

Assessment of the effects of below-zero temperatures on photosynthesis and chlorophyll *a* fluorescence in leaf discs of *Eucalyptus globulus*

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Introduction

The sensitivity of plants to low temperatures has been assessed by a number of methods, including measurements of visible symptoms of injury, vital staining techniques, protoplasmic streaming, plasmolysis and changes in the pattern of chlorophyll fluorescence kinetics (Baker *et al.*, 1983; MacRae *et al.*, 1986). The aim of our study was to test the possibility of using changes in photosynthetic capacity and in slow fluorescence kinetics in leaf discs of *Eucalyptus globulus* to screen resistance to below-zero temperatures, which we compared with the classic tissue necrosis method.

Materials and Methods

Leaf discs (10 cm²) of *E. globulus* potted plants were subjected to low temperature treatments. They were placed in the dark, inside an aluminium box floating in a 10 l bath containing ethylene glycol. After 2 h of exposure at -2, -3, -4

and -5°C, we measured slow fluorescence kinetics of chlorophyll *a* and photosynthetic capacity at 25°C with saturating light and CO₂ concentration (provided by a bicarbonate/carbonate buffer, pH 8.7, giving rise to a CO₂ concentration of approximately 5%), using an LD-2 Hansatech oxygen electrode + fluorometer. Leaf discs were illuminated with an LS-2 Hansatech light source. Fluorescence was induced with red light at 650 nm and was detected at 760 nm. Control discs were kept in the dark at 25°C for the same periods.

When the methods of injury assessment were to be compared, whole plants were frozen and the leaf discs collected for measurements of photosynthetic oxygen evolution and fluorescence. Tissue necrosis was expressed in terms of mean % injury per leaf per plant.

Results

The effects of the temperature treatments on photosynthetic capacity (P_N) of the eucalypt leaf discs are shown in Fig. 1. Values of P_N measured either at 700 or 2500 $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ are slightly increased in treatments of 2 h at -2 and -3°C, whereas in treatments at -4 and

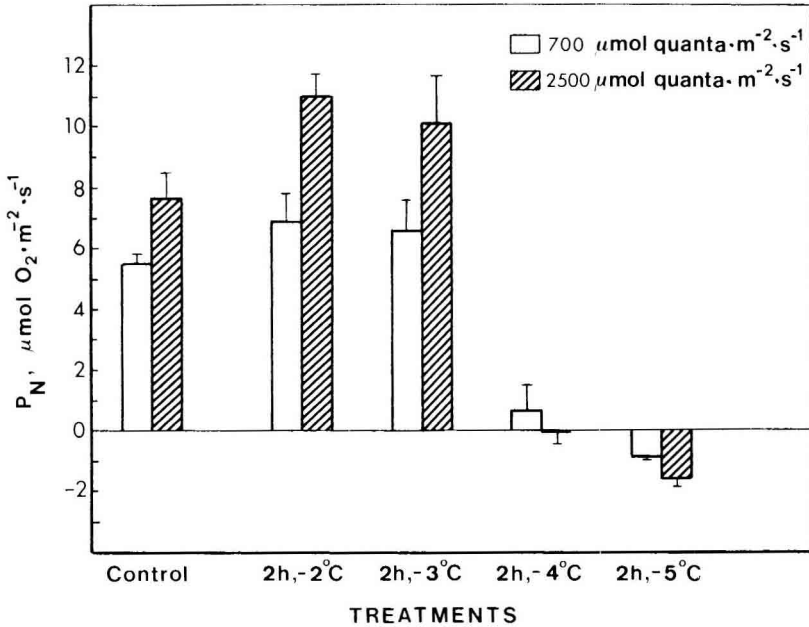


Fig. 1. Net photosynthesis in mature leaf discs of eucalypts subjected to different temperature treatments. Measurements with 700 and 2500 $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, at 25°C and saturating CO_2 with an oxygen electrode. Mean values \pm SE of 3 or 4 replicates.

-5°C photosynthesis dropped to values close to or below zero.

Measurements in young and old leaves confirm the results obtained with mature leaves (Fig. 2), with no significant differences in sensitivity among leaf ages.

The ratio of fluorescence decrease to steady-state fluorescence, termed R_{fd} or $(F_p - F_T)/F_T$ according to Lichtenthaler and Rinderle (1988), showed some decline in eucalypt leaf discs after 2 h of treatment at -4°C (Fig. 3). Values of about 50% of the controls were recorded when the treatment was at -5°C.

Comparing the percentage of tissue necrosis, measured 1 wk after the treatment, with R_{fd} values gave rise to a linear regression $Y = 3.527 - 0.026 x$, ($R^2 =$

0.71, $P < 0.001$), with R_{fd} being the dependent variable.

Discussion and Conclusions

Two hours of exposure at -4 and -5°C reduced the photosynthetic capacity by 90 and 130%, respectively, in comparison to the control. Inhibitory effects of low temperatures were consistently more pronounced at 2500 than at 750 $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This seems to indicate that photoinhibition took place at high photon flux density in low temperature-stressed leaves.

