Chapter 2
General literature review

2.1 Waratah production in Australia

Australia's native plant species provide the worldwide floriculture industry with unusual and exotic flower and foliage products, with an increasing number of species being developed for commercial production (Gollnow *et al.,* 2003). The NSW waratah *Telopea speciosissima* (Smith) R.Br is one such species, known for its large attractive red inflorescence, subtended by showy floral bracts. The striking appearance of the species is evident from the Latin name for waratah, with *Telopea* meaning "seen from a distance" and *speciosissima* meaning "most beautiful" (Wrigley and Fagg, 1989). The genus *Telopea* belongs to the family Proteaceae and includes five species restricted to southeastern Australia (Harden *et al.*, 2000).

Waratahs are a display or feature flower, included in the category of 'wildflowers' with other Australian native species, as well as South African Proteaceae (Yencken, 1999; Gollnow *et al.,* 2003). Waratahs are well suited for use as a cut flower, with a decorative three-dimensional bloom, strong stems from 0.2 to 1.0 m long, and a vase life of more than thirteen days (Offord, 1996). However, the large floral bracts, particularly in *Telopea speciosissima* and its hybrids, often suffer from browning prior to harvest, also known as 'bract burn'. This disorder considerably reduces their market value and potential for export (Mullins, 1987). Bract browning is regarded as a major weakness in the production of high quality blooms by both Australian and international researchers (Gollnow and Worrall, 2000; Criley, 2001).

The vast majority (95%) of flowers exported from Australia are wildflowers, from total flower exports worth A$55 million in 2002 (Gollnow, 2003). Flower exports have increased significantly since 1997/98, when flower exports were estimated at A$27.1 million, comprising approximately 50% fresh Australian native flowers and foliage (Yencken, 1999). Production of Australian species worldwide (including those grown in California, Africa, Israel and South America) is estimated at A$400 million per year, with products grown in Australia making up approximately 12.5% of the total ($50 million) (Gollnow *et al.*, 2003). Wildflowers make up approximately 10-15% of domestic floriculture sales (Gollnow *et al.*, 2003), an increase on the estimate of 8-10% suggested by Yencken (1999).

Waratahs are particularly popular in New South Wales, where it is the state floral emblem (Radcliffe-Smith, 1998; Gollnow *et al.*, 2003). Annual production of waratahs was estimated to be between 0.6 and 1.7 million stems in 1994, supplied by approximately 28 000 cultivated plants, with prices between $2 - 4 per stem (Offord, 1996). A more recent survey conducted by Gollnow and Fishpool (2001) confirmed that more than 20 000 waratah plants were cultivated in NSW in 1998. Their conservative estimate indicates that up to 160 000 waratah plants may come into full production in the near future, as new growers become established and recent commercial plantings mature (Gollnow and Fishpool, 2001).

The domestic market favours large-headed waratah varieties, while smaller headed waratahs achieve better returns on the export market, particularly considering the relatively high freight cost per stem (Gollnow *et al.,* 2003). White waratahs, and waratahs of a high quality, give the best returns on both domestic and export markets (Gollnow *et al.,* 2003), emphasising the need to produce waratahs free from bract browning and other blemishes.

Waratahs, like many other wildflower crops, were initially bush picked to establish market acceptance and provide an adequate supply of blooms (Mackenzie, 1987; Gollnow *et al.,* 2003). However, the quality and quantity of bush picked waratahs could not be guaranteed (Mackenzie, 1987) and flowers picked were likely to be of inferior quality (NSW NPWS, 2002). Bush picking also raises serious conservation concerns, as it reduces the potential for the species to persist at the site of picking (Mackenzie, 1987; Denham and Auld, 2002). Excessive harvest of waratahs from wild populations can potentially reduce seed production and seed bank size, putting populations of the species at risk of decline (Offord and Campbell, 1994; Denham and Auld, 2002). Commercial plantations of waratahs have now been developed and further harvest from wild populations is prohibited under the National Parks and Wildlife Act 1974 (NSW NPWS, 2002), although illegal harvesting still occurs (Offord, 2003). In the last five years, commercial waratah growers have planted tens of thousands of waratahs, indicating renewed interest in the crop (Gollnow *et al.,* 2003). Waratah cultivars with proven market appeal, such as those described by Dennis (1991), Mathews (1993) and Nixon and Payne (1996) are increasingly planted (Gollnow *et al.,* 2003), although many established growers continue to rely on seedling grown plants or their own selections.

