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Exposure- and flux-based assessment of ozone risk to sugarcane plants

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ABSTRACT

Ozone (O_3) is a toxic oxidative air pollutant, with significant detrimental effects on crops. Sugarcane (*Saccharum* spp.) is an important crop with no O_3 risk assessment performed so far. This study aimed to assess O_3 risk to sugarcane plants by using exposure-based indices (AOT40 and W126) based on O_3 concentrations in the air, and the flux-based index (POD_y, where y is a threshold of uptake) that considers leaf O_3 uptake and the influence of environmental conditions on stomatal conductance (g_{sto}). Two sugarcane genotypes (IACSP94-2094 and IACSP95-5000) were subjected to a 90-day Free-Air Controlled Experiment (FACE) exposure at three levels of O_3 concentrations: ambient (Amb); Amb x1.2; and Amb x1.4. Total above-ground biomass (AGB), stalk biomass (SB) and leaf biomass (LB) were evaluated and the potential biomass production in a clean air was estimated by assuming a theoretical clean atmosphere at 10 ppb as 24 h O_3 average. The Jarvis-type multiplicative algorithm was used to parametrize g_{sto} including environmental factors i.e. air temperature, light intensity, air vapor pressure deficit, and minimum night-time temperature. Ozone exposure caused a negative impact on AGB, SB and LB. The O_3 sensitivity of sugarcane may be related to its high g_{sto} ($\sim$535 mmol H_2O m^{-2} s^{-1}). As sugarcane is adapted to hot climate conditions, g_{sto} was restricted when the current minimum air temperature (T_{min}) was below $\sim$14 °C and the minimum night-time air temperature of the previous day (T_{nmin}) was below $\sim$7.5 °C. The flux-based index (POD_y) performed better than the exposure-based indices in estimating O_3 effect on biomass losses. We recommend a y threshold of 2 nmol m^{-2} s^{-1} to incorporate O_3 effects on both AGB and SB and 1 nmol m^{-2} s^{-1} on LB. In order not to exceed 4% reduction in the growth of these two sugarcane genotypes, we recommend the following critical levels: 1.09 and 1.04 mmol m^{-2} POD_2 for AGB, 0.91 and 0.96 mmol m^{-2} POD_2 for SB, and 3.00 and 2.36 mmol m^{-2} POD_1 for LB of IACSP95-5000 and IACSP94-2094, respectively.

1. Introduction

Tropospheric ozone (O_3) is one of the most toxic oxidative air pollutants, with significant detrimental effects on human and plant health (Ashmore, 2005; Matyssek et al., 2013). The levels of O_3 concentration have increased since the industrial revolution, when background concentrations were $\sim$10 ppb (Volz and Kley, 1988). Nowadays, O_3 levels may range between 35 and 50 ppb as annual average (Cooper et al., 2010, 2012, 2014). Little information is available about O_3 background concentrations in the Southern Hemisphere (Sofen et al., 2016). However, industrialization has increased on densely populated regions of developing countries, so that trends of O_3 concentration appear to be similar to those in the rest of the world (Emberson et al., 2001; Cooper et al., 2014).

Important crop species are affected by O_3-induced oxidative stress, with a global economic cost estimated to be in the order of US$11 to US $26 billion (Mills and Harmens, 2011). Methods for quantifying the effects of O_3 on vegetation have considerably developed (CLRTAP, 2015) and the United Nations Convention on Long-Range Transboundary Air Pollution has adopted O_3 critical levels (CLs). Such CLs use two main metric types: exposure- and flux-based indexes, which use the O_3 concentrations in the air and the stomatal O_3 flux (F_{st}), respectively. CL defines the O_3 cumulative exposure or cumulative F_{st} at a level that causes direct injury to vegetation (i.e. 4% decrease in biomass, according to CLRTAP, 2015).

