Chapter 5
Light environment (sun/shade) effects on browning

5.1 Introduction

5.1.1 Assessing the light environment of waratahs

Several researchers and growers have suggested that waratahs of a higher quality, with less bract browning, are produced in the shade (Offord, 1996; Nixon, 1997) or by physically protecting the developing bud, for example, by bagging (Burnett and Nixon, 1990). However, the reduction in browning under shade has not been proven experimentally. Waratahs are generally considered to be shade-adapted, as they grow as an understorey shrub in their natural environment (Offord, 1996), and have the relatively low CO_2 assimilation rate, transpiration rate and stomatal conductance typical of an understorey species (Reynoso *et al.,* 2000). However, the light environment in which Proteaceous species grow has not been well characterised in terms of light intensity or quality (Myerscough *et al.,* 2000) and commercially grown waratahs are cultivated in diverse light environments. They may be grown in exposed areas with little protection, surrounded by natural bush or exotic trees, or protected by artificial windbreaks or shade cloth (Chapter 3.2). Summer shading of waratahs in southwestern Japan has been recommended by Reynoso *et al.* (2000), to minimise solar radiation damage to differentiating flowers and new vegetative growth. In this chapter, the aim is to measure the effect of full sun and shaded environments on the occurrence and severity of bract browning during bud development for several cultivars, and to assess the severity of browning of waratahs in their natural environment.

5.1.2 Altering the light environment using shade cloth

Shading is standard practice for many crops, including coffee and cacao (Demmig-Adams *et al.,* 1997). It is not known whether these species have an inherently lower capacity for photoprotection or are unable to cope directly with other stressors, such as heat or water stress (Demmig-Adams *et al.,* 1997). Shade is also used to protect plants from dynamic or chronic photoinhibition, including *Dendrobium* orchids (He *et al.,* 1998), tea (Mohotti and Lawlor, 2002) and Chilean guava (Pastenes *et al.,* 2003).

Shade cloth has the potential to change light intensity, the proportion of diffuse radiation and light quality compared to full sun, depending on the density (shade factor) and colour (Yates, 1989; Healey and Rickert, 1998). The shade factor of a material is a measure of the incident radiation not transmitted through it (Yates, 1989). Black shade cloth is a neutral filter, with almost no change in shade factor from 300-1100 nm. However, in shade cloth with green warp fibres and black weft fibres, there is a significant reduction in shade factor between 450-550 nm (green wavelengths), so more green light is transmitted (Yates, 1989). Oren-Shamir *et al.* (2001) found a peak in transmittance around 520 nm under green shade net. However, green wavelengths are the least photosynthetically effective, so photosynthetic rates under green shade cloth are likely to be less than under black (Yates, 1989). Green shade nets also transmitted light with a significantly lower UV-A/PAR ratio than black neutral nets (Oren-Shamir *et al.,* 2001). The proportion of diffuse to direct PAR was significantly higher under green shade net compared to black (Oren-Shamir *et al.,* 2001). Compared to shade cloth, natural shade is enhanced in green wavelengths and influences NIR effects to a greater degree, due to low red/far red ratios (Anderson, 1964; Yates, 1989).

The design of the shade enclosure can also change the temperature and relative humidity around the plants (Andersson and Skov, 1991). For example, one layer of black plastic netting and one standard layer of shade netting decreased temperatures and vapour pressure deficit, compared to no netting (Mesen *et al.,* 1997). The shade shelter may also be designed to minimise temperature differences between shade and full sun environments (for example, Valladares and Pearcy, 1997, Close *et al.,* 2001).

The first experiments in this chapter (section 5.2) aim to describe the light environment of naturally occurring waratahs growing in the Royal National Park and quantify the severity of bract browning in the natural habitat. Sun and shade effects on bract browning are then described for waratahs cultivated at Mount Annan from 2001-2003 (section 5.3). The light environment of the cultivated waratahs is described in section 5.3.1 Initially, shade (50% black shade cloth) and sun treatments were applied to waratahs of various cultivars at Mount Annan in a factorial experiment with irrigation frequency, to determine whether shade and frequent irrigation effectively reduced browning (section 5.3.2). In subsequent years, shade treatments were applied at different stages of bud development, and bract browning in sun and shade treatments were compared (section 5.3.3 and 5.3.4). Finally, sun and shade (50% green shade cloth) treatments were applied on a commercial waratah plantation at Jervis Bay and the effect on bract browning was monitored in 2002 and 2003 (section 5.4).

5.2 Describing the natural light environment and bract browning of waratahs in the Royal National Park

5.2.1 Study site - Royal National Park

Waratah plants growing in their natural environment in the Royal National Park were observed at three sites (Figures 5.1a and 5.1b) in spring 2003, with permission from the NSW National Parks and Wildlife Service. Four to five plants, each with one to seven flowers, were chosen for observations at each site.

Site 1 (relatively open canopy): Sir Bertram Stevens Drive, between wildlife signs after Warnumbul Rd on left, on right hand side when driving from Audley weir.

S 34^{o}05.953”
E 151^{o}03.518”
Elevation 220m

Site 2 (intermediate canopy cover): Heathcote East site – Bottle Forest Trail off Bottle Forest Rd, Heathcote East.

S 34^{o}05.252”
E 151^{o}01.113”
Elevation 217m

Site 3 (relatively dense canopy cover): McKell Avenue between Waterfall Flat and Gunjulla Flat Picnic areas.

S 34^{o}08.838”
E 151^{o}00.330”
Elevation 143m

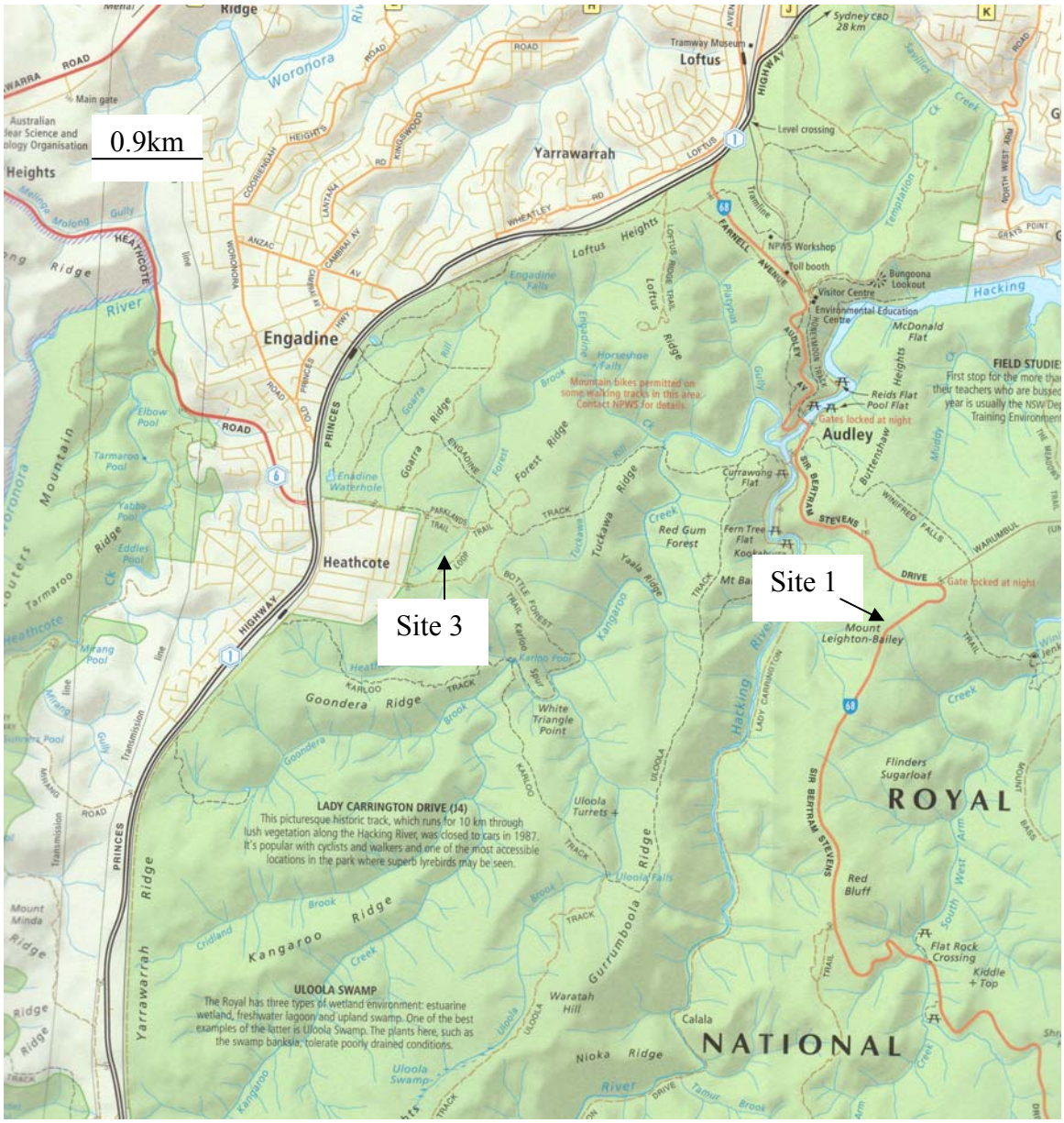

Figure 5.1a: Map of sites 1 and 3 in the Royal National Park, where natural waratah populations were studied (Pringle, 2003).

