Ecological and biological features of adaptation of deciduous plants to chemical stress

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Abstract. In the urbanized environment, young plants lack moisture due to the limited space. This violates photosynthesis and respiration. To normalize respiration, the plant reduces excessive evaporation and evens out the water balance showing signs of xeromorphy. For the human environment, decorative and appearance of greenery are crucial. The purpose of the article is to study adaptive abilities of deciduous plants when using glyphosate-based herbicidal preparations, 2,4-D, dicamba, triasulfuron, and clopyralid. Anatomical and morphological changes at the cellular and organismic levels in young Tilia cordata Mill plants were identified: the size and density of stomata; frequency of stop cells of stomata; plant height; the number of shoots. The use of glyphosate, 2,4-D, dicamba, triasulfuron, clopyralid stimulated the height of shoots and increased the frequency of occurrence of guard cells with a length “0.15-0.19” mm. When using herbicides as a factor of chemical stress, the number of stomata per 1 mm² of the leaf surface increased. Ecological and biological plasticity of Tilia cordata Mill under environmental pollution (chemical stress) was identified. It is useful for directed formation of xeromorphic signs in young plants and further planting in urbanized conditions.

1. Introduction
Small-leaved linden young plants are an excellent model of woody plants resistant to urbanized conditions. They have unique gas and smoke resistance, shade tolerance, frost resistance and durability. They are promising plants for biomonitoring and remediation of anthropogenic and man-made territories [1, 2]. Heart-shaped linden, or small-leaved linden (Tilia cordata Mill.) is widespread in Europe and Western Asia.

During a period of leaf growth and development, the stomatal apparatus forms. Its state characterizes the response of the plant to changes in environmental conditions [3]. The size and density of the stomata are associated with the regulation of transpiration which ensures the maintenance of turgor and thermoregulation, the exchange of air masses and carbon dioxide absorption, carbon assimilation during photosynthesis [4]. According to researchers, xeromorphic signs are characterized by small adaxial and abaxial epidermis cells; an increased number and a decreasing size of stomata; a reduced stomatal
conduction in the movement of water vapor through the stomata [5]. An increase in the number of stomata is considered as a method of adapting a plant to unfavorable abiotic factors [4]. Therefore, observations of xeromorphic signs are required to regulate the water regime and gas exchange of plants under aggressive chemical stress (use of chemicals).

The purpose of the article is to study adaptive abilities of small-leaved linden plants under chemical stress (use of chemicals).

2. Materials and methods
The experiment was carried out in Bogorodskoe, Vladimir region. The soil of the experimental plot was medium-loamy, sod-podzolic, well-cultivated, with a pronounced lumpy-grainy structure characterized by a pH value equal to 6.1 (close to neutral); humus content was 2.9%.

The experiments were carried out on two-year old linden plants (T. cordata) in 2015-2016. In the experiment scheme, herbicidal preparations of different chemical nature were used: glyphosate (1.5 l / ha in ai); 2,4-D (0.91 l / ha for ai); dicamba dimethylamine salt, then dicamba (0.96 l / ha ai); triasulfuron (7.5 g / ha in the ai); clopyralid (0.06 l / ha for ai). Manual weeding followed by cultivation of the rows was used as a control variant. Location of the plots was randomized, the experiment was repeated four times. During the growing season, descriptions, photographic recording of plants and phenological observations were carried out.

Weather conditions were favorable for the growth of plants. The average annual temperature was + 14.1 °C; the amount of precipitation was 51.6 mm; relative humidity was 71%. Years of observation had different aridity levels; the amount of precipitation was 2-3 times lower than the average annual data: 2015 - 19.2 mm, 2016 - 25.6 mm. In general, the first year (2015) was the warmest year in the history of meteorological observations (+ 2.4 °C to the average annual rate). The period with the sum of temperatures above +10 °C was 163 days in comparison with the average annual data (144 days).

Annually, 5–7 pieces of leaves were taken from the middle of the crown from each plant (repetition - 10 plants) which differed in maturity [6]. The anatomical and morphological structure of leaves was studied using imprints made from the surface of uncut leaves. The method for preparing a plant object to study the state of stomata involved production of a thin transparent film of stomata imprints (replicas) using polymer compounds in an organic solvent (nitrocellulose, polymethyl methacrylate in acetone) followed by removal of the imprint and studying it using a light biological microscope [7]. The imprint of the leaf surface was placed in a drop of water on a glass slide; the object was fixed with a cover glass. Microscopic examination was performed using a light biological microscope at a magnification of 40x (a combination of a 4x objective and a 10x eyepiece) and 800x (40x and 20x). The division value for the 4x objective and 10x eyepiece was 0.025 mm. The The number of repetitions was 10. At a 80x magnification of the microscope, the field of view was 2.24 mm².

