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Short communication

Elevated atmospheric CO_2 increases *Eucalyptus urophylla* S. T. Blake stem diameter by stimulating cell proliferation and reducing lignin deposition

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ABSTRACT

In 2019, the atmospheric CO_2 concentration exceeded the 415 ppm milestone for the first time in the human history. According to projections of the Intergovernmental Panel on Climate Change (IPCC), CO_2 levels will continue to rise in the future, potentially affecting all living organisms. Plants with C3 metabolism may benefit from rising CO_2 levels because the significant losses of photosynthesis, driven by photorespiration could be diminished under this scenario. This study addressed the anatomical changes in the stems of young *Eucalyptus urophylla* plants induced through cultivation in elevated CO_2 (eCO_2). Plants cultivated under eCO_2 showed increased stem diameter (*i.e.*, radial width of the secondary xylem, secondary phloem and cortex tissues). Periodic acid–Schiff (PAS)/Toluidine Blue staining suggested a decrease in the lignification content in the newly formed tissues of eCO_2 stimulated plants. Levels of caffeate/5-hydroxyferulate O-methyltransferase form 1 (COMT1), a lignin biosynthesis specific proteoform, were significantly reduced in stem sections, supporting our findings: eCO_2 induces plant growth, but reduces lignified tissues.

Keywords: carbon dioxide, climate change, *Eucalyptus*, lignin, plant stress, stem anatomy

The Fifth Assessment Report (AR5) of the International Panel for Climate Change (IPCC) predicts an increase in the global concentration of carbon dioxide gas (CO_2) reaching 550-1200 ppm by the year 2100 (IPCC 2014). In 2019, the 415 ppm CO_2 milestone was reached and registered at the Mauna Loa Observatory in Hawaii (Le Page 2019). In the absence of environmentally friendly climate policies adopted by major emitting countries, the atmospheric CO_2 levels may rise to alarming concentrations with detrimental outcomes to many life forms. Many species will have to promptly respond to environmental fluctuations in order to avoid damage and, ultimately, ensure survival. Unlike other forms of life, elevated CO_2 (eCO_2) levels may positively affect plant growth. One of the most consistent responses to this environmental stimulus is the increase in photosynthetic rates and yield in crop species (Ainsworth & Long 2005; Ainsworth & Rogers 2007). This effect is even more pronounced for plants employing the C_3 photosynthetic pathway, as they depend on a high CO_2:O_2 ratio to counterbalance losses caused by photorespiration. In Brazil, *Eucalyptus* plants are important material sources for pulp and paper production (Stape *et al.* 2010). Due to

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large-scale breeding programs and advances in forestry biotechnology, several species are used nationwide, and their growth may be impacted by changes in the atmosphere. The first investigation into growth and photosynthetic performance of *Eucalyptus* upon CO_2 stimulus dates back to the 1990s, when Roden & Ball (1996) showed that these parameters were affected by cultivation in eCO_2 atmosphere. It has been recently reported that eCO_2 stimulates biomass production, and reduces the photorespiration process and the total leaf content of the RuBisCO enzyme (Aspinwall *et al.* 2018; Sharwood *et al.* 2017; Wujeska-Klause *et al.* 2019). Although significant advances in understanding the physiological responses of *Eucalyptus* species to eCO_2 have been achieved, little is known about the structural changes imposed by cultivation under this condition. This study addresses the anatomical changes in the stems of young *Eucalyptus urophylla* S. T. Blake plants cultivated under eCO_2.

