Notas Científicas

Plant growth and leaf-spot severity on eucalypt at different CO_2 concentrations in the air

Carlos Eduardo Oliveira da Silva[1] and Raquel Ghini[2]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Agronômicas, Fazenda Experimental Lageado, CEP 18610-307 Botucatu, SP, Brazil. E-mail: du_oliveira007@live.com [2]Embrapa Meio Ambiente, Caixa Postal 69, CEP 13820-000 Jaguariúna, SP, Brazil. E-mail: raquel.ghini@embrapa.br

Abstract – The objective of this work was to evaluate the effects of increased air-CO_2 concentration on plant growth and on leaf-spot caused by *Cylindrocladium candelabrum* in *Eucalyptus urophylla.* Seedlings were cultivated for 30 days at 451, 645, 904, and 1,147 µmol mol^{-1} CO_2; then, they were inoculated with the pathogen and kept under the same conditions for seven days. Increased CO_2 concentration increased plant height and shoot dry matter mass, and decreased disease incidence and severity. Stem diameter was not affected by the treatments. Increased concentrations of atmospheric CO_2 favorably affect eucalypt growth and reduce leaf-spot severity.

Index terms: *Cylindrocladium candelabrum*, *Eucalyptus urophylla*, beneficial effects, climate change, disease incidence.

Crescimento de plantas e severidade da mancha foliar em eucalipto a diferentes concentrações de CO_2 no ar

Resumo – O objetivo deste trabalho foi avaliar os efeitos do aumento da concentração de CO_2 do ar sobre o crescimento de plantas e sobre a mancha foliar causada por *Cylindrocladium candelabrum* em *Eucalyptus urophylla*. As mudas foram cultivadas durante 30 dias, a 451, 645, 904, 1.147 µmol mol^{-1} de CO_2; em seguida, elas foram inoculadas com o patógeno e mantidas nas mesmas condições por sete dias. O aumento da concentração de CO_2 aumentou a altura de plantas e a massa de matéria seca da parte aérea, e diminuiu a incidência e a severidade da doença. O diâmetro do caule não foi afetado pelos tratamentos. O aumento das concentrações de CO_2 atmosférico afeta favoravelmente o crescimento de plântulas de eucalipto e reduz a severidade da mancha foliar.

Termos para indexação: *Cylindrocladium candelabrum*, *Eucalyptus urophylla*, efeitos benéficos, mudança climática, incidência de doença.

The concentration of carbon dioxide (CO_2) in Earth's atmosphere has gradually increased in the last 800 thousand years, with actual values exceeding 350 µmol mol^{-1} (Lüthi et al., 2008). Rockström et al. (2009) proposed 350 µmol mol^{-1} as the CO_2 concentration limit for climate change, besides a radiative forcing limit of 1 W m^{-2}. In 2011, the CO_2 concentration was 391 µmol mol^{-1}, which exceeded the pre-industrial levels in ~40% (Climate change 2013, 2013). CO_2 is a greenhouse gas that has a direct impact on plant growth and on disease epidemics, causing changes in pathogen-host relationships. Above- and belowground biomass accumulation exhibits strong and consistent increases under elevated CO_2 (Eastburn et al., 2011). However, the effects on plant diseases may differ according to the pathogen, the host, the environmental conditions, and the methodology used in the studies (Luck et al., 2011).

Climate change can alter susceptibility of trees to certain pathogens and change the geographical distribution of pathogens (Sturrock et al., 2011). Studying the impacts of rising CO_2 concentrations, Santos et al. (2013) observed that at high air-CO_2 concentrations the plantlets of two eucalyptus clones had longer disease incubation period and lower severity of wilt caused by *Ceratocystis fimbriata*. Ghini et al. (2014) observed that increasing CO_2 concentration decreased rust severity caused by *Puccinia psidii* on eucalypt clonal plantlets. Both Santos et al. (2013)

and Ghini et al. (2014) verified that plantlet growth increased with rising air-CO_2 concentration.

Species of *Cylindrocladium* have been described as important fungal pathogens of various *Eucalyptus* and *Pinus*, causing considerable lost in tropical and subtropical regions, commonly associated with nursery diseases such as root rot, damping-off, and leaf-spot (Alfenas et al., 2004).

