

# A photobleaching event at the North Forest Products' Somerset nursery reduces growth of *Eucalyptus globulus* seedlings

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## Abstract

*On 10 July 1998, seven-week-old Eucalyptus globulus seedlings at the North Forest Products' Somerset nursery were not protected with shade cloth covers. There was a mild overnight frost followed by a cold morning with bright sunlight. Within a week, visible leaf yellowing and leaf death (photobleaching) were observed. These effects were restricted to discrete areas of the tray beds, with alternate strips of damaged and undamaged seedlings. The undamaged strips corresponded to areas of the bed shaded from direct sunlight between 1000 h and 1030 h by the steel frames that supported the shade cloth. Chlorophyll fluorescence measurements made on 22 July 1998 demonstrated that damaged seedlings had experienced levels of photoinhibition indicative of photobleaching while shaded seedlings had not. Seedling height of damaged seedlings was significantly less than that of shaded seedlings three months after the photobleaching event occurred.*

## Introduction

Light is essential for plant growth but in excess can cause severe physiological stress. This stress occurs when other factors (e.g. water stress or extreme temperatures) reduce rates of photosynthesis and the leaf is unable to utilise the absorbed light energy. This phenomenon is termed 'photoinhibition' and, if associated with low temperatures, cold-induced photoinhibition. Cold-induced photoinhibition can occur at temperatures above 0°C (Greer and Laing 1988).

Plants have mechanisms that minimise photoinhibition. For example, plants adapted to cold environments have low levels of chlorophyll to reduce light absorption and high levels of carotenoids to dissipate excess light energy (Haldimann 1999). In such ways, plants balance the absorption and utilisation of light. Nevertheless, even in plants that are acclimated to low temperature, their capacity to absorb energy can exceed their capacity to dissipate that energy. The production of free oxygen radicals under these conditions can cause visible tissue damage termed

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'photobleaching'. Eucalypt seedlings are inherently pre-disposed to cold-induced photoinhibition and photobleaching because young foliage has low rates of photosynthesis and low levels of chlorophylls and carotenoids relative to mature eucalypt foliage (Close *et al.* 1999).

During early morning and particularly after an overnight frost, leaf temperatures are low but the leaves may be exposed to bright light. Conditions conducive to cold-induced photoinhibition of regenerating seedlings of *Eucalyptus pauciflora* growing at high altitude sites in New South Wales were associated with mornings following clear night skies that induced radiation frosts (Ball *et al.* 1991). These seedlings were observed to regenerate under the south-western side of a mature tree canopy. In effect, the canopy minimises the intensity and severity of the frost. On the north-eastern side, the ground was exposed to early sunlight (due to the sun's low winter azimuth) while the south-western side was shaded. Thus, seedlings regenerated in the niche where irradiance was minimised early in the morning. In a parallel experiment on a high altitude pasture site in New South Wales, Holly *et al.* (1994) found that 50% shade cloth tree-shelters minimised photoinhibition and maximised growth of seedlings of *Eucalyptus polyanthemos* compared to non-shaded controls.

Non-destructive, rapid measures of cold-induced photoinhibition are possible with an instrument called the chlorophyll fluorometer. The levels of light energy emitted as fluorescence from chlorophylls are in proportion to the levels of light energy utilised in the photosynthetic photosystems. A baseline level of chlorophyll fluorescence ( $F_0$ ) under ambient light conditions is measured. The photosystems are then saturated with light and an induced higher level of fluorescence measured ( $F_m$ ). The proportion of intercepted light utilised in photochemistry (reported as optimal quantum yield) can then be calculated ( $\Delta F/F_m$ ). Light energy not utilised by photosynthesis but dissipated by carotenoids

can also be calculated (reported as non-photochemical quenching [NPQ] and  $F_0$  quenching).

