Calandrinia sp. (Mt Clere)

Brunonia australis

An evaluation of the temperature and daylength requirements of Australian potted colour species

FINAL REPORT TO THE AUSTRALIAN FLORA FOUNDATION

Dr Margaret Johnston
Land Crop and Food Sciences
The University of Queensland Gatton
December 2010

Rhodanthe floribunda

Pycnosorus thompsonianus

Table of Contents

Summary

The Asteraceae species have been the focus of much of the Australian research on flowering physiology. Information on the flowering responses of several species has been published (Mott and McComb 1975; Sharman and Sedgley 1988; 1989 a, b; Sharman et al. 1990; Bunker 1995). Species have been classified into facultative long day plants (LDP), facultative short day plants (SDP) and day neutral species. For some species the importance of low temperatures for flowering has been reported (Mott and McComb 1975; Sharman and Sedgley (1988; 1989a, b; Sharman et al. 1990). Flowering of *Rhodanthe chlorocephala* subsp. *rosea* (syn. *Helipterum roseum*) was inhibited at constant 25°C under a 12 h photoperiod at 250 Wm^{-2} (Sharman and Sedgley 1989b: Sharman et al 1990). Halevy et al. (2001) reported that temperature influenced the daylength response of the woody cut flower species *Ozothamnus diosmifolius* (syn. *Helichrysum diosmifolium)* in Israel. A summary of environmental conditions imposed in these experiments and flowering responses can be found in Appendix 1.

Many studies have reported a range of diverse flowering responses of Australian species to temperature, daylength and light intensity including *Acacia* (Sedgley 1985), *Chamelaucium* (Shillo et al 1984; Dawson and King 1993), *Anigozanthos* (Motum and Goodwin 1987), *Eucalyptus* (Moncur 1992), *Pimelea* (King et al. 1992; King et al. 1995; Seaton and Plummer 2004), *Boronia* and *Hypocalymma* (Day et al. 1994), *Hardenbergia* (King 1998), *Crowea, Lechenaultia* and *Verticordia* (King et al. 2008), *Brunonia* and *Calandrinia* (Cave et al 2010a and b; Wahyuni et al. 2011 in press).

The diversity of flowering responses reported means that each species requires investigation of the effect of environmental factors on flowering to enable manipulation of flowering time especially if species are to be used as potted colour products. In addition it is important to understand juvenility and determine when the seedling is able to perceive the flowering signal as well as quantifying the effect of environmental factors on flower induction, initiation and development. Environmental factors also influence plant habit particularly height and branching and this can influence the number of flowers per plant

and hence plant quality. Plant growth regulators can be used to modify plant habit and may also influence flowering (King et al. 2008).

This study investigated the flowering responses of *Brunonia australis*, *Calandrinia* sp. (Mt Clere; not yet fully classified), *Pycnosorus thompsonianus and Rhodanthe floribunda.*

The first study on *Brunonia australis* R. Br (Goodeniaceae) and *Calandrinia* (Portulacaceae) investigated the role of daylength and growth regulators, Gibberellic acid (GA_3) and paclobutrazol (Pac), to control vegetative growth, peduncle elongation and flowering of *Brunonia* and *Calandrinia.* Plants were grown under long days (16h), short days (11h) and 8 weeks under short day then transferred to long day (SDLDs). Plants in each daylength were treated with GA_3, Pac, and GA_3+ Pac. GA_3 was applied as 10 µL drop of 500 mg L^{-1} concentration to the newest mature leaf. A single application of Pac was applied as a soil drench at 0.25 mg a.i. dose per plant.

Both *Brunonia* and *Calandrinia* flowered earlier in long days but still flowered in short days, so both can be classified as facultative LD plants. *Brunonia* under SDLDs were more vigorous and attractive than plants under LDs while still being more compact than plants under SDs. In *Brunonia*, GA_3 promoted earlier flowering and increased the number of inflorescences under SDs. Pac at 0.25 mg a.i. per plant applied alone or in combination with GA_3 delayed flower development in *Brunonia*, and resulted in a reduced number of inflorescences per plant compared to the control plants. Vegetative growth of *Calandrinia* was similar under LDs, SDs and SDLDs, whereas GA_3 application increased plant size. Pac-treated *Calandrinia* looked compact and attractive, and Pac application did not affect time to flower and flower number.

