BRIEF COMMUNICATION

Photosynthetic responses of tropical trees to short-term exposure to ozone

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Abstract

Saplings of the tropical trees *Tibouchina pulchra* (Cham.) Cogn., *Caesalpinia echinata* Lam., and *Psidium guajava* L. cv. *Paluma* were exposed in open-top chambers with charcoal filtered air and measurements of gas exchange and chlorophyll fluorescence were made before (t_1) and after exposure to non-filtered air plus O_3 (t_2), simulating 6-h peaks of O_3 similar to those observed in São Paulo city (SE Brazil, reaching an *AOT40* of 641 nmol mol^{-1}). After the fumigation, the net photosynthetic rate, stomatal conductance, transpiration rate, and F_v/F_m were reduced (p<0.05) for the three species. *C. echinata* was the most sensitive species and *P. guajava* cv. *Paluma* the most resistant.

Additional key words: *Caesalpinia echinata*; chlorophyll fluorescence induction; net photosynthetic rate; photosystem 2; *Psidium guajava*; stomatal conductance; *Tibouchina pulchra*; transpiration rate.

In the city of São Paulo, Southeast Brazil, the largest in territory and in population, approximately six million of motor vehicles are circulating and emitting pollutant precursors of ozone. Besides, climate conditions that favour the formation of ozone (O_3), such as bright days with high irradiance and elevated temperatures, commonly occur during the year at that region. As a result, O_3 concentrations in São Paulo are frequently over the standard limits established by the Brazilian legislation (hourly concentration > 82 nmol mol^{-1}).

Taking into account that acute episodes of O_3 generally cause serious disturbances to the plant species (Heath 1994), it is possible to suppose that native plant species from urban fragments of forest as well as ornamental and cultivated species growing around the city might suffer the deleterious effects of O_3. Among so many tropical trees growing in São Paulo, *Tibouchina pulchra* (Cham) Cogn., *Caesalpinia echinata* Lam., and *Psidium guajava* L. cv. *Paluma* deserve special attention in relation to their sensitivity to O_3.

T. pulchra (Melastomataceae) and *C. echinata* (Leguminosae) are important native trees of the Atlantic forest along the seacoast of the Southeastern and Northeastern regions of Brazil. *T. pulchra* occurs at different environmental conditions, including the stressing situations around industries and has great potential for biomonitoring studies (Klumpp *et al.* 2000). *C. echinata* is the symbol tree of Brazil, being regionally known as *pau-brasil* or *pernambuco* (brazilwood). *P. guajava* (*Myrtaceae*) is an important commercial fruit tree in Brazil, frequently cultivated in São Paulo State and its wild variety revealed to be accumulator of sulphur and fluorine (Moraes *et al.* 2002).

The present study tested the hypothesis that short-term exposures of O_3 similar to those frequently registered in the city of São Paulo may cause photosynthetic disturbances, creating different risk situations and threatening growth of these species.

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Abbreviations: *AOT40* – accumulated ozone doses above the threshold of 40 mm^3 m^{-3} h^{-1} or approximately 43 nmol mol^{-1}; E – transpiration rate; F_v/F_m – potential photosystem 2 quantum efficiency; g_s – stomatal conductance; P_N – net photosynthetic rate.
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The experiment was conducted at "Campo Experimental La Peira" (39°6'N–00°26'W, 30 m above sea level), belonging to the Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), at Valencia, Spain. Saplings of *T. pulchra*, *C. echinata,* and *P.guajava* cv. *Paluma* were planted in pots (1 600 cm^3) with standardized substrate (coconut fibre, peat, vermiculite, sand, and granulated fertilizer – 5 : 3 : 1 : 1 : 0.1). After 75 d of acclimation in an open-top chamber (OTC) with filtered air [*Purafil* activated charcoal filter, *Purafil*, Doraville, CA, USA; average hour concentration of 12 nmol(O_3) mol^{-1}], net photosynthetic rate (P_N), stomatal conductance to water vapour (g_s), transpiration rate (E), and the potential photosystem 2 (PS2) quantum efficiency (F_v/F_m) were determined in the second leaflet of five plants (t_1). The plants were fumigated during 6 h, from 10:00 to 16:00 GMT, with non-filtered air plus O_3, which simulated a realistic episode of high concentrations of ozone during springtime in São Paulo city, according to air quality data from CETESB (2002). Ozone was generated from pure oxygen through a high voltage discharge generator (*SIR S.A*., Madrid, Spain) and the concentrations of O_3 fumigated were controlled by dilution with filtered air. A *DASIBI 1008 RS* (*Dasibi Environmental Corp*., Glendale, CA, USA) monitor continuously measured the concentrations of O_3 before and during fumigation, and sensors measured the temperature and the relative air humidity inside the open top chambers.

