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MORPHO-ANATOMICAL AND ECOLOGICAL FEATURES OF THE SPECIES OF THE GENUS *JUGLANS* L. IN THE CONDITIONS OF BELGOROD REGION

Abstract: For the first time, the study of morphology and biology of six species of the genus *Juglans* L.: *J. regia* L., *J. mandshurica* Maxim., *J. sieboldiana* Maxim., *J. cinerea* L., *J. nigra* L., *J. rupestris* Engelm was conducted in the conditions of the Belgorod region. Rhythm of seasonal development, drought tolerance and yields were identified. Leaf trichomes of six species of the genus *Juglans* L. were described. A preparation method of leaf epidermis of the genus *Juglans* L was modified. New data on quantitative and qualitative indicators of fruits was observed including: morpho-structural components, productivity and tolerance to unfavorable conditions in the Belgorod region. Morpho-anatomical structure of the leaves was studied by using the SEM Quanta 200 3D, the Carl Zeiss microscope.

Key words: genus *Juglans* L, leaf, trichomes, stomata, drought tolerance.

Language: English

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Introduction

Breeding tolerant and high-yielding species with high quality fruits of the genus *Juglans* L walnut are topical issues [2, p.60-62; 15, p.179-180; 16, p.444-448]. Walnut plantations and tree improvement are parts of measurement systems to increase food production. Walnut trees are valuable, due to their content of biologically active substances such as fats, proteins, carbohydrates, vitamins and minerals. Additionally, the fruits are ideal for use in a healthy balanced diet. They also have technologically important features. Walnut oil is highly valued in the food and confectionery industries. Wood of the species of the genus *Juglans* L is unsurpassed in beauty and quality, used for production of high-grade furniture, decoration of office space, and the airplanes' cabin, yachts, parquet [5; 15, p.179-180].

To increase the diversity of food production, especially in years with abnormal weather conditions, the introduction of plants was held in the Belgorod region. In the Belgorod region, there are no walnut plantations; walnuts are mainly found in private households, and occasionally used in landscaping streets. The purpose of this study is to identify morpho-anatomical and ecological characteristics of some species of the genus *Juglans* L in the conditions of Belgorod oblast.

Materials and methods

Experimental studies were carried out in the Belgorod State University in the period 2009-2013. Field and pot experiments were carried out in the botanical garden of Belgorod State University. The study of the leaf anatomy was conducted in the nanocenter "BSU".

The objects of study were six species of the genus *Juglans* L., growing in the botanical garden NSU "BSU": *J. regia* L., *J. manshurica* Maxim, *J. sieboldiana* Maxim, *J. cinerea* L., *J. nigra* L., *J. rupestris* Engelm. Species names are given in accordance with the nomenclature «GRIN Taxonomy for Plants». Morphological measurements were made, according to the "Method for studying age-related changes in plants by morphological signs"[10-14].

The basis of anatomical studies is a technique of collecting material and anatomical research methods, a plan describing the structure of the leaf and data processing method. A study of leaf structures was conducted by using a light microscope Biola Lomo D11U11, Micromed-5, binocular IAS 10; morphometric measurements were made using a micrometer screw MOB-1-16×. Morphological and anatomical description of trichomes was performed using CM "Biola C 13" and SEM Quanta 200 3D in the center of collective use "BSU".

Results and discussions

Morpho-anatomical features of leaves of the species of the genus *Juglans* L

Characteristics of leaf mesophyll: Leaf of plants of the genus *Juglans* has dorsoventral structure. The degree of differentiation of palisade mesophyll; the ratio of palisade and spongy mesophylls differ, depending on the plant species and habitat features. Xeromorphic leaves tend to have more highly developed palisade tissue than mesomorphic leaves do. The walnuts *Juglans regia* and *Juglans rupestris* have the greatest thickness of the leaf blades. For this reason, they have the greatest thickness of palisade and spongy mesophylls, comparing with that of other species (Table-1). Mesophyll of the all walnuts including: *J.regia*, *J.rupestris*, *J. mandshurica*, *J. sieboldiana*, *J.cinerea*, *J. nigra* – multilayer. Coefficients of palisade mesophylls of the walnuts *Juglans regia* and *Juglans rupestris* are significantly smaller than the other species' coefficient. Volumes of columnar mesophyll cells of the walnuts *J. regia*, *J. cinerea*, *J. nigra* are the significantly smallest one. However, the shape of the palisade mesophyll cells has more important significance. The more elongate cell shape, the larger area per unit cell volume occurs. The columnar cells of the walnut *Juglans regia* have more elongate shapes than cells of other species have (Table-2).