The second study investigated the flowering responses of *Pycnosorus thompsonianus* (Asteraceae), to daylength and temperature regimes. Plants were cooled at 20/10°C or kept at 30/20°C for 21 or 42 days under short day (SD), long day (LD), or short day for six weeks before transfer to long day (SDLD).

LDs promoted earlier flowering and plants under LDs flowered regardless of temperature regimes. Cool temperatures and cooling periods were required for flowering of plants under SDs, but this was not important for plants under LDs and SDLDs. Plants under SDs without cooling only produced 3 inflorescences per plant whereas plants which received 21 or 42 days of cooling had 19. Forty-two percent of the SD plants under 30/20°C remained vegetative after a 20 week growing period. Extending the cooling period from 21 to 42 days induced earlier flowering of plants in all daylengths but did not increase number of inflorescences per plant. Daylength was more effective than temperatures for promoting earlier flowering and for increasing the flower production.

Similar to *Pycnosorus*, plants of *R. floribunda* flowered without chilling showing a facultative requirement for low temperature. Plants were competent to perceive chilling as one day old seedlings. Chilling for 21 days was the most effective treatment, reducing time to first visible floral bud (FVFB) and anthesis and increasing inflorescence number by 100% at 23 weeks. Chilling for seedlings at 4 week old stage increase flowering of by 4-5-fold (Table 3.1).

The number of growing degree days (GDD) from transplanting to FVFB and anthesis of 3 week chilled plants of *R. floribunda* was found to be shortest among chilling treatments with 419 and 628 degree days, respectively. These could be used to guide for commercial production to reduce production time by chilling plants at seedling stage (4 weeks old) for 3 weeks under $20/10^{0}$C.

Section 1: Regulation of Flowering of *Brunonia australis* and *Calandrinia* sp.

S. Wahyuni [A], S. Krisantini [B], M. E. Johnston [AC]

[A] The University of Queensland, School of Land, Food and Crop Sciences, Gatton, Australia 4343

[B] Bogor Agricultural University, Department of Agronomy and Horticulture, Bogor, Indonesia 16680

[C] Corresponding author; email: m.johnston@uq.edu.au

Abstract

Brunonia australis R. Br (Goodeniaceae) and *Calandrinia* (Portulacaceae), native to Australia, are potential new flowering potted plants. This research investigated the role of daylength and growth regulators, Gibberellic acid (GA_3) and paclobutrazol (Pac), to control vegetative growth, peduncle elongation and flowering of *Brunonia* and *Calandrinia.* Plants were grown under long days (16h), short days (11h) and 8 weeks under short day then transferred to long day (SDLDs). Plants in each daylength were treated with GA_3, Pac, and GA_3+ Pac. GA_3 was applied as 10 µL drop of 500 mg L^{-1} concentration to the newest mature leaf. A single application of Pac was applied as a soil drench at 0.25 mg a.i. dose per plant. Both *Brunonia* and *Calandrinia* flowered earlier in long days but still flowered in short days, so both can be classified as facultative LD plants. *Brunonia* under SDLDs were more vigorous and attractive than plants under LDs while still being more compact than plants under SDs. In *Brunonia*, GA_3 promoted earlier flowering and increased the number of inflorescences under SDs. Pac at 0.25 mg a.i. per plant applied alone or in combination with GA_3 had extended flower development in *Brunonia*, and resulted in a reduced number of inflorescences per plant compared to the control plants. Vegetative growth of *Calandrinia* was similar under LDs, SDs and SDLDs, whereas GA_3 application increased plant size. Pac-treated *Calandrinia* looked compact and attractive, and Pac application did not affect time to flower and flower number.

Keywords: Australian native species, gibberellic acid, growth regulator, paclobutrazol, photoperiod.

