Chapter 10
General discussion and conclusions

10.1 Causes of bract browning in waratahs

Bract browning occurred when waratah plants were grown in full sun, exposed to high light intensities for most of the day (Chapter 5 and Appendix A1). Browning of floral bracts increased significantly at bud opening and worsened towards commercial flower maturity. However, bract browning did not appear to be a calcium-related disorder, as the incidence of browning was not significantly reduced by calcium applied to the bracts as soluble sprays or to the potting medium (Chapter 4). The data suggest that bract browning, like lettuce tipburn, is more likely to be a result of environmental stress. However, calcium may play a secondary role in such browning disorders, by facilitating the plant's response to external signals such as light (Marme, 1983). Interestingly, recent studies on PSII membrane fragments have shown that calcium-depleted photosystem II complexes have a higher susceptibility to photoinhibition than active PSII complexes, due to the accumulation of highly oxidising species such as $P680^+$ (Arato *et al.*, 2004). However, this discussion will focus on the primary cause of browning identified in this study, that is, chronic photoinhibition resulting from exposure to high intensity light.

The browning of waratah bracts was positively correlated with chronic photoinhibition, measured as a decrease in predawn photosynthetic efficiency (Chapters 6 and 8). While waratah bracts, particularly those in high sun environments, experienced chronic photoinhibition, leaves were more resilient and were able to maintain a high photosynthetic efficiency in both shade and full sun (Chapter 6). Naturally occurring waratahs displayed little or no browning of floral bracts and seemed to experience high

light intensities for only a relatively short period each day (Chapter 5). Consistent and significant reductions in bract browning were observed over three years when waratah plants of various cultivars were grown under 50% shade cloth (Chapters 5 and 6).

Bracts maintained a higher concentration of chlorophylls, carotenoids and anthocyanins in the shade than in full sun (Chapter 7), and experienced saturation of non-photochemical quenching at relatively low light intensities (Chapter 6). These traits suggest that bract tissue is adapted to a lower light environment than leaf tissue, similar to *Dendrobium* floral tissue (He *et al.*, 1998) and *Petunia* corolla tissue (Weiss *et al.*, 1988).

10.2 Effect of shade on waratahs

Shading relieved photoinhibition by decreasing light intensity (Chapter 6), as well as reducing the maximum daily temperature and increasing the minimum relative humidity around the plants (Chapter 5). The design of the shade shelter at Mount Annan was also likely to reduce wind speed, as waratahs were protected by shade cloth on three sides of the enclosure.

10.2.1 Wind

Bract browning in waratahs is likely to be significantly reduced at lower wind speeds, with cultural techniques to minimise wind damage to waratahs described by Offord (1996) and Nixon (1997). Although the effect of wind on waratah bract browning has not been investigated, wind damage to leaves of other species such as *Acer* (Wilson, 1980) and birch (Hoad *et al.*, 1998) resulted in light or dark brown lesions, tearing and distortion of leaf lamina, chlorosis, blistering, necrosis and tissue death. Damage could

result from wind borne particles or the impact of leaf tips and edges on vulnerable leaves and organs such as buds. Wind sheltering reduced visible damage from 10-22% of leaf cover to less than 3% in birch plants (Hoad *et al.*, 1998). Thus, minimising wind speed around waratahs by shading is likely to minimise bract browning caused by wind borne particles and abrasion of bracts by leaves.

10.2.2 Inflorescence numbers

While shading of waratahs significantly reduced bract browning, covering waratah plants with shade cloth throughout the year may adversely affect flower set. For example, in *Leucospermum*, heavy shade reduced flower initiation and flower quality, as flower failed to develop their natural orange colour (Jacobs, 1983; Napier and Jacobs, 1989). While shading did not adversely affect the development of flower colour in waratah (Chapter 7), commercial growers are still concerned about the number of flowers initiated under shade.

