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CHANGES IN THE MORPHOMETRIC AND ANATOMICAL PARAMETERS OF SHOOTS AND LEAVES OF ACER PLATANOIDES L. AFTER REJUVENATION PRUNING

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Abstract. The influence of rejuvenation pruning on changes in anatomical and morphometric characteristics of annual shoots and leaves of Acer platanoides L. in the conditions of Dnipro (Ukraine) has been investigated. In the first two years after pruning, a significant intensification of growth processes is observed. The length and diameter of annual shoots, the number of internodes and leaves, and the area of the leaf blade increased. Pruning has a particularly noticeable effect on the increase in the length of annual shoots and the area of the assimilation surface of the shoot – in the first year after pruning, they increased by 4.6 and 3.2 times, respectively. In rejuvenated plants, a thickening of the leaf blade occurs, mainly due to the development of a spongy parenchyma. In the fourth year after pruning, the intensity of growth processes decreases and differs little from the control (unpruned) plants. The leaves of crowned plants are damaged more significantly than those of unpruned ones. In four years, the rejuvenated plants restore their crowns well.

Key words: *Acer platanoides L.*; Rejuvenation pruning; Annual shoots; Leaf area; Biometric parameters; Stomatal index.

INTRODUCTION

Pruning is one of the methods to properly maintain the urban tree plantations. It is carried out to rejuvenate plants, to improve crown decorativeness (Брикелл, 1992), in order to prevent the spread of disease damage (Marciulynienea et al., 2017), to increase drought resistance of plants and improve the quality of wood (Maurin & Desrochers, 2013). It has been established that proper pruning of the tree crowns in field-protective forest belts increases the yield of grain crops (Jones et al., 1998; Bayala et al., 2002). In urban areas, pruning is usually done to reduce potential contact between trees and power lines (Lecignea et al., 2018). The following pruning practices are recommended: crown reduction pruning and planting on the „trunk", cutting into the „trunk" with planting on a tree stump, crown reduction and canopy raising, shaping the tree crown by fork pruning (Кругляк, 2006).

Tree pruning is used to remove old and dying skeletal branches, after which the aesthetic appeal of renewed plants is significantly improved, which is primarily necessary for urban plantations, whose growth conditions are unfavorable and therefore they do not achieve natural longevity (Казарян, 1969). Tree vitality depends on the degree and method of pruning. For example, annual shaping in roadside plantations in

Krasnoyarsk had a positive effect on the tree condition of the small elm (Latin name of the tree) (Татаринцев, 2012), and pruning trees “under the trunk” in the Tyumen plantations reduced their vitality and decorativeness (Казанцев & Казанцев, 2009).

Relatively recently, attention has been paid to studying the response of urban trees to hard rejuvenation pruning, which is widely used in street plantations. (Головинская, 2000; Горбенко, 2006; Капелюш & Бессонова, 2006; Kristoffersen et al., 2010; Ponomaryova & Bessonova, 2010; Курницька & Пахолюк, 2012; Олексійченко & Матковская, 2015; Fini, et al. 2015). Many authors point to a number of violations of the tree pruning rules in large cities, namely, the failure to comply with the terms and technology of this procedure (Shigo, 1984; Курницька & Пахолюк, 2012; Олексійченко & Матковская, 2015), as well as the fact of not taking into account plant age and species specificity (Горбенко, 2006).

At the same time, in Malaysia, in order to improve plant maintenance practices, not only tree health is taken into account, but also the skill level of workers who prune trees in urban areas (Badrulhisham & Othman, 2016). Therefore, it is necessary to pay more attention to compliance with the rules and proper time for pruning in urban settings.

A number of international publications (Sharma, 1975; Fini et al., 2009; Sjöman et al. 2015; Percival, 2017) have also been devoted to the study of species resistance of the genus *Acer L.* under urban conditions, including the effect of pruning on this species (Dănescu et al., 2015).

The purpose of this paper is to analyze the effect of hard rejuvenation pruning of Norway maple (*Acer platanoides L.*) on the growth of annual shoots and leaf area, as well as their anatomical parameters during three years of tree crown restoration.