Introduction

Brunonia australis R. Br and *Calandrinia*, native to Australia, are potential new flowering potted plants. *Brunonia* is a perennial herb endemic to Australia, with a cluster of elliptical leaves at the base of the plant from which 50 cm flowering stems arise. The blue flowers occur in an inflorescence of 2-3 cm in diameter. *Brunonia* makes an attractive pot plant and display when mass-planted as annual bedding or border plants. *Calandrinia* (not yet fully classified) is a drought tolerant succulent (Harrison et al. 2009) with pink to purple flowers of ca 7 cm diameter (Harrison et al. 2009) about 40-50 cm in size. Both species have a rosette growth habit when juvenile, are found in semi-arid areas and flower mainly in spring to early summer (Gray and Knight 2001).

Two major issues are under investigation in order to commercialise these species: regulation of flowering, and control of vegetative growth and elongation of peduncle. Photoperiod plays an important role in the plant's transition to flowering (Mouradov et al. 2002). Gibberellin has been reported to induce flowering of long day and/or cold requiring species grown under non-inductive conditions, including rosette long day (LD) plants such as *Arabidopsis* (Zeevart 2006) and of commercial ornamental plants such of the Araceae family such as *Philodendron* (Chen et al, 2003), *Zantedeschia* (Kozlowska et al. 2007), and *Spathiphyllum* (Henny et al. 2000). Gibberellin may cause stem elongation, an undesirable feature for ornamentals, and interacted with other hormones in affecting plant growth and development (reviewed by Ross and O'Neill, 2001).

Paclobutrazol, a gibberellin biosynthesis inhibitor, is a growth regulator frequently applied to enhance flowering (Thompson et al. 2005; Mishra et al. 2005) and to reduce plant height of ornamentals to produce more compact plants with higher ornamental values (Faust et al., 2001, Gibson & Whipker 2001, Karaguzel et al., 2004, Warner & Erwin 2003). Paclobutrazol has been reported to reduce peduncle length of *Dicentra* (Kim et al, 1999), *Ixia* (Demeulemeester et al. 1995) and *Cichorium* (Wulster & Ombrello 2000).

The objectives of this study were (1) to investigate the role of daylength on *Brunonia* and *Calandrinia* flowering; (2) to determine if GA_3 could be used to stimulate rapid and uniform flowering of *Brunonia* and *Calandrinia* grown at several daylengths, (3) to determine if paclobutrazol or GA_3 + paclobutrazol can reduce plant size and peduncle length.

Materials and methods

Plant materials

Seeds were surface sterilized by immersion in 0.2% sodium hypochlorite solution for 10 minutes then triple rinsed with distilled water before sowing in 50 mm (0.125 L) tubes containing propagation media of peat, perlite and vermiculite (1:1:1) with 2 g L^{-1} Osmocote® Exact Mini 3-4 month [N:P:K 16:3.5:9.1 + 1.2 Mg] (Scotts International B.V., The Netherlands). Seed were kept for 5 days in a greenhouse with a mean temperature of 27.5°C and light intensity ranged from 277 to 1018 µmol/sec.m^2.
Nine weeks after emergence *Brunonia* seedlings were transplanted to individual 100 mm (0.5 L) diameter plastic pots containing growth media of 100% pine bark with 2 g L^{-1} Osmocote® plus 8-9 month [N:P:K:Mg:: 15:4:7.5:1.8], 2 g L^{-1} Osmocote® plus 3-4 month [N:P:K:Mg:: 16:5:9.2: 1.8], 2 g L^{-1} Nutricote® [N:P:K 16:4.4:8.3] (Chisso-Asahi Fertilizer Co.,Ltd. Tokyo, Japan), 1.3 g L^{-1} Osmoform® [N:P:K:Mg:: 18:2.2:11:1.2] (Scotts Australia, Baulkham Hills, NSW, Australia), 1.3 g L^{-1} Coated iron [Fe:S 28:17], 1.2 g L^{-1} Dolomite® [Ca:Mg:: 14:8] (Yates, Australia) and 1.2 g L^{-1} Saturaid® (Debco, Melbourne, Australia). Plants were treated with 1g L^{-1} Banrot® (a.i. Thiophanate-methyl etridiazole, Scotts Australia Pty. Ltd., Australia) shortly after transplanting. *Calandrinia* was kept in 50 mm tubes (0.125 L) throughout the duration of the experiment. Twelve weeks after emergence,

2 g per tube of Basacote® Mini 3-4 month [N:P:K:S:Mg :: 13:6:16:10:1.4] (Compo GmbH & Co.KG, Germany) was applied as a top-dressing.

