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Increased atmospheric carbon dioxide concentration: effects on eucalypt rust (*Puccinia psidii*), C:N ratio and essential oils in eucalypt clonal plantlets

By R. Ghini[1,6], R. E. de O. Mac Leod[2], A. Torre Neto[3], D. C. Cardoso[4], W. Bettiol[1], L. A. S. de Morais[1] and B. Vique[5]

[1]Embrapa Environment, C. P. 69, CEP 13820-000 Jaguariúna, SP, Brazil; [2]Faculdade de Ciências Agronômicas de Botucatu (UNESP – FCA), Botucatu, SP, Brazil; [3]Embrapa Instrumentation, São Carlos, SP, Brazil; [4]Agronomic Institute of Campinas, Campinas, SP, Brazil; [5]Vallourec Florestal Ltda, Curvelo, MG, Brazil; [6]E-mail: raquel.ghini@embrapa.br (for correspondence)

Summary

Evaluation of impacts of high CO_2 atmospheric concentration is strategically important for the development of adaptation measures and sustainable crop management. The objective of this study was to evaluate the effects of increased atmospheric CO_2 concentration on eucalypt rust (*Puccinia psidii*), C:N ratio, yield and chemical composition of essential oils and growth of eucalypt clonal plantlets. Two clones with different levels of rust resistance were studied: a *Eucalyptus urophylla* × *E. camaldulensis* hybrid (VM 01) and an *E. urophylla* (clone MN 463). The experiments were performed in open-top chambers (OTCs) with CO_2 mean concentrations (μmol mol^{-1}) of 399 (unenclosed control), 412 (OTCs with ambient CO_2 concentration) and 508 (OTCs with high CO_2 concentration) and in closed chambers (CCs) with CO_2 mean concentrations of 390, 405, 520 and 700. Increased atmospheric CO_2 concentrations resulted in a decrease in rust pustules per leaf, uredinia per leaf area, spores per uredinia and area under the disease progress curve in VM 01 (hybrid) clonal plantlets. The disease did not occur in MN 463 clonal plantlets, which demonstrated that high CO_2 concentrations did not change the level of rust resistance. Plant growth of the two clones was stimulated by up to 23% in height and 26% in stem diameter in OTCs and by 18% for both clones in CCs. An increased C:N ratio in leaves, stems and roots was observed only for the VM 01 clonal plantlets. Essential oils produced by VM 01 (2.8 g 100 g^{-1}) and MN 463 (1.4 g 100 g^{-1}), as well as the major essential oil compounds (80% 1.8-cineole for VM 01; 50% 1.8-cineole and 32% α-pinene for MN 463), were not altered. In this study, increased concentrations of atmospheric CO_2 favourably impacted eucalypt growth and reduced rust severity, while not influencing the production of essential oils.

1 Introduction

The impacts from increased atmospheric carbon dioxide (CO_2) concentrations on forest systems must be evaluated, so that adequate management strategies can be developed for adapting crops to new environmental conditions. Inputs of CO_2 in the atmosphere from anthropogenic activities have increased significantly, and it is predicted that CO_2 concentration could reach 550 μmol mol^{-1} in 2050 and 730–1000 μmol mol^{-1} in 2100, if mitigation measures are not adopted (IPCC 2007). Because CO_2 is a basic component of photosynthesis, high concentrations of this gas could alter physiological processes in plants. Plants generally have higher growth rates in high CO_2 environments, although this response may vary between species and locations (Ainsworth and Long 2005). Many aspects regarding the response of eucalypt plantations to increased CO_2 concentrations must be determined to develop appropriate management strategies (Chmura et al. 2011; Booth 2013).

Secondary plant metabolites could be altered by increased atmospheric CO_2, which may lead to changes in the occurrence of pests and diseases (Braga et al. 2006; Kretzschmar et al. 2009). Eucalypt plantations are not only used for pulp production, but also used to produce essential oils, a process that could be altered by climate change and may be related to disease incidence (Sangwan et al. 2001; Braga et al. 2006). However, a previous study showed that the production of secondary metabolites, essential oils, phenolic compounds and condensed tannins within leaves of *Eucalyptus globulus* Labill. and *E. pauciflora* Sieber ex Spreng. was unaffected by increased atmospheric CO_2 concentrations (McKiernan et al. 2012).

Despite the economic importance of the eucalypt plantations, few studies have addressed CO_2 influences on eucalypt diseases. With perennial wood crops, disease increases under a changing environment are generally more difficult to address because of a greater difficulty to replace plants, reduced efficacy of chemical control and low availability of biological control methods. Loblolly pine (*Pinus taeda* L.) seedlings cultivated in twice ambient levels of atmospheric CO_2 exhibited reduced incidence of fusiform rust [caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedgc. & Hunt) Burdsall & G. Snow] and pitch canker (caused by *Fusarium circinatum* Nirenberg and O'Donnell), but the severity of these diseases did not change under increased CO_2 (Runion et al. 2010). In a controlled environment, high CO_2 concentrations led to an increase in incubation period and decrease in wilt severity caused by *Ceratocystis fimbriata* Ellis and Halst. in two *Eucalyptus* clones (Silva et al. 2013).

Modelling has been used to project the current and future potential distributions of Mycosphaerella leaf disease, which is an important disease of *E. globulus*, with no or full acclimation of photosynthesis in an environment with high CO_2 concentration (Pinkard et al. 2010). Any growth enhancement by CO_2 must be included in the model, so that total stem volume at harvest is accurately predicted, which is critical for assessing most interactions.