Waratahs have a relatively short cultivation and selection history, compared to flower crops such as roses and carnations, although they are a relatively well-known Australian native species. Significant progress has been made in defining the agronomic requirements for cultivated waratahs in the last fifteen years (compare Mackenzie, 1987; Mullins, 1987 and Offord, 1996). However, some aspects of waratah production require further investigation, particularly management techniques to maximise flower quality, reduce bract browning, and increase the duration of the flowering season (Gollnow, 1996; Gollnow and Worrall, 2000; Criley, 2001).

Waratahs are usually grown as field crops in a relatively open environment, with windbreaks for protection in some plantations (Dennis, 1991; Tranter, 1998). However, information describing the optimal light requirements for waratah flower initiation and flower quality is contradictory. Worrall (1994) suggests that waratahs "grow and flower best in the full sun", with shaded plants displaying less vigour, producing fewer inflorescences and flowering two to four weeks later than those in full sun. Offord (1996) suggests that growing waratahs under shade cloth or filtered light provided by trees, similar to conditions in their natural habitat, is likely to reduce bract browning. Shade cloth has the additional advantage of providing physical protection from wind, hail and pests (Offord, 1996; Nixon, 1997). Nixon (1997) states that while more flowers are produced in full sunlight, better quality flowers are produced in the shade. Criley (2001) reports that waratahs "flower best in full sun or light shade". Shading has the potential to increase waratah flower quality, although care must be taken to avoid adverse effects on flower set and colour. For example, shading of *Leucospermum cordifolium*, another cut flower species belonging to the family Proteaceae, resulted in a paler flower colour (Jacobs and Minnaar, 1980). The response of flower and fruit set

and size to shading appears to be species dependent, and is discussed in relation to quality by He *et al.* (1998) and Tyas *et al.* (1998).

The expansion of the Australian wildflower industry in the last ten years (Gollnow *et al.*, 2003) and the increasing proportion of Australian natives in total flower exports, indicate a need to maintain a steady supply of high quality Australian native cut flowers. Yencken (1999) emphasises that quality assurance is pivotal to the future success of the Australian wildflower industry, and Gollnow (2003) concurs, stating that "customers demand a product free of blemishes". The demand for high quality flowers extends to waratahs (Offord, 1996), with a reduction in bract browning in commercial plantations considered a priority for waratahs to reach their full potential (Offord, 1996; Criley, 2001). The extensive planting of waratahs in the five years prior to 2003 (Gollnow *et al.*, 2003) indicates that the value of lost production due to bract browning is likely to increase in the future. This project investigated the cause and control of bract browning in waratahs, in order to improve the quality of flowers for domestic and export markets.

2.2 Waratah flower initiation and development

Waratah flower initiation has been observed from mid December, with flower primordia emerging from mid January to February (Dupee and Goodwin, 1990a). The flower develops in bud form for seven to eight months, protected by an outer layer of bracts (Nixon, 1997). The prolonged development of waratah flowers leaves the bracts susceptible to damage, including browning, for a period of more than six months (Nixon, 1997).

Nixon (1997) described three waratah bract types: (1) floral bracts in one or more whorls, (2) protective bracts and (3) small, scale-like basal bracts with no [red] colouration that hold larger bracts in position. Bract size varies between species and between flowers, some being "small and insignificant" and others "voluminous", with colours from white to pink, red, and green (Nixon, 1997). Crisp and Weston (1993) describe a range of bract characteristics in naturally occurring southern (*T. speciosissima*) and Gibraltar Range populations (now *T. aspera*; Harden *et al.*, 2000) of waratahs (Table 2.1). Accurate description of bract types, expanding on the categories described by Nixon (1997), will be necessary during this project to evaluate the severity of bract browning in waratahs.