The objective of this work was to evaluate the effects of increased air-CO_2 concentration on plant growth and on leaf-spot caused by *Cylindrocladium candelabrum* in *Eucalyptus urophylla*.

The *C. candelabrum* isolate was obtained from the microbial culture collection of Embrapa Meio Ambiente (record: CCMA-1191). Culture-medium discs containing the pathogen mycelia were placed on young leaves of castor-bean plants (*Ricinus communis*) and incubated for seven days in plastic trays (43x24x8 cm) covered with clear glass lids (Alfenas & Mafia, 2007). Incubation was carried out at 28°C with 12-hour photoperiod. After seven days, the castor-bean leaves were rinsed with sterile, distilled water, and the conidia suspension was filtered. The concentration was adjusted to 2×10^5 conidia mL^{-1} and mixed with 0.5 mL L^{-1} of tween 20.

Eucalyptus urophylla seedlings, of approximately 45 days old, were cultivated in 50 cm^3 tubes containing a substrate based on *Pinus* bark and rice husk. Then, they were pruned to 10-cm height. Immediately after pruning, 10 seedlings were placed in plastic boxes (34x24x32 cm) which were covered with a clear glass lid. Each box contained sterile vermiculite at the bottom, to support the tubes and maintain the moisture.

CO_2 concentrations inside the boxes were kept at 451±35, 645±118, 904±116, and 1,147±216 µmol mol^{-1}. For this, pure CO_2 was periodically injected inside the boxes (0.5 s, every 50 min) through tubes. To homogenize the CO_2 concentration, air from outside of the room was injected under controlled conditions using an electromagnetic air compressor (Boyu ACQ-007, Raoping Guangdong, China) programmed to turn on and off in 15 min intervals. The boxes were kept at 28±1.9ºC and 12-hour photoperiod, with 20,000 lux of illumination, provided by daylight and Grolux lamps (40 W) (Sylvania, São Paulo, SP, Brazil). Concentrations were daily monitored with an infrared gas analyzer, Hand-Held Carbon Dioxide Meter GM70, (Vaisala, Vantaa, Finland).

The seedlings were grown for 30 days. After this period, pathogen inoculation was performed by spraying the conidial suspension, to the point of run-off, on the leaves of *E. urophylla* seedlings (Alfenas & Mafia, 2007). Throughout the assays, seedlings were daily irrigated with 15 mL nondistilled water (without chlorine) per plant, without fertilization.

The disease severity was assessed with a diagrammatic scale three days after the inoculation, as proposed by Carvalho Filho et al. (2008), with modifications. Higher percentages of lesioned leaf area (55, 70, 85, and 99%) were added to the diagrammatic scale due to the high disease severity observed. The number of lesioned leaves and of leaves containing sporulated lesions per plant were also evaluated. Leaves with sporulated lesions were observed by a stereoscopic microscope (40× magnification), to confirm the presence of conidiophores and conidia. The assessments were performed every two days, for seven days after inoculation (three assessments).

At the end of the assays, eight days after inoculation, it was also evaluated stem diameter, plant height, and dry weight of shoots and roots.

The experiment followed a randomized complete block design, with four treatments (air-CO_2 concentrations) and five blocks. The assay was repeated twice. Plant growth and disease variables were analyzed by analysis of variance, and the treatments were compared by Tukey's test, at 5% probability. The data were normally distributed and had homogeneity of variances; therefore, the results from experimental replicates were grouped for the statistical analyses.

Increasing air-CO_2 concentration reduced the incidence and severity of *C. candelabrum* leaf-spot, as well as the incidence of leaves with sporulated lesions

Table 1. Lesioned leaf incidence, leaf spot severity, and incidence of *Cylindrocladium candelabrum* conidia on leaves of *Eucalyptus urophylla* seedlings grown at different CO_2 concentrations in the air[1].

CO_2 concentration (µmol mol^{-1})	Incidence on lesioned leaves[2]	Severity[2]	Incidence on leaves with sporulated lesions[2]
451	324.35a	200.22a	90.09a
645	201.39b	78.95b	59.46b
904	227.18b	93.45b	52.34b
1,147	207.01b	85.77b	54.81b

[1]Values followed by equal letters do not differ, by Tukey's test, at 5% probability (averages from two assays). [2]Area under the disease progress curve (Madden et al., 2007).

