

Chapter 6

Light environment (shade/sun) effects on photoinhibition

6.1 Introduction

6.1.1 Waratah bracts may be susceptible to light damage (photoinhibition)

Browning of waratah bracts may be due to excess light, leading to damage of the photosynthetic reaction centre and development of chlorotic and necrotic lesions. Excess photon flux density (PFD) is defined as the difference between light absorbed by the plant and light energy that can be processed by the electron transport chain, through photosystems II and I, during photosynthesis (Figure 6.4; Foyer *et al.*, 1994; Demmig-Adams *et al.*, 1997). Excess PFD can be experienced as a result of chlorophyll loss, an increase in light intensity, or a reduction in photosynthetic capacity (photoinhibition, either dynamic or chronic). Chronic photoinhibition indicates inactivation of the reaction centres of photosystem II (PSII) (Osmond, 1994), leading to oxidation reactions, chlorosis and necrosis. Dynamic photoinhibition indicates protective down-regulation of photosynthesis by diversion of electrons away from PSII (Osmond, 1994).

Photoinhibition causes browning in other species, such as orchids (He *et al.*, 1996) and *Illicium* species (star anise, Olsen *et al.*, 2002). Shading also reduces photoinhibition and subsequent light damage in leaves and flowers of other species. Such species include *Heliconia* (He *et al.*, 1996), orchids (He *et al.*, 1998), mangoes (Nir *et al.*, 1997), *Schefflera* (Schiefthaler *et al.*, 1999), Eucalypts (Close *et al.*, 2001) and Chilean guava (Pastenes *et al.*, 2003). Hence, photoinhibition warrants investigation as a cause of bract browning in waratahs.

Waratahs are considered a shade-adapted species, growing under eucalypt trees in their natural habitat (Offord, 1996; Chapter 6). Shade plants and those grown in low light have adaptations to maximise light absorption. For example, the antenna within the light harvesting complexes (LHCII) are larger in shade plants, making the reaction centres more vulnerable to excess photons due to more effective exciton transfer (Osmond, 1994). Thus, at any given irradiance shade plants tend to absorb more light in excess of photosynthesis than sun-adapted plants and then need to dissipate more excess light, leaving them susceptible to chronic photoinhibition (Osmond, 1994). As an example, exposing shade-grown tropical plants to full sunlight caused an abrupt decrease in the quantum yield of photosynthesis (measured as the ratio of variable to maximal fluorescence yield, F_v/F_m) (Lovell *et al.*, 1994). This decrease in quantum yield is characteristic of photoinhibition, as is leaf bleaching and senescence.

The aim of the studies in this chapter is to determine whether photoinhibition occurs in waratah bracts, particularly in the sun, and whether the reduction in browning of waratah bracts under shade (Chapter 5) is due to a reduction in chronic photoinhibition. Chlorophyll fluorescence measurements were used to monitor photoinhibition in waratah leaves and bracts throughout floral development.

6.1.2 What happens to absorbed light? What is photoinhibition?

Light harvesting is the process of light absorption and subsequent energy transfer to the photosynthetic reaction centre (Horton *et al.*, 1996). The presence of anthocyanins (Chapter 8), leaf and chloroplast movement and waxy cuticles can diminish the light falling on PSII (Anderson and Chow, 2002). Absorbed light energy elevates electrons to higher unstable energy states, which then return rapidly to their ground level by

releasing energy. Energy is released (1) by transfer to a neighbouring chlorophyll molecule and ultimately into the reaction centre chlorophyll P680 for use in photosynthesis (including photorespiration and the Mehler reaction), (2) as heat (non-photochemical quenching) or (3) as fluorescence (Salisbury and Ross, 1992). These pathways are in competition (Lichtenthaler, 1996), such that:

Equation 6.1 (see also Figure 6.3):

$$E_{\text{absorbed}} = E_{\text{photochemistry}} + E_{\text{heat (non-photochemical quenching)}} + E_{\text{fluorescence}}$$

Plants are often exposed to conditions that limit photosynthetic electron transport (Alscher *et al.*, 1997). For example, limited water availability or low temperatures can lead to stomatal closure, and a decrease in CO₂ available for carbon assimilation (Nilsen and Orcutt, 1996; Alscher *et al.*, 1997; Pastenes *et al.*, 2003). If light intensity remains high while the plant is stressed, carbon assimilation decreases or stops while light absorption continues (Alscher *et al.*, 1997; Tsonev *et al.*, 2003). This can lead to photoinhibition, described below, or photorespiration (the Mehler-ascorbate peroxidase reaction), which also has a photoprotective role (Alscher *et al.*, 1997).

Short-term dynamic photoinhibition is a ‘readily reversible’ protective down-regulation mechanism, dissipating excess photons via non-photochemical quenching (Demmig-Adams and Adams, 1992; Osmond, 1994; Osmond and Grace, 1995). Non-photochemical quenching (NPQ, also known as thermal or non-radiative dissipation) involves the transfer of light energy absorbed by chlorophyll to xanthophyll cycle carotenoids, thus quenching the excited state of chlorophyll (Taiz and Zeiger, 1998). The xanthophyll cycle involves conversion of the carotenoid violaxanthin to zeaxanthin, via the intermediate antheraxanthin, at high light intensities (Figure 6.1, Hall and Rao, 1999). Therefore, a major component of non-photochemical quenching relies on

zeaxanthin formation (Hall and Rao, 1999). The excited state of carotenoid, unlike chlorophyll, has insufficient energy to form highly reactive singlet oxygen, so it decays to its ground state while losing energy as heat. Increases in NPQ are mirrored by decreases in the efficiency of PSII, as removal of excess excitation energy before it reaches the PSII reaction centres decreases the efficiency of the conversion of absorbed light into photochemistry.

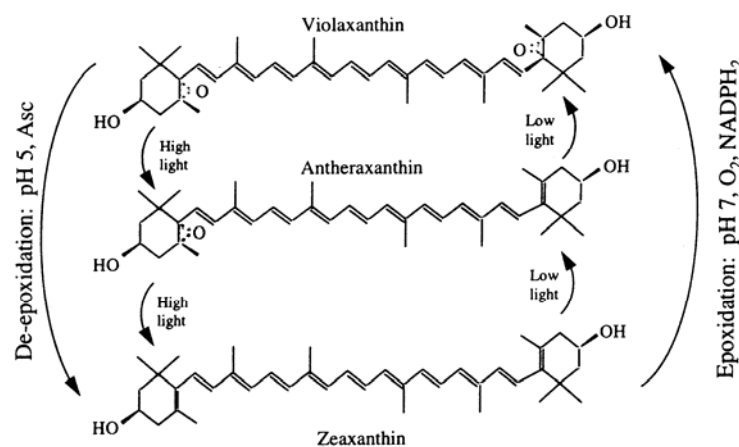


Figure 6.1: Changes in light intensity result in the carotenoids of the xanthophyll cycle (violaxanthin, antheraxanthin and zeaxanthin), facilitating non-photochemical quenching (Hall and Rao, 1999).

Chronic photoinhibition is the light-induced ‘slowly reversible loss of PSII reaction centre function’, lessening the ability of PSII to evolve oxygen (Chow *et al.*, 2002). This photoinactivation of PSII occurs when electron flow is blocked and molecules designed for oxidation/reduction reactions become reduced. PSII reaction centres are inactivated after 10^6 - 10^7 turnovers, and inactivation is therefore dependent on light dosage rather than rate of photon absorption (Anderson *et al.*, 1997). Thus, inactivation of PSII can occur at all light levels, although up to saturating light levels the turnover of the D1 protein of the PSII reaction centre is fast enough to prevent net loss of PSII function (Anderson *et al.*, 1997). Restoration of PSII function requires partial

disassembly, degradation of the photodamaged D1 protein, biosynthesis and reinsertion of a new D1 protein and reassembly of the PSII complex (Melis, 1999).

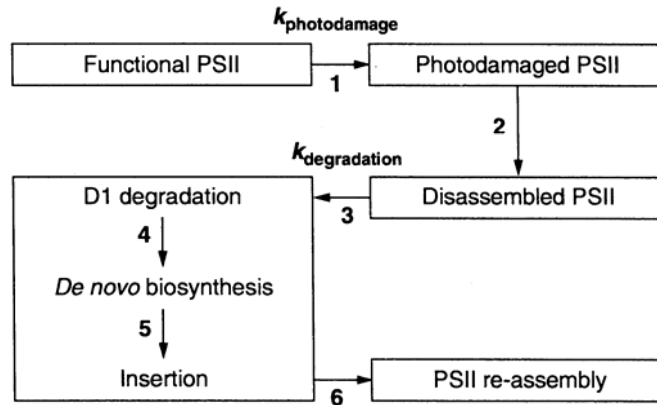


Figure 6.2: Sequence of events in PSII damage, disassembly and repair (Melis, 1999).

The rate-limiting step of PSII repair is the rate of D1 protein degradation and replacement (Melis, 1999). Thus, permanent damage occurs when the rate of D1 repair fails to keep pace with the rate of damage, or when photoinactivation is accelerated under high light, especially in combination with other stressors (Long *et al.*, 1994; Anderson and Chow, 2002). Loss of function of PSII is one of the most sensitive indicators of stress in plants (Ball *et al.*, 1994) and is observed as a decline in quantum yield and the light-saturated rate of photosynthesis.

Degradation of PSII and subsequent damage to other cellular components such as lipids, proteins and pigments are mediated by the action of free radicals and reactive oxygen species such as the reaction centre chlorophyll P680⁺, singlet oxygen, superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radicals (HO[•]), as described in the literature review of Chapter 9.

6.1.3 Factors other than high light increase photoinhibition and necrosis

Stressors in addition to high light such as high or low temperatures, water stress and nutritional disorders can slow down photosynthesis (Koeniger *et al.*, 1998) and increase photoinhibition (Powles, 1984). These stressors may possibly increase bract browning of waratahs, if the cause of browning is photoinhibition.

6.1.3.1 Temperature

Both high and low temperatures combined with light exposure can cause photoinhibition (Powles, 1984), although the mechanism for damage at each extreme differs. Photoinhibition at low temperatures is dependent on oxygen and may affect PSI rather than PSII (Powles, 1984; Sonoike, 1996), while high temperatures affect the function and organisation of the photosynthetic apparatus, particularly PSII and electron transport in chloroplasts (Pastenes *et al.*, 2003). High and low temperatures also induce stomatal closure, reducing the availability of CO₂ for photosynthesis and therefore increasing over-excitation of PSII (Sonoike, 1998; Pastenes *et al.*, 2003). Both high and low temperature stress is associated with increased leakiness of membranes (Powles, 1984; Sonoike, 1996). Responses to high temperatures in other species resulting in chlorosis and necrosis will be considered first in this discussion, followed by low temperatures.

Waratahs are likely to experience high light and temperature combinations frequently during flowering, increasing their susceptibility to photoinhibition. *Dendrobium* orchids, like waratahs, have floral parts more susceptible to photoinhibition, bleaching and wilting than leaves, particularly at high temperatures. At low light intensity, decreases in quantum yield (F_v/F_m) and chlorophyll content of *Dendrobium* leaves and

flowers were observed at 38°C but not 28°C (He *et al.*, 1998). At high light intensity, there was a significant decrease in quantum yield and chlorophyll content at both temperatures, although *Dendrobium* leaves and flowers were more susceptible to photoinhibition at 38°C. Leaf and petal temperatures of *Dendrobium* orchids were similar in the shade, but in intermediate and full sun, petal temperatures were higher than leaves, contributing to photoinhibition (He *et al.*, 1998). Similarly, shade significantly reduced the temperature of Chilean guava leaves compared to leaves on plants grown in full sun (Pastenes *et al.*, 2003).

Stressed *Heteromeles* plants had a maximum leaf temperature up to 10°C higher than air temperatures, exceeding 50°C on the hottest days. High light and high temperatures caused the greatest reduction in quantum yield within the first ten minutes of exposure (Valladares and Pearcy, 1997). Low light intensities prevented damage resulting from high temperatures (Valladares and Pearcy, 1997). Sun and shade leaves of the tropical forest species *Alocasia macrorrhiza* both developed necrotic spots the day after exposure to high temperatures (45°C) combined with high light, particularly on horizontally oriented leaf tips (Koeniger *et al.*, 1998). However, high light combined with moderate temperatures of 30°C, or low light combined with temperatures of 45°C were less detrimental. *Alocasia* plants lost about 10% of their total leaf area to necrotic spots, although some leaves survived despite necrosis and even increased photosynthetic rates in the undamaged leaf tissue (Koeniger *et al.*, 1998). In contrast, high light had a greater effect on photoinhibition than high temperatures in leaves of young tropical forest species, even though leaf temperatures in the forest canopy often rose above 40°C (Krause *et al.*, 1995).