Table 2.1: Median bract number, length and width of naturally occurring *Telopea speciosissima* and *T. aspera* waratahs, with range of values observed for each character in brackets (Crisp and Weston, 1993).

	Telopea population	
Character	Southern (*T. speciosissima*)	Gibraltar Range (*T. aspera*)
Number of bracts	25 (15-36)	21 (17-27)
Bract length (cm)	7.4 (4.2-11.6)	5.5 (3.5-7.8)
Bract width (cm)	2.6 (1.4-5.3)	2.4 (1.4-3.5)

The date of waratah flowering depends on geographic location and climatic differences, occurring in early August in coastal Queensland and up to December in Tasmania (Offord, 1996). In 1988, flowering of *T. speciosissima* seedlings at five sites in NSW occurred over a period of fifty to eighty days from early August to late September (Dupee and Goodwin, 1990b). Waratah inflorescences are harvested when 0 - 50% of flowers are open, although inflorescences with 0 - 5% of flower open have the longest vase life (Faragher, 1989) and least opportunity for bract damage in the field (Nixon, 1997).

2.3 Differences between bracts and leaves

Leaves associated with flowers, often modified in size relative to vegetative leaves, are referred to as bracts or hypsophylls (Bell, 1991). Bracts may function as a screen to minimise UV damage to developing ovules, particularly if the bracts contain flavonoids (Caldwell *et al.*, 1983).

While bracts are essentially modified leaves, there are significant differences between the two organs. For example, poinsettia (*Euphorbia pulcherrima* Willd.) leaves and bracts are morphologically similar tissue types that differ in plastid and pigment development and associated photosynthetic capacity (Woodrow and Grodzinski, 1987). Poinsettia bracts have fifty times less chlorophyll per unit area than leaves (Woodrow and Grodzinski, 1987). Bracts tend to have a lower photosynthetic rate than leaves, for example, poinsettia bracts have a photosynthetic rate 5 to 10% that of leaves (Woodrow and Grodzinski, 1987; Wissemeier and Marienfeld, 1998). On some bracts, only CO_2 evolution (net respiration) rather than CO_2 consumption (net photosynthesis) could be measured. The low stomatal density of poinsettia bracts (Nell and Barrett, 1986) translates to a low transpiration rate, about 17% that of leaves (Wissemeier and Marienfeld, 1998). Similarly, Bondada *et al.* (1994) found that cotton bracts have a lower rate of photosynthesis than leaves. It is not known whether waratah bracts have the ability to photosynthesise, and if so, how their photosynthetic efficiency compares to that of waratah leaves.

The pigmentation of waratah bracts is likely to change as the flower matures. In this respect, leaves with red anthocyanic pigmentation, such as those of some *Syzygium* species (Dodd *et al.*, 1998), may resemble waratah bracts more closely than leaves

pigmented primarily with chlorophyll and carotenoids. Waratah bracts may also bear some similarity to petals and other floral tissues. Bracts, petals and sepals can all contain chlorophyll, although the red colour of anthocyanins may mask the green of chlorophyll. For example, the chlorophyll content of *Petunia* corollas increased during development, reaching a maximum just before anthesis (Weiss *et al.,* 1988), while the chlorophyll content of *Dendrobium* sepals, petals and the labellum were highest in the bud and decreased as flowers developed (Khoo *et al.,* 1997). Anthocyanins accumulated in the upper corolla tube of *Petunia* at later stages of development, leading to a pink flower (Weiss *et al.,* 1988). Chlorophyllous tissues, particularly bracts, may therefore make a significant contribution to flower photosynthesis. In *Arctium* flowers, 90% of the chlorophyll in *Arctium* flower heads was present in bracts, which supplied 15-30% of the carbon required by flower heads (Heilmeier and Whale, 1987). *Dendrobium* sepals had similar chlorophyll contents to petals but had a significantly higher rate of CO_2 fixation throughout flower development (Khoo *et al.,* 1997). *Petunia* corolla chloroplasts were also able to photosynthesise, although the quantum yield for electron transport and the light required for saturation of photosynthesis was lower than in leaves (Weiss *et al.,* 1988). The low light intensity for saturation of photosynthesis in corolla tissue is similar to that observed in shade plants (Weiss *et al.,* 1988), suggesting that floral tissues, and possibly bracts, are more susceptible to damage at high light intensities. Indeed, Khoo *et al.* (1997) found that *Dendrobium* sepals are likely to be photoinhibited above $100\mu molm^{-2}s^{-1}$, as non-photochemical quenching, that is, photoprotection via the xanthophyll cycle, reached a maximum at this light intensity. Maturation of the waratah inflorescence may result in loss of chlorophyll and possibly senescence of bracts, as described by Hendry *et al.* (1987).