MATERIAL AND METHODS

Research was carried out in a large industrial city (Dnipro) located in the steppe zone of Ukraine. The city is located in a climatic zone with insufficient moisture and high summer temperatures. The anthropogenic load in the area where the experimental plants are located is mainly caused by the high traffic congestion levels. There are no industrial enterprises in this area. Experimental and control trees grow in linear street plantings along Gagarin avenue directly at the roadbed (the average traffic intensity is 1900 cars/hour). The coordinates of the research site are 48º42’70 „N, 35º03’40” E.

The objects of the study were 25-year-old trees of *Acer platanoides L.*, 10 sample plants in each option. Experimental plants were subjected to hard rejuvenation pruning (option 2), while the control variants were not (option 1).

We observed the dynamics of tree crown formation, measured the growth of annual shoots in the growing branches, as well as the size of leaves on these shoots. Samples were taken in early September from the southeastern side under the same lighting conditions at a height of 2 m from the soil level. The morphometric parameters of the shoots were measured by conventional methods (Молчанов & Смирнов, 1967). The leaf area was determined by the contour-weight method (Карманова, 1976; Бессонова, 2006).

For anatomical study, the 3rd-4th leaf from the start of the shoot of the current year was used. Fragments of a leaf in its middle part, enclosed between the main vein and the edge, were selected for analysis (Васильев, 1988). In order to study the changes in the indices of the anatomical components of a one-year-old shoot, the authors used its section at a distance of 1.0 cm from its base. Shoots were cross-sectioned using a hand-held microtome (Клейн & Клейн, 1974).

To study the size of cells and tissues, a Biomed-4 microscope and an eyepiece micrometer were used, the division value of which was determined by an object micrometer. The measurements were repeated 30 times. Stomata density was determined on the prints obtained by the method of G. Kh. Molotkovsky (1935). The stomatal index (*Ui*) was calculated using the formula: $Ui = \frac{Nu \times 100}{Nne + Nu}$, where *Nu* is the number of stomata per 1 mm^2 of leaf surface area, and *Nne* is the number of epidermal cells per 1 mm^2 (Gupta, 1961). The xeromorphic index (*Ixr*) was calculated using the formula: $Ixr = \frac{Ne + Nn}{100}$, where *Ne* is the density of epidermal cells, pcs/mm^2; *Nn* – stomata density, pcs/mm^2. The palisade index was determined as the ratio of the palisade mesophyll size to the total leaf thickness and was expressed as a percentage (Brailko, 2013).

The research results were processed using Microsoft Office Excel 2007. The arithmetic mean error was calculated. To compare the morphological and anatomical parameters of the control and experimental variants, Student's t-test was used ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

In maple plants, after hard rejuvenation pruning, more intensive shoot growth occurs than in the trees without rejuvenation (Table 1). Length excess of annual shoots of plants that underwent rejuvenation in the second year after this procedure is 460.66%, in the third – 306.31%, in the fourth – 222.12%.

In pruned trees, internodes of annual shoots are longer than in the control option. This pattern is observed in the second, third and fourth years after rejuvenation (Table 1).

The greatest difference between the variants, both in the length of annual shoots and internodes, is observed in the second year after pruning. The number of internodes on annual shoots in rejuvenated plants also increases, most of all in the second year after this procedure. However, in the fourth year, no significant difference was established in their number in both options.

Table 1. *The effect of pruning on the length of annual shoots and internodes*

Option	After 1 year	After 2 years	After 3 years
Length of annual shoots, cm			
With no pruning (control)	16.12±1.09	15.84±0.96	13.38±0.85
With pruning (experiment)	74.26±2.33*	48.52±1.10*	29.72±1.15*
% to control	460.66	306.31	222.12
Length of internodes of annual shoots, cm			
With no pruning (control)	4.27±0.31	3.27±0.27	3.11±0.34
With pruning (experiment)	8.50±0.42*	8.32±0.39*	6.57±0.50*
% to control	199.06	223.85	211.25
Number of internodes, pcs.			
With no pruning (control)	3.77±0.21	4.84±0.42	4.30±0.32
With pruning (experiment)	8.71±0.52*	7.62±0.51*	5.12±0.47*
% to control	231.83	157.43	119.06

Note: * - differences between the control and the experimental variant are significant of p≤0.05

Tree pruning had a similar effect on the number of leaves on annual shoots (Table 2). An increase in their number is significant, especially one year and two years after tree pruning; there are no statistical differences between the variants three years later (i.e., in the fourth year after pruning).

