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Response of ethylene and chlorophyll in two eucalyptus clones during drought

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Application. Understanding the relationship between ethylene and chlorophyll concentration during water stress could provide a means for developing a method to determine drought susceptibility in *Eucalyptus* and perhaps other important species. Drought tolerance appeared to be associated with higher chlorophyll concentration and lower ethylene production rate.

Abstract. A study was conducted with seven-month-old plantlets, of the clone 2814 of *Eucalyptus grandis* × *E. camaldulensis* Dehnh. hybrid and of the clone 2798 of *E. grandis* × *E. robusta* Sm., subjected to sublethal water stress. During the imposed drought, leaf water potentials and transpiration rates decreased, while ethylene production and leaf chlorophyll concentration increased to a maximum during the onset of water stress, and then declined. Stomatal closure coincided with or preceded maximum ethylene production. A saturation type relationship between ethylene production and chlorophyll concentration was observed. Genotypic differences in the response to water stress occurred between clones with clone 2814 appearing to be a drought avoider and clone 2798 a drought tolerator.

Introduction

Water is the most important factor limiting the distribution of forests, their species composition and the growth of the trees throughout the world (Kramer 1983). When plants are subjected to water stress many physiological processes are affected. Drought reduces photosynthesis by causing stomatal closure, by inhibiting chlorophyll formation (Bourque and Naylor 1971), by increasing chlorophyll breakdown, and by changing conformation of chloroplast membranes (Schapendonk 1987). Many stresses including drought trigger premature senescence, and are characterized by the breakdown of the chlorophyll molecule (Johnson 1988).

The relationships between water stress and plant hormones are complex. For example, ethylene production increases in plants subjected to water stress (Apelbaum and Yang 1981; Beyer et al. 1984; Hoffman et al. 1983; Kimmerer and Kozlowski 1982; Stumpff and Johnson 1987), but the role of ethylene in stomatal closure during water stress is unclear. Stumpff and Johnson (1987) reported that the modulation of ethylene synthesis by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ethylene-forming enzyme (EFE) was

influenced by water stress level, organ and genetic source in loblolly pine (*Pinus taeda* L.). Water stress may accelerate leaf senescence and induce abscission in older leaves during intense or prolonged stress, consequently reducing transpirational area and water loss (Beyer et al. 1984; Johnson 1988).

The objective of this study was to investigate the effect of water stress on ethylene and chlorophyll production by two *Eucalyptus grandis* hybrid clones.

Materials and methods

Seven-month-old plantlets from *Eucalyptus grandis* × *E. camaldulensis* hybrid (clone 2814) and from *E. grandis* × *E. robusta* hybrid (clone 2794) each represented by three plantlets, were grown under controlled environment (25°/20 °C day/night temperature; 70% relative humidity; 16 hour photoperiod at 450 μmole m^{-2} s^{-1} photosynthetic photon flux density) and then subjected to sublethal water stress. When transpiration fell to zero (foliar water potentials of −3 MPa for clone 2798 and of −4.3 MPa for clone 2814), the stressed plants were watered regularly for a week before making the final sample.

Xylem water potential, transpiration, ethylene production and chlorophyll concentration were measured on each sample date. Xylem water potential was determined with a pressure chamber on one leaf per plant collected at the same location and position in the crown of each plantlet. Transpiration was measured with a LI-COR LI 1600 steady-state porometer on the same leaves subjected to the ethylene and chlorophyll analysis.

Ethylene evolution was measured in two leaves, one removed from the basal portion and one from the upper part of each plantlet and placed into separate 10 ml vials. A 0.5 mL gas sample was withdrawn from the head space of each vial after two and three hours of incubation in light at 25 °C and analyzed by means of a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector and a 1 m alumina-packed glass column at 100 °C (Stumpff and Johnson 1987). The ethylene peak was identified by comparison of its retention time with that of an ethylene standard. Ethylene production rates were expressed as pL ethylene g leaf dry $weight^{-1}$ h^{-1}.

About 0.5 g of each single leaf was homogenized with cold 80% acetone avoiding direct light. Leaf sample extracts were centrifuged at 2500 rpm for 10 min at 0 °C, and were analyzed spectrophotometrically for chlorophyll concentration (Arnon 1949). The remaining foliar tissue was put in the oven for the dry weight determination. Dry weight of roots was measured at the end of the experimental period.

