6.6 Discussion

Both chronic and dynamic photoinhibition occur in waratah bracts in full sun. Photoinhibition can be reduced by shading plants, either from flower initiation in late summer or from bud expansion in late winter. In contrast, waratah leaves are subject to dynamic photoinhibition throughout the day, but do not develop chronic photoinhibition in either shaded or sun-exposed environments.

6.6.1 Shade can reduce photoinhibition in waratah bracts

Waratah bract tissues exposed to the sun had a significantly lower quantum yield than early or late shaded tissues, indicative of chronic photoinhibition (Figure 6.11). Waratah bracts were unable to maintain efficient photosynthesis in full sun, suggesting that they are adapted to the low light intensity provided by the shade environment, rather than the full sun environment. Similarly, when shade adapted Chilean guava plants are grown in full sun, leaves attain a maximum quantum yield of 0.6, compared to 0.8 or above in shaded leaves. Understorey or sub-canopy *Illicium* spp. (star anise) also suffer from reduced net CO_2 assimilation in full sun environments, resulting in bleaching, necrosis and, in the most susceptible species, plant death (Olsen *et al.*, 2002). The sclerophyllous shrub *Heteromeles arbutifolia*, also exhibits a chronic decrease in quantum yield of leaves, indicating photoinhibition, when grown in full sun (Valladares and Pearcy, 1997). Quantum yield for sun leaves of *Heliconia* falls within the range of 0.643 - 0.781, with visible yellowing, compared to 0.8 or above for intermediate and deep shade leaves (He *et al.*, 1996). However, in waratahs, leaf tissue is more photosynthetically resilient than bract tissue.

Therefore, shade can be used to reduce chronic photoinhibition in susceptible tissue, such as waratah bracts. In other crops, such as *Dendrobium*, photosynthetic rates are maximised in the vegetative stage by growing plants under higher light. Plants are then moved to a lower irradiance once flowers are inititated to prevent photodamage (He *et al.*, 1998). Chilean guava plants are also susceptible to photoinhibition, and Pastenes *et al.* (2003) recommend growing plants under shade or as an intercrop under tree canopies to prevent serious photoinhibition. Shade also prevented dynamic photoinhibition of tea plants during the day, although chronic photoinhibition was not present (Mohotti and Lawlor, 2002). Some species of *Illicium* (star anise) used in landscaping are highly susceptible to photoinhibition and photooxidative bleaching, which can be prevented by growing plants in a shaded environment (Olsen *et al.*, 2002). Thus, waratah bract quality may be improved by growing plants in the shade, at least from bud opening onwards, to minimise photoinhibition and subsequent visible damage.

6.6.2 Differences in optical properties and photoinhibition of waratah bracts and leaves

Waratah bracts were found to reflect and transmit more light and absorb less light than leaves. However, this adaptation did not prevent bracts from absorbing more light than they could utilise in photosynthesis or dissipate via non-photochemical quenching. Although leaves absorbed more light, they also had a higher quantum yield than bracts and were able to maintain a higher effective quantum yield during the day.

6.6.2.1 Optical properties

Waratah leaf absorbance was much higher than expected, with a mean of 95%, compared to approximately 85% absorbance of leaves of many other species. Absorbance may be higher due to the relatively thick leaves of waratah, as thick leaves tend to absorb more light than thin leaves, as light entering leaves is attenuated with depth (Atwell *et al*., 1999 p. 26). However, another species of Proteaceae, *Macadamia*, is noted as having a high transmittance (implying a lower absorbance) despite relatively thick leaves (Syvertsen *et al.*, 1995).

Waratah bracts had a lower absorbance than leaves (62-76%, compared to 95%), which may be a tissue age effect, due to bract tissue developing later than leaf tissue. For example, young apical grapevine leaves had an absorbance of 60-70%, while mature non-senescent leaves had an absorbance of 85-90% (Schultz, 1996). Similarly, *Syzygium* leaves at 50% expansion absorb 5-20% less PAR than mature leaves, which absorb more than 80% PAR (Woodall *et al.*, 1998).