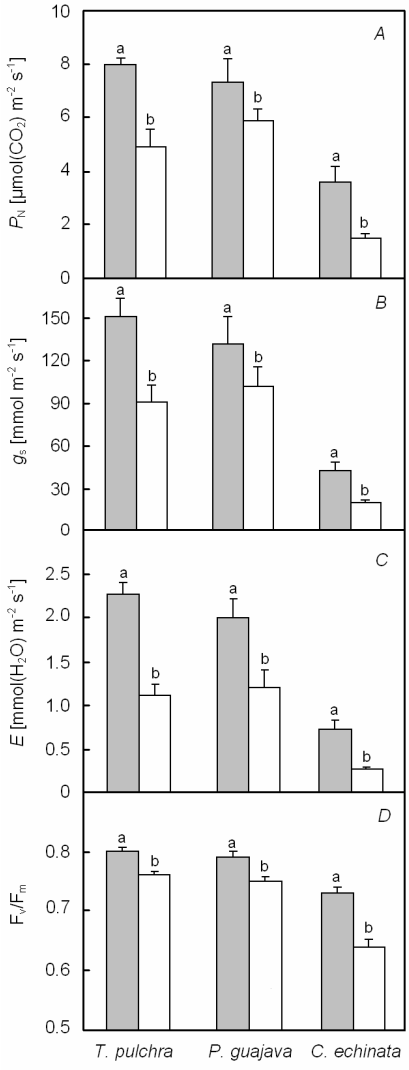

Fig. 1. Mean net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and potential photosystem 2 quantum efficiency (F_v/F_m) in saplings of *T. pulchra*, *P. guajava* cv. *Paluma*, and *C. echinata* before (t_1, *full columns*) and after 6 h of exposure to O_3 (t_2, *empty columns*). *Bars* represent S.D. (n = 5). For each species *different letters* indicate significant differences from t_1 measurements (p<0.05).

The *AOT40* reached 641 nmol mol^{-1} (t_2), with maximum hourly concentration of 181 nmol mol^{-1} and minimum of 45 nmol mol^{-1}. Mean relative air humidity in the OTCs was 50 % and the mean temperature was 18 °C.

Just after finishing fumigation, P_N, g_s, E, and F_v/F_m were determined in the same leaflet of all plants (t_2). The gas exchange measurements were made through a portable infrared gas analyzer system (*HCM-1000, Walz*, Effeltrich, Germany), which was connected to a leaf chamber with control of temperature and irradiance and monitoring of the relative air humidity and CO_2 concentration. P_N, g_s, and E were determined at ambient CO_2 concentration, with leaf temperature 23–28 °C and saturating photosynthetic photon flux density of 800 µmol m^{-2} s^{-1}. This irradiance was saturating for the three species as previously determined by the establishment of irradiance response curves. Ambient temperature was 28 °C. Potential PS2 quantum efficiency values (F_v/F_m) were obtained by means of a modulated chlorophyll fluorescence analyzer (*PAM-2000, Walz*, Effeltrich, Germany), after a 45 min period of adaptation of leaves to the dark.

The results for each photosynthetic parameter, obtained before and after the fumigation for each species, were compared by *t*-test (p<0.05). Analyses of variance (F-test) followed by a multiple comparison test (*Tukey* test) were employed for comparisons among the species (p<0.05).