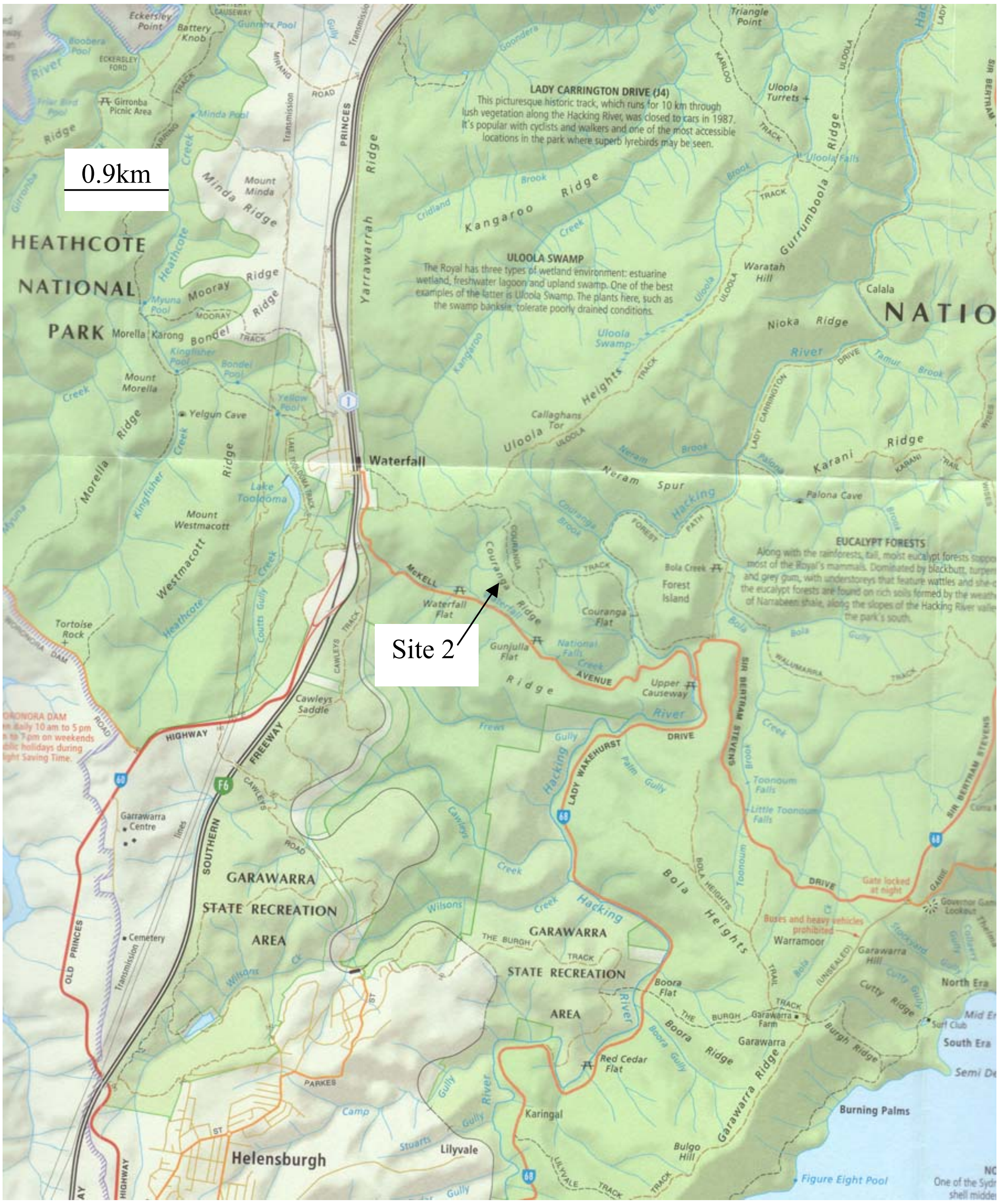

Figure 5.1b: Map of site 2 in the Royal National Park, where natural waratah populations were studied (Pringle, 2003).

Figure 5.2: Waratah plant 3 tagged at Site 1 in the Royal National Park at date of initial bract browning assessment (7/9/03).

5.2.2 Methods

5.2.2.1 Measurements of the natural light environment

The light intensity directly above waratah flowers and along a transect of surrounding vegetation at 1 m above the ground was measured at Site 1 in the Royal National Park (Figure 5.1a). Measurements were taken on days with clear skies, to reduce the effect of variable cloud cover (Duarte *et al.,* 2002) and therefore, a greater proportion of diffuse radiation and increased light scattering (Healey and Rickert, 1998). Measurements directly over flowers were made with a hand held quantum sensor (Licor Li470, Nebraska, USA), as described by Duarte *et al.* (2002) for data logger measurements above Brazilian pine seedlings. Data loggers for light intensity measurements were not available during this study. Measurements in surrounding vegetation were made every metre along 25 m transects using a 1 m long sunfleck ceptometer (Delta T Devices, Cambridge, UK) held 1 m from the ground, as described for Nicotra *et al.* (1999) for canopy analyser measurements. Three measurements were made over each flower, or at each 1 m interval, at each time point. Both the quantum sensor and the ceptometer were calibrated against a recently calibrated quantum sensor (Li-Cor Li189, Nebraska, USA) prior to use.

5.2.2.2 Bract browning in the natural environment in 2003

The number of basal and floral bracts browned and senesced was recorded on tagged flowers at each of three sites in the Royal National Park (Figure 5.3). Flowers chosen for assessment were at the tight bud (TB) or juvenile open (JO) stage of development on 7th September. Flowers were assessed on two subsequent dates, until all flowers were mature.

5.2.3 Results

5.2.3.1 Measurements of the natural light environment

Data collection in the Royal National Park was limited by cloud cover on days set aside for field measurements. For this reason, the light intensity directly above waratahs and along a transect in the understorey were only measured for one full day at site 1 during spring 2003, rather than at all three sites over several days as intended.

Waratah flowers were exposed to light intensities between 1400-1600 μmol m^{-2} s^{-1}, for up to two hours per day in their natural environment (Figure 5.3a-d). This is higher than the average daily maximum light intensity in full sun at Mount Annan (1200 μmol m^{-2} s^{-1} during October 2003). However, the duration of exposure to light above 1000 μmol m^{-2} s^{-1} was much shorter in the natural environment (up to two hours, compared to five hours in a full sun environment), reducing the probability of light damage.

The light intensity along an understorey transect, between waratah plants 3, 4 and 5 around midday (Figure 5.4), showed a similar range of light intensities, with some areas between 1200-1600 μmol m^{-2} s^{-1}, but a greater proportion of areas between 200 and 800 μmol m^{-2} s^{-1}.

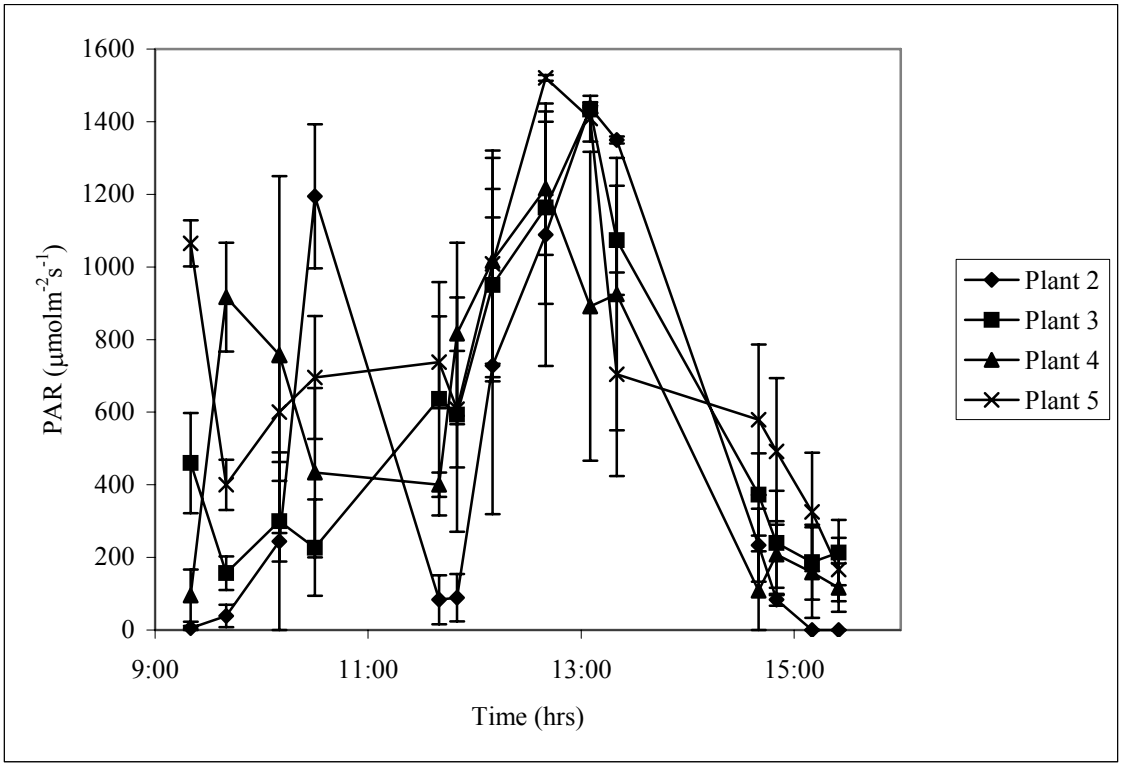

Figure 5.3: Mean light intensity (Photosynthetically Active Radiation (PAR) µmol m^{-2} s^{-1} ± SE) from 9am to 3.30pm directly above waratah buds on plant 2 (n = 3 buds), plant 3 (n = 5), plant 4 (n = 2) and plant 5 (n = 4) at site 1 in the Royal National Park on 15th September 2003.

