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Psidium guajava 'Paluma' (the guava plant) as a new bio-indicator of ozone in the tropics

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Psidium guajava 'Paluma', a tropical species widely used in Brazilian food industry, is a potential sensitive bio-indicator of ozone.

Abstract

Psidium guajava 'Paluma' saplings were exposed to carbon filtered air (CF), ambient non-filtered air (NF), and ambient non-filtered air + 40 ppb ozone ($NF + O_3$) 8 h per day during two months. The AOT40 values at the end of the experiment were 48, 910 and 12 895 ppb h^{-1}, respectively for the three treatments. After 5 days of exposure (AOT40 = 1497 ppb h^{-1}), interveinal red stippling appeared in plants in the $NF + O_3$ chamber. In the NF chamber, symptoms were observed only after 40 days of exposure (AOT40 = 880 ppb h^{-1}). After 60 days, injured leaves per plant corresponded to 86% in $NF + O_3$ and 25% in the NF treatment, and the average leaf area injured was 45% in $NF + O_3$ and 5% in the NF treatment. The extent of leaf area injured (leaf injury index) was explained mainly by the accumulated exposure of ozone ($r^2 = 0.91$; $p < 0.05$).

Keywords: Ozone sensitivity; Foliar injury; *Psidium*; Tropical tree species, Bio-monitoring

1. Introduction

Ozone (O_3) is currently assumed worldwide as the most important air pollutant. Along the past decades, there has been a global increase in the lower tropospheric O_3 levels (Emberson et al., 2001; Krupa et al., 2001) attributed primarily to increases in anthropogenic O_3 precursors (Derwent et al., 2002). Concentrations in rural or forested areas are generally as high as or higher than in urban regions (Millán et al., 1992).

In the Northern Hemisphere a characteristic problem in the summer is ozone formation, maximum values occurring towards solar noon (ozone photolysis being more intense at higher radiation) and minimum values at night (Fleming et al., 2006). Air contamination by ozone is not different in Brazil, a Southern Hemisphere country. Potentially, the problem is even worse, due to several reasons. First, the phenomenon is not confined to the summer season, since bright days, with high solar radiation and elevated temperatures are common all over the year, favoring the O_3 formation. Second, about half of the Brazilian car fleet in important metropolitan regions, such as São Paulo, is over 10 years old, which contributes to enhance substantially the atmospheric levels of ozone precursors. Consequently, the Brazilian air quality standards for O_3 are frequently above the standard limits. In the city of São Paulo for example, the largest in area and population in Brazil, 265 episodes of ozone concentration exceeding the Brazilian standard of 80 ppb and 88 episodes exceeding the attention threshold (>100 ppb) were registered per year along the last 5 years, totaling 74 days with acute episodes (CETESB, 2005). Emberson et al. (2001) predicted that ozone

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tropospheric levels in Brazil tend to increase or at best remain unchanged over the next 30 years. Third, the monitoring of air quality by physical and chemical methods is still very limited in the country, even in the more developed South and Southeast regions. Alternative methods, such as bio-monitoring with sensitive plant species, are thought to be effective means for alleviating this problem.

These biological methods are feasible because O_3, being a strong oxidant, affects plants in several ways, including visible foliar injury, inhibition of photosynthesis and alterations of carbon allocation, growth and productivity reduction, changes in crop quality and increased sensitivity to abiotic and biotic stresses (Chappelka et al., 1997; Krupa et al., 2001; Zhang et al., 2001).

The identification and characterization of the O_3-induced foliar injury symptoms in well adapted plant species to a certain environment, by means of controlled or field experiments, are of major interest for assessing realistically the risks imposed by air pollutants on local species or vegetation, defining areas with phytotoxic concentrations and detecting levels of chronic pollution (Innes et al., 2001; Krupa et al., 2001; Sanz et al., 2001; Zhang et al., 2001). In fact, the bio-indicator organisms react to both atmospheric concentration of pollutants and climatic condition during exposure, the intensity of responses depending on the biological characteristics of each species, enabling the determination of the real amplitude of stress to which the plants and vegetation are exposed (Smith et al., 2003). Field surveys have recorded O_3-like injury symptoms on numerous tree, shrub, and forbs species in Europe and North America (Innes et al., 1996; Skelly et al., 1998; Van der Heyden et al., 2001; Sanz et al., 2002; de Vries et al., 2003). However, little information is available on the effects of ozone on the multitude of native plant species throughout Europe or even North America, and much less in tropical regions where O_3 concentrations have been increasing. The necessity of modeling bio-indicator plants to a regional scale, such as to the tropics, becomes prominent.

