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Ozone affects leaf physiology and causes injury to foliage of native tree species from the tropical Atlantic Forest of southern Brazil

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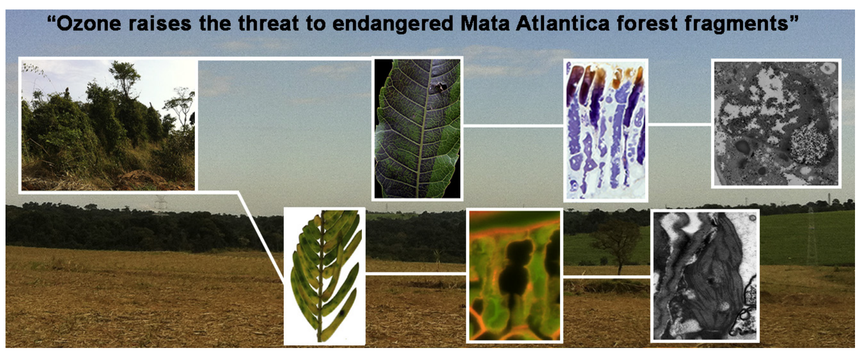
HIGHLIGHTS

- Ozone levels in southern Brazil affect the remnants of the tropical Atlantic Forest.
- Macro and microscopic injury in foliage was reproducible and similar to that observed in other biomes.
- The high tree biodiversity contributed to the wide spectrum of symptoms.
- More than one third of trees from two sensitive species showed leaf injury.

GRAPHICAL ABSTRACT



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ABSTRACT

In southern Brazil, the recent increase in tropospheric ozone (O_3) concentrations poses an additional threat to the biodiverse but endangered and fragmented remnants of the Atlantic Forest. Given the mostly unknown sensitivity of tropical species to oxidative stress, the principal objective of this study was to determine whether the current O_3 levels in the Metropolitan Region of Campinas (MRC), downwind of São Paulo, affect the native vegetation of forest remnants. Foliar responses to O_3 of three tree species typical of the MRC forests were investigated using indoor chamber exposure experiments under controlled conditions and a field survey. Exposure to 70 ppb O_3 reduced assimilation and leaf conductance but increased respiration in *Astronium graveolens* while gas exchange in *Croton floribundus* was little affected. Both *A. graveolens* and *Piptadenia gonoacantha* developed characteristic O_3-induced injury in the foliage, similar to visible symptoms observed in >30% of trees assessed in the MRC, while *C. floribundus* remained asymptomatic. The underlying structural symptoms in both O_3-exposed and field samples were indicative of oxidative burst, hypersensitive responses, accelerated cell senescence and, primarily in field samples, interaction with photo-oxidative stress. The markers of O_3 stress were thus mostly similar to those observed in other regions of the world. Further research is needed, to estimate the proportion of sensitive forest species, the O_3 impact on tree growth and stand stability and to detect O_3 hot spots where woody species in the Atlantic Forest are mostly affected.

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1. Introduction

During the pre-Columbian era, the Atlantic Forest covered approximately 150 million ha in tropical and subtropical areas of eastern Brazil. Nowadays, the forest coverage amounts to 16,380 ha (approximately 11 to 16% of the former extension), distributed across *ca.* 245,000 forest fragments (Ribeiro et al., 2009). Forest fragmentation increases inland, such as in the Metropolitan Region of Campinas (MRC) of central eastern São Paulo state where 15% of the state area is still forested (Fig. S1).

Forest stands in the MRC belong to the semi-deciduous forest type, including 450 species of evergreen (45%), semi-deciduous (13%) and deciduous (42%) tree species (Morellato, 1991; Oliveira-Filho and Fontes, 2000; Santin, 1999). All year round, however, the forest canopy remains foliated, and the foliage turnover is highly species specific. The mostly discontinuous forest fragments of MRC are under heavy pressure by intensive agriculture (mostly sugarcane), forest fires, selective logging on the one side and abandonment on the other, leading to a shrinking coverage (Candido and Nunes, 2010; Domingos et al., 2015). As a consequence, these areas show physiognomic signs of disturbance, including open canopies, lush understory and high dominance of climbing vines and bamboos (Filho and Santin, 2002). Two-thirds of the mid- and late-successional species are endangered (Pütz et al., 2011). However, the conservation of these precious remnants of Atlantic Forest is still not a priority. In addition to land use-related pressure, elevated ambient concentrations of tropospheric ozone (O_3) - which is regarded as the air pollutant most detrimental to vegetation (Matyssek et al., 2012; Garthwaite et al., 2009; Matyssek and Sandermann, 2003) - have recently reached phytotoxic levels in the MRC (Moura et al., 2014a). In addition to pollutants from local sources, a high supply of O_3 and its precursors is transported to the MRC from the Metropolitan Region of São Paulo (MRSP) by dominant winds (Boian and Andrade, 2012).

