ACTIVE BIOMONITORING OF ECOTOXICOLOGICAL EFFECTS FROM TROPOSPHERIC OZONE IMPACT ON REPRESENTATIVE TREE SPECIES

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ABSTRACT
In order to access the occurrence of phytotoxic ozone effect at urban and rural sites, two-year old seedlings of ozone-sensitive (Quercus robur L.) and ozone-tolerant (Quercus rubra Michx.) tree species were observed. The seedlings were exposed to ambient air for three month period (June-August), 2008 in two monitoring sites - in the city of Sofia, beside a big road with dense traffic, and in the Plana Mountain. The relationship between ozone pollution and ozone-induced effects on leaf area index and water regime were examined. The results for leaf area parameters are too preliminary due to the short period of the examinations. Strong ozone impact was found at the urban site with higher ozone exposure, corresponding to transpiration rate. The ozone induced alterations in physiological activity of tree seedlings may serve as yearly indication for leaf injuries, which could be expected in the further studies. The obtained results are discussed in relation to AOT40 index.

Keywords: biomonitoring, leaf area, transpiration, tropospheric ozone

Introduction
Ozone is recognized as the most phytotoxic air pollution (2). The negative effects of ozone on trees have been well documented at biochemical, physiological, and morphological levels, including growth reduction and the onset of typical visible leaf injury (5). Plant response to ozone may vary with species, varieties, and physiological age. Comparison between sensitive and tolerant species has a key role in assessing ozone damage, investigating the sites of cellular injury, and identifying ozone tolerance mechanism. Injury varies between years in relation to ozone concentrations and water availability (8). Relatively little is known about the influences of growing-season background ozone concentrations on foliar water loss (4). Changes in cuticular properties due to ozone exposure is one of the main reasons for increased cuticular transpiration (12), which may lead to greater stress during summer drought. This can have negative effect on whole tree water balance. Ozone may also damage the guard cells adjacent to the stomata and causes an impairment of the stomatal regulation of transpiration. When transpiration is increased, plants would be stressed by the excess water loss.

To understand consequences on whole tree water balance, effects of ozone exposure on foliar biomass and water loss need to be considered. Leaf is associated with such key physiological processes as photosynthesis and transpiration. That is why the estimation of leaf area is an essential part of plant growth analysis (1). The response of deciduous trees to air pollution is of particular concern, because these species are of critical ecological importance within most national parks and forests in Bulgaria. The aim of the current study was to assess the ozone induced changes in leaf area and transpiration rate for seedlings of two oak species differing in ozone sensitivity exposed to ambient tropospheric ozone.

Materials and methods
Study site and plot design
The study took place from June to August of 2008 in urban and rural regions of western Bulgaria. Monitoring station Drujba is situated in the eastern part of the Sofia city - at an altitude of 580 m. This site is in a close vicinity of high road with very heavy traffic. The climate is moderate-continental. The average annual sum of precipitations amounted to 572 mm. The dominate vegetation here in presented mainly by
deciduous trees and shrub plants. Station Plana is situated at the Plana Mountain in the western part of the country - at 42° 28’34.65” N latitude, 23° 25’39” E longitude and at an altitude of 1234 m a. s. l. Climate is moderate-continental and the average sum of precipitations is 800-850 mm/year. The vegetation of the mountain consists of deciduous and coniferous forests. Local areas with birch, alder and hornbeam are also typical for this region and hazel-bush, briar, hornbeam and hawthorn are often met here.

Nursery grown one-year-old seedlings of *Quercus robur* L. and *Quercus rubra* Michx. are transplanted at October of 2007 into 6-l plastic pots with a mixture of sand and potting soil substrata – four uniform plants in each pot. Seedlings were grown in greenhouse and are daily watered. In May to September of 2008 pots with 2-year-old seedlings were exposed to ambient air at two open plots – a kilometer away from station Drujba and in station Plana. Each plot contained six individuals of each species. Common oak (*Quercus robur* L.) has been well documented as ozone-sensitive trees (3). Red oak (*Quercus rubra* Michx.) is tolerant of tropospheric ozone and urban conditions (9). Pollution exposure, lasting around 90 days, is designed to compare the tree's response to ambient ozone.