In Tasmania, *Eucalyptus globulus* seed is usually sown in late February and March to allow for good establishment, growth and acclimation of seedlings before winter (I.C. Ravenwood, unpublished). Seed was germinated on 25 May 1998 (unusually late in the season for *E. globulus*) at the Somerset nursery of North Forest Products. Unintentionally, shade cloth covers were not in place to shelter unhardened seven-week-old seedlings. The seedlings were exposed to freezing overnight temperatures and bright sunlight the following morning (10 July 1998). Approximately three days after this event, leaf tissue damage, expressed as visible yellowing, was observed. Only certain areas of the tray bed were affected and it was noted that there were 'strips' of undamaged seedlings. These strips were approximately 4 m apart, the same distance apart as the steel frames that support the shade cloth. These frames cast a moving band of shade onto the tray bed under clear sky conditions. The majority of damage was to expanded leaves rather than growing tips. It was hypothesised that photoinhibition had caused the tissue damage (i.e. photodamage) and not frost, as growing tips are more susceptible to frost damage than mature leaves (Steponkus 1984).

Twelve days after the frost event, chlorophyll fluorescence (optimal quantum yield, NPQ and  $F_0$  quenching) and leaf area with symptoms of photobleaching were estimated in both damaged and undamaged seedling strips. The precise period during which tissue damage occurred was investigated and the effects on subsequent seedling performance assessed.

## Materials and methods

### *Plant material*

Seedlings of *E. globulus* were raised from seedlot G2490 (North Forest Products

improved seed) at the Somerset nursery, 10 km west of Burnie. Seed was sown on 25 May 1998 in 'Lannen 64' plug trays and germinated in a glasshouse at approximately 20°C. After four weeks, the seedlings were moved to an open area sheltered by movable 50% shade cloth covers but otherwise exposed to ambient conditions.

#### *Environment*

Air temperature (°C) at bench height (800 mm) and total incident shortwave radiation ( $W/m^2$ ) on a horizontal surface were measured by a nursery climate control and weather station system ('PlantPlan', Levin, New Zealand). The area of the tray bed shaded by the steel support frames was recorded every half hour from 0800 h until 1500 h.

#### *Chlorophyll fluorescence*

Measurements of steady state fluorescence (optimal quantum yield; NPQ;  $F_o'$  quenching according to Demmig-Adams and Adams 1996) were made at hourly intervals from 0900 h to 1500 h on 22 July 1998 (the first cloud-free day since 10 July) using a PAM-2000 Fluorometer (Walz 1993). A quantum sensor attached to the leaf clamp of the PAM-2000 measured the incident photosynthetically active radiation (PAR,  $\mu mol/m^2/s$ ) perpendicular to the surface of the leaf for each measurement. Fully expanded leaves of three randomly selected seedlings from each of the damaged and undamaged areas (respectively the exposed and shaded area between 1000 h and 1030 h on 10 July) were measured. All leaves selected for measurement were intact. In the damaged treatment, only green areas of the leaves were used where visible yellowing did not exceed 30%.

#### *Photobleaching and height growth*

The percentage leaf area showing symptoms of photobleaching was estimated visually (in increments of 10%) on ten randomly selected seedlings from the damaged and undamaged strips. Seedling height on 60 randomly

selected seedlings within each strip was measured on the 22 September.

#### *Analysis of results*

Differences in chlorophyll fluorescence variables, percentage leaf area damage and height were analysed using the paired *t*-test for means of two populations (Steel and Torrie 1980).

## **Results**

#### *Environment*

Diurnal patterns of total incident radiation (irradiance) on 10 July and 22 July were similar and generally uninterrupted by cloud (Figures 1a, b). Sunlight was first recorded at 0750 h. Irradiance peaked at 250  $W/m^2$  at 1200 h on both days. On 10 July, air temperature was 0.5°C at 0750 h and reached 10°C at 1115 h (Figure 1c). On 22 July, air temperature was 3°C at 0750 h and reached 10°C at 0940 h (Figure 1d).

The shadow caused by the steel support frame coincided with the area of undamaged seedlings between 1000 h and 1030 h. During this period on 10 July, air temperature was 7.8–8.5°C and irradiance 170–200  $W/m^2$ .

Maximum incident PAR at the leaf surfaces varied between 650 and 830  $\mu mol/m^2/s$ . The shadow reduced PAR to between 45–65  $\mu mol/m^2/s$  (Figure 2).

#### *Chlorophyll fluorescence*

Optimal quantum yields were significantly higher ( $P < 0.001$ ) in undamaged compared to damaged seedlings but remained relatively constant within treatments throughout the day (Figure 3a). In the undamaged seedlings, yields varied between 0.4 and 0.6, and in the damaged seedlings between 0.1 and 0.2.