In other crops such as chrysanthemum, shading reduces flower number (Cermeno *et al.*, 2001) and therefore productivity, by decreasing photosynthesis. However, in understorey crops such as orchids, shade has the potential to increase photosynthesis and flower yield by minimising photoinhibition (He *et al.*, 1998). The Australian native *Boronia* is another understorey species grown for cut flower and oil production, naturally occurring under paperbark (*Melaleuca parviflora*) or jarrah (*Eucalyptus marginata*) trees (Plummer *et al.*, 1998a and b). Field shading trials revealed that flower number was significantly higher in *B. heterophylla* stems grown under 75% full sunlight after five months of shading, although differences between 75% and 100% sunlight were not significant after seventeen months (Plummer *et al.*, 1998a). Photon flux

densities less than 75% full sunlight also significantly reduced photosynthesis, transpiration rate and stomatal conductance (Plummer *et al.*, 1998a). Shade was not recommended for commercial production of *Boronia*, as the temporary enhancement of flower production is not likely to compensate for the cost of erecting shade shelters (Plummer *et al.*, 1998a).

The potential detrimental effects of shading on waratah production are likely to be minimised by shading only from bud expansion to flower maturity, as described in Chapter 5. Shaded waratahs were similar in size to sun grown flowers, with the additional commercial benefit of a more intense bract colour due to maintenance of chlorophyll and anthocyanin pigmentation (Chapter 7). Similarly, He *et al.* (1998) recommended that *Dendrobium* orchids be grown under high light during the vegetative phase to maximise photosynthesis, then transferred to low light after flower initiation to prevent photoinhibition and visible damage. However, shading for only part of the year may not be practical or economically viable due to the cost of shade structures, particularly those with retractable shade screens.

The optimal intensity of shade for minimising bract browning of waratahs was not investigated, due to the limited number of potted waratahs with flowers available for experimentation, although 50% green or black shade cloth significantly minimised bract browning (Chapter 5). In the future, studies of waratah shading would be best conducted with field grown plants, rather than the potted plants studied at Mount Annan, as photosynthesis can be limited in potted plants (Plummer *et al.*, 1998a) and results of long term shading experiments may be confounded by the limitation of resources in potted plants.

10.3 Pigmentation and bract browning in waratahs

While bracts with high pigment contents were less likely to experience photoinhibition, the total chlorophyll content of bracts was not directly related to their susceptibility to photoinhibition and browning, with the exception of 'Wirrimbirra White' waratah bracts (Chapter 8). 'Wirrimbirra White' inner bracts appear to be particularly susceptible to photoinhibition, as they had minimal photosynthetic capacity (Chapter 6), low chlorophyll content (Chapter 7) and experienced high levels of browning in full sun (Chapter 5). If these inner bracts absorbed a similar proportion of light to outer bracts, but had less ability to dissipate the light energy via photochemical and non-photochemical quenching, they would be likely to experience severe photoinhibition.

10.3.1 Chloroplast structure

The inner bracts of red waratah cultivars have a high quantum yield (Chapter 6) and chlorophyll concentrations that increase significantly during flower development (Chapter 7). In tissues turning green, such as inner bracts, it may be the organisation of chloroplasts rather than the absolute concentration of chlorophyll that alters the susceptibility of the tissue to photoinhibition. In these greening tissues, chlorophyll molecules are not well organised into antenna complexes, therefore, the excitation energy of chlorophyll molecules cannot be directed to the reaction centre for dissipation (Caspi *et al.*, 2000). The lifetime of the excited chlorophyll molecule may thus be long enough to generate triplet state chlorophyll and increase the probability of singlet oxygen generation (Caspi *et al.*, 2000), as described in the literature review in Chapter 9.1.