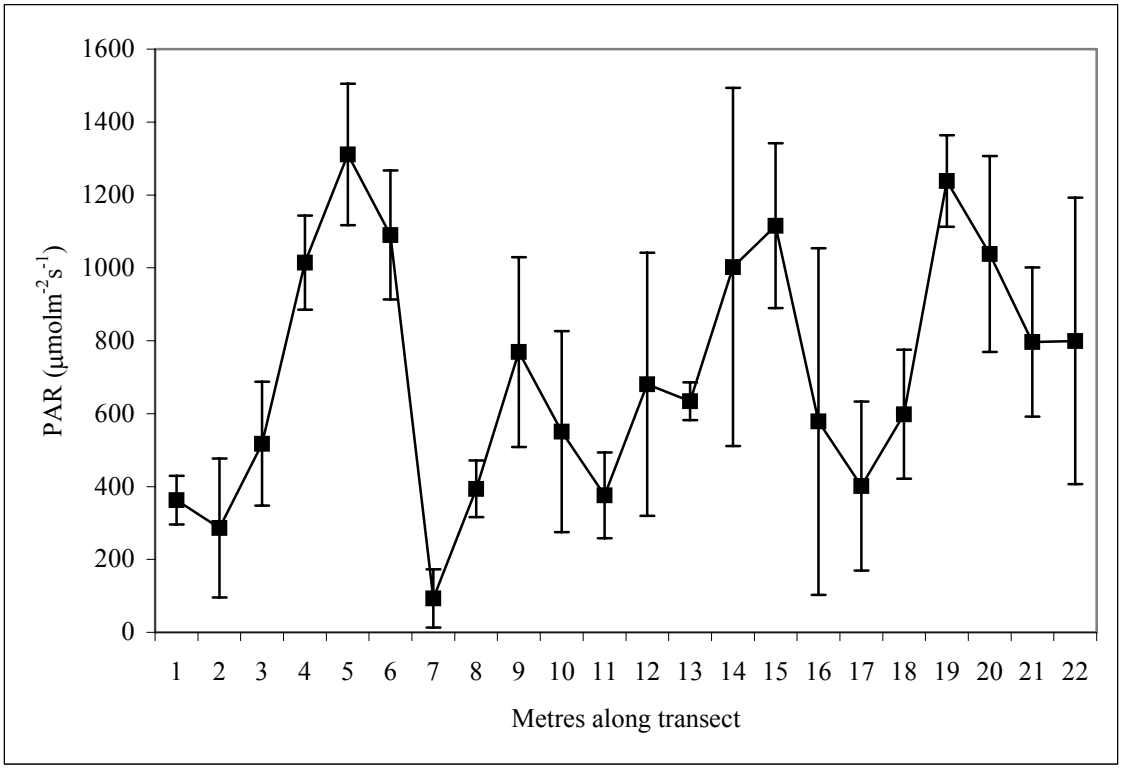

Figure 5.4: Mean light intensity (Photosynthetically Active Radiation (PAR) µmol m^{-2} s^{-1} ± SE) approximately every metre along a transect between plants 3, 4 and 5 at site 1 in the Royal National Park, presented as mean of measurements at three times around maximum solar altitude (11.25am, 11.55am and 12.12pm) on 15th September 2003.

5.2.3.2 Bract browning in the natural environment in 2003

In the Royal National Park, only 2 - 4 floral bracts from a total of 15 - 25 bracts on each flower were brown or senesced (Figure 5.5). However, most basal bracts browned and senesced between early September and mid-October (Figure 5.6). Although the number of brown and senesced bracts stayed the same or increased over time, the mean number of brown basal bracts decreased at site 1 on 6/10/03 because there were fewer flowers remaining, with less bract browning and senescence than flowers that matured earlier.

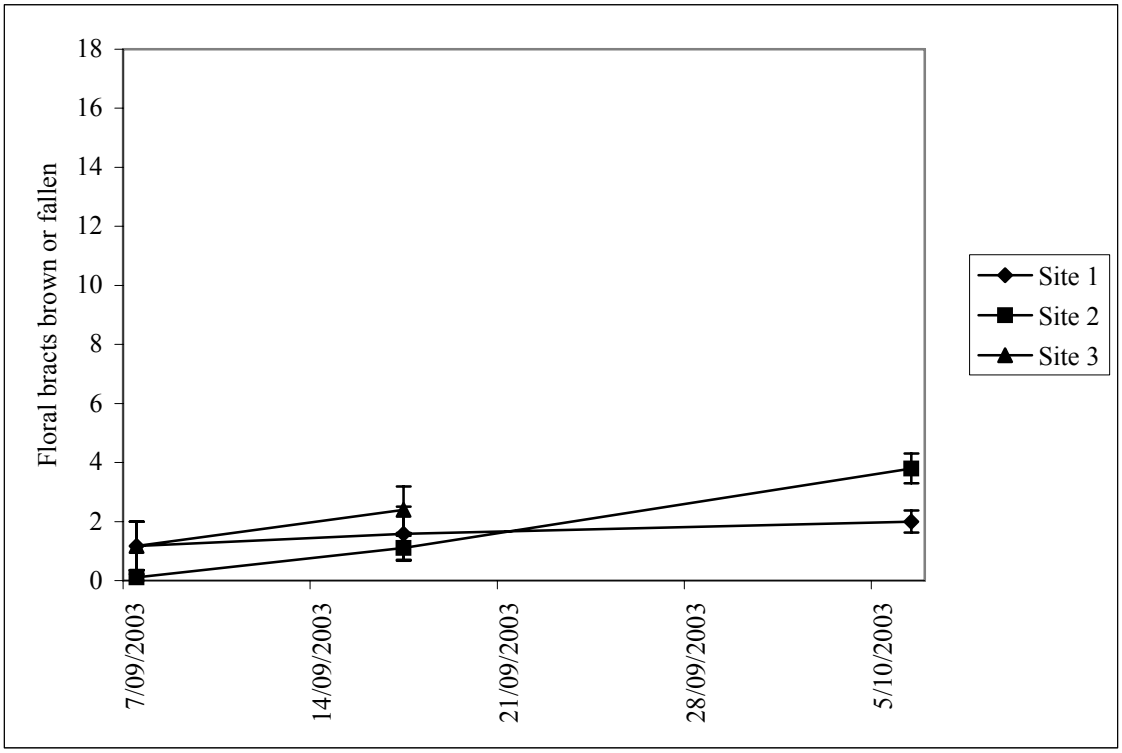

Figure 5.5: Mean browning and loss of floral bracts (± SE) of *Telopea speciosissima* waratahs in the Royal National Park. At site 1 (♦), n = 5 plants each with 2-5 buds, at site 2 (■), n = 4 plants each with 2-7 buds and at site 3 (▲), n = 5 plants each with 1-4 buds. However, n decreases at later sampling dates due to flower maturation.

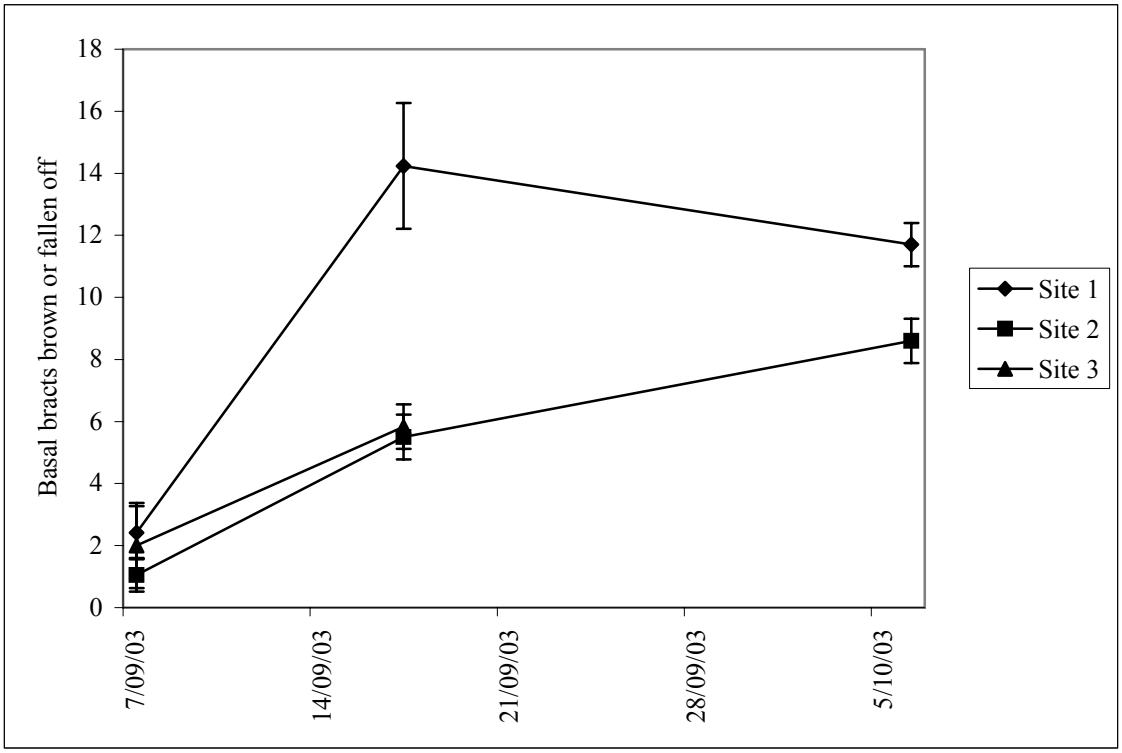

Figure 5.6: Mean browning and loss of basal bracts (± SE) of *Telopea speciosissima* waratahs in the Royal National Park. At site 1 (♦), n = 5 plants each with 2-5 buds, at site 2 (■), n = 4 plants each with 2-7 buds and at site 3 (▲), n = 5 plants each with 1-4 buds. However, n decreases at later sampling dates due to flower maturation.

In the following experiment (section 5.3.1), the light environment of cultivated waratahs grown in full sun and under 50% shade cloth at Mount Annan will be measured for comparison with the natural light environment described in sections 5.3 and 5.4. The bract browning of waratahs under sun and shade conditions will also be measured over three years from 2001 to 2003 (section 5.3.2 and 5.3.3). The initial browning experiments in 2001 examined cultivar differences in bract browning with weekly irrigation only, as well as combined light and irrigation frequency effects on browning for two cultivars 'Olympic Flame' and 'Sunflare' (section 5.3.2). Experiments in 2002 and 2003 investigated the effect of time of shading on bract browning for several cultivars (section 5.3.3).