Non-photochemical quenching (NPQ) was significantly higher ( $P < 0.05$ ) in damaged compared to undamaged seedlings

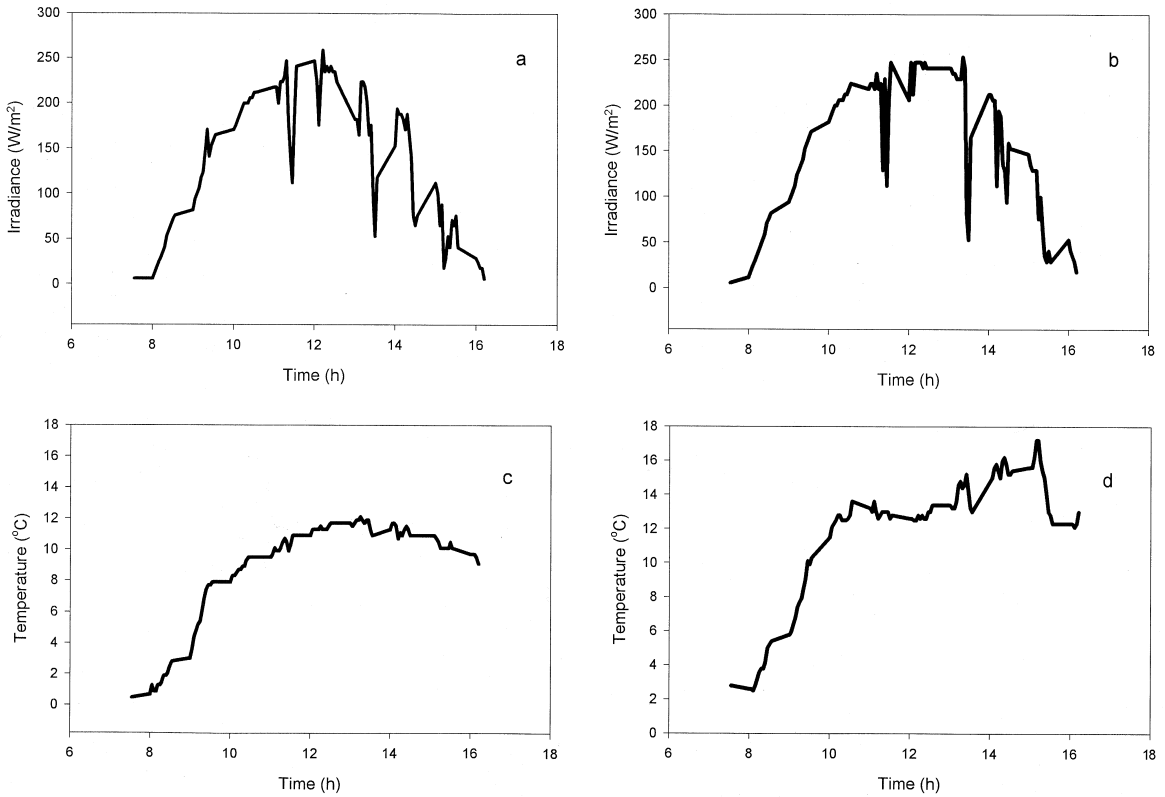


Figure 1. Diurnal changes in total incident shortwave radiation (W/m<sup>2</sup>) at the Somerset nursery on 10 July 1998 (a) and 22 July 1998 (b); and air temperature (°C) profile at tray bed height on 10 July 1998 (c), and 22 July 1998 (d).

