Differences in histological and physiological traits of ozone sensitive and resistant bean strains

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Abstract: An examination of possible histological causes of differences in ozone sensitivity between ozone sensitive (R123) and resistant (S156) Phaseolus vulgaris strains was carried out. A distinction between the causes and effects of ozone sensitivity was also performed. We studied several morphological and histological traits, which included stomata number and size and also looked at different cell characteristics, such as stomatal index; leaf tissue thickness, fraction and gaseous conductance of intercellular air spaces. Together with this, we made gas-exchange measurements and found inner CO₂ levels to be higher in the ozone sensitive strain. We also found several quantitative morphological parameters between the two strains to be initially different, however, these differences changed after exposure to summer climate and ozone. Stomatal function between the two strains was also differently altered by the pollutant, which was apparent from differences in stomatal openness when investigated in summer. According to our histological data, epidermal cells of the ozone sensitive strain grew larger on leaves that developed after exposure to cumulative considerable phytotoxic ozone doses; moderately decreasing the number of stomata and epidermal cells per mm² epidermal area despite the originally higher number of epidermal cells in sensitive plants. Cross sections of injured sensitive leaves revealed disorganisation of mesophyllum tissues.

Keywords: Ozone sensitivity • Phaseolus vulgaris • Leaf morphology • CO₂ exchange • Stomatal index

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1. Introduction

Ozone effects cultivated plants in two ways: 1) visible symptoms and impairment of photosynthetic efficiency can be named as short-term effects, while 2) long term exposure to ozone causes a decrease in growth and yield, leading to premature senescence [1]. The significance of ozone pollution and its effects have been clearly demonstrated by Sandermann et al. [2], Hayes et al. [3] and Holland et al. [4]: ozone concentrations exceed the phytotoxic thresholds all over Europe. At phytotoxic levels, ozone reduces both crop yield and biomass of sensitive species and affects crop quality, thus causing significant economic losses. Another effect of ozone is the reduction of net primary production and the net carbon exchange of plants, consequently increasing the carbon losses from different cropping systems; increase in CO₂ emissions and groundwater nitrate pollution, and decrease of crop nitrate content. Thus, examination of the effects of ozone on sensitive plant species is extremely important from an economical and environmental aspect.

Crous et al. [5] concluded that the ozone sensitive clone of the bioindicator clover clone pair is more vulnerable to reactive oxygen species that are harmful for cell membranes, making plants much more liable to develop visible symptoms. Fiscus et al. [6] describes ozone sensitivity as accelerated senescence and ozone symptoms by numerous dying or dead cells in the tissues of ozone exposed leaves. Visible symptoms are explained by degradation of palisade cells [7], but in bean leaf tissues it was shown that ozone caused cell death among spongy as well as palisade parenchyma cells [8]. In accordance with the recognition that an abiotic stress which has some oxidative type characteristic (e.g.: metal stress) can influence stomatal operation [9], more open stomata of ozone sensitive plants is a presumable consequence of the oxidative stress that they are exposed to under elevated ozone levels. Numerous studies suggest that ozone directly affects the operation of guard cells [10] through H₂O₂, which easily develops when ozone reacts with different molecules and takes part in the signal pathways of stomatal operation [6,11]. Reactive oxygen species such as hydrogen

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peroxide and superoxide are described as secondary messengers of stomatal closure regulation [12], and by reacting with these active oxygen species, thus changing the polarization grade of guard cell membrane, ozone molecules can influence stomatal closure. Experiments conducted by Elagöz et al. [10] on bean plants showed ozone pollution to affect stomatal conductance as well as stomatal density, and these parameters together with stomatal size mutually affected each other.

As an ozone effect, stomatal density has been shown to increase [11,12] while stomatal or aperture size to decrease [13,14]. Ferdinand et al. [7] found ozone sensitive clone of *Prunus serotina* to have greater stomatal density and smaller palisade parenchyma ratio. The authors claim that resistance can be explained by different gas permeabilities, which is based on varying leaf structure, since gas resistance of palisade tissue is greater than that of spongy tissue.

It has been shown in several studies [15–17], that regulation of stomatal density and cell division in developing leaves is connected with the response that the plant gives to environmental factors or stresses. Study of Navea et al. [18] demonstrates a certain defence mechanism against drought stress by which the plant reduces the number of stomata and refers to it as ‘stomatal abortion’. Stomatal density is shown to be related to water conditions. Beside that, development of stomata during cell differentiation is verified to be conducted by genes that are simultaneously regulating physiological parameters such as stomatal conductance. Stomatal development is also dependent on ambient CO₂ concentration [19]. As part of the regulation of CO₂ exchange and water balance, this genetic/hormonal control is maintained during the entire vegetation period. This control mechanism is able to change stomatal distribution and density of newly developed leaves according to environmental changes [20]. Modification to stomata abundance and size as a stress response is also stated an important pollutant absorption-controlling mechanism [9].

Stengleina et al. [21] contended that stomatal density is essentially affected by both initiation of stomatal development and the expansion of epidermal cells. Expansion of epidermal cells is in turn a function of many environmental and developmental variables: even the altitude of experimental site can influence stomatal index and density. Ozone unbalances the redox state of plant cells, which leads to modifications in metabolic processes and gene expression, which in turn effect cell growth and development. Cell division is particularly sensible to the redox state of cells whereby oxidative stress impedes cell cycle hence proliferation [22].

There are differences in ozone sensitivity among plant species. However, a lot of unanswered questions emerge regarding the possible causes of ozone sensitivity or resistance. Ozone bioindicator bean strains (*Phaseolus vulgaris* R123 and S156) were created by artificial selection [23] to the developed typical ozone symptoms, thus the basis for the difference in ozone sensitivity of the two strains remained unknown. By comparative investigation of the two strains we tried to get closer to the recognition of the causes of ozone sensitivity, and we also sought some histological explanation of different physiology of ozone sensitive and resistant plant genotypes.

### 2. Experimental Procedures

#### 2.1 Plant material

*Phaseolus vulgaris* ozone-sensitive (S156) and ozone-resistant (R123) strains were planted in mould in pots. The volume of pots was 10 liters. Planting and cultivation were implemented according to the protocol of ‘The International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops’ [24]. In winter, plants were grown inside the botanical garden greenhouse of Szent István University, (Gödöllő, Hungary) at approximately 18°C and irrigated regularly. In summer, plants were grown in pots in the same botanical garden, but shaded (according to the ICP protocol) and regularly irrigated. Samples for epidermal impressions and cross-sections were taken twice in 2009, firstly in winter, without considerable ozone levels in ambient air. We used this data as a reference for comparative analysis. While experimental conditions were obviously different in the two seasons, the two genotypes grew under identical conditions in both summer and also in winter. The difference of the measured parameters between the two genotypes in winter could be the base for evaluating the dissimilarities in the impacts of altered environmental conditions and higher ozone levels on the two genotypes. Second sampling was made in summer (August) after plants had been exposed to high ozone pollution in ambient air for an extended period. Seeds from winter plants were sown on 20° December, 2008; seeds of summer generation were sown in May 2008. Samples were taken three months after sowings. In winter, plants were grown in the greenhouse and a sample of 3-5 young and 3-5 mature leaves of 3-3 plants from each genotype were taken for epidermal imprints and cross sections, respectively.

In summer, samples were taken from plants at three different developmental states: 1) young leaves, 2) mature leaves and 3) old/symptomatic leaves. Prior to taking samples, stomatal conductance and gas exchange rates were measured on the respective leaves.