Table 1
 Leaf anatomy of the species of the genus *Juglans* L in the conditions of Belgorod oblast.

| Specie | Leaf thickness, μm | Number of the palisade tissue layers | Number of the spongy tissue layers | Thickness of palisade mesophyll, μm | Thickness of spongy mesophyll, μm | Coefficient of palisade mesophyll,% |
|-----------------------|--------------------|--------------------------------------|------------------------------------|-------------------------------------|-----------------------------------|-------------------------------------|
| <i>J. mandshurica</i> | 102.64±1.94** | 2.0±0* | 3.43±0.053** | 50.80±0.627** | 28.86±0.583** | 92.38±2.74** |
| <i>J. sieboldiana</i> | 109.04±1.27** | 2.35±0.050 | 4.0±0.058** | 63.63±0.851** | 35.84±0.773** | 92.37±2.97** |
| <i>J. regia</i> | 149.13±2.05 | 2.45±0.067 | 4.8±0.092 | 83.55±1.41 | 63.92±1.78 | 56.88±0.683 |
| <i>J. rupestris</i> | 151.19±1.39 | 2.1±0.026* | 4.05±0.045** | 71.34±1.62** | 61.17±0.979 | 58.77±1.32 |
| <i>J. cinerea</i> | 96.83±0.540** | 2.0±0* | 3.6±0.04** | 57.70±0.514** | 35.06±0.341** | 83.33±1.17** |
| <i>J. nigra</i> | 86.83±0.977** | 2.0±0.05* | 4.0±0.047** | 55.73±0.757** | 34.29±0.461** | 84.04±2.21** |

* – significant differences at a probability level 0.95;

** – significant differences at a probability level 0.99, in comparison with *J. regia*.

Table 2
 Characteristics of palisade mesophyll cells of the species of the genus *Juglans* L in the conditions of Belgorod oblast.

| Specie | Diameter of palisade mesophyll cell, μm | Height of palisade mesophyll cell, μm | Volume of palisade mesophyll cell, μm ³ |
|-----------------------|---|---------------------------------------|--|
| <i>J. mandshurica</i> | 35.15±6.34** | 25.40±1.18** | 12162.7±3900.35** |
| <i>J. sieboldiana</i> | 40.15±3.88** | 27.64±1.45** | 17421.3±2696.34** |

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| | | | |
|---------------------|--------------|--------------|-------------------|
| <i>J. regia</i> | 11.13±1.29 | 35.46±1.0 | 4672.4±1187.79 |
| <i>J. rupestris</i> | 36.04±5.62** | 34.13±1.97 | 25665.8±6468.32** |
| <i>J. cinerea</i> | 42.15±7.30** | 28.85±1.41** | 9508.93±2870.77 |
| <i>J. nigra</i> | 57.84±3.34** | 22.77±1.38** | 9014.59±2136.07 |

* – significant differences at a probability level 0.95;

** – significant differences at a probability level 0.99, in comparison with *J. regia*.

Adaptive features of leaf structure.
Classification of trichomes: Non-glandular unicellular trichomes (Fig. 1) are found on the adaxial and abaxial leaf epidermis of the walnut *J. cinerea*. They are sickle-shaped with length $319.23 \pm 44.13 \mu\text{m}$. (Fig.2, a). The unicellular trichomes of the walnut *J. sieboldiana* are rarely found on the

abaxial and adaxial epidermis, but only found on the veins (length: $327.39 \pm 25.84 \mu\text{m}$). Trichomes of the walnut *J. mandshurica* evenly arrange on the abaxial and adaxial epidermis (length $373.88 \pm 44.32 \mu\text{m}$). Unicellular conical –shaped trichomes of *J. nigra* are rarely found on the abaxial epidermis (length: $91.19 \pm 10.11 \mu\text{m}$).

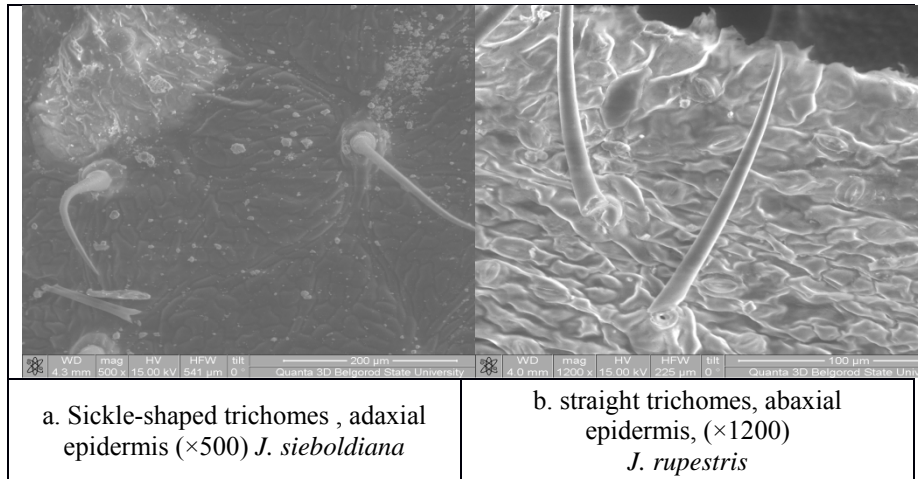


Figure 1 - The shape of non-glandular unicellular trichomes of the species of the genus *Juglans L.*