5.3 Light intensity and irrigation frequency effects on bract browning at Mount Annan

5.3.1 Light intensity measurements at Mount Annan

5.3.1.1 Methods

The intensity of photosynthetically active radiation (PAR) in full sun and under black 50% shade cloth at Mount Annan was monitored using a Middleton PAR sensor (SK01-DP, Carter-Scott design, Melbourne) connected to a TGXP logger using a 9V battery source. The sensor was calibrated by the manufacturer prior to the experiment. In both light environments, the sensor and data logger were placed on the bench well away from plants. Since only one sensor was available, measurements were made under shade cloth in September 2003 and in full sun in October 2003. As the light intensity in October was higher than in September, the shade cloth data was corrected using solar radiation data from both months from a fixed Environdata (Warwick, Queensland) weather station in full sun at Mount Annan. Correction factors and solar radiation data are presented in Appendix Table A3.1.

Changes to the microclimate under shade cloth were also investigated for the main experimental site at Mount Annan from 10th September to 29th October 2003. Temperature and relative humidity were measured in full sun and under 50% shade cloth using TinyTag data loggers (Hastings Data Loggers, Port Macquarie, NSW). Data loggers were taped into an upturned plastic container, to reduce exposure to rain, and placed on poles approximately 1 m above the bench, at a similar height to waratah flowers. The minimum and maximum temperature and relative humidity were analysed for each site using a *t*-test in Minitab (Release 14.1, Minitab Inc. 2003), with log transformation for non-normally distributed data.

5.3.1.2 Results

The light intensity in full sun reached an average maximum around 1200 μmol m^{-2} s^{-1} at midday in October 2003, while in shade, the corrected average was around 800 μmol m^{-2} s^{-1} (Figure 5.7). The approximate shade factor of shade cloth used in this experiment (nominally 50%) was between 20-40% from 7 am to around 3 pm, but was much higher in early morning and late afternoon.

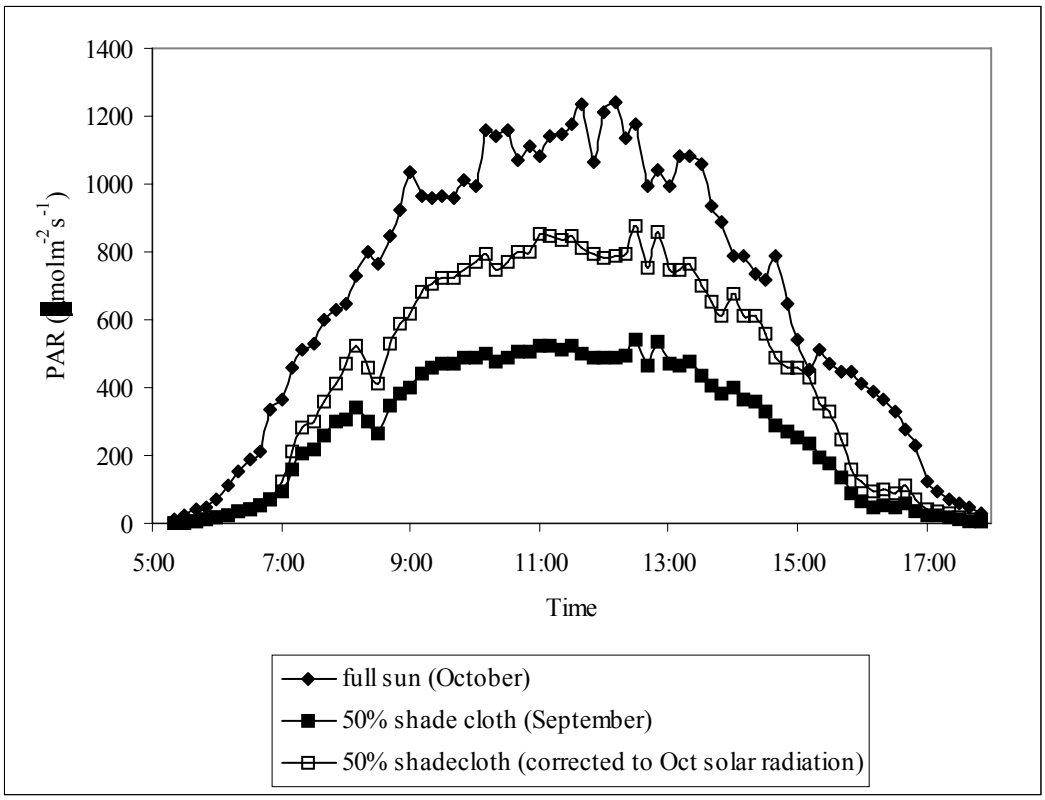

Figure 5.7: Photosynthetically active radiation (Photosynthetically Active Radiation (PAR), μmol m^{-2} s^{-1}) in full sun (♦) and under 50% shade cloth at Mount Annan during flower maturation in 2003. PAR under shade cloth is presented as raw data, collected in September (■), and with correction to allow direct comparison to full sun data, collected in October (□).

The mean daily maximum temperature was significantly higher by 5ºC in full sun than under shade cloth (sun 33.9ºC, shade 28.7ºC; P = 0.000). The minimum relative humidity was significantly lower in full sun than under 50% shade cloth (P = 0.002 for log transformed data), with back transformed means of 14.9% in the sun and 20.9% under shade cloth. The maximum relative humidity and minimum temperature were similar in the sun and shade.

Plants in full sun at Mount Annan are exposed to high light intensities for a longer duration than plants in natural populations, although in the natural environment light intensity is highly variable over the course of a day. The shade cloth treatment at Mount Annan approximately halves the light intensity experienced by waratah plants, as well as moderating temperature and relative humidity. The bract browning response of waratahs grown in the artificial shade environment and in full sun will investigated in detail in the next sections (5.3 and 5.4).

5.3.2 Cultivar, irrigation and light environment experiments in 2001

5.3.2.1 Methods

'Olympic Flame', 'Sunflare', 'Fire and Brimstone' and pink waratah cultivars were moved to full sun or 50% shade cloth on 29 and 30th April, from their previous position (either full sun or 50% shade cloth). Initially, ten plants of each cultivar were included in experiment 5.3.2.2a (for comparison of browning in different cultivars, with weekly irrigation only) and twenty plants of each cultivar for each light and irrigation combination in experiment 5.3.2.2b (for comparison of bract browning under daily or weekly irrigation for two cultivars). However, some plants had vegetative rather than floral buds, so fewer plants had flowers for assessment at commercial maturity than anticipated. Plants were irrigated to saturation either daily or weekly from 12th June onwards.

The severity of bract browning was assessed using the scale described in Chapter 3 (Figure 3.1). Plants in experiment 5.3.2.2a were assessed on 14th September and at commercial maturity (between 29th September and 17th October), while plants in

experiment 5.3.2.2b were assessed at commercial maturity only. The date of commercial maturity varied within and between cultivars and treatments.

The browning scores to be analysed are categorical variables with a natural ordering (hence, ordinal), and are not likely to be normally distributed or have constant variance (Agresti, 1996). Therefore, the distribution of scores was modelled using the Ordinal Logistic Regression procedure in Minitab (Release 14.1, Minitab Inc. 2003) with *light environment* (full sun or 50% shade cloth), *cultivar* and *date of assessment* included as predictors of browning score. Pink waratahs were grouped as one cultivar, although their genetic backgrounds were different. The chi-square test for deviance difference was used to remove predictors that did not have a significant impact on browning score. Main effects, rather than interactions, were assessed, as a solution could not be reached in Minitab if interactions were included.

5.3.2.2a Results of sun/shade experiment, with weekly irrigation only

Light environment (sun or shade) was a significant predictor of browning score for inner bracts ($P = 0.000$). Shade cloth significantly reduced bract browning, shown as a higher proportion of low scores (0-1), compared to plants grown in full sun (Figure 5.8) All waratahs grown in full sun showed some degree of bract browning, while 60% of waratahs grown under shade cloth showed no signs of browning. Cultivar and date of maturity were not significant predictors of bract browning, suggesting that all cultivars examined were equally susceptible to bract browning at flower maturity. Outer bracts generally had high scores, and could not be analysed using Ordinal Logistic Regression.

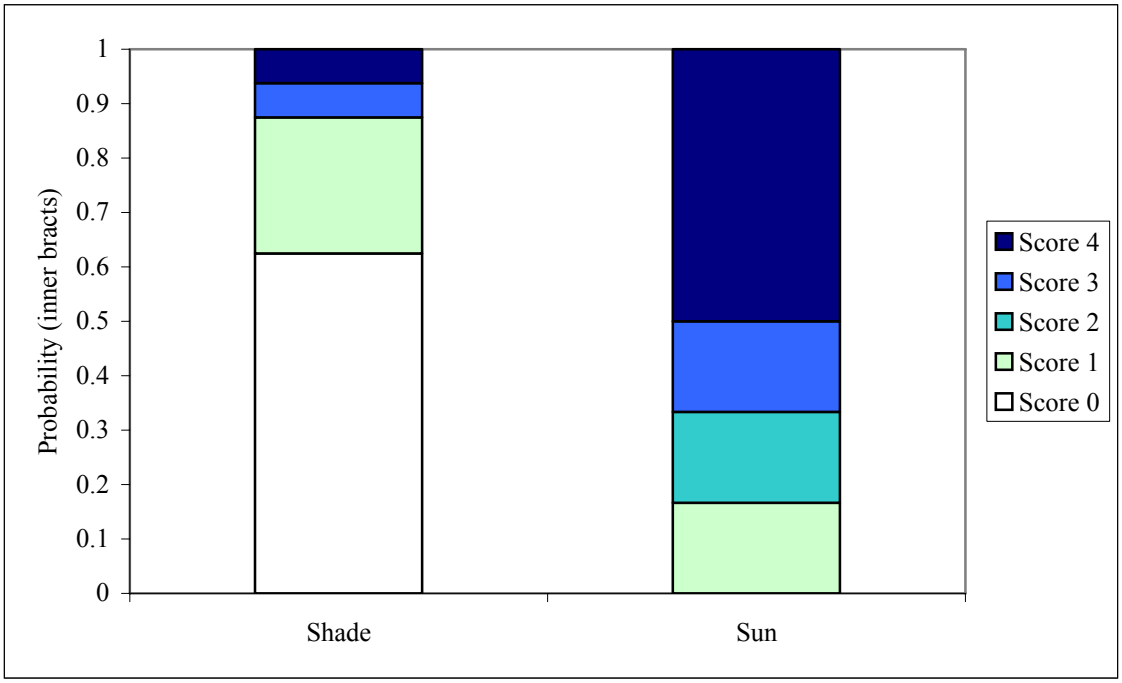

Figure 5.8: Probability of bract browning score (0-4) for inner bracts; pooled for cultivars 'Olympic Flame', 'Fire and Brimstone', 'Sunflare' and pink waratahs at commercial maturity. Plants were grown under 50% shade cloth or in full sun (P = 0.000). n ≥ 2 plants for all cultivars in each treatment, except for 'Fire and Brimstone' in the shade, with n = 1.