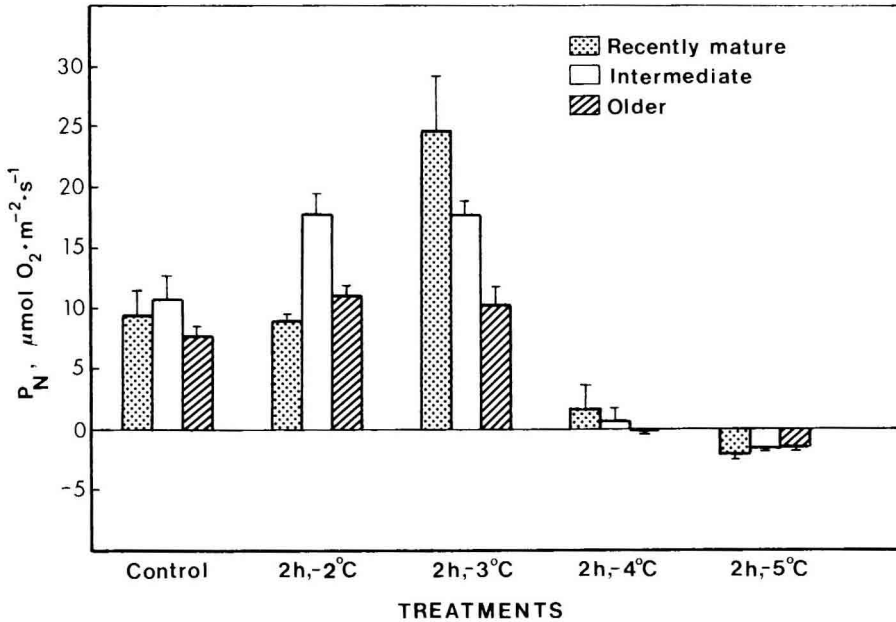


Fig. 2. Effects of leaf age on photosynthetic response to treatments at low temperature, measured at 25°C and saturating conditions of CO_2 and light with an oxygen electrode in leaf discs of eucalypts. Mean values \pm SE of 3 or 4 replicates.

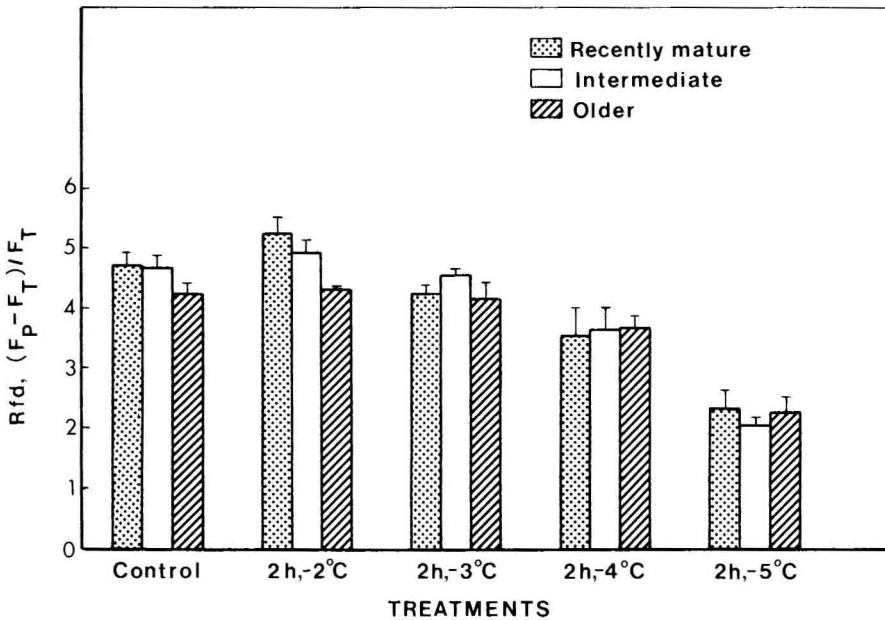


Fig. 3. Ratio of fluorescence decline of chlorophyll *a* ($R_{id} = F_p - F_T/F_T$) in eucalypt leaf discs of different ages, subjected to low temperature treatments. Means \pm SE of 3 or 4 replicates are given.

The increasing delay of the fluorescence decrease kinetics to reach the final steady-state fluorescence, which is well expressed by the decrease in F_{fd} values when treatment temperatures declined, is in accordance with results reported by several authors (MacRae *et al.*, 1986; Smillie *et al.*, 1987). Such alterations in the fluorescence kinetics indicate damage of the photosynthetic function. However, we cannot tell whether the disturbances occurred during the induction period, the state I–state II transitions or the photosynthetic CO_2 fixation, since F_{fd} values cover the whole process of photosynthesis (Lichtenthaler and Rinderle, 1988).

These results obtained using leaf discs are in agreement with earlier work with intact *E. globulus* plants. The degree of correlation obtained between the percentage of tissue necrosis and either photosynthetic capacity or fluorescence quenching indicates that both techniques may be

used as reliable screening tests for the detection of low temperature effects on leaves of *E. globulus*.

References

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