Sophisticated single-row unbranched conical hairs are found on the adaxial and abaxial epidermis of the walnut *Juglans sieboldiana* (Fig. 1, 2) (length $122-200 \mu\text{m}$). They are uniformly distributed on adaxial epidermis. They are mostly found on the veins of the abaxial epidermis. Multicellular single-row trichomes of the *Juglans rupestris* are only found on the abaxial epidermis. They are straight, evenly distributed (length $150.56 \pm 15.5 \mu\text{m}$). Non-glandular sophisticated tufted trichomes have the length of branches varying from 125 to $560 \mu\text{m}$, the diameter of the multicellular base is $47.24 \pm 2.50 \mu\text{m}$. (Fig.2d). Number of branches of the sophisticated tufted trichomes is 2-8, those of branched trichomes- 8-15 (Fig 2.c). Tufted trichomes are found on the abaxial epidermis of the walnuts *J. cinerea*,

Juglans sieboldiana, *J. mandshurica*. Branched trichomes are found on the vein of the adaxial and abaxial surfaces of the walnut *Juglans sieboldiana*. Highly significant linear correlation between the length and the number of branches ($r = 0,833$) was identified. We observed only sessile tufted trichomes for all species. Basic epidermal cells adjacent to the base of trichomes exhibit more different shape and size than the remaining cells of the epidermis. They have a trapezoidal shape. The cells at the base of hair covered with cuticle, outwardly resembling "cushion". According to the SEM, the surface of the hair does not form folds; however it well detected microrelief helically oriented relative to the vertical axis of the cell (Fig. 3).

| Class | Type | Subtype | | |
|---------------|---------------------------|--|--|---|
| Non glandular | Unbranched hairs | | | |
| | | Simple (unicellular) conical curved a (×2240) | Simple (unicellular) conical straight b (×1120) | Sophisticated single-row unbranched conical (×800) |
| | Tufted | | | |
| | | Branched c (×800) | Sophisticated tufted d (×1000) | Sophisticated tufted d (×1600) |
| Glandular | Capitate Unbranched Hairs | | | |
| | | Stalk consists of 2 the conical cells, Head – of 2-4 cells G1(×2100) | Stalk consists of 5 the conical cells, Head – of 8-9 cells G2(×2100) | Stalk consists of 8-9 the conical cells, Head – of 4-6 cells G3 (×1000) |
| | Peltate | | | |
| | | 4-cellular F1 (×2000) | 6-8-cellular F2 (×2000) | multicellular F3 (×1600) |

Figure 2 - Morphological classification of trichomes of the genus *Juglans* L.

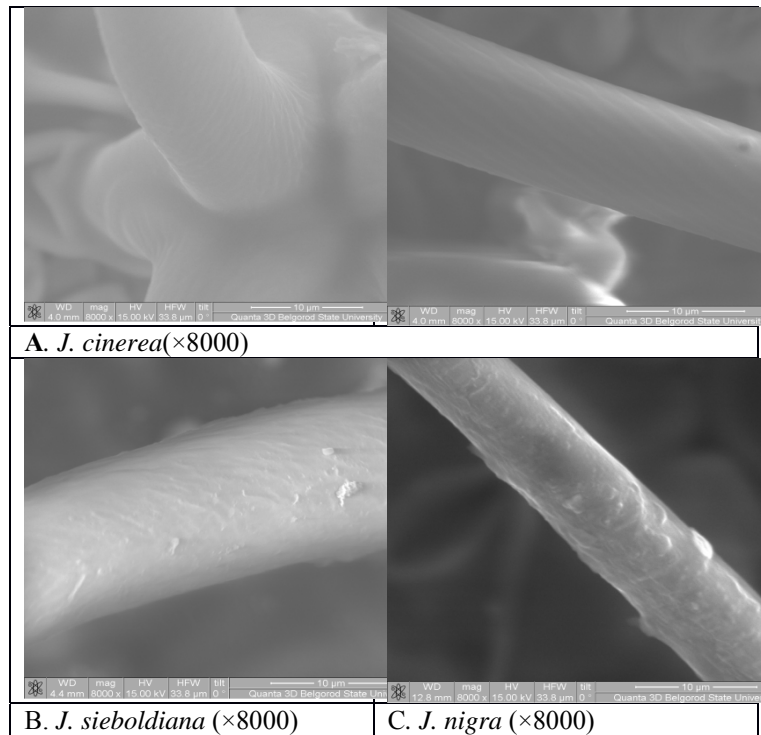


Figure 3 - The surface microrelief of non-glandular trichomes of the genus *Juglans* L.

Capitate trichomes are found on the adaxial and abaxial epidermis of all species of nuts, except *J. regia*. Capitate trichomes are on short stalk (Fig. 2, G1). Small capitate trichomes of the walnut *J. cinerea* consisting of 2-cellular stalk and 2-4 cellular head, are found on adaxial and abaxial leaf surface. The capitate trichomes of the walnut *J. sieboldiana* on the two-cell stalk are found on the veins of abaxial epidermis. Their heads contain of 6 to 12 cells; a large part thereof is damaged. Capitate trichomes of the walnut *J. cinerea* (Fig. 2, G2) on the long 5-cellular stalk (length of $120.42 \pm 15.70 \mu\text{m}$), 3-4 cell head (diameter $93.1 \pm 3.17 \mu\text{m}$) are located on the abaxial and adaxial epidermis. They are found only in the veins on the abaxial epidermis.

