6.5 Do quantum yield and dynamic photoinhibition vary in leaves and bracts grown under different light environments?

6.5.1 The principles of fluorescence measurement (continued)

While measurements of quantum yield before dawn are useful in determining the presence or absence of chronic photoinhibition, measurements of fluorescence in illuminated leaves or bracts can describe effective quantum yield over a day, as well as non-photochemical quenching (dynamic photoinhibition), as described in 6.1.3. The quantum yield during illumination (hereafter referred to as effective quantum yield) can be calculated using equation 6.7.

Equation 6.7 Quantum yield during illumination (effective quantum yield)

$$(Fm'-Ft)/Fm' = Fv'/Fm' \text{ (alternatively, denoted by } \Delta F/Fm')$$

Where Ft = steady-state fluorescence just before light flash
Fm' = maximal fluorescence (all PSII centres closed) in a light-adapted state

Another parameter measuring photochemical quenching (qP, Equation 6.8) can be calculated with Ft and Fm' as described above and measurement of Fo', which is fluorescence in the absence of actinic (photosynthetic) light measured with a simultaneous flash of far-red light to open all PSII reaction centres (van Kooten and Snel, 1990). qP indicates the proportion of open PSII centres, so a change in qP is due to the closure of reaction centres caused by saturation of photosynthesis (Maxwell and Johnson, 2000).

Equation 6.8 Photochemical quenching

$$qP = (Fm'-Ft)/(Fm'-Fo')$$

Non-photochemical quenching (NPQ, Equation 6.9) is linearly related to heat dissipation and can be calculated from the above fluorescence parameters without having to measure the minimum fluorescence yield after illumination (Fo') (Maxwell and Johnson, 2000), which can be difficult in the field. Values of NPQ vary between 0 and infinity, although 0.5-3.5 is usual for plants at saturating light intensities (Maxwell and Johnson, 2000). Another parameter, qN, has also been used in the past to quantify non-photochemical quenching (Equation 6.10). However, NPQ and the related parameter qN measure changes in dissipation relative to the dark-adapted state, so if dark-adapted values of Fv/Fm are markedly different, comparison of NPQ or qN can be ambiguous (Maxwell and Johnson, 2000).

Equation 6.9 Non-photochemical quenching

$$NPQ = (Fm-Fm')/Fm'$$

Equation 6.10 Non-photochemical quenching

$$qN = 1-(Fm'-Fo')/(Fm-Fo)$$

In experiments 6.5.2 to 6.5.6, the effective quantum yield and capacity for non-photochemical quenching in sun and shade grown waratah bracts and leaves will be investigated, during daylight hours and under artificial illumination. Initially, the aim of the experiment was to determine whether waratah leaves and bracts differ with respect to yield and non-photochemical quenching during daylight hours, and whether this is influenced by shade or sun treatments.

6.5.2 Diurnal measurements of effective quantum yield and NPQ

6.5.2.1 Method

Plants used for measurement were the same as those described in Experiment 5.3.4, grown at Mount Annan in 2003 and exposed to full sun or shaded early or late during bud development. Leaf and bract areas to be measured were circled with a black permanent marker and numbered, in order to return to the same area for subsequent measurements throughout the day. Measurements of quantum yield were made before dawn and measurements of effective quantum yield were made throughout the day without dark adaptation. The intensity of measuring and actinic light and saturating pulses was varied at the TB stage, therefore only yield measurements (change in F/Fm', i.e. a ratio that is independent of light intensity) are presented. At the MF stage, light intensities were kept constant throughout the day, therefore NPQ was calculated in relation to predawn measurements. Missing values during the day are the result of equipment failure (low battery of fluorometer or laptop at TB stage) or outer bract senescence and damage to the measured flower (MF stage).

Data were analysed using the Linear Mixed model option of the REML procedure. *Time* (hours) was introduced as a fixed effect, as well as a random effect (*Plant/Tissue/Time*) with correlated error structures for *Tissue* (diagonal) and *Time* (AR1). The first order autoregressive structure (AR1) models the random error as linearly dependent on the error associated with previous time points.