Table 2. *Changes in the leaf area in Acer platanoides due to pruning*

Option	After 1 year	After 2 years	After 3 years
Number of leaves, pcs.			
With no pruning (control)	5.20±0.47	7.10±0.42	6.60±0.34
With pruning (experiment)	11.70±0.62*	9.52±0.31*	7.23±0.45
% to control	225.00	134.08	109.54
Area of leaves, cm^2			
With no pruning (control)	102.3±3.12	110.5±2.14	92.8±3.55
With pruning (experiment)	142.2±2.25*	146.0±2.54*	134.0±4.33*
% to control	139.14	132.29	144.42
Assimilation area of one-year shoot, cm^2			
With no pruning (control)	531.96±23.21	784.76±30.42	612.68±25.32
With pruning (experiment)	1665.38±79.52*	1391.92±45.51*	969.33±54.47*
% to control	313.06	177.48.43	158.21

Note: * - differences between the control and the experimental variant are significant of p≤0.05

In trees, after deep rejuvenation, larger leaves formed on annual shoots. The average leaf area is 139.14% after one year, 132.29% after two years and 144.42% after three years following the pruning, compared to unpruned trees. Increased degree of leaf size in comparison with the control values over the years changes less than other morphometric parameters. However, the area of the assimilation surface of the annual shoot in plants after hard rejuvenation pruning is especially larger than in the control variant after one year (Table 2). This is related, as already noted, to the formation of the largest number of leaves on the tree shoots of this variant in this period compared to the control option. The size of the leaf surface of the shoot is determined by both the leaves' area and their number. In the 3rd year, their number decreases, and in the 4th year, it is almost the same as in unpruned trees.

The leaves of pruned plants were damaged more significantly than those of the unpruned ones. The damage caused to leaves of roadside plantings increases from June to September, while the proportion of damage by marginal and interveinal necrosis in leaves of rejuvenated plants is higher, especially in the first years after pruning.

It should be noted that the crowns of trees with pruned skeletal branches usually restore in 4 years. During this period, 1.8–2.5 m long branches are formed, and their average number is 41 pieces. The crown formed in this case is much larger than that of 5-year-old trees of the same species.

Annual shoots that form on branches in the first years after the pruning have a larger diameter than the annual shoots of unpruned trees. However, they develop a thinner periderm due to weaker phellem development. The phellogen layer is the same in both variants. Collenchyma is better developed in the shoots of pruned trees. The thickness of the parenchyma of the primary cortex is not statistically different in both variants. The secondary bark of pruned trees is thicker. Similar changes occur in the wood layer. Characteristically, the values of the xylem/phloem ratio are close in plants of both variants, which indicates a relatively proportional growth of these tissues after pruning. The core diameter is 33.84% larger than that of the control plants. (Table 3).

According to the differentiation of the mesophyll, the leaves of *Acer platanoides L.* are dorsiventral, hypostomatic (the stomata are located on the leaf underside).

Table 3. *Influence of rejuvenation pruning on the thickness of histological elements of the annual shoots of Acer platanoides, μm*

Tissue	Unpruned trees (control)	Pruned trees (experiment)	% to unpruned samples
Cork (felema)	68.31±1.15*	57.29±1.20*	86.79
Phelloderm	38.25±1.20*	32.11±0.78*	83.86
Collenchyme	47.14±0.60*	61.73±1.40*	130.95
Parenchyma of the primary bark	49.72±0.65*	48.26±1.32	97.06
Primary bark	96.86±3.46	109.99±2.01*	113.55
Secondary bark (phloem)	220.26±1.25*	290.47±2.24*	131.87
Wood (xylem)	337.63±2.71*	475.25±3.21*	140.76
Core (diameter)	1136.21±13.16*	1520.77±14.81*	133.84

Note: * - differences between the control and the experimental variant are significant of p≤0.05

Leaves of greater thickness are formed on the annual shoots of branches of rejuvenated trees compared to those formed on unpruned trees (Table 4). At the same time, on the transverse section of the leaf, the thickness of some histological elements is greater, of others is less or does not change in comparison with the indicators established in the plants that have not undergone anti-aging tree pruning. Consequently, the cuticle layer is much thinner, the bottom and upper epidermis is somewhat thicker.

