Chapter 8
Relationship between browning, photoinhibition and pigmentation

8.1 Introduction

This chapter describes the relationship between parameters affecting bract browning measured in Chapters 5-7, particularly the relationship between pigmentation, photoinhibition (as measured using chlorophyll fluorescence) and browning. The relationship between these factors and lipid peroxidation will be discussed in Chapter 9.

8.1.1 Chlorophylls, carotenoids and susceptibility to photoinhibition

Differences in the chlorophyll and carotenoid concentrations of waratah bracts and leaves may be directly related to their susceptibility to photoinhibition. However, the relationship between chlorophyll concentration and photoinhibition appears to differ with tissue age, and also with species. The consequences for photoinhibition of tissue with a low chlorophyll content are also interpreted differently by researchers. For example, Krause *et al.* (1995) suggest that the low chlorophyll content of young leaves in a tropical forest results in absorbance of a higher fraction of excess light compared to mature leaves, and greater susceptibility to photoinhibition, while Khoo *et al.* (1997) suggest that a low chlorophyll content in mature *Dendrobium* flowers leads to less absorbed radiation and minimisation of photoinhibition. Carotenoid pigments such as xanthophylls also have the ability to reduce photoinhibition, by quenching incoming excitation energy (Niyogi, 1999; Anderson and Chow, 2002) and promoting non-photochemical quenching (Demmig-Adams and Adams, 1992).

8.1.2 Anthocyanins, flavonoids and susceptibility to photoinhibition

Many authors have suggested that anthocyanins protect against photoinhibition, by intercepting light energy and reducing photooxidative damage. A layer of anthocyanins in the palisade mesophyll of senescing red-osier dogwood *(Cornus stolonifera*) leaves decreased light capture by chloroplasts (Feild *et al.*, 2001). By contrast, Gould *et al.* (2000) suggest that anthocyanins in the lowermost tissues may enhance light capture by internal reflection. Anthocyanins also reduce photoinhibition in several species. For example, anthocyanic (red-senescing) leaves of dogwood also absorbed more light between 495-644 nm and recovered from a high light stress treatment (1500 μmol m^{-2} s^{-1} for 30 mins), while yellow-senescing leaves with no anthocyanins did not recover (Feild *et al.* 2001). A reduction in photoinhibition was found in reddish-purple *Bauhinia* pods compared to green (Smillie and Hetherington, 1999), red leaves of *Begonia pavonina* and *Triolena hirsuta* (Gould *et al.,* 1995), red *Quintinia serrata* leaves (Gould *et al.,* 2000) and red *Quercus coccifera* leaves (Manetas *et al.,* 2003). However, anthocyanic leaves of some species appear to be more susceptible to photoinhibition than green leaves, including red juvenile *Syzygium* leaves, possibly due to their much lower photosynthetic capacity than green juvenile leaves (Dodd *et al.* 1998).

The reduction in light intensity and likelihood of photooxidative damage is termed photoabatement. In practice, it is difficult to separate photoabatement by anthocyanins from their antioxidant function (Gould *et al.,* 2002b), but Neill and Gould (2003) demonstrated that both photoabatement and scavenging of active oxygen species by anthocyanins operate independently in red lettuce leaves. Anthocyanins function as antioxidants through two mechanisms: (1) Chelation of anthocyanins to transition metals, especially copper and iron, and thus, a decrease in the formation of hydroxyl

radicals via the Fenton reaction and (2) A direct antioxidant action by donation of protons. Both anthocyanins and flavonoids have the ability to directly scavenge active oxygen species, including hydrogen peroxide, singlet oxygen, and superoxide, hydroxyl and peroxyl radicals, as well as peroxynitrite (Gould *et al.,* 2002b). The superoxide scavenging effect of anthocyanins and flavonoids are dependent on the number of hydroxyl groups on the B ring (Tsuda *et al.,* 1996). UV-induced lipid peroxidation was found to decrease following anthocyanin addition to a liposome system (Tsuda *et al.,* 1996).