6.5.2.2 Results

At the tight bud (TB) stage, effective quantum yield of leaves and outer bracts was compared in 'Fire and Brimstone' and 'Olympic Flame' cultivars grown in the sun or

shaded early or late. Significant interactions were found between tissue type and time of day ($P < 0.001$) and between cultivar, treatment and time of day ($P = 0.020$). At the mature flower (MF) stage, effective quantum yield of leaves and inner bracts was compared in 'Fire and Brimstone' and 'Olympic Flame' cultivars grown in the sun or shaded late (early shaded plants matured prior to measurement). Significant interactions were again found between tissue type and time of day ($P < 0.001$) and between cultivar, treatment and time of day ($P = 0.024$).

At both the TB and MF stages, significant decreases in effective quantum yield were apparent for leaf and bract tissue within two hours of dawn, although the effective quantum yield of leaves was higher than that of bracts throughout the day (Figures 6.26 and 6.29).

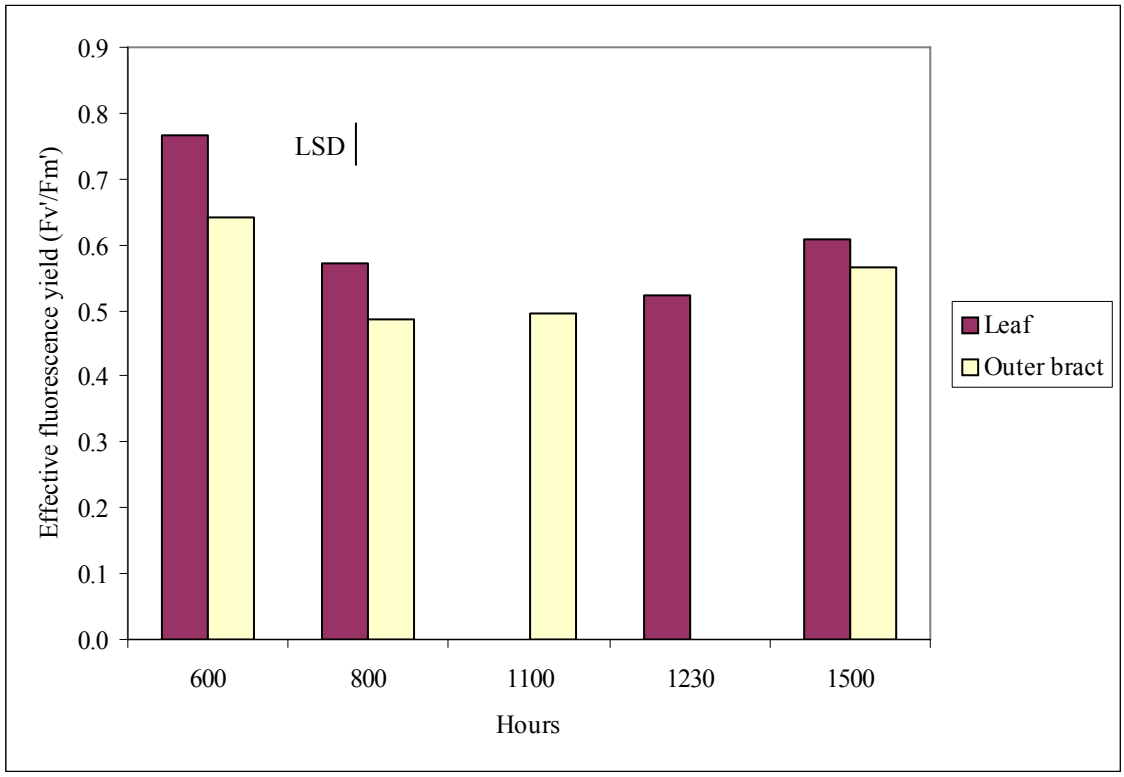

Figure 6.26: Effective fluorescence yield (Fv'/Fm') during the day (0600 to 1500 hrs) for leaves and outer bracts pooled across cultivars 'Fire and Brimstone' and 'Olympic Flame' at the tight bud (TB) stage. LSD = 0.048. n = 4 plants of each cultivar in each light treatment.

