (*a, d, g, j*), Figure 2 (*a, d, g*), and Figure 3 (*a, d*).

Figure 1. *Degree of tissue damage in cultivars of Ligustrum ovalifolium Hassk*

Note: *a, d, g, j* – control; *b, e, h, k* – freezing temperature -20°C; *c, f, i, l* – freezing temperature -25°C

Source: photos taken by the authors

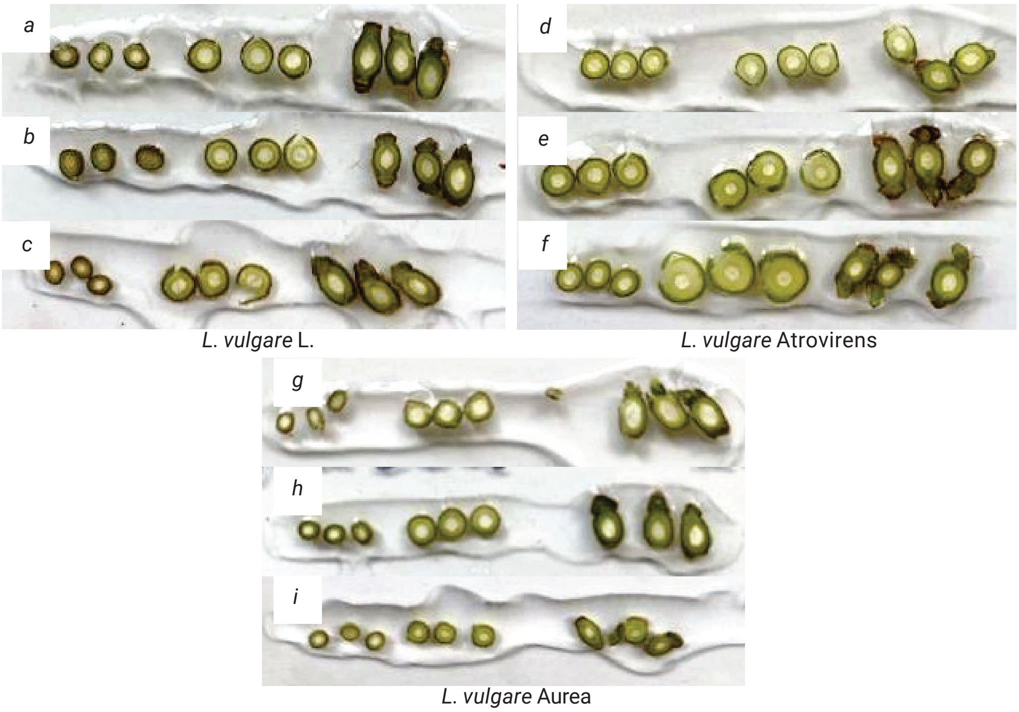

Figure 2. *Degree of tissue damage in cultivars of Ligustrum vulgare L*
Note: *a, d, g* – control; *b, e, h* – freezing temperature -20°C; *c, f, i* – freezing temperature -25°C
Source: photos taken by the authors

Figure 3. *Degree of tissue damage in cultivars of Ligustrum japonicum Green Century, Ligustrum ibota Musli*
Note: *a, d, g, j* – control; *b, e, h, k* – freezing temperature -20°C; *c, f, i, l* – freezing temperature -25°C
Source: photos taken by the authors

Analysis of the data at -20°C revealed that the majority of the experimental plant samples showed minimal damage, with scores not exceeding 20 points. This suggests a relatively high tolerance to temperatures down to -20°C. The observed damage primarily consisted of browning in certain areas of the shoot's anatomical sections, as shown in Figure 1 (*b, e, h, k*), Figure 2 (*b, e, h*), and Figure 3 (*b, e*). *Ligustrum japonicum* Green Century showed the highest damage score at 22 points. Browning was observed in various parts of the shoot (Fig. 3*b*), with the bud area being particularly affected. All other species and cultivars showed only slight browning. The least amount of damage was observed in *L. vulgare* Atrovirens, with a total damage score of 3.7 points.

Further lowering the temperature to -25°C resulted in more significant damage to almost all the samples. The total damage scores for most species and cultivars increased considerably. This indicates a potential sensitivity of certain plants to this temperature. The lowest level of damage was recorded in *L. vulgare* Atrovirens (7.0 points), while the highest levels were observed in *Ligustrum japonicum* Green Century (29.9 points) and *Ligustrum ibota* Musli (26.3 points).