2.4 Bract browning in waratahs

While the trigger for bract browning is unknown, many environmental factors have been implicated in its development (Table 2.2).

Table 2.2: Suggested causes of bract browning in waratahs (*Telopea* spp.)

Suggested Cause of Bract Browning	Reference
Water deficit stress	Worrall, 1983
	Nixon, 1997 p23
	Worrall, 1994
	Offord, 1996
Wind damage	Mullins, 1987
	Nixon, 1997 p23
	Burnett and Nixon, 1990
	Offord, 1996
Heat stress	Burnett and Nixon, 1990
Rubbing of leaves and branches	Offord, 1996
Early morning frost followed by strong sunlight	Offord, 1996

Bract browning in waratahs has been reported to occur prior to and during anthesis (Offord, 1996). Some cultivars are more susceptible to browning than others (Offord, 1996), with light coloured cultivars such as 'Wirrimbirra White' tending to show more obvious bract browning under adverse conditions (Nixon, 1997).

Worrall (1994) reported that covering flowers with light shade cloth may reduce the incidence of bract browning and Nixon (1997) reported better quality flowers under shade cloth. Bract browning may be reduced by increased watering prior to and during flowering, pruning bushes low, planting rows parallel to the prevailing wind, protection by wind breaks or shade cloth and picking flowers as early as possible (Offord, 1996; Nixon, 1997). Burnett and Nixon (1990) reported control of bract browning by covering blooms with clear cellophane bags with small ventilation holes from bud colouring onwards.

Bract browning may be minimised by selecting resistant cultivars (Worrall, 1994) or cultivars that tend to abort bracts (Dawson, 1996). Dennis (1991) and Offord (1996) considered susceptibility to bract browning an important characteristic when choosing clones for cultivation. Although selection of waratah cultivars that abort their bracts may minimise the problem of bract browning (Dawson, 1996), floral bracts were noted to be an important attribute determining flower quality, with 55% of consumers preferring large obvious bracts and only 1% of consumers suggesting that bracts should be insignificant (Offord and Campbell, 1994).

2.5 Browning in other crops

Browning and necrosis of leaf and bract tissue in other crops has been attributed to a cascade of events that leads to cell breakdown. The environmental triggers and physiological cause of browning disorders including *Protea* leaf blackening, bract necrosis of poinsettia, tipburn of lettuce, sun damage of fruit, blackleaf in grapevines and bleaching and necrosis of leaf and floral tissue in a variety of species are briefly reviewed here.

2.5.1 Protea *leaf blackening*

Protea leaf blackening is a postharvest disorder, unlike bract browning of waratahs, which occurs prior to harvest. However, cell breakdown in *Protea* leaves may occur in a similar way to browning of waratah bracts. Leaf blackening in cut *Protea* flowers usually occurs within 3-7 days of harvest and is triggered by rapid transport of carbohydrates from the leaves to the inflorescence. Leaf blackening is reduced by constant illumination and inhibited by active photosynthesis (Jones and Clayton-Greene, 1992) and delayed by removal of the inflorescence (Dai and Paull, 1995).