Treatments

Five days after emergence, seedlings were transferred to a controlled-environment research greenhouse for daylength treatments. The plants were randomly allocated to one of the three groups for the daylength treatments a, i.e. LD, SD and SD for 8 weeks then transferred to LD (SDLD) in the temperature controlled greenhouses with set points at 25/10°C (day/night) operating on a 12 h cycle (6:00 – 18:00h daily) under an 11 (SD) or 16 h (LD) photoperiod. Variation in temperature from the set point was ± 2 °C. The SD treatment (6:00-17:00h daily) was provided by ambient light (380 ± 44 µmol $m^{-2}sec^{-1}$) and regulated by blackout curtains. The LD treatment consisted of 11 h of ambient light (described above) plus a 5 h night break from 21:00 to 2:00 h daily (4.5 µmol $m^{-2}sec^{-1}$) supplied by 100 W incandescent lamps spaced 125 cm apart, 90 cm above the plants (Sylvania, Indonesia).

The plants in each daylength treatment were randomly allocated to treatment with gibberellin (GA_3), paclobutrazol (Pac) or GA_3 + Pac. The first GA_3 (GA_3, 67645-1G, Sigma-Aldrich Inc., USA) treatment was performed three weeks after the mean date of emergence. GA_3 was applied as a 10 µl drop of 500 mg L^{-1} concentration solution to the centre of the uppermost expanded leaf blade of the plants by using Finnpipette® (Themo Fisher Scientific, Finland) micropipette as described by King et al. (2001) and MacMillan et al. (2005). The application of GA_3 was repeated every two weeks for the duration of the experiment applied onto the youngest mature leaf. Five applications of GA_3 were applied to *Brunonia* while *Calandrinia* received six applications.

Paclobutrazol (Condense®, Crop Care Australasia Pty. Ltd., Australia) was applied as a single soil drench application at 0.25 mg a.i. per plant (Rademacher, 2000) when 50% of the plants in each daylength treatment had initiated flowers buds. The control plants were treated the same way with distilled water.

The experiment was conducted at The University of Queensland, Gatton (27° 34’S, 152° 20’E) from January to July 2008. Once plants were transferred to the controlled

environment research greenhouse, plants were observed every two days and the number of days to first visible flower bud (FVFB) and to anthesis was recorded. The peduncle length (*Brunonia*) was measured at anthesis of the first floret, and number of leaves at the FVFB and at anthesis was recorded. The total number of inflorescences was recorded at the completion of the experiment 15 weeks after plant emergence. Plant height was measured from the medium to the apical tip. Data on plant size (height and width) were collected at transplanting date and at the end of the experiment.

Experimental design and statistical analysis

A completely randomized design was used within each three daylength (LD, SD and SDLD). The plants were randomly allocated to treatment with GA_3, paclobutrazol (Pac) and GA_3 + Pac, or distilled water (control) and plants were re-randomised every 3 days. There were 15 plant replicates of *Brunonia* and 7 plant replicates of *Calandrinia* for each treatment. Data obtained were subjected to analysis of variance using the GLM procedure in Minitab® version 15.

Results

Brunonia

GA_3-treated Brunonia under SDs or SDLDs initiated the FVFB about 7 days earlier than the control plants (Table 1.1) and reached anthesis 7 to 8.5 days, respectively for SDs and SDLDs (Table 1.1). In addition, GA_3-treated plants had fewer leaves at FVFB (Table 1.1) and at anthesis (Table 1.1).