The freezing process at -25°C revealed that different parts of the *Ligustrum* L. shoot experienced varying degrees of damage. This indicates that the tissues have differing levels of resistance to low-temperature stress. As shown in Table 1, the pith was the most vulnerable to low temperatures across almost all the tested plants. Notably, *Ligustrum japonicum* Green Century and *Ligustrum ibota* Musli showed maximum pith damage, particularly at the bud and in the middle section of the shoot. For example, in *Ligustrum japonicum* Green Century, the pith damage score at the bud was 10.8 points, the highest recorded (Fig. 3*c*). The wood tissue also sustained significant damage, especially in the cultivars *L. ovalifolium* Vicari (6.5 points in the middle section of the shoot) and *L. ovalifolium* Aureum (6.5 points in the upper section of the shoot). *L. vulgare* Atrovirens exhibited the least amount of wood damage (1.4 points). The cambium was less susceptible to low temperatures compared to the pith and wood. However, some plants, such as *L. ovalifolium* Green Diamond (5.2 points in the middle section), showed significant cambial damage (Fig. 1*f*). The lowest level of cambium damage was observed in *L. vulgare* Atrovirens (0.5 points in the middle section). Similar to the cambium, the bark showed relatively less damage, as indicated in Table 1. For instance, *L. vulgare* L. and *L. vulgare* Atrovirens had the lowest bark damage scores, ranging from 0.5 to 0.8 points.

Table 1. *Damage to shoots and buds from low temperatures in Ligustrum L. species and cultivars, 2024*

No.	Species, cultivar	Variant	Object of freezing																
			Apex, points				Middle, points				Through the bud, points					Total Points			
			Bark	Cambium	Wood	Pith	Bark	Cambium	Wood	Pith	Bark	Cambium	Wood	Pith	Bud	Apex	Middle	Through bud	Total
1	*Ligustrum ovalifolium* Hassk.	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		-20	0.5	0.5	1.2	1.2	0.5	0.4	1.0	1.0	0.8	0.5	1.0	1.0	1.2	3.4	2.9	3.3	9.6
		-25	1.5	1.8	1.8	1.5	0.5	0.5	0.4	2.0	1.2	1.1	1.5	2.2	2.0	6.6	3.4	6.0	16.0
2	*L. ovalifolium* Green Diamond	K	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2
		-20	0.9	0.8	1.8	1.2	0.8	0.8	1.5	1.0	0.8	0.9	1.5	0.9	1.2	4.7	4.1	4.1	12.9
		-25	1.2	1.1	2.0	1.5	1.1	1.1	1.8	1.2	1.0	1.0	2.5	1.5	4.0	5.8	5.2	6.0	17.0
3	*L. ovalifolium* Vicari	C	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	1.0	0.0	1.0	2.0
		-20	0.0	0.0	1.0	0.0	0.5	0.5	1.0	1.1	0.5	1.1	1.4	2.0	1.0	1.0	3.1	5.0	9.1
		-25	3.5	3.5	2.5	3.0	0.8	1.0	2.0	1.5	1.0	1.5	2.5	2.0	3.0	12.5	5.3	7.0	24.8
4	*L. ovalifolium* Aureum	K	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.5	0.5	0.5	0.5	1.4	1.0	2.9
		-20	0.5	1.5	1.5	1.0	0.5	1.4	1.4	1.2	0.5	1.8	2.2	2.0	2.8	4.5	4.5	6.5	15.5
		-25	1.5	2.5	2.2	2.0	0.8	2.0	2.5	2.0	0.8	1.5	2.0	2.2	3.5	8.2	7.3	6.5	22.0
5	*Ligustrum vulgare* L.	C	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.2	0.6
		-20	0.5	0.5	0.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.3	2.3	0.0	0.5	2.8
		-25	2.5	2.5	3.0	1.0	0.5	0.5	0.8	0.5	0.5	0.5	1.0	0.6	1.0	9.0	2.3	2.6	13.9