**Ozone and meteorological monitoring**

Ozone concentrations were continuously monitored throughout the vegetation season of 2008 using ML®9812 ozone monitor, which was calibrated biweekly. Meteorological data, including air temperature (°C), relative humidity (%) and global radiation (W/m²) were provided by the measurement stations at the open plots.

**Leaf area and transpiration measurements**

Leaf transpiration rate was measured from June to August in 2008 using a LiCor 6400 portable photosynthesis system (LiCor Inc., USA). The average environmental conditions inside the cuvette throughout the measurement periods were 25 °C for air temperature, 50 % for relative humidity and 1100 µmol m⁻² s⁻¹ for photosynthetically active radiation (PAR). Measurements were conducted between 10:00 and 14:00 on suitable weather conditions. Within each plot, one plant per species was selected and one fully sun-exposed leaf was tagged and measured throughout the experimental period. At least 20 readings per plant were made during each measurement.

The leaf area was estimated for each plant from the empirical relationship (10):

$$LA = 2.1371 \times (L^{1.9642}) - 2.7013,$$

where LA is leaf area (cm²) and L is the maximal width of the central leaflet of each leaf (cm).

**Data analysis**

Transpiration rate data were checked for outliers and values outside the determined physiological limits were omitted. Ozone effects on leaf transpiration were tested by ANOVA analysis for each species and sample plot (Systat 7.0).

**Results**

**Ozone exposure and climatic conditions**

Accumulated ozone exposure over 40 ppb AOT40 for vegetation season of 2008 was 21 635 ppb and 27 684 ppb at Plana and Drujba, respectively. These values are more than 2 times above the threshold of 10 000 ppb.h for tree plants. Monthly levels of cumulative ozone doses increased from June to August and both at two sites the highest values of AOT40 were observed in August (Fig. 1).

![Graph of AOT40](image)

**Fig.1. Index AOT40 for June – August period of 2008 in urban (Drujba) and mountain station (Plana)**

The greatest differences between two sites in relation to ozone exposure were found in July. Courses of ozone doses during the growth period tightly followed global radiation and air temperature at that time of the year (Table 1). Monthly mean air temperature in Drujba was with 3-4 °C higher than in Plana. Such trend was observed also for the relative humidity – with a difference of 8 %. Almost equal are the season means of global radiation in two stations.

**Transpiration**

Significant differences in transpiration rate (T) were observed between oak species and sites. Higher ozone exposure at station Drujba, as compared to station Plana, reduced monthly mean T of the leaves for ozone-tolerant *Q. rubra* Michx. as the season progressed. Ozone-sensitive seedlings of *Q. robur* L in station Drujba exhibited the highest rate of
transpiration. Both in two sites T of *Q. robur* L. had a similar dynamics – with a maximum in July and decrease in August when ozone exposure increased markedly. Leaf T levels of *Q. rubra* Michx. in Plana remained fairly constant during the season.

### TABLE 1.

Monthly average values of climatic parameters and transpiration rate at two experimental sites during June-August period of 2008

<table>
<thead>
<tr>
<th>Station</th>
<th>Temperature °C</th>
<th>Air Humidity %</th>
<th>Radiation W/m²</th>
<th>Transpiration (mmol H₂O/m².sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Q. robur</em> L.</td>
<td><em>Q. rubra</em> Michx.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Drujba</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td>20.4</td>
<td>64.6</td>
<td>269, 6</td>
<td>1,095±0,071 0,734±0,080</td>
</tr>
<tr>
<td>Jul</td>
<td>21.7</td>
<td>57.5</td>
<td>259, 3</td>
<td>2,065±0,144 0,057±0,027</td>
</tr>
<tr>
<td>Aug</td>
<td>23.6</td>
<td>51.0</td>
<td>302, 4</td>
<td>1,151±0,054 0,059±0,020</td>
</tr>
<tr>
<td><strong>Plana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td>16, 5</td>
<td>73, 1</td>
<td>285, 1</td>
<td>0,170±0,020* 0,362±0,006*</td>
</tr>
<tr>
<td>Jul</td>
<td>18, 2</td>
<td>65, 8</td>
<td>274, 1</td>
<td>0,468±0,038* 0,353±0,064*</td>
</tr>
<tr>
<td>Aug</td>
<td>20, 4</td>
<td>59, 6</td>
<td>268, 0</td>
<td>0,166±0,022* 0,414±0,075*</td>
</tr>
</tbody>
</table>