Daylength interacted with PGR application to affect the number of inflorescences per plant (Table 1.1). GA_3-treated plants had 30 % more inflorescences per plant under SDs compared to control plants, but GA_3 did not affect number of inflorescences under LDs or SDLDs (Table 1.1). In general under LDs and SDLDs plants had significantly more inflorescences than plants under SDs (Table 1.1), e.g. the control plants under SDs had 25 inflorescences whereas plants under LD and SDLDs had more than 45 inflorescences per plant (Table 1.1).

Table 1.1. Effects of daylength (DL) and growth regulator (GR) on days to the first visible flower buds (VB) and to anthesis, leaf number at VB and at anthesis and number of inflorescence in *Brunonia*.

Daylength (DL)	Growth Regulator (GR)	Days to VB	Leaf Number at VB	Days to Anthesis	Leaf Number at Anthesis	Total Number of Inflorescences
LD						
	Control	32.6 [a]	9.7 [b]	65.1 [a]	38.3 [c]	44.9 [c]
	GA_3	29.8 [a]	8.4 [ab]	62.7 [a]	26.7 [a]	46.2 [c]
	Pac	32.8 [a]	9.9 [b]	72.8 [b]	35.8 [bc]	28.3 [a]
	GA_3+Pac	29.9 [a]	8.0 [a]	66.8 [ab]	29.0 [ab]	36.5 [b]
SDLD						
	Control	45.7 [b]	18.5 [b]	86.1 [b]	70.3 [b]	49.3 [c]
	GA_3	38.2 [a]	11.3 [a]	77.7 [a]	46.3 [a]	52.2 [c]
	Pac	60.9 [c]	29.0 [c]	94.7 [c]	67.0 [b]	11.5 [a]
	GA_3+Pac	39.8 [a]	13.0 [a]	81.4 [ab]	41.4 [a]	35.1 [b]
SD						
	Control	47.6 [b]	21.0 [b]	89.5 [b]	78.3 [b]	25.5 [c]
	GA_3	40.6 [a]	13.4 [a]	82.4 [a]	49.7 [a]	38.1 [d]
	Pac	65.3 [c]	33.8 [c]	97.0 [c]	70.3 [b]	2.8 [a]
	GA_3+Pac	37.6 [a]	12.7 [a]	79.6 [a]	44.1 [a]	12.6 [b]
DL		**	**	**	**	**
GR		**	**	*	*	**
DL x GR		**	**	ns	ns	**

Note: Values followed by different letters within a column and daylength treatment are significantly different at 95% LSD. Significance of interaction between LD and GR is given; n.s. : not significant, *P < 0.05, ** P<0.01, *** P< 0.001

Under LDs GA_3 application did not promote earlier flowering (Table 1.1). The time taken to the FVFB and to anthesis, leaf number at FVFB and number of inflorescences per plant under LDs were similar to the control plants (Table 1.1).

Plants treated with GA_3 + Pac initiated the FVFB and commenced anthesis at a similar time with the GA_3-treated plants. GA_3+Pac-treated plants had number of inflorescences intermediate to the GA_3 and the Pac-treated plants in all daylengths, and had fewer inflorescences per plant than the control plants (Table 1.1).

When compared to the GA_3 treated plants, Pac applied alone when 50% of the plants had initiated floral buds delayed the average time taken to FVFB and to anthesis of plants, especially under SDLDs and SDs. The Pac-treated plants had significantly more leaves at

FVFB, particularly under SDs and SDLDs, but at anthesis the leaf number of Pac treated plants were similar to the control (Table 1.1).

The initial height and width of the *Brunonia* plants were similar (Table 1.2). GA_3 applied alone did not affect final *Brunonia* height and width compared to the control plants. Pac-treated plants were the shortest and the smallest in all daylengths (Table 1.2), whereas GA_3 + Pac-treated plants had plant height and width intermediate to the GA_3 and the Pac-treated plants in all daylengths (Table 1.2).