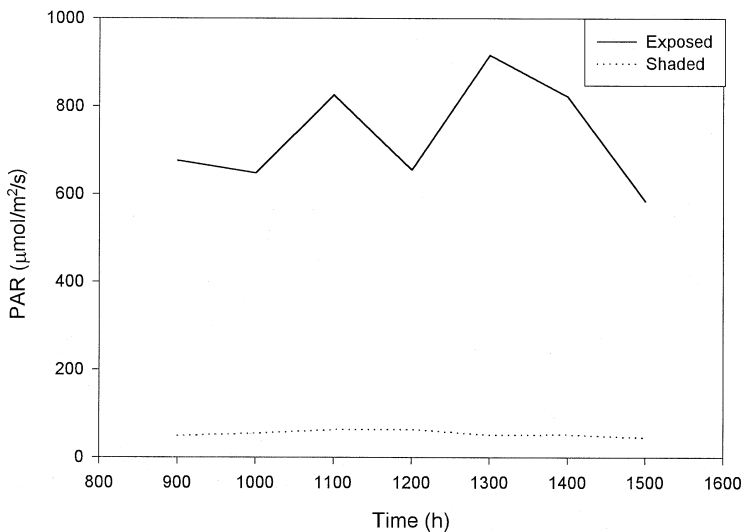


Figure 2. Diurnal changes in photosynthetically active radiation (PAR, μmol/m<sup>2</sup>/s) incident on leaves of *Eucalyptus globulus* seedlings on 22 July 1998. Note 'shade' relates to measurements made of seedlings covered by the moving shadow projected by the steel support beams.

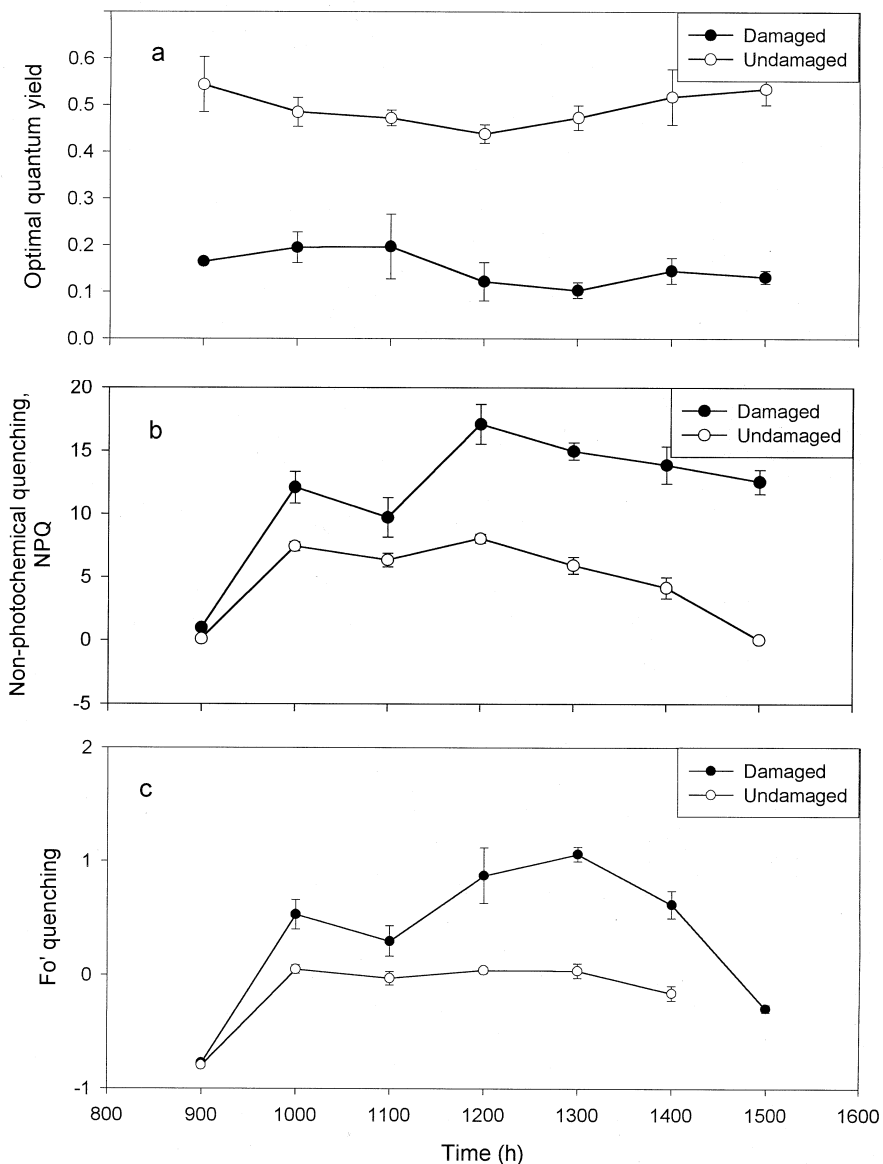


Figure 3. Diurnal changes in optimal quantum yield (a), non-photochemical quenching (NPQ) (b), and  $F_o'$  quenching (c) of *Eucalyptus globulus* leaves on 22 July 1998. All variables are dimensionless.