The unusual high light absorbance of waratah leaves and lower absorbance of bracts means that calculations of electron transport rate (ETR, Equation 6.5) assuming 84% absorbance (for example, Heinz Walz, 1993) are not accurate for waratah leaves or bracts. While electron transport rate was not calculated during this project, in future experiments the absorbance of leaves and bracts will need to be measured for accurate calculation of ETR.

Waratah leaf absorbance and reflectance were independent of light environment, as observed by Schultz (1996) for grapevine leaves, suggesting similar chlorophyll contents. However, the optical properties of waratah bracts varied with light treatment. Differences between leaves and bracts may be due to different pigment profiles, as bracts have less chlorophyll than leaves and contain higher concentrations of anthocyanins (Chapter 7). Woodall *et al.* (1998) found that anthocyanin-containing *Syzygium* leaves at 50% expansion had higher absorbance and reflectance than green leaves of the same age. Higher absorbance in the green region of the spectrum was also noted for red Coleus leaves compared to green varieties (Burger and Edwards, 1996) and senescing dogwood leaves accumulating anthocyanins compared to those not accumulating anthocyanins (Feild *et al.*, 2001). Neill and Gould (1999) found that the concentration of anthocyanins, rather than their location, had a greater impact on optical properties of *Quintinia serrata* leaves.

6.6.2.2 Quantum yield and photoinhibition

Waratah leaves had a consistently higher quantum yield than bracts (Figures 6.12, 6.13, 6.16, 6.17, 6.20 and 6.21). Waratah bract tissue was also more severely affected by the sun than leaf tissue (Figures 6.14, 6.15, 6.20 and 6.21). Similarly, leaves of *Dendrobium* orchid were also found to be more photosynthetically efficient than floral tissues (Khoo *et al.*, 1997) and *Petunia* corollas had a lower quantum yield than leaves (Weiss *et al.*, 1988). *Dendrobium* leaves were also more resilient than sepals or petals when exposed to high light, maintaining a higher quantum yield before dawn and effective quantum yield throughout the day (He *et al.*, 1998). Intermediate light or shade protected *Dendrobium* sepals and petals from the symptoms of photoinhibition (decreased quantum yield,

bleaching and wilting) that occurred in full sun (He *et al.*, 1998), similar to the protection of waratah bracts on plants grown under 50% shade cloth.

Outer waratah bracts were susceptible to photoinhibition from the early stages of flower development, indicated by a reduction in quantum yield (Figure 6.12). Inner bracts showed severe decreases in quantum yield at the intermediate (JO) stage of development (Figures 6.13 and 6.17), indicating susceptibility to photoinhibition when inner bracts are no longer protected by outer bracts. Similar decreases in quantum yield during flower development were observed for floral tissues of *Dendrobium* orchid (Khoo *et al.*, 1997), with the greatest differences apparent between the TB and JO stages. The most significant differences between waratah leaves and bracts were observed at the JO stage of development (Figures 6.13 and 6.18), suggesting that bracts at the intermediate stage of development are particularly susceptible to light damage.

At the TB stage, inner bracts not yet exposed to light or other environmental stressors maintained a high quantum yield, similar to that of leaves (Figure 12). This appears to be similar to the protection of developing conifer needles by surrounding bud scales (DeLucia *et al.,* 1992). However, outer bracts that were not protected during development had a significantly lower quantum yield than leaves. The lower quantum yield of outer bracts compared to other tissues may be senescence related, as outer bracts often abscise particularly in the sun (Chapter 5). Wingler *et al.* (2004) found that quantum yield declined in senescing *Arabidopsis* leaves.

Inner bracts of waratah may function similarly to shade tissues, as they are shielded from light by the outer bracts during early development. For example, Weiss *et al.* (1988) suggested that Petunia corollas have a similar photosynthetic response to shade plants. This parallel may be extended to waratah bracts, particularly inner bracts, which are enclosed by the outer bracts until the JO stage of floral development, and thus, are shaded. Petunia corollas had large photosynthetic units (that is, a low concentration of reaction centres per chlorophyll) (Weiss *et al.*, 1988). Such large photosynthetic units may be needed early in flower development, if the photosynthetic system is required to operate while the corolla is still covered by the sepals (and therefore light intensity reaching the corolla is significantly reduced). Larger antennae would also predispose waratah inner bract tissue to photoinhibition, as more light would be absorbed in excess of photosynthetic requirements, particularly in full sun. The size of the chlorophyll antennae is approximated by the ratio of chlorophyll *a* to *b* (Neidhardt *et al.*, 1998), and will be discussed further in Chapter 8.