Table 4. *Influence of the rejuvenation pruning on the anatomical structure of leaves on annual shoots, μm*

Tissue	Unpruned trees (control)	Pruned trees (experiment)	% to unpruned samples
Thickness, micron:			
leaf	157.38±2.45*	186.62±4.26*	118.58
cuticles	3.21±0.04	2.16±0.02*	67.29
epidermis			
upper	18.17±0.45*	20.56±0.51*	113.15
bottom	12.60±0.30*	14.50±1.06*	115.08
columnar parenchyma	53.34±1.02*	60.11±0.97*	112.69
spongy parenchyma	70.06±1.96*	89.29±2.12*	127.45
Cell size, μm:			
columnar parenchyma			
length	45.03±0.83*	49.21±0.91*	109.28
width	8.65±0.94*	12.34±1.24*	142.65
spongy parenchyma			
length	16.81±0.61*	24.09±0.85*	143.30
width	15.11±0.45*	22.38±0.29*	148.11
Palisade coefficient	5.20±0.11	3.98±0.10	76.54
Columnar/spongy	0.76±0.02	0.67±0.03	88.15

Note: * - differences between the control and the experimental variant are significant of p≤0.05

The thickness of the columnar and spongy mesophylls is greater in the shoot leaves of the pruned trees. The value of their ratio decreases, which indicates that in the shoot leaves of rejuvenated plants, the development of spongy parenchyma is more significantly stimulated than that of columnar. The spongy mesophyll of the leaves of this variant has larger intercellular spaces.

The outlines of epidermal cells are sinuous, and the stomata are relatively evenly distributed over the leaf area in both variants. According to the structural features of the stomata and adjacent cells, they belong to the anamocytic (disordered) type. Their density per 1 mm2 in the leaves of annual shoots of rejuvenated plants is lower than in the unpruned trees (Table 5). Stomatographic analysis indicates that the size of the stomatal guard cells (length and width) and the degree of their openness are greater in pruned trees. The stomatal-epidermal index is practically the same in both variants. The xeromorphic index is lower in the leaves of annual shoots of pruned trees.

Analysis of the obtained results shows that the annual shoots of the branches of hardly rejuvenated *Acer platanoides L.* trees are characterized by more intensive growth due to reparative regeneration than the shoots of unpruned plants. A significantly larger assimilation surface is formed after two years due to an increase in both the number of leaves and their average area, and after three years only due to a larger area, since there are almost no differences in the number of leaves.

Table 5. *Changes in the parameters of the stomatal apparatus after pruning*

Index	Unpruned trees (control)	Pruned trees (experiment)	% to unpruned samples
Number of stomata per 1mm2, pcs	273.22±3.41	221.10±5.33*	80.92
Number of epidermal cells per 1 mm2, pcs	893.87±5.33	748.31±7.25*	83.71
Stomatal-epidermal index	23.15±0.61	22.80±0.58	98.49
Guard cell length, µm	20.51±0.48	25.08±0.51*	122.28
Guard cell width, µm	4.21±0.15	5.48±0.28*	130.16
Stoma openness, µm	3.05±0.10	7.12±0.21*	233.44
Xeromorphic index,%	11.67±0.53	9.69±0.31*	83.03

K.E. Bakhtadze (1948) highlighted an increase in the leaf size of the newly formed shoots after pruning the plants of tea. M.N. Kazantseva and A.A. Soloviova (2009) obtained similar data on the increase in the leaf area of Populus balsamifera on shoots formed after radical pruning. N.P. Krenke (1950) believed that the increase in leaf parameters during the regeneration period is explained from the point of view of the theory of cyclic aging and rejuvenation. It is the rejuvenation of the newly formed shoots after pruning that, according to this researcher, represents the reason explaining the more active growth of leaves.

More intensive growth processes in the annual shoots of pruned *Acer platanoides L.* plants were observed in the process of crown restoration after hard rejuvenation pruning also in other objects - *Platanus orientalis* and *acerifolia* (Капелюш & Бессонова, 2006), as well as *Tilia cordata* and *T. platyphyllos* (Ponomaryova & Bessonova, 2010). Also N.P. Krenke (1950) pointed out that in the process of plant regeneration, it is of considerable interest to assess the correlative relationships of separate parts of an individual. There is a strong correlation between root functions and assimilation mass. The deciduous surface is formed depending on the thickness of the sucking roots (Казарян, 1969). A change in the ratio of underground and aboveground mass of trees in favor of the former leads to intensive growth of shoots and leaves.