5.3.2.2b Results of sun/shade and irrigation experiment

Scores for inner bracts

Light environment (P = 0.000), irrigation frequency (P = 0.022) and date of assessment (P = 0.017) were significant predictors of browning scores for inner bracts. Sun exposure, weekly irrigation and assessment at commercial maturity, rather than in mid-September, resulted in higher bract browning scores. Scores for browning of inner bracts were not significantly different between cultivars.

Inner bract scores were also analysed separately for sun and shade treatments, to determine whether irrigation frequency or harvest date significantly affected browning within these light environments. In full sun, irrigation frequency did not have a significant effect on browning score for either cultivar, although browning scores increased significantly from mid-September to commercial maturity (P = 0.019).

Under 50% shade cloth, responses to irrigation frequency differed between cultivars (P = 0.019, Figure 5.9). In shaded 'Sunflare', daily irrigation resulted in lower bract browning scores (often scores of 0), compared to weekly irrigation, although differences were not significant. In shaded 'Olympic Flame', daily and weekly irrigation resulted in similar scores for bract browning. These results show that light environment has a greater effect on bract browning than irrigation frequency. However, there seems to be an interaction between light intensity and irrigation frequency on browning severity.

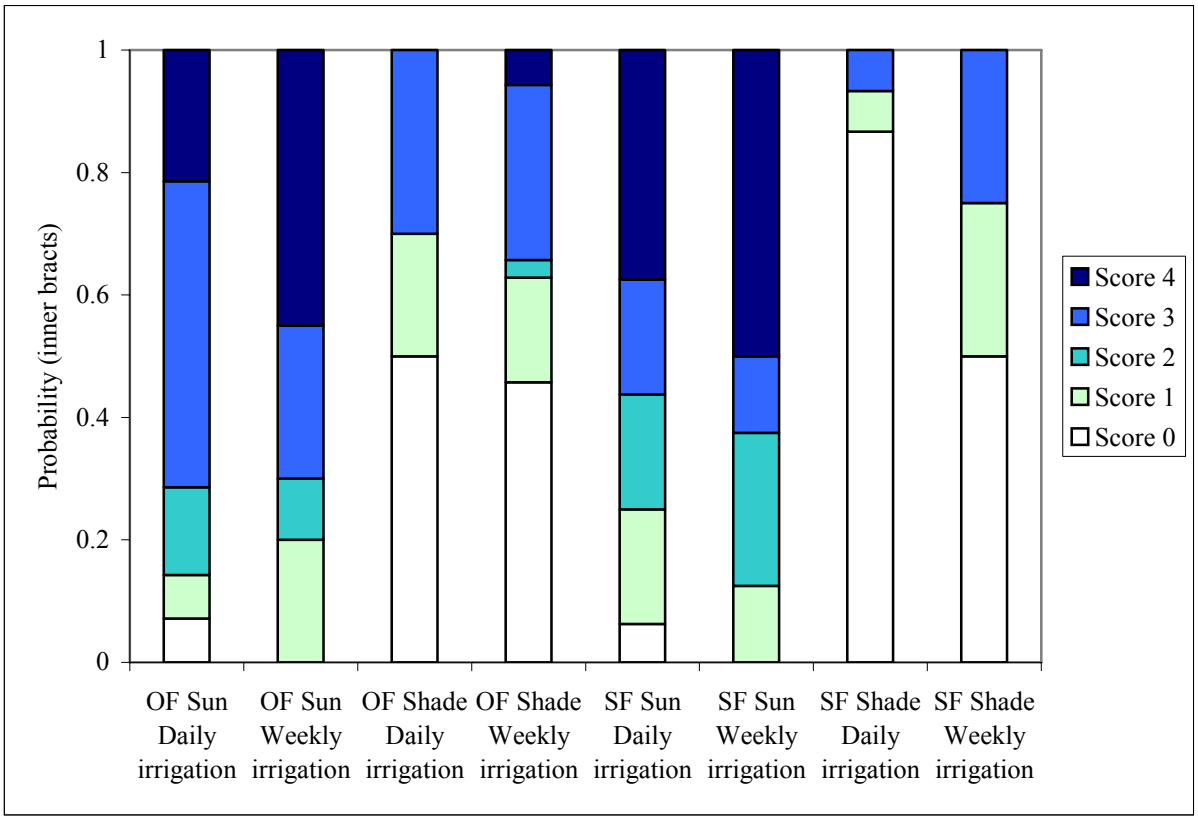

Figure 5.9: Probability of bract browning scores (0-4) for inner bracts of 'Sunflare' (SF) and 'Olympic Flame' (OF) waratahs at commercial maturity, irrigated daily or weekly and grown in full sun or under 50% shadecloth. n = 4-12 plants with 1-3 buds per plant, for all cultivars in each light and irrigation treatment, except for 'Sunflare' in the shade with weekly irrigation (n = 1).

Scores for outer bracts

Light environment (P = 0.040) and cultivar (P = 0.002) were significant predictors of browning scores for outer bracts. Shading waratahs resulted in significantly lower bract browning scores, with fewer high scores of 4, compared to full sun (Figure 5.10). 'Sunflare' waratahs also had a significantly higher outer bract browning score than 'Olympic Flame' waratahs. Browning scores did not increase significantly between assessment in mid-September and commercial harvest, indicating that damage generally occurs earlier than in inner bracts.

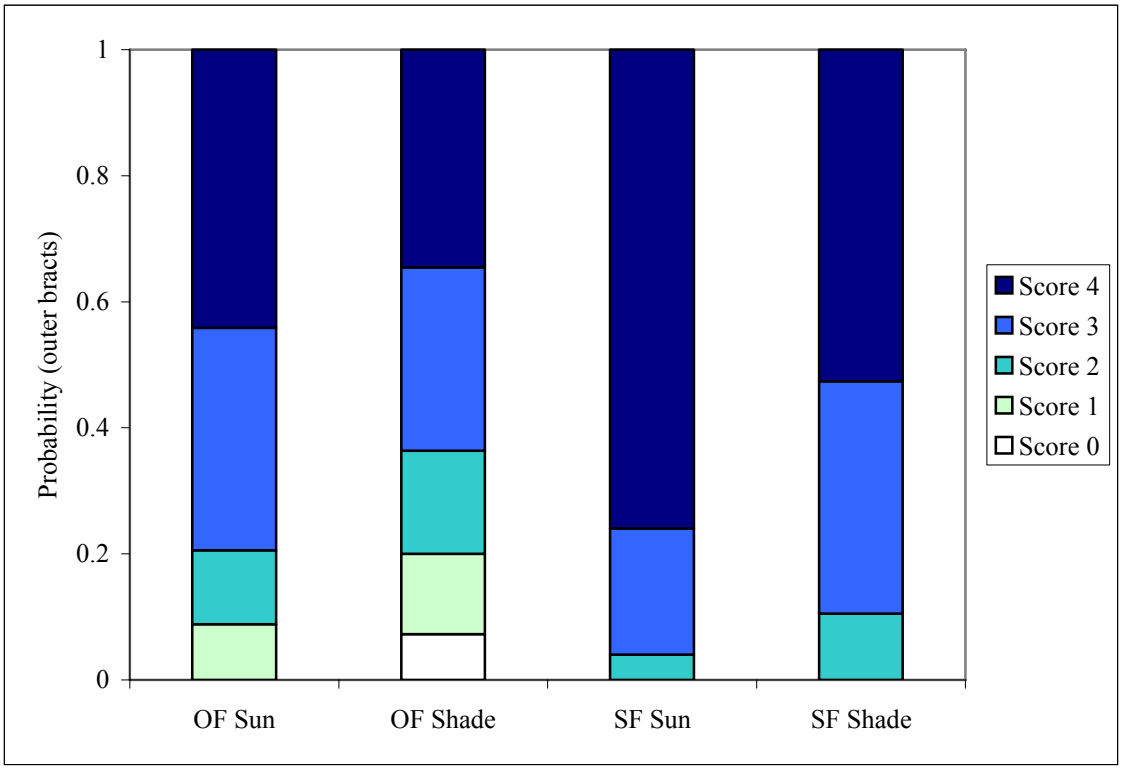

Figure 5.10: Probability of bract browning scores (0-4) for outer bracts of 'Sunflare' (SF) and 'Olympic Flame' (OF) waratahs, irrigated daily or weekly and grown in full sun or under 50% shade cloth. Scores for assessment dates in mid-September and at commercial maturity were not significantly different, therefore, pooled assessment dates are presented. n = 4-12 plants with 1-3 buds per plant, for all cultivars in each light and irrigation treatment, except for 'Sunflare' in the shade with weekly irrigation (n = 1).

These experiments show that daily irrigation did not exacerbate browning in waratah bracts, and therefore daily irrigation was used during late bud development in all other experiments. Bract browning responses did not differ significantly between cultivars. A

major reduction in bract browning was evident in waratahs grown under shade cloth. The aim of subsequent experiments in 2002 was to determine whether the reduction in bract browning with shade cloth was consistent from year to year in 'Fire and Brimstone' waratahs, and to determine whether the time of shading is critical in the reduction in browning. The method of assessing bract browning severity was changed, as analysis of data sets with large numbers of high scores was not possible in some experiments in 2001, for example, outer bracts in experiment 5.3.2. Therefore, a more sensitive method of describing bract browning severity was sought in 2002.