Glandular trichomes of the walnut *J. sieboldiana* are found on the veins of abaxial epidermis (the length of the stalks $54.37 \pm 3.14 \mu\text{m}$, diameter of the head $77.10 \pm 5.58 \mu\text{m}$). Capitate trichomes of the walnut *J. nigra* characterized by the 6-cellular stalk (length $115.17 \pm 20.15 \mu\text{m}$), 7-8 cellular head (diameter of $57.48 \pm 5.87 \mu\text{m}$) on the adaxial epidermis (only in veins) and abaxial epidermis (evenly spaced) (Fig. 2, G3).

The walnut *J. rupestris* has the subtype of glandular trichomes on the abaxial and adaxial epidermis. The number of stalk cells in a range from

3 to 7 (length $56.35 \pm 8.24 \mu\text{m}$), most heads destroyed. Capitate trichomes of the walnut *J. mandshurica* having a long stalk on the adaxial and abaxial epidermis are located at the veins. The length of the stalks $44.69 \pm 5.45 \mu\text{m}$, diameter of the head $64.49 \pm 11.11 \mu\text{m}$.

Peltate trichomes are characteristics of plants of the family *Juglandaceae*. They are 4, 8-cellular, as well as multicellular trichomes. 4-cellular trichomes are rarely found on the adaxial epidermis, but they are evenly arranged on the abaxial epidermis. Small peltate glands of *J. sieboldiana* are rarely observed on the adaxial epidermis, but found on the abaxial epidermis - 4-8 cellular (Table-3). Small peltate glands of the walnut *J. nigra* are found on the abaxial and adaxial epidermis with the diameter $400.74 \pm 39.20 \mu\text{m}$. For the walnut *J. mandshurica*, small peltate glands are rarely located on the adaxial and abaxial epidermis. Multicellular peltate trichomes are observed in all the studied species of the genus *Juglans* L. The largest areas of multicellular peltate trichomes are observed in the walnut *J. mandshurica*, *J. sieboldiana*, *J. cinerea* and the smallest areas - the walnut *J. regia* and *J. rupestris*. Peltate trichomes of the walnuts *J. regia* and *J. sieboldiana* have the most rounded shape, which corresponds to a greater fullness of their secret.

Table 3

Characteristics of multicellular peltate trichomes of plants of the genus *Juglans L.*

| Specie | Area of a peltate gland $\times 10^3 \mu\text{m}^2$ | Area of a subcuticular cavity of peltate gland. μm^2 | Coefficient of eccentricity of peltate gland | Coefficient of eccentricity of subcuticular cavity of peltate gland |
|-----------------------|---|---|--|---|
| <i>J. cinerea</i> | 9.845 \pm 0.321 * | 5.5095 \pm 0.244 ** | 0.495 \pm 0.027* | 0.505 \pm 0.032** |
| <i>J. sieboldiana</i> | 10.045 \pm 0.203 ** | 4.259 \pm 0.290 ** | 0.325 \pm 0.030 | 0.420 \pm 0.035 |
| <i>J. mandshurica</i> | 11.876 \pm 0.131 ** | 3.592 \pm 0.129 ** | 0.424 \pm 0.017 | 0.227 \pm 0.016 |
| <i>J. rupestris</i> | 3.363 \pm 0.410 ** | 1.112 \pm 0.133 * | 0.560 \pm 0.039** | 0.558 \pm 0.046 |
| <i>J. regia</i> | 1.858 \pm 1.338 | 0.162 \pm 0.117 | 0.389 \pm 0.032 | 0.494 \pm 0.047 |

Cuticular layer on the surface of peltate trichomes is thicker, it forms a special relief. Multicellular peltate trichomes often have radial folds along the anticlinal walls (*J. cinerea*,

J. sieboldiana). However, cuticle of the walnut *J. nigra* is thinner, waxy film on the surface of the epidermis is not expressed. Cuticle largely subsides around subcuticular cavity (Fig. 4 B)

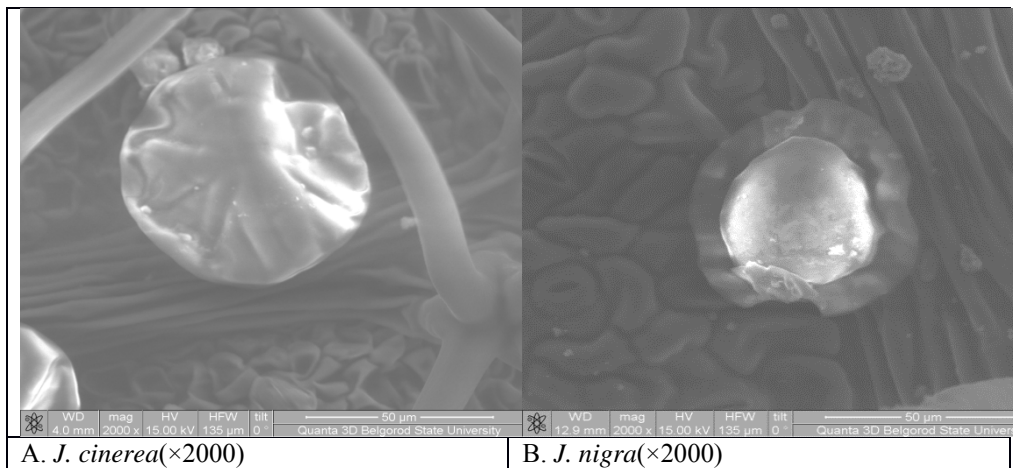


Figure 4 - Relief surface of cuticular layer of peltate trichomes of abaxial leaf surface of the species of the genus *Juglans L.*

