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Snejana B. Dineva

Comparative studies of the leaf morphology and structure of white ash *Fraxinus americana* L. and London plane tree *Platanus acerifolia* Willd growing in polluted area

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Abstract: The leaf blades of white ash *Fraxinus americana* L. and London plane tree *Platanus acerifolia* Willd. growing in heavy polluted industrial areas were studied for morphological and anatomical changes developed under the influence of industrial contamination. The aim of the investigation was to determine and compare the influences of air polluted with SO_2, N_xO_x, Pb, As, Zn, Cu etc. on the morphology and the structure of the leaves of these deciduous trees. Both species are tolerant to environmental changes but with different environmental characteristics and tolerances and they are widely used for planting. Under polluted conditions, the trees strengthened the anatomic xeromorphic characteristics of their leaf structures, which gave them the opportunity to mitigate the stressful conditions of the environment. The observed responses are regarded as adaptive and compensative to the adverse effects of air pollution.

Additional key words: air pollution, morphology, anatomy of leaf, *Fraxinus americana* L., *Platanus acerifolia* Willd.

Address: S. B. Dineva, Technical College – Yambol, Thracian University “Stara Zagora”, “Gr. Ignatiev” No. 38 str., Yambol 8600, Bulgaria, e-mail: sbdineva@abv.bg

Introduction

Environmental conditions, including polluted air, water, soil etc., place trees under numerous stresses and contribute to their decline. Wood species are utilized to mitigate air pollution in highly polluted areas. It has been observed that plants growing in heavily polluted industrial areas are affected particularly by varieties of pollutants (oxides of nitrogen and sulphur, hydrocarbon, ozone, particulate matters (MP10), hydrogen fluoride, peroxyacyl nitrates (PAN) etc.). Studies show that under polluted conditions, plants develop different morphological and anatomical changes (Inamdar and Chaudahri 1984; Iqbal 1985; Karenlampi 1986; Gupta and Ghouse 1988; Bhatti and Iqbal 1988; Veselkin 2004). Plant adaptation to changing environmental factors involves both short-term physiological responses and long-term physiological, structural, and morphological modifications (Gravano et al. 2003; Novak et al. 2003; Vander et al. 2001). These changes help plants minimize stress and maximize use of internal and external resources.

This work was designed to investigate and compare the effects of heavily polluted air on the morphology and the anatomy of the leaves of two tree species, *Fraxinus americana* L. and *Platanus acerifolia* Willd., growing in the industrial region of the metallurgical plant, Kremikovtzi.

London plane tree *Platanus acerifolia* Willd. is a deciduous tree, 21–30 m long, spreading with age. The name *acerifolia* comes from Greek and means leaves like those of *Acer*. The tree was first found in London in 1663 as a cross between *Platanus orientalis* (Oriental plane tree) and *Platanus occidentalis* (American sycamore). The tree grows in full sunlight or light shade. It prefers deep, rich, moist, well-drained soil, but it will grow in most soils. The London plane tree can withstand the high pH, pollution, and grime of cities. The tree is widely planted in London. It withstands heavy pruning and is frequently pollarded.

The currently accepted scientific name of white ash is *Fraxinus americana* L. (*Oleaceae*). *Fraxinus americana* L. is a tree up to 18 m tall and 50 cm in diameter, with a conical or rounded dense crown. Although it is native to moist locations and prefers a sunny exposure, it is resistant to heat. The tree grows rapidly and is almost pyramidal when young, but gradually its growth slows down and it develops a more spreading round or oval shape. White ash is tolerant to the ozone, soil salt and drought.

Both, London plane tree and white ash are ornamental deciduous trees widely used for planting but with different environmental features and tolerances. London plane tree is strongly recommended as a street plant suitable for urban areas, while ash tree has better net effect on air quality. However, white ash is not suitable for urban areas and is mostly recommended for planting in large landscape areas.