Significant decreases in effective quantum yield were observed for both cultivars within two hrs of dawn, particularly in full sun environments at both TB (Figures 6.27 and 6.28) and MF stages (Figure 6.30 and 6.31). Early and late shaded tissues had a higher effective quantum yield throughout the day compared to sun tissues. Significant recovery was made between the middle of the day and late afternoon (1500 or 1600 hours) for both cultivars at both stages. However, differences in effective quantum yield recovery depended on the stage of flower development. Effective quantum yield of 'Olympic Flame' at the TB stage recovered to predawn levels in early or late shaded tissues but not sun-exposed tissues (Figure 6.27), while 'Fire and Brimstone' effective quantum yield at this stage did not recover to predawn levels with any treatment (Figure 6.28). At the MF stage, effective quantum yield of both cultivars recovered to predawn levels in the late shade treatment but not in the sun (Figure 6.30 and 6.31).

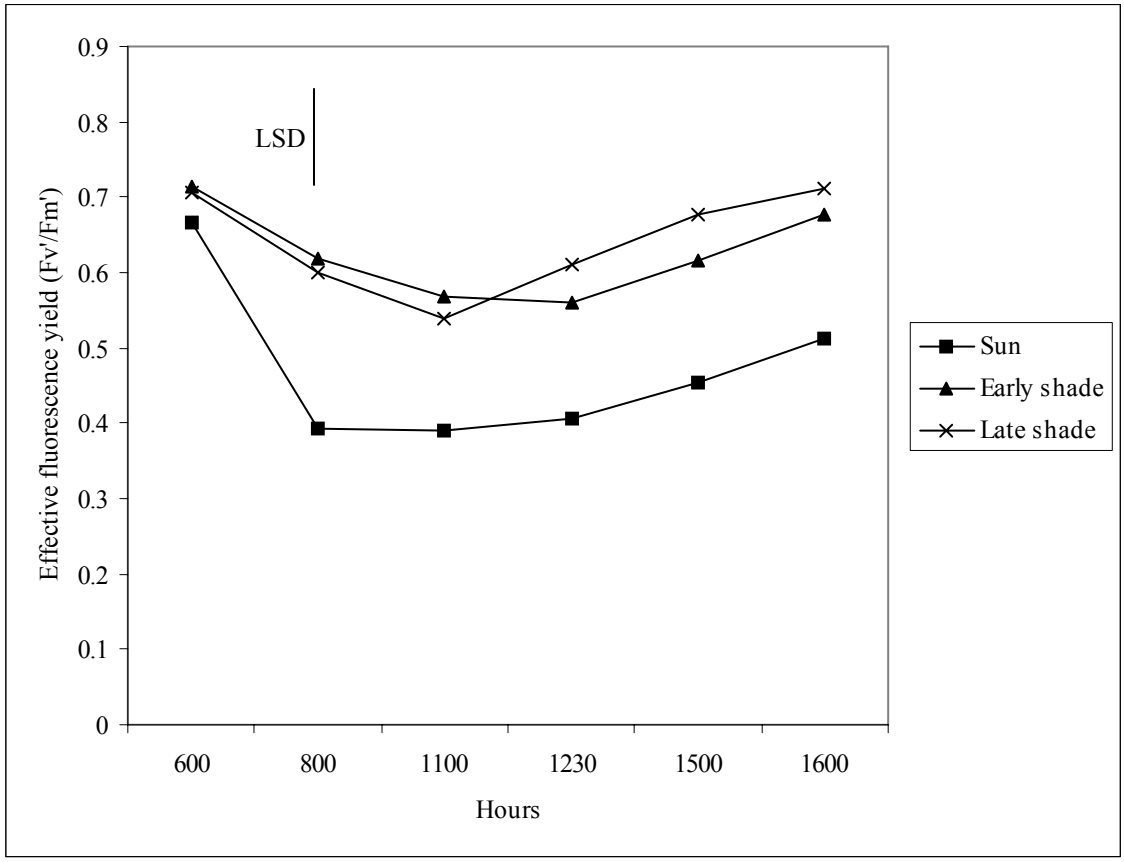

Figure 6.27: Effective fluorescence yield (Fv'/Fm') of sun, early and late shade treatments in 'Olympic Flame' tissues during the day, pooled across leaf and outer bract tissue at the tight bud (TB) stage. LSD = 0.104. n = 4 plants in each light treatment.