P_N, g_s, E, and the F_v/F_m ratio in *C. echinata* were lower than in the other two species before and after fumigation. On the other hand, those parameters were similar in *T. pulchra* and *P. guajava* before and after fumigation (p<0.05). All the plants presented significant reductions in the photosynthetic parameters after the exposure to O_3 (Fig. 1). However, these reductions were significantly less intense in *P. guajava* than in the other species (Table 1). *C. echinata* showed the highest decrease in g_s and F_v/F_m.

The photosynthetic responses reflected the inherent growth characteristics of each species. *P. guajava*, with faster growth, not only showed higher P_N, g_s, and E, but also less intense decreases in the parameters measured after fumigation than *C. echinata*, the species with very

low growth rates. Even considering that this last species should have been exposed to a lower dose of O_3, due to the low g_s, its photosynthetic responses to the short exposure to O_3 were more exacerbated, showing to be the most sensitive among the three species studied. *P. guajava* was the least responsive to O_3, taking into account the photosynthetic parameters measured.

Table 1. Percent reduction in relation to t_1 measurements in net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and potential photosystem 2 quantum efficiency (F_v/F_m) in saplings of *T. pulchra*, *P. guajava* cv. *Paluma*, and *C. echinata* after 6 h of exposure to O_3. Means ± S.D., n = 5. *Different letters* indicate significant differences between species (p<0.05).

	T. pulchra	*P. guajava*	*C. echinata*
P_N	38.6 ± 6.3 ab	20.9 ± 4.2 b	50.8 ± 4.7 a
g_s	46.1 ± 3.6 ab	26.4 ± 5.1 b	49.4 ± 8.8 a
E	50.1 ± 6.4 c	37.2 ± 7.1 b	65.0 ± 4.7 a
F_v/F_m	4.83 ± 0.66 b	6.57 ± 0.41 b	13.70 ± 2.20 a

The concomitant reductions of g_s and P_N indicated that g_s possibly restrained the CO_2 uptake, contributing to the decrease of P_N during the period of fumigation. But, the decrease in P_N could also have been a result of the less efficient process of carboxylation, due to the depression caused by O_3 in the synthesis and the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase, which can precede the stomatal responses (Farage and Long 1999, Castagna *et al.* 2001). Anyway, if the studied species are exposed to successive peaks of O_3 under urban field conditions, their growth will supposedly be restrained.

The decrease of F_v/F_m indicated a photochemical loss in the efficiency of excitation capture of PS2 in leaves adapted to the darkness. Ozone, as well as other environmental stresses, can limit the plant capacity of using the photon energy (Pell *et al.* 1992). In the absence of any mechanism to avoid the potentially harmful energy of excitation stored in the photochemical apparatus, a decrease in P_N is expected, causing a reduction of the reaction centres, which results in photoinhibition (Castagna *et al.* 2001, Ort 2001). This phenomenon was observed for the three tropical tree species studied, but more intensively in *C. echinata*, once the saplings revealed the strongest reduction in that ratio.

Our study demonstrated that the restrictions in photosynthesis process of the studied species could be observed just after short-term exposure to O_3, forming the following gradient of photosynthetic damage: *C. echinata* > *T. pulchra* > *P. guajava*.

Episodic peaks of high concentrations of O_3 during only some hours, accounting similar *AOT40* simulated in the present study, are frequently observed along sunny days of spring and summertime in São Paulo. During 2001, for example, the hourly standard limit of O_3 concentration (82 nmol mol^{-1}) was surpassed 1 139 times, 377 of the values were over 102 nmol mol^{-1}, totalling 78 d of the year with episodic peaks of ozone (CETESB 2002). In this manner, the decreases in the measured photosynthetic parameters in young trees of all three species are expected in the urbanized environments of São Paulo, confirming the existence of a risk situation imposed by O_3 and a potential restriction to their growth and survival, mainly as concerns *C. echinata*.

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