Crops in temperate regions often experience high light intensity and low temperatures on cold spring mornings (Tsonev *et al.*, 2002), coinciding with the season of flower maturation in waratahs. Bract browning of waratahs following frost in combination with strong sunlight has been reported by Offord (1996) and growers in the Blue Mountains of NSW (F. Allatt, personal communication). Such low temperatures and strong light affect PSII, while low temperatures in combination with weak light appear to affect PSI (Sonoike, 1998). The reactive oxygen species responsible for photooxidation following chilling injury, generated in both PSI and PSII, have been reviewed in detail by Wise (1995) and include singlet oxygen, the superoxide anion radical, hydrogen peroxide, the hydroxyl radical and the monodehydroascorbate radical. Both tropical and temperate species may be affected by photoinhibition at low temperatures, as described below.

Nir *et al.* (1997) found that chilling at night followed by high light resulted in chlorosis and necrosis of mango leaves, with photoinhibition proportional to temperature of chilling. Repetitive cycles of night chilling resulted in accumulation of photoinhibition. Shading with 50% shade cloth prevented chlorosis and necrosis on control trees and prevented the daily drop in F_v/F_m in control and pre-chilled trees. In sun-exposed mango leaves partially shaded by adjacent leaves, the exposed portion turned brown while the shaded portion remained green (Nir *et al.*, 1997). Nir *et al.* (1997) suggest that the avoidance of sun exposure by shading is easier than the avoidance of chilling. Similarly, shade cloth shelters reduced the cold induced photoinhibition of *Eucalyptus polyanthemos* and *E. nitens* seedlings (Holly *et al.*, 1994; Close *et al.*, 2001).

6.1.3.2 Water stress

Water stress often interacts with light and temperature stress, although species respond differently to combinations of stressors. Water stress can cause stomatal (CO₂ restricted) and non-stomatal limitation of photosynthesis, that is, direct photoinhibition (Powles, 1984). Water stress directly inhibited PSII electron transport in *Nerium*, rather than indirectly through stomatal limitation or high temperatures (Bjorkman and Powles, 1984). Shading of *Nerium* plants prevented development of photoinhibition, even with extreme water stress (Bjorkman and Powles, 1984). In contrast, onset of water stress in naturally shaded plants of *Heteromeles arbutifolia* resulted in greater reductions in predawn Fv/Fm and carbon limitation due to stomatal closure, than in sun-exposed plants (Valladares and Pearcy, 2002). This unexpected result was attributed to increased competition for water resources in shaded plants. Water stress, in combination with the high light and high temperatures experienced by horizontally-oriented *Heteromeles* leaves, led to decreased transpirational cooling, resulting in photoinhibition and necrotic spots (Valladares and Pearcy, 1997).

The susceptibility of evergreen sclerophylls and semi-deciduous shrubs to photoinhibition was strongly increased by water stress (Werner *et al.*, 2002). However, the extent of chronic and dynamic photoinhibition was dependent on leaf orientation (Werner *et al.*, 2002). The increasing horizontal orientation of waratah bracts towards flower maturity may increase their susceptibility to photoinhibition caused by combined high light and high temperatures, as observed in *Heliconia* and *Alocasia* leaves (He *et al.*, 1996; Koeniger *et al.*, 1998).

6.1.3.3 Nutrition

Poor nutrition, particularly nitrogen deficiency, directly impacts photosynthesis and photoinhibition (Powles, 1984), leading to decreased photosynthetic capacity, increased excess excitation energy and decreased protein synthesis, including PSII repair (Close *et al.*, 2001). Low nitrogen availability exacerbated the photoinhibition experienced by coffee (Nunes *et al.*, 1993; Ramalho *et al.*, 1997) and tea (Mohotti and Lawlor, 2002) plants on exposure to high light. The decrease in quantum yield of *Eucalyptus nitens* seedlings was more pronounced in non-fertilised seedlings compared to fertilised seedlings, on exposure to low temperatures and high light (Close *et al.*, 2001). Nutrient deprived *Eucalypt* seedlings had a lower chlorophyll content, but a higher concentration of xanthophylls per chlorophyll, which would help harmlessly dissipate excess light energy (Close *et al.*, 2001). Similarly, harmless dissipation of excess energy has been suggested as a photoprotective mechanism in nitrogen deprived *Chenopodium* plants (Kato *et al.*, 2002). However, in *Chenopodium album*, susceptibility to photoinhibition and the rate of recovery from photoinhibition were more dependent on acclimation to growth irradiance than nitrogen availability (Kato *et al.*, 2002). Shading may minimise the effects of nutrient deprivation by minimising absorption of excess light energy (Close *et al.*, 2001; Mohotti and Lawlor, 2002).

Although these environmental stressors (temperature extremes, water stress and poor nutrition) may impact on photoinhibition, the primary cause of browning appears to be high light intensity (Chapter 6). Due to constraints on time and plant numbers, this chapter will therefore concentrate on the primary cause of potential photoinhibition – high light.

6.1.4 How can photoinhibition be measured? The principles of fluorescence measurement

Fluorescence is the re-emission of a small percentage of the light absorbed by chlorophyll and competes with photochemistry (photochemical charge separation) and heat dissipation (non-photochemical quenching) (Maxwell and Johnson, 2000). Fluorescence emission occurs at a longer wavelength than light absorption, so a leaf or bract can be exposed to light at a defined wavelength and the amount of light re-emitted as fluorescence can be easily distinguished. Therefore, measurement of chlorophyll fluorescence yield can be used to monitor the efficiency of photochemistry and heat dissipation (Figure 6.3).

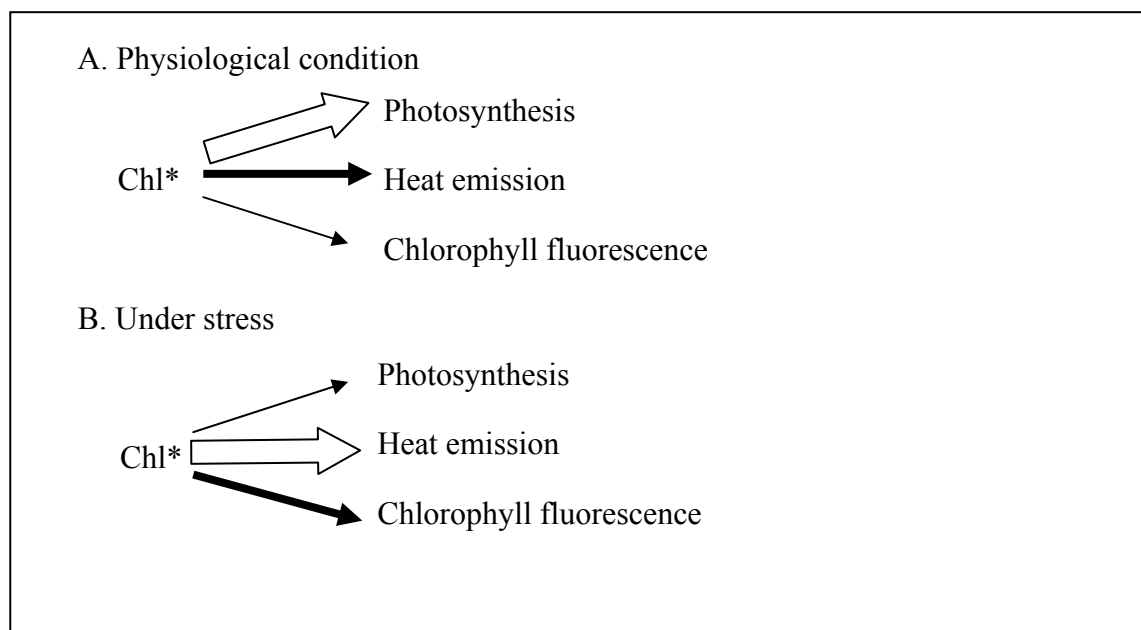


Figure 6.3: Pathways for de-excitation of chlorophyll a via photosynthesis, heat emission (NPQ) and chlorophyll fluorescence under non-stress (A) and stress (B) conditions (from Lichtenthaler, 1996).

The transfer of photosynthetic material from darkness to light leads to a rapid increase in chlorophyll fluorescence. This increase is due to reduction of electron acceptors in the photosynthetic pathway downstream of PSII, particularly PQ and Q_A (Maxwell and Johnson, 2000; Figure 6.4). After accepting one electron, Q_A cannot accept any more electrons until the first electron has been passed onto Q_B and the reaction centre is described as 'closed'.

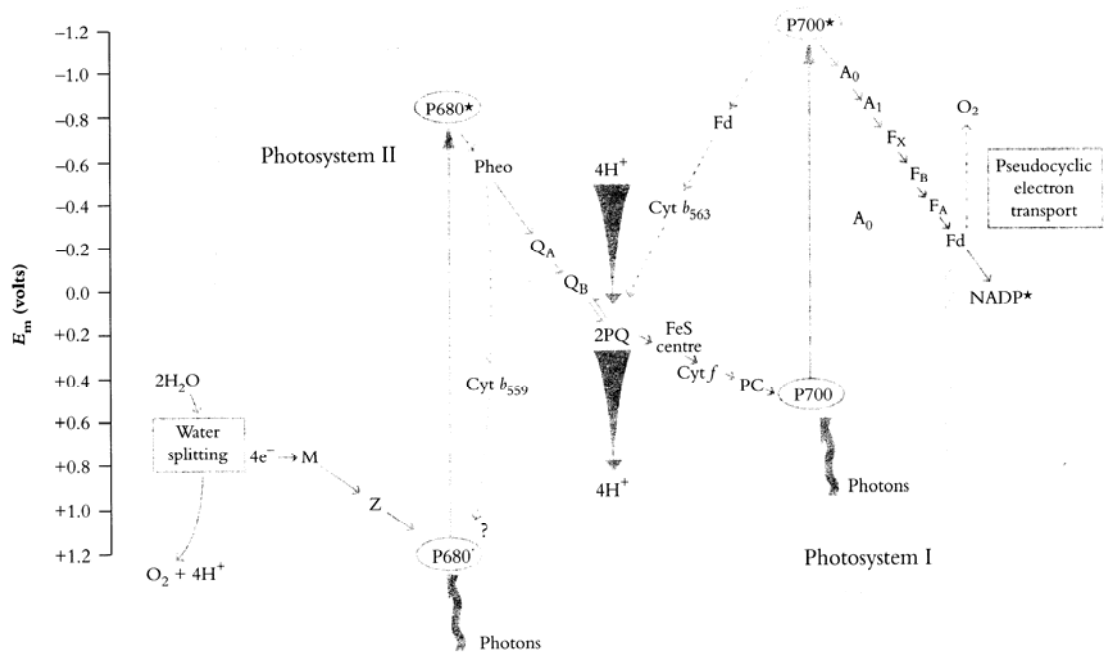


Figure 6. 4: Photosynthetic electron transport chain (Atwell *et al.*, 1999 p.38)

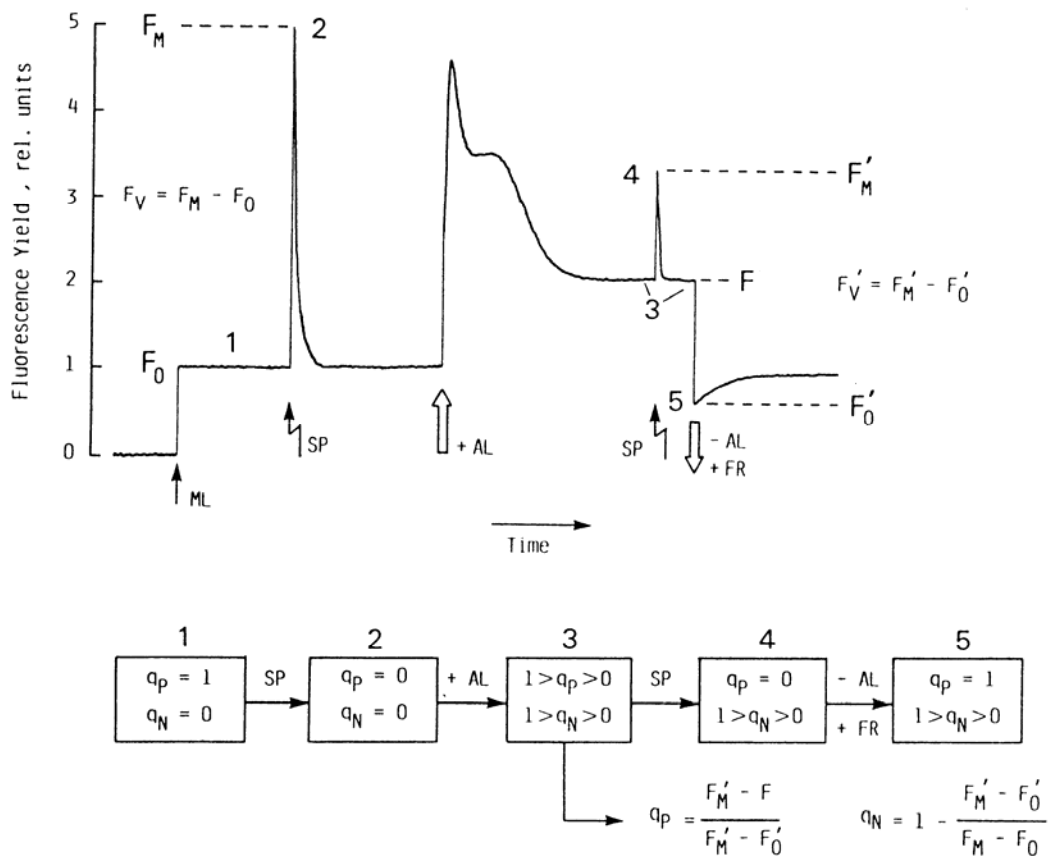


Figure 6.5: Fluorescence trace (van Kooten and Snel, 1990). ML is weak modulated measuring light, SP is saturating pulse light, AL is continuous actinic (photosynthetic) light, FR is far-red light. Fluorescence notation is described in the text.