Eucalypt rust caused by *Puccinia psidii* winter is a serious threat to the yield of eucalypts and of other species of the Myrtaceae family (Carnegie and Lidbetter 2012). *Puccinia psidii* is believed to be native to South and Central America (Alfenas et al. 2005) and represents an invasive pathogen threat that was detected in Australia in 2010 (Carnegie et al.

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2010). Eucalypt rust occurs mainly in seedlings, nurseries and young trees in the field. The disease threatens the biodiversity and represents a high economic risk to eucalypt plantations (Carnegie and Cooper 2011; Silva et al. 2013). The objective of this study was to evaluate the effects of increased atmospheric CO_2 concentrations on the life cycle of the eucalypt rust pathogen, plantlet growth, C:N ratio and essential oil production in two eucalypt clones with different levels of rust resistance.

2 Material and methods

The experiments were performed in open-top chambers (OTCs) and closed chambers (CCs) at Embrapa Environment, Jaguariúna, state of São Paulo (SP), Brazil (22°41′S and 47°00′W; altitude of 570 m). The plantlets used in the experiments originated from two eucalypt clones [one derived from *E. urophylla* S. T. Blake (MN 463) and another from the interspecific crossing of *E. urophylla* × *E. camaldulensis* Dehnh. (VM 01)] and were produced by mini-cuttings placed in conical plastic tubes (50-ml capacity) containing pine-bark substrate.

2.1 Open-top chambers

The OTCs had a cylindrical shape with a diameter of 1.9 m and height of 0.9 m. Three OTCs were injected with CO_2 (OTCs with high CO_2 concentration), and three OTCs were not injected with CO_2 (OTCs with ambient CO_2 concentration). In addition, three controls (unenclosed controls) were used. The chambers were constructed of galvanized-steel frame with sides covered with clear polyethylene film (150 μm thick) with an additive for protection against UV radiation. The CO_2 distribution system included annealed copper tubing (6.35 mm diameter) for the primary and secondary lines. Drip irrigation tubing, with non-compensating emitters at a spacing of 10 cm, was adapted for the injection system and it was installed around the interior of the OTCs at a 0.4 m height. The monitoring instrumentation consisted of commercial devices, which were adapted to operate with wireless sensor technology that was compatible with the IEEE protocol 802.15.4. The commercial devices had the following components: (i) a CO_2 flow regulator in the main CO_2 distribution line (model GFC 17 with optional RS 232; Aalborg, Orangeburg, SC, USA); (ii) latching valves on the injection system at the entrance of each OTC (model BA222-70; Jefferson Buenos Aires, Argentina); (iii) an infrared gas analyser (IRGA) CO_2 sensor in the centre of each OTC (model GMP 343; Vaisala, Helsinki City, Finland); and (iv) a complete weather station in the centre of one of the OTCs with injection (model WXT 520; Vaisala). Dedicated software was developed to maintain the target CO_2 concentration and collect data, which were provided in the form of text files separated by tabulation.

The experiment was performed with the following CO_2 concentrations: 399 ± 28 (unenclosed control), 412 ± 62 (OTCs with ambient CO_2 concentration) and 508 ± 109 (OTCs with high CO_2 concentration) μmol mol^{-1}. During the assay, mean temperature was 21°C, minimum temperature was 15.7°C, and maximum temperature was 25.6°C with a mean relative humidity of 63% (winter/spring).

Plantlets were derived from rooted cutting in 60-day-old minigarden and transplanted into the OTCs. The soil possessed the following attributes: pH (H_2O) 5.0, 36 g l^{-1} organic material, 1.0 mg dm^{-3} phosphorus (P), 14 mg dm^{-3} sulphur (S), 0.7 mmolc dm^{-3} potassium (K), 9 mmolc dm^{-3} calcium (Ca), 7 mmolc dm^{-3} magnesium (Mg), 19 mmolc dm^{-3} aluminium (Al), 53 mmolc dm^{-3} hydrogen (H) and 88.7 mmolc dm^{-3} cation exchange capacity (CEC)]. The plantlets were fertilized every 15 days with 25 g of KCl, 10 g of $CaCl_2$, 6 g of $NH_4H_2PO_4$ and 5 g of $(NH_4)_2SO_4$ dissolved in 12 l of water and equally distributed between the plots. Irrigation was performed by drip irrigation when necessary. Each plot consisted of 17 plantlets of each clone.

2.2 Closed chambers

The CCs were made of plastic boxes (20 × 32 × 32 cm) covered by a clear glass sheet. Uninterrupted injection of pure CO_2 into the CCs occurred for 0.5 seconds every 50 min throughout the experiment. To homogenize the gas concentration inside the boxes, a compressor was programmed to turn on and off every 15 min and inject external air through a tube inside the boxes. The CCs were kept at a mean temperature of 23 ± 2°C, relative humidity of 50% and 12-h photoperiod with 20 000 lux from daylight and grow lamps.

The following CO_2 concentrations were tested: 390 ± 21, 405 ± 30, 520 ± 75 and 700 ± 93 μmol mol^{-1}. Atmospheric CO_2 concentrations inside the boxes were monitored with an infrared gas analyzer (model GMP343; Vaisala).

Rooted cuttings (45-day-old) in tubes were pruned for standardization leaving two pairs of leaves, and they were then incubated in the CCs. The CCs contained 4 l of vermiculite to maintain humidity and support the tubes. During the experiment, fertilizer was added every 2 days by adding 2 ml of an aqueous nutrient solution to each plant [750 mg $Ca(NO_3)_2$, 500 mg KNO_3, 150 mg $NH_4H_2PO_3$, 400 mg $MgSO_4$, 13.8 mg chelated iron, 1.5 mg H_3BO_3, 1.5 mg $MnSO_4$, 0.5 mg $ZnSO_4$, 0.15 mg $CuSO_4$ and 0.15 mg Na_2MoO_4 in 1 l with an electrical conductivity of 2.3 dS m^{-1} (Qualifértil®; Qualifértil, São Paulo, SP, Brazil)].