In greening barley thylakoids, disorganised chloroplasts were more susceptible to singlet oxygen-mediated photodamage (Caspi *et al.*, 2000). Etiolated barley leaves transferred to the light were particularly sensitive to photodamage, leading to oxidation of lipids, phenols, amino acids and pigments and potentially, membrane breakdown, protein denaturation and chlorophyll bleaching (Caspi *et al.*, 2000). A similar mechanism for photodamage may occur in inner bracts of waratah, which have a low pigment concentration and possibly, disorganised chlorophyll molecules, while they are protected by outer bracts (Chapter 7). Inner waratah bracts are exposed to direct light for the first time during the juvenile open stage of flower development, coinciding with the most significant reduction in quantum yield compared to leaves (Chapter 6).

10.3.2 Anthocyanins and UV-absorbing compounds

The presence of anthocyanins did not reduce the susceptibility of waratah bracts to browning (Figure 6.11 showing 'Wirrimbirra White' waratah with browning and Chapter 8), although destruction of anthocyanins occurred in sun-exposed bracts (Chapter 7). Anthocyanins in waratah bracts may have functions other than photoabatement, for example, attracting pollinators such as birds (Willis, 1959; Mol *et al.*, 1998). The epidermal location of anthocyanins (Chapter 7) also suggests they function as a screen, rather than being located in mesophyll tissue to screen chloroplasts from excess light (Field *et al.*, 2001) or directly scavenge active oxygen species (Gould *et al.*, 2002; Neill and Gould, 2003). Bracts had significantly higher concentrations of UV absorbing compounds than anthocyanins (Chapter 7), and the positive correlation between UV-B absorbing compounds and quantum yield was much stronger than the relationship between anthocyanins and quantum yield (Chapter 8).

The data suggests that in red waratahs, UV absorbing compounds (possibly flavonoids) made a greater relative contribution to photoprotection than anthocyanins. Bract and floral tissues of other species also have high UV absorbance – the corollas of many flowers protect the pollen grains from UV-B radiation prior to flower opening (Caldwell *et al.*, 1983), while in the Himalayan plant *Rheum nobile*, bracts absorb UV radiation to protect and warm the reproductive tissues (Omori *et al*., 2000). The greater potential for UV absorbing compounds, rather than anthocyanins, to protect against light damage in waratahs agrees the results of Havaux and Kloppstech (2001), who found that *Arabidopsis* mutants expressing flavonoids experience less photooxidation than mutants expressing anthocyanins. Waratah bracts also had lower concentrations of UV absorbing compounds than waratah leaves (Chapter 7), another factor perhaps contributing to their differing susceptibility to photoinhibition and browning.

The contribution of UV absorbing compounds such as flavonoids to photoprotection is particularly important if photoinhibition results from UV damage, rather than visible light damage alone, as flavonoids other than anthocyanins have high absorbance in the UV region and little or no absorbance in the visible region (Caldwell *et al.*, 1983). The role of UV light in photoinhibition has not been resolved (Anderson and Chow, 2002), with some researchers suggesting that photoinhibition is a UV-light phenomenon and others suggesting that different mechanisms trigger photoinhibition caused by visible and UV light (for example, Nogues *et al.*, 1998).

Most studies of UV damage have concentrated on UV-B (280-315 nm) rather than UV-A or UV-C, as UV-B wavelengths are the shortest to reach ground level and are highly energetic (Atwell *et al.*, 1999). Thinning of the ozone layer and changes in ozone layer