Anthocyanins of red lettuce located in the cytosol reduced the concentration of superoxide (O_2^-) in the cytosol (Neill and Gould, 2003). Oxidised anthocyanins (aryloxyl radicals) are then formed and stabilised through electron delocalisation or self-association, preventing further radical reactions. Bleaching of anthocyanins can result from their non-enzymatic reaction with superoxide (Yamasaki *et al.,* 1996). Vacuolar anthocyanins are also likely to scavenge hydroperoxyl radicals or hydrogen peroxide, particularly under severe stress conditions (Neill and Gould, 2003). Gould *et al.* (2002) found that red regions of *Pseudowintera colorata* leaves maintained relatively low levels of H_2O_2 following mechanical injury, while green regions accumulated H_2O_2 for up to ten minutes. A band of anthocyanins surrounded the necrotic area during the ninety two hours after wounding, encircling the wounded region (Gould *et al.*, 2002).

8.1.3 Pigmentation, photoinhibition and bract browning

Brown areas of ‘Olympic Flame’ waratah bracts were found to have greater photoinhibition, evident as lower quantum yield (Fv/Fm), compared to green areas of sun bracts, when measured using an Imaging PAM fluorometer (Table 7.5). Browning

and necrosis of shade adapted and floral tissues are attributed to photoinhibition and pigment loss in many crops, for example, *Dendrobium* and *Illicium*, but are not often quantified or compared directly. The severity of bract browning in waratahs, measured as counts of brown bracts, may be related to photoinhibition (quantum yield, Fv/Fm) and pigmentation of inner and outer bracts. This will be explored in section 8. 2 using correlation techniques applied to experimental data collected at Mount Annan in 2002 and 2003 (section 8.2.2) and at Jervis Bay in 2002 (section 8.2.3).

8.2 Correlations between browning, photoinhibition and pigmentation

8.2.1 Statistical analysis

Measurements of browning, photoinhibition and pigmentation (total chlorophyll, total carotenoids and anthocyanins and UV-B absorbance) are described in Chapters 5-7, respectively. The data for each parameter was averaged over replicates, to obtain one value for each parameter for each tissue on each plant. Only inner and outer bract tissues (not leaves) were included in the analysis. Data were pooled over cultivars, sun and shade treatments and stages of sampling, as the effect of these 'treatments' was examined in detail in preceding chapters. Browning data described the severity of browning and senescence of floral bracts on one bud of each plant, rather than inner and outer bracts separately, so browning data was matched to both inner and outer bracts. Relationships between parameters were investigated using a correlation matrix in Genstat (Lawes Agricultural Trust, 2003). Scatter plot matrices were also constructed in Genstat to visualise the relationship between most of the parameters tested.

8.2.2 Relationship between photoinhibition, pigmentation and browning at Mount Annan

Data on pigmentation and quantum yield were pooled over sampling stages (TB, JO and MF in 2002 and JO and MF only in 2003) and treatments (sun, early and late shading) for the cultivar 'Fire and Brimstone' grown at Mount Annan in 2002 and 2003, as well as an additional cultivar 'Olympic Flame' in 2003. Browning and quantum yield (Fv/Fm) were negatively correlated (-0.564 in 2002, -0.242 in 2003), demonstrating that browning increased as quantum yield decreased (Figures 8.1 and 9.3). Hence, browning was positively correlated with photoinhibition, measured as a decrease in quantum yield.

However, the strength of the relationship between browning and photoinhibition varied from year to year. The stronger correlation in 2002 may be due to more severe bract browning and loss (maximum of 27 bracts) and lower quantum yields (below 0.4 in one instance), while in 2003, bract browning and loss was less severe (maximum of 15 bracts) and quantum yields were slightly higher (only one data point below 0.5). The variation in the severity of browning from year to year may be explained by environmental factors that alter the susceptibility of bracts to photoinhibition and browning, for example, changes in the proportion of light absorbed or the pool of electron chain components. Variation in browning severity has been noted in other physiological browning disorders such as tipburn from season to season and year to year (Collier and Tibbitts, 1982; Saure, 2001; Wissemeier and Zuehlke, 2002). Differences in response from one year to the next may be the result of including a second cultivar 'Olympic Flame' in the analysis, and only sampling later stages of flower development in 2003.