Each plot comprised 20 plants. Eight plants per plot were used for non-destructive analyses and 12 were used for destructive analyses.

2.3 Inoculation

Urediniospores of *P. psidii* were collected from infected leaves of *E. grandis* and multiplied in Malabar plum/rose apple [*Syzygium jambos* (L.) Alston] plants. A urediniospore suspension was prepared in distilled water with Tween 80 (0.05%)

at a concentration of 2×10^4 urediniospores ml^{-1}. The suspension was sprayed on both sides of the leaves, and the plants were kept in humid chambers for 12 h in the dark (Ruiz et al. 1989). Plant inoculations occurred at 62 and 68 days after the establishment of the experiment in the OTCs and CCs, respectively.

2.4 Evaluations

Severity of the eucalypt rust was evaluated 18 days after inoculation (d.a.i.) in four plants of each clone per treatment using the second and third pairs of leaves from previously marked branches. For this purpose, the percentage of lesioned leaf area was tested by image analysis with ASSESS 2.0 software (American Phytopathological Society, Saint Paul, MN, USA). These data were used to calculate the area under the disease progress curve (AUDPC).

At 18 d.a.i., the numbers of pustules per leaf, uredinia per leaf area (1.13 cm^{-2}) and spores per uredinia were evaluated according to the methodology described by Ruiz et al. (1989) in a single pathogen cycle on the second and third pairs of leaves. The analyses were performed for four and two plants per plot in the OTCs and CCs, respectively.

To evaluate *in vitro* germination of *P. psidii* urediniospores, an aliquot (100 μl) of urediniospore suspension (2×10^4 urediniospores ml^{-1}) was plated in Petri dishes (60 × 16 mm) containing 2% water agar containing streptomycin (30 mg l^{-1}) and tetracycline (30 mg l^{-1}) within three open Petri dishes per plot with five replicates. The Petri dishes were incubated in the dark in CCs with damp filter paper at a temperature of 20 ± 2°C and four CO_2 concentrations (390, 405, 520 and 700 μmol mol^{-1}). After 3, 6, 9, 12 and 24 h, one drop of lactophenol was added to each dish to stop germination, and the germination of 100 spores was analysed. Spores were considered germinated when the length of the germ tube was greater than urediniospore diameter.

Plant height (distance between soil/substrate to tip of main stem) was measured every 15 days, and the area under the height progress curve (AUHPC) was calculated. Stem diameter of the plants was measured with a digital calliper in 17 plants of each clone per plot in OTCs and four of each clone per plot in CCs.

To determine the levels of carbon (C) and nitrogen (N) in the leaves, stem and roots, a composite sample for each plant organ was prepared by gathering four plantlets of each clone all from the same treatment. Each composite sample was dried in a convection oven at 50°C until a constant weight was reached. After drying, the samples were ground and passed through a sieve (0.42-mm mesh). Analyses of the levels of C and N were performed by dry combustion in a TruSpec CHN elemental analyzer (LECO Corporation, Saint Joseph, MI, USA), which uses TRUSPEC CN® (LECO Corporation, Saint Joseph, MI, USA) software to present results. The device was calibrated with 0.2 g of BARLEY 502-277 standard sample (C = 45.20 ± 0.28%, N = 1.69 ± 0.03% and LOT = 1007), and the C:N ratio was then calculated for each composite sample.

The essential oils were extracted by hydrodistillation in a modified Clevenger-type apparatus for 2 h. Differing amounts (ca. 30 g) of dry leaf samples were used for the extraction according to the phytomass originating from the OTC trial. The extracted essential oils were transferred into clear glass flasks with stoppers and screw caps, and they were then weighed, wrapped in aluminium foil and stored in the freezer until analysis to avoid degradation of chemical compounds. The essential oils were analysed by gas chromatography–mass spectrometry (GC–MS) in a Shimadzu mass spectrometer (QP 5050) using a fused silica capillary DB-5 column (30 m long, 0.25 mm inner diameter and 0.25 μm thickness). Helium was used as the carrier gas (1.7 ml min^{-1}), and the injector and detector temperatures were 240 and 230°C, respectively. The essential oils were solubilized in ethyl acetate (5 mg essential oil 1 ml^{-1} solvent), and 1 μl of solution was injected using a 1:20 split with the following temperature programme: 60–240°C at a rate of 3°C min^{-1}). The analyses for each sample were performed in triplicate, and the constituents were identified by comparing mass spectrums with the GC–MS system database (Nist 62 Libr) and Kovats retention index (KI) (Adams 1995).

2.5 Statistical analysis

The experimental design was a randomized block design with three and five replications in the OTC and CC experiments, respectively. Analysis of variance was used to compare the effect of the CO_2 concentrations on the eucalypt rust and plant characteristics between and within the cultivars. Tukey's test (5% probability) was used to compare the means. Data were analysed using SISVAR® (version. 5.3; UFLA, Lavras, Brazil) statistical software. Regression analyses were applied to the data obtained in the CCs using EXCEL® (ver. 2013; Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1 Rust

Increased CO_2 concentration resulted in reduced pathogen multiplication and disease severity in VM 01 clonal plantlets under both conditions studied (OTC and CC) (Fig. 1). In the OTC trial, OTC + CO_2 treatment significantly reduced the number of rust pustules per leaf, uredinia per leaf area, spores per uredinia and AUDPC. In the CC experiment, the evaluated variables significantly differed when comparing the CO_2 concentrations of 390 and 405 μmol mol^{-1} to the CO_2 concentrations of 520 and 700 μmol mol^{-1}. The disease did not occur in MN 463 clonal plantlets in either of the trials. The control (C) and OTC-A treatments did not differ, which indicated that the structure of the OTC did not affect pathogen and disease development (Fig. 1).