(Table 1). Braga et al. (2006) reported a significant increase in phytoalexin production in two soybean cultivars at 720 µmol mol^{-1} CO_2, in comparison to an environment with 360 µmol mol^{-1}. However, McKiernan et al. (2012) verified that elevated CO_2 concentration did not affect contents of secondary metabolites, total phenolics, condensed tannins, or the total oil yield of *Eucalyptus globulus* and *Eucalyptus pauciflora*.

As to growth, seedling height in the treatments with higher CO_2 concentrations was higher than in the treatment with 451±35 µmol mol^{-1} CO_2 (Table 2). Likewise, higher seedling shoot dry weight was observed in the treatments with high CO_2 concentrations. For the root dry matter weight, only the treatment with 1,147±216 µmol mol^{-1} CO_2 had significantly higher values than the other treatments. Otherwise, Novriyanti et al. (2012) observed no positive effects of elevated CO_2 concentrations on growth of eucalypts, despite increases on net photosynthetic rate. The increase in plant height and shoot dry weight, as observed in the present study, was also observed by Santos et al. (2013) and Ghini et al. (2014), in clonal eucalypt plantlets grown under high air-CO_2 concentrations for approximately 60 days.

The main effect of the treatments was on the host plant, which might imply alterations on plant disease. However, the high leaf-spot severity in the plants grown at 451±35 µmol mol^{-1} CO_2 caused severe defoliation, which may have been the cause of the reduction of shoot dry mass observed in this treatment (Table 2).

Stem diameter of the plants was not altered by treatments (Table 2), most likely due to the short exposure time of the plants to high CO_2 concentrations. This short time period was used because the pathogen quickly leads to damage on leaves and to intense defoliation.

Increased CO_2 concentration in the air decreases the incidence and severity of *C. candelabrum* leaf-spot in *E. urophylla* seedlings, as well the incidence of leaves with sporulated lesions. Moreover, it also increases plant growth, based on the height and shoot dry matter weight of the seedlings. These favorable effects of rising atmospheric CO_2 concentration on the severity of *C. candelabrum* leaf-spot and eucalypt growth agree with the results obtained by Santos et al. (2013) and Ghini et al. (2014) for *Ceratocystis* wilt and rust, respectively.

Table 2. Plant height, stem diameter, and shoot and root dry weight of *Eucalyptus urophylla* seedlings, grown at different CO_2 concentrations in the air and infected with *Cylindrocladium candelabrum*[(1)].

CO_2 concentration (µmol mol^{-1})	Plant height (cm)	Stem diameter (mm)	Shoot dry (g)	Root dry (g)
451	11.2b	2.04a	0.19b	0.21b
645	12.7a	2.11a	0.25a	0.19b
904	12.5a	2.12a	0.27a	0.21b
1,147	12.5a	2.14a	0.28a	0.26a

[(1)]Values followed by equal letters do not differ, by Tukey's test, at 5% probability. Averages from two assays.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for scholarships granted; to Flora Cantareira, for the donation of seedlings; and to Dr. Ricardo Harakava, from the Biological Institute of São Paulo, for the molecular classification of the pathogen.

References

ALFENAS, A.C.; MAFIA, R.G. (Ed.). **Métodos em fitopatologia**. Lavras: UFV, 2007. 382p.

ALFENAS, A.C.; ZAUZA, E.A.V.; MAFIA, R.G.; ASSIS, T.F. de. **Clonagem e doenças de eucalipto**. Viçosa: Ed. da UFV, 2004. 442p.

BRAGA, M.R.; AIDAR, M.P.M.; MARABESI, M.A.; GODOY, J.R.L. Effects of elevated CO2 on the phytoalexin production of two soybean cultivars differing in the resistance to stem canker disease. **Environmental and Experimental Botany**, v.58, p.85-92, 2006. DOI: 10.1016/j.envexpbot.2005.06.018.

CARVALHO FILHO, M.R.; MENÊZES, J.E.; MELLO, S.C.M. de; SANTOS, R.P. dos. **Avaliação de isolados de *Trichoderma* no controle da mancha foliar do eucalipto in vitro e quanto à esporulação em dois substratos sólidos**. Brasília: Embrapa Recursos Genéticos e Biotecnologia, 2008. 22p. (Embrapa Recursos Genéticos e Biotecnologia. Boletim de pesquisa e desenvolvimento, 225).