Waratah bracts have a similar quantum yield to floral tissues such as sepals or petals of other species, possibly due to their colouration (Chapter 8) and, for inner bracts, early protection by outer bracts. Waratah bracts, particularly those grown in the sun, had a reduced quantum yield and these bracts maintained a lower effective quantum yield throughout the day than leaves. They also required lower light intensities for saturation of photosynthesis, particularly at the intermediate (JO) stage of development. Waratah bracts, like *Dendrobium* sepals (Khoo *et al.*, 1997), showed greater decreases in quantum yield and photochemical quenching (q_P) as irradiance increased. Bracts also reached saturation of non-photochemical quenching (qN) at a lower light intensity compared to leaves, comparable to Petunia petals (Weiss *et al.*, 1988) and *Dendrobium* sepals (Khoo *et al.*,

1997). Inner bracts appeared to experience high levels of stress once they are exposed to the environment, particularly in full sun, which was evident as a significantly reduced quantum yield and chronic photoinhibition.

6.6.3 Linking changes in quantum yield (Fv/Fm) to its components of minimum fluorescence yield (Fo) and maximum fluorescence yield (Fm)

The reduction in quantum yield of photoinhibited waratah bract tissue in the sun (Figure 6.21) is a consequence of reduced minimum and maximum fluorescence yield (Fo and Fm respectively) (Table 6.3). This decrease in quantum yield as a result of reduced maximum fluorescence yield (Fm) is expected in photoinhibited tissue, with Wingler *et al.* (2004) suggesting that the maximum fluorescence yield is a measure of the capacity to reduce the electron acceptor. Trends in the minimum fluorescence yield (Fo) are more difficult to interpret, and Lovelock *et al.* (1994) found that changes in Fo of tropical species exposed to high light differ between species. Many authors suggest that the minimum fluorescence yield generally increases as quantum yield decreases during photoinhibition (Balachandran and Osmond, 1994, Maxwell and Johnson, 2000, Bertamini and Nedunchezhian, 2003), although decreases in minimum fluorescence yield have been attributed to senescence in kangaroo paw (Miranda *et al.*, 2000) and *Arabidopsis* (Wingler *et al.*, 2004). Lang *et al.* (1998) noted that a decrease in chlorophyll results in decreased minimum and maximum fluorescence yield.

Outer bracts have a significantly higher minimum and maximum fluorescence yield than inner bracts and leaves (Table 6.4), although quantum yield is lower than leaves. The increased minimum fluorescence yield in bracts compared to leaves may indicate larger

photosynthetic units (light harvesting antennae), similar to Petunia corollas (Weiss *et al.*, 1988). An increase in minimum fluorescence yield, as observed in outer bracts, has also been variously attributed to an increase in the number of permanently closed reaction centres (Lovelock *et al.* 1994), increased photoinhibitory damage to the D1 protein and other reaction centre components (Balachandran and Osmond, 1994; Bertamini and Nedunchezhian, 2003) and the release of free chlorophyll from pigment-protein complexes (Wingler *et al.*, 2004). Leaves, with their higher concentration of chlorophyll (Chapter 8) and lower minimum fluorescence yield, are likely to have smaller photosynthetic units than bracts. The minimum fluorescence yield is generally accepted as an emission by antenna chlorophyll *a* molecules (Krause and Weis, 1991), and thus reflects the size of the antenna chlorophyll (Lovelock *et al.*, 1994). The higher maximum fluorescence yield in outer bracts of waratah suggests photosynthetically efficient tissue, although quantum yield values do not support this observation, particularly in comparison with leaves (Figure 6.21).