The correlation between browning and pigmentation also varied from year to year, with similar possible causes to those described above. In 2002, browning and quantum yield were not well correlated with the anthocyanin, carotenoid or chlorophyll concentration of bracts, showing that a low pigment concentration could result in either high or low photoinhibition and a large or small number of brown bracts (Figure 8.1).However in 2003, a strong relationship between quantum yield and pigmentation was revealed, with positive correlations between quantum yield and carotenoid concentration (0.522) and UV-B absorbing compounds (0.574). Total chlorophyll and anthocyanin concentrations showed a weaker positive correlation with quantum yield in 2003 (Figure 8.2). These results indicate a lower potential for photoinhibition at high pigment concentrations, with carotenoids and UV-B absorbing compounds having the greatest protective effect. Even in 2002, high pigment concentrations were unlikely to result in photoinhibition (low Fv/Fm) or large numbers of brown bracts, as there were few data points in these regions. Although browning tended to decrease as anthocyanins increased (Figures 8.1 and 9.3), the correlation between these parameters was not particularly strong and UV-B absorbing compounds such as flavonoids may play a more significant role in photoprotection, based on the strength of their correlation with quantum yield (Figures 8.2 and 9.3)

Total chlorophyll was strongly correlated with total carotenoid concentration in both years (0.909 in 2002, 0.846 in 2003), indicating that carotenoids are found along with chlorophyll in the photosynthetic antennae, rather than in other leaf structures (Middleton and Teramura, 1993). The relationship between anthocyanin and chlorophyll or carotenoid was not strong (Figures 8.1 and 8.2).