In order to understand how the anatomy of leaf blades associate with the different ecological recommends of the trees, we measured the most common anatomic characteristics of leaf blades, expecting that the diverse tolerances will correlate with the dissimilar changes in the structure of blades developed under the influence of air contamination.

Materials and methods

Characteristic of the region

The study examined leaf blades from *Fraxinus americana* L. and *Platanus acerifolia* Willd. The plant material was collected from two fields – the heavily polluted area of metallurgical factory “Kremikovtzi” (42°47’ N; 23°30’ E) and, as a control, from the National Park Vitosha (42°30’ N; 23°15’ E, Bulgaria). Both places are located in the Sofia valley with temperate continental climate.

The region of the metallurgical plant, Kremikovtzi, is heavily polluted with SO_2, N_xO_x, Pb, As, Zn, Cu etc. Major industrial processes in the region generate dust particles. Particulates are not extremely damaging, but they can inhibit or reduce photosynthesis by plugging stomates. Particles are usually washed from leaves by rain or irrigation, and are therefore more harmful during dry periods. The main air pollutant is sulfur dioxide. During the investigation period the amount of sulfur dioxide in the observed area was 0.5 mg/m^3.

Plant material and methods

Samples were taken randomly (30 leaf blades from species) from the south side of the crown at 160–200 cm of the trees (10–15 trees of species) from both regions. The trees were of a similar age (15 years), sun exposure and had uniform height and growth form. The surface area of the leaves was measured. The middle parts of the leaf blades were cut and fixed in 90% ethanol – 90 cm^3, ice acetic acids – 5 cm^3 and formalin – 5 cm^3. Standard histological techniques and light microscopy were used to examine the anatomical characteristics of the leaf blades. The cross-sections of the leaf blades were prepared and observed under light microscopy, measured, drawn and photographs were taken. The measurements were repeated 30 times per one parameter. Cell size and thickness of the layers were assessed statistically with t-test.

Results

The study revealed that the leaf blades from both species showed a decrease in their surfaces (Table 1). The average leaf area from the National Park Vitosha for *Platanus acerifolia* Willd. (204.66 cm^2 ; $\sigma = 50.00$; n =30) is approximately twice the size of that from the plants growing in the polluted region (117.66 cm^2; $\sigma = 48.49$; n = 30).

On the surfaces of the leaves of London plane tree there were no visible injuries (chlorosis or necrosis), while on the surface of the blades of white ash, collected in September, were many small spots of necrosis. Sulfur dioxide is classified as a point-source pollutant. Acute sulfur dioxide damage causes severe leaf scorching, usually on upper interveinal leaf surfaces. Younger leaves are generally more sensitive. Chronic sulfur dioxide damage results in leaf chlorosis (colors of the chlorosis ranging from white to red). Moisture in the air or on leaf surfaces may combine with sulfur dioxide to form sulphur acid. Sulphur acid causes leaf scorch; spotting, defoliation, and can also cause tree death over a large geographic area with many affected species. Whether or not a pollutant causes acute or chronic damage depends upon tree species. Even individual trees within a species often display varying degrees of tolerance to the same pollutant. In most of the tolerant plants, changes can be observed in the leaf structure as a response to the air contamination. If the plant species develops under the stresses of air pollution, morphological or anatomical changes depend on its genetic ability to tolerate different limitations of the environment.