Using a fluorometer, a low intensity of modulated measuring light is applied, sufficient to excite fluorescence but avoid a photosynthetic response (Stage 1, Figure 6.5). The PAM 2000 fluorometer (Heinz Walz GmbH, Effeltrich, Germany) uses modulated light (light switched on and off at a high frequency) and a detector that measures only the fluorescence response excited by modulated light, thus avoiding interference from external light sources, including full sunlight in the field (Heinz Walz GmbH, 1993; Maxwell and Johnson, 2000).

Subsequently, a flash of high intensity light can be applied to transiently close all PSII reaction centres. The presence of closed reaction centres leads to a decrease in photochemical efficiency and an increase in fluorescence yield (Stage 2, Figure 6.5). The distinctive curve produced on illumination of a dark adapted sample is known as the Kautsky induction curve (Maxwell and Johnson, 2000). The resulting maximum fluorescence yield (F_m) is equivalent to yield in the absence of any photochemical quenching. The efficiency of photochemical quenching of PSII for dark adapted samples can be described by the ratio F_v/F_m (Equation 6.2). The parameter F_v/F_m is hereafter referred to as 'quantum yield' (Maxwell and Johnson, 2000), inferring that measurements were made or samples were harvested predawn.

Equation 6.2: Dark adapted (optimal) quantum yield

$$(F_m - F_o) / F_m = F_v / F_m$$

Where:

F_o = instantaneous fluorescence yield/ dark adapted initial fluorescence

F_v = dark adapted variable fluorescence

F_m = dark adapted maximum fluorescence yield

F_v / F_m = dark adapted ratio of variable to maximal fluorescence

Many studies of chlorophyll fluorescence emphasise the quantum yield parameter (F_v/F_m), which is well correlated with the quantum efficiency of photosynthetic gas exchange (Ball *et al.*, 1994). Sustained depression of the quantum yield measured pre-dawn indicates chronic photoinhibition (Osmond, 1994). The quantum yield indicative of chronic photoinhibition is fairly arbitrary and depends on the species in question (Dodd *et al.*, 1998), although a quantum yield (F_v/F_m) < 0.8 is often considered to indicate chronic photoinhibition.

After the initial increase to F_m , fluorescence yield begins to decline (Figure 6.5). The actinic (photosynthetic) light is then switched on and 'quenching' is caused by an increase in the rate of electron movement away from PSII (photochemical quenching) and an increase in the conversion of energy to heat (non-photochemical quenching).

Steady-state fluorescence (Stage 3, Figure 6.5) is reached under field conditions in response to the prevailing light intensity, and can be induced using a fluorometer for a chosen intensity of illumination. The fluorescence of illuminated samples in a steady state cannot be directly compared to dark adapted samples, however, the efficiency of photochemical quenching can still be determined by probing the system with a saturating pulse of light (Stage 4, Figure 6.5). The saturating pulse (approx. 1 second) fully reduces the electron transport chain, so for the duration of the pulse, photochemical quenching is reduced to zero and all remaining quenching must be non-photochemical (Heinz Walz GmbH, 1993). The quantum yield during illumination (Equation 6.3) will hereafter be referred to as the 'effective quantum yield'.

Equation 6.3: Quantum yield during illumination (effective quantum yield)

$$(F_m' - F_t) / F_m' = \Delta F / F_m' \text{ or } F_v' / F_m'$$

Where:

F_t = steady-state fluorescence just before light flash

F_m' = maximal fluorescence (all PSII centres closed) in a light-adapted state

Non-photochemical quenching (NPQ) can also be calculated from the above fluorescence parameters without having to measure the minimum fluorescence yield after illumination (F_o') (Stage 5, Figure 6.5; Equation 6.4).

Equation 6.4: Non-photochemical quenching

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

6.2 Background to chlorophyll fluorescence measurements

Experiments with the Photobioenergetics group at the Research School of Biological Sciences (RSBS, supervised by Dr W.S. (Fred) Chow) at the Australian National University, Canberra, provided background information for experiments at field sites. The experiments at RSBS describe the differences in the optical properties of leaves and bracts and define the relationship between chlorophyll fluorescence measurements (used throughout this chapter) and oxygen evolution as a measure of photosynthesis. Two experiments were completed over six days in August-September 2003.

6.2.1 Comparison of optical properties of waratah leaves and bracts

6.2.1.1 Aim

Mature green leaves of many species absorb approximately 85% of incident radiation, but absorbance can change with leaf age, time of season, epidermal characteristics and

chlorophyll content (Schultz, 1996). It is not known whether waratah leaves absorb a similar proportion of incident radiation to leaves of other species. It is also likely that the optical properties of waratah bracts differ from leaves, as bracts have less chlorophyll (Chapter 7) and develop later than waratah leaves.

Measurements of light absorption by photosynthetic tissue are necessary for accurate calculation of electron transport rate (Equation 6.5; Krall and Edwards, 1992), incorporating the equation of Genty *et al.* (1989) for the quantum yield of photochemistry. The rate of electron transport is likely to decrease with photoinhibition and non-photochemical quenching (for example, Krause *et al.*, 1995).

Equation 6.5: Electron transport rate

$$ETR = \Phi_{PSII} \times PFD_{abs} \times (0.5)$$

Where ETR is the rate of PSII-driven electron transport, also known as J_F

Φ_{PSII} is the quantum yield of PSII photochemistry, defined as $1-F_t/F_m'$

PFD_{abs} is the light absorbed by a photosynthetic tissue

(0.5) assumes that energy is equally partitioned between PSI and PSII

6.2.1.2 Methods

Absorbance, reflectance and transmittance of leaf and bract discs were measured using an integrating sphere with a quantum meter (Licor, Nebraska, USA) for light detection. A slide projection lamp provided a source of white light, focused at a fixed distance to maximize incident light. Transmittance (T) was calculated as I_t/I_o where I_t is intensity of light transmitted through a leaf or bract sample and I_o is the intensity of incident light (i.e. no sample). Cardboard discs of known reflectance were used to construct a calibration curve for reflectance and allow calculation of leaf or bract reflectance (R) (Appendix Figure A4.1). Absorbance (A) was calculated using equation 6.6.

Equation 6.6: Absorbance

$$\text{Absorbance} = 1 - T - R$$

Where T = transmittance
R = reflectance

Waratahs plants of cultivar ‘Olympic Flame’ grown at Mount Annan in 2003 were subject to three light treatments - early shade, late shade and sun - as described in experiment 6.5. Waratah stems (flowers and leaves) were harvested at Mount Annan on 21/09/03, when flowers were at the juvenile open or mature flower stage. Stems were transported in a cool environment to the Australian National University, where they were placed in water and held at 4°C in the dark for up to six days.

Measurements of optical properties were carried out on 25/09/03 and 26/09/03 on leaves or bracts at room temperature. Two discs of each tissue type — mature leaf, inner bract and outer bract — were measured from each of two plants in each light treatment, except for the shade treatment (one plant only).

Results were analysed in Genstat (Lawes Agricultural Trust, 2002) using ANOVA and plotted in Microsoft Excel. Where data points represent a mean of several replicates, variance is expressed with standard error (s.e.) bars.

6.2.1.3 Results

Significant interactions existed between treatment (light environment) and tissue type for all optical properties (absorbance $P = 0.006$, reflectance $P = 0.039$, transmittance $P < 0.001$), while orientation of tissue (abaxial or adaxial surface) did not significantly affect optical properties (absorbance $P = 0.190$, reflectance $P = 0.426$, transmittance $P = 0.020$). Therefore, results are presented for adaxial surfaces only.

Within each treatment, leaves had significantly higher absorbance and lower transmittance and reflectance than bracts (Figures 6.6 – 6.8). Light treatment had no effect on any optical parameter measured in leaves, or on inner bract absorbance or reflectance. Inner bract transmittance was significantly higher in the early shade treatment than in late shade or sun treatments. Outer bracts had significantly higher absorbance and lower reflectance and transmittance in sun compared to early or late shading treatments.

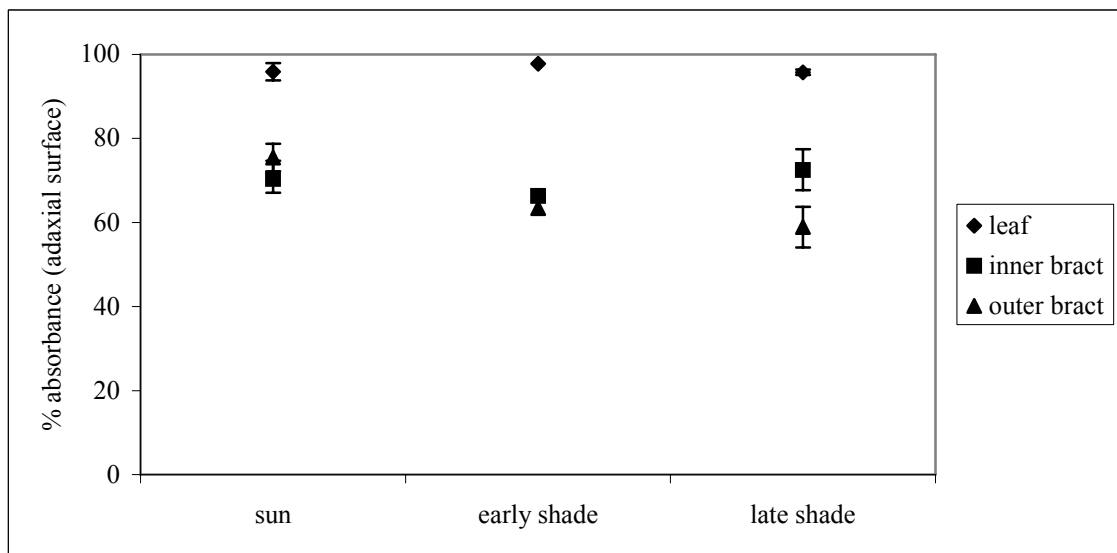


Figure 6.6: Absorbance of incident light applied to the adaxial surface of leaves (◆) and inner bract (■) and outer bract (▲) discs of waratah plant grown in the sun or shaded early or late during bud development. n = 2 plants in each light environment, except for early shade n = 1.