The data were analyzed using analysis of variance available on the statistical system SAS (1985). For the response variables measured, the two clones

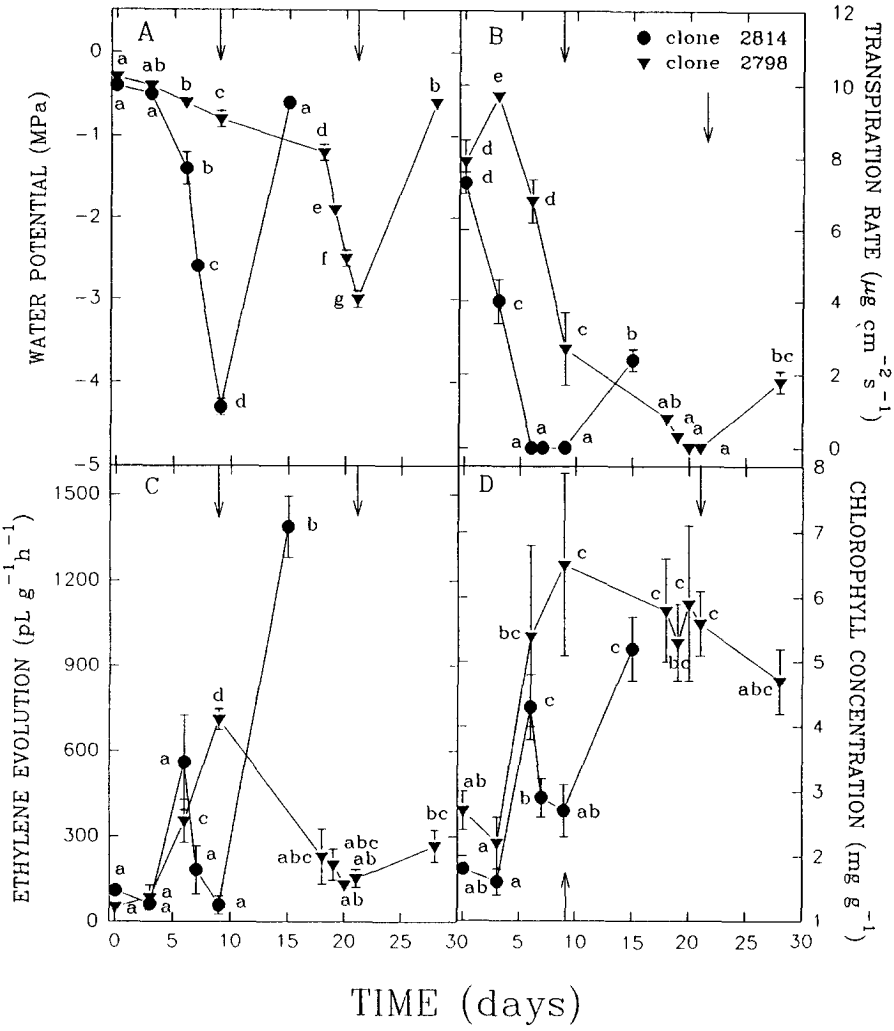

Fig. 1. Physiological responses of two *Eucalyptus* clones as affected by sublethal water stress: A) leaf water potential; B) transpiration rate; C) ethylene evolution, and D) chlorophyll concentration. Means within each clone followed by the same letter do not differ significantly (alpha = 0.05). Arrows indicate when each clone was rewatered.

were found to be significantly different, at least during some part of the study, and were, therefore, analyzed separately to determine treatment effects within each clone. For significant treatment effects, means were separated using Duncan's multiple range test.