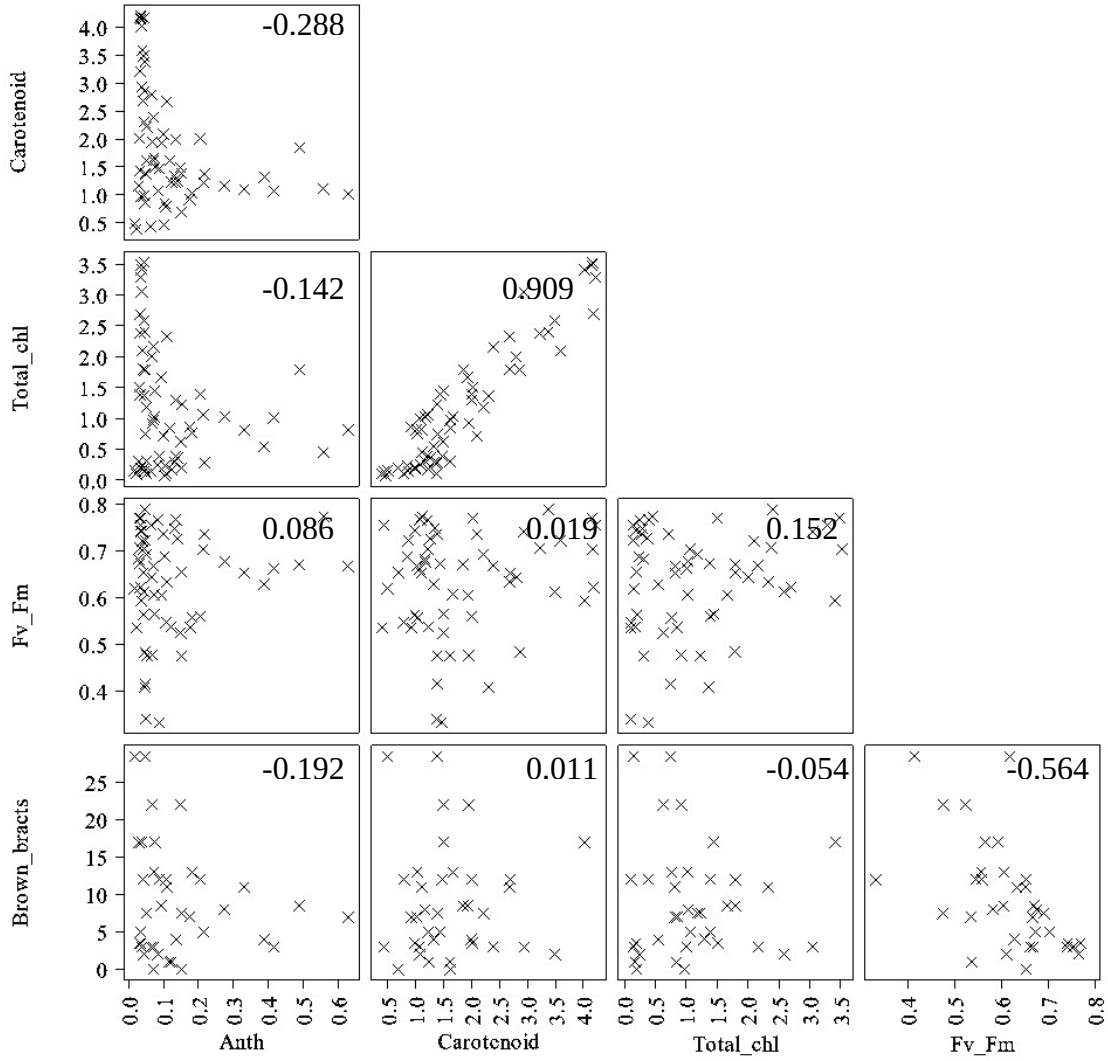

Figure 8.1: Correlation matrix for chlorophyll (μg/cm^2), carotenoid (μg/cm^2), anthocyanin (abs/cm^2), quantum yield (Fv/Fm) and browning of floral bracts (including bracts browned and senesced) of 'Fire and Brimstone' waratahs at Mount Annan in 2002. Correlation coefficients describing the strength of the relationship between parameters are given in the top right hand corner of each plot.