thickness with season, meteorological conditions and latitude also exacerbate biological damage caused by UV-B (Jansen *et al.*, 1998). The UV-index for the Sydney area during late winter and spring, when waratah flowers mature, is rated as high to very high (Australian Radiation Protection and Nuclear Safety Agency, 2004), suggesting that increasing UV-radiation in this period may possibly contribute to bract browning. Potential targets for UV-B damage include DNA, proteins, membranes, photosynthetic pigments and phytohormones (Strid *et al.*, 1994; Greenberg *et al.*, 1997; Jansen *et al.*, 1998). Similar responses are noted for photoinhibition (Chapters 6 and 9) and UV light damage: UV-B damage responses include degradation of chlorophyll and carotenoids, photoinhibition and lipid peroxidation caused by active oxygen, photooxidation and free radical reactions (Teramura and Sullivan, 1994; Greenberg *et al.*, 1997). Waratah bracts experienced reductions in chlorophyll and carotenoid content, as well as photoinhibition in full sun (Chapters 6 and 7), which may be the result of either visible light or UV-B damage. However, the strong link made between browning and photoinhibition of waratah bracts in this study is sufficient to manage the disorder, without the need to determine whether photoinhibition is the result of UV or visible light damage.

10.3.3 Manipulating waratah bract colour

The relative proportions of pigments in waratah bracts, described in Chapter 7, may be manipulated during breeding of waratahs to obtain particular bract or flower colours. The genetics of waratah breeding have been recently investigated by Offord (2003), with flower colour likely to be under monogenic control, and hence, relatively easily manipulated. Flower and bract colour are determined not only by the combination of anthocyanins, carotenoids and chlorophyll, but also by co-pigmentation with flavonols or flavones, vacuolar pH and cell shape (Mol *et al.*, 1998). For example, *Dendrobium*

petals and sepals with flat cells and a tightly packed mesophyll have a glossy texture, while dome-shaped cells and a loosely packed mesophyll result in a velvety texture (Mudalige *et al.*, 2003). Epidermal cell shape may also change as the flower develops (Weston and Pyke, 1999), and therefore examination of cell shape at waratah flower maturation would be of greatest commercial interest.

The intensity of anthocyanic coloration is determined by the location of anthocyanins in tissue, with mesophyll, epidermal and subepidermal anthocyanins imparting a more intense colour than epidermal or subepidermal anthocyanins alone (Mudalige *et al.*, 2003). In 'Olympic Flame' waratahs, anthocyanins appeared to be restricted to epidermal and subepidermal layers (Chapter 7); crossing this cultivar with others may result in additional layers of anthocyanins and more intense red coloration.

Red waratah cultivars display partial dominance over white cultivars, resulting in pink offspring (Offord, 2003). White cultivars such as 'Wirrimbirra White' have a similar pigment profile to red cultivars, with the exception of relatively low anthocyanin concentrations in all bracts (Chapter 7). This pattern of pigmentation is similar to the white 'Alba' cultivar of *Chamelaucium* and the magenta or red cultivars (Klyne *et al.*, 2003). The white waratah cultivar appeared to have higher concentrations of UV-absorbing compounds than red cultivars, although to confirm this, cultivars of both colours should be grown at the same site using the same cultural practices to ensure this response is consistent (Chapter 7).

Identification of the UV absorbing compounds and anthocyanins present in red and white waratah cultivars would allow more precise manipulation of bract and flower

colours during waratah breeding, as the flavonoid and anthocyanin synthesis pathway is well understood. The acylation or methylation of anthocyanins also changes their absorption spectra (Chalker-Scott, 1999) and may alter the susceptibility of waratah flowers to damage by visible or UV light, as discussed above. Most of the genes encoding anthocyanin biosynthesis have been isolated, and these genes can be manipulated to promote accumulation of pathway intermediates, resulting in new flower colours (Mol *et al.*, 1998). However, manipulating these pigments in long-lived woody species such as waratahs is likely to be considerably more labour intense than in short lived herbaceous crops, due to the extended period from planting to full flowering (years rather than months).

10.4 Recommendations

10.4.1 Examine photoinhibition and browning responses of wider range of cultivars

A key recommendation of this project is the future examination of the susceptibility of a wider range of cultivars with respect to photoinhibition and browning. This would allow identification of more resilient cultivars, as well as cultivars that have a predisposition towards browning. For example, novel pink waratah cultivars attract consumer interest, but may be susceptible to browning which is particularly noticeable against a pale background, like in 'Wirrimbirra White' waratahs. In contrast, cultivars with yellow or orange hues may have higher concentrations of carotenoids than existing cultivars, which may improve their resilience to bract browning through increased non-photochemical quenching. Further research is also recommended to identify the anthocyanins and UV-absorbing compounds responsible for bract colour, to allow more precise manipulation of bract and flower colour during breeding and minimise susceptibility to photoinhibition and browning.