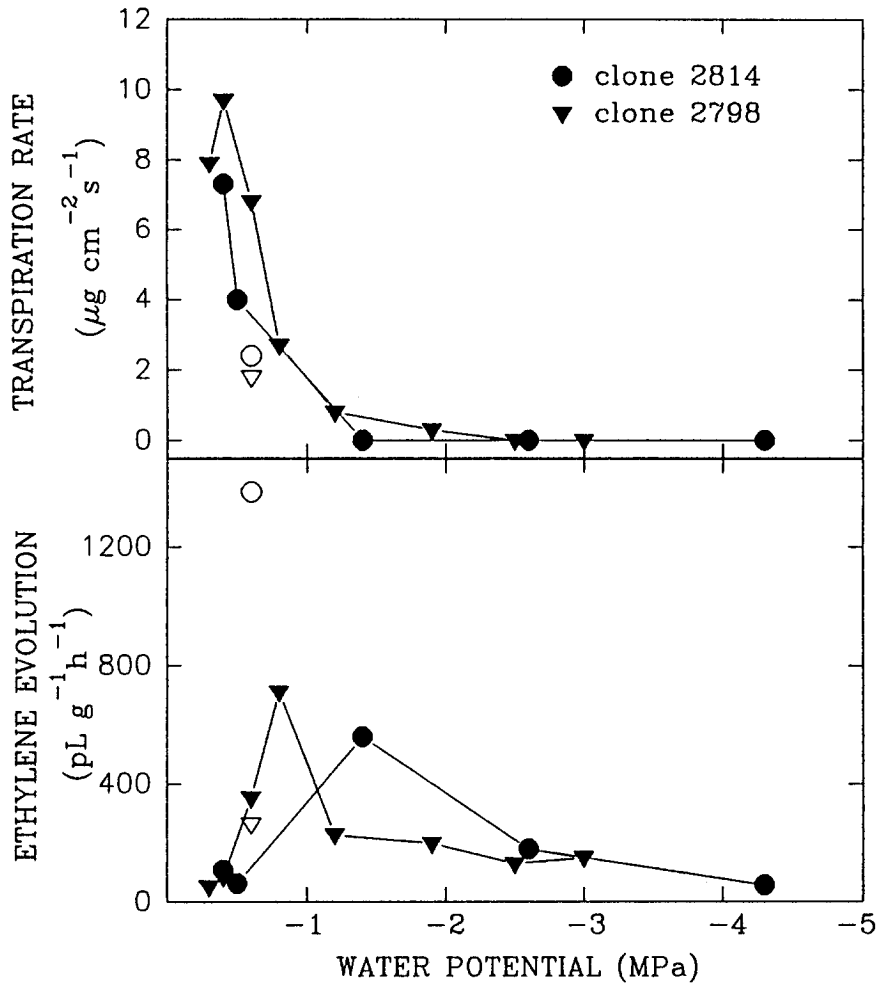

Fig. 2. Transpiration and ethylene evolution as affected by water stress in two *Eucalyptus* clones. Open symbols indicate values one week after rewatering. Statistics are provided in Fig. 1.

Results

Transpiration rate (E) decreased as leaf water potential (LWP) decreased from control value of −0.4 and −0.3 MPa to minimums of −4.3 and −3.0 MPa for clones 2814 and 2798, respectively (Fig. 1A and B). At the onset of water stress the two clones responded differently. Stomata (as measured by transpiration rate) of clone 2814 began closing immediately at the onset of stress while stomata in clone 2798 exhibited opening on day 3. Transpiration rate fell to zero for both clones when LWP was ca −1.3 MPa (Fig. 2). Clone

2814, however, reached the point of zero transpiration on a day 6 whereas clone 2798 required about 20 days to reach the same value of E (Fig. 1B). Upon rewatering, LWP recovered to −0.5 MPa in both clones while transpiration rate only achieved a value 20 to 25 percent of initial values even after a week of watering (open symbols, Fig. 2).

Total chlorophyll concentration increased with increasing water stress, reaching a maximum at or slightly after stomatal closure and then declined only slightly with intensifying stress (Fig. 1D). Although both clones had similar chlorophyll concentrations at the beginning of the stress period, clone 2798 had nearly double the concentration of clone 2814 after stomatal closure. After rewatering, chlorophyll concentration decreased slightly in clone 2798, remaining well above pre-stress concentration. In clone 2814, which dropped most of its leaves as a result of the imposed water stress, chlorophyll concentration actually increased after watering in the few remaining leaves. Significant differences were found for both the clones in chlorophyll concentration.

Ethylene production by the leaves increased with initial water stress (Figs. 1C and 2) with clone 2798 exhibiting a significant 7-fold increase as LWP decreased from −0.3 to −0.8 MPa, coinciding with the drop in E. Ethylene production rate in clone 2814 increased about 6-fold between LWP of −0.4 and −1.3 MPa and appeared to lag behind stomatal closure (Fig. 2). Once LWP decreased below −1.2 and −2.5 MPa, ethylene production decreased to near pre-stress levels in clone 2798 and 2814, respectively. Rewatering resulted in the largest clonal difference (open symbols, Fig. 2). In clone 2798, ethylene production increased slightly from 200 to 270 pL/g DW/h whereas in clone 2814, ethylene went from 100 to nearly 1400 pL/g DW/h. It is noteworthy that clone 2814 exhibited significant leaf drop during this period.

Similar responses in total chlorophyll concentration and ethylene production rates to decreasing LWP suggested that the two may be related (Fig. 1C and D). Saturation-type relationships between chlorophyll and ethylene were observed for both clones suggesting a possible role of ethylene in the observed chlorophyll increase (Fig. 3).

Root dry weight measured at the end of the study showed that plantlets of clone 2814 had a greater root biomass than the plantlets of clone 2794.