Stomata: The regulation of water metabolism of plants is due to change in stomatal conductance [5-9]. Guard cells dominated the principal cells of the epidermis are found on the epidermis of the walnut

J. mandshurica, *J. sieboldiana*, *J. cinerea*. Stomata of the walnut *Juglans regia*, *Juglans nigra* are located in the same level with the main cells of the epidermis (Fig. 5).

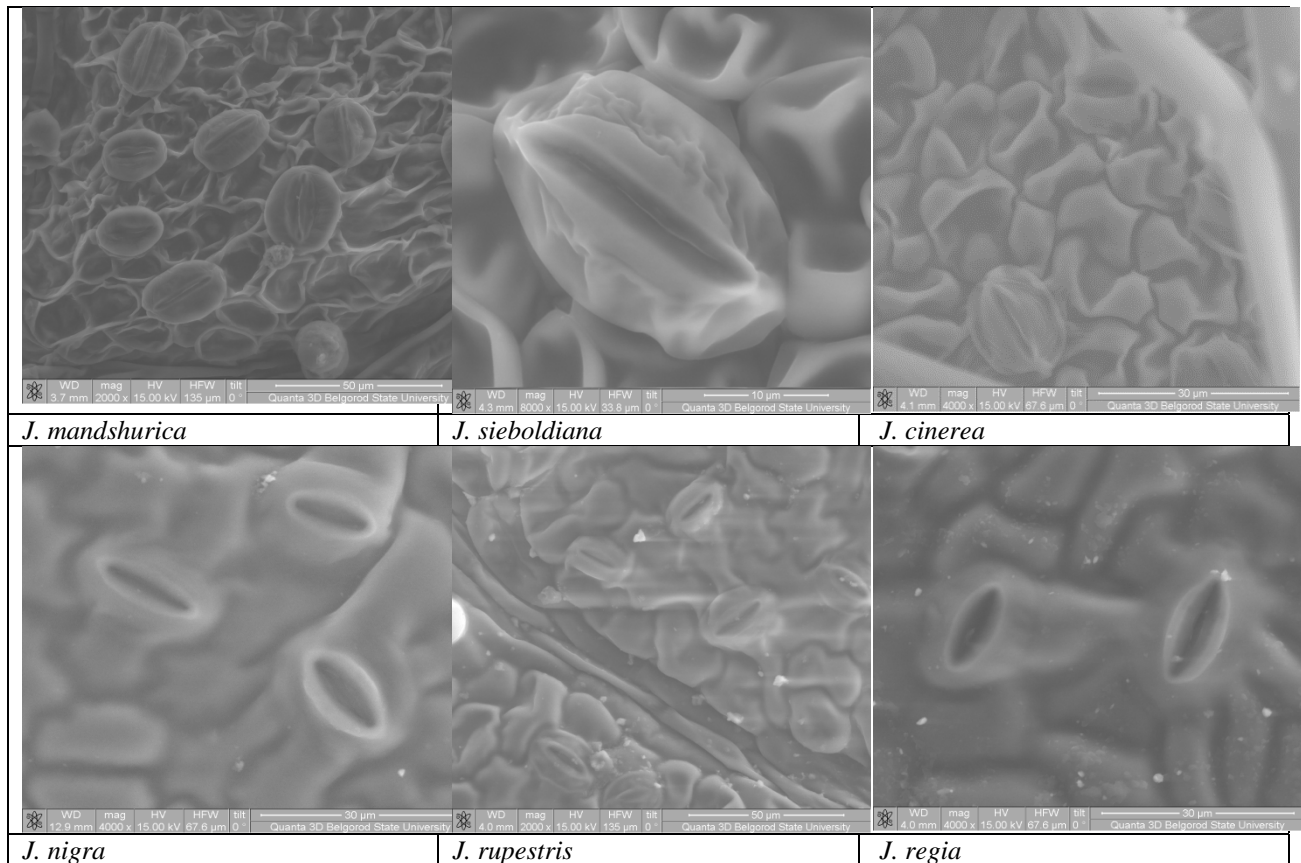


Figure 5 - Abaxial leaf epidermis of the genus *Juglans*. L.