10.4.2 Examine effect of visible and UV light on browning and photoinhibition

The response of waratahs, particularly in terms of bract browning, to visible and UV radiation should be further investigated by growing plants under light sources of a known intensity and spectra, with and without supplementary UV radiation, as described by Greenberg *et al.* (1997) and Rozema *et al.* (1997). The photooxidation of cutins under visible light, described by Rontani *et al.* (2005), is also worth investigating in waratah bracts as a possible cause of the browning of epidermal cells shown in Chapter 7.4.

In the field, shade cloth or protection by a canopy of vegetatation would reduce both visible and UV light intensity, to minimise photoinhibition and browning of waratah bracts. Damage induced by UV or visible light may also be minimised by selecting waratah cultivars for breeding programs with smaller, thicker, waxy bracts; high concentrations of UV-B screening pigments; and high concentrations of compounds to scavenge active oxygen and other free radicals (Day, 1993; Greenberg *et al.*, 1997; Jansen *et al.*, 1998). The presence of hairs on waratahs bracts, as observed on some seedling waratahs during this study, could possibly reduce their susceptibility to UV-B damage, as observed for *Olea, Verbascum* and *Quercus* leaves (Karabourniotis *et al.*, 1992; Skaltsa *et al.*, 1994), although this hypothesis would need to be tested experimentally.

10.4.3 Examine chloroplast organisation and identify cellular events leading to bract browning

Studies of bract anatomy during waratah flower development would reveal whether chloroplasts are well-structured, and thus able to direct light energy into the photosynthetic reaction centres. Chloroplast organisation may be poor in bracts that are increasing in chlorophyll (greening tissues, as discussed in 10.3.1) or bracts that are maturing or senescing. Chloroplasts may degenerate to chromoplasts as flowers reach maturity, as observed in *Dendrobium* orchids (Khoo and Hew, 1999) and wallflower petals (Weston and Pyke, 1999). However, deterioration of chloroplast to chromoplast is more likely in senescing outer bracts of waratah, as inner floral bracts of waratah appear to have separate cells containing either chloroplasts or anthocyanic pigments (Figure 8.8). The chloroplast structure of inner and outer waratah bracts could be investigated to determine whether bract chloroplasts are similar to those in leaves, as observed in *Petunia* corollas (Weiss *et al.*, 1988) and cotton bracts (Bondada *et al.*, 1994) or whether inherent differences exist that may increase the susceptibility of bracts to browning.

It would also be useful to examine the cellular events leading to bract browning in more detail, using direct measurements of oxidative damage rather than measuring subsequent lipid peroxidation. Exposing waratah bracts to intense light and monitoring the concentration of reactive oxygen species and scavenging enzymes or using high resolution imaging following fluorescence staining in the laboratory may further elucidate the chain of damaging reactions leading to bract browning in waratahs.

Recent studies have confirmed that hydrogen peroxide and superoxide play a key role in photooxidative damage, as outlined in Chapter 9. Hydrogen peroxide at high concentrations triggers programmed cell death, while at low levels it is involved in acclimation to oxidative stress (Vandenabeele *et al.*, 2003). Kim and Lee (2005) confirm that hydrogen peroxide induces photooxidative damage targeted at photosystem II (PSII). The cellular and molecular processes involved in hydrogen peroxide-mediated cell death are discussed in detail by Vandenabeele *et al.* (2003), who have identified several candidate genes for oxidative stress sensing and signal transduction, as well as heat shock proteins involved in the regulation of cell death. Mitochondrial disruption was also correlated with oxidative stress during hydrogen-peroxide induced cell death. Vandenabeele *et al.* (2003) anticipate that signalling molecules other than hydrogen peroxide are involved in acclimation to oxidative stress and cell death, such as oxylipins, ethylene, salicylic acid, jasmonic acid and nitric oxide.