(Figure 3b). NPQ was near zero in both treatments at 0900 h but significantly higher ( $P = 0.042$ ) in damaged compared to undamaged seedlings. NPQ increased rapidly over the next hour to about 12 and 7 in damaged and undamaged seedlings, respectively. NPQ continued to increase between 1100 h and 1200 h in damaged seedlings to reach a maximum of 16.5. After 1200 h, NPQ decreased gradually to 12 and 0

at 1500 h in damaged and undamaged seedlings, respectively.

$F_o'$  quenching was also significantly higher ( $P < 0.05$ ) in damaged compared to undamaged seedlings at all measurement times except 0900 h.  $F_o'$  quenching of undamaged seedlings was only positive at 1000, 1200 and 1300 h.  $F_o'$  quenching of damaged seedlings increased rapidly from

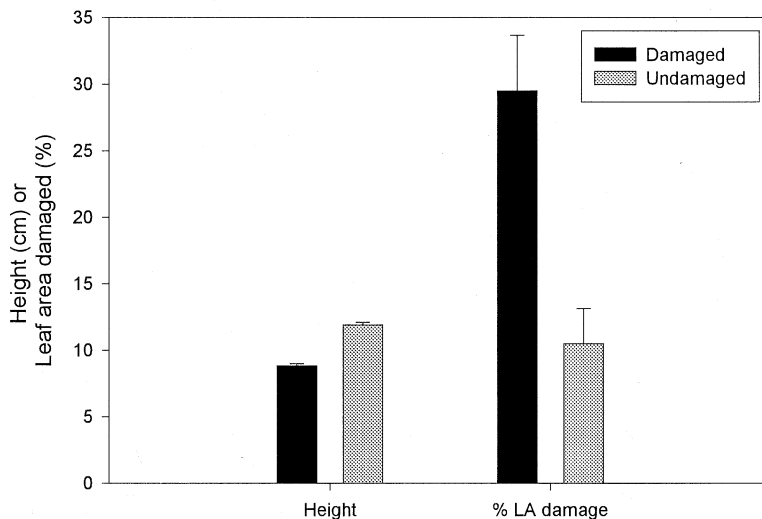


Figure 4. Leaf area (LA) damage estimated on 22 July 1998 and height growth measured on 22 September 1998, for damaged and undamaged seedling strips at the Somerset nursery.

0900 to 1000 h and again from 1100 to 1200 h before returning to negative values by 1500 h.

#### Leaf area damage and seedling height growth

Percentage leaf area photobleached was significantly greater ( $P < 0.01$ ) in damaged (29%) compared to undamaged (11%) seedlings (Figure 4). Two months after the frost event, undamaged seedlings had significantly ( $P < 0.001$ ) greater height (12.5 cm) compared to damaged seedlings (8.0 cm).

#### Discussion

Photoinhibition of unhardened *E. globulus* seedlings in a nursery has been demonstrated. In the absence of shade, levels of photobleaching were sufficient to significantly reduce subsequent height growth.

This conclusion was reached because the photobleaching was in clearly defined strips of seedlings interspersed with undamaged strips. The undamaged strips contained seedlings with significantly less photobleaching (Figure 4). These strips coincided with the areas shaded by the steel support frames between 1000 h and 1030 h on 22 July

(Photo 1). As this was only 12 days after the event, it can be assumed that the strips were shaded at similar times on both days. Thus, only a narrow window of the combined variables of temperature and radiation was required for extensive photodamage to occur. The photobleaching occurred as a consequence of the combined effects of high incident PAR (700–800  $\mu\text{mol}/\text{m}^2/\text{s}$ ) and low temperature (8°C) between 1000 h and 1030 h on 10 July 1998. From the comparison of temperature profiles of 10 July and 22 July (Figure 1), it appears that if the air temperature is less than 10°C, young seedlings may be susceptible to photobleaching at the highest incident PAR levels associated with winter sunlight.