While xeromorphic leaves increase, number of stomata per unit area decreases. The highest concentrations of stomata are observed on the lower leaf surface of the walnut *J. mandshurica*, *J. sieboldiana*, *J. rupestris*, and the smallest concentrations of stomata – the walnuts *J. regia*, *J. cinerea*. Increasing xerophyte structure of the leaf blade is marked reduction in the size of the stomata. In our experiment, no significant direct relationship was obtained between the size of the stomata and drought tolerance parameters. The

smallest areas of stomata are observed in the walnut *J. nigra*, *J. sieboldiana*, *J. regia* and *J. mandshurica*. The decrease in stomatal resistance is associated with an increase in the rate of photosynthesis. The lowest capacities stomata are observed in the walnut *J. regia*, *J. nigra* and *J. rupestris* (Table-4). The greatest degrees of openness of the stomata are observed in the walnut *J. mandshurica* and *J. sieboldiana*, which have high transpiration rate and moisture loss during a six-hour wilting.

Table 4
 Stomatal parameters of the leaf of the species of the genus *Juglans* L in the conditions of Belgorod oblast.

| Specie | Stomatal area. μm^2 | DOS | Stomatal number per unit leaf area 1 mm^2 |
|-----------------------|--------------------------------|-------------|---|
| <i>J. mandshurica</i> | 265.82±8.64 | 10.47±1.35 | 150.0±13.50 |
| <i>J. sieboldiana</i> | 278.50±17.29 | 15.35±3.30* | 155.5±33.0 |
| <i>J. regia</i> | 274.55±17.25 | 6.17±1.65 | 81.3±33.0 |
| <i>J. rupestris</i> | 375.57±13.43** | 8.74±0.466 | 120.3±4.66 |
| <i>J. cinerea</i> | 357.54±16.89** | 7.91±1.27 | 83.33±12.7 |
| <i>J. nigra</i> | 250.62±11.27 | 8.12±0.958 | 101.5±9.58 |

* – significant differences at a probability level 0.95;

** – significant differences at a probability level 0.99, in comparison with *J. regia*;

DOS – the degree of openness of the stomata.

Features of cuticle layer: The leaves of *J. regia*, *J. nigra*, *J. rupestris* are covered with a thick layer of cuticle, which thickens on the epidermal cells adjoining to the guard cells of stomata. Longitudinally oriented microfibrils of the walnut *J. mandshurica*, *J. cinerea*, *J. sieboldiana* were detected in the study of the ultrastructure of the guard cell walls. Their diameters are in the range 430.37-677.26 nm. Microfibrils of the walnut *J. regia*, *J. rupestris*, *J. nigra* on the surface of guard cells are hardly distinguishable due to the thick cuticle layer (Fig. 3). Cuticle forms radial folding from the base of trichomes and around large stomata. Cuticle on the adaxial leaf surface is thicker than one on the abaxial (Fig. 4). It is mostly observed in the walnut *J. regia*, *J. rupestris*, *J. nigra*, *J. sieboldiana*. The waxy coating is unevenly distributed on the leaf surface of the walnut *J. nigra*. However, it is evenly distributed on the leaf surface of the walnut *J. regia* and *J. sieboldiana*.

Stomata form differs in various species of the genus *Juglans* L: the walnut *J. mandshurica* has almost circular shape of stomata; the walnut *J. cinerea*, *J. nigra* and *J. sieboldiana* – oval shape; the walnut *J. regia* - elongated oval shape.

Ecological features of some species of the genus *Juglans* L introducing in conditions of Belgorod oblast.

Phenophases of some species of the genus Juglans L: In 2011, the vegetation period of the walnuts *J. rupestris*, *J. nigra*, *J. cinerea*, *J. regia*, *J. manshurica* began in early May, and *J. sieboldiana* - in the third decade of April (Table-5). Phenological observations revealed the plant with only staminate, pistillate flowers or with bisexual floweres. 8% of the test plants *J. manshurica* had only pistillate flowers, 83% - the staminate flowers, 8% - did not have flowers. The walnuts *J. rupestris* had only staminate flowers, *J.nigra* - pistillate flowers. 10% of the walnut *J. sieboldiana* had only staminate flowers, 33% - only pistillate flowers, 44% - did not bloom this year. 25% of the walnut *J. regia* did not bloom, 23% - had only pistillate flowers, 3% had only staminate flowers, 49% plants had both pistillate and staminate flowers.

Budding phase in most species began in early first or second decade of May, flowering in early second or third decade of May. Staminate flowers bloomed earlier than pistillate flowers did (Tab-5).

Table 5

Phenology of the species of the genus *Juglans* L.