More intensive growth processes in the annual shoots of pruned Acer platanoides plants were observed in the process of crown restoration after deep rejuvenating pruning also in other objects - *Platanus orientalis* and *acerifolia* (Капелюш & Бессонова, 2006), as well as *Tilia cordata* and *T. platyphyllos* (Ponomaryova & Bessonova, 2010). Also N.P. Krenke (1950) pointed out that in the process of plant regeneration, it is of considerable interest to assess the correlative relationships of separate parts of an individual. There is a strong correlation between root functions and assimilation mass. The deciduous surface is formed depending on the thickness of the sucking roots (Казарян, 1969). A change in the ratio of underground and aboveground mass of trees in favor of the former leads to intensive growth of shoots and leaves.

Currently, a large number of various compounds are known that move into shoots together with mineral salts in the sap (a large set of amino acids, their amides, ureides, peptides, proteins, organic acids, organophosphorus compounds, growth activators, etc.) (Казарян, 1969; Кулаева, 1973). It has been shown that stem and leaf growth regulation by the root system, as well as the leaf aging process, is carried out with the participation of cytokinins, which can be considered as a hormonal factor of the root system affecting the vital activity of aboveground organs (Mothes & Engelbrecht, 1963; Кулаева, 1973). Cytokinins, along with auxins, are involved in the regulation of cell division and growth, their differentiation, metabolism of organs that have completed growth, and influence some organs in the whole plant (Werner & Schmülling, 2009; Schäfer et al., 2015; Jameson & Song, 2016).

Thus, the activation of the growth processes of shoots and leaves can be explained by a decrease in the aboveground / underground mass ratio after the pruning procedure of skeletal branches and regrowth of a new tree crown. This leads to a significantly better supply of organs, which are formed as a result of regeneration, with water, nutrients, as well as with growth stimulants, which are synthesized in the roots, primarily with cytokinins.

Rejuvenation pruning of *Acer platanoides L.* trees significantly affects the formation of the histological elements of the shoot. Thus, the thickness of the secondary bark (phloem), wood (xylem), collenchyme and the diameter of the core increase. The size of the secondary bark does not differ in both variants, and the layer of cork and fellem is thinner in the shoots of pruned trees. Better development of xylem elements promotes greater flow of water and dissolved minerals into the leaves, which may also be one of the reasons for the increase in their surface area, and the phloem – for the outflow of assimilates, their movement in a downward direction.

Changes in leaf anatomical characteristics present a structural reflection of plant response to external factors (Капелюш & Бессонова, 2006; Georgy, 2009; Sack et al., 2003), primarily to water availability (Torres et al., 2003; Крохмаль, 2015). The leaves of annual shoots of newly growing branches of pruned trees have a more mesomorphic structure than those of unpruned ones. This is highlighted by a decrease in the xeromorphic index, the thickness ratio of the columnar parenchyma to the spongy parenchyma, an increase in the stomata size with a smaller amount per unit area of the epidermis (mm2), and a greater openness of the stomatal fissures. It is known that such changes in the structure of leaves may be observed under conditions of their better water supply.

CONCLUSIONS

In the first 2–4 years after hard rejuvenation pruning of trees, a very significant intensification in the growth of annual shoots and the formation of leaves of a larger area in comparison with unpruned plants was observed, which can be explained by an increase in the ratio of the root system to the aboveground part.

Annual shoots of pruned trees have larger diameters in comparison with unpruned trees due to the thickening of such histological elements as secondary bark, wood and pith.

The anatomical structure of leaves in pruned trees changes towards mesomorphism: there is a decrease in the index of prosenchymality and xeromorphism, a more significant development of spongy parenchyma, and a decrease in the number of stomata per unit area of the epidermis with an increase in their size. Xeromorphization of leaves can be considered as an adaptive response to environmental pollution, since this helps to reduce the intake of toxic gases into their tissues and to reduce the area of damage.

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Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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