Table 1. Differences of morphological and anatomical measurements between means of polluted and control samples

Morphological and anatomical measurements	Difference of means between polluted and control for *Fraxinus americana* L. (μm)	Difference of means between polluted and control for *Platanus acerifolia* Willd (μm)
Leaf surface (cm^2)	–44.1**	–87***
Common thickness of the leaf (S)	–9.39*	100.81***
Thickness of the upper cuticle (a)	0.49	5.15*
Thickness of the upper epidermis (b)	–3.41	11.78*
Thickness of the palisade mesophyll (c)	2.79	31***
Thickness of the spongy mesophyll (d)	–12.4	43.71***
Thickness of the lower epidermis (e)	1.9	8.99***
Thickness of the lower cuticle (f)	1.24	0.18

* $p<0.05$; ** $p<0.001$; *** $p<0.0001$

Both tree species have completely different thickness of their leaf blades (Fig. 3). The common thickness of the leaf and the width of the mesophyll layer of *Fraxinus americana* L. is twice that of *Platanus acerifolia* Willd. *Fraxinus americana L.* have leaf width of about 529.48 μm (529.48; σ = 6.88), while *Platanus acerifolia* Willd is 298.36 μm (298.36; σ = 6.38) for the control and 399.17 μm (399.17; σ = 4.82) for the polluted region. In the same way, the mesophyll layer of *Fraxinus americana* L. is about 444.85 μm (444.85; σ = 13.76) for the control and 435.24 μm (435.24; σ = 14.28) for polluted, while the size of the mesophyll from the control area for the London plane tree is about 214.54 μm (214.54; σ = 12.89) and from the polluted 289.2 μm (289.2; σ = 5.68). We can assume that these main differences in the leaf structures contribute to the poles apart net effects of these plant trees on air quality.

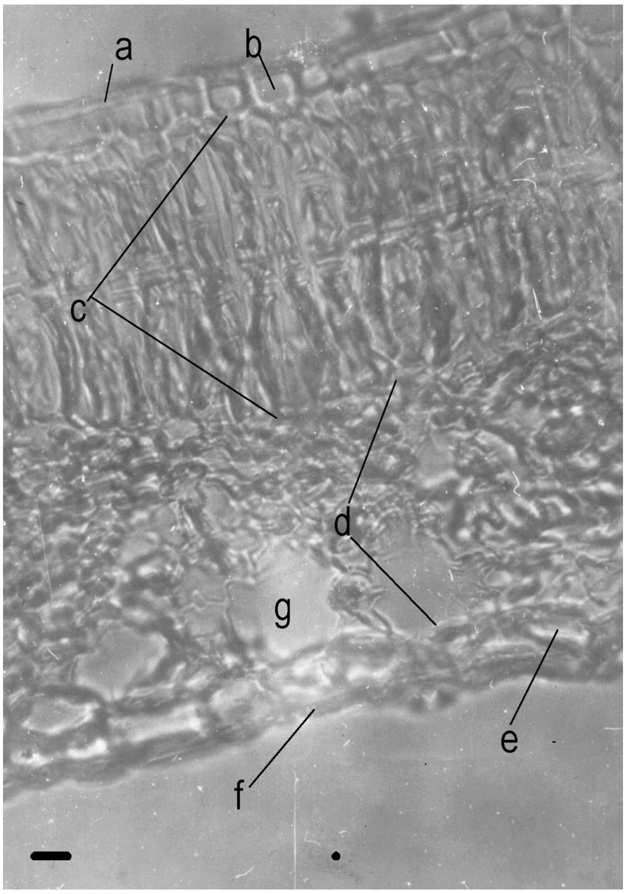

Fig. 1. *Fraxinus americana* L. Control (July)
a – upper cuticle; b – upper epidermis; c – palisade mesophyll; d – spongy mesophyll; e – lower epidermis; f – lower cuticle; g – intercellular air space; bar = 30 μm