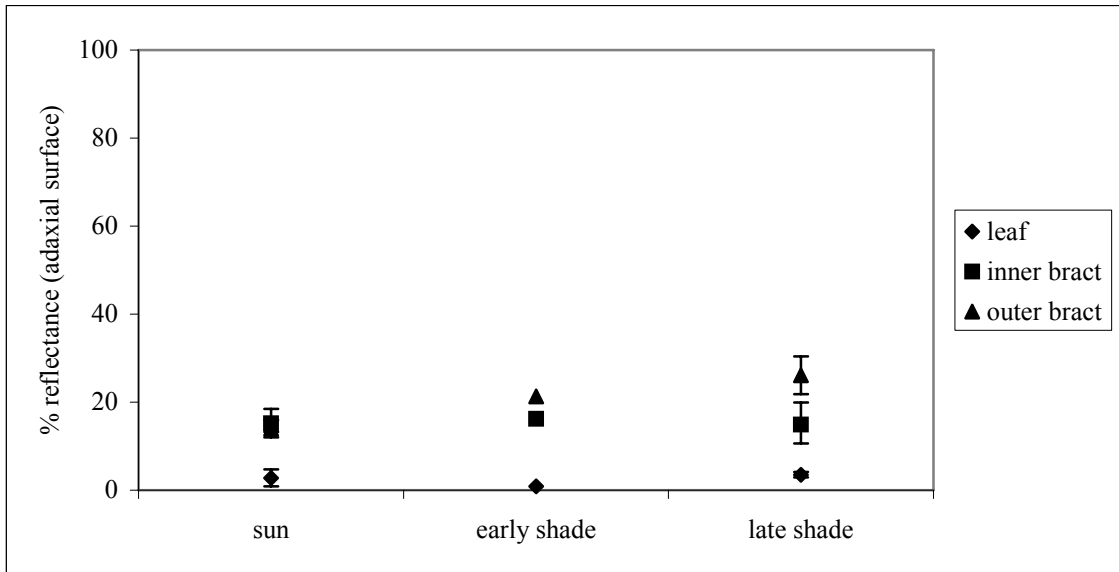


Figure 6.7: Reflectance of incident light applied to the adaxial surface of leaves (◆) and inner bract (■) and outer bract (▲) discs of waratah plant grown in the sun or shaded early or late during bud development. n = 2 plants in each light environment, except for early shade n = 1.

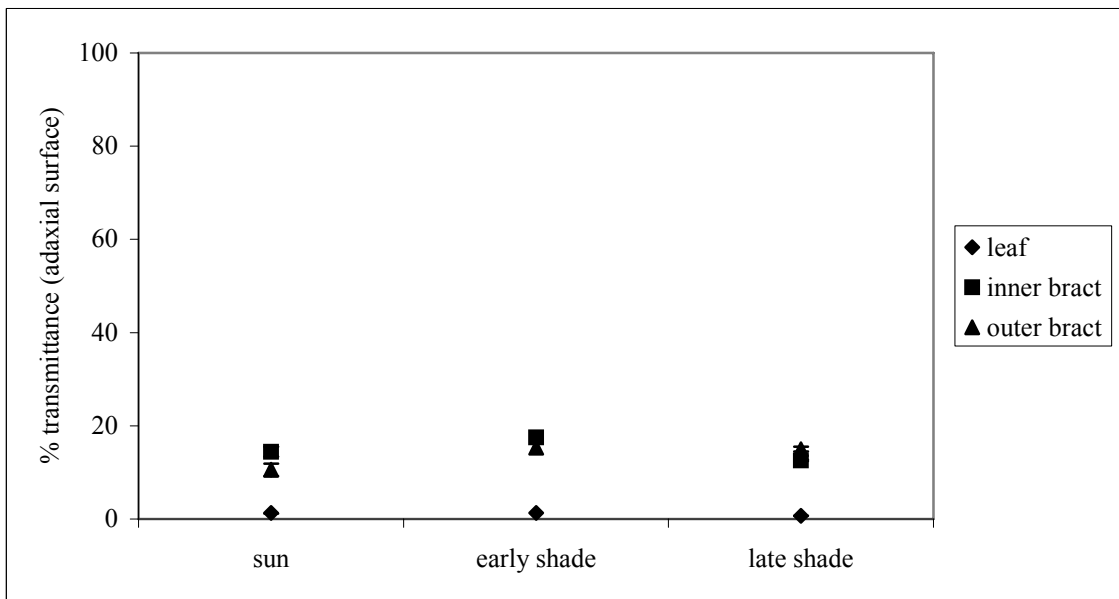


Figure 6.8: Transmittance of incident light applied to the adaxial surface of leaves (◆) and inner bract (■) and outer bract (▲) discs of waratah plant grown in the sun or shaded early or late during bud development. n = 2 plants in each light environment, except for early shade n = 1.

6.2.2 Relationship between functional PSII and quantum yield (F_v/F_m) in waratah bracts

6.2.2.1 Aim

Understorey species such as waratah generally require low light intensities for saturation of electron transport (Anderson *et al.*, 1988). Excess light can cause damage to the reaction center of photosystem II (PSII) through degradation of the D1 protein by free radicals, singlet oxygen and $P680^+$ (Chow, 2001). If the rate of D1 degradation (photoinactivation) exceeds the rate of repair, then chronic photoinhibition can result (Osmond, 1994). Dark-adapted quantum yield measurements approximate the number of functional PSII reaction centers (Oquist *et al.*, 1992), although the relationship is not always linear (Hendrickson *et al.*, 2003).

Fluorescence measurements are preferred because they are faster and non-destructive, while oxygen evolution measurements are destructive and time consuming (Chow *et al.*, 2002) (approx. 20 seconds compared to 40 minutes per sample, respectively). This experiment aims to determine the relationship between functional PSII and quantum yield for waratah bracts, with the number of functional PSII expected to be proportional to the quantum yield (F_v/F_m).

6.2.2.2 General methods

The number of functional PSII reaction centres was calculated from the yield of flash-induced oxygen evolution in leaf or bracts discs (3.72 cm^2), as described by Chow *et al.* (1991), Balachandran and Osmond (1994), Park *et al.* (1996), Yamane *et al.* (1998), He and Chow (2003) and Hendrickson *et al.* (2003). A Clark-type oxygen electrode (Hansatech, King's Lynn, UK) in the gas phase was used to monitor photosynthetic O_2 evolution from leaf discs in 1% CO_2 . Following 10 minutes dark equilibration,

repetitive single-turnover saturating xenon flashes (10 Hz, 2.5 μ s full width at half peak intensity; type FX200, E.G. and G. ElectroOptics, Salem, MA, USA) were applied for 4 min, followed by 4 min darkness. This cycle of flashes and darkness was repeated 2-3 times for each sample. Background far-red light was used to prevent limitation of electron transport by PSI. The calculation of functional PSII complexes takes into account the slight heating artifact due to flashes and was expressed on a leaf area basis.

Fluorescence yield (F_v/F_m) was measured with a Hansatech Plant Efficiency Analyser (King's Lynn, UK) after 10 min dark adaptation following each photoinhibitory treatment and O_2 measurement.

Results were analysed using linear regression with groups (i.e. shade treatment or sun treatment) in Genstat (Lawes Agricultural Trust, 2002) to establish whether a linear relationship existed between the two variables measured (functional PSII and quantum yield). The percentage of variance accounted for by the regression model was expressed as an R^2 value (Quinn and Keough, 2002).

The source of waratah material used, and further description of methods in initial (6.2.2a) and subsequent (6.2.2b) experiments are described below. Waratah flowers were examined at different stages of development – tight bud (TB), juvenile open bud (JO) and mature flower (MF) (described in detail in Chapter 3).

Experiment 6.2.2a

The initial experiment examined the number of functional PSII reaction centres and the quantum yield in a range of leaf and bract samples. Waratah flowers of cultivars ‘Wirrimbirra White’ and ‘Shady Lady’ were harvested from a commercial population at Jervis Bay on 01/08/03. Flowers were at the tight bud stage. Stems were transported in a cool environment to the Australian National University, where they were placed in water and held at 4°C in the dark. Measurements of oxygen evolution and fluorescence were carried out on 04/08/03 and 05/08/03 at room temperature.

Experiment 6.2.2b

This experiment further examined the decrease in functional PSII and quantum yield following photoinhibitory light treatments. Treatments involved floating bract discs in a water bath at 10°C or 25°C under a theatre lamp of 700-900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for cumulative treatments of 40 minutes each, to a total treatment time up to 200 minutes to induce photoinactivation.

Waratah flowers of cultivar ‘Olympic Flame’ were harvested at Mount Annan on 21/09/03. Light treatments were shade from flower initiation (shade) or sun from flower initiation (sun). Flowers were at the juvenile open or mature flower stages. Flowers were transported in a cool environment to the Australian National University, where they were placed in water and held at 4°C in the dark. Measurements of oxygen evolution and fluorescence were carried out between 23/09/03 and 26/09/03 on bracts at room temperature. Results from bracts measured in experiment 6.2.2a are also incorporated in the figures.

6.2.2.3 Results

Table 6.1 shows the number of functional PSII and the quantum yield (Fv/Fm) for a range of leaf and bract discs. Leaves and outer bracts gave satisfactory measurements, but inner bracts produced a signal too small to be distinguished from the heating artefact. Brown parts of bracts did not produce a fluorescence signal and did not evolve oxygen, however, areas adjacent to brown areas (often chlorotic) showed reduced quantum yield and fewer functional PSII. Therefore, areas adjacent to browning could be compared to bracts with no browning.

Table 6.1: Comparison of functional PSII and quantum yield in different waratah bract and leaf types from Experiment 6.2.2a

Cultivar	Tissue type and colour	Functional PSII ($\mu\text{mol}/\text{m}^2$)	Quantum yield (Fv/Fm)
	Typical healthy green leaf	Approx. 2-3	Approx. 0.8-0.85
'Wirrimbirra White'	Yellow-green leaf	0.927	0.760
'Wirrimbirra White'	White inner bract	0.012*	0.486
'Wirrimbirra White'	Green outer bract	0.280	0.630
'Wirrimbirra White'	Brown outer bract	No signal	No signal
'Wirrimbirra White'	Yellow section of brown outer bract	0.076	0.529
'Shady Lady'	Green-red outer bract	0.250	0.686

* hardly distinguishable from heating artefact

The number of functional PSII calculated from flash-induced oxygen evolution increased with the quantum yield of bract discs, following photoinhibitory treatments (Figure 6.9). A linear relationship existed between functional PSII and average quantum yield ($R^2 = 80.2$); however results for sun and shade treatments needed to be considered separately ($P < 0.001$ for change in deviance from one fitted line to separate fitted lines for each treatment).

Since the relationship between functional PSII and quantum yield has been confirmed for waratah bracts, future experiments focussed on whether chronic photoinhibition occurred in waratah bracts and whether leaves were similarly affected (section 6.3). The experiment also investigated the impact of shading on photoinhibition.

Fitted and observed relationship

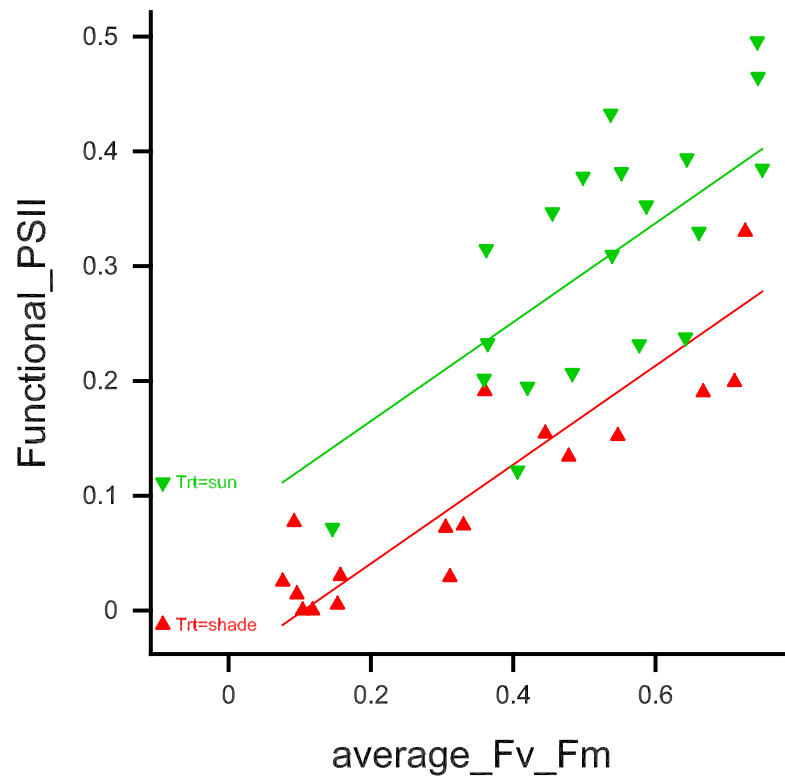


Figure 6.9: Average quantum yield (Fv/Fm) plotted against number of functional photosystem II (PSII) centres ($\mu\text{mol PSII m}^{-2}$). Shade values in red and sun values in green (dots for observed values, line for values fitted according to regression output). $n = 3$ plants in shade and 1 plant in sun, each with 2 colours of bract tested.

6.3 Does chronic photoinhibition occur in waratahs? Is it reduced by shading?

6.3.1 Study sites

Experiments were conducted at Mount Annan on ‘Fire and Brimstone’ waratahs (6.3.3) and Jervis Bay on ‘Wirrimbirra White’ waratahs (6.3.4) in 2002 and at Mount Annan on ‘Fire and Brimstone’ and ‘Olympic Flame’ waratahs in 2003 (6.3.5). Following the general methods for examining chronic photoinhibition, the aims, methods and results for each of these experiments (6.3.3-6.3.5) are presented separately, followed by discussion of all three experiments.