Superoxide radicals also play a key role in photooxidation, although the damage appears to be targeted at photosystem I (PSI) (Kim and Lee, 2005). Recent studies have confirmed that PSI is very sensitive to photodamage under chilling conditions (Scheller and Haldrup, 2005), as suggested by Wise (1995) and Sonoike (1998). PSI damage involves oxidation of the iron-sulfur clusters at the reducing side of PSI, with P700 being relatively resistant to photooxidation (Scheller and Haldrup, 2005). In some species, chilling damage occurs exclusively in PSI, although in other species, both PSI and PSII are affected (Scheller and Haldrup, 2005). For example, under chilling stress in maize, the quantum efficiency of PSII and the concentration of superoxide radicals had a strong negative correlation (Ke *et al.*, 2004). It is possible that waratah bracts are susceptible to photoinhibition under chilling conditions (reviewed in section 6.1.3),

possibly involving superoxide and PSI as described above, and studies of this phenomenon are recommended to obtain experimental evidence.

10.4.4 Examine wider range of environmental stressors linked to photoinhibition and browning

Further research into the interaction between high light and high temperatures, low temperatures and water stress is recommended, as this may reveal an increased range of environmental conditions that contribute to photoinhibition and bract browning. An understanding of the environmental limitations to the cultivation of high quality waratahs would provide further information for the modelling of existing and potential waratah plantations, similar to the study of Mackenzie (1987). The impact of environmental stresses on bract browning may become increasingly important if the range of waratah cultivation expands, potentially into Queensland and inland NSW. These situations could be particularly unsuitable for waratah cultivars incorporating genetic material from *Telopea* species adapted to cooler areas such as *T. oreades* and *T. truncata***.** The influence of water stress on bract browning is likely to be particularly important, due to continuing cycles of drought in Australia and increasing water restrictions in agricultural and horticultural enterprises. Thus, irrigation frequency and light intensity experiments, similar to those described in Chapter 5.3.2, could be repeated with a wider range of cultivars and monitored for photoinhibition using chlorophyll fluorescence techniques.

The critical temperature above which irreversible damage occurs should be determined for waratah leaves and bracts using chlorophyll fluorescence techniques (Bilger *et al.*, 1984); a method that correlates well with the temperature at which CO_2 uptake ceases and necrotic spots develop (Koeniger *et al.*, 1998). Waratahs are likely to experience

frequent high light and temperature combinations. For example, during peak waratah flowering in 2003, the mean daily maximum temperature in full sun was over 30°C (Chapter 5.3). Shading during flowering in 2003 may have reduced the maximum bract temperature by up to 5°C, provided waratah bract temperatures were similar to air temperatures. Sun leaves and bracts are likely to have slightly higher critical temperatures for damage than shade leaves, due to acclimation to higher temperatures (Koeniger *et al.*, 1998). 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).

10.4.5 Quantify economic impact of shading

Long-term experiments to examine the number of flowers initiated in shaded conditions and other commercially important traits such as stem length are recommended. Different levels of shade cloth should be trialled, to optimise the reduction in bract browning, since only 50% shade cloth was used in this project. Other colours of shade cloth may also produce differences in pigmentation, photoinhibition and browning, due to the reflectance and transmittance of radiation of different wavelengths (discussed in Chapter 5.1.2), although in experiments in Chapter 5 both green and black shade cloth were equally effective in reducing bract browning and maintaining bract pigmentation. The production of high quality waratahs free from bract browning will become increasingly important, as the volume of waratahs produced multiplies due to recently planted waratahs and consumers in domestic and international cut flower markets continue to demand attractive products free from blemishes.