No effect was observed on the *in vitro* germination of *P. psidii* urediniospores (Tukey's 5%). Urediniospore germination rates at 390, 405, 520 and 700 μmol mol^{-1} CO_2 were as follows: 33, 33, 32 and 33% after 3-h incubation; 53, 52, 52 and

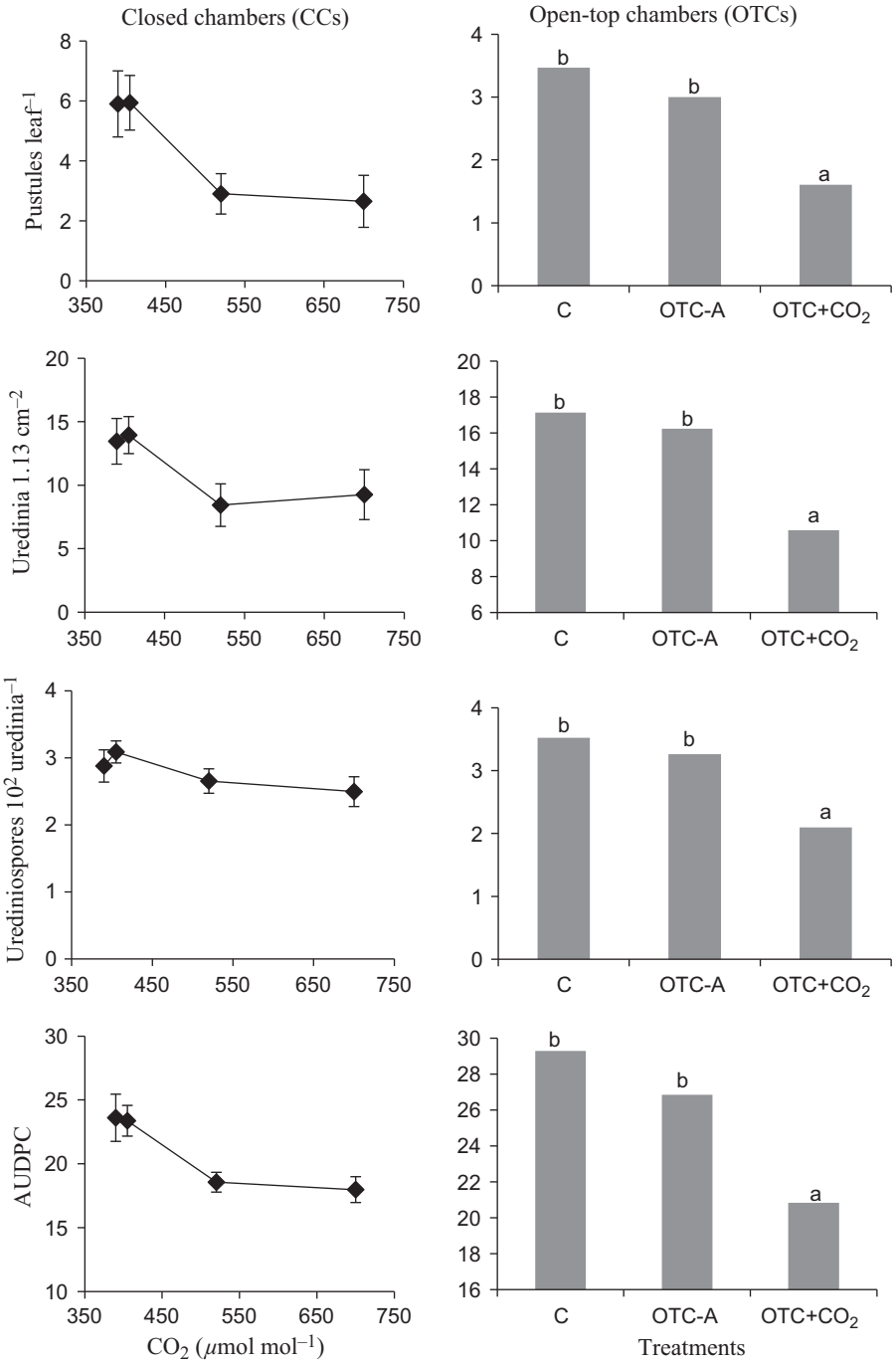

Fig. 1. Number of rust pustules per leaf, number of uredinia per leaf area (1.13 cm^2), number of urediniospores per uredinia and area under the disease progress curve (AUDPC) of VM 01 eucalypt plantlets cultivated in closed chambers (CCs) and open-top chambers (C, unenclosed control; OTC-A, OTCs with ambient CO_2 concentration; and OTC + CO_2, OTCs with high CO_2 concentration) 18 days after pathogen inoculation. Bars represent standard error. Means followed by the same letter do not differ from each other (Tukey's 5%).

53% after 6-h incubation; 67, 65, 68 and 64% after 9-h incubation; 75, 76, 73 and 75% after 12-h incubation; and 83, 84, 85 and 86% after 24-h incubation, respectively.

3.2. Growth, C:N ratio and essential oils of eucalypt plantlets

Increased atmospheric CO_2 concentrations stimulated growth in the two eucalypt clones (VM 01 and MN 463) by up to 23% in height and 26% in stem diameter in plants in OTCs and by 18% in height and stem diameter in plants in CCs (Fig. 2). The two growth variables (height and stem diameter) did not significantly differ between the two clones.