Table 1. *Continued*

No.	Species, cultivar	Variant	Object of freezing																
			Apex, points				Middle, points				Through the bud, points					Total Points			
			Bark	Cambium	Wood	Pith	Bark	Cambium	Wood	Pith	Bark	Cambium	Wood	Pith	Bud	Apex	Middle	Through bud	Total
6	*L. vulgare* Atrovirens	K	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.2	0.2	0.2	0.6
		-20	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.6	0.5	0.8	0.8	1.2	0.5	0.5	2.7	3.7
		-25	0.5	0.5	0.8	0.5	0.5	0.8	0.7	0.5	0.4	0.5	0.7	0.6	1.4	2.3	2.5	2.2	7.0
7	*L. vulgare* Aurea	C	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.4
		-20	0.5	0.5	0.4	0.0	0.4	0.4	0.5	0.0	0.4	0.4	0.5	0.0	0.5	1.4	1.3	1.3	4.0
		-25	0.6	0.5	0.8	0.5	0.5	0.5	0.6	0.5	0.6	0.6	1.5	0.5	1.0	2.4	2.1	3.2	7.7
8	*Ligustrum japonicum* Green Century	C	0.8	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.5	1.2	1.8	0.0	1.3	3.1
		-20	2.2	2.0	1.5	1.4	1.8	1.8	1.5	1.8	1.8	1.5	2.5	2.2	2.3	7.1	6.9	8.0	22.0
		-25	2.5	2.7	2.5	1.8	2.2	2.4	2.5	2.5	2.2	2.4	3.2	3.0	3.5	9.5	9.6	10.8	29.9
9	*Ligustrum ibota* Musli	C	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.5	0.5	0.9	0.5	0.0	1.4	1.9
		-20	1.3	1.4	1.8	0.8	1.2	1.3	1.8	0.9	2.2	2.2	2.8	1.0	2.4	5.3	5.2	8.2	18.7
		-25	2.5	2.5	2.0	1.5	2.2	2.6	2.0	2.0	2.5	2.5	2.2	1.8	3.3	8.5	8.8	9.0	26.3

Source: developed by the authors

Thus, analysing all the data, it can be seen that *L. vulgare* Atrovirens is the most resistant to low temperatures. The overall damage level was minimal, with a total of 7.0 points, and the most damaged part was the pith in the middle section of the shoot. In contrast, *Ligustrum japonicum* Green Century and *Ligustrum ibota* Musli showed the highest levels of damage, with the wood and pith being the most affected. The results of the study show that at -25°C, the pith of the shoots sustains the most damage, particularly in the upper part and through the bud. Accordingly, the bark and cambium suffer less damage, although the extent of the damage also depends on the species and cultivar. Among the plants studied, *L. vulgare* Atrovirens can be considered potentially resistant to low temperatures, while *L. japonicum* Green Century and *L. ibota* Musli are less resistant. Water deficit is a crucial indicator of a plant's ability to retain moisture, which helps determine its potential drought resistance. The analysis of water deficit data for *Ligustrum* L. species and cultivars (Table 2) revealed that the values of this parameter varied significantly depending on the species and cultivar.

Table 2. *Determination of water deficit in Ligustrum L. species and cultivars*

Species, cultivar	Before saturation, g	After saturation, g	Water deficit, %	Average value, %
Ligustrum vulgare L.	0.702	0.813	13.65	$16.25^{\pm 2.8}$
	0.307	0.38	19.21	
	0.545	0.648	15.90	
L. vulgare Atrovirens	0.538	0.604	10.93	$11.77^{\pm 1.35}$
	0.39	0.45	13.33	
	0.37	0.416	11.06	
L. vulgare Aurea	0.334	0.396	15.66	$14.00^{\pm 1.47}$
	0.38	0.436	12.84	
	0.359	0.415	13.49	
Ligustrum ovalifolium Hassk.	0.927	1.112	16.64	$16.14^{\pm 0.48}$
	0.886	1.056	16.10	
	0.828	0.982	15.68	

Table 2. Continued

Species, cultivar	Before saturation, g	After saturation, g	Water deficit, %	Average value, %
L. ovalifolium Aureum	0.43	0.486	11.52	$11.39^{\pm 0.74}$
	0.414	0.463	10.58	
	0.343	0.39	12.05	
L. ovalifolium Green Diamond	0.343	0.386	11.14	$11.34^{\pm 0.3}$
	0.325	0.368	11.68	
	0.254	0.286	11.19	
L. ovalifolium Vicari	0.17	0.184	7.61	$6.16^{\pm 1.36}$
	0.11	0.117	5.98	
	0.097	0.102	4.90	
Ligustrum ibota Musli	0.227	0.245	7.35	$10.65^{\pm 3.17}$
	0.22	0.247	10.93	
	0.202	0.234	13.68	
Ligustrum japonicum Green Century	0.33	0.352	6.25	$6.42^{\pm 1.97}$
	0.443	0.484	8.47	
	0.442	0.463	4.54	

Source: developed by the authors

Thus, the highest water deficit values were observed in *L. vulgare* L. (16.25%), *L. vulgare* Aurea (14.00%), and *L. ovalifolium* Hassk. (16.14%). Among the other plants, those with a moderate level of water deficit can be highlighted, including *L. vulgare* Atrovirens (11.77%), *L. ovalifolium* Aureum (11.39%), *L. ibota* Musli (10.67%), and *L. ovalifolium* Green Diamond (11.34%). *L. ovalifolium* Vicari (6.16%) and *L. japonicum* Green Century (6.42%) showed the lowest water deficit levels, as shown in Figure 4.

