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Snejana B. Dineva

Leaf blade structure and the tolerance of *Acer negundo* L. (Box elder) to the polluted environment

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Abstract: Polluted air is a stress factor that contributes to the decline of urban trees. Air pollution may cause short-term (acute) damage, which is immediately visible and long-term (chronic) damage, which can lead to gradual tree decline. Long-term damage may predispose trees to other disorders. The impact of technogenic factors on the leaf's anatomical structure of *Acer negundo L.* (Box elder) was studied. The thickness of the upper cuticle is increased when compared to those from an ecologically pure area. A change in the rate of mesophyll tissue is due to the enlargement of the palisade parenchyma. The thickness of lower epidermal cells is decreased. All changes of the leaf blade structure are significant and are in direction of increasing the xerophyte characteristics of the leaves. Box elder is native to much of temperate North America. This is a tree of lowlands and wet hardwood forests. As such, we can assume that the registered changes are adaptive responses of the tree to the contaminated environment and that the tree can be considered to be relatively tolerant.

Additional key words: *polluted air, sulphur dioxide,* anatomical structure

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

Introduction

Trees sequester many pollutants from the atmosphere, including sulphur dioxide, ozone, carbon monoxide and particulate matter of ten microns or less (PM_{10}) and therefore the assimilative organs of the plants are the first, directly affected organs of industrially polluted air (Thomas 1951; Kummer 1983).

Sulphur dioxide is a typical industrial emission, which may be injurious to tree foliage directly or through acidic rains (Havas 1971; Havas & Huttunen 1980; Thiel 1985; Traca 1985; Chappelka & Freer-Smith 1995; Sivakumar et al. 2001; Driscoll et al. 2001; James et al. 2003). Acid rain can harm terrestrial plants by damaging outer leaf surfaces and by changing the root environment. Acid rain can strip away the waxy protective coating from plant leaves. Important nutrients such as calcium, magnesium, and potassium can then more readily leach out of leaf tissue by acid rain (James et al. 2003).

The obvious effects of pollution on leaf blades of plants include mottled foliage, "burning" at leaf tips or margins, and stunted growth. In general, the visible injury to plants is of three types: (1) collapse of leaf tissue with the development of necrotic patterns, (2) yellowing or other color changes, and (3) alterations in growth or premature loss of foliage.

As a rule, plants are more severely affected by mixtures of pollutants than individual pollutants. Pollutant mixtures may produce synergistic, additive or antagonistic responses. The extent of leaf injury depends upon the plant species and environmental conditions prior to and during exposure. Ozone and sulphur dioxide often combine to cause plant injury before either of these pollutants alone would cause damage (Edward & Chappelka 2004). Thus the threshold limit of tobacco Bel-W3 to O_3 is reduced if low concentrations of SO_2 are present (Heggestad 1991). Closure of stomata occurs at lower concentrations of O_3 if O_3 and SO_2 are present together (Csintalan & Tuba 1992). A combination of O_3 and HF enhanced senescence in maize that was not apparent when similar concentrations of the pollutants on their own were applied (MacLean 1990). Heck (1989) summarized a number of studies involving pollutant mixtures including exposure information and plant responses. Most work has been in relation to mixtures of O_3 and SO_2 (Fialho & Bucker 1995; Mcleod 1995). To a lesser extent SO_2 and NO_2 combinations have been investigated (Kasana & Lea 1994) and sometimes a combination of all three are studied (Bucker & Guderian 1994). Some studies have investigated metal and gaseous pollutant mixtures (Dueck et al. 1987; Keller & Matysseck 1990; Edwards et al. 1992). Others have focused on the effects of gaseous pollutants and acid rain mixtures on vegetation (Blank et al. 1990; Blaschke & Weiss 1990; Ashenden et al. 1996; Shan et al. 1996). Many studies show that under polluted conditions, plants developed different physiological, morphological and anatomical changes (Evans L.S. & Curry T.M. 1979; Paparozzi & Tukey 1983; Inamdar & Chaudahri 1984; Iqbal 1985; Karenlampi 1986; Gupta & Ghouse 1988; Bhatti and Iqbal 1988; Jahan & Iqbal 1992; Gravano et al. 2003; Novak et al. 2003; Dineva, 2004).

In nature pollutants often occur in combination with other pollutants. It is therefore necessary to investigate the effects of pollutant mixtures on plants. Studies using natural conditions and pollutant levels are therefore more useful. In general, individual trees exhibit considerable variation in their ability to withstand pollutant stress (Sheppard et al. 1989). This work was designed to appraise the structure of the leaf blades of Box elder in relation to its tolerance to an industrially polluted environment.