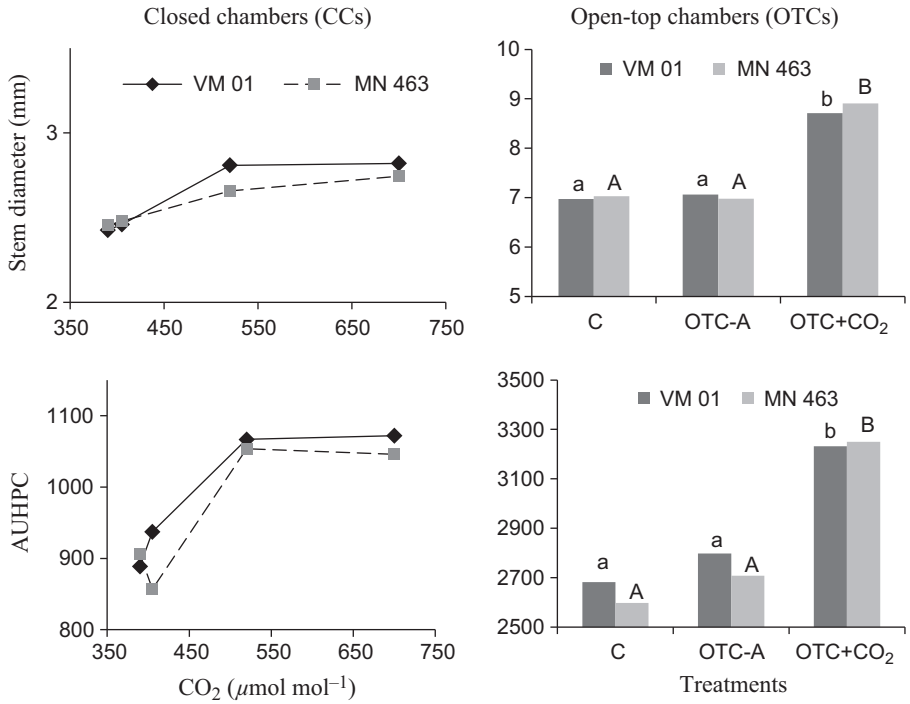

Fig. 2. Stem diameter and area under the height progress curve (AUHPC) of VM 01 and MN 463 eucalypt clonal plantlets cultivated in closed chambers (CCs) and open-top chambers (C, unenclosed control; OTC-A, OTCs with ambient CO_2 concentration; and OTC + CO_2, OTCs with high CO_2 concentration). Means followed by the same letter do not differ within each clone (Tukey's 5%).

The C and OTC-A treatments did not differ on almost all variables evaluated, which indicated that the OTC structure did not affect plant growth.

In CCs, C:N ratio increased in the leaves, stems and roots of VM 01 clonal plantlets with increased CO_2 concentrations (Table 1). In the OTC trial, C:N ratio increased only in leaves of VM 01 clonal plantlets in the OTC + CO_2 treatment (Table 2). The C:N ratio did not change in the plant parts of MN 463 clonal plantlets in any of the treatments in the CCs and in treatments OTC-A and OTC + CO_2 in the OTC trial. The increased C:N ratio was a result of the decreased level of N in the plants because the level of C was not altered with the treatments (Tables 1 and 2).

Table 1. Nitrogen (N), carbon (C) and C:N ratios in leaves, stems and roots of VM 01 and MN 463 eucalypt clonal plantlets cultivated in closed chambers (CCs) under different concentrations of atmospheric CO_2.

CO_2 concentration (μmol mol^{-1})	N (g kg^{-1})				C (g kg^{-1})				C:N ratio			
	VM 01		MN 463		VM 01		MN 463		VM 01		MN 463	
Leaves												
390	25.84	bcA	26.79	aA	491.60	aA	503.40	aB	19.20	abA	18.91	aB
405	27.00	cA	25.15	aA	488.80	aA	503.80	aB	18.16	aA	20.22	aA
520	24.00	abA	25.88	aA	493.20	aA	506.00	aB	20.69	bcA	19.65	aA
700	22.60	aA	25.84	aB	493.30	aA	503.80	aB	21.86	cB	19.55	aA
CV (%)	6.81				0.93				7.14			
Stem												
390	10.13	bA	10.44	aA	469.00	aA	469.20	aA	46.91	aA	45.81	aA
405	10.29	bA	9.34	aA	467.40	aA	472.60	aA	45.70	aA	52.13	aA
520	8.81	abA	9.99	aA	466.60	aA	471.40	aA	53.64	abA	47.89	aA
700	8.39	aA	9.40	aA	467.00	aA	471.60	aA	56.18	bA	50.63	aA
CV (%)	10.02				0.89				10.08			
Roots												
390	12.20	bA	12.01	bA	474.40	aA	479.60	aB	39.35	abA	40.11	aA
405	12.16	bB	11.12	abA	473.20	aA	477.20	aB	39.08	aA	43.15	aB
520	10.86	aA	11.17	abA	475.80	aA	477.40	aA	43.91	bcA	43.90	aA
700	9.99	aA	10.69	aA	477.40	aA	477.60	aA	47.90	cA	44.72	aA
CV (%)	6.52				0.63				6.37			

Means followed by the same lowercase letter in the columns and uppercase letter in the rows do not differ from each other (Tukey's 5%).

Table 2. Nitrogen (N), carbon (C) and C:N ratios in leaves, stems and roots of VM 01 and MN 463 eucalypt clonal plantlets cultivated in open-top chambers.