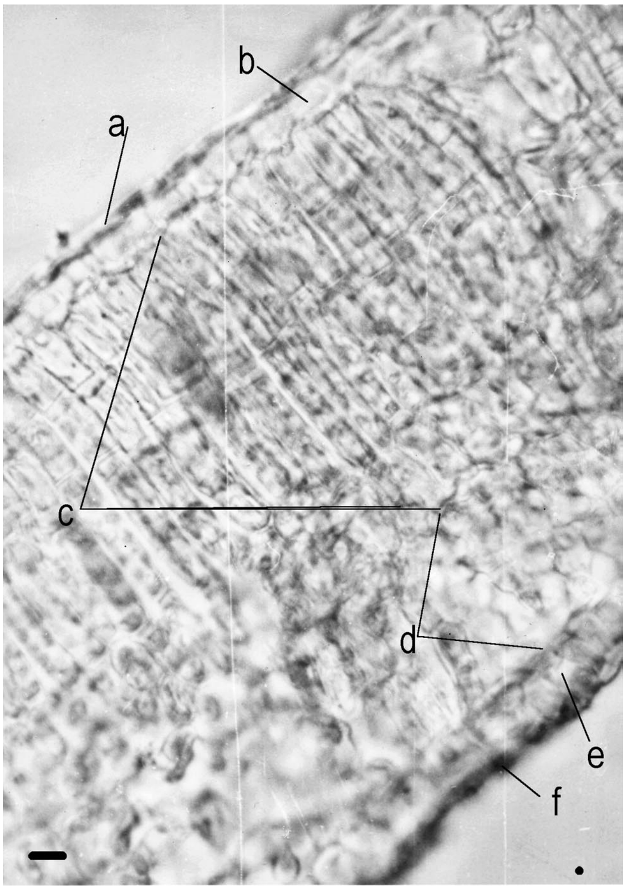

Fig. 2. *Fraxinus americana* L. – Polluted (July)
bar = 30 μm

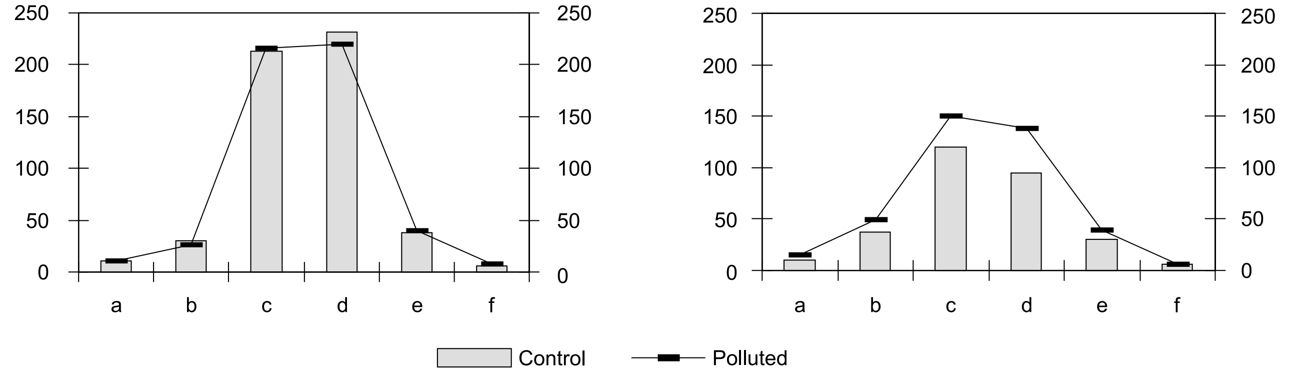

Fig. 3. Anatomical measurements of the cross sections of leaf blades (July). (a) *Fraxinus americana L.;* (b) *Platanus acerifolia* Willd.

a – upper cuticle; b – upper epidermis; c – palisade mesophyll; d – spongy mesophyll; e – lower epidermis; f – lower cuticle

Under polluted conditions, *Fraxinus americana* L. shows insignificant changes in the anatomy of its leaf blades (Table 1, Figs 1, 2).

The palisade mesophyll of *Fraxinus americana L.* consists of two layers of long-shaped flat cells, situated next to each other, as is shown in both photos (Fig. 1 and 2). The upper part of the spongy mesophyll of the leaves from Kremikovtzi (Fig. 2) is formed from small, flat cells without empty spaces of air chambers. Even though there are no significant differences between the obtained measurements of the structures of the blades for the control and polluted trees of *Fraxinus americana L.* (Table 1, Fig. 3), the rearrangement of the cells suggested an adaptation response of the plant to the polluted environment.