5.3.3 Light environment effects on bract browning at Mount Annan in 2002

5.3.3.1 Methods

Experimental treatments

'Fire and Brimstone' waratahs were subject to one of three treatments, the first with buds initiated in the shade and kept in the shade until harvest (early shade), the second with buds initiated in the sun and kept in the sun until harvest (sun) and the third with buds initiated in the sun then moved into the shade on 17th July, just prior to bud swelling (late shade). Plants were irrigated to saturation every day or every second day, as necessary.

Assessment of bract browning severity

The severity of bract browning was monitored weekly from mid-August to commercial harvest stage. Bract browning severity was quantified by counting the number of bracts with more than 10% browning, as described by McConchie *et al.* (1991) for protea leaf blackening, rather than using a score for individual flowers. Only outer and inner floral bracts were counted (not basal bracts), as these are the most commercially significant,

following the method of Dennis (1991) who counted only red-pigmented bracts of two new waratah cultivars. Bracts that are severely browned often senesce, therefore the number of floral bracts senesced, as well as brown bracts remaining on the flower, were taken into account when describing the severity of browning. Since basal bracts also senesce but are not commercially significant, the average number of basal bract scars for each cultivar was subtracted from the total number of bract scars counted, to calculate the approximate number of floral bracts browned and/or senesced at each time interval (see Equations 5.1 and 5.2). 'Fire and Brimstone' and 'Wirrimbirra White' cultivars have an average of seven basal bracts per flower head (Table 3.2). 'Olympic Flame' basal bracts were not counted, but were assumed to average seven bracts per flower.

Equation 5.1:

$$\text{Number of floral bracts senesced} = \text{number of bract scars} - \text{number of basal bracts (if bract scars} > \text{number of basal bracts)}$$

Equation 5.2:

$$\text{Number of floral bracts brown or senesced} = \text{number of bracts with } >10\% \text{ browning} + \text{number of floral bracts senesced}$$

Statistical analysis

The browning data are repeated measurements on the same experimental unit over time (also known as longitudinal data), and measurements at each time are not independent (an assumption of ANOVA) (Hand and Crowder, 1996). Therefore, data were analysed using the Repeated Measures (AREPMEAS) Analysis of Variance procedure in Genstat (Lawes Agricultural Trust, 2003), to take into account the correlation between measurements at subsequent time points. Data were square root transformed prior to analysis, due to the likely Poisson distribution of the data (counts), in order to improve normality and homogeneity of variance (Quinn and Keough, 2003). In the AREPMEAS

analysis, *Sun/shade treatment* was included as the Treatment term and *Bud* nested in *Plant* as the Block term. The last two sampling dates included a large number of missing values due to maturation of most flowers prior to these dates. Therefore, these dates were not included in the analysis, as a solution could not be reached in Genstat (Lawes Agricultural Trust, 2003).

5.3.3.2 Results

Shade cloth reduced browning and bract loss in 'Fire and Brimstone' waratahs by 30 - 60% compared to sun exposed flowers at maturity in 2002. There was a significant interaction of time of measurement and treatment (P = 0.008), with significant increases in browning observed between the beginning of monitoring (21/8/02) and the middle of September for all treatments (Table 5.1). Initially, only 1 - 3% of the total floral bracts in a flower head showed browning, based on an average of 34 floral bracts per 'Fire and Brimstone' flower. This increased to 16%, 29% and 42% in late shaded, early shaded and sun exposed waratahs, respectively, at flower maturity.

From the middle of September (the juvenile open (JO) stage), browning of bracts in the sun was significantly higher than bracts shaded late, and higher than bracts shaded early (Table 5.1, from 12/9/02 onwards). However, differences between sun and early shading were only significant on 18/9 and 24/10. Late shading reduced browning more effectively than early shading (Table 5.1, 24/10/02), with significant differences between shade treatments from the juvenile open stage.

Although the number of brown and senesced bracts increased on an individual flower head over time, the predicted mean of brown or fallen bracts on early and late shaded flowers decreased on 10/10/02 (Table 5.1). This occurred because flowers matured or were harvested from this time point, reducing the subsequent number of flowers measured. The remaining flowers had less browning, leading to a decrease in the mean bract browning count.

Table 5.1: Square root and back transformed number of 'Fire and Brimstone' floral bracts brown or senesced for sun, early and late shade treatments during floral development in 2002. Lower case letters indicate significant differences between treatments for each sampling date, while upper case letters indicate significant differences between sampling dates within a treatment. Time by Treatment interaction $P = 0.008$; LSD for square root transformed data = 0.62. n ≥17 plants for all treatments, with 1-5 buds per plant, although n decreases at later sampling dates due to flower maturation.

		Square root transformation						Back transformed		
		Treatment								
Date	Time (days)	Sun		Early shade		Late shade		Sun	Early shade	Late shade
21/8/02	0	0.99	Aa	0.85	Aa	0.54	Aa	0.97	0.72	0.29
28/8/02	7	1.09	Aa	1.16	ABa	0.77	Aa	1.20	1.34	0.60
4/9/02	14	1.46	ABa	1.34	ABCa	0.86	Aa	2.13	1.78	0.73
12/9/02	22	2.04	BCa	1.51	BCab	0.97	Ab	4.16	2.29	0.94
18/9/02	28	2.50	CDa	1.77	Cb	1.16	ABb	5.24	3.13	1.34
25/9/02	35	3.03	DEa	2.44	Da	1.75	Bb	9.18	5.94	3.05
3/10/02	43	3.19	EFa	2.89	DEa	2.08	BCb	10.17	8.33	4.33
10/10/02	50	3.32	EFa	2.72	DEa	1.90	BCb	11.02	7.38	3.62
16/10/02	56	3.71	Fa	3.17	Ea	2.42	Cb	13.76	10.04	5.85
24/10/02	64	3.78	Fa	3.14	Eb	2.30	BCc	14.31	9.88	5.27

A similar experiment examining light environment, time of shading and bract browning response was run again in 2003, to confirm the reduction in browning under shade cloth, determine whether trends resulting from early or late shading are consistent from year to year and expand the cultivars tested to include both 'Fire and Brimstone' and 'Olympic Flame'.

5.3.4 Cultivar and light environment effects on bract browning at Mount Annan in 2003

5.3.4.1 Methods

'Fire and Brimstone' and 'Olympic Flame' waratahs were subject to the same three treatments as in 2002, except that plants in the late shade treatment were moved into the shade on 29th July. Plants were again irrigated to saturation every day or every second day, as necessary. The severity of bract browning was described in the same way as in 2002.

The data were analysed using the AREPMEAS procedure in Genstat, as described in Experiment 7.4, with *Light treatment* and *Cultivar* as Treatments terms and *Plant* as a Block term. Sufficient measurements were made at each time to allow all dates to be included in the analysis.

5.3.4.2 Results

Shading resulted in a decrease in bract browning and senescence (53% and 70% reduction due to early and late shading, respectively) of 'Fire and Brimstone' and 'Olympic Flame' waratahs compared to those grown in full sun (Figures 5.11- 5.14). Significant interactions between light treatment and time ($P < 0.001$) were found to influence floral bract browning and senescence. Early and late shaded waratahs had significantly less bract browning and loss compared to sun exposed plants from late September (Table 5.2, 27/9/03 onwards). There were no significant differences between browning resulting from early or late shading (Figure 5.12 and 5.14), or between cultivars (Figure 5.11 and 5.13). Browning and bract loss increased significantly from early August in sun and late shaded bracts and from mid September in early shaded bracts (Table 5.2). A decrease in browning of early shaded bracts occurred on 27/9, due

to measurement of fewer flowers with less browning than at previous time points, as described in experiment 5.4.

Table 5.2: Square root and back transformed number of pooled 'Fire and Brimstone' and 'Olympic Flame' floral bracts brown or senesced for sun, early and late shade treatments during floral development in 2003. Lower case letters indicate significant differences between treatments for each sampling date, while upper case letters indicate significant differences between sampling dates within a treatment. Time by treatment interaction $P < 0.001$; LSD for square root transformed data = 0.80. n = 3-8 for all treatments, with 1 bud measured per plant, although n decreases at later sampling dates due to flower maturation.

		Square root transformation			Back transformed		
		Treatment					
Date	Time (days)	Sun	Early shade	Late shade	Sun	Early shade	Late shade
29/7/03	0	0.93 Aa	1.04 Aa	1.42 Aa	0.86	1.09	2.02
7/8/03	9	1.16 ABa	1.07 Aa	1.57 ABa	1.34	1.14	2.47
14/8/03	16	1.35 ABa	1.21 Aa	1.71 ABa	1.82	1.47	2.94
19/8/03	21	1.44 ABCa	1.21 Aa	1.71 ABa	2.06	1.47	2.94
28/8/03	30	1.58 ABCDa	1.24 Aa	1.71 ABa	2.49	1.53	2.94
5/9/03	38	1.75 BCDa	1.28 Aa	1.82 ABa	3.05	1.63	3.29
10/9/03	43	1.86 BCDEa	1.43 Aa	1.84 ABa	3.46	2.05	3.40
21/9/03	54	2.17 DEFa	1.75 ABa	1.86 ABa	4.69	3.08	3.45
27/9/03	60	2.56 EFGa	1.66 ABb	1.99 ABab	5.55	2.77	3.96
1/10/03	64	3.00 GHa	1.76 ABb	2.11 ABb	8.99	3.08	4.47
7/10/03	70	3.48 HIa	2.37 Bb	2.09 ABb	12.08	5.63	4.38
15/10/03	78	4.28 Ia	2.91 Bb	2.36 Bb	18.28	8.44	5.56

In parallel to the experiments on red waratah cultivars at Mount Annan, experiments were conducted on 'Wirrimbirra White' waratahs on a commercial waratah plantation at Jervis Bay in 2002 and 2003. The experiment at Jervis Bay examined sun and shade effects on browning in a cultivar thought to be very susceptible to bract browning (Appendix A1), and determine whether any reduction in browning was consistent from year to year in a commercial plantation. Green shade cloth (50%) was used in these experiments.

Figure 5.11: Mature ‘Fire and Brimstone’ waratah in full sun at Mount Annan in 2003, showing severe bract browning.