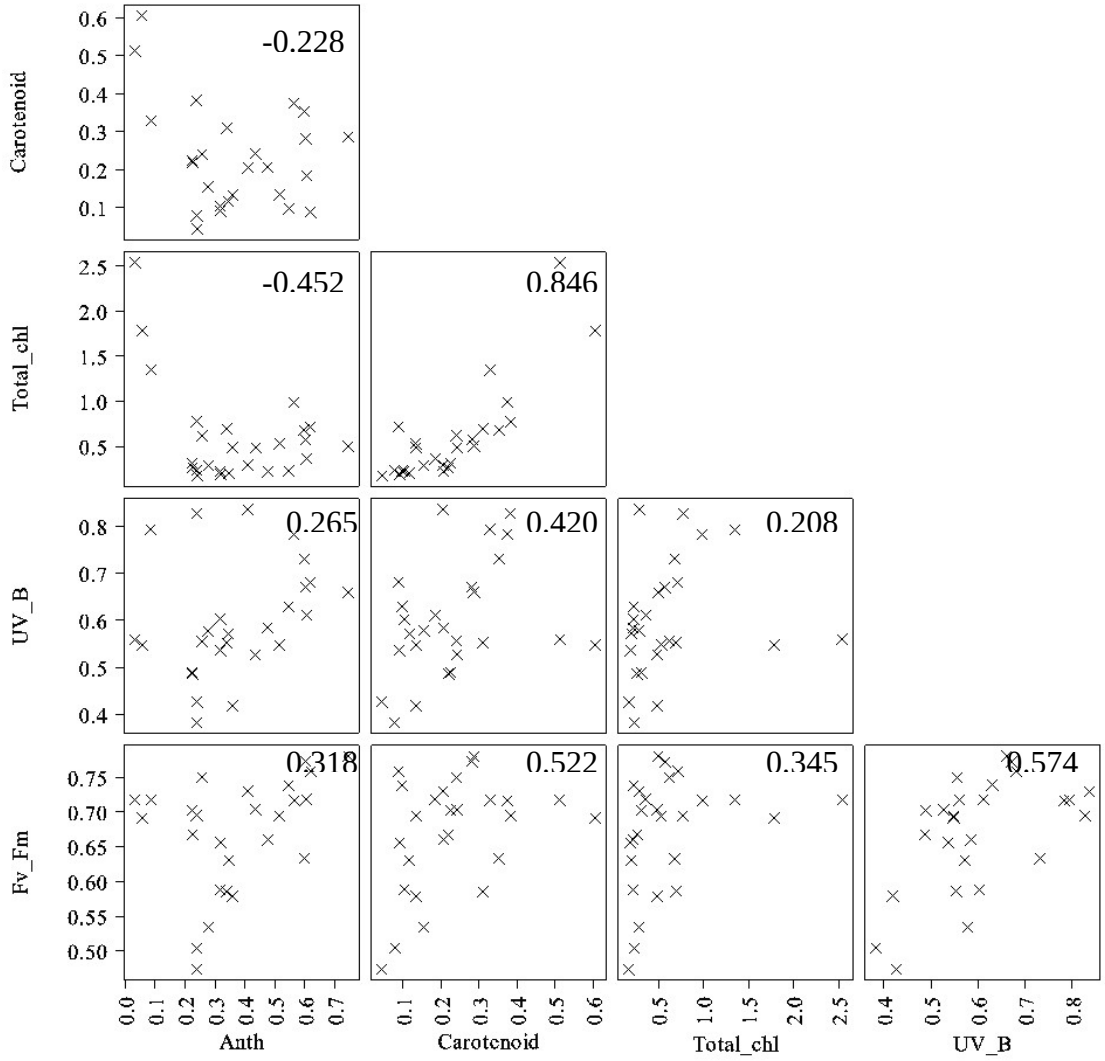

Figure 8.2: Correlation matrix for chlorophyll (μg/cm^2), carotenoid (μg/cm^2), anthocyanins (abs/cm^2), UV-B absorbing compounds (abs/cm^2) and quantum yield (Fv/Fm) of 'Fire and Brimstone' and 'Olympic Flame' waratahs at Mount Annan in 2003. Correlation coefficients describing the strength of the relationship between parameters are given in the top right hand corner of each plot.

8.2.3 Relationship between photoinhibition, pigmentation and browning at Jervis Bay

Data on pigmentation and quantum yield were pooled over treatments (sun and shade) for the cultivar 'Wirrimbirra White' at flower maturity. Quantum yield (Fv/Fm) was strongly correlated with the chlorophyll content of bracts (0.814, Figure 8.3). The relationship between quantum yield and carotenoid content was much weaker than that of chlorophyll. Quantum yield and browning had a weak positive correlation, but the results were confounded by the lack of measurements from inner bracts and the inability to measure fluorescence on brown areas of bracts (Chapter 6).

Anthocyanins, while strongly correlated with quantum yield (0.919), were present in very low concentrations in comparison to red waratah cultivars with a range of absorbance from 0.003 to 0.055 per cm^2, so this relationship was more likely to describe increasing quantum yield with increasing bract pigmentation.

The cultivar 'Wirrimbirra White' showed different relationships between pigmentation and fluorescence to other cultivars, although this may be due to the measurement of quantum yield at floral maturity only, rather than at all stages of development. Quantum yield was strongly correlated with bract chlorophyll concentrations, with yield increasing as chlorophyll concentration increased. This relationship reinforces the observation in Chapter 6, that tissues with low chlorophyll content have low photosynthesis and may not produce a measurable fluorescence signal. It also suggests that tissue with minimal pigmentation may be particularly sensitive to photoinhibition and browning.