| Species | Phenophases | | | | | | | | |
|-----------------------|--------------------|------------|------------|-----------------|-------------|---------------|-------------|-------------|-------------|
| | Breaking leaf buds | First leaf | Third leaf | Budding | Flowering | | Fruits | Ripe fruits | Defoliation |
| | beginning | beginning | beginning | beginning | beginning | ending | beginning | beginning | |
| <i>J. mandshurica</i> | 1.05-5.05 | 2.05-7.05 | 3.05-4.05 | ♂ 7.05-15.05 | 10.05-19.05 | 19.05-24.05 | 24.05-29.05 | 15.09 | 21.07 |
| | | | | ♀ 17.05 | 20.05 | 29.05 | | | |
| <i>J. rupestris</i> | 3.05 | 1.05-4.05 | 11.05 | ♂ 12.05 | 15.05-25.05 | | 2.06 | 15.09 | 7.10 |
| <i>J. nigra</i> | 2.05 | | 3.05 | ♀ 21.05 | 25.05-13.06 | | 13.06 | - | 1.10 |
| <i>J. cinerea</i> | 1.05-4.05 | 3.05 | 4.05 | ♂ 5.05-6.05 | 10.05-14.05 | 16.05-19.05 | 28.05-2.06 | 25.09 | 23.09 |
| | | | | ♀ 16.05 - 17.05 | 16.05-17.05 | 28.05-3.06 | | | |
| <i>J. sieboldiana</i> | 28.04 | 28.04-1.05 | 1.05-2.05 | ♂ 6.05 | 8.05 | 29.05 | 28.05-30.05 | 15.09 | 23.09 |
| | | | | ♀ 17.05 | 19.05 | 28.05 - 30.05 | | | |
| <i>J. regia</i> | 2.05-6.05 | 2.05-9.05 | 4.05-11.05 | ♂ 1.05-13.05 | 7.05-15.05 | 15.05-30.05 | 20.05-5.06 | 7.10 | 10.10 |
| | | | | ♀ 13.05-20.05 | 16.05-22.05 | 20.05-5.06 | | | |

Defoliation of the species of the walnut *Juglans* L varied considerably. We observed that the leaves of the walnut *J. mandshurica* early started yellowing

and falling, due to its low drought tolerance. Defoliation of the walnuts *J. cinerea*, *J. sieboldiana* were observed in the second, third decades of

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September and *J. regia*, *J. nigra*, *J. rupestris* – in early October.

Abiotic factors: Drought tolerance is the ability of a plant body as little as possible to modify the metabolism in inadequate water supply [1-4]. As can be seen from (Table-6) that water content of leaves of the species *J. regia*, *J. nigra* exceeds 70%, which is typical for plants with a high degree of drought tolerance. Loss of water of leaves from wilting after

six hours of the walnut *J. regia* does not exceed 30%, which corresponds to plants with high drought resistance ; the walnut *J. nigra*, *J. rupestris* (not exceed 40%) - an average degree of drought tolerance. One of the key indicators in assessing the degree of drought tolerance is the degree of leaf damage after a six-hour wilting. The smallest degree of leaf damage is observed in the walnuts *J. regia*, *J. nigra*, *J. rupestris*.

Table 6
Characteristics of the water regime of the leaves and the degree of relative drought tolerance of the species of the genus *Juglans L.*

| Specie | Water content,% | Water deficit,% | Water lost after leaf wilting , % of water content | | | | Drought resistance, point |
|-----------------------|-------------------|-----------------|--|------------------|------------------|--------------------|---------------------------|
| | | | After 2 h. | After 4h. | After 6h. | Degree of damage,% | |
| <i>J. mandshurica</i> | 65.82± 1.78* | 12.96± 2.44 | 40.33± 1.65** | 66.59± 1.88** | 76.28± 1.94** | 90.33± 4.38** | 4 |
| <i>J. sieboldiana</i> | 65.23± 1.42* | 3.45± 0.998* | 27.40± 3.29** | 51.12± 4.32** | 68.11± 4.08** | 84.0± 3.56** | 5 |
| <i>J. regia</i> | 70.59± 1.12 | 17.86± 6.19 | 9.10± 0.687 | 17.23± 1.30 | 25.55± 1.86 | 25.55± 6.46 | 8 |
| <i>J. rupestris</i> | 65.70±** 0.521 | 8.95± 0.601 | 16.88± 1.82** | 27.88± 2.98 | 39.60± 3.79** | 14.0± 4.99 | 6 |
| <i>J. cinerea</i> | 73.98± 3.69 | 25.45± 3.91 | 24.42± 0.947** | 43.04± 1.46** | 59.65± 1.79** | 92.33± 2.58** | 4 |
| <i>J. nigra</i> | 82.50± 5.98 | 28.93± 1.47 | 10.63± 0.81 | 21.61± 1.61 | 31.57± 2.23 | 6.8± 1.49 | 6 |

* – significant differences at a probability level 0.95;

** – significant differences at a probability level 0.99, in comparison with *J. regia*

Leaves of the *J. mandshurica* had the greatest moisture loss, as a result, their leaf blades were damaged up to 90% of the total area. During the period of wilting, the walnuts *J. regia*, *J. nigra*, *J. rupestris* remained at the same level of water lost. The walnut *J. regia*, *J. nigra*, *J. rupestris* had

significantly greater proportion of bound water than the other plant species (Fig. 6). The largest percentage of free water was observed in the leaves of *J. mandshurica*, which was characterized by the lowest water-holding capacity.