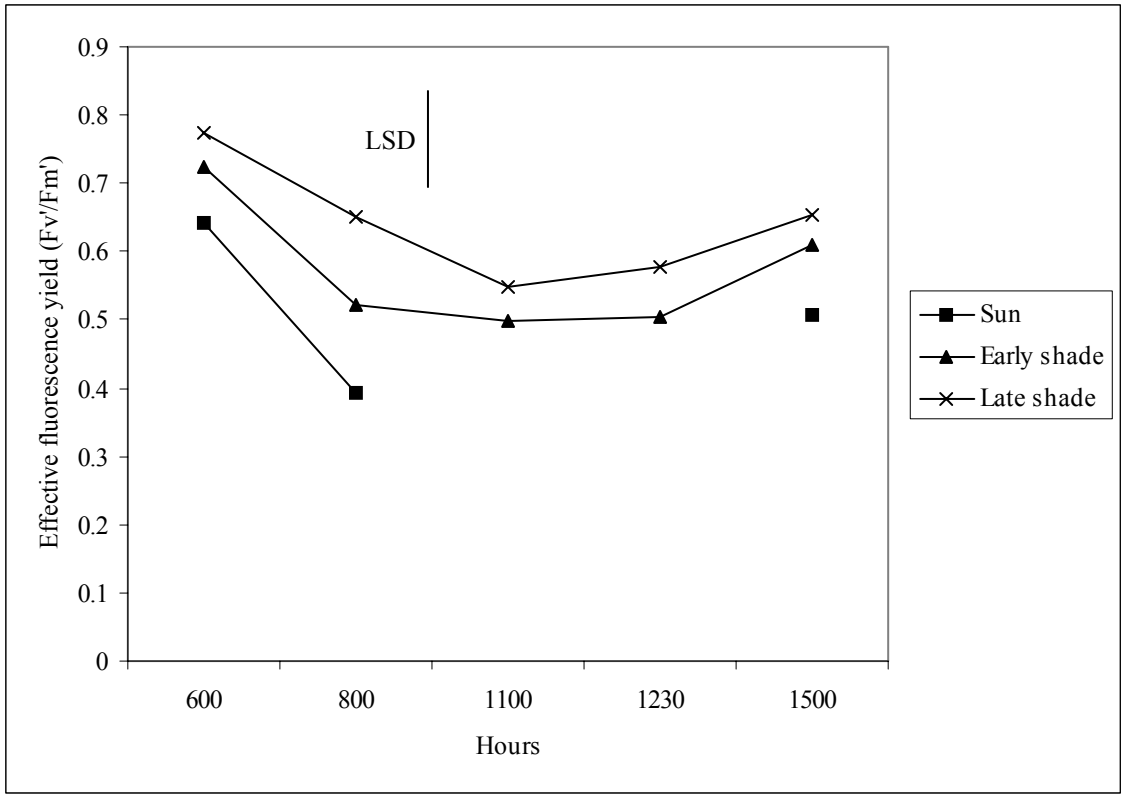

Figure 6.28: Effective fluorescence yield (Fv'/Fm') of sun, early and late shade treatments in 'Fire and Brimstone' during the day, pooled across leaf and outer bract tissue at the tight bud (TB) stage. LSD = 0.104. n = 4 plants in each light treatment.