Protea inflorescence development leads to high demand for respiratory substrates and sugars for nectar production (McConchie *et al.,* 1991; Dai and Paull, 1995). Although the link between cellular breakdown and high carbohydrate demand is not clear, initial investigations suggested that membrane damage allows phenols to be released from the vacuole. Phenols could then come into contact with oxidative enzymes such as polyphenol oxidase or peroxidase from the chloroplast or cytoplasm (Jones and Clayton-Greene, 1992). McConchie *et al.* (1993 and 1994) suggest that leaf blackening is the result of non-enzymic oxidation of free phenolics, as carbohydrate stress resulted in increased production of polyphenols and cleavage of glycosylated phenolics.

2.5.2 Poinsettia bract necrosis

The initial symptoms of bract necrosis on poinsettia (*Euphorbia pulcherrima*) appear as small necrotic lesions at the looped end of lateral veins on bracts (McAvoy and Bible, 1996). Small necrotic dots along the margin expand and can lead to a necrotic border several centimetres wide on bracts (Wissemeier, 1993). Bract necrosis occurs most frequently on transitional bracts compared to leaves or true bracts, that is, those with axillary cyathia (Nell and Barrett, 1986). Dark green leafed poinsettia cultivars appear to be less susceptible to bract necrosis (McAvoy and Bible, 1997), suggesting that low pigment content may increase susceptibility to necrosis. While Bierman *et al*. (1990) found that poinsettia leaves are also susceptible to burn along their margins, Wissemeier and Marienfeld (1998) suggest that under normal circumstances, marginal necrosis is never seen on leaves, even when bracts are heavily affected. Similarly, browning appears to affect waratah bracts, rather than leaves.

Poinsettia bract necrosis has been attributed to a localised calcium deficiency (Stromme *et al.* 1994). Calcium deficiencies are often observed in organs with a low transpiration rate, such as flowers (Gislerod, 1999). However, a threshold level of calcium preventing necrosis cannot be identified, suggesting other factors are involved in the disorder (Wissemeier, 1993). For example, high light intensities late in poinsettia bract development led to exponential increases in bract necrosis (Wissemeier *et al.*, 2000). Condensed tannins identified in the vacuole of affected poinsettia tissues (McAvoy *et al.*, 1998) may be responsible for the discoloration associated with bract necrosis.

Bract necrosis has also been observed on sunflower (*Helianthus annuus*) bracts and disk flowers in Texas, South Africa and Spain, and has been linked to moisture and heat stress during budding and anthesis (Yang and Berry, 1983).

2.5.3 Tipburn of lettuce and other leafy vegetables

Tipburn is a disorder describing necrosis of the leaf margin of lettuce and other leafy vegetable crops (Saure, 1998). Tipburn is generally accepted as a calcium-related disorder, caused by a localised calcium deficiency of leaves and leaf margins (Collier and Tibbitts, 1982; Saure, 1998). Localised calcium deficiency causing loss of membrane integrity seems to cause the initial damage (Collier and Tibbitts, 1982), with further symptom development documented by Tibbitts *et al.* (1965). Prior to tipburn development, the larger veins near the lettuce leaf margin darken as a result of laticifer rupture, which releases latex into the surrounding tissue. The released latex causes collapse of parenchyma, occlusion of xylem elements and coagulation of latex between the site of rupture and the leaf margin. The marginal tissue loses turgor and scattered mesophyll cells become necrotic. The collapsed areas on young leaves become necrotic

and restrict further leaf enlargement. Bangerth (1979) and Kirkby and Pilbeam (1984) attribute the formation of brown melanin compounds in localised calcium deficiency to leakage of phenolic precursors from the vacuole into the cytoplasm, followed by oxidation by polyphenoloxidases.