In the middle part of the leaf between the veins, the number of stomata was calculated in terms of 1 mm² of the surface of the leaf plate, and the length of the stomatal fissure formed by the stomata stop cells was measured. Using a digital camera, photos of the main (colorless) cells of the covering tissue of the leaf or the upper (adaxial) epidermis were produced. Using a mechanical odometer, we measured a contour of the area of a polygon shaped like five main cells of the epidermis lying side by side on the surface [5]. The area was calculated in mm² for one cell. The number of repetitions was 10.

The experimental data were analyzed in Microsoft Excel (2013). Standard statistics data were calculated for each variational series: (X ± σ) arithmetic mean and a standard deviation, a coefficient of variation (Kv). Correlation and regression analysis was used to interpret the results. To establish the reliability of differences between the compared signs, the Student’s t-test was used. When testing statistical hypotheses, a 5% level of significance or P ≤ 0.05 was used.

3. The study of the structure of the modified lead-tin-base bronze
According to the averaged data for two years, a significant increase in the number of stomata per 1 mm² of the surface of the leaf plate was established when processing the studied herbicides from 34% to 73% relative to the control variant.
Adaptation of the stomatal apparatus. The maximum number of cells per unit area was determined for the option with dicamba (402 ± 141 pcs./mm²), glyphosate (373 ± 24 pcs./mm²), triasulfuron (365 ± 104 pcs./mm²), 2,4-D (350 ± 39 pcs./mm²) clopyralid (310 ± 75 pcs./mm²).

Dicamba, 2,4-D and triasulfuron are drugs of systemic action against dicotyledons, including perennial plants, which include small-leaved linden. In the options with dicamba and triasulfuron, a sharp increase in dispersion values was observed. It is characterized by an increase in the average number of stomata due to the effective adaptation of linden plants in response to chemical stress.

According to the averaged data for two years, a significant increase in the number of stomata per 1 mm² of the leaf plate surface by 34-73% was established when using herbicides.

The comparative analysis of the area of the main cells of the adaxial epidermis identified deviations for all options (1.22-1.34 mm²) with the exception of glyphosate (a decrease by 1.04 ± 0.8 mm²). Glyphosate inhibits an enzyme used in the synthesis of aromatic amino acids tryptophan, tyrosine and phenylalanine. It is able to penetrate through into leaves and move throughout the plant to the growth points acting on the whole vegetative plant, including woody plants. Therefore, the inhibitory effect of glyphosate is manifested in a decrease in the area of adaxial side cells of the epidermis (from 20% and higher compared with the control option) which indicates signs of xeromorphism and is aimed at reducing excessive evaporation and loss of plant moisture. When using glyphosate, a decrease in the area of adaxial side cells of the epidermis is a reaction (adaptation) of linden plants to chemical stress.

Extensive experimental material made it possible to analyze the frequency of occurrence of stomatal closure cells of various lengths. The data array was divided into dimension groups by the method of scaling: 0.05-0.09 mm; 0.10-0.14 mm; 0.15-0.19 mm with a measurement accuracy of 0.002 mm. Analysis of a sample of experimental data taking into account the chemical nature of the substance showed that in the options with glyphosate, 2,4-D, dicamba, there was an increase in the frequency of occurrence of stomatal closure cells with a size of “0.15-0.19 mm” by 40% compared to the control option, triasulfuron and clopyralid, where the frequency of occurrence did not exceed 30%.

Stomatal movements and the length of stomata cells are associated with redistribution of potassium ions between trailing and accompanying cells and production of abscisic acid (ABA). Potassium affects the physical state of colloids of plasma and cell walls, metabolism and the flow of water with solutes. But plant protection from drying by stopping stomata occurs at the early stages of water deficiency; this phytohormone, an antagonist of growth stimulants or auxins, enters the plant with water including dicamba and 2,4-D. An increase in the size of cells is due to an increase in evaporation or a decrease in the amount of available water entering from the inner parts of the plant [7, 8]. As an adaptation effect, an increase in the amount of bound water over the amount of free water occurs [8].