Materials and methods

Characteristics of the regions

The study examined leaf blades from *Acer negundo L. (Negundo aceroides,* Box elder*)*. The plant material was collected from two regions – one heavily polluted (metallurgical factory "Kremikovtzi", 42°47' N; 23°30'E) and another, as a control (National Park Vitosha, 42°30'N; 23°15'E).

Heat power stations and the "Kremikovtzi" metallurgic plant emit 90% of the total pollution amount in the region under investigation. The field from where the plant material was collected stayed in distance of 2 km from the point source of main contamination, in the region of metallurgical plant. Table 1 gives data on the highest pollution concentration at ground surface level measured in the "Kremikovtzi" district (Tzekova and Delev 2004).

Description of the plant tree – *Acer negundo* L. – Box elder, ash-leaf maple, Manitoba maple

Box elder is native too much of temperate North America (Little 1979). There are also isolated stands of Box elder growing naturally in the mountains of Mexico and Central America. Box elder is cultivated as an ornamental in Europe and has escaped there and become widely naturalized. This is a tree of lowlands and wet hardwood forests. It grows along rivers and streams, often in the subcanopy beneath larger maples, oaks, sycamores, and elms (Bellah and Hulbert 1974).

The Box elder is a maple with compound leaves, similar to those of ash (genus *Fraxinus*). The leaves are pinnately compound with 3 or 5 (rarely 7 or 9) leaflets, each about 5–10 cm long. Each leaf is borne on the end of a slender petiole that is 5.1–7.6 cm. Box elder leaves are often light yellow when they first come out, aging to pale green in summer, and turning yellow-brown in fall before they drop (Hosie 1969).

For 10–20 years Box elder is a very fast-growing tree then it slows down and rarely lives beyond a century (Loehle 1988; Sutton & Johnson 1974). It can grow to 4.6 m in just four or five years. When growing among other trees, Box elder forms a high, open crown, with the undivided portion of the trunk much longer and usually straighter than that of an

Table 1. Concentration of pollution components in the industrial district "Kremikovtzi"

Year	SO_2 [Mg]	NO × [kg]	CO [kg]	Pb [kg]	Dust [Mg]
1994	8203,8	4480525	67359862	15897341	70869
1995	8302,2	4523238	73042454	16570430	74668
1996	11897,5	5493854	66379819	12501979	56679
1997	11869,1	5493854	74094740	16090905	72371
1998	11905,6	5441284	63205819	13981770	62811

open-grown tree (Hosie 1969). This variable-sized tree may reach 21 m in height and 0.92 m in diameter but is more often medium sized, from 12 to 15 m high and from 0.3 to 0.6 m in diameter (Hosie 1969). Box elder requires a lot of pruning to remove broken and dead branches. Box elder does best in full sun exposure. Although it grows naturally in moist soils, Box elder does well in dry, upland sites with well-drained soils. It benefits from watering during prolonged dry spells. Box elder is one of the hardiest, cold tolerant of all broad-leaved shade trees.

Box elder probably is the most robust and aggressive of all the maples, and suitable for use mainly where most other trees cannot survive. It tolerates air pollution, compacted soils, and a wide range of soil pH's, extreme cold, and even brief periods of standing water. Box elder was commonly planted as a street tree (especially in Europe and the American Great Plains) but it is relatively short-lived, tends to break branches in strong winds and under the weight of ice and snow, and drops a lot of litter (Hosie 1969; Szaro 1989; Welsh et al. 1987; Lanner 1983). However, a row of Box elder will make a screen and wind break in a short period.

Plant material and methods

Plant material was collected on 27 and 28 July 2004 year, when the leaves are fully developed and mature. Samples were taken randomly (30 leaf blades from tree) from the south side of the crown at 160–200 cm of the trees (10–15 trees) from each field. The trees were of a similar age (15 years old), sun exposure and had uniform height and growth form. The middle parts of the leaf blades were cut and fixed in 90% ethanol – 90 cm^3, ice acetic acid – 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 leaf blades from the polluted region were entirely covered with necrosis spots, yellow-green and scorched. The results from the anatomical measurements are shown in Table 2.

Under microscopy the difference between the leaves of polluted region and non-polluted was easy differentiate from the cell's size of the lower epidermis layer (Fig. 1). This is only one significant difference in which the polluted leaf blades showed decreasing of the cell size 9.1 μm with σ = 0.5 compared to the control 11.2 μm and σ = 0.5. In the cross sections of the leaves from the pure region the cells from the lower epidermis (11.2 μm and σ = 0.5) have a similar size to that from the upper layer (13.79 μm with σ =1.3).