GA_3 did not affect the peduncle length whereas Pac significantly inhibited peduncle elongation; Pac-treated plants had peduncle length of about 10% of the control in all daylength treatments (Table 1.2). Plants treated with GA_3 + Pac had peduncle length intermediate to the GA_3 and the Pac treated plants in all daylength treatments (Table 1.2).

Table 1.2. The effect of daylength (DL) and growth regulator (GR) on peduncle length, plant height dan plant width of *Brunonia*.

Treatment	Plant Height (mm)		Plant Width (mm)		Peduncle Length (cm)
	Initial	Final	Initial	Final	
Daylength (DL)					
LD	19.5	49.4 [x]	29.5	101.9 [x]	13.6 [x]
SDLD	19.5	62.9 [y]	27.7	138.7 [y]	15.8 [y]
SD	20.2	59.9 [y]	27.6	132.7 [y]	17.2 [y]
Growth Regulator (GR)					
Control	19.7	71.9 [a]	26.8	147.1 [a]	24.7 [a]
GA_3	20.1	71.8 [a]	29.9	146.3 [a]	24.1 [a]
Pac	19.8	36.0 [c]	28.2	88.5 [c]	2.3 [c]
GA_3+Pac	19.4	49.8 [b]	28.1	116.0 [b]	11.0 [b]
DL	ns	**	ns	**	**
GR	ns	**	ns	**	**
DL x GR	ns	ns	ns	ns	ns

Note: Values followed by different letters within a column are significantly different at 95% LSD. Significance of interaction between LD and GR is given; n.s. : not significant, *P < 0.05, ** P<0.01, *** P< 0.001

Calandrinia

Calandrinia reached FVFB earlier in LD with fewer leaves than in SD and SDLD treatments (Table 1.3). Daylength had no significant effect on final *Calandrinia* height and width (Table 1.3).

GA_3 or GA_3 + Pac-treated plants initiated the FVFB about 5 days earlier with fewer leaves than the control plants (Table 1.3), but the number of floral buds formed were similar. The plants in each of PGR and daylength treatment had about 30 floral buds per plant when the experiment was terminated. GA_3-treated plants were 1.4 cm taller and 2 cm wider than the control plants (Table 1.3).

Pac applied alone when 50% of the plants had initiated floral buds did not affect the average time taken to FVFB in *Calandrinia*. Pac-treated plants had similar number of leaves at FVFB and plant sizes to the control and GA_3 + Pac-treated plants (Table 1.3).

Table 1.3. The effects of daylength (DL) and growth regulator (GR) on days to first visible bud (VB), leaf number at VB and number of flower buds of *Calandrinia*.

Treatment	Days to VB	Leaf Number at VB	Number of Flower Buds	Plant Height (mm)	Plant Width (cm)
Daylength (DL)					
LD	81.2 ^{x}	71.6 ^{x}	29.5 ^{x}	28.4 ^{x}	22.4 ^{x}
SDLD	87.1 ^{y}	88.2 ^{y}	28.0 ^{x}	33.5 ^{x}	21.1 ^{x}
SD	85.4 ^{y}	82.1 ^{y}	29.8 ^{x}	32.4 ^{x}	23.7 ^{x}
Growth Regulator (GR)					
Control	87.8 ^{a}	92.1 ^{b}	26.3 ^{a}	27.2 ^{a}	22.8 ^{a}
GA_3	81.9 ^{b}	69.7 ^{a}	30.1 ^{a}	41.3 ^{b}	25.1 ^{b}
Pac	86.3 ^{a}	87.6 ^{b}	30.4 ^{a}	23.2 ^{a}	18.7 ^{c}
GA_3+Pac	82.2 ^{b}	73.0 ^{a}	29.7 ^{a}	31.4 ^{a}	22.9 ^{a}
DL	**	**	ns	ns	ns
GR	**	**	ns	**	**
DL x GR	ns	ns	ns	ns	ns

Note: Values followed by different letters within a column are significantly different at 95% LSD.