CLIMATE change 2013: the physical science basis. Cambridge: Intergovernmental Panel on Climate Change, 2013. 1535p.

EASTBURN, D.M.; MCELRONE, A.J.; BILGIN, D.D. Influence of atmospheric and climatic change on plant-pathogen interactions. **Plant Pathology**, v.60, p.54-69, 2011. DOI: 10.1111/j.1365-3059.2010.02402.x.

GHINI, R.; MAC LEOD, R.E. de O.; TORRE NETO, A.; CARDOSO, D.C.; BETTIOL, W.; MORAIS, L.A.S. de; VIQUE,

B. Increased atmospheric carbon dioxide concentration: effects on eucalypt rust (*Puccinia psidii*), C:N ratio and essential oils in eucalypt clonal plantlets. **Forest Pathology**, 2014. DOI: 10.1111/efp.12117.

LUCK, J.; SPACKMAN, M.; FREEMAN, A.; TREBICKI, P.; GRIFFITHS, W.; FINLAY, K.; CHAKRABORTY, S. Climate change and diseases of food crops. **Plant Pathology**, v.60, p.113-121, 2011. DOI: 10.1111/j.1365-3059.2010.02414.x.

LÜTHI, D.; LE FLOCH, M.; BEREITER, B.; BLUNIER, T.; BARNOLA, J.-M.; SIEGENTHALER, U.; RAYNAUD, D.; JOUZEL, J.; FISCHER, H.; KAWAMURA, K.; STOCKER, T.F. High-resolution carbon dioxide concentration record 650,000-800,000 years before present. **Nature**, v.453, p.379-382, 2008. DOI: 10.1038/nature06949.

MADDEN, L.V.; HUGHES, G.; BOSCH, F. van den. **The study of plant disease epidemics**. St. Paul: The American Phytopathological Society, 2007. 432p.

MCKIERNAN, A.B.; O'REILLY-WAPSTRA, J.M.; PRICE, C.; DAVIES, N.W.; POTTS, B.M.; HOVENDEN, M.J. Stability of plant defensive traits among populations in two Eucalyptus species under elevated carbon dioxide. **Journal of Chemical Ecology**, v.38, p.204-212, 2012. DOI: 10.1007/s10886-012-0071-4.

NOVRIYANTI, E.; WATANABE, M.; KITAO, M. High nitrogen and elevated CO2 effects on the growth, defense and photosynthetic performance of two eucalypt species. **Environmental Pollution**, v.170, p.124-130, 2012. DOI: 10.1016/j.envpol.2012.06.011.

ROCKSTRÖM, J.; STEFFEN, W.; NOONE, K.; PERSSON, Å.; CHAPIN, F.S.; LAMBIN, E.F.; LENTON, T.M.; SCHEFFER, M.; FOLKE, C.; SCHELLNHUBER, H.J.; NYKVIST, B.; WIT, C.A. de; HUGHES, T.; LEEUW, S. van der; RODHE, H.; SÖRLIN, S.; SNYDER, P.K.; COSTANZA, R.; SVEDIN, U.; FALKENMARK, M.; KARLBERG, L.; CORELL, R.W.; FABRY, V.J.; HANSEN, J.; WALKER, B.; LIVERMAN, D.; RICHARDSON, K.; CRUTZEN, P.; FOLEY, J.A. A safe operating space for humanity. **Nature**, v.461, p.472-475, 2009. DOI: 10.1038/461472a.

SANTOS, M. de S. dos; GHINI, R.; FERNANDES, B.V.; SILVA, C.A. Increased carbon dioxide concentration in the air reduces the severity of Ceratocystis wilt in *Eucalyptus* clonal plantlets. **Australasian Plant Pathology**, v.42, p.595-599, 2013. DOI: 10.1007/s13313-013-0223-1.

STURROCK, R.N.; FRANKEL, S.J.; BROWN, A.V.; HENNON, P.E.; KLIEJUNAS, J.T.; LEWIS, K.J.; WORRALL, J.J.; WOODS, A.J. Climate change and forest diseases. **Plant Pathology**, v.60, p.133-149, 2011. DOI: 10.1111/j.1365-3059.2010.02406.x.

Received on October 23, 2013 and accepted on February 21, 2014