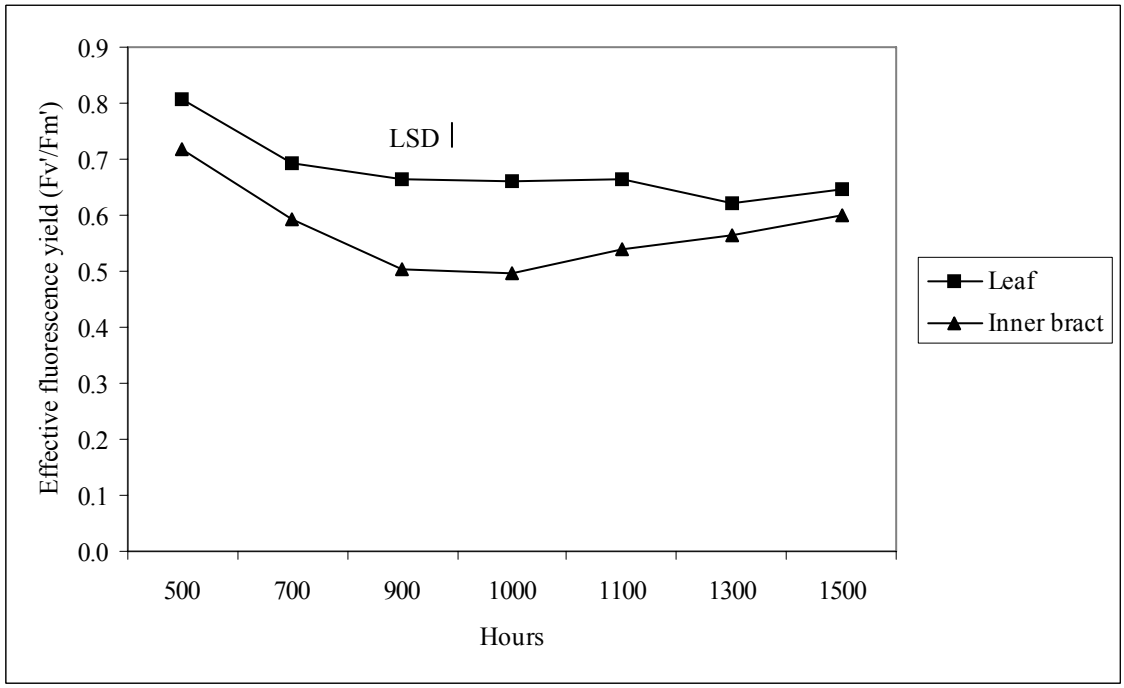

Figure 6.29: Effective fluorescence yield (Fv'/Fm') during the day for leaves and inner bracts, pooled across light treatments (sun, early and late shade) and cultivars 'Fire and Brimstone' and 'Olympic Flame' at the mature flower (MF) stage. LSD = 0.055. n = 2 plants of each cultivar in full sun and 3 plants in late shade treatments.