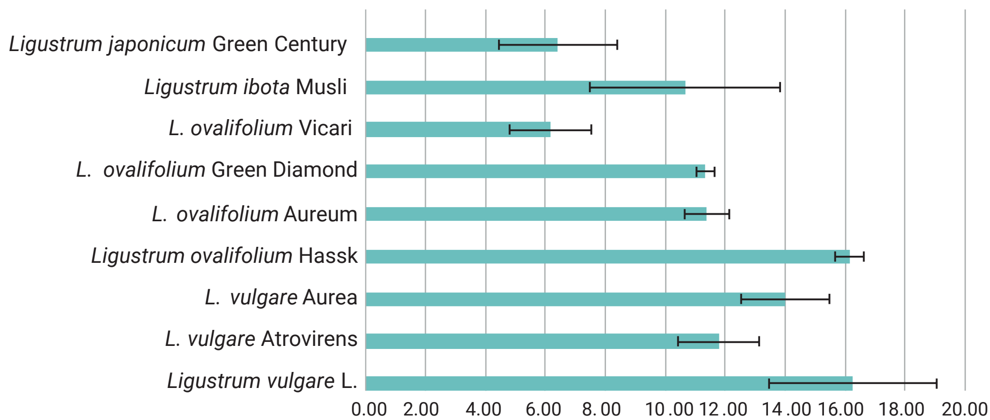

***Figure 4.** Water deficit values of leaves in Ligustrum L. species and cultivars*
Source: developed by the authors

High saturation levels were observed in *L. vulgare* L., *L. vulgare* Aurea, and *L. ovalifolium* Hassk. (Fig. 5). This suggests that these plants may be less adaptable to conditions with limited moisture. Conversely, *L. ovalifolium* Vicari and *L. japonicum* Green Century exhibited the lowest saturation levels, indicating a potential ability to withstand drought.

Figure 5. *Consequences of moisture oversaturation in the leaf blades of L. ovalifolium Hassk*
Source: photo taken by the authors

Water content is a key parameter that reflects the level of moisture retention in plant tissues, particularly leaf blades. This indicator allows for the assessment of a plant's ability to adapt to its growing conditions, especially drought. In this study, the water content of *Ligustrum* L. species and cultivars was determined. Similar to previous findings, significant variability in results was observed depending on the species and cultivar. Among the plants with the highest water content, exceeding 70%, *L. vulgare* Aurea (71.3%), *L. ovalifolium* Aureum (77.9%), *L. ovalifolium* Vicari (74.1%), and *L. ibota* Musli (76.3%) stand out. Of these, *L. ovalifolium* Aureum and *Ligustrum ibota* Musli have the highest values, exceeding 75%, indicating their high moisture retention capacity even under potentially unfavourable conditions (Fig. 6). All other plants exhibited moderate water content, suggesting their ability to maintain a stable water balance within their tissues. It is also worth noting that cultivars such as *L. vulgare* Atrovirens and *L. japonicum* Green Century had relatively lower water content, suggesting a lesser adaptive ability to conditions of insufficient moisture.

An interesting observation is that cultivars with variegated leaves, such as white-green or yellow-green (*L. vulgare* Aurea, *L. ovalifolium* Aureum, *L. ovalifolium* Vicari, and *Ligustrum ibota* Musli), tend to have comparatively higher water content than species and cultivars with typical green leaves.

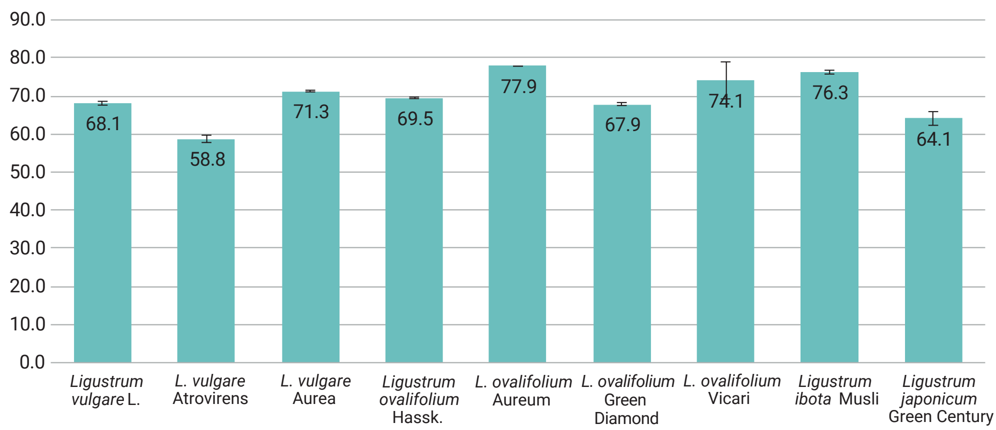

Figure 6. *Water content of the leaves of Ligustrum L. species and cultivars*
Source: developed by the authors