	N (g kg^{-1})				C (g kg^{-1})				C:N ratio			
Treatment	VM 01		MN 463		VM 01		MN 463		VM 01		MN 463	
Leaves												
C	25.30	bA	26.79	bA	501.83	aA	502.83	aA	19.91	aA	18.85	aA
OTC-A	22.63	abA	23.23	aA	501.00	aA	502.67	aA	22.16	bA	21.70	bA
OTC + CO_2	20.51	aB	23.58	aA	498.00	aA	500.00	aA	24.29	cB	21.25	bA
CV (%)	5.78				0.51				5.16			
Stem												
C	6.57	bA	6.36	bA	480.67	aB	474.67	aA	73.17	aA	75.60	aA
OTC-A	6.40	abB	5.78	aA	479.67	aA	474.00	aA	74.67	abA	82.33	bB
OTC + CO_2	5.93	aA	5.86	aA	481.33	aA	478.33	aA	81.67	bA	81.67	bA
CV (%)	5.04				0.74				5.43			
Roots												
C	3.45	bA	4.00	bB	479.50	aA	468.33	aA	141.55	aB	118.47	aA
OTC-A	2.88	aA	3.09	aA	463.33	aA	474.67	aA	162.25	aA	153.95	bA
OTC + CO_2	2.89	aA	3.52	abB	478.00	aA	458.33	aB	165.42	aB	132.31	abA
CV (%)	12.80				2.27				11.94			

Means followed by the same lowercase letter in the columns and uppercase letter in the rows do not differ from each other (Tukey's 5%).
C, unenclosed control; OTC-A, OTCs with ambient CO_2 concentration; OTC + CO_2, OTCs with high CO_2 concentration.

The amounts of essential oils produced by VM 01 (approximately 28 mg g^{-1} leaves) and MN 463 (approximately 14 mg g^{-1} leaves) clonal plantlets in addition to the major compounds of the essential oils (80% 1.8-cineole for VM 01; 50% 1.8-cineole and 32% α-pinene for MN 463) were not altered by increased CO_2 concentrations (Fig. 3).

4 Discussion

The results obtained in the present study in OTCs and CCs demonstrated that increased atmospheric CO_2 reduced the severity of rust, increased plant growth and did not alter the concentration of essential oils in eucalypt plantlets (Figs 1–3). Increased plant growth as a result of increased CO_2 concentrations has been demonstrated in various species and is related to increased photosynthesis and other metabolic processes (Duff et al. 1994; Pangga et al. 2004; Cheng 2009; Runion et al. 2010; Niu et al. 2011). Few studies have evaluated the changes in the severity of diseases and pathogen life cycles resulting from increased atmospheric CO_2 concentrations (Eastburn et al. 2011). This is the first report on the effect of increased CO_2 concentration on eucalypt rust. For the *Acer rubrum* L. × *Phyllosticta minima* (Berk. & M.A. Curtis) Underw. and Earle pathosystem, a reduction of 35, 50 and 10% of damaged leaf area has been observed in three consecutive years of testing in plants subjected to CO_2 concentrations of 200 μmol mol^{-1} above the ambient level in a free-air carbon dioxide enrichment (FACE) experiment (McElrone et al. 2005).

Biotrophic pathogens, such as *P. psidii*, only exist in association with living host plant tissue, and their fitness is easily measured by their ability to reproduce (Chakraborty 2013). The direct influence of CO_2 on plant physiology could also alter the colonization of host tissues by biotrophic pathogens (Eastburn et al. 2011). In the present study, we verified that increased CO_2 concentrations reduced the number of pustules per leaf and the production of *P. psidii* propagules (Fig. 1). One consequence of increased CO_2 concentration is the reduced production of pathogen inoculum for secondary infestations, which could decrease the severity of the disease over time when considering the multiplication cycles of the pathogen in the field. The severity of the wilt caused by *C. fimbriata* was also reduced with increased atmospheric CO_2 concentrations in the same eucalypt clones (Santos et al. 2013). This information indicates that integrated management to control these diseases could become easier under conditions of elevated CO_2, but interactions with other environmental factors could also influence disease development. In contrast, increased CO_2 concentrations (586 μmol mol^{-1}) increased leaf damage caused by *Cercospora liquidambaris* Cooke and Ellis and *Cercospora cercidicola* Ellis (syn. *Passalora cercidicola*) in American sweetgum (*Liquidambar styraciflua* L.) and eastern redbud (*Cercis canadensis* L.) (McElrone et al. 2010). Thus, more studies are needed to determine the influence of CO_2 concentrations and interactions with other environmental factors on eucalypt diseases.

Previous studies have shown that increased CO_2 concentration does not affect conidia germination of plant pathogens (Manning and Tiedemann 1995; Hibberd et al. 1996), and these findings were supported in the present study where increased CO_2 concentration did not affect urediniospore germination.

Reduced N levels in plant tissues grown under increased CO_2 have been reported by various authors (Ainsworth and Long 2005; McElrone et al. 2005; Eastburn et al. 2011; Santos et al. 2013), and this trend was also observed in the VM 01 eucalypt clonal plantlets (Tables 1 and 2). Despite the tendency for reduction, the N levels in MN 463 plants did not significantly decrease, which is consistent with other previous reports (Eastburn et al. 2010; McElrone et al. 2010). These results demonstrate that not only do species but also clones and other factors, such as the conditions in which the experiments are performed, can result in different responses to changes in atmospheric CO_2 levels (Ainsworth and Long 2005). Greater susceptibility of plants to diseases could be related to low N fertilization in high CO_2 environments (Fleischmann et al.