The effects of phytotoxic O_3 levels on the native vegetation have been assessed using field surveys of foliar symptoms, based on the relative specificity of O_3 stress injury (Innes et al., 2001; Novak et al., 2003; Sanz and Calatayud, 2010; Skelly et al., 1999). So far however, inventories have been primarily conducted at mid- and higher latitudes (Bussotti et al., 2003; Feng et al., 2014; Schaub and Calatayud, 2013; Smith, 2012). In angiosperms, O_3 injury is indicated by dot-like or confluent whitish to dark-brown stippling associated with interveinal discoloration in the form of bleaching, bronzing or reddening patterns. These symptoms form gradients of increasing severity basipetally, and their development is prevented by shading. In addition to these common traits, however, the morphology of symptoms largely varies between species, and there is a risk of confusion with symptoms caused by several biotic and abiotic stress factors (Günthardt-Goerg and Vollenweider, 2007; Vollenweider and Gunthardt-Goerg, 2006). In Brazil, only a few studies using standard bioindicators (Sant'Anna et al., 2008) or native trees (Furlan et al., 2008) have provided some preliminary indications of harmful O_3 effects on species growing under tropical conditions. Under experimental O_3 exposure, *Caesalpinia echinata* showed a reduction in net carbon assimilation, stomatal conductance and transpiration but no visible injury (Moraes et al., 2006). However, the foliage of *Tibouchina pulchra* responded to O_3 exposure with visible injury, developing interveinal, adaxial, dark–reddish-colored stippling, primarily in older foliage (Furlan et al., 2008). However, the O_3 sensitivity and types of symptom development in trees from the Atlantic Forest are still mostly unknown, and the responses of tropical ecosystems to current O_3 levels need to be specifically assessed (Sitch et al., 2007).

The assessment of O_3-like visible injury being conducted on new species and ecosystems requires, in particular, the use of thorough validation techniques. Microscopic methods provide tools for 1) confirming the diagnosed stress factor, 2) excluding the causal role of other stressors and 3) characterizing the synergies between O_3 and other environmental constraints (Alves et al., 2011; Faoro and Iriti, 2009; Günthardt-Goerg and Vollenweider, 2007; Kivimäenpää et al., 2004). Recent research has demonstrated the link between the plurality of structural changes and the numerous processes and plant responses that are triggered or amplified by O_3 stress within foliage. These processes and responses include oxidative burst (OB) in the apoplast (Günthardt-Goerg et al., 1997; Pasqualini et al., 2003) and symplast (Moura et al., 2014b; Pellinen et al., 1999), photo-oxidative stress (Calderòn Guerrero et al., 2013; Foyer et al., 1994), accelerated cell senescence (ACS; Günthardt-Goerg and Vollenweider, 2007; Inada et al., 1998; Mikkelsen and Heide-Jørgensen, 1996) and hypersensitive response-like (HR-like) (Levine et al., 1996; Pasqualini et al., 2003; Vollenweider et al., 2013). Considering the large biodiversity of Atlantic Forest and rather stable tropical climate or O_3 regime (Moura et al., 2014a), the similarity of structural markers of O_3 stress in the native trees of MRC *versus* those recorded in forests at higher latitudes still requires confirmation.

The present study was launched while the first evidence of phytotoxic O_3 levels (Boian and Andrade, 2012; Moura et al., 2014a) and visible foliar injury (Alves et al., 2011; Klumpp et al., 2000) was reported. The principal objective was to determine whether the current O_3 levels in the rural region downwind of São Paulo affect the native vegetation of endangered forest fragments from the once continuous Atlantic Forest. The tested hypotheses included: 1) in response to current O_3 levels, the foliage of tropical trees shows injury comparable to that observed in foliage of plants from biomes at higher latitudes (1.1) and resulting from changes in the leaf physiology induced by oxidative stress (1.2); 2) the sensitivity of native trees from the Atlantic Forest fragments to O_3 stress is species-specific. Therefore, the impact of O_3 stress in the MRC was studied by means of a) exposure experiments under controlled conditions, and b) a field survey of O_3 injury performed along selected forest fragments. Both studies were ascertained using microscopical validation and by comparing O_3 injury, developed during exposure experiments, *versus* that occurring under field conditions. Three native tree species from different plant families, distributed throughout the MRC and with contrasting functional traits, were selected as exemplary representatives of the MRC forest fragments. Seedlings were exposed to O_3 levels slightly higher than those measured in the MRC (Moura et al., 2014a). For the field survey, we applied a methodology similar to that in use in Europe (Schaub and Calatayud, 2013). Changes in leaf physiology and visible injury in samples from the exposure experiment and MRC forest fragments were mechanistically related to stress responses at the tissue and cell level using several microscopical techniques (Fink, 1999; Günthardt-Goerg and Vollenweider, 2007).

2. Materials and methods

2.1. Environmental conditions, vegetation and ozone pollution at the study sites

The study sites were located along the eastern and northern edge of four forest fragments in the gently sloping highlands of MRC, west of São Paulo (550–690 m a.s.l.; Fig. S1). The MRC covers an area of approximately 3647 km^2, includes 20 cities and is home to >3 million inhabitants (Moura et al., 2014a). It is thus one of the largest urban areas in Brazil. It experiences a humid subtropical climate (Cfa, according to the Köppen classification, Alvares et al., 2013), with a hot and rainy season from October to March and a dry season from April to September (Moura et al., 2014a). During the wet season, the monthly rainfall and average temperature reach 200 mm and 24 °C but drop to 30 mm and 20 °C, during the dry season (Franchito et al., 2008). The forest fragments grow on oxisol soils derived from various types of parent bedrock (sandstone to diabase; Lopes et al., 2015). The land surrounding the fragments supports a somewhat dense and mixed network of agricultural, industrial and urban activity. Sugarcane is the main cash crop (Lopes et al., 2015).