The analysis of water content in the *Ligustrum* L. genus allows for the identification of species and cultivars with a greater ability to retain moisture, such as *L. vulgare* Aurea, *L. ovalifolium* Aureum, *L. ovalifolium* Vicari, and *L. ibota* Musli. It also highlights those with a lower capacity, including *L. vulgare* L., *L. vulgare* Atrovirens, *L. ovalifolium* Hassk., *L. ovalifolium* Green Diamond, and *L. japonicum* Green Century. The results suggest the potential drought resistance of these plants and their suitability for urban landscaping, particularly in areas with limited water availability.

The study of water retention capacity in the leaf blades of *Ligustrum* L. species and cultivars is a crucial aspect for assessing their potential drought resistance. The experiment was conducted under stable laboratory conditions (temperature 23.8°C, relative humidity 44.5%), allowing for a more accurate evaluation of moisture loss dynamics over 24 hours. Similar to previous experiments, some variability in results was observed, depending on the species and cultivar of the tested plants. The highest average rate of moisture loss was observed in *L. ovalifolium* Aureum (44.66%) and *L. ibota* Musli (42.71%) (Table 3, Fig. 7). Notably lower moisture loss was observed in *L. ovalifolium* Vicari (37.59%) and *L. ovalifolium* Green Diamond (36.12%).

Table 3. *Water retention capacity of Ligustrum L. leaves*

Plant name	Sample weight by exposure time, g				Average water loss rate, %
	2 hours	4 hours	6 hours	24 hours	
Laboratory results	**24°C – 44%**	**24.2°C – 43%**	**23.7°C – 49%**	**22.8°C – 45%**	
Ligustrum vulgare L.	10.13	15.95	20.43	47.67	23.26
	11.11	16.78	19.61	42.27	
	9.27	15.12	20.49	50.24	
L. vulgare Atrovirens	12.97	22.87	32.08	57.00	31.26
	11.11	20.94	31.20	53.85	
	14.07	25.48	35.74	57.79	
L. vulgare Aurea	8.93	13.75	17.87	45.70	24.73
	8.28	16.55	21.38	57.93	
	8.97	16.67	21.15	59.62	
Ligustrum ovalifolium Hassk.	2.24	4.17	5.77	19.55	10.22
	3.82	7.64	10.62	36.73	
	1.80	4.09	6.22	19.97	
L. ovalifolium Aureum	19.46	32.89	44.30	79.19	44.66
	21.13	38.03	52.82	78.87	
	17.44	29.65	43.02	79.07	
L. ovalifolium Green Diamond	10.24	27.56	40.16	64.57	36.12
	11.76	27.73	39.50	68.07	
	12.23	26.62	36.69	68.35	
L. ovalifolium Vicari	9.09	25.76	33.33	71.21	37.59
	12.16	39.19	37.84	70.27	
	8.70	27.54	37.68	78.26	
Ligustrum ibota Musli	15.60	31.21	41.13	79.43	42.71
	17.58	36.26	45.05	76.92	
	17.74	29.03	43.55	79.03	
Ligustrum japonicum Green Century	8.12	16.24	18.78	40.44	22.51
	7.86	17.30	21.38	48.74	
	9.69	16.25	19.69	45.63	

Source: developed by the authors

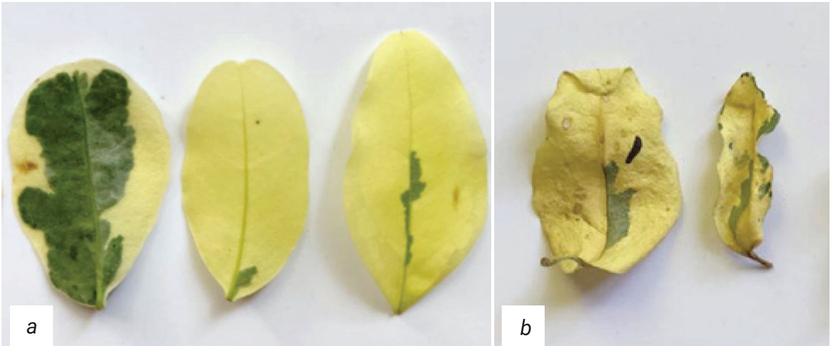

Figure 7. *Dynamics of moisture loss from leaves, illustrated by Ligustrum ibota Musli.*
Note: *a* – before the experiment; *b* – after 24 hours
Source: photos taken by the authors