In lettuce grown in glasshouses, tipburn develops when enlarging leaves partially enclose the growing points of young leaves, thus restricting their transpiration (Collier and Tibbitts, 1982). These enclosed leaves, whose transpiration is restricted by the outer leaves, do not accumulate calcium as they expand (Barta and Tibbitts, 2000). By contrast, exposed leaves with unrestricted transpiration accumulate calcium rapidly as the leaves enlarge (Barta and Tibbitts, 2000). However, tipburn incidence varies between years, locations, and planting dates and appears to be significantly influenced by the growing environment (Saure, 1998), suggesting that factors other than calcium are involved.

Saure (1998) suggests that tipburn may be a stress-related disorder, with rapidly growing plants particularly susceptible to damage. Other disorders generally attributed to calcium deficiency such as bitter pit in apple and blossom end rot of tomato cannot simply be explained by calcium concentrations (Perring, 1986; Saure, 1996; Saure, 2001). Other stresses causing cell membrane deterioration may be involved, with calcium as a secondary factor (Saure, 1996 and 2001). Stresses implicated include heat, drought and water stress in bitter pit development (Perring, 1986); high light intensity, high temperatures and high relative humidity in tipburn development (Saure, 1998); and water stress, high daily radiation and high temperatures in blossom end rot development (Saure, 2001). Lettuce tipburn occurs when temperature and radiation levels are high,

particularly in glasshouses in spring and field crops in summer (Collier and Tibbitts, 1982). Recently Wissemeier and Zuehlke (2002) found that tipburn in field grown lettuce was significantly correlated with the sum of irradiation from planting to harvest over three years. High irradiation three to four weeks prior to harvest also seemed to cause tipburn (Wissemeier and Zuehlke, 2002). Documenting the degree of susceptibility and stress intensity during lettuce head development may help relate the occurrence of tipburn to environmental factors (Saure, 1998). The rate of waratah flower development in late winter and early spring, and the timing of browning need to be further investigated, but similar stress-related phenomena may be involved in bract browning.

2.5.4 Sun damage and browning of fruit

Solar radiation induces sunscald of apple fruit, through excitation of chlorophyll molecules and production of reactive oxygen species (Merzlyak *et al.*, 2002). Sunscald symptoms were associated with a dramatic loss of chlorophyll and carotenoid pigments, and accumulation of phenolic compounds (Merzlyak *et al.*, 2002). Similarly, raspberry fruit is prone to white drupelet disorder on the sun-exposed side of the fruit, attributed to solar injury or sunscald due to high temperatures and UV radiation (Renquist *et al.*, 1987). The disorder has been reported in Colorado, California, New Mexico and southeastern Australia. A significant reduction in solar injury of raspberries occurred when plants were covered with 25 - 30% shade during the last few days of ripening (Renquist *et al.*, 1987).

Pomegranates are also susceptible to sunburn, visible as large dark spots on the fruit skin exposed to intense sunlight (Melgarejo *et al.,* 2004). Damage increases as previously shaded pomegranate fruit are exposed to the sun when branches bearing the fruit bend as fruit weight increases (Melgarejo *et al.,* 2004). Sunburn of pomegranates was reduced when sprayed with a commercially available refined kaolin product, likely to be due to a reduction in fruit temperature and exposure to UV radiation (Melgarejo *et al.,* 2004). Similar results may be achieved by shading the entire crop (Melgarejo *et al.,* 2004).

Lychee fruit are susceptible to browning in the field and after harvest, particularly if the fruit are subject to postharvest dessication (Underhill, 1992). In the field, lychee fruit on panicles on the northwest side were of lower quality, due to light and dark brown skin blemishes (Tyas *et al.,* 1998). Covering fruit with paper bags improved fruit quality and red colouration with no negative impact on yield (Tyas *et al.,* 1998). The anthocyanin content of lychee fruit decreases as browning increases (Zhang *et al.,* 2001), suggesting that anthocyanin degradation or changes in anthocyanin coloration due to pH (Underhill, 1992) may be involved.

Maturity bronzing of banana fruit involves cracking, red-brown discoloration and necrosis of the peel, particularly in rapidly growing fruit produced in the wet season in North Queensland (Williams *et al.,* 1990). Covering banana bunches with open covers during early fruit development is recommended to reduce maturity bronzing (Daniells *et al.,* 1992).