Figure 5.12: Mature ‘Fire and Brimstone’ waratah shaded from bud initiation (early shading) at Mount Annan in 2003, showing no bract browning.

Figure 5.13: Mature ‘Olympic Flame’ waratah in full sun at Mount Annan in 2003, showing severe bract browning.


Figure 5.14: Mature ‘Olympic Flame’ waratah shaded from bud swelling (late shading) at Mount Annan in 2003, showing no bract browning.

5.4 Light environment effects on bract browning at Jervis Bay in 2002 and 2003

5.4.1 Study site - Jervis Bay

Experiments were conducted on 'Wirrimbirra White' waratah plants grown commercially at Crooby Cottage Wildflowers on Jervis Bay Rd, Woollamia (approximately 175 km south of Sydney, 35º 00' latitude, 150º 38' longitude; Geoscience Australia, 2004).

Experimental plants were chosen at random from a row of 30 mature white waratahs oriented northwest to southwest. Plants in the center of the row were more exposed to wind and sun than plants at either end, due to gaps in surrounding vegetation. Five plants were covered with individual shade shelters constructed from a galvanised steel tube frame covered with knitted green 50% shade cloth (Sarlon, Brunswick, Victoria) on all sides (Figure 5.15). A further five plants were chosen as untreated controls.

Plants were sprayed with Eco oil, Aminogro (L amino acids and crustacean waste), Acadian (soluble seaweed extract), all from Organic Crop Protectants (Lilyfield, NSW) with Chemwet (Nufarm Australia, Laverton, Victoria) or Agral (Syngenta Australia, Pendle Hill, NSW) surfactant every one to two weeks during the experiment in both 2002 and 2003. Drip irrigation was applied as necessary.

Figure 5.15: Commercially grown 'Wirrimbirra White' waratah plants in full sun and under 50% shade cloth at Jervis Bay.

5.4.2 Methods

Plants were shaded from 16th August 2002 and bract browning was monitored every week until 20th September, using the same method for describing browning severity as in experiment 5.3.

In 2003, the experiment was repeated with plants shaded from 1st August, although significant browning was already present, possibly due to irrigation problems earlier in the year. Bract browning was monitored every two weeks, until 30th September.

Data were analysed using the AREPMEAS procedure as described in Experiment 5.3. The last sampling dates (20/9/02 and 30/9/02) were not included in the analysis, as a solution could not be reached in Genstat (Lawes Agricultural Trust, 2003) due to a large number of missing values.

5.4.3 Results

Figure 5.16 shows the severe bract browning of 'Wirrimbirra White' waratah flowers in the sun, compared to shade-grown flowers with minimal browning. Shading resulted in a 30% reduction in the number of 'Wirrimbirra White' bracts browned or lost in both 2002 and 2003, compared to those grown in the sun (Tables 5.3 and 5.4). Significant interactions between treatment and sampling date were evident in both years, although differences between light treatments as a main effect were not significant.

Figure 5.16: Mature (MF stage) 'Wirrimbirra White' waratah flowers grown in full sun (left) or under 50% shade cloth (right) at Jervis Bay in 2002.

5.4.3.1 Bract browning in 2002

There was a significant interaction of time of measurement and treatment in 2002 (P = 0.008), although differences between light treatments were not significant. Browning and bract loss initially affected 6% of the total number of floral bracts. Browning and senescence increased significantly between the first and second sampling dates, that is, from the middle to end of August (Table 5.3). Browning continued to increase at a similar rate in both sun and shade plants from the end of August. By mid-August, browning and bract loss affected 39% of floral bracts on shade plants and 55% of bracts on sun plants.

Table 5.3: Square root and back transformed number of 'Wirrimbirra White' floral bracts brown or senesced for sun and shade treatments during floral development in 2002. Lower case letters indicate significant differences between treatments for each sampling date, while upper case letters indicate significant differences between sampling dates within a treatment. n =5 plants in each treatment, with 12-25 buds per plant, although n decreases at later sampling dates due to flower maturation. Time by treatment interaction P = 0.002; LSD for square root transformed data = 0.61.

		Square root transformation		Back transformed	
		Treatment			
Date	Time (days)	Sun	Shade	Sun	Shade
17/8/02	0	1.14 Aa	1.08 Aa	1.31	1.16
23/8/02	6	2.51 Ba	1.98 Ba	5.32	3.94
30/8/02	13	2.77 BCa	2.22 BCa	7.67	4.92
6/9/02	20	3.15 CDa	2.62 CDa	9.92	5.84
11/9/02	25	3.39 Da	2.88 Da	11.49	8.28

5.4.3.2 Bract browning in 2003

In 2003, significant interactions between light treatment and time (P < 0.001) were again found to influence floral bract browning and senescence in 'Wirrimbirra White' waratahs. However, bracts in the sun only had more severe browning than shade at the last sampling date (affecting 43% of total floral bracts in the shade and 65% in the sun), although differences were not statistically significant. Browning of sun bracts increased significantly from late August to mid September, while browning of shade bracts did not increase significantly over time (Table 5.4). In 2003, bract browning was already high in early August, affecting about 25% of the total number of floral bracts. This high value at the tight bud stage was attributed to problems with irrigation management early in the season, although this affected all plants equally.

Table 5.4: Square root and back transformed number of 'Wirrimbirra White' floral bracts brown or senesced for sun and shade treatments during floral development in 2003. Lower case letters indicate significant differences between treatments for each sampling date, while upper case letters indicate significant differences between sampling dates within a treatment. Time by Treatment interaction $P < 0.01$; LSD for square root transformed data = 0.85. n =5 plants in each treatment, with 7-17 buds per plant, although n decreases at later sampling dates due to flower maturation.

		Square root transformation				Back transformed	
		Treatment					
Date	Time (days)	Sun		Shade		Sun	Shade
1/08/03	0	2.25	Aa	2.25	Aa	5.07	5.04
16/8/03	6	2.46	Aa	2.44	Aa	5.05	5.93
29/8/03	13	2.76	Aa	2.75	Aa	7.62	7.57
13/9/03	20	3.69	Ba	3.02	Aa	13.59	9.12

5.5 Discussion

5.5.1 Effects of natural and artificial shade on light intensity and microclimate

5.5.1.1 Effect of shade cloth on light intensity, light quality and microclimate of cultivated waratahs

The shade factor of the black shade cloth used in experiments at Mount Annan is nominally 50%, but the light intensity under the shade cloth appears to be lower than 50% for most of the day. The actual shade factor varies with the design of the shade house and the angle of incident radiation, decreasing as solar altitude increases (Yates, 1989). The shade house also had a significantly lower maximum daily temperature and higher relative humidity, compared to the full sun environment. Temperature is an important factor in some disorders associated with high light intensity, and shade cloth may reduce the temperature of shaded waratah bracts. For example, unshaded raspberry fruit prone to solar injury was approximately 7ºC above the air temperature, while 30% or 60% shade cloth reduced raspberry fruit temperature by over 4ºC (Renquist *et al.,* 1987). The reduction in temperature and increase in relative humidity may combine with the reduced light intensity to minimise any stress experienced by waratah plants

under shade cloth. However, the effects of light intensity alone will be further investigated in Chapter 7.

While black shade cloth is a neutral filter, coloured shade cloths can be used to manipulate light quality and scattering and therefore manipulate plant responses to red/far red light. Green shade cloth (used at Jervis Bay in experiment 5.4) reduced the red/far red ratio, but had little effect on branch length and number of new shoots in *Pittosporum* (Oren-Shamir *et al.,* 2001). Red shade net had increased transmittance at wavelengths greater than 590nm and a smaller peak at 400nm, with a slight reduction in red/far red ratio, resulting in enhanced branch elongation. Blue shade net had a peak transmittance around 470nm and very low transmittance between 580 and 750nm and a similar red/far red ratio to natural light (Oren-Shamir *et al.,* 2001). The proportion of diffuse to direct PAR was significantly higher under white shade mesh (Healey and Rickert, 1998) and blue, red and green shade nets, and slightly higher under grey shade net and Aluminet compared to black (Oren-Shamir *et al.,* 2001).

5.5.1.2 Characterising the natural light environment of waratahs

Measurements of the light intensities above waratah flowers in their natural habitat are presented as an indication of the conditions experienced, since data was collected on one day only. Our measurements along a transect (Figure 5.4) support the observation that the light environment in the understorey is highly variable (Engelbrecht and Herz, 2001). However, spatial and temporal variability require further characterisation to accurately describe the light environment of natural waratah populations.

The structure of the canopy and variability in foliage height has a profound impact on understorey light availability and distribution (Nicotra *et al.,* 1999). The canopy structure at site 1 in the Royal National Park meant that waratahs were protected from full sunlight for most of the day (Figure 5.3), only experiencing high light intensities for a short time while sunlight directly penetrated canopy gaps. These small openings in the canopy, known as sunflecks, can have an intensity of up to 200 times the normal understorey PFD (Watling *et al.*, 1997) and are therefore responsible for extreme variation in light intensity (Anderson, 1964) and possibly, light damage. However, understorey plants exposed to sunflecks are capable of rapidly dissipating excess light energy through the xanthophyll cycle, thus minimising photodamage (Watling *et al.,* 1997). However, plants at Mount Annan are exposed to high light intensities for a longer duration than plants in natural populations, and may exceed their capacity for photoprotection and experience chronic photoinhibition.

Further experimentation in the natural environment would ideally use several data loggers above flowers at each site to measure light intensity at least one day per month during waratah bud maturation. Short-term direct measurements of this kind have been described as a "powerful means to rank long-term light conditions at different sites in the understorey" (Engelbrecht and Herz, 2001). Indirect measurements (for example, hemispherical fisheye photographs and plant canopy analysis) are not suitable for this study, where light conditions within a single site need to be assessed, although they are useful for ranking understorey light conditions between a large number of sites (Engelbrecht and Herz, 2001).

5.5.2 Bract browning and the natural light environment

Browning and senescence of floral bracts on waratahs (an indicator of browning severity) was relatively low in the natural environment (Figure 5.5), although the smaller basal bracts browned and senesced as flowers matured (Figure 5.6). This suggests that the natural canopy cover at the three sites assessed provided sufficient shade to avoid the browning observed in full sun situations at Mount Annan and Jervis Bay. At other sites visited in the Royal National Park, early maturing flowers with high levels of browning and necrosis were observed when flowers were exposed above the canopy at sites regenerating after fire (Figure 5.17).