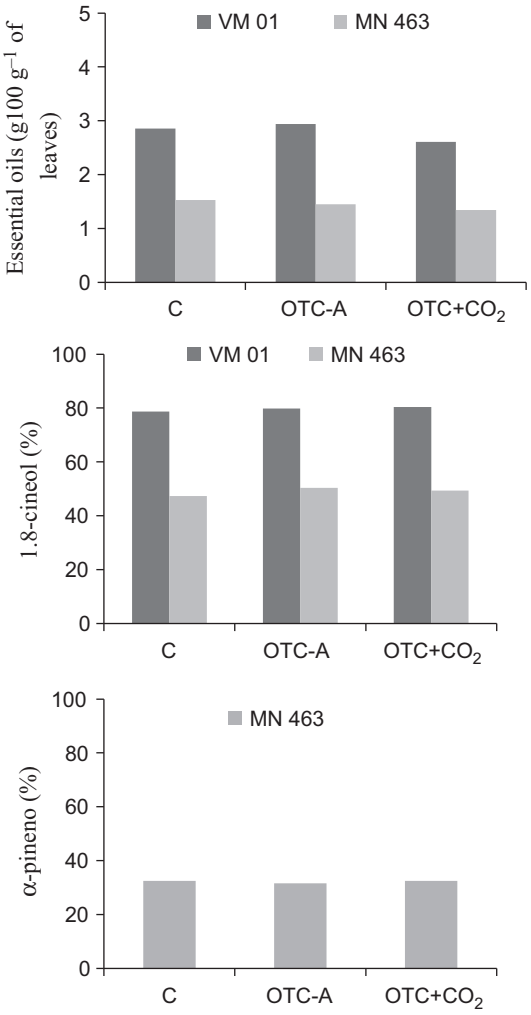

Fig. 3. Yield and chemical composition of essential oils of VM 01 and MN 463 eucalypt clonal plantlets cultivated in open-top chambers (C, unenclosed control; OTC-A, OTCs with ambient CO_2 concentration; and OTC + CO_2, OTCs with high CO_2 concentration). Means are not significantly different (Tukey's 5%).

2010). The data obtained in the present study did not allow definitive conclusions about the influence of reduced N concentration in tissues on the severity of eucalypt rust. However, the results indicated that the nutritional management of crops should be targeted towards any new environmental conditions to avoid imbalances.

The chemical composition of essential oils is determined by genetic factors, but other factors could lead to significant alterations in the production of secondary metabolites (Sangwan et al. 2001). Secondary metabolites represent a chemical interface between plants and the environment. Stimuli from the environment where plants are grown could redirect metabolic pathways and alter biosynthesis of different compounds. Diverse interacting factors, such as plant/microorganism, plant/insect and plant/plant interactions, plant age, plant developmental stage and abiotic factors, such as light, temperature, rainfall, nutrition, season, harvest time, harvest techniques and post-harvest techniques, could all potentially influence secondary metabolites. These factors could be inter-related or independent, yet they may jointly influence secondary metabolism. In the present study, increased atmospheric CO_2 concentrations did not affect the yield and chemical composition of essential oils in the eucalypt clones (Fig. 3) as has been observed in previous studies on secondary plant metabolites (McKiernan et al. 2012).

The information obtained in the present study about the effects of increased concentrations of atmospheric CO_2 on rust and other characteristics of eucalypt plants demonstrates the importance of screening eucalypt clones in CO_2-rich environments (Tausz et al. 2013). Regarding the variables evaluated, increased atmospheric CO_2 concentrations can favourably impact eucalypt growth while reducing rust severity under the environmental test conditions. This effect could potentially compensate for negative impacts from other environmental variables that are affected by climate change and should be considered in the development of adaptation strategies to address climate change. However, genetic resistance to disease, especially quantitative resistance, can be influenced by diverse environmental factors. Therefore, continued studies that consider combined effects of rising atmospheric CO_2, higher temperatures, drought and other variables affecting ecophysiological responses of plants and pathogens cycle are necessary to better understand the potential impacts of climate change on diseases.

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References

Adams, R. P., 1995: Identification of Essential oil Components by gas Chromatography/Mass Spectroscopy. Carol Streams: Allured Publ, 69p.

Ainsworth, E. A.; Long, S. P., 2005: What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. **165**, 351–372.

Alfenas, A. C.; Zauza, E. A. V.; Wingfield, M. J.; Roux, J.; Glen, M., 2005: *Heteropyxis natalensis*, a new host of *Puccinia psidii* rust. Australas. Plant Pathol. **34**, 285–286.

Booth, T. H., 2013: Eucalypt plantations and climate change. For. Ecol. Manage. **301**, 28–34.

Braga, M. R.; Aidar, M. P. M.; Marabesi, M. A.; Godoy, J. R. L., 2006: Effects of elevated CO_2 on the phytoalexin production of two soybean cultivars differing in the resistance to stem canker disease. Environ. Exp. Bot. **58**, 85–92.

Carnegie, A. J.; Cooper, K., 2011: Emergency response to the incursion of an exotic myrtaceous rust in Australia. Australas. Plant Pathol. **40**, 346–359.

Carnegie, A. J.; Lidbetter, J. R., 2012: Rapidly expanding host range for *Puccinia psidii* sensu lato in Australia. Australas. Plant Path. **41**, 13–29.

Carnegie, A. J.; Lidbetter, J. R.; Walker, J.; Horwood, M. A.; Tesoriero, L.; Glen, M.; Priest, M. J., 2010: *Uredo rangelii*, a taxon in the guava rust complex, newly recorded on Myrtaceae in Australia. Australas. Plant Pathol. **39**, 366–463.

Chakraborty, S., 2013: Migrate or evolve: options for plant pathogens under climate change. Glob. Change Biol. **19**, 1985–2000.

Cheng, S., 2009: Changes in root growth and relationships between plant organs and root hydraulic traits in American elm (*Ulmus americana* L.) and red oak (*Quercus rubra* L.) seedlings due to elevated CO_2 level. For. Stud. China **11**, 65–76.