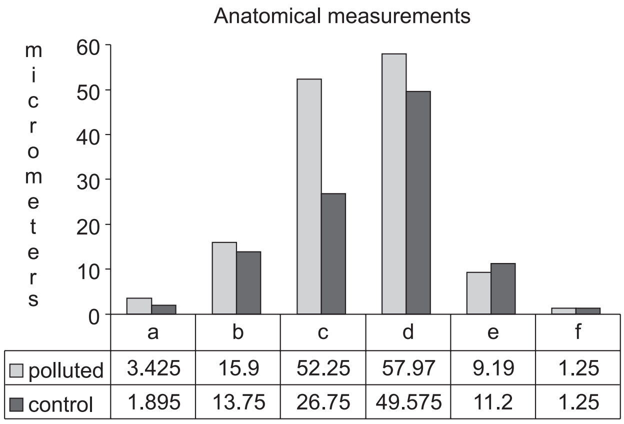

Fig. 1. Anatomical measurements of leaf blades of *Acer negundo* L. a – upper cuticle; b – upper epidermis; c – palisade mesophyll; d – spongy mesophyll; e – lower epidermis; f – lower cuticle

In all other anatomical aspects of the leaves (Table 2) the samples from contaminated field showed higher values in conducted measurements. The leaf blades from polluted region have thicker upper cuticle layer 3.425 μm (σ = 0.45). The upper epidermal cells have mean value 15.925 μm (σ = 1.25), while from the control area is 13.79 μm (σ =1.3). The palisade parenchyma is expanded 52.25 μm and with

Table 2. Differences of anatomical measurements between means of polluted and control samples

Anatomical measurements	Difference of means between polluted and control (μm)
Common thickness of the leaf (S)	36.96***
Thickness of the upper cuticle (a)	1.55***
Thickness of the upper epidermis (b)	2.1**
Thickness of the palisade mesophyll (c)	25.5***
Thickness of the spongy mesophyll (d)	5.75***
Thickness of the lower epidermis (e)	–2***
Thickness of the lower cuticle (f)	0

*: p<0,05; **: p<0,001; ***: p<0,0001

bi-layers cells, in pure field the mean value is only 26.75 μm ($\sigma = 2.67$).

The spongy mesophyll is built up of closely packed cells resembling palisade parenchyma. The intracellular spaces of the spongy mesophyll are extremely small in the leaf blades from the industrial area.

Discussion and conclusion

The region of the metallurgical plant, Kremikovtzi, is heavily polluted with SO_2, N_xO_x, and Pb, As, Zn, Cu etc. Sulphur dioxide is a typical industrial emission which may cause injuries to tree foliage directly or through acid rain. Foliar injury is typically most severe near the source of sulphur dioxide and decreases with increasing distance from the industry (Skelly et al. 1987, Hanisch and Kilz 1990; Turunen and Huttunen 1996). Compound leaves show a greater damage than narrow simple leaves (Aggarwal 2000 a). The Box elder is a maple with compound leaves; our observation revealed that on their surfaces there are many small spots of necrosis and chlorosis.

Studies of the foliar epidermal traits conducted on *Cassia simea L* showed increase in densities of stomata, trichomes and epidermal cells, longer trichomes and reduction in size of epidermal cells at polluted sites as compared to that at reference site (Aggarwal 2000 b). The registered changes in the anatomical measurements of the leaves of Box elder from the industrial contaminated field showed the same tendency with great significance.

Bennett et al. (1992) showed a negative relationship between the thickness of palisade mesophyll and sensitivity to O_3. Palisade mesophyll of sensitive individuals was thinner than that of tolerant individuals by 17% to 27%. Under polluted environment, Box elder developed a thicker mesophyll layer than that in non-polluted conditions. A change in the rate of mesophyll tissue is due to the enlargement of the palisade parenchyma. Thus the coefficient of palisadeness in the leaf blades from the polluted region is 47%, while from the pure ones is only 35%.

The obvious changes in the structure of the leaves suggested that *Acer negundo L.* is under high pressure from the pollution conditions. In our previous studies with other deciduous trees the resistant tree plants have insignificant changes in the anatomy of their leaf blades. The tolerant trees strengthen the anatomic xeromorphic characteristics of their leaf structures under polluted conditions. Both species that were under investigation in our previous work 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 (Dineva 2004). Based on our previous results and those presented here, it can be concluded that Box elder is relatively tolerant to the contaminated environment.

Similar results are obtained by other authors, and describe Box elder *(Acer negundo L.)* as having a range of ozone and sulphur dioxide tolerance and sensitivity (Bonnie and Joel 2000) and relatively tolerant to common air pollutants (Forest Service 1974). Growth of Box elder is poor on saline, sodic, sodic-saline, and most acidic soils; it is not recommended for use in rehabilitation of disturbed sites. This tree's potential for erosion control and for long-term revegetationis is low to medium (Dittberner and Olson 1983).

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