Significantly lower rates of moisture loss were observed in *L. japonicum* Green Century (22.51%), *L. vulgare* L. (23.26%), and *L. vulgare* Aurea (24.73%). Among these, *L. ovalifolium* Hassk. exhibited the lowest rate of water loss (10.22%). The best water retention capacity was found in *L. ovalifolium* Hassk. and *L. japonicum* Green Century, while the fastest rate of moisture loss was observed in *L. ovalifolium* Aureum and *L. ibota* Musli. It is also worth noting that in all tested plants, the most intense moisture evaporation occurred between 6 and 24 hours into the experiment. At a temperature of 40°C, all species and cultivars showed no damage to the leaf blades, even after treatment with hydrochloric acid for 5 minutes (Table 4, Fig. 8*a*). This indicates their high resistance at this temperature.

Table 4. *Dynamics of the effect of high temperatures on leaf condition*

No.	Plant name	Time of hydrochloric acid treatment, min	Temperature of roasting, °C		
			40	50	60
			Percentage of leaf damage, %		
1	*Ligustrum vulgare* L.	1	0	4	81
2		3	0	5	85
3		5	0	7	97
4	*L. vulgare* Atrovirens	1	0	8	68
5		3	0	14	74
6		5	0	13	89
7	*L. vulgare* Aurea	1	0	4	80
8		3	0	6	82
9		5	0	6	88
10	*Ligustrum ovalifolium* Hassk.	1	0	5	90
11		3	0	2	96
12		5	0	3	100
13	*L. ovalifolium* Aureum	1	0	7	72
14		3	0	8	84
15		5	0	9	92
16	*L. ovalifolium* Green Diamond	1	0	0	85
17		3	0	0	90
18		5	0	0	96

Table 4. *Continued*

No.	Plant name	Time of hydrochloric acid treatment, min	Temperature of roasting, °C		
			40	50	60
			Percentage of leaf damage, %		
19	*L. ovalifolium* Vicari	1	0	0	73
20		3	0	1	84
21		5	0	2	95
22	*Ligustrum ibota* Musli	1	0	5	62
23		3	0	6	77
24		5	0	6	89
25	*Ligustrum japonicum* Green Century	1	0	2	93
26		3	0	5	95
27		5	0	6	100

Note: 0 – no damage; 0-15 – light damage; 16-50 – moderate damage; 50-100 – severe damage or complete leaf death
Source: developed by the authors

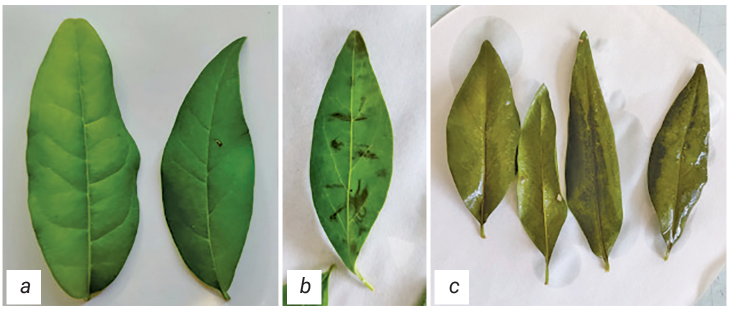

Figure 8. *Leaf blades of Ligustrum vulgare L. after roasting*
Note: *a* – 40°C, *b* – 50°C, *c* – 60°C
Source: photos taken by the authors

At elevated temperatures of up to 50°C, *L. vulgare* L. and its cultivars exhibited slight damage to the leaf blades, which was only 1-5%. When *L. vulgare* Atrovirens (Fig. 8*b*) was soaked in hydrochloric acid for 3-5 minutes, the damage increased to 20%. In contrast, the cultivars of *L. ovalifolium* Hassk. remained almost undamaged, with the damage percentage ranging from 0-1%, as shown in Figure 9*a*. Similarly, the leaf blades of *L. ibota* Musli and *L. japonicum* Green Century showed minimal damage, with browning percentages of 5-6%. Increasing the temperature to 60°C resulted in critical leaf damage. High levels of damage were observed in all *L. vulgare* L. cultivars (Fig. 8*c*), with damage ranging from 80% to 100%. The cultivars of *L. ovalifolium* (Fig. 9*b*) showed similar results, with leaf damage between 70% and 100%.