Platanus acerifolia Willd. strengthen the anatomic xeromorphic characteristics of their leaf structures under these polluted conditions (Table 1, Fig. 3). The

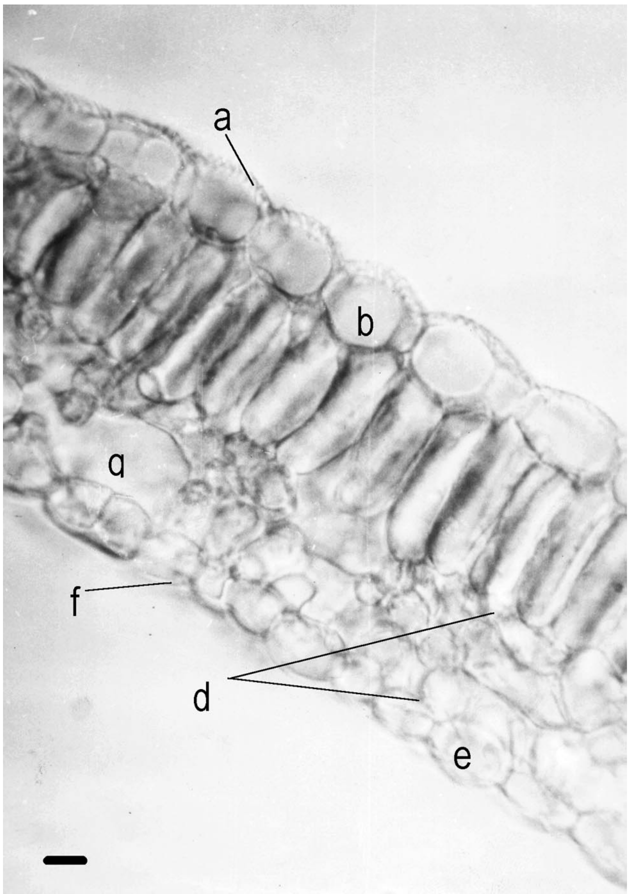

Fig. 4. *Platanus acerifolia* Willd – Control (July)

a – upper cuticle; b – upper epidermis; c – palisade mesophyll; d – spongy mesophyll; e – lower epidermis; f – lower cuticle; g – intercellular air space; bar = 30 μm

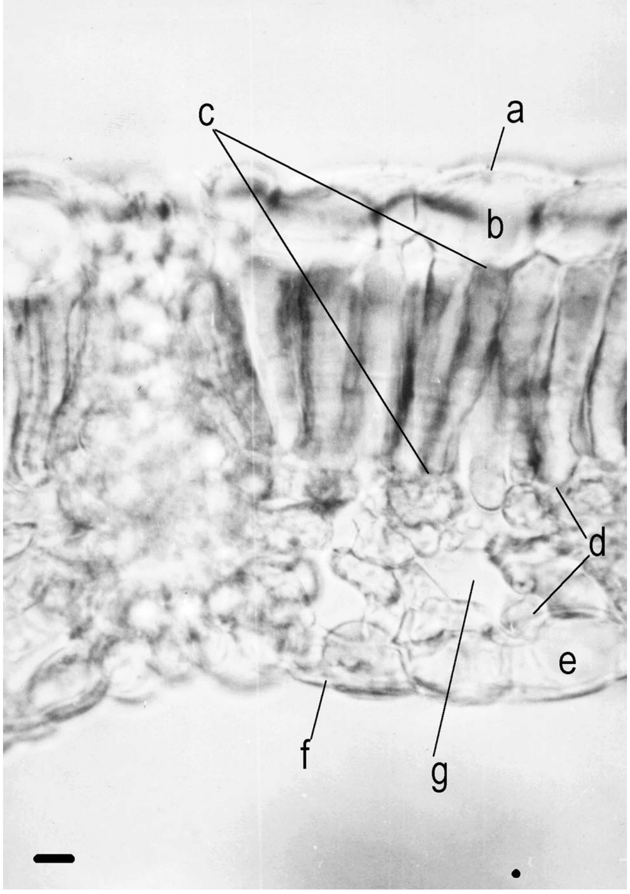

Fig. 5. *Platanus acerifolia* Willd – Polluted (July)

