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Current ambient concentrations of ozone in Panama modulate the leaf chemistry of the tropical tree *Ficus insipida*

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HIGHLIGHTS

- Elevated [O_3] was recorded at three forests near NO_x emission hotspots in Panama.
- This [O_3] affected the physiology of the widespread native tree *Ficus insipida*.
- The effects of O_3 on *F. insipida* included a decrease in leaf chemical defenses.
- O_3 also led to decreased lipid content in mature leaves.
- AOT was below critical levels for temperate trees, but *F. insipida* has high g_{s_max}.

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ABSTRACT

Tropospheric ozone (O_3) is a major air pollutant and greenhouse gas, affecting carbon dynamics, ecological interactions, and agricultural productivity across continents and biomes. Elevated [O_3] has been documented in tropical evergreen forests, the epicenters of terrestrial primary productivity and plant-consumer interactions. However, the effects of O_3 on vegetation have not previously been studied in these forests. In this study, we quantified ambient O_3 in a region shared by forests and urban/commercial zones in Panama and found levels two to three times greater than in remote tropical sites. We examined the effects of these ambient O_3 levels on the growth and chemistry of seedlings of *Ficus insipida*, a regionally widespread tree with high stomatal conductance, using open-top chambers supplied with ozone-free or ambient air. We evaluated the differences across treatments in biomass and, using UPLC-MS-MS, leaf secondary metabolites and membrane lipids. Mean [O_3] in ambient air was below the levels that induce chronic stress in temperate broadleaved trees, and biomass did not differ across treatments. However, leaf secondary metabolites – including phenolics and a terpenoid – were significantly downregulated in the ambient air treatment. Membrane lipids were present at lower concentrations in older leaves grown in ambient air, suggesting accelerated senescence. Thus, in a tree species with high O_3 uptake via high stomatal conductance, current ambient [O_3] in Panamanian forests are sufficient to induce chronic effects on leaf chemistry.

1. Introduction

Tropospheric ozone (O_3) is a major globally relevant secondary air pollutant and greenhouse gas, and has a suite of adverse effects on plant growth and physiology. Ozone forms naturally as a product of photochemical reactions with the precursors NO_x, CH_4, CO, and volatile organic compounds (VOCs). Over the industrial period, anthropogenic emissions of these precursors from fossil fuel and biomass burning have increased ambient tropospheric O_3 concentrations over a large portion of the Earth's surface (Vingarzan, 2004). As a secondary air pollutant, O_3 is often present at high concentrations in VOC-emitting vegetated areas that are downwind

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from sources of NO_x precursors. Thus, tropospheric O_3 has become a global air pollutant: many regions are already experiencing near-surface O_3 concentrations that can cause visible plant damage and/or reduce primary productivity in natural and agricultural ecosystems (Sandermann, 1996; Fuhrer et al., 1997; Chappelka and Samuelson, 1998; Skarby et al., 1998; Fowler et al., 1999; Vingarzan, 2004; Mills et al., 2011a,b; Ainsworth et al., 2012). Further, regional and global models have forecasted near-surface O_3 concentrations to reach two to eight times their pre-industrial levels by the year 2100 (Fowler et al., 1999; Vingarzan, 2004; Wild et al., 2012; Naik et al., 2013), with the potential to significantly reduce the land carbon sink and thus contribute to climate warming (Sitch et al., 2007).

The influence of O_3 on plants has generally been assessed on the basis of reductions in plant growth and productivity (Ashmore, 2005). Ozone generates reactive oxygen species and causes oxidative stress within leaves, which in turn decreases photosynthesis, plant growth, and biomass accumulation (reviewed by e.g. Skarby et al., 1998; Chappelka and Samuelson, 1998; Karnosky et al., 2007; Ainsworth et al., 2012). Early experiments on the impacts of O_3 on herbaceous crop species led to an adoption of $[O_3] = 40$ ppb as an approximate threshold, or critical level, for adverse effects on plant productivity. However, it was subsequently recognized that this critical level varies widely among plant species and even among genotypes within species, due mainly to differences in stomatal conductance and thus differences in the flux of O_3 into the leaf at a given value of ambient $[O_3]$ (reviewed by Fuhrer et al., 1997; Chappelka and Samuelson, 1998; Musselman et al., 2006; Karnosky et al., 2007; Mills et al., 2011a; Ainsworth et al., 2012; Anav et al., 2016). A key finding from these studies is that stomatal conductance and $[O_3]$ both exhibit a linear relationship to leaf-level O_3 dose (dose $\propto g_s * [O_3]$). Consequently, plant biomass or photosynthesis reductions in response to O_3 are generally correlated more strongly with cumulative O_3 uptake than with accumulated O_3 exposure (reviewed by Karlsson et al., 2004; Büker et al., 2015). In addition to stomatal conductance, the leaf-level capacity for detoxification of reactive oxygen species also plays a role in determining the critical level for a species or genotype.