The research demonstrated a significant correlation between temperature and the susceptibility of *Ligustrum* L. species and cultivars to leaf blade damage. While the plants remained resistant to damage at 40°C, a rise in temperature to 50°C, and particularly 60°C, led to increased and critical damage across most cultivars. Similar studies focused on determining frost resistance, with the aim of identifying potentially resilient species and cultivars, provided a foundational basis for this investigation. These studies, such as those conducted by M.S. Kuznetsova & O.I. Kitaiev (2018), assessed the frost and winter hardiness of *Calluna vulgaris* (L.) Hill. and its cultivars. This involved laboratory freezing methods, as well as visual damage assessments under field conditions. Additionally, O. Babyn *et al.* (2024) detailed a research process examining the potential frost resistance of *Cercis* L. species and cultivars, which also employed laboratory freezing of experimental plant shoots at -20°C and -25°C.

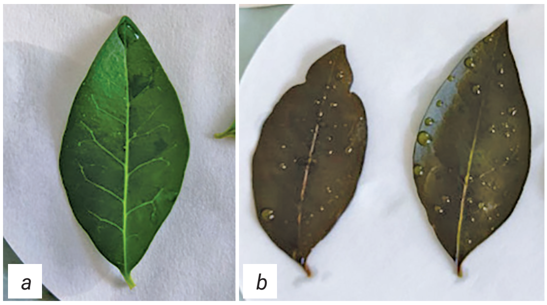

Figure 9. *Leaf blades of Ligustrum ovalifolium Hassk. after roasting*
Note: *a* – 50°C, *b* – 60°C
Source: photos taken by the authors

When determining drought resistance indicators, including heat resistance and water retention capacity, the data obtained were similar to those reported by V.M. Novosad (2014). It was established that *L. vulgare* L. exhibits higher resistance to high temperatures. However, when the temperature rises to 60°C, necrosis occurs in 80-90% of the leaf blade. *L. ovalifolium* Aureum showed a significantly lower tolerance to high temperatures, and upon reaching 60°C, the leaf blades discoloured, indicating complete tissue death. Furthermore, the water retention capacity study revealed that *L. ovalifolium* Aureum is less drought-resistant than *L. vulgare* L. After just 30 minutes, the maximum moisture content in the tested leaves was 15%, increasing to 25% after 90 minutes. In contrast, the maximum moisture content in *L. vulgare* L. leaves was 13.4%, and 23.4% at the end of the experiment. The findings of V.M. Novosad (2014) were analysed and expanded to include a wider range of studied plants. Certain aspects of the research were corroborated.

Researchers, including O. Babyn *et al.* (2024), have explored drought resistance indicators such as water retention capacity, water deficit, and heat resistance. Their study, focusing on the *Cercis* L. genus, aimed to determine the plants' ability to withstand dry periods in urban environments. Y. Telepenko (2018) assessed the drought tolerance of 25 blackberry (*Rubus* L.) varieties in the western Forest-Steppe region of Ukraine. This involved analysing the water-physical properties of leaves using varietal study methods and employing analysis of variance. The study revealed variations in water retention capacity, water deficit, and tissue water content, allowing for the classification of varieties based on their drought tolerance levels. S. Toscano *et al.* (2018) investigated the responses of *Lantana camara* L. and *Ligustrum lucidum* L. to four different water deficit regimes. The results indicated that the water deficit significantly reduced the biomass of both species. However, *Ligustrum lucidum* L. exhibited superior adaptive capabilities and more rapid stomatal regulation.

Research by V.Ya. Zayachuk (2014) indicated that approximately 40 *Ligustrum* L. species exist globally, primarily distributed across Asia, Europe, Australia, and North Africa, with *L. vulgare* L. being native to Ukraine. An examination of the ornamental plant market, particularly the plants cultivated and sold by leading Ukrainian nurseries and garden centres – such as Natalis nursery, Proxima garden centre, and Zelena Kraina ornamental plant nursery – reveals a significant expansion in plant variety to meet landscaping demands.

Consequently, over the past 5-10 years, there has been a notable increase in the range of *Ligustrum* L. species and cultivars. This expansion provides opportunities to utilise not only the indigenous *L. vulgare* L. but also around five other species and numerous cultivars. The *Ligustrum* L. genus is gaining popularity due to its adaptability, evident in both its aesthetic qualities and its tolerance to diverse growing conditions.

Similar research on drought resistance has been carried out by Y.M. Kruhlyak (2010), focusing on various *Salix* L. species and hybrids. Additionally, T. Kopylova (2016) examined the water balance in *Pyracantha* M. Roem. leaf blades and assessed the plants' ability to withstand high temperatures, subsequently evaluating their drought resistance. These methodological approaches were applied in this study to analyse the water regime and drought tolerance of *Ligustrum* L. species, confirming the effectiveness of using physiological parameters to evaluate plant adaptive potential in urban environments.