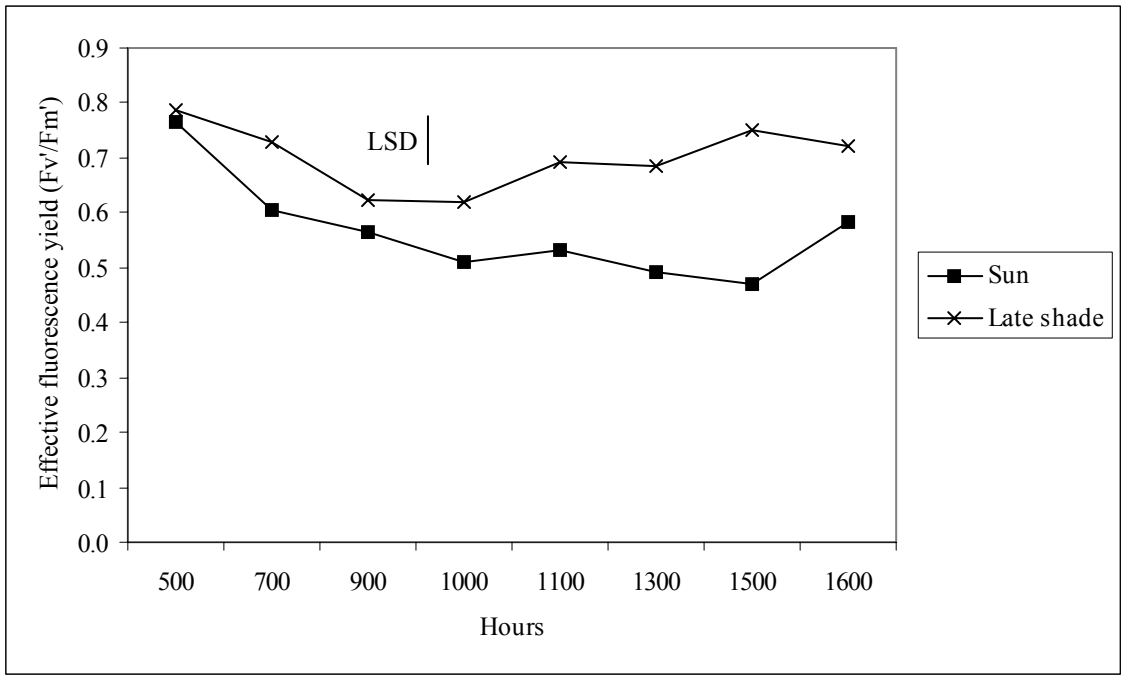

Figure 6.30: Effective fluorescence yield (Fv'/Fm') of sun exposed and late shaded 'Olympic Flame' tissues during the day, pooled across leaf and inner bract tissue at the mature flower (MF) stage. LSD = 0.100. n = 2 plants of each cultivar in full sun and 3 plants in late shade treatments.

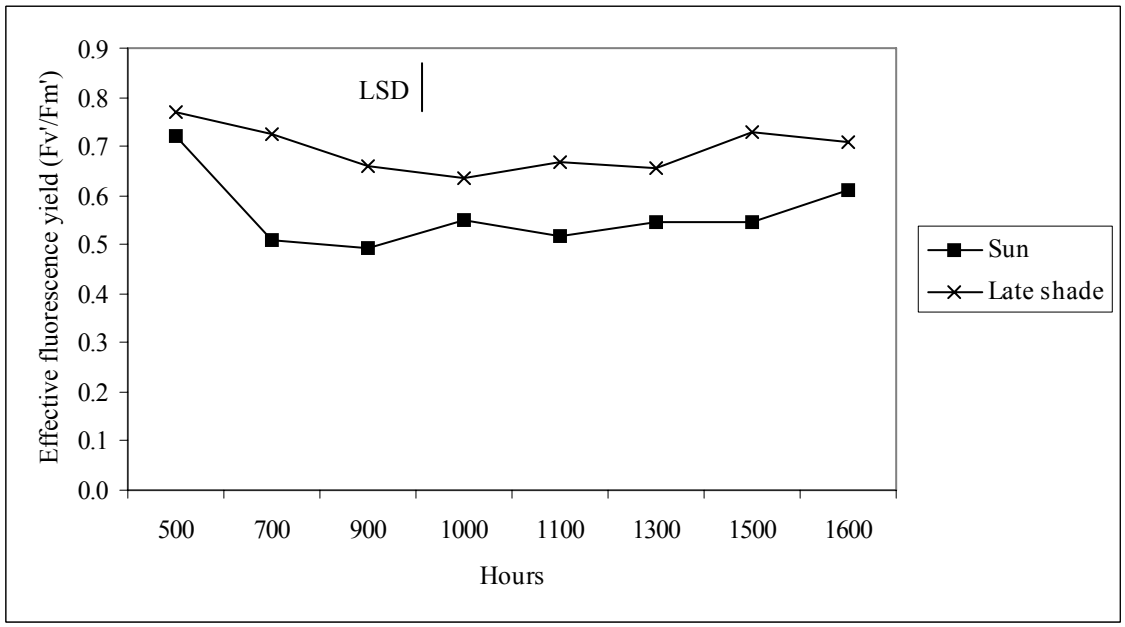

Figure 6.31: Effective fluorescence yield (Fv'/Fm') of sun exposed and late shaded 'Fire and Brimstone' tissues during the day, pooled across leaf and inner bract tissue at the mature flower (MF) stage. LSD = 0.100. n = 2 plants of each cultivar in full sun and 3 plants in late shade treatments.

Analysis of non-photochemical quenching (NPQ) at flower maturity revealed significant interactions between tissue type, cultivar and time of day ($P < 0.001$). Leaves had lower NPQ (Figure 6.32a) than inner bracts in the sun (Figure 6.32b) or outer bracts (Figure 6.32c). NPQ was also higher in sun exposed than shaded tissues, although differences are only significant for outer bracts at 700 and 900 hours. In bracts, NPQ increased significantly between dawn and 700 (outer bracts) or 1000 (inner bracts). Effective quantum yield during the day was higher in leaves than bracts (Figure 6.26 and 6.29), due to higher NPQ in bracts (compare Figure 6.32a to 6.32b,c).