Discussion

Water stress affected ethylene production in both clones (Fig. 2) with an increase coinciding with stomatal closure at least in clone 2798. Stomatal response to endogenous (Erkan and Bangerth 1980) and exogenous ethylene (Squier et al. 1985, Vitagliano and Hoad 1978) has been shown in different species. Ethylene has been reported to change the permeability of guard cell

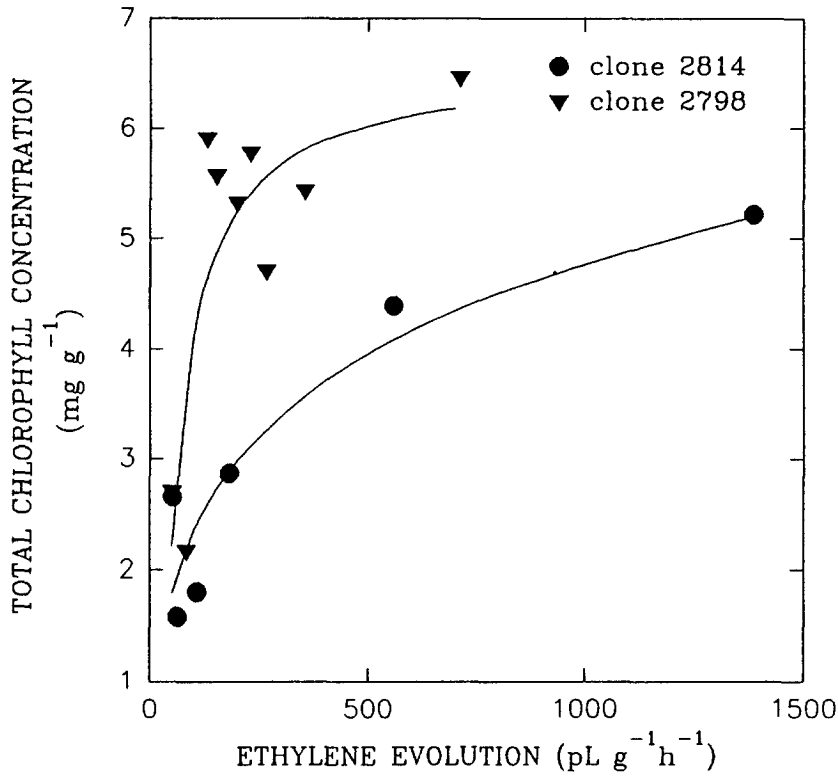

Fig. 3. The relationship between total chlorophyll concentration and ethylene production rate in two *Eucalyptus* clones subjected to sublethal water stress. The regression equation shown by the solid lines are:

clone 2798: $Y = (2.563 - 53.89/x^2, R^2 = 0.67$
clone 2814: $Y = -2.309 + 1.027\ln(x), R^2 = 0.85$

membranes, altering ion and/or water flux (Hanson and Kende 1975). In other species, however, stomata appear to be insensitive to endogenous (Bradford 1983; El-Beltagy and Hall 1974; Johnson 1984), and exogenous ethylene (Pallaghy and Raschke 1972; Pallas and Kays 1982). The subsequent decrease in ethylene production as water stress intensified indicates that ethylene is not involved in maintaining stomatal closure, and ethylene decrease is probably a result of inhibition of ACC synthase (Stumpff and Johnson 1987). Ethylene production by both *Eucalyptus* clones was similar to the trend reported for a drought-hardy source of loblolly pine subjected to water stress (Stumpff and Johnson 1987).

Chlorophyll concentration reached a maximum at or just following stomatal closure and decreased slightly as water stress intensified (Fig. 1). The

initial increase in chlorophyll concentration could be an adaptive response to maintain photosynthesis during the time stomatal closure begins to restrict CO_2 influx or it could be part of a general stress response that imparts some other adaptive advantage. Chlorophyll content of leaves and photosynthesis are well correlated (Kozlowski 1982; Reich et al. 1986). It is noteworthy that the highest chlorophyll concentration in clone 2814 was observed after rewatering when the plantlets had lost most of their leaves due to stress-induced senescence and abscission. This resulting decrease in plantlet leaf area is considered an adaptive drought avoidance mechanism (Kramer 1983). In this instance, increased chlorophyll concentration may enhance photosynthetic efficiency of the remaining leaves. Although the cause of this is unknown, increased cytokinin transport from the regenerating root system (Johnson 1988) or the availability of nitrogen translocated from the senescing leaves could provide plausible explanations. Water stress affects many metabolic pathways in plants. The positive relationship between chlorophyll concentration and ethylene production (Fig. 3) could be due to stomatal closure and the need for maintaining photosynthesis, or a direct regulation of chlorophyll biosynthesis. Despite the good fit of equations to these relationships, the study was not designed to determine causality and it is quite possible that both ethylene and chlorophyll were independently but similarly affected by water stress.

Differences in response to water stress were evident between clones. Plantlets of clone 2814 were more sensitive to water stress and avoided the imposed drought by rapidly closing their stomata within 6 days, decreasing leaf area by massive leaf abscission, and having greater root biomass. In contrast, clone 2798 plantlets appeared to tolerate drought by keeping stomata open an additional 3 days before closing, maintaining a higher chlorophyll concentration and leaf area throughout the imposed drought.

In conclusion, water stress resulted in increased ethylene production and chlorophyll concentration with maxima coinciding with or following shortly after stomatal closure. The saturating-type relationship found between chlorophyll and ethylene warrants further study in order to determine causality.

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