Chmura, D. J.; Anderson, P. D.; Howe, G. T.; Harrington, C. A.; Halofsky, J. E.; Peterson, D. L.; Shaw, D. C.; St Clair, J. B., 2011: Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. For. Ecol. Manage. **261**, 1121–1142.

Duff, G. A.; Berryman, C. A.; Eamus, D., 1994: Growth, biomass allocation and foliar nutrient contents of two *Eucalyptus* species of the wet-dry tropics of Australia grown under CO_2 enrichment. Funct. Ecol. **8**, 502–508.

Eastburn, D. M.; Degennaro, M. M.; Delucia, E. H.; Dermody, O.; McElrone, A. J., 2010: Elevated atmospheric carbon dioxide and ozone alter soybean diseases at SoyFACE. Glob. Change Biol. **16**, 320–330.

Eastburn, D. M.; McElrone, A. J.; Bilgin, D. D., 2011: Influence of atmospheric and climatic change on plant–pathogen interactions. Plant. Pathol. **60**, 54–69.

Fleischmann, F.; Raidl, S.; Oßwald, W. F., 2010: Changes in susceptibility of beech (*Fagus sylvatica*) seedlings towards *Phytophthora citricola* under the influence of elevated atmospheric CO_2 and nitrogen fertilization. Environ. Pollut. **158**, 1051–1060.

Hibberd, J. M.; Whitbread, R.; Farrar, J. F., 1996: Effect of elevated concentrations of CO_2 on infection of barley by *Erysiphe graminis*. Physiol. Mol. Plant Pathol. **48**, 37–53.

IPCC 2007: Climate Change 2007: The Physical Science Basis. Geneva: Intergovernmental Panel on Climate Change, p. 996.

Kretzschmar, F. S.; Aidar, M. P. M.; Salgado, I.; Braga, M. R., 2009: Elevated CO_2 atmosphere enhances production of defense-related flavonoids in soybean elicited by NO and a fungal elicitor. Environ. Exp. Bot. **65**, 319–329.

Manning, W. J.; Tiedemann, A. V., 1995: Climate change: potential effects of increased atmospheric carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. Environ. Pollut. **88**, 219–245.

McElrone, A. J.; Reid, C. D.; Hoye, K. A.; Hart, E.; Jackson, R. B., 2005: Elevated CO_2 reduces disease incidence and severity of a red maple fungal pathogen via changes in host physiology and leaf chemistry. Glob. Change Biol. **11**, 1828–1836.

McElrone, A. J.; Hamilton, J. G.; Krafnick, A. J.; Aldea, M.; Knepp, R. G.; DeLucia, E. H., 2010: Combined effects of elevated CO_2 and natural climatic variation on leaf spot diseases of redbud and sweetgum trees. Environ. Pollut. **158**, 108–114.

McKiernan, A. B.; O'Reilly-Wapstra, J. M.; Price, C.; Davies, N. W.; Potts, B. M.; Hovenden, M. J., 2012: Stability of plant defensive traits among populations in two *Eucalyptus* species under elevated carbon dioxide. J. Chem. Ecol. **38**, 204–212.

Niu, Y.; Jin, C.; Jin, G.; Zhou, Q.; Lin, X.; Tang, C.; Zhang, Y., 2011: Auxin modulates the enhanced development of root hairs in *Arabidopsis thaliana* (L.) Heynh. under elevated CO_2. Plant, Cell Environ. **34**, 1304–1317.

Pangga, I. B.; Chakraborty, S.; Yates, D., 2004: Canopy size and induced resistance in *Stylosanthes scabra* determine anthracnose severity at high CO_2. Phytopathology **94**, 221–227.

Pinkard, E. A.; Battaglia, M.; Bruce, J.; Leriche, A.; Kriticos, D. J., 2010: Process-based modelling of the severity and impact of foliar pest attack on eucalypt plantation productivity under current and future climates. For. Ecol. Manage. **259**, 839–847.

Ruiz, R. A. R.; Alfenas, A. C.; Ferreira, F. A., 1989: Influência da temperatura, luz e origem do inóculo sobre a produção de uredosporos e teliosporos de *Puccinia psidii*. Fitopatol. Bras. **14**, 70–73.

Runion, G. B.; Prior, S. A.; Rogers, H. H.; Mitchell, R. J., 2010: Effects of elevated atmospheric CO_2 on two southern forest diseases. New Forest. **39**, 275–285.

Sangwan, N. S.; Farooqi, A. H. A.; Shabih, F.; Sangwan, R. S., 2001: Regulation of essential oil production in plants. Plant Growth Regul. **34**, 3–21.

Santos, M. S.; Ghini, R.; Fernandes, B. V.; Silva, C. A., 2013: Increased carbon dioxide concentration in the air reduces the severity of *Ceratocystis* wilt in *Eucalyptus* clonal plantlets. Australas. Plant Path. **42**, 595–599.

Silva, P. H. M.; Miranda, A. C.; Moraes, M. L. T.; Furtado, E. L.; Stape, J. L.; Alvares, C. A.; Sentelhas, P. C.; Mori, E. S.; Sebbenn, A. M., 2013: Selecting for rust (*Puccinia psidii*) resistance in *Eucalyptus grandis* in São Paulo State, Brazil. For. Ecol. Manage. **303**, 91–97.

Tausz, M.; Tausz-Posch, S.; Norton, R. M.; Fitzgerald, G. J.; Nicolas, M. E.; Seneweera, S., 2013: Understanding crop physiology to select breeding targets and improve crop management under increasing atmospheric CO_2 concentrations. Environ. Exp. Bot. **88**, 71–80.