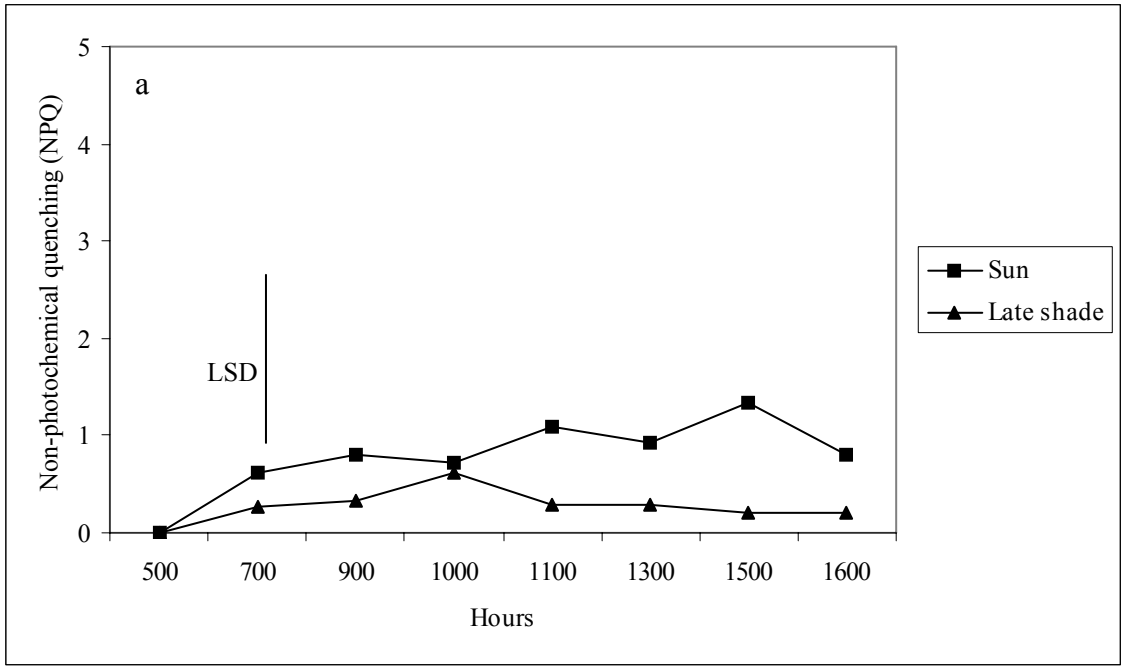

Figure 6.32a: Non-photochemical quenching (NPQ) of sun and late shade waratah leaves during the day at flower maturity, pooled across 'Fire and Brimstone' and 'Olympic Flame' cultivars.