We cultivated young plants in ambient CO_2 (aCO_2) and eCO_2 (410 and 980 ppm, respectively) for 30 days in plant growth chambers and analyzed transverse stem sections. Growth under eCO_2 resulted in an increased radial width of the secondary xylem, secondary phloem and cortex tissues, and a decreased pith width (Fig. 1). This increase in the stem diameter of *E. urophylla* plants corroborates the findings of

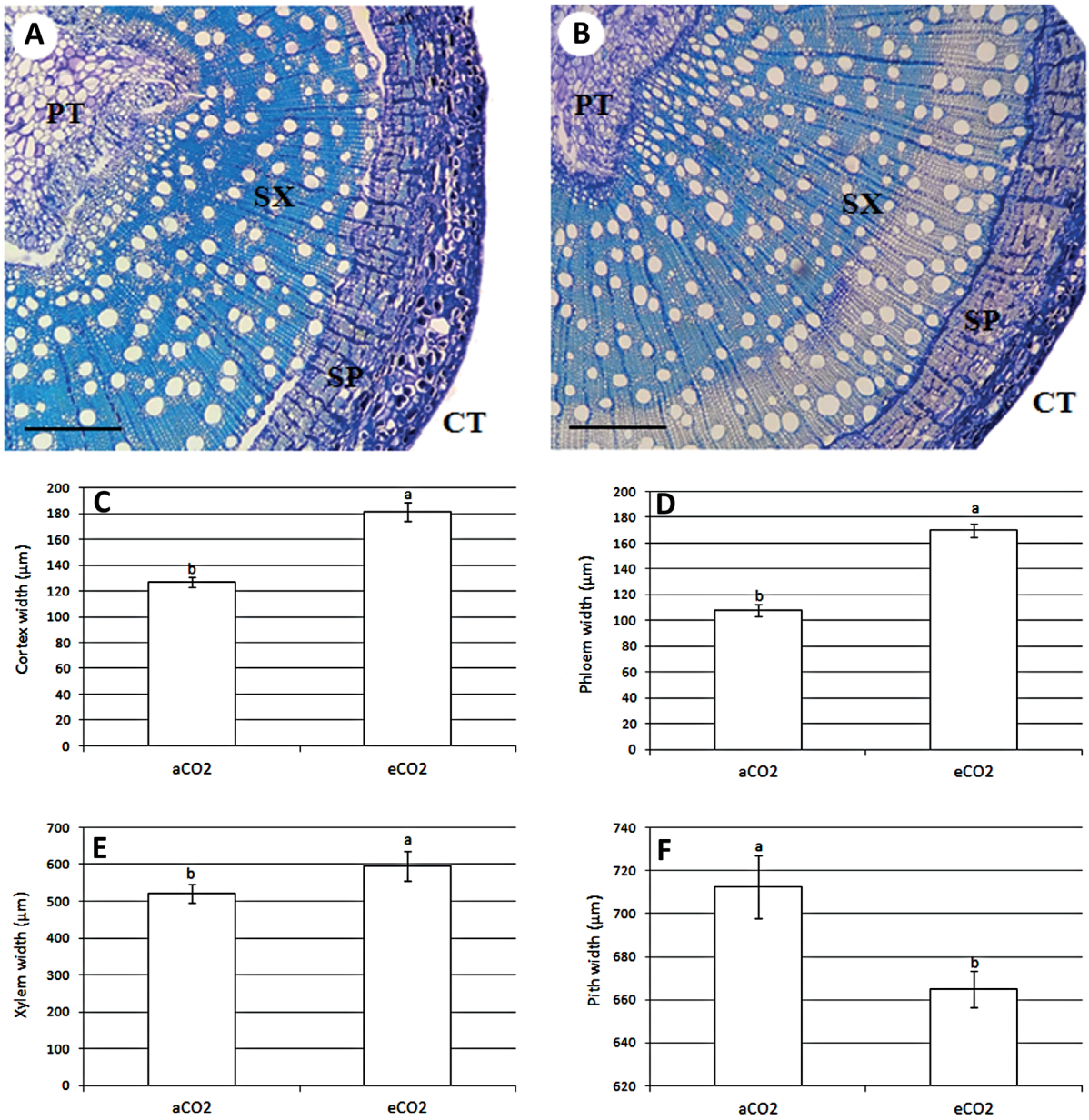

Figure 1. PAS/Toluidine Blue staining of transverse sections of stems from young *Eucalyptus urophylla* plants cultivated at 410 (**A**) and 980 (**B**) ppm of CO_2. Radial width of the cortex (**C**), secondary phloem (**D**), secondary xylem (**E**) and pith (**F**) of plants cultivated under these conditions are also shown. PT: pith; SX: secondary xylem; SP: secondary phloem; CT: cortex. Different letters indicate statistically significant differences according to Mann-Whitney test ($p<0.05$).

most studies, which have reported that exposure to eCO_2 affects secondary growth (Pritchard *et al.* 1999), and indicates higher availability of sugar molecules for plant stem growth under this condition.