### Table 1. Observation data on linden plants

| Option, parameter | Winning for one plant (piece/plant) after using chemicals |
|-------------------|--------------------------------------------------------|
|                   | single       | double      |
| control           |             |             |
| (without herbicides) |             |             |
| $X \pm \sigma$    | 5.0±1.6     | 4.6±0.3     |
| $Kv, \%$          | 32.7        | 5.4         |
| clopyralid        | 2.6-7.7     | 5.4-6.4     |
| 2,4-D             | 3.7-5.1     | 3.8-5.9     |
| triasulfuron      | 3.8-7.8     | 4.2-8.8     |
| glyphosate        | 3.7-5.2     | 3.5-5.2     |
| dicamba           | 3.8-8.6     | 3.8-7.1     |
| $X \pm \sigma$    | 5.2±1.9     | 5.4±1.6     |
| $Kv, \%$          | 38.3        | 29.7        |
Phenological development under stress - spawn formation. The highest growth rates were observed in 2-4 year old plants. In the control option, the growth height was 74-89 cm. The stress factor (herbicide) influenced the dynamics of linden growth: regardless of the frequency of use, the height increased by 2-12% compared to the control plants. Analysis of the growth rate identified an increase in the growth rate when using herbicides by 20-51%; in the scheme of twofold application, the growth rate slowed down and amounted to 45-63% of the control one.

Among the changes in response to chemical stress, the shoot growth was studied (Table 1). In all options, formation of shoots was 5 ± 1 pieces per plant and did not depend on the frequency of applications of the preparations.

The maximum shoot formation (7-9 shoots/plant) was observed when using dicamba. The result is related to the chemical nature of the active ingredient. Dicamba stimulates the growth of younger plant tissues. In addition, dimethylamine salt of dicamba is distinguished by its high solubility in water (above 75 g/100 ml); sodium salt of dicamba and other herbicides have worse solubility (38 g/100 ml). Therefore, active formation of shoots is a stress reaction to moisture entering with a dissolved herbicide through the p-vascular system stimulating the development of young tissues.

4. Conclusion
In general, the use of herbicides (glyphosate, 2,4-D, dicamba, triasulfuron, clopyralid) caused adaptive changes (reaction) in small-leaved linden plants. An increase in the number of stomata per 1 mm² of the leaf plate surface by 34-73% was observed. A significant decrease in the size (area) of the adaxial side of the epidermis when using glyphosate (more than 20%) was determined. It characterizes the development of xeromorphism signs in young linden plants. It was suggested using the parameter “length of the stomata guard cells” as a response (adaptation) of linden plants to chemical stress. In young deciduous plants, an increase in the frequency of occurrence (40% and above) of guard cells with a length of “0.15–0.19 mm” (with a measurement accuracy of 0.002 mm) can be a signal of prolonged exposure to chemical stress.

In general, adaptive anatomical and morphological changes in young Tilia cordata plants can be assessed as an adaptation of linden plants to chemical stress. Signs of xeromorphy aimed at normalizing respiration, reducing excessive evaporation and equalizing water balance are indicators of this process. Thus, planting of linden in the conditions of environmental pollution (chemical stress) are promising in urbanized conditions characterized by the lack of moisture.

References
[1] Mozolevskaya E G, Sokolova E S, Kuzmichev E P, Belova N K and Kulikova E E 1996 Scientific works of MGUL 283 36–64
[2] Gorelova S V and Frontasyeva M V 2017 The Use of Higher Phytoremediation (Springer: Cham.) pp 103–155
[3] Sellin A and Kupper P 2007 European J. of forest research 126(2) 241–251
[4] Aasamaa K, Sõber A and Rahi M 2001 Functional Plant Biology 28(8) 765–774
[5] Ziyatdinova K Z and Urazgildin R V 2013 Bulletin of the Chelyabinsk state university 7(298) 181–184
[6] Anatomical research methods of cultivated plants 1989 ed G.I. Moskaleva (Leningrad: All-Union Academy of Agricultural Sciences, All-Russian Research Institute of Plants named after N I Vavilov)
[7] Tretyakov N N et al 1990 A workshop on physiology of plants (Moscow: Agropromizdat) pp 135–271
[8] Borzenkova R A and Hramtsova E V 2006 The management to laboratory researches of a big special workshop on physiology and biochemistry of plants (Yekaterinburg: USU publishing house) pp 14–26