Figure 6.10: PAM 2000 fluorometer, for monitoring photoinhibition using chlorophyll fluorescence measurements, and laptop in use at Mount Annan Botanic Garden.

6.3.2 General methods

Chlorophyll fluorescence was measured with a Teaching PAM or PAM-2000 fluorometer (Figure 6.10) (Heinz Walz GmbH, Effeltrich, Germany) for each stage of flower development as shown in Table 6.2. The more exposed surface of each tissue was selected for measurement, that is, the adaxial leaf surface and abaxial bract surface. This ensured that the maximal reduction in photosynthetic efficiency was measured for each tissue type. In situations where measuring light or gain were changed to avoid signal saturation, only quantum yield (F_v/F_m) or effective quantum yield ($\Delta F/F_m'$) measurements are presented, as absolute F_o and F_m values are dependent on the intensity of measuring light. Poor fluorescence results, due to extensive browning of tissue, low signal intensity or signal overload were noted as missing values.

Table 6.2: Timing of fluorescence measurements and equipment used in 2002 and 2003 for waratah tissues at different stages of flower development. Stages are TB = tight bud, JO = juvenile open bud, MF = mature flower.

Year and stage	Date of fluorescence measurement	Equipment used
2002 TB	28/8/02 leaves	Teaching PAM
	2/9/02 outer bracts	
	4/9/02 inner bracts	
2002 JO	18/9/02 all tissues	Teaching PAM
2002 MF	10/10/02 all tissues	PAM 2000
2003 TB	27/8/03 F&B	PAM 2000
	28/8/03 Olympic Flame	
2003 JO	2/10/03 both cultivars	PAM 2000
2003 MF	23/10/03 both cultivars	PAM 2000

The number of samples varied for each stage and each treatment in both 2002 and 2003, due to different numbers of plants being at an appropriate stage for measurement. The resulting unbalanced treatment structure was analysed in Genstat (7th edition, Lawes Agricultural Trust) using the linear mixed model option of the restricted maximum likelihood (REML) procedure, as described in Chapter 5. The fixed model included all interactions of *Treatment*, *Stage*, *Tissue* and *Cultivar* (as appropriate), with *Tissue* nested in *Plant* as the random model. The *Tissue* term was expected to have a higher

variance than other terms, so a diagonal correlated error structure for *Tissue* was included in the model.

The following experiments describe measurements of the quantum yield of leaves and bracts during waratah flower development to identify whether chronic photoinhibition occurred. Experiments were conducted on waratahs at Mount Annan and Jervis Bay in 2002 (6.3.3 and 6.3.4) and Mount Annan in 2003, using standard chlorophyll fluorescence techniques (6.3.5) and imaging fluorescence techniques (6.3.6). The initial experiment at Mount Annan in 2002 compares the quantum yield of leaves and bracts of waratah cultivar ‘Fire and Brimstone’ given three different light treatments – sun, early shading and late shading – at three stages of floral development. These stages are the tight bud (TB), juvenile open bud (JO) and mature flower (MF) stages.

6.3.3 Quantum yield measurements at Mount Annan 2002

6.3.3.1 Method

In 2002, fluorescence measurements of ‘Fire and Brimstone’ leaves and bracts were taken using a PAM 2000 fluorometer with a leaf clip (Leaf Clip Holder 2030-B, Heinz Walz GmbH, Effeltrich, Germany), while Teaching PAM fluorometer measurements were taken with leaf or bract discs placed directly on the sample holder. Measurements for all stages were taken in the lab, following harvest in the hour before sunrise and holding in a darkened room. Fluorescence measurements on leaves harvested in this way were stable for five hours after harvest, so the timing of subsequent measurements was not critical (Appendix Figure A4.2). For each plant available at each stage, one or two areas either side of the midrib on each of two leaves were selected, as well as one or two distal areas on each of two bracts.

Due to missing values of late shaded flowers at the JO stage, data was analysed in two subsets: (1) all sun and early shading treatments at all stages of flower development and (2) all sun, early and late shading treatments at TB and MF stages of flower development.

6.3.3.2 Results

Sun, early and late shading treatments were compared at the initial (TB) and final (MF) stages of flower development. Sun tissues had a significantly lower quantum yield compared to early and late shaded tissue ($P < 0.001$, Figure 6.11), indicating that chronic photoinhibition had occurred.

Significant interactions existed between tissue type and stage ($P = 0.002$), as inner bract quantum yield decreased significantly during flower development while outer bract quantum yield was significantly lower than leaf quantum yield from early (TB) to late (MF) floral development. At the TB stage, inner bracts had a high quantum yield similar to leaves, while outer bracts had a significantly lower quantum yield than leaves, indicating that chronic photoinhibition had occurred. Leaf quantum yield did not change significantly over time (Figure 6.12).

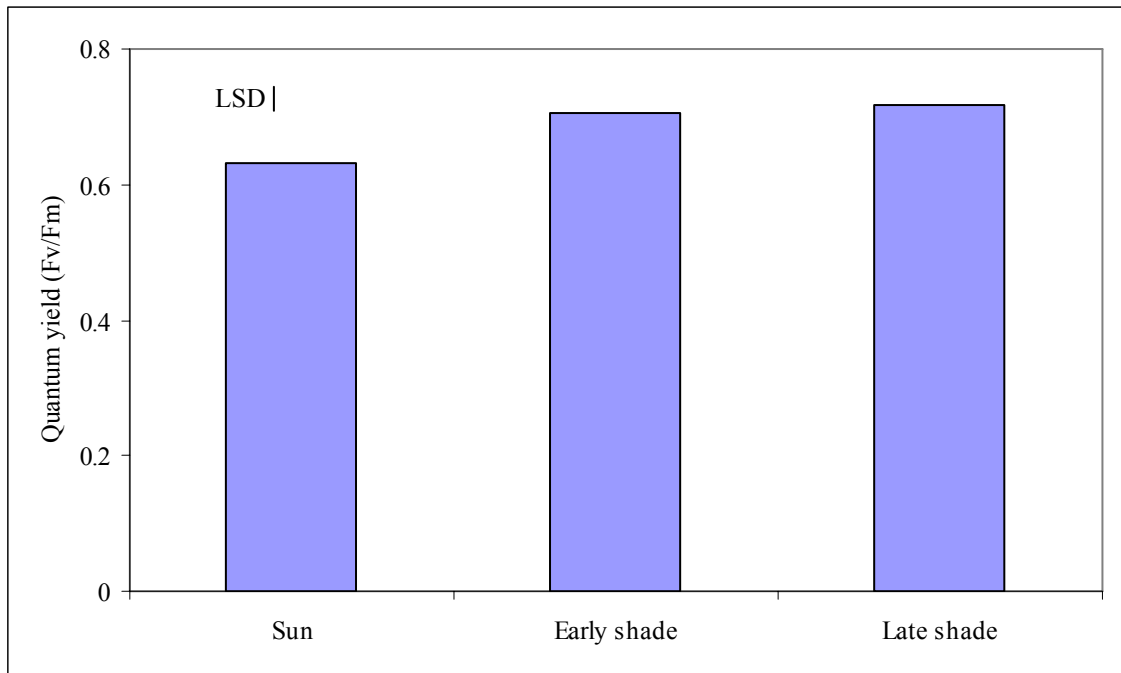


Figure 6.11: Differences in quantum yield (Fv/Fm) for each treatment pooled for leaves, inner and outer bracts at tight bud (TB) and mature flower (MF) stages of floral development in 'Fire and Brimstone' waratahs. LSD = 0.035. n = 3-4 plants for each tissue type, treatment and stage combination except inner bracts in early shade and inner bracts in late shade both with n = 2 and JO shade treatments (not measured).

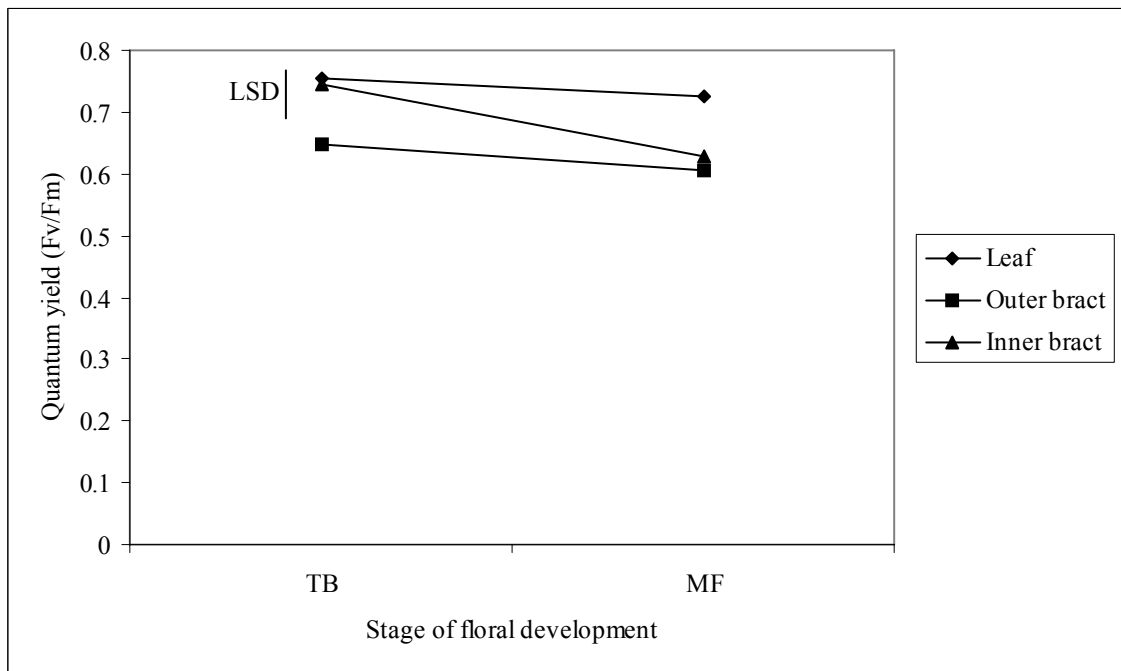


Figure 6.12: Differences in quantum yield (Fv/Fm) pooled across all treatments for leaves (◆), inner bracts (▲) and outer bracts (■) at tight bud (TB) and mature flower (MF) stages of floral development in 'Fire and Brimstone' waratahs. LSD = 0.064. n = 3-4 plants for each tissue type, treatment and stage combination except inner bracts in early shade and inner bracts in late shade both with n = 2 and JO shade treatments (not measured).

Early shading and sun treatments were also compared at all stages of flower development (TB, JO and MF) to examine the effects of light treatments on the intermediate stage of floral development. Significant interactions existed between Tissue type and Stage ($P = 0.016$) (Figure 6.13), with trends at the TB and MF stages similar to those described above. At the intermediate (JO) stage of flower development, both inner and outer bracts had a significantly lower quantum yield than leaves, indicating chronic photoinhibition. Bract quantum yield did not change significantly from the intermediate to final stages of flower maturity, although inner bract quantum yield tended to decrease and outer bract quantum yield increased slightly.