Although tropospheric O_3 is a global air pollutant, its effects on plant productivity and trophic interactions have mainly been studied in temperate regions of the northern hemisphere. This is despite the potential for elevated $[O_3]$ to have deleterious impacts on plant productivity and the tropical carbon cycle (Pacifico et al., 2015). In forested regions of the tropics, numerous monitoring campaigns have already studied $[O_3]$ at and near the atmosphere-biosphere interface (Kaplan et al., 1988; Fan et al., 1990; Jacob and Wofsy, 1990; Kirchhoff et al., 1990; Cros et al., 1992, 2000; Andreae et al., 2002; Gut et al., 2002; Rummel et al., 2007; Karl et al., 2009; Jardine et al., 2011; Andreae et al., 2015). During the LBA-EUSTACH project (Rummel et al., 2007), $[O_3]$ within the forest canopy exceeded 40 ppb, the exposure-based critical level for many temperate plant species (reviews by Fuhrer et al., 1997; Chappelka and Samuelson, 1998).

Modeling efforts over the past decade have explored the ramifications of elevated O_3 on tropical forest ecosystems. Sitch et al. (2007) simulate a large negative impact of projections of increasing O_3 concentrations on the tropical carbon sink. In addition, Pacifico et al. (2015) were able to reproduce the Rummel et al. (2007) deposition velocities in an Earth System Model, and thus we have confidence in the ability to simulate the O_3 flux into leaves. However, these global modeling efforts rely on dose response relationship based on temperate and boreal species (Sitch et al., 2007; Pacifico et al., 2015). Few studies have investigated the effects of O_3 pollution on tropical tree species (Cassimiro and Moraes, 2016; Furlan et al., 2008), and with notable exceptions (Assis et al., 2015) little work has been done in integrating leaf-physiology and exposure levels to determine critical levels for adverse effects in tropical tree species. The main leaf-level physiological determinants of a plant species' O_3 critical level are detoxification capacity and stomatal flux (Karlsson et al., 2004; Musselman et al., 2006; Mills et al., 2011b; Büker et al., 2015). Evaluations of leaf-level detoxification capacity have not been conducted for tropical trees, but stomatal flux data have been collected from an array of species (Zotz and Winter, 1996; Poorter and Bongers, 2006). For trees of tropical lowland moist forests, maximal stomatal conductance is approximately 85% higher than that for temperate deciduous trees (Kelliher et al., 1995; Körner, 1995; Poorter and Bongers, 2006), suggesting that many species of tropical trees may be sensitive to O_3 pollution given the linear relationship between stomatal conductance and O_3 dose.

In addition to its well-characterized effects on plant physiology, O_3 pollution can influence plant chemical defenses against biotic and abiotic stressors (reviewed by Sandermann, 1996; Kangasjärvi et al., 2005; Valkama et al., 2007; Bidart-Bouzat and Imeh-Nathaniel, 2008; Lindroth, 2010). After O_3 is taken up through the stomata, O_3 and O_3-generated reactive oxygen species (ROS) activate hormones involved in all major defense signaling pathways, including ethylene, abscisic acid, jasmonic acid, and salicylic acid. The activation of these hormones influences the synthesis of secondary metabolites, which can in turn alter the dynamics of plant-herbivore interactions. Available data suggest that the dose-responses of secondary metabolic pathways to O_3 vary in magnitude and direction across types of secondary metabolites, plant developmental stage, and plant and herbivore species (Valkama et al., 2007; Lindroth, 2010). While these effects of O_3 uptake on secondary metabolic pathways have been noted in conjunction with the aforementioned effects on primary physiology in many studies of chronic low-level O_3 exposure, it is not known whether these two types of effects share a critical level.

The present study is motivated by considering the potential impacts of O_3 on the contribution of tropical forest primary productivity to the global carbon budget, as well as on the plant-consumer interactions integral to tropical forest biodiversity. Satellite-based measurements and modeling of emissions have indicated a hotspot of NO_x (precursors to O_3) in Panama, specifically within the region surrounding the Panama Canal and Panama City (Hietz et al., 2011). We conducted the present study to measure O_3 concentrations within forested areas surrounding this hotspot of O_3-precursors and to investigate the effects of ambient O_3 concentrations in Panama on a common species of tropical tree. Our research questions are: 1) how do O_3 concentrations vary across the Pacific-Caribbean gradient of forests within the Panama Canal watershed, 2) how do O_3 concentrations vary across the forest strata, from the canopy to the understory, within a given site, and 3) for a tropical tree species with relatively high stomatal conductance, does exposure to ambient concentrations of O_3 in the Panama Canal watershed impact primary productivity, secondary metabolic pathways, and/or leaf senescence? To address these questions, we conducted an O_3 monitoring campaign at four sites over two years and an open-top chamber experiment with potted tree seedlings.

2. Materials and methods

2.1. Regional O_3 monitoring

Since tropospheric $[O_3]$ had not previously been measured in the vicinity of the Panama Canal and surrounding urban areas, and precursor emissions were known to be present (Hietz et al., 2011), we conducted monitoring to assess the distribution of O_3 across