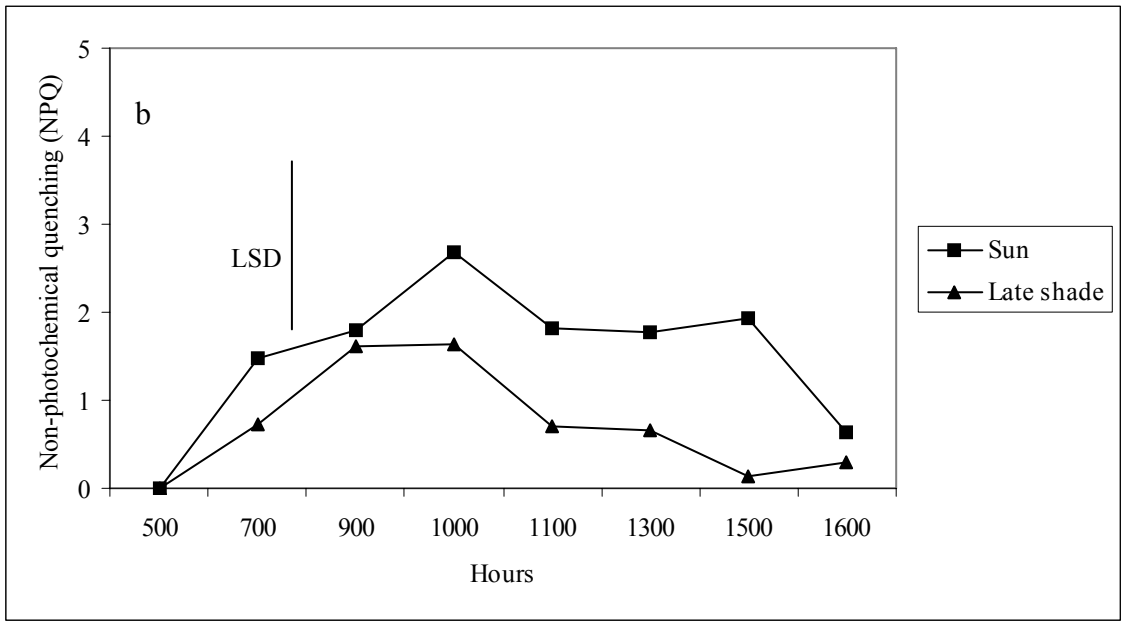

Figure 6.32b: Non-photochemical quenching (NPQ) of sun and late shade inner waratah bracts during the day at flower maturity, pooled across 'Fire and Brimstone' and 'Olympic Flame' cultivars.

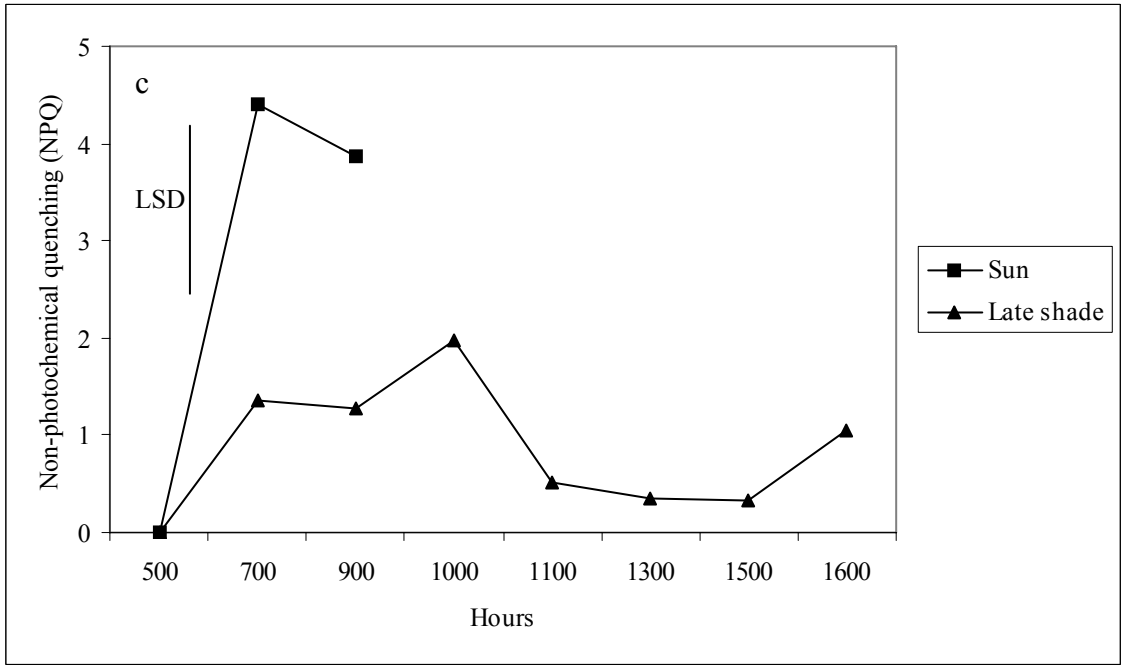

Figure 6.32c: Non-photochemical quenching (NPQ) of sun and late shade outer waratah bracts during the day at flower maturity, pooled across 'Fire and Brimstone' and 'Olympic Flame' cultivars. LSD = 1.9314. n = 2 plants of each cultivar in full sun and 3 plants in late shade treatments.