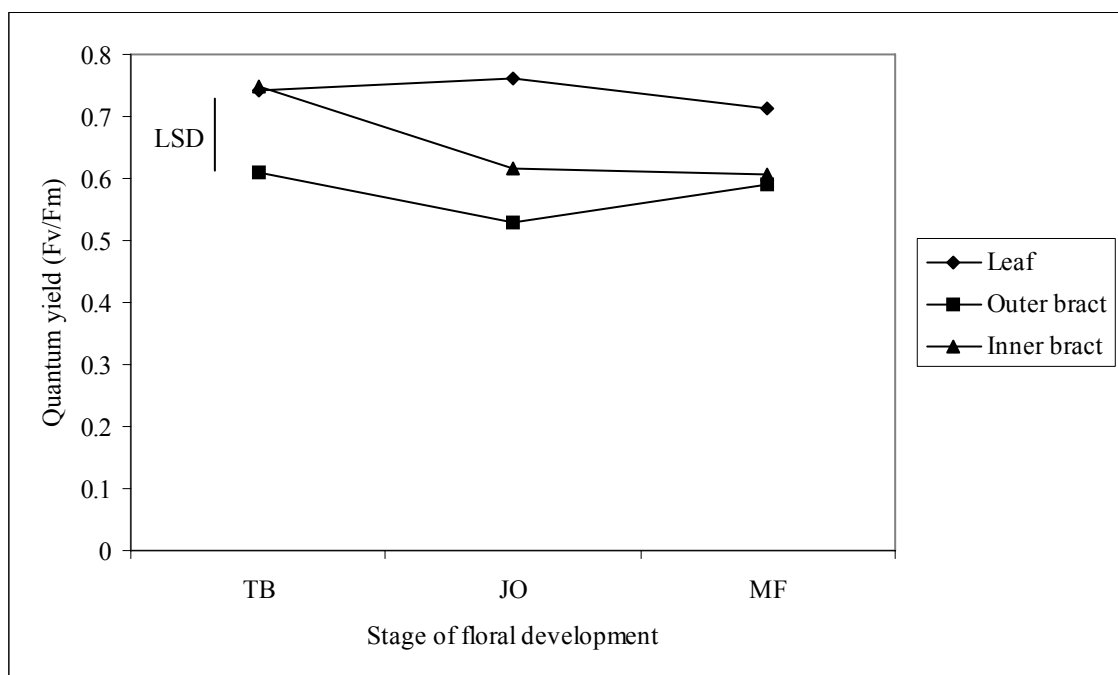


Figure 6.13: Differences in quantum yield (Fv/Fm) across early shade and sun treatments, for leaves (◆), inner bracts (▲) and outer bracts (■) at tight bud (TB), juvenile open bud (JO) and mature flower (MF) stages of floral development in ‘Fire and Brimstone’ waratahs. LSD = 0.131. $n = 3-4$ plants for each tissue type, treatment and stage combination except inner bracts in early shade and inner bracts in late shade both with $n = 2$ and JO shade treatments (not measured).

Measurements of chronic photoinhibition made on red ‘Fire and Brimstone’ waratahs at Mount Annan were similarly made on ‘Wirrimbirra White’ waratahs at the Jervis Bay study site in the same year, as described in section 6.3.5 below. Again, the aim of this experiment was to determine whether white waratahs (‘Wirrimbirra White’) at flower maturity are susceptible to photoinhibition, and if photoinhibition can be reduced by shade.

6.3.4 Quantum yield measurements at Jervis Bay 2002

6.3.4.1 Method

In 2002, leaves and bracts of ‘Wirrimbirra White’ waratahs from Jervis Bay were collected before dawn at the mature flower stage and covered in aluminium foil. Measurements of quantum yield were then made indoors using the same sampling technique as described in experiment 6.3.3.

6.3.4.2 Results

The quantum yield of leaves and bracts of shaded and sun exposed ‘Wirrimbirra White’ waratahs was compared, with significant interactions between tissue and treatment ($P = 0.013$). Outer bracts in the shade had a similar quantum yield to leaves in the sun or shade (Figure 6.14), indicating photosynthetically efficient, healthy tissue. However, outer bracts in the sun had a significantly lower quantum yield, showing that chronic photoinhibition had occurred. The quantum yield of inner bracts was significantly lower again (Figure 6.14), and could not be measured in sun exposed inner bracts.

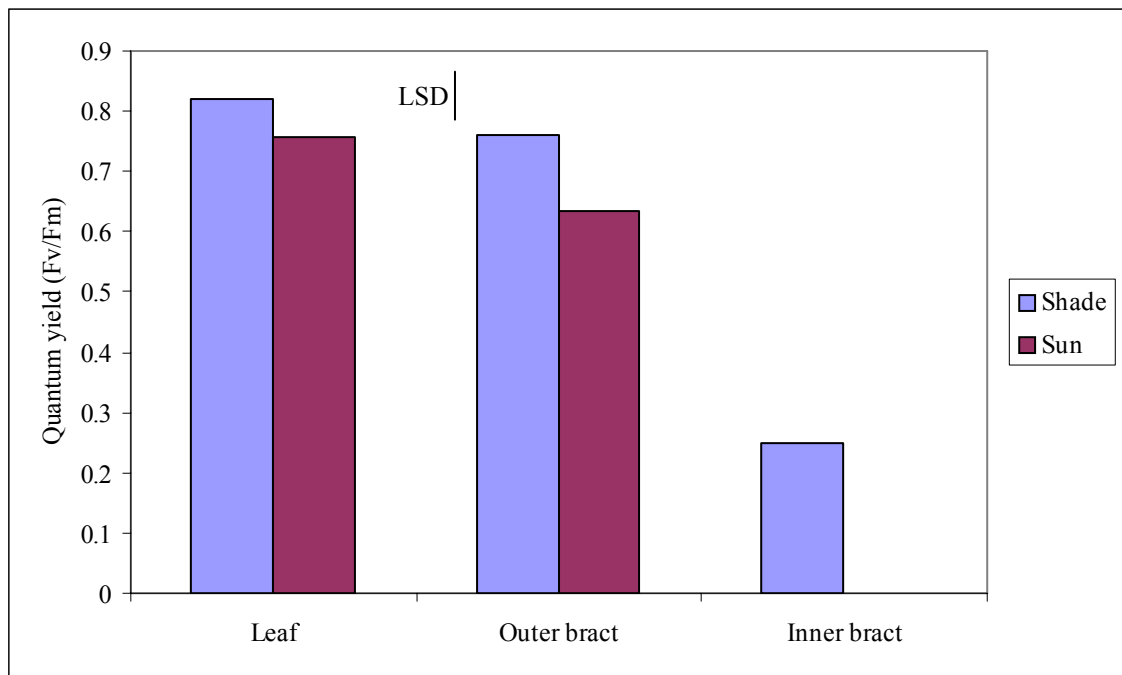


Figure 6.14: Quantum yield (Fv/Fm) of leaves, outer and inner bracts of ‘Wirrimbirra White’ waratahs at the mature flower (MF) stage of development. LSD = 0.078. n = 5 plants.

Experiments that revealed chronic photoinhibition in exposed waratah bracts were repeated again at Mount Annan in 2003 (section 6.3.5). These experiments were used to determine whether photoinhibition in ‘Fire and Brimstone’ was consistent from year to year over three different light treatments – sun, early shading and late shading – at three stages of floral development and to measure a second cultivar ‘Olympic Flame’. The experiment also aims to quantify differences in Fo and Fm across different cultivars, tissues and light treatments at the mature flower stage.

6.3.5 Quantum yield measurements at Mount Annan 2003

6.3.5.1 Method

In 2003, fluorescence responses were measured on ‘Fire and Brimstone’ and ‘Olympic Flame’ waratahs. Leaf measurements were made with a leaf clip as in 2002, while bract measurements were made with a dark leaf clip (Dark Leaf Clip DLC-8, Heinz Walz GmbH, Effeltrich, Germany) to keep sensor orientation and distance constant, except for bracts at TB stage, as the dark leaf clip was unavailable at this time.

In this case, measurements were made by holding the sensor head as still as possible and close to but not touching the bract surface. Predawn measurements were made in the hour preceding and half hour following sunrise. TB and MF stage measurements were made in the field, while JO stage measurements were made in the lab, following predawn harvest and holding in a darkened room. Field and lab measurements on leaves and bracts showed a strong relationship ($R^2 = 0.8361$), suggesting that the location of predawn measurements did not significantly influence the results (Appendix Figure A4.3).

For each plant at each stage, two areas either side of the midrib on each of two leaves were selected, as well as one distal area on each of four bracts. Where distinct brown areas were noted, measurements were made on both brown and non-brown areas of the bract. At the TB stage, only outer bracts and leaves were able to be measured without destructive harvest, while at JO and MF stages, inner bracts, outer bracts (if not fallen off) and leaves were measured. Due to these missing values, and other missing values at later stages of measurement for ‘Fire and Brimstone’, data were analysed in four subsets: (1) TB stage of flower development to compare leaves and outer bracts of both ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars; (2) JO stage of flower

development for ‘Olympic Flame’, to compare all treatments and all tissues; (3) JO and MF stages for ‘Olympic Flame’, to compare inner and outer bracts over all treatments at both stages and (4) Fo and Fm data from the MF stage of flower development to compare sun and late shading treatments across all tissues of ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars .

6.3.5.2 Results

The quantum yield of leaves and outer bracts in all treatments was compared at the early (TB) stage of flower development. Light treatment had a significant effect ($P < 0.001$) on the quantum yield of outer bracts, with sun quantum yield $<$ early shading $<$ late shading (Figure 6.15). Leaves had a significantly higher quantum yield ($P = 0.016$) than outer bracts of both Olympic Flame and Fire and Brimstone cultivars (Figure 6.16). Leaves were not affected by light treatment (Figure 6.15).

Leaves and outer bracts of ‘Olympic Flame’ waratahs had a significantly higher quantum yield than inner bracts at the intermediate (JO) stage of flower development ($P < 0.001$, Figure 6.17). *Tissue by Treatment* interactions and the *Treatment* main effect were not significant, although quantum yield was reduced in sun bracts compared to early and late shaded bracts (Figure 6.18).

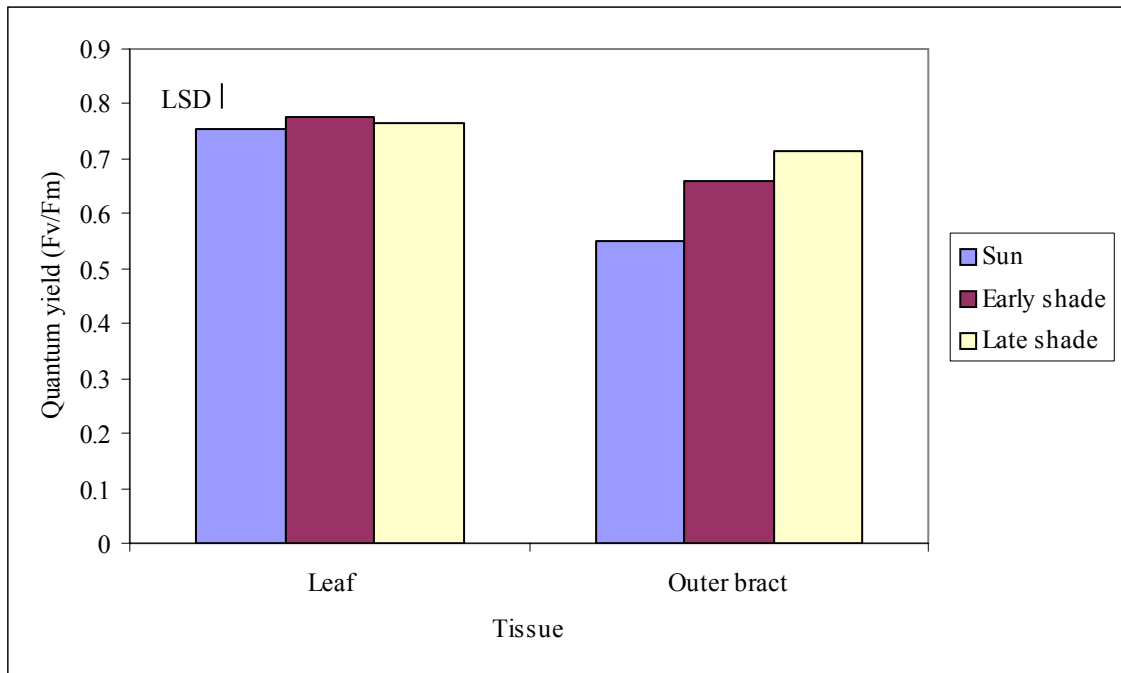


Figure 6.15: Quantum yield (Fv/Fm) of leaves and outer bracts combined for ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars for each light treatment (sun, early and late shade) at the tight bud (TB) stage. LSD = 0.049. n = 3-4 plants for each cultivar, treatment and tissue combination.