Irradiance increased steadily from 0800 h to 1130 h (Figures 1a, b) at the nursery but PAR at the leaf surface was consistently high, between 650 and 850  $\mu\text{mol}/\text{m}^2/\text{s}$  (Figure 2). Leaves oriented towards the sun in the morning maximised their interception of incident light and had higher levels of photobleaching than other leaves. There was also evidence of some photobleaching (10%) of such leaves in the areas shaded between 1000 h and 1030 h indicating that the period of physiological stress had extended beyond this time period.



Photo 1. 'Strips' of shaded and non-shaded areas at approximately 1000 h on 22 July 1998. Note the shadows cast by the steel support frames.

The weather station records indicate that air temperatures prevailing between 11 July and 22 July were considerably higher than those on 10 July. The differences between treatments measured on 22 July were therefore a manifestation of the severe photoinhibitory conditions present on 10 July. Cold-induced photoinhibition is more commonly associated with cooler climates (Öquist 1987; Ögren and Sjöström 1990). If severe, it is associated with the loss of chlorophyll and photobleaching (Oberhuber and Bauer 1991; Haldimann 1999). Visible yellowing of the leaves of the *E. globulus* seedlings was indicative of severe photobleaching. However, areas of leaves that showed no visible damage on 22 July may have been under physiological stress since the 10 July. The low optimal quantum yields in the damaged compared to the undamaged seedlings indicated that this was the case. The low yields led to increased levels of excess light energy and a greater requirement for NPQ and Fo' quenching which was greater for damaged than

undamaged seedlings (Figures 3b, c). In general, the diurnal profiles of NPQ and Fo' paralleled the rise and fall of incident irradiance: the lower levels at 1100 h coincided with a short period of cloud cover. The rapid increase of NPQ and Fo' between 0900 h and 1000 h is consistent with the steep rise in available light that occurred during the period and with seedlings being most vulnerable to photoinhibition between 1000 h and 1030 h under the conditions prevailing on 10 July 1998. Yield, NPQ and Fo' quenching of undamaged seedlings were similar to those found on other healthy plants (Bilger *et al.* 1995; Massacci *et al.* 1995; Barker *et al.* 1998). In contrast, NPQ of the damaged seedlings was high compared to levels reported for cold-induced photoinhibition of *E. nitens* seedlings (Warren *et al.* 1998). However, Warren *et al.* (1998) only exposed seedlings to low temperature for around 55 minutes. It has been demonstrated that longer periods of exposure to low temperature increase the incidence and extent of photobleaching (Ögren and Sjöström 1990).

Immature leaves have been found to have lower chlorophyll levels, lower rates of photosynthesis and higher levels of photoinhibition compared to mature foliage (Krause *et al.* 1995; Dodd *et al.* 1998). Young seedlings of *E. globulus* and *E. nitens* planted into the field have similar characteristics as well as lower levels of carotenoids (relative to mature foliage) to dissipate excess energy (Close *et al.* 1999). The very high levels of NPQ observed in the *E. globulus* seedlings in the nursery are consistent with physiologically immature leaves that are highly susceptible to photoinhibition.

When mechanisms for energy dissipation are superseded, reactive oxygen species are produced (Niyogi 1999). This gives rise to photobleaching (Asada 1992). Twenty-nine per cent of leaf area was visibly damaged in the seedlings exposed to high irradiance on 10 July and the fluorescence variables indicated that the undamaged foliage was under physiological stress on 22 July. Thus, photoinhibition was responsible for the significant reduction in height growth of the damaged compared to undamaged seedlings (expressed two months after the photodamage event). Quantitative reductions in growth following cold-induced

photoinhibition have been reported previously for *Brassica napus* (Farage and Long 1991) and *Eucalyptus polyanthemos* (Holly *et al.* 1994).

## Conclusions

Young *E. globulus* seedlings were found to be inherently susceptible to cold-induced photoinhibition. A severe photoinhibitory event can lead to photobleaching that significantly reduces seedling growth. The results indicated that young seedlings in a nursery should be protected by shade cloth in the winter at least until the air temperature is greater than 10°C, particularly on days after low overnight temperatures followed by clear skies and bright sunlight.

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