The objectives of the present study were to identify and characterize exposure−response relationships for O_3-induced foliar injury on a Brazilian tree species, *Psidium guajava* 'Paluma' (the guava plant), and to examine its utility in bio-monitoring studies in the tropics. The cultivar Paluma was selected due its high economical importance.

2. Material and methods

2.1. Plant material

P. guajava L. (Myrtaceae) is a native fruit tree in the Tropical Rain Forest. Its fruits are widely consumed either fresh or processed (beverages, syrup, ice cream and jams) and it is also used in ethno-medicine because of anti-malarial and anti-oxidant properties (Olajide et al., 1999; Jaiarj et al., 2000). Previously, this species was also found to be a good accumulator of sulfur and fluoride in bio-monitoring studies (Pandey and Pandey, 1994; Singh et al., 1994; Moraes et al., 2002).

P. guajava 'Paluma' is a new fruit cultivar for the fresh market and processing industry. In Brazil, its plantations cover over 13 500 ha, with an annual fruit production of 300 000 tons (IBGE). Saplings of *P. guajava* 'Paluma' were obtained from Agromillora Taperão Ltda., a Brazilian producer specializing in shoot production by rooting semi-herbaceous guava cuttings.

2.2. Experimental design

The experiment was conducted at "La Peira experimental field" (39°6′N−00°26′W, 30 m above sea level) of the Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), Valencia, Spain. Saplings of *P. guajava* (~30 cm tall) were planted in pots (1.6 L) with standardized substrate (coconut fiber, peat, vermiculite, sand and granulated fertilizer − 5:3:1:1:0.1). For acclimation, they were kept for 45 days in an open top chamber (OTC) with filtered air (Purafil activated charcoal filter). The ozone exposure was carried out from August 26 to October 21, 2002. During the period, mean temperature, relative humidity and rainfall at La Peira were, respectively, 26.6 °C, 40% and 50 mm. During both the acclimation and exposure periods, water supply was provided to the plants on a daily basis and fertilizer on a weekly basis, by aspiration. The plants were observed throughout the experiment in order to ensure protection against insect and pests. Spraying specific products against pests was not necessary.

After 45 days of acclimation, 10 plants were transferred to each controlled ozone exposure. One open top chamber (OTC) was used to each of the following treatments: CF − ambient air filtered through activated charcoal (Purafil®, Purafil Inc, USA), NF − non-filtered ambient air, and NF + O_3 − non-filtered air plus ozone. Ozone was generated by passing a stream of pure oxygen through a high voltage discharge generator. Blowers (0.5 CV or 2500 rpm) connected to each OTC guaranteed a similar flux of air and climatic conditions for all of them. The chambers were equipped with particulate filters. Every day, from 8:00 to 16:00 GMT, O_3 concentrations in the NF + O_3 treatment were hourly raised above the ambient by 40 ppb. The levels of ozone in the NF + O_3 chambers were controlled as required by dilution with filtered air. The concentrations of ozone were sequentially measured in each OTC by an ozone analyzer (DASIBI 1008-RS, Dasibi Environmental Corp., USA), and sensors installed inside each OTC monitored the temperature and relative humidity. Hourly O_3 concentrations were used to estimate AOT40 (accumulated ozone exposure over a threshold of 40 ppb h^{-1}).

2.3. Visible foliar injury assessment

Whole-plant foliar injury was assessed weekly during the two months on 10 saplings in each exposure treatment. All plants were observed macroscopically. Symptoms usually appeared as dark-colored stipple (reddish) on the upper leaf surface only; they did not occur on leaf veins or veinlets; and initially appeared with greater severity on older leaves towards the base of the plant (Innes et al., 2001).

On each plant, the percentage of leaves showing symptoms was quantified. In addition, the percentage leaf area showing symptoms was estimated in 5% increments and used to calculate the Leaf Injury Index (LII) modified from El-Khatib (2003):

$$\mathrm{LII}(\%) = \frac{(N_1 \times 1) + (N_2 \times 2) + (N_3 \times 3) + (N_4 \times 4) + (N_5 \times 5)}{(N_0 + N_1 + N_2 + N_3 + N_4 + N_5) \times 5} \times 100$$

where N_0, N_1, N_2, N_3, N_4 and N_5 are numbers of leaves with zero, first, second, third, fourth and fifth degree symptoms of ozone injury, respectively. The degree of symptoms is represented by: 0: no symptoms; 1: 0−5%; 2: 6−25%; 3: 26−50%; 4: 51−75%; 5: more than 75% of foliar area with symptoms.

2.4. Statistical analysis

The AOT40 values determined during the experiment were plotted versus the results of LII and a regression analysis was performed (after testing normality and homoscedasticity) with AOT40 as the predictor.