Discussion

LD treatment promoted earlier flowering of both species (Table 1.1 and 1.3). *Brunonia* under LDs initiated the FVFB 15 and 16 days earlier than under SDs and SDLDs, respectively (Table 1.1) and produced double the number inflorescences per plant compared to SDs (Table 1.1). In addition, *Brunonia* reached anthesis 19 days earlier under LD compared to SD and SDLD (Table 1.1). The effect of LD on time to FVFB in *Calandrinia* was not as pronounced, i.e. 4 to 5 days earlier than under SDs or SDLDs (Table 1.3). In addition, number of flower buds in *Calandrinia* was not affected by daylengths or growth regulator treatments (Table 1.3).

Even though flowering was promoted by LDs both *Brunonia* and *Calandrinia* still flowered in SDs, implying that both species can be classified as facultative LD plants. These results are consistent with the recent reports on *Brunonia* and *Calandrinia* regulation of flowering (Cave and Johnston, 2010).

GA_3 promoted earlier flowering and anthesis in *Brunonia* in SDLDs and in SDs (Table 1.1) and increased the number of inflorescences per plant in SDs by 30% (Table 1.1), but had no effect in LDs. GA application might replace long day requirement for flowering (Wilson et al, 1992). A possible correlation between gibberellin and photoperiod pathways of flowering has been described by Boss et al. (2004). The gibberellin pathway has a minor effect on flowering under LD, whereas under SD the gibberellin pathway is the key promotion pathway of flowering (Boss et al. 2004, Mouradov et al. 2002).

A certain level of endogenous GA might be important for flowering; an *A. thaliana* mutant that was severely defective in gibberellin biosynthesis failed to flower under SD unless GA_3 was applied (Wilson et al. 1992). In another rosette LD plant, *Silene armeria*, the endogenous GA content increased several fold following transfer of plants from SD to LD (Talon and Zeevaart, 1992). Other long day, rosette plants such as *Agrostemma githago* (Jones and Zeevaart, 1980), *Spinacia oleracea* (Wu et al. 1996) and *A. thaliana* (Gocal et al. 2001) have been reported to have a similar stem elongation and flowering response to GA and daylength. This might explain, at least partly, the promotion of *Brunonia* flowering by GA_3 application under SD.

Pac application at 0.25 mg a.i delayed the average time taken to FVFB and to anthesis and had severely reduced the number of inflorescences in *Brunonia* in all daylengths (Table 1.1). Since Pac was applied when 50% of the plants had initiated floral buds, Pac obviously had delayed the time to FVFB of the rest 50% of the *Brunonia* plants in each daylength treatment which had delayed floral development resulting in fewer inflorescences per plant (when the final scoring was conducted at 15 weeks after plant emergence). In contrast to *Brunonia*, Pac had no effect on time to flower and number of flower buds in *Calandrinia*, and the Pac-treated *Calandrinia* had similar number of leaves at FVFB and plant size to the control and GA_3 + Pac-treated plants (Table 1.3). Similar results were reported in azalea (Wilfret and Barrett, 1994); Pac-treated plants at 0.8 mg a.i. flowered at a similar time with untreated plants (Wilfret and Barrett, 1994).

One of the aims of this research is to obtain pot plants with compact growth and with shorter peduncles. The peduncle length in *Brunonia* grown under LDs was about 3 cm shorter compared to inflorescences in SDLDs and SDs (Table 2), but the vegetative growth was poor and the plants were less attractive (Fig 1). *Brunonia* plants grown under SDs were most vigorous, whereas plants under SDLDs were more vigorous and attractive than plants under LDs while still being more compact than plants under SDs. Therefore, 8 weeks in SDs to enhance the vegetative growth, followed by transfer to LDs to reduce the peduncle length can be used for commercial production of *Brunonia*.

Pac was more effective than LD in reducing *Brunonia* peduncle length (Table 1.2). However, Pac application severely inhibited *Brunonia* vegetative growth (Table 1.2, Fig. 1.1), delayed flowering under SDs and SDLDs and reduced the number of inflorescences per plant in all daylengths (Table 1.1). The rate of Pac applied in this study, i.e. 0.25 mg a.i. per plant, was the