Among the many exposure indices (Paoletti et al., 2007), AOT40 is possibly the most widely used in Europe (Fuhrer et al., 1997; CLRTAP, 2015) and Asia (Kohno et al., 2005). AOT40 is the accumulated hourly O_3 concentrations in the air above a threshold of 40 ppb over the growing season and it does not account for the role of stomatal aperture on regulating leaf gas exchange (Paoletti and Manning, 2007; EU Directive, 2008/50/EC). In North America, W126 has been developed and is based on the sum of hourly concentrations, where each concentration is weighted by a function that emphasizes the highest O_3

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values (Lefohn et al., 1988; U.S. EPA, 1996). Only one F_{st} index has been developed, i.e. the phytotoxic ozone dose (POD_y) accumulated above an hourly uptake threshold '*y*' over the growing season (CLRTAP, 2015). POD_y is based on the Jarvis-type multiplicative stomatal conductance model (Jarvis, 1976), which is used to estimate F_{st} considering the effects of environmental conditions on stomatal conductance (Emberson et al., 2000). As O_3 is injurious to plants only after uptake through stomata, POD_y may be more relevant for assessing O_3 risk to plants. On the other hand, POD_y calculation demands more input variables than AOT40 and W126 (De Marco et al., 2015).

Establishing the most appropriate indices, thresholds and CLs for the protection of vegetation is a key issue of modern O_3 research (Sicard et al., 2016). Most of the studies on O_3 effect on crops have been done with species growing under temperate climate conditions (Betzelberger et al., 2010; Feng et al., 2012). Ozone impacts on tropical plant species are less understood (Klumpp et al., 1994; The Royal Society, 2008; Moura et al., 2014) and no CLs for O_3 effects have been suggested for crops growing in tropical or subtropical climates. Overall, knowledge about O_3 responses in tropical crop species growing in South America and Africa is insufficient (The Royal Society, 2008). Ozone impacts were described for several C_3 crop species, such as soybean (Morgan et al., 2003), wheat (González-Fernández et al., 2013) and rice (Ainsworth, 2008; Pang et al., 2009). For C_4 species, impaired photosynthesis, visible leaf injury and reduced growth were reported in maize (Leitão et al., 2007; Singh et al., 2014) in a genotype-dependent manner (Singh et al., 2014), indicating differential O_3 sensitivities (González-Fernández et al., 2014).

Among the bioenergy crops, sugarcane (*Saccharum* spp.) is a C_4 species cultivated worldwide that yielded around 1.8 million tons in 2013, with an expectation of yield increase of 21% until 2024 (FAOSTAT, 2015). Sugarcane is mainly produced in South America, with Brazil being the main producer (~460 thousand tons/year) followed by India (~290 thousand tons/year) and China (~90 thousand tons/year) (FAOSTAT, 2015). In addition to sugar production, sugarcane also plays a key role in bioethanol production, currently produced from the sucrose stored in sugarcane stalks (Souza et al., 2013). To produce the so-called first-generation (1G) bioethanol, sugar is extracted mainly from the sucrose stored in the stalks while leaves serve as raw material for the second-generation (2G) bioethanol (Souza et al., 2013). Ozone impacts on sugarcane yield (Grantz and Vu, 2009) and gas exchange (Grantz et al., 2012) were already investigated in an experiment carried out in continuous stirred-tank reactors. Such reports demonstrated that this species is O_3 sensitive and that effects vary among genotypes, as also found for sugarcane responses to drought (Ribeiro et al., 2013).

Based on the hypotheses that O_3 effects on sugarcane correlates with the O_3 indices and that POD_y is more biologically meaningful than AOT40 or W126, the aim of the present study was to assess O_3 risk to sugarcane plants using AOT40, W126 and POD_y as metrics of injury.