Bract browning in waratahs may bear some similarity to fruit burn that occurs with intense sunlight including UV radiation, and high temperatures. Control of bract browning may also be possible by shading the whole crop or physically covering individual waratah flowers.

2.5.5 Grapevine blackleaf

Blackleaf in grapevines is characterised by deep-blue, purple and/or brown discolouration in discreet, irregular regions of leaves in the outer canopy exposed to full sunlight (Lang *et al.,* 1998). Cells in the affected region of the leaf die, then the leaf becomes brittle, shatters and falls off. Previously shaded leaves are then exposed to direct sunlight and develop symptoms. Complete defoliation of the vine can occur. Lang *et al.* (1998) rejected the idea that low potassium concentrations are responsible for blackleaf, and suggest that light may be a significant trigger for the disorder.

Chlorophyll fluorescence and photosynthetic values for green and black portions of affected leaves suggest that damage to the photosynthetic system may occur prior to visible discoloration (Lang *et al.,* 1998). The decrease in photosynthesis, known as photoinhibition, leads to production of highly reactive oxygen species that may cause cell damage and further reduce photosynthetic efficiency (Smithyman *et al.,* 2001). Some grapevine species are particularly susceptible to photoinhibition, for example, ‘Concord’ grapevines that evolved in heavily forested areas with less exposure to drought and light stress (Smithyman *et al.,* 2001).

2.5.6 Bleaching and necrosis of leaf and floral tissue

Leaves and floral tissues of other species are also susceptible to pigment bleaching and necrosis, often caused by chronic photoinhibition after exposure to high light and temperature stress, as described for grapevine leaves. For example, chronic photoinhibition (measured as a decrease in the chlorophyll fluorescence parameter Fv/Fm) occurred in *Dendrobium* petals, sepals and leaves in full sun (He *et al.*, 1998). Intermediate sunlight reduced photoinhibition in leaves, but not sepals or petals. Photoinhibition, and subsequent bleaching and wilt, were only relieved when sepals and petals were fully shaded (He *et al.*, 1998). Similarly, leaf tissue of the orchid *Oeceoclades maculata* became photoinhibited with visible chlorosis and necrosis and/or accelerated senescence at high light intensity (Johnson, 1993).

Illicium species (star anise), like many orchids, are shade adapted (Olsen *et al.*, 2002). There are conflicting views of the ability of *Illicium* species to survive in full sun, with some authors reporting better flowering in full sun and others reporting yellowing and bleaching of foliage (Olsen *et al.*, 2002). Similarly, the light tolerance of waratah species has not yet been resolved. Pigment loss occurred in *Illicium* species exposed to full sun, as a result of photooxidation, with *I. henryi* exhibiting bleaching and necrosis prior to plant death (Olsen *et al.* 2002).

Chilean guava (*Ugni molinae*, Myrtaceae) is another understorey species that suffers from chronic photoinhibition and pigment loss in full sun, due to higher leaf temperatures and high light intensity (Pastenes *et al.*, 2003). Cultivation of Chilean guava under shade cloth reduces photoinhibition, although production of the species as

an intercrop may be more economically viable than growing under shade cloth (Pastenes *et al.*, 2003).

Leaves of *Schefflera actinophylla*, an understorey shrub, grown in full sun experienced a decrease in photosynthetic efficiency during the day, while shade grown plants maintained a high efficiency throughout the day (Schiefthaler *et al.*, 1999). However, this decrease was due to protective dissipation of excess light via the xanthophyll cycle. In contrast, *Schefflera* plants grown in the greenhouse and then exposed to full sun showed a large and rapid decrease in photosynthetic efficiency (Schiefthaler *et al.*, 1999), indicative of chronic photoinhibition. Waratah bracts, particularly those protected during flower development, may experience a similar decrease in photosynthetic efficiency on exposure to full sun.