Variability in minimum and maximum fluorescence yield values under different light treatments were further explored through fluorescence imaging measurements (Experiment 6.5.5). The minimum fluorescence yield was not significantly affected by sun and shade treatments in waratah leaves and bracts at the juvenile open (JO) stage, in contrast to measurements of the same parameters at flower maturity (Tables 6.3 and 6.4). The variability of minimum and maximum fluorescence yield was relatively high for undamaged areas (Figure 6.23), indicating that some of these areas may be suffering from chronic photoinhibition without showing visible symptoms.

Sun bracts of waratah at the juvenile open stage exhibited a decrease in quantum yield in the centre of brown areas, as a consequence of reduced minimum fluorescence yield as well as maximum fluorescence yield (Table 5). A decrease in minimum and maximum fluorescence yield has also been noted in the senescing outer leaves of *Arabidopsis*, resulting in a drastic reduction in quantum yield (Wingler *et al.*, 2004). Both minimum and maximum fluorescence yield were reduced in black areas of blackleaf affected grapevine leaves, compared to green portions of leaves (Lang *et al.*, 1998). Balachandran *et al.* (1994) noted a decrease in fluorescence intensity in areas of tobacco leaves affected by tobacco mosaic virus. A more severe decrease in fluorescence at the edge of affected areas, as noted by Balachandran *et al.* (1994), was not found in areas adjacent to bract browning in waratahs. The variability in fluorescence parameters was found to increase as *Arabidopsis* leaves senescence, caused by variations between and within individual leaves (Wingler *et al.*, 2004). However, waratah leaves in the sun did not have significantly different quantum yield (Figure 6.22), minimum or maximum fluorescence yield compared to leaves in the shade. These results contrast those of Lichtenthaler *et al.* (2000), whose group used chlorophyll fluorescence imaging to demonstrate a higher yield in sun leaves of beech, compared to shade leaves.

6.6.4 Effective quantum yield and non-photochemical quenching during the day

Waratah leaves were able to maintain a higher effective quantum yield during the day than bracts, indicating a lower susceptibility to photoinhibition. However, waratah bracts had a higher capacity than leaves for harmless dissipation of excess light via non-photochemical quenching. For example, the effective quantum yield of bracts did not decrease significantly from mid-morning (Figure 6.29), coinciding with attainment of maximal non-

photochemical quenching (NPQ) (Figure 6.32b,c). However, the contribution of non-photochemical quenching and photoinhibition to decreased effective quantum yield could only be determined by measurements of dark-adapted tissues throughout day.

Tissue and treatment combinations that showed greatest recovery of effective quantum yield by late afternoon were more resilient to high light (for example, Figure 27), but comparisons need to be made with predawn values of quantum yield to determine whether damage is chronic (section 6.6).

Sun tissues of waratah had a higher capacity for harmless dissipation of light via non-photochemical quenching (Figure 6.32) and therefore lower effective quantum yield than shade tissues (Figure 6.30 and 6.31), although the low effective quantum yield in sun tissue may also result from photoinhibition. Shaded *Heteromeles* shrubs had a smaller reduction in the effective quantum yield of leaves during the day and significantly higher quantum yield before dawn than shrubs in full sun (Valladares and Pearcy, 1997), similar to shaded waratah bracts. The capacity of waratah bracts to dissipate heat via non-photochemical quenching (qN) may increase over time, as qN for saturation of bracts at the JO stage is lower than qN for saturation at the MF stage of flower development. However, changes in xanthophyll pool size occur in a time scale of days (Demmig-Adams *et al.*, 1997), so rapid exposure of shaded tissue to full sun, as occurs with bracts at the intermediate (JO) stage, may only allow part of the excess radiation to be dissipated.

Although leaf angle was not measured in this study, bracts are more vertically oriented and would be exposed to higher irradiance in the early morning, while horizontally oriented

leaves would be exposed to higher irradiance at midday. Murchie *et al.* (1999) found that quantum yield and photochemical quenching also decline sharply as tissue is exposed to increased irradiance, a property that is dependant on leaf angle. For waratahs, vertically-oriented bracts would receive maximal radiation between 0900 to 1000 hrs and horizontally oriented leaves would receive maximal radiation at 1300 hrs, corresponding with the periods of lowest effective quantum yield (Figure 6.29).