Figure 5.17: Waratah with bract browning and necrosis above regenerating vegetation in Royal National Park (11th August 2003)

The light requirements of a species under natural conditions may provide a guide to the light environment required for the same species grown for regeneration (for example, Eucalypt seedlings; Holly *et al.,* 1994) or cropping purposes (for example, Chilean guava; Pastenes *et al.,* 2003). In the natural environment, waratahs appear to experience

high light intensities (up to 1600 μmol m^{-2} s^{-1}) for a relatively short period of time during the day (Figure 5.2). In contrast, the full sun environment at Mount Annan had relatively high light intensities for an extended period during the day (>1000 μmol m^{-2} s^{-1} for approximately five hours per day) (Figure 5.1). The results obtained for waratahs in Experiment 5.7 indicate that simulating natural canopy cover in a commercial growing environment (for example, using shade cloth as described in Experiments 5.3 to 5.6) may be a key factor in reducing bract browning.

5.5.3 Bract browning of cultivated waratahs

5.5.3.1 Light and irrigation frequency effects

Growing waratahs under 50% shade cloth reduced browning of inner bracts in four cultivars receiving weekly irrigation, as well as reducing browning of inner and outer bracts of two of those cultivars subjected to daily or weekly irrigation. This suggests that bract browning in waratahs is mediated by prolonged exposure to high light intensities, and can be significantly reduced under shade cloth. Reduced irrigation frequency in general, resulted in a significant increase in bract browning.

A large number of plants with vegetative buds, rather than floral buds, were included in each treatment when the experiment was initially designed in late April. This resulted in fewer flowers being available for assessment at commercial maturity, and low numbers of replicates for some treatments. In future years, only plants with floral buds should be included in experiments. Therefore, experiments need to be designed when buds are more mature and floral buds can be distinguished from vegetative buds more easily.

Analysis of data sets with large numbers of high scores was not possible in some experiments, for example, outer bracts in experiment 5.3.3.1. Therefore, a more sensitive method of describing bract browning severity was sought in 2002.

5.5.3.2 Sun and shade effects

Shade cloth (50%) significantly reduced browning and senescence of floral bracts in 'Fire and Brimstone' and 'Olympic Flame' cultivars, compared to flowers grown in full sun (Experiment 5.3). The most significant differences between treatments occurred at commercial harvest time. Similarly, shade reduced browning of 'Wirrimbirra White' waratah bracts, with significant interactions between sampling time and shade treatment (Experiment 5.4). Waratahs shaded later in the season (from bud opening) had significantly less browning than those shaded early (from bud initiation) at Mount Annan in 2002, although this trend was not consistent across years. Browning prior to measurements, initiated between late July and mid August in both years, was generally low, affecting less than two bracts per flower; except at Jervis Bay in 2003 where irrigation problems occurred earlier in the season. This confirms anecdotal evidence from growers (Appendix A1) and observations made in 2001 (Chapter 3), that browning generally occurs as flower buds begin to swell and open, in the eight weeks preceding commercial harvest.

Browning in other crops often increases with high light intensity. Higher solar radiation and low rainfall resulted in a greater proportion of broccoli heads with symptoms of brown bead, which is a physiological disorder (Jenni *et al.*, 2001b). Exponential increases in marginal bract necrosis of poinsettia were observed with increasing supplementary light intensity during late flower development (Wissemeier *et al.*, 2000).

Saure (1998) cites many instances of higher tipburn occurrence and severity in vegetables with increasing light intensities and extended photoperiods.

In contrast, postharvest leaf blackening in Protea is delayed or reduced when cut flowers are placed under bright light (McConchie *et al.,* 1991). This occurs because protea leaf blackening is a result of carbohydrate depletion by the inflorescence, and bright light allows leaves to continue photosynthesis and hence, maintain their carbohydrate supply, preventing blackening (McConchie *et al.,* 1991; Dai and Paull, 1995).

In some species, browning and necrosis under high light is linked to the development of photoinhibition, which can be relieved by shading. For example, exposure of the understorey orchid *Oeceoclades maculata* to light intensities greater than 250 μmol m^{-2} s^{-1} for a month, led to chlorosis, partial tissue necrosis and/or accelerated senescence (Johnson, 1993). Shading reduced photoinhibition and subsequent light damage in leaves and flowers of many species, including *Heliconia* (He *et al.,* 1996), *Dendrobium* orchids (He *et al.,* 1998), mangoes (Nir *et al.,* 1997), *Schefflera* (Schiefthaler *et al.,* 1999), Eucalypts (Holly *et al.,* 1994; Close *et al.,* 2001) and Chilean guava (Pastenes *et al.,* 2003). Shade also reduced solar injury in red raspberries from 41% to 8 - 16% compared to full sun, minimising the effects of UV radiation and high temperatures thought to cause injury (Renquist *et al.,* 1987). The potential for photoinhibition of waratahs in full sun, and a reduction in photoinhibition in shade, will be investigated in Chapter 6.

Alternatives to shading have also been applied in some crops to reduce browning. Maturity bronzing (a superficial bronze-red blemish) was reduced in bananas by covering the banana bunch, leaving the cover open at the bottom (Daniells *et al.,* 1992). Similarly, enclosing lychee fruit in paper bags improved fruit quality by reducing light and dark brown blemishes while maintaining red colouration (Tyas *et al.,* 1998). Bagging of lychee fruit (Tyas *et al.,* 1998) and waratah buds (Burnett and Nixon, 1990) is likely to increase relative humidity and reduce water loss from tissues. Melgarejo *et al.* (2004) used a kaolin-based spray as a cheaper alternative to shading to reduce sunburn of pomegranate fruit, caused by high temperatures and intense sunlight. However, this treatment is not suitable for waratah flowers, due to the likelihood of white residue remaining on the bracts.

5.5.3.3 Time of shade application

The optimal time of shading in waratahs (from bud initiation or from bud swelling) was investigated at Mount Annan in 2002 and 2003 (section 5.3). However, results are not consistent from year to year, with late shading in 2002 reducing browning more effectively than early shading, while in 2003, early and late shade effects on bract browning were not significantly different. However, late shading (from bud swelling) reduced bract browning at both Mount Annan and Jervis Bay in 2002 and 2003.

These results corroborate those of Tyas *et al.* (1998) who found that lychee fruit quality was improved by bagging from half to three quarter fruit fill. Earlier bagging (from early fruit set) did not further improve lychee quality (Tyas *et al.,* 1998). In contrast, early shading resulted in the greatest reduction in browning in other crops. For example, the effect of bunch covering on reducing maturity bronzing in bananas was greatest

when bunches were covered early in fruit development, i.e. about eleven days after bunch emergence (Daniells *et al.,* 1992). Late shading of waratahs is sufficient to significantly reduce bract browning, although the economic feasibility of applying shade in commercial plantations for a period of several months requires further investigation.

5.5.3.4 Cultivar differences in bract browning

There were no significant cultivar differences evident when 'Fire and Brimstone', 'Olympic Flame', 'Sunflare' and pink waratahs were compared under shade and sun regimes with weekly irrigation in 2001. All cultivars showed severe bract browning in full sun at flower maturity and significantly reduced browning in the shade (Figure 5.8 for all cultivars pooled). This is not surprising, as all the cultivars chosen for study are described by growers as susceptible to bract browning, with the exception of 'Olympic Flame' (Appendix A1). Similarly, when 'Fire and Brimstone' and 'Olympic Flame' were compared in experiments in 2003, with a more sensitive assessment for browning severity, no cultivars differences were apparent (Experiment 5.3.4). Comparisons between red and white cultivars were not made in the present study, because differences in plant age, maintenance and environmental effects between Mount Annan and Jervis Bay would have confounded treatment effects.

Interestingly, when additional factors such as irrigation frequency were added as treatments, cultivar differences became apparent. For example, in experiment 5.3.2, outer bracts of 'Sunflare' had a higher score for browning than 'Olympic Flame'. Inner bract scores for 'Sunflare' on daily irrigation were lower than inner bract scores for 'Sunflare' weekly irrigation, while shaded 'Olympic Flame' did not show significant

differences under daily or weekly irrigation. These differences between cultivars may indicate variation in water use efficiency and other physiological processes between cultivars. However, responses to sun and shade appear consistent between cultivars and it is this light response that appears to have the greatest effect on bract browning and will be investigated further.

5.6 Conclusions

Bract browning and senescence generally occurred in the four to six weeks prior to commercial flower maturity, coinciding with bud swelling and opening (the juvenile open stage of development). Covering waratah plants with 50% shade cloth reduced bract browning over several years in red cultivars 'Fire and Brimstone' (2001-2003), 'Olympic Flame' (2001, 2003), 'Sunflare' (2001) and pink cultivars of varying genetic backgrounds (2001) at Mount Annan and the white cultivar 'Wirrimbirra White' (2002, 2003) on a commercial property at Jervis Bay. The design of the shade shelter at Mount Annan increased the minimum relative humidity and decreased the maximum daily temperature around plants, as well as reducing light intensity.

Waratahs in their natural environment appear to experience high intensity radiation for a shorter period of time than plants grown in full sun conditions, although the natural light environment requires further characterisation. Waratahs growing in a natural understorey environment experienced little browning of floral bracts, although basal bracts often browned and senesced. This suggests that simulating natural canopy cover in a commercial growing environment (for example, using shade cloth) may be a key factor in reducing browning of the commercially significant floral bracts. However, the

economic benefits of improving waratah quality by shading need to be assessed against the cost of shading the plants.

Since high light is a trigger for bract browning and shade treatments reduced browning, the mechanism of browning under high light was investigated further. This involved measurements of photoinhibition (Chapter 6), pigment destruction under high light (Chapter 7) and evidence of membrane damage by lipid peroxidation (Chapter 10). The relationship between these parameters is examined in Chapters 9 and 10.