a – upper cuticle; b – upper epidermis; c – palisade mesophyll; d – spongy mesophyll; e – lower epidermis; f – lower cuticle; g – intercellular air space; bar = 30 μm leaves from the polluted area have thicker upper cuticle (15.19 μm; σ = 3.41) and larger width of the palisade mesophyll (150.68 μm; σ = 14.57), and all changes in the leaf blades are significant (Table 1; Figs 3, 4, 5).

Both species have high coefficient of palisadeness; 47% – 49% for control and polluted sample of *Fraxinus americana L.*, and 56% – 52% for *Platanus acerifolia* Willd. control and polluted respectively.

Discussion

Many authors have obtained similar results for the morphological parameters of the leaves among plants that are developed in air-contaminated regions. Jahan and Iqbal (1992) reported reduction of leaf length, breadth and area of leaves and length of petiole for *Ficus bengalenis, Guaiacum officinale, Eucalyptus sp.*, growing in polluted urban territories. *Euphorbia hirta* growing in a coal smoked environment showed a decrease in leaf area (Gupta and Ghouse 1988).

The structure of the leaf blades is important place in determines the response of the tree to the air contamination. Gaseous pollutants, such as ozone and sulfur dioxide, enter plants through natural openings, usually stomates. After passing through the stomates of the lower epidermis, the toxic gas meets the spongy mesophyll (Nikolaevski 1963; Ilkun 1971; Ilkun 1978). The wide intercellular spaces provide faster penetration and absorption of the phytotoxicant toward the palisade cells. That is why Nikolaevski (Nikolaevski 1963) believe one of the main criteria for resistance to air pollution is higher Rp/Rs ratio (coefficient of palisadeness), where Rp – is the thickness of the palisade mesophyll and Rs – is the thickness of the spongy mesophyll.

Ferdinand et al. (2000) found in two black cherry families that the O_3-sensitive genotype had significantly more stomata, a thinner palisade mesophyll layer and a thicker spongy mesophyll layer, lower ratio of palisade to spongy mesophyll, and greater leaf weight and area. In addition, total leaf thickness, guard cells, and specific leaf mass were also slightly greater for the ozone sensitive genotype. The representative cross-sections of the leaf blades of ozone sensitive genotype note shorter palisade mesophyll cells and large intercellular air space. Ferdinand demonstrated longer palisade mesophyll cells and little intercellular air space, on the representative cross-sections of the leaves of O_3-tolerant genotype (MO-7).

The thickness of the upper cuticle, the width of palisade mesophyll, and the greatest number of palisade coefficient are the main properties that distinguish the tolerant and resistant plant species from the sensitive ones to atmospheric pollution (Kulagin 1968; Nikolaevski 1963; Ninova 1970; Ferdinand, 2000).

Conclusion

The comparative analysis of the results from the cross-sections of the leaves of the London plane tree and white ash explains the different recommendation for their practical uses. The great breadth of the mesophyll and bi-layer of palisade mesophyll of *Fraxinus americana* L. correlated with the positive net effect on air quality. Both trees species have high palisade coefficient and are tolerant to the air contamination. Ash tree has better net effect on air quality against ozone, sulfur dioxide, nitrogen oxides, particulate matter, carbon monoxide, but it is not suitable for urban areas and it is mostly recommended for planting in large landscape areas.