6.6.5 Effective quantum yield, non-photochemical (qN) and photochemical quenching (qP) to determine light intensities for likely damage

Leaf and bract effective quantum yield at flower maturity was similar at light intensities up to 100 μmol m^{-2} s^{-1} (Figure 6.33), however, leaves had a higher yield at higher light intensities (120-306 $\mu molm^{-2}s^{-1}$) (Figure 6.37). Similarly, Khoo *et al.* (1997) found that effective quantum yield was higher in *Dendrobium* leaves than in flower petals or sepals, although these responses were evident at a lower light intensity (50 $\mu molm^{-2}s^{-1}$).

Leaves and bracts at flower maturity had a similar capacity for photochemical (qP) and non-photochemical (qN) quenching at light intensities up to 306 μmol m^{-2} s^{-1} (Figure 6.38), indicating a similar capacity for photosynthesis and photoprotection by heat dissipation. However, Imaging PAM measurements at an earlier stage of development (JO stage) indicated that qP of bracts dropped to zero at 461 μmol m^{-2} s^{-1} and qN of bracts reached saturation between 100 and 200 μmol m^{-2} s^{-1}. Alternatively, bracts may be more susceptible to high light intensities at the intermediate (JO) stage of development.

Waratah bracts, like *Dendrobium* floral tissue, were not as efficient at utilising radiant energy as leaf tissue. The photochemical quenching (qP) of *Dendrobium* flowers dropped as low as 0.4 at 100 μmol m^{-2} s^{-1} (Khoo *et al.*, 1997), while waratah bract tissue exhibited more severe damage (qP of zero) at irradiances up to 461 μmol m^{-2} s^{-1} in both sun and shade bracts. Non-photochemical quenching (qN) of *Dendrobium* flower parts reached saturation at 100 μmol m^{-2} s^{-1} (Khoo *et al.*, 1997), as did waratah bracts in the sun at the JO stage. This suggests a similar low capacity for photoprotection above this intensity (Khoo *et al.*, 1997). However, at the MF stage waratah bracts did not reach a saturating qN up to 306 μmol m^{-2} s^{-1}, suggesting that they begin to acclimate to higher light intensities.

Although non-photochemical quenching of waratah bracts appeared lower than that of leaves when measured as qN (Figure 6.35), values of NPQ over a day were higher for bracts (Figures 6.31b,c) than leaves (Figure 6.32a). This apparent contradiction may be due to the differing predawn quantum yield of bracts and leaves, which provide a reference point for qN and NPQ (Maxwell and Williams, 2000).

Leaves were more resilient than bracts at light intensities up to 461 μmol m^{-2} s^{-1}, with photochemical quenching (qP) of about 0.3 and an effective quantum yield of about 0.15. 'Olympic Flame' leaves have lower qP and qN than 'Fire and Brimstone' leaves, even at low light intensities (Figure 6.34). This indicates fewer PSII reaction centres were available to accept electrons and thus, possible photoinhibition on exposure to light. However, quantum yield values for 'Olympic Flames' leaves were similar to those of 'Fire and Brimstone' (Figure 6.20), indicating that the photoinhibition was not chronic.

6.6.6 Damage and repair to PSII

Even when damage to PSII occurs through chronic photoinhibition, repair is possible, as "unlike Humpty Dumpty, PSII can be put together again with relatively little energy cost" (Anderson and Chow, 2002). The PSII repair cycle involves migration of damaged PSII centres without their light harvesting complexes (LHCII) out of the granal thylakoids and migration of undamaged but inactive PSII centres without LHCII into the stromal lamellae (Demmig-Adams and Adams, 1992). Non-functional PSII centres possessing D1 protein accumulate in stacked grana (Anderson and Aro, 1994), allowing for restoration without disassembly of PSII on return to non-stressed conditions (Anderson *et al.*, 1997). Shade plants tend to accumulate more non-functional PSII's that have a protective capacity (Anderson and Aro, 1994) but have a lower D1 turnover (Anderson *et al.*, 1997). However, high light intensities limit the degradation and biosynthesis of the D1 protein (Melis, 1999). Thus, repair of PSII is likely to occur only at low light intensities (29 μmol photons $m^{-2}s^{-1}$ in *Capsicum* leaves) (He and Chow, 2003), so opportunities for reassembly of PSII are limited.