Conclusions

The ability of *Ligustrum* L. species and cultivars to withstand low temperatures varies significantly. In particular, the *Ligustrum vulgare* Atrovirens cultivar demonstrated the highest resistance to low temperatures, evidenced by minimal damage levels (7.0 points at -25°C). Conversely, *Ligustrum japonicum* Green Century and *Ligustrum ibota* Musli exhibited the lowest resistance, displaying the highest damage scores, especially in the wood and pith regions. The nature of tissue damage indicates that the pith of the shoots is the most vulnerable, particularly at the shoot tip and through the buds, while the cambium and bark proved more resilient to low-temperature effects. Indicators characterising potential resistance to drought and elevated temperatures, including water retention capacity, water content, water deficit, and heat resistance, also showed considerable variability depending on the species and cultivar. The highest water deficit values were observed in *L. vulgare* L. (16.25%), *L. vulgare* Aurea (14.00%), and *L. ovalifolium* Hassk. (16.14%). Moderately low water deficit values were recorded for *L. ovalifolium* Vicari (6.16%) and *L. japonicum* Green Century (6.42%). Among the plants exhibiting the highest water content, variegated cultivars such as *L. vulgare* Aurea, *L. ovalifolium* Aureum, *L. ovalifolium* Vicari, and *L. ibota* Musli stood out, with levels exceeding 75%. The best water retention capabilities were observed in *L. ovalifolium* Hassk. and *L. japonicum* Green Century, attributed to the denser structure of their leaf blades compared to other varieties.

The heat resistance study revealed that at 40°C, none of the experimental plants suffered leaf blade damage. When the temperature was raised to 50°C, most plants experienced minor leaf blade damage, not exceeding 10-15% of the total leaf area. This suggests that these temperatures do not significantly impact the plants' leaf mass. However, an increase to 60°C resulted in severe damage to the majority of leaf blades across all cultivars or even complete tissue death. Determining resistance to adverse factors enables the selection of resilient *Ligustrum* L. species and cultivars for nursery stock propagation. This, in turn, facilitates the scientifically sound application of these plants for urban landscaping needs in Kyiv.

Acknowledgements

None.

Conflict of Interest

None.

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Оцінка морозо- та посухостійкості видів та культиварів роду *Ligustrum* L. при виробництві садивного матеріалу

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Анотація. Актуальність дослідження обумовлена необхідністю вдосконалення оцінки адаптивного потенціалу рослин для озеленення міських просторів, зокрема в умовах Києва, де фактори, як нестача вологи та коливання температур, є ключовими при виборі рослин, здатних витримувати екологічні навантаження та зберігати декоративні властивості. Метою роботи стали дослідження морозо- та посухостійкості видів та культиварів роду *Ligustrum* L., що дозволило оцінити їх адаптивні можливості для використання в умовах урбанізованого середовища. У рамках дослідження були використані фізіологічні методи для оцінки таких показників, як водоутримуюча здатність, водний дефіцит, оводненість, жаростійкість та морозостійкість. За результатами дослідження встановлено, що *Ligustrum vulgare* L. та її культивари мають високі показники морозостійкості. Це проявлялося у найменшій кількості балів ушкодження тканин за температури -25°C, в середньому 7,0-13,9 балів. Інші ж рослини за результатами дослідження при температури -20 °C мали незначний бал ушкодження (в середньому менше 2 балів), це вказує на те що дана температура не є критичною. Водночас сумарний бал при пониженні температури до -25 °C значно зростає, зокрема в *Ligustrum ovalifolium* Hassk. та її культиваріів сумарний бал коливається в межах 14,2-24,8, тоді як *Ligustrum japonicum* 'Green century' та *Ligustrum Ibota* 'Musli' мали 30,7 та 26,3 бали відповідно, що є близьким до критичного значення, але упродовж вегетації, клітини та тканини здатні відновитись. *Ligustrum vulgare* L., *Ligustrum ovalifolium* Hassk. та деякі культивари мали досить високу посухостійкість, що відображається у відносно невеликих відсотках зміни кількості вологи в листових пластинках. Отримані результати також показують стійку водорегулюючу функцію даних рослин, а також високі показники жаростійкості зокрема за температури 50 °C. Слід зазначити, що підвищення температури до 60 °C було критичне для всіх рослин. Це відображалося у частковому побурінні або повному відмиранні листових пластинок. Оцінка вищезгаданих показників дає змогу для відбору вихідного матеріалу з метою отримання стійкого садивного матеріалу до факторів урбанізованого середовища

Ключові слова: садивний матеріал; листова пластинка; озеленення; водний режим; стійкість; морозне ушкодження