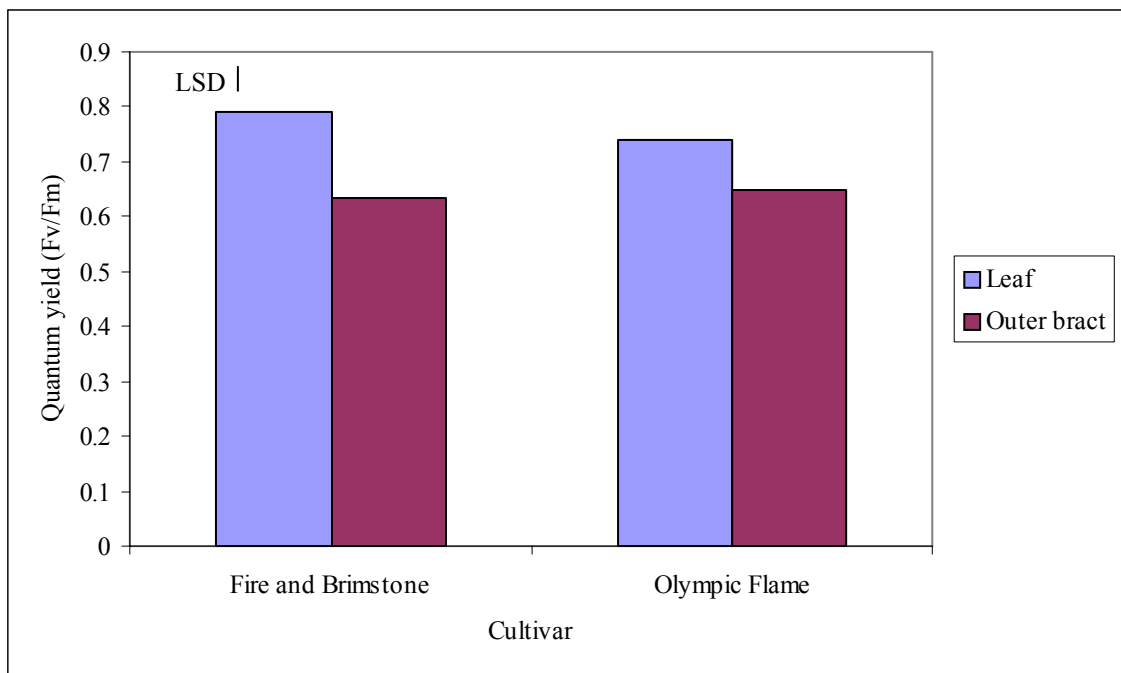


Figure 6.16: Quantum yield (Fv/Fm) of ‘Fire and Brimstone’ and ‘Olympic Flame’ leaves and outer bracts pooled across all light treatments at the tight bud (TB) stage. LSD = 0.039. n = 3-4 plants for each cultivar, treatment and tissue combination.

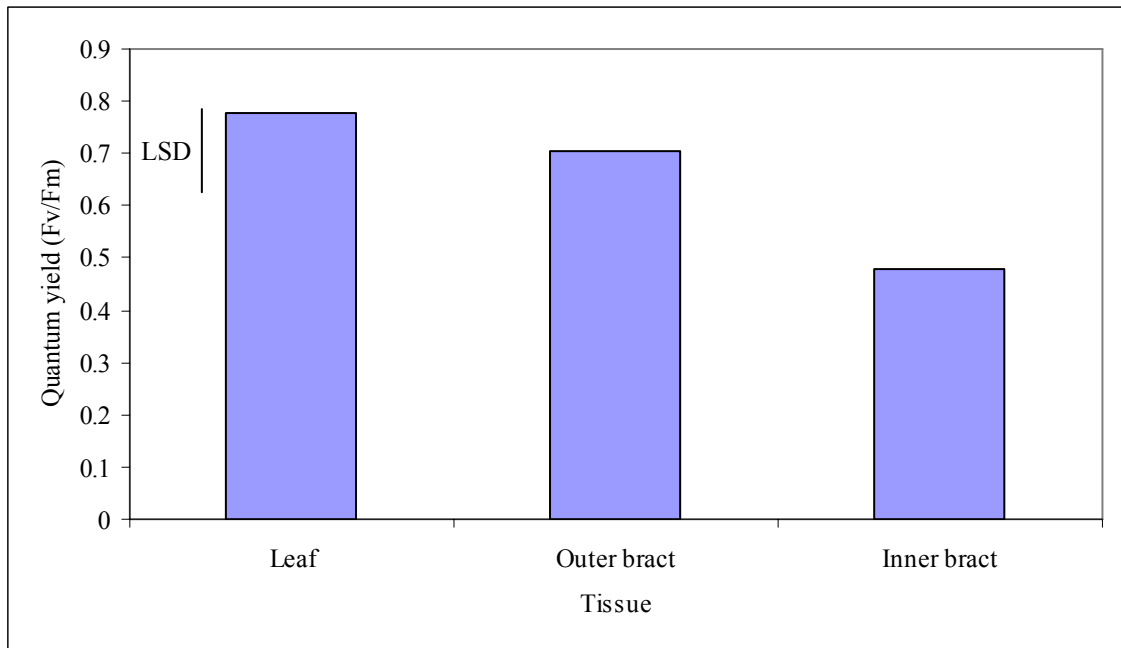


Figure 6.17: Quantum yield (Fv/Fm) of leaf, outer and inner bract tissues on ‘Olympic Flame’ waratahs, pooled across all treatments at the juvenile open flower (JO) stage. LSD = 0.130. n = 2 plants for each cultivar, treatment and tissue combination except for tissues of ‘Olympic Flame’ in the shade with n = 1.

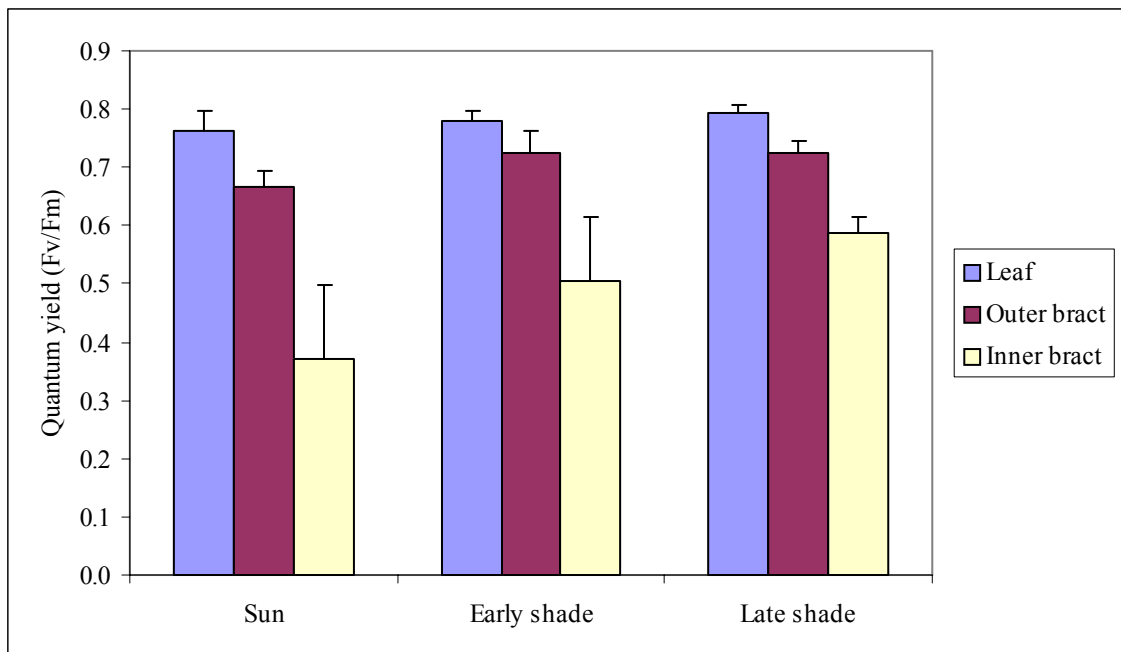


Figure 6.18: Quantum yield (Fv/Fm+ SE mean for each tissue and treatment combination) of leaf, outer and inner bract tissues on ‘Olympic Flame’ waratahs in each treatment (sun, early and late shade) at the juvenile open flower (JO) stage. n = 2 plants for each cultivar, treatment and tissue combination except for tissues of ‘Olympic Flame’ in the shade with n = 1.

0

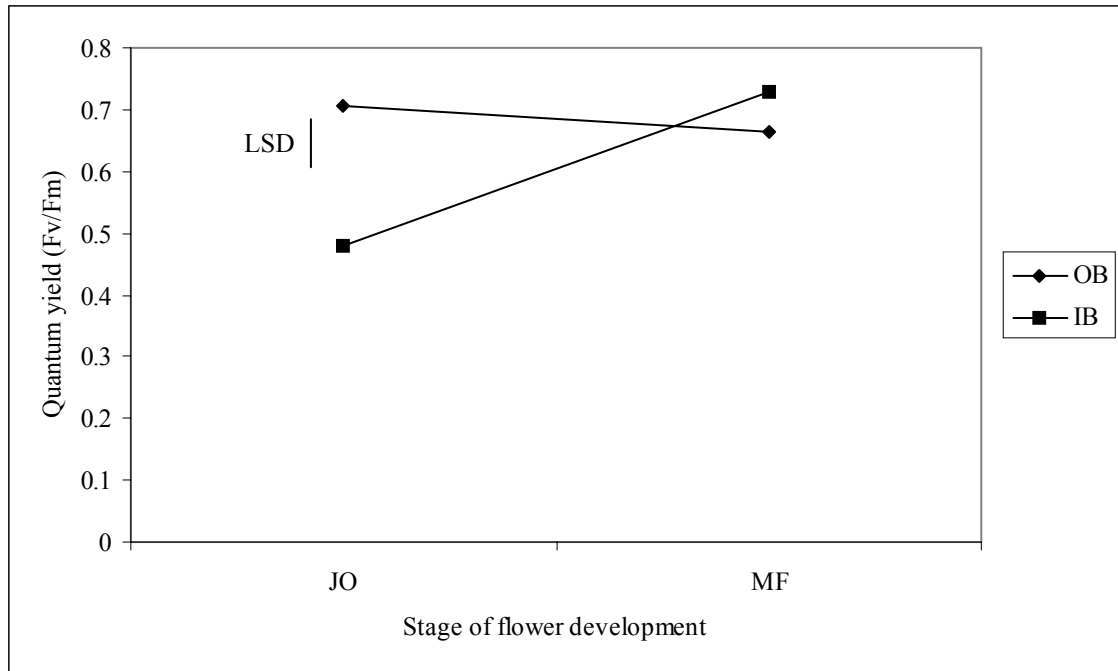


Figure 6.19: Differences in quantum yield (Fv/Fm) of inner and outer bracts of 'Olympic Flame' waratahs between juvenile open (JO) and mature flower (MF) stages. LSD = 0.094. n = 2-4 plants for each cultivar, treatment and tissue combination except 'Olympic Flame' tissues at JO stage and outer bracts at MF stage in the shade, all with n = 1.

Between the intermediate (JO) and final (MF) stages of flower development, outer bract quantum yield decreased slightly, while inner bract quantum yield increased significantly ($P < 0.001$, Figure 6.19).

Responses in quantum yield (Fv/Fm) and its components, minimum and maximum fluorescence yield (Fo and Fm), were investigated at flower maturity. Significant interactions in quantum yield were found between Tissue and Cultivar ($P = 0.031$) (Figure 6.20) and between Tissue and Treatment ($P = 0.002$) (Figure 6.21). Leaves had a higher quantum yield than bracts, although differences in the quantum yield of inner and outer bracts of different cultivars were not significantly different (Figure 6.20). Leaf tissue remained unaffected by shading, although in the sun the quantum yield of both inner and outer bracts was significantly lower than that of leaves (Figure 6.21).

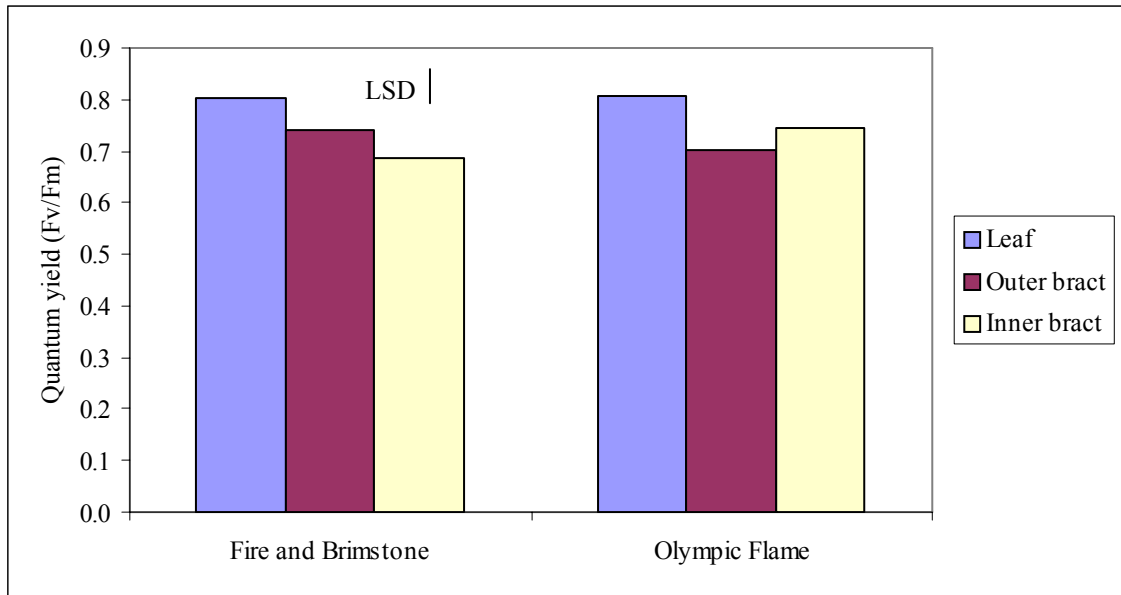


Figure 6.20: Quantum yield (Fv/Fm) of leaves, outer and inner bracts in 'Fire and Brimstone' and 'Olympic Flame' cultivars pooled across light treatments at the mature flower (MF) stage. LSD = 0.077. n = 2-4 plants for each cultivar, treatment and tissue combination except 'Fire and Brimstone' outer bracts in the sun, 'Fire and Brimstone' leaves and inner bracts in early shade, and 'Olympic Flame' outer bracts in the shade, all with n = 1. 'Fire and Brimstone' early shade outer bracts not measured at MF stage.