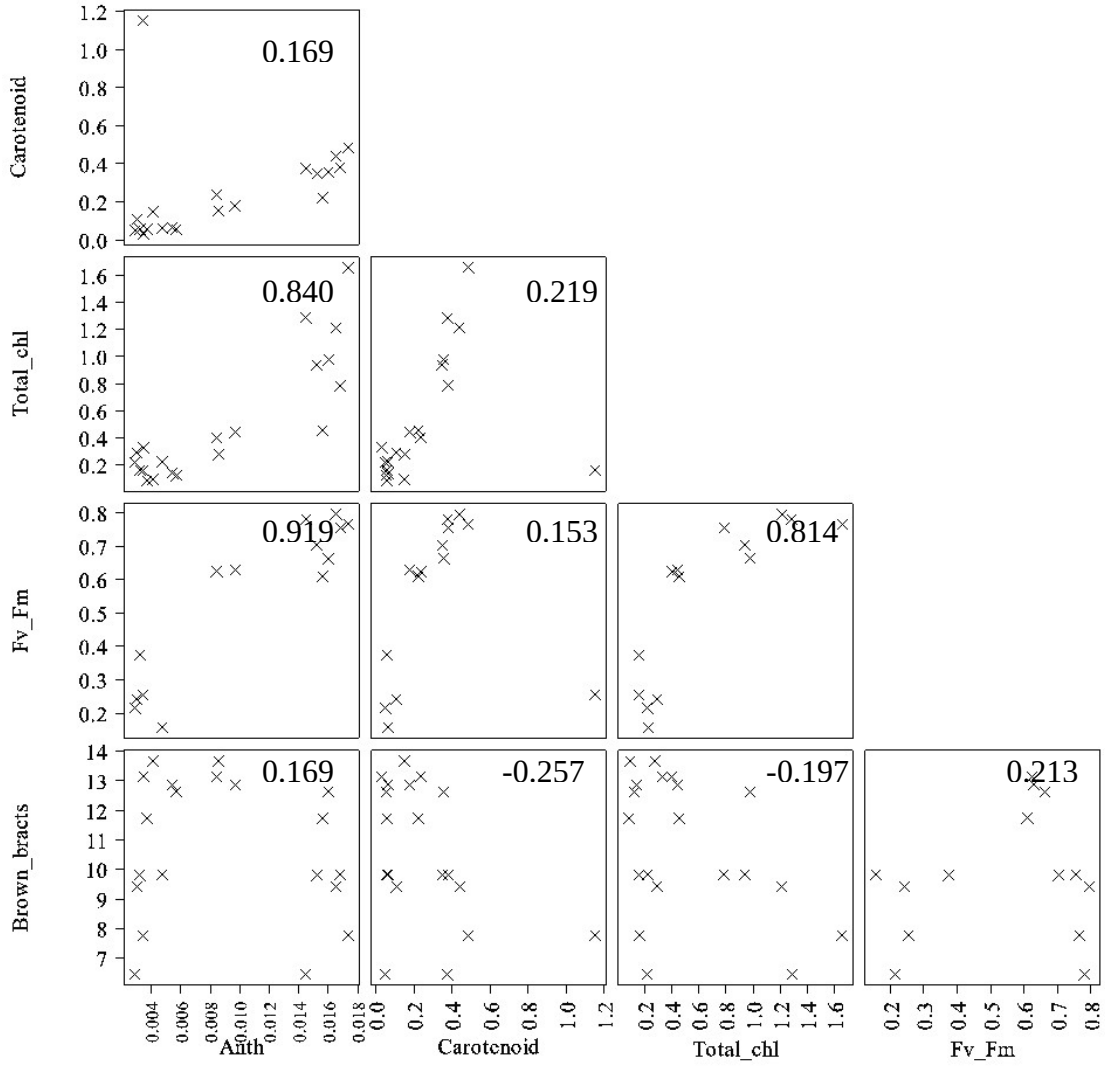

Figure 8.2: Correlation matrix for chlorophyll (μg/cm^2), carotenoid (μg/cm^2), anthocyanin (abs/cm^2), quantum yield (Fv/Fm) and browning of floral bracts (including bracts browned and senesced) of 'Wirrimbirra White' waratahs at flower maturity in 2002. Correlation coefficients describing the strength of the relationship between parameters are given in the top right hand corner of each plot.

8.3 Conclusion

Browning and bract senescence decreased as quantum yield increased in red waratah cultivars ‘Fire and Brimstone’ and ‘Olympic Flame’. Conversely, photoinhibition (low quantum yield) was correlated with browning, although the strength of the relationship varied from year to year, possibly due to environmental variation. These results corroborate measurements made with the Imaging PAM fluorometers, which showed greater photoinhibition in brown areas of bracts (Chapter 6). Browning and photoinhibition could not be consistently linked with low pigment concentrations and anthocyanins in waratah bracts do not appear to have a strong role in photoprotection. Measurement of fluorescence in the cultivar ‘Wirrimbirra White’ was difficult due to low pigment concentrations, with Fv/Fm increasing as pigmentation increased.

Having established the link between photoinhibition and browning in this chapter, the possibility of oxidative damage occurring as a result of chronic photoinhibition in waratah bracts will be investigated in the following chapter (Chapter 9). In this study, lipid peroxidation will be used to estimate oxidative damage.