* P< 0.05

**Leaf area**

Leaf area was measured for 30 leaves of *Quercus rubra* Michx. at station Plana. The average leaf area amounted to 193, 5 cm². At station Drujba the total number of leaves was 41 and the average leaf area is 178, 5 cm². For *Quercus robur* L. at station Plana 112 leaves were measured with a total area of 101, 6 cm² and at station Drujba - 120 leaves with 95, 3 cm² total leaf area. The total leaf area for both two species examined is greater in station Plana compared to station Drujba.

**Discussion**

The strongest and most consistent negative effects of ozone exposure on water loss were found for *Q. robur*, typically considered to be more ozone sensitive. Thus, ozone tolerance may depend on the response of transpiration rate and capability for water balance maintenance.

Nevertheless *Q. robur* reduced T during the later part of the exposure period, common oak seedlings in Drujba had the highest T in comparison to the other tree species. Thus an exclusive water loss may lead to high water stress and deeper negative effect of ozone action. The tolerant *Q. rubra* maintained low rate of T and the difference between two stations was much less compared to *Q. robur*.

A number of researchers note changes in transpiration rates, usually increases, upon exposure to ozone (6). This effect might be offset by decreased foliar biomass. If stomatal regulation is affected and transpiration increased, plants will allow the additional uptake of pollutants through open stomata and suffer from excess water loss (11). When ozone exposure results in a systemic water deficit, turgor pressure in plant cells may be so reduced that abscission of leaves occurs. Leaf drop appear to be one of the symptoms of ambient ozone exposure. It is the additional factor for leaf area reduction. Many factors such as leaf age and increases of some secondary compounds (phenolics, ethylene) may influence plant response to ozone (6). The soil water deficit also may influence plant response. Besides the high level of ozone exposure at two stations, far above the threshold, adverse effects of ozone exposure to tree seedlings may also occur at concentrations more characteristic over a whole growing season, which are considerably lower than peak concentrations (7).

Significantly more water loss of ozone-sensitive seedlings exposed to urban air conditions than seedlings exposed to mountain air demonstrated that stomatal behavior altered seedling responses to ambient ozone exposures. The observed increased water loss from leaves of sensitive *Q. robur*, exposed to higher ozone dose, could be due to both increased
permeability of the cuticle to water vapour and increased water loss via residual stomatal conductance (11). Many authors suggest that stomatal conduction to water vapor was reduced in the ambient ozone (6). The extent to which increased rates of foliar water loss are compensated will have important role for whole plant water balance and susceptibility to drought stress.

The results for leaf area changes are preliminary, since the test seedlings were examined during the only one vegetation season. It is typical for ozone effect that visible changes in leaves appear during the second year of the exposure. However, some hidden injury or physiological disturbance might have occurred which caused reduction in morphological and anatomical characters of all the plants. The variation of transpiration rate causal by O₃ could be examined as stress signal to the detection of any changes in leaf area.

The results of this work suggest that ozone concentrations commonly observed during a typical growing season, in both urban and montane environments, are often above the critical value of 40 ppb. Cumulative ozone dose AOT40 is more than 2 time above the threshold. Ozone exposures in urban sites lead to higher foliar water loss for Q. robur seedlings, which may have implications for tree water balance. However, ozone exposure also reduced leaf size through effects on new leaf growth, leaf excision and foliar biomass. Importantly, responses were highly species-dependent. Future research needs to address the effects of ozone in field conditions and for trees of different sizes.

Acknowledgements

The study is funded by the DO 02 - 127/2008 and BY H3-03/05 Projects at the Ministry of Education and Science (Bulgaria). Many thanks to the Executive Environment Agency at the Ministry of Environment and Water – Bulgaria and Prof. Donev from the Sofia University for giving of the ozone and climatic data.

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