Thus, at high light intensities photoinhibition can occur more quickly than PSII repair, resulting in chronic photoinhibition and oxidative damage. Absorption of excess light leads to increases in strong oxidants in the thylakoids, while hydrogen peroxide and superoxide in the stroma tend to deactivate enzymes through oxidation of thiol functional groups (Asada, 1994). Visible symptoms of light-induced oxidation include yellowing/pigment bleaching (He *et al.*, 1998), development of necrotic lesions (Lang *et al.*, 1998; Koniger *et*

al., 1998) and senescence (Schiefthaler *et al.*, 1999), as seen in sun-exposed bracts (Chapter 10).

6.6.7 Fluorescence measurement techniques for waratah tissues

Changes in predawn and diurnal fluorescence yield were monitored using a pulse-modulated fluorometer (Teaching PAM or PAM 2000). This technique allowed for rapid and non-destructive probing of PSII function, as quantum yield (Fv/Fm) was linearly related to the quantum yield of oxygen evolution.

6.6.7.1 Relationship between quantum yield and functional PSII

A linear relationship was established between quantum yield and functional PSII for the waratah bracts studied, with the number of functional PSII centres increasing as quantum yield increases (Figure 6.9). Conversely, quantum yield and functional PSII declined with increasing exposure to high light intensities and, for samples at 10ºC, with limitation of D1 repair due to low temperatures. The relationship between functional PSII and quantum yield of oxygen evolution in shade bracts did not extrapolate to the origin (Figure 6.9), indicating that measurements of quantum yield may have underestimated changes in oxygen evolution (Balachandran and Osmond, 1994).

When quantum yield was normalised to the number of functional PSII present prior to photoinhibitory treatments, the relationship was less strong. This decrease may be due to variation in the initial functional PSII content, which ranges from 0.077 to 0.496 μmol PSII m^{-2}. Varying chlorophyll content in the bracts (Chapter 8) may account for this wide range

of functional PSII centres prior to treatment, particularly when compared to results for healthy leaves (generally 2 - 3 μmol PSII m^{-2}, W.S. Chow, personal communication). In future experiments, minimum fluorescence yield (Fo) could be normalized to allow for differing initial chlorophyll contents and functional PSII expressed as mmol PSII mol Chl^{-1}, as presented by Park *et al.* (1996).

The results may also be improved by measuring quantum yield after a longer period of dark adaptation, as some photoprotective processes (non-photochemical quenching) that reduce quantum yield below its maximum may remain. However, the period of dark relaxation (40 minutes after removal of samples from high light) is considered adequate for elimination of rapidly relaxing quenching (Maxwell and Johnson, 2000).

The nature of the relationship between functional PSII and quantum yield may vary between species and also within species for different growth conditions and treatments (Hendrickson *et al.*, 2003). For example in shaded waratah bracts, quantum yield may underestimate oxygen evolution under stress conditions. This corroborates measurements in other species, where oxygen evolution has been shown to be more sensitive than quantum yield as a measure of PSII function at high temperatures (Yamane *et al.*, 1998) and low photon exposure (Park *et al.*, 1996).

6.6.7.2 Fluorescence techniques

Fluorescence techniques were suitable for measurement of leaves and bracts, although both tissue types were moved from their natural orientation during field measurements, so incident PAR could not be accurately recorded along with quantum yield_and hence,

electron transport rates were not calculated. The high quantum yield of red inner bracts (Figure 6.12) shows that the anthocyanins responsible for bract pigmentation did not interfere with measurement of fluorescence parameters. However, tissues with a low chlorophyll concentration (for example, 'Wirrimbirra White' inner bracts) or high levels of browning did not register a fluorescence signal or evolve detectable concentrations of oxygen.