To obtain more detailed information about the structural changes induced by CO_2, we performed Periodic acid-Schiff (PAS)/Toluidine Blue staining of the stem transverse sections. The data suggest that the radial growth observed in the *E. urophylla* stems is mainly driven by cell proliferation and increased number of cell layers (Fig. 1). Additionally, cells of the outer layers of xylem from eCO_2 cultivated plants showed less intense PAS/Toluidine Blue staining (Fig. 1), suggesting a decrease in the lignin deposition rates in newly formed tissues (see O'Brien *et al.* 1964). Pairwise comparisons revealed a decrease of approximately 30 % in the optical density (OD) of the xylem outer layer when compared to that of the inner layer, whereas no difference was observed between the xylem inner layers from eCO_2 and aCO_2 stimulated plants (data not shown). We hypothesize based on our anatomical data that the decrease in lignin deposition in the newly formed tissues of *E. urophylla* stems occurs as a way to compensate for its dampening effect on cell growth and expansion.

Due to the rapid and ongoing advances in resolution, sensitivity and accuracy, mass spectrometry (MS) analysis has proven to be a powerful analytical tool to identify and quantify proteins and their different proteoforms in almost any tissue. We used this technology to assay the relative quantity of caffeate/5-hydroxyferulate O-methyltransferase form 1 (COMT1, Eucgr. A01397) – a key enzyme specifically involved in lignin (monolignol) biosynthesis (Carocha *et al.* 2015). We observed a decrease in the abundance of COMT1 in *E. urophylla* plants grown under eCO_2 compared to those cultivated under aCO_2 (Fig. 2). As COMT1 catalyzes one of the last reactions in the production of the guaiacyl (G) and syringyl (S) lignin monomeric units, this finding corroborates the observed reduction of lignin deposition in the newly formed xylem cells.

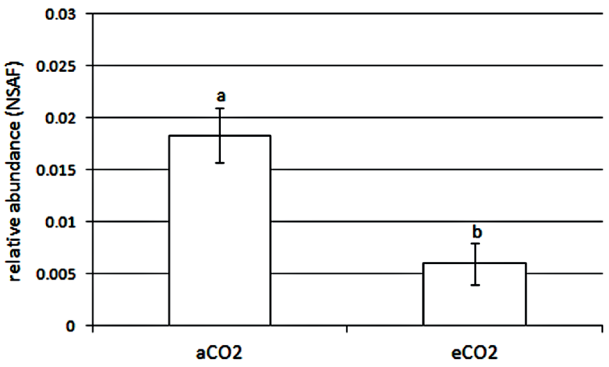

Figure 2. Relative abundance, in terms of the normalized spectral abundance factor (NSAF, Paoletti *et al.* 2006) of the proteoform caffeate/5-hydroxyferulate O-methyltransferase 1 (COMT1), in *Eucalyptus urophylla* stems cultivated at 410 (aCO_2) and 980 (eCO_2) ppm of CO_2. Different letters indicate statistically significant differences according to Student´s *t*-test ($p<0.05$).

The anatomical differences in the stems of young *E. urophylla* plants cultivated at 980 ppm of CO_2 are described in the present brief communication. Using different staining techniques, we observed that the stem diameter of the plants increased when stimulated by eCO_2 concentrations, and that the newly formed tissues, most notably xylem cells, presented decreased deposition of the organic polymer lignin. Quantification of a key enzyme of the lignin biosynthesis pathway corroborated these findings.

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