London plane tree is strongly recommended as a street plant and it is suitable for planting in urban areas. The representative cross-section shows visible changes in the structure of the leaves from trees growing under polluted conditions. The plant develops adaptive reactions, which causes strengthened anatomic xeromorphic characteristics of the leaf.

The observed changes in the morphology and anatomy of leaves of both species are regarded as adaptive responses, aimed at combating the adverse effects of pollution.

References

Bhatti G.H., Iqbal M. Z. 1988. Investigations into the effect of automobile exhausts on the phenology, periodicity and productivity of some roadside trees. Acta Sociotatis Botanicorum Poloniae, p. 57.

Ferdinand J.A., Fredericksen T.S., Kouterick K.B., Skelly J.M. 2000 Leaf morphology and ozone sensitivity of two open pollinated genotypes of black cherry (*Prunus serotina*) seedlings. Environmental Pollution 108 (2): 297–302.

Gravano E., Giulietti V., Desotgiu R., Bussotti F., Grossoni P., Gerosa G., Tani C., 2003. Foliar response of an *Ailanthus altissima* clone in two sites with different levels of ozone-pollution. Environmental Pollution 121 (1): 137–146.

Gupta M.C., Ghouse A.K.M. 1988. Effects of coal smoke pollutants from different sources in the growth, chlorophyll content, sten anatomy and cuticular traits of *Euphorbia hirta* L. Environmental Pollution 47: 221–230.

Ilkun G.M. 1971. Gazoustoĭchivost' rasteniĭ., Kiev, p. 240.

Ilkun G.M. 1978. Zagriazniteli atmosfery i rasteniĭa, Kiev, Naukovaia dumka, p. 246.

Inamdar JA, Chaudahri GS 1984. Effects of environmental pollutants of leaf epidermis and leaf architecture of Peristrophe bicalyculata. Journal of Plant Anatomy and Morphology 1: 1–8.

Iqbal M.Z. 1985. Cuticular and anatomical studies of white clover leaves from clean and air-polluted areas. Pollution Research 4: 59–61.

Jahan S., Iqbal M.Z. 1992. Morphological and anatomical studies of leaves of different plants affected by motor vehicles exhaust. Journal of Islamic Academy of Sciences 5: 1, 21–23.

Karenlampi L. 1986. Relationship between macroscopic symptoms of injury and cell structure changes in needle of ponderosa pine exposed to air pollution in California (USA). Annales Botanici Fennici 23: 255–264.

Kulagin Z. 1968. O gazoustoĭchivosti drevesnykh rasteniĭ kak ekologicheskaia problema. V sb.: Rastitel'nost' i promyshlennye zagriazneniai, Okhrana prirody na Urale, Vyp. 5, Svredlovsk, p. 25.

Nikolaevski V. S. 1963. O pokazateliakh gazoustoĭchivosti drevesnykh rasteniĭ. INTA Biologii UFAN, Vyp. 31, Svredlovsk, p. 74.

Ninova D. 1970. Izsledvaniia vŭrkhu anatomichnite pokazateli za dimoustoĭchivost pri niakoi dŭrvesni rasteniia, Gorskostopanska nauka No. 4, p. 7.

Novak K., Skelly J., Schaub M., Kraeuchi N., Hug C., Landolt W., Bleuler P. 2003. Ozone air pollution and foliar injury on native plants of Switzerland. Environmental Pollution 125 (1): 41–52.

Vander Heyden D., Skelly J., Innes J., Hug C., Zhang J., Landolt W., Bleuler P., 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environmental Pollution 111: 321–331.

Veselkin D. V. 2004. Anatomical Structure of Ectomycorrhiza in *Abies sibirica* Ledeb. and *Picea obovata* Ledeb. under Conditions of Forest Ecosystems Polluted with Emissions from Copper-Smelting Works *Russian Journal of Ecology* (ISSN 1067–4136), Vol. 35: 71.