Brown bead of broccoli is a disorder causing yellowing and browning of floral buds as they approach maturity (Jenni *et al.*, 2001a). The affected flower buds often become necrotic and fall off, with secondary damage caused by soft rot bacteria. Low levels of calcium, high solar radiation and low precipitation were all associated with a higher incidence of brown bead (Jenni *et al.*, 2001b).

Photoinhibition can also occur with high light stress and low temperatures, for example, in non-shaded *Eucalyptus* seedlings, causing visible damage, leaf abscission and in susceptible species, plant death (Close *et al.*, 2002). Such cold-induced photoinhibition may occur in waratahs, which exhibit bract browning following frost and strong sunlight (Offord, 1996).

Environmental factors such as drought, high or low temperatures, in combination with high light, have been related to many physiological disorders involving pigment bleaching and tissue necrosis, as described above. Such environmental stresses have the potential to reduce photosynthetic efficiency, therefore increasing photoinhibition (Powles, 1984). Shade-adapted plants are particularly susceptible to photoinhibition and subsequent visible damage (Powles, 1984; Osmond, 1994). Solar radiation, alone or through photoinhibition, can contribute to free radical formation and oxidative stress (Levine, 1999). This increases lipid peroxidation and results in visible necrotic damage (Levine, 1999). Oxidative stress has been cited as a direct or indirect cause of physiological leaf spot disorders resulting in necrosis (Staples, 2002), for example, in barley (Wu and von Tiedemann, 2002). Photoinhibition and oxidative damage caused by high light or other stresses may play a role in the development of bract browning in waratahs, although photoprotective mechanisms such as non-photochemical quenching (Demmig-Adams *et al.,* 1997) may also be active.

The presence of anthocyanins and flavonoids in leaf tissue, and presumably, in waratah bract tissue, may offer some protection against light stress. Anthocyanins can shield chloroplasts from high light (Steyn *et al.,* 2002) and scavenge reactive oxygen species (Neill and Gould, 2002). For example, in senescing red-osier dogwood (*Cornus stolonifera*) leaves, anthocyanins decrease light capture by chloroplasts and appear to enhance recovery from high light stress (Feild *et al.,* 2001). Anthocyanins improve the antioxidant capacity of red leaves of *Quintinia serrata* compared to green leaves (Neill *et al.,* 2002). Anthocyanins may also be produced in response to photoinhibition, for example, in cold-induced photoinhibition of *Eucalyptus* seedlings (Close *et al.,* 2002).

Flavonoids and other related compounds also offer protection against UV-B radiation, and are present in relatively high concentrations in several genera of Proteaceae (Wand, 1995).

2.6 Conclusion

Minimisation of bract browning is necessary to improve the quality of waratah flowers sold on domestic and export markets. Waratah bract browning seems to have similar environmental triggers to browning in other crops, suggesting commonality with the disorders reviewed in this chapter.

Browning responses in other crops are often attributed to localised calcium deficiency. However, the incidence of calcium-related disorders is not predictable solely from calcium content, suggesting that other factors may be involved (Wissemeier, 1993; Saure, 1998). Environmental factors such as high light, alone or in combination with other stresses such as high or low temperatures and water stress, can result in browning through photoinhibition, pigment loss and oxidative damage, particularly in shade adapted species. Environmental stresses may also interact with the stage of flower and bract development in waratahs to cause browning. For example, early picking may reduce the likelihood of bract browning (Offord, 1996; Nixon, 1997), suggesting that browning may increase as flowers mature.

The aim of this project is to characterise the bract browning disorder in waratahs, and identify the physiological cause of the disorder. The hypotheses that localised calcium deficiency, a full sun environment or water stress cause bract browning will be tested experimentally during the first season. Subsequently, other potential causes of bract

browning such as photoinhibition, pigment loss and photooxidation will be investigated, and the physiological response of bracts and leaves quantified in each experiment. However, it is necessary to characterise waratah bract development and the incidence, timing and severity of bract browning in different cultivars prior to any experimental treatments. These aspects of the bract browning disorder are investigated in Chapter 3.