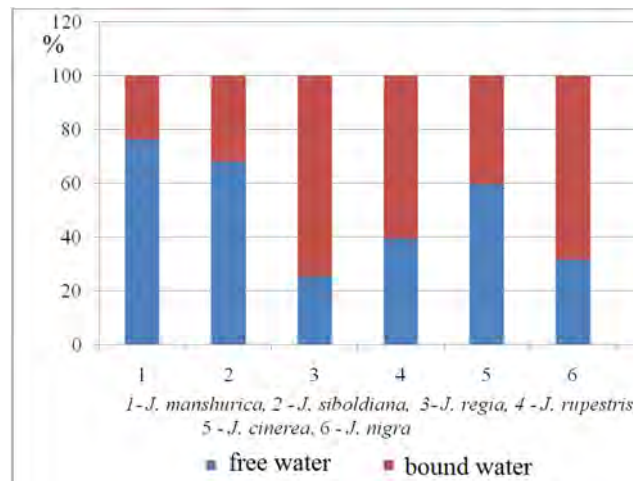


Figure 6 - The ratio of free and bound water in the leaves of the genus *Juglans L.*

Drawings on a complex of indicators (method Nikitsky botanical garden), an average point of drought tolerance of various species were found. The highest degree of drought tolerance was observed in the walnut *J. regia*, *J. nigra*, and *J. rupestris*. Thus, *J. regia* had high water-holding capacity low transpiration rate and high water potential. The walnuts *J. sieboldiana*, *J. nigra* and *J. rupestris* characterized with an average degree of drought tolerance. The walnuts *J. Mandshurica*, *J. cinerea* had indicators of drought tolerance, relating them to the plants with a low degree of drought tolerance. In the conditions of Belgorod oblast, they need watering.

Harvest was observed in five species of the genus *Juglans* L: *J. regia*, *J. manshurica*, *J. sieboldiana*, *J. cinerea* and *J. rupestris*. All immature fruits of the *J. nigra* aborted. The walnut *J. regia* has rounded-cordate shape, while other walnuts such as *J. manshurica*, *J. rupestris* and *J. cinerea* are oval-heart-shaped form. They differ both in size and shape of the fruit. The shapes of all studied species are not

perfectly rounded, so we decided to use multiple measurements in different directions (Table-7). Size of walnut kernel and a small thickness of the shell have economic value. Nuts of the walnut *J. regia* have a small mass, but the kernel mass is significantly high, due to its light shell (Table-7). The thickest shells are observed in the *J. manshurica*, and the kernel mass is low, a small mass of the walnut kernel was observed in walnut *J. cinerea*. The walnut *J. sieboldiana* differs in significantly high mass of the kernel and small thickness of the shell. The walnuts *J. rupestris* and *J. manshurica* have single fruitlets with a small number of fruits (2-3). The walnut *J. sieboldiana* differs in the significantly highest number of fruits in the stems, compared with other species, while the walnut *J. regia* differs in a large number of fruits on the trees. The most productive forms are the walnut *J. regia* with harvest - 1.5-2.0 kg, the number of fruitlets on tree is 83-150. The walnut *J. sieboldiana* has fruitlets with 6-7 fully maturing nuts.

Table 7

Characteristics of fruiting plants of the genus *Juglans* L.

| Specie | Fruit weight (g) | | | | Kernel weight (g) | Shell thickness (cm) |
|-----------------------|------------------|--------------|-------------|----------------|-------------------|----------------------|
| | | Height (cm) | Width (cm) | Thickness (cm) | | |
| <i>J. mandshurica</i> | 7.85±0.382 | 3.84±0.089** | 2.65±0.037 | 2.67±0.040 | 1.32±0.271** | 0.70±0.05** |
| <i>J. rupestris</i> | 9.66±0.401** | 3.59±0.054** | 2.46±0.037* | 2.48±0.033* | 1.63±0.033** | 0.23±0.01* |
| <i>J. cinerea</i> | 9.10±0.144** | 4.46±0.042** | 2.66±0.023 | 2.61±0.019 | 1.21±0.042** | 0.27±0.01** |
| <i>J. sieboldiana</i> | 11.65±3.60 | 4.09±0.289** | 2.99±0.414 | 3.31±0.495 | 3.20±1.79 | 0.25±0.01** |
| <i>J. regia</i> | 7.78±0.348 | 3.21±0.054 | 2.70±0.077 | 2.68±0.065 | 2.98±0.172 | 0.20±0 |

* – significant differences at a probability level 0.95;

** – significant differences at a probability level 0.99, in comparison with *J. regia*

Conclusions

1. In the Belgorod region, the genus *Juglans* L has full rhythm of seasonal development, allowing them to cultivate in the local conditions. Phenological observations revealed plants with staminate, pistillate and bisexual flowers. Budding phase in most species begins in early first - second of May, flowering in early second –third of May. Staminate flowers bloom earlier than pistillate flowers.

2. Morphological characteristics of trichomes of some species complement the existing classification of structural formations of the epidermis of the genus *Juglans* L.

3. *J. regia* differs in high water-holding capacity, low transpiration rate and high water potential differs: the walnut *J. regia* is the nuts with a high degree of drought tolerance. Average degrees

of drought tolerance are *J. sieboldiana*, *J. nigra* and *J. rupestris*. The walnuts *J. mandshurica*, *J. cinerea* belong to plants with a low degree of drought tolerance in conditions of Belgorod oblast.

4. The most promising for breeding are the walnuts *J. regia* and *J. sieboldiana* in the conditions of Belgorod region because *J. rupestris*, *J. mandshurica*, *J. cinerea* have fewer fruitlets on the trees with a small number of fruits.

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