10.4.6 Application of results to browning events in other crops

The lack of involvement of calcium as the primary cause of bract browning in waratahs corroborates evidence that factors other than calcium are the cause of browning disorders such as poinsettia bract necrosis (Wissemeier, 1993) and lettuce tipburn (Wissmeier and Zuehlke, 2002). In particular, high intensity light, which leads to chronic photoinhibition and browning in waratah bracts and a range of leaf and floral tissues in other crops (reviewed in Chapter 2.5.5 and 2.5.6), is worth reinvestigating as the cause of pre-harvest physiological browning disorders previously attributed to localised calcium deficiency, using chlorophyll fluorescence techniques to monitor photoinhibition. If chronic photoinhibition is found to cause browning in these other crops, the use of shade as a control for photoinhibition and browning should be investigated. Shade has been successful in reducing waratah bract browning by minimising photoinhibition and has also been used to protect other crops such as *Dendrobium* orchids (He *et al.,* 1998), tea (Mohotti and Lawlor, 2002) and Chilean guava (Pastenes *et al.,* 2003), from dynamic or chronic photoinhibition.

The investigation of cellular and molecular processes causing photoinhibition and subsequent cell death for a wide range of plants including waratahs is recommended, although the refinement of techniques for investigation *in planta* may be necessary. At present, experiments to elucidate the processes of cellular and molecular damage from photooxidation are limited to widely grown crop plants such as rice and maize, often at the seedling stage (for example, Jiao *et al.* 2003; Ke *et al.,* 2004; Kim and Lee, 2005) or mutant strains of *Arabidopsis* and tobacco (for example, Vandenabeele *et al.,* 2003; Wingler *et al.,* 2004).

10.5 Conclusions

Bract browning in the NSW waratah occurred predominantly during bud expansion and opening of the inflorescence in the six to eight weeks prior to harvest. The disorder generally affected bract tips and margins, similar to calcium-related disorders such as lettuce tipburn and poinsettia bract necrosis. However, calcium applied as sprays to the bracts or as gypsum to the potting media did not reduce browning. Browning also affected exposed bracts, rather than the enclosed tissues affected in calcium-related disorders. These results and reports of reduced bract browning in shaded environments suggested that light intensity may play a greater role than calcium in the development of bract browning in waratahs.

Experiments in 2001 showed that shade reduced bract browning more effectively than frequent irrigation in two waratah cultivars grown in pots at Mount Annan. Experiments on other red cultivars at Mount Annan and on the 'Wirrimbirra White' cultivar on a commercial waratah plantation during the following two years showed that 50% shade cloth consistently reduced bract browning. The bract browning disorder was linked to the development of chronic photoinhibition in waratah bracts, which was exacerbated in a full sun environment. Outer bracts were susceptible to browning due to their prolonged exposure to the environment during flower development, with some outer bracts senescing towards flower maturity. Inner bracts were susceptible to browning from the intermediate stage of development, when they emerged from the outer bracts that initially protected them. In contrast, waratah leaves did not suffer from browning and were able to maintain a high maximal photosynthetic efficiency.

Full sun exposure of waratah bracts resulted in a chronic reduction in photosynthetic efficiency, pigment destruction and the development of brown and necrotic areas. These events suggest bract browning is the result of oxidative damage following photoinhibition. However, lipid peroxidation measurements did not confirm oxidative damage in brown waratah bracts, possibly due to interference from anthocyanins, flavonoids and sugars other than sucrose.

Commercial waratah growers may achieve significant reductions in bract browning by shading plants after flower bud initiation until harvest. Waratahs grown in the shade were a consistently higher quality due to enhanced pigment development and reduced photoinhibition and browning. However, further research is required to optimise the percentage of shade cloth required to minimise bract browning and the economic impact of shading on flower set and quality.