2. Materials and methods

2.1. Plant material

Sugarcane genotypes IACSP95-5000, a high yield genotype (Magalhães Filho et al., 2015), and IACSP94-2094, a drought resistant genotype (Ribeiro et al., 2013; Sales et al., 2013) were used. Both genotypes were developed by the Sugarcane Breeding Program of the Agronomic Institute (ProCana, IAC, Brazil). Stalk segments containing an individual bud were sown in 5-L plastic pots, containing peat substrate (Lithuania Peat Moss) and gravel (3:1), and placed inside a growth room with controlled air temperature (30 °C) and photosynthetic photon flux density (PPFD) of 300–400 μmol m^{-2} s^{-1}. Thirty-five days after emergence, the plants were transferred to 50-L pots containing the same substrate, and placed under the free-air controlled exposure (FACE) conditions. After one month, when new leaves flushed, plants were moved to the experimental plots, and maintained well-watered by dripping every afternoon until maximum water holding capacity of the soil was reached. Nutrient supply was provided as 20 g of NPK (8:8:8) per pot, once a month, until the end of the experimental period.

2.2. Free-air O_3 exposure

The experiment was carried out in an O_3 FACE facility located at Florence, Italy (43°48′N, 11°12′E and 55 m a.s.l.). Ozone was generated from pure oxygen by an O_3 generator (TGOC13X, Triogen Ltd., Glasgow, UK). The air containing O_3 was then diluted with ambient air in a mixing tank and injected into the canopies through 25 teflon tubes hanging down from a fixed grid above the plants (2 m height) in each plot. The O_3 concentration at canopy height was monitored continuously (Mod. 202, 2B Technologies, Boulder CO, USA), and the observed value was used as feedback to the valves to regulate O_3 emission, using the proportional–integral–derivative algorithm (PID). Details on the ozone FACE are described in Paoletti et al. (2017).

The O_3 exposure lasted 90 days (from 10th June to 8th September 2015 - summer season), which is a reasonable time span for sugarcane growth. The experimental period was restricted to the summer season in order to guarantee the ideal temperature for sugarcane, a perennial species with most of its vegetative growth occurring during warmest seasons (Sage and Monson, 1998). Environmental conditions during the experimental period were close to ones found in Brazilian subtropical conditions, where sugarcane is extensively cultivated (Pagani et al., 2017). Sugarcane plants were exposed to three levels of O_3 concentrations: ambient (Amb); ambient x1.2 (x1.2); and ambient x1.4 (x1.4), with three plots per each O_3 treatment and three individuals of each genotype per plot, with each plot being considered as a replicate (n = 3).

All main environmental variables were continuously recorded (from 1 min to 1 h), subject to manual and statistical quality assurance procedures, and stored in a local FTP server. Photosynthetic active radiation (PAR), wind speed and direction, precipitation, air temperature and relative humidity (RH) were recorded by a Watchdog station (Mod. 2000; Spectrum Technology, Inc., Aurora, IL, USA) at 2.5 m a.g.l.

2.3. Biomass assessment

At the end of the exposure period, stalks and leaves were harvested and dried in an oven at 60 °C until constant weight. Leaf (LB) and stalk (SB) biomasses were used to calculate the total above-ground biomass (AGB) production.

2.4. Measuring and modeling the stomatal conductance

Stomatal conductance to water vapor (g_{sto}) was measured along the exposure period, always on the newest fully expanded leaf (leaf +1). Diurnal courses of g_{sto} as well as nighttime measurements were carried out under natural conditions of air temperature, air relative humidity, and PPFD using a portable infra-red gas analyzer (CIRAS-2, PP Systems, Herts, UK). Leaves selected for the measurements were fully exposed to the direct irradiance (Muraoka et al., 2000). A final database of 395 measurements of g_{sto} for IACSP95-5000 and 398 measurements for IACSP94-2094 was used to parameterize the multiplicative model (Jarvis, 1976).

The maximum (g_{max}; mmol H_2O m^{-2} s^{-1}) and minimum (f_{min}, relative to g_{max}) g_{sto} values were calculated by averaging g_{sto} above the 95th and below the 5th percentiles, respectively, considering the whole data set (Alonso et al., 2008). Terms describing modification of g_{sto} by soil moisture (i.e., f_{swc}) were not used in this study, as the substrate was maintained well-irrigated and close to the maximum water holding capacity throughout the exposure period, and no reductions in g_{sto} due to changes in substrate water content were recorded (data not shown).