The definition of a larger number of sample areas using fluorescence imaging meant that variability across bracts could be described with greater accuracy. Data captured from a larger number of areas of interest in Imaging PAM measurements also showed more significant trends than data captured from fewer data points using the PAM 2000. However, the geometry of the curved bracts had more influence in Imaging PAM measurements due to the larger area measured. Thus, interpretation of fluorescence images from bract b in Figure 6.23 and bracts b and d in Figure 6.24 are difficult to interpret, as the sample surface was not flat and measurable fluorescence intensity changed with the angle of irradiance and detection (Balachandran *et al.*, 1994, Buschmann *et al.*, 2000). For this reason, measurements from these bracts were not included in the data set analysed.

6.6.7.3 Variability in bract fluorescence measurements

The low quantum yield of 'Wirrimbirra White' inner bract tissue (Figure 6.14), similar to the results obtained in Experiment 6.2.2, shows that this tissue has an inherently low photosynthetic efficiency. Such tissue is likely to be highly susceptible to chronic photoinhibition; this may be the reason the fluorescence measurements were unable to be made on sun exposed inner bract tissue.

The quantum yield of inner bracts at the juvenile open stage (Figure 6.18) showed greater variability than other bract types and the number of measurements taken in this experiment was insufficient to reveal significant differences between treatments. However, imaging fluorescence data for this cultivar (Olympic Flame) at this stage (JO) supported data presented in Experiment 6.4, illustrated the variation of quantum yield across bracts and revealed significant differences between treatments.

Inner bracts showed a similar (Figure 6.12) or increased (Figure 6.18) quantum yield between the JO and MF stages of development. This response may be an artefact of measuring less exposed tissue following senescence of bracts measured at earlier stages. Alternatively this response may indicate repair of D1 protein leading to increased photosynthetic yield, adaptation of tissue to a higher light environment, or compensation of surrounding undamaged tissue. The yield response between the intermediate (JO) stage and flower maturity (MF stage) may be dependent on environmental conditions.

6.7 Conclusions from photoinhibition studies

Chronic photoinhibition (reduced quantum yield) was observed in inner and outer bracts of waratahs in 2002 and 2003, particularly when grown in a full sun environment. The reduction in quantum yield in sun tissues was a consequence of decreased minimum and maximum fluorescence yield, suggesting chlorophyll destruction or senescence. Shade cloth (50%) reduced chronic photoinhibition in ‘Fire and Brimstone’ and ‘Wirrimbirra White’ cultivars in 2002 and ‘Fire and Brimstone’, ‘Olympic Flame’ and ‘Wirrimbirra

White' cultivars in 2003. Waratah bracts showed greater decreases in effective quantum yield and photochemical quenching than leaves when exposed to light intensities up to 461 $\mu molm^{-2}s^{-1}$, and reached saturation of non-photochemical quenching at a lower light intensity than leaves.

Leaves had a higher quantum yield before dawn and effective quantum yield during the day, than bracts at all stages of flower development. Inner bracts had a high quantum yield while covered by outer bracts, although this decreased during flower development, as bracts were increasingly exposed to the environment. Inner bracts have similar photosynthetic responses to shade tissues and petals of other species. Outer bracts had suboptimal quantum yields even at early stages of flower development. The most significant reduction in quantum yield, relative to leaf values, was observed at an intermediate (JO) stage of flower development.

Fluorescence techniques were suitable for monitoring PSII function in waratah leaves and bracts. A positive linear relationship between the fluorescence parameter for quantum yield (Fv/Fm) and the quantum yield of oxygen evolution was established for waratah bracts.

There is a clear link between photoinhibition and the pigment content of floral tissues and leaves of other species, in that photoinhibition can leaf to pigment destruction but pigments can protect plant cells from photoinhibition. Hence, to understand the process of bract browning in waratahs, it is essential to examine the pigments in leaves and bracts at varying stages of development for different cultivars and light regimes. The results of these studies are reported in the next chapter (Chapter 7).