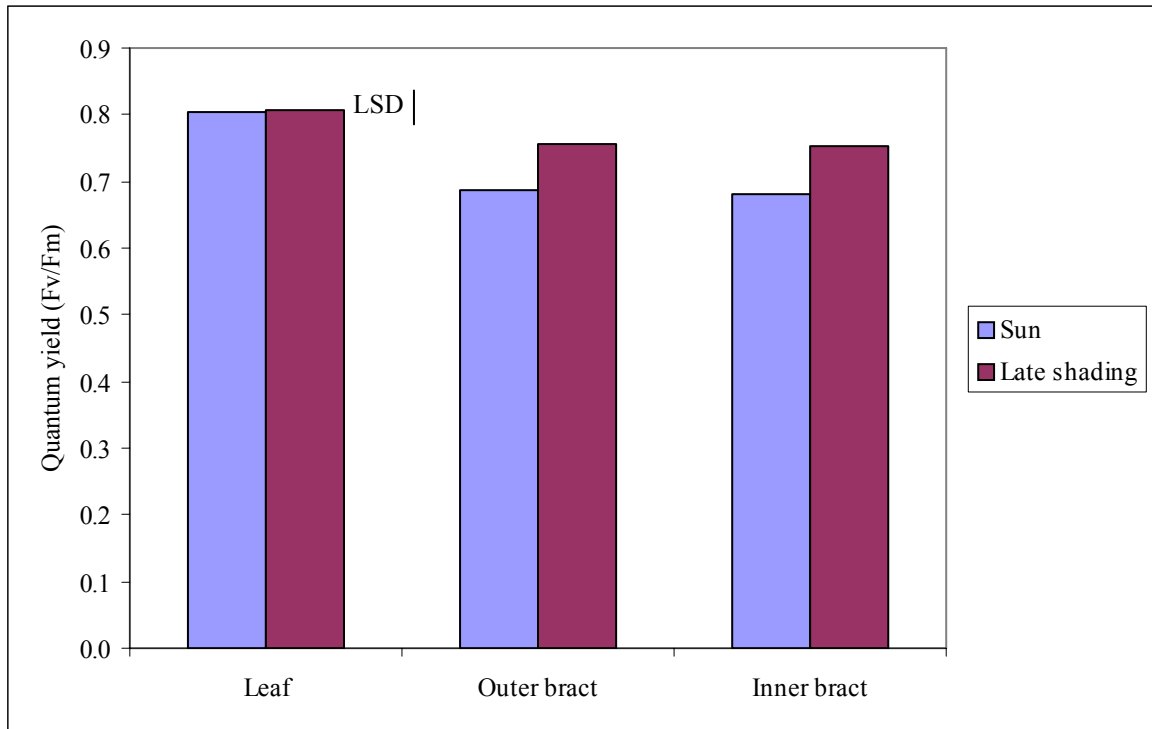


Figure 6.21: Quantum yield (Fv/Fm) of leaves, outer and inner bracts in sun and late shading treatments, pooled across 'Fire and Brimstone' and 'Olympic Flame' cultivars at the mature flower (MF) stage. LSD = 0.076. n = 2-4 plants for each cultivar, treatment and tissue combination except 'Fire and Brimstone' outer bracts in the sun, 'Fire and Brimstone' leaves and inner bracts in early shade, and 'Olympic Flame' outer bracts in the shade, all with n = 1. 'Fire and Brimstone' early shade outer bracts not measured at MF stage.

The minimum predawn fluorescence yield (F_o) of tissues at flower maturity varied significantly with treatment ($P = 0.021$) and tissue type ($P < 0.001$). The maximum fluorescence yield (F_m) showed similar significant trends, with treatment ($P = 0.008$) and tissue type ($P < 0.001$). Sun tissues had a significantly lower minimum and maximum fluorescence yield than shade tissues (Table 6.3). Outer bracts had a significantly higher minimum and maximum fluorescence yield than inner bracts or leaves (Table 6.4).

Table 6.3: Minimum (F_o) and maximum (F_m) predawn fluorescence yield of sun and late shaded tissues at flower maturity, pooled across ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars.

Fluorescence parameter	Sun	Late shading	LSD value
F_o	0.226	0.273	0.039
F_m	0.864	1.230	0.033

Table 6.4: Minimum (F_o) and maximum (F_m) predawn fluorescence yield of leaf, outer and inner bract tissues at flower maturity, pooled across ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars and sun and late shading treatments.

Fluorescence parameter	Leaf	Outer bract	Inner bract	LSD value
F_o	0.165	0.375	0.208	0.053
F_m	0.859	1.489	0.793	0.480

The differences in quantum yield, particularly at the juvenile open stage, indicate that chronic photoinhibition is occurring in waratah bracts. The variability of dark-adapted fluorescence parameters in ‘Olympic Flame’ leaves and bracts is now investigated further at the juvenile open (JO) stage of development. The experiments will also determine whether quantum yield differences exist between sun and shade treatments over a larger area of bracts than is visibly browned. In the following experiment (6.4), macroscopic chlorophyll fluorescence imaging is used, allowing for quantitative, non-destructive mapping of photosynthetic heterogeneity in leaves and other tissues (Genty and Meyer, 1994; Buschmann *et al.*, 2000).

6.4 Imaging-PAM fluorescence measurements of F_o , F_m and quantum yield (F_v/F_m)

6.4.1 Method

'Olympic Flame' waratahs at the JO (juvenile open) stage were harvested on 10/9/03, cooled and transported to the lab for measurement the following day. The Imaging-PAM chlorophyll fluorometer, like the PAM-2000 fluorometer, was used to apply a pulse of saturating light in order to measure fluorescence parameters. However, the Imaging-PAM captures fluorescence information from an area of tissue and displays it as a colour image, with colours coding for numerical values between 0 (black) and 1 (purple) (see colour bar Figure 6.23). The blue LED lamps of the Imaging PAM, with peak emission at 470 nm, were used to supply measuring light (intensity 3), actinic light (intensity 3) and saturating pulses (intensity 10).

Bracts and leaves from waratahs grown in the sun or shade were selected and mounted on a 26 x 34 mm frame to keep the tissue flat. For each tissue, 6-8 areas of interest (1.3 mm², averaging values from approx. 20 pixels) including undamaged areas, brown areas and areas adjacent to browning were defined for collection of numerical data from images. Images of near infra-red (NIR) absorbance, dark-adapted F_o , F_m , quantum yield (F_v/F_m) and light curves were recorded as .pim files and later exported as .jpg images. NIR images show the reflectance of light at 780 nm that is 'not absorbed by photosynthetically active pigments' (Heinz Walz, 2003). Therefore, NIR images are only used to visualise the margins of the tissue in the frame (black areas in NIR images of Figures 6.23 and 6.24) and indicate areas of low chlorophyll content (ie brown areas). Light absorption estimates using the ratio of red (R) to near infra red (NIR) light re-emitted are likely to be incorrect for anthocyanin-containing tissue (Heinz Walz, 2003), such as waratah bracts and are not presented here.

6.4.2 Results

Analysis of numerical data from defined areas of interest revealed significant *Tissue* by *Treatment* interactions ($P < 0.001$). However, these differences were not statistically significant for the smaller number of sub-samples measured using the PAM-2000 (Figure 6.18). Bracts in the sun had a significantly lower quantum yield than bracts in the shade or leaves in either light environment (Figure 6.22). This lower quantum yield is seen in fluorescence images as a higher proportion of green, yellow and black areas (decreasing yield) in sun bracts (Figure 6.23) compared to blue areas (high yield) in shade bracts and leaves (Figures 6.24 and 6.25).

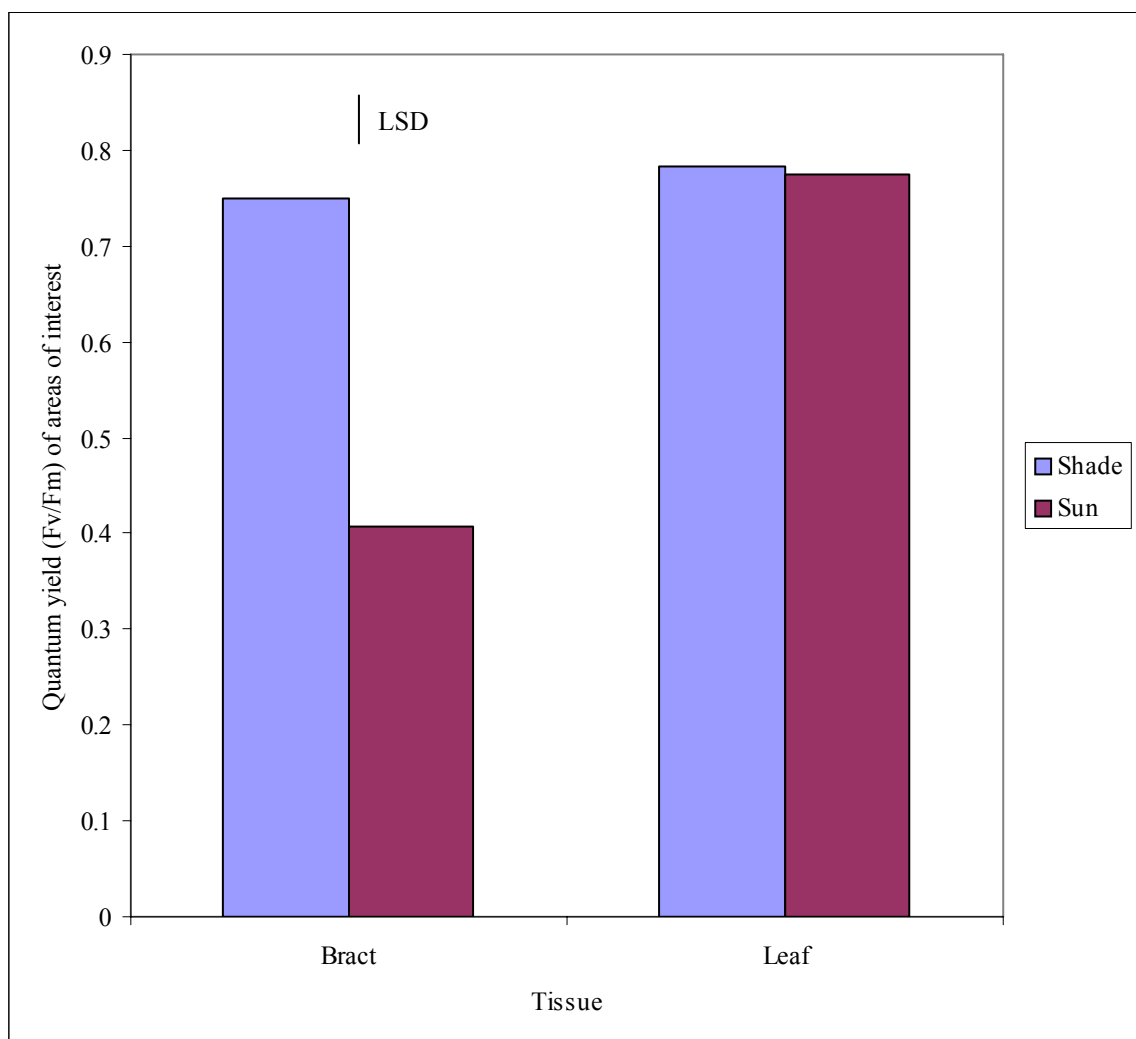


Figure 6.22: Quantum yield (Fv/Fm) of bracts and leaves of 'Olympic Flame' waratahs grown in the shade or sun, and harvested at the JO stage of development. LSD = 0.099. n = 2 plants in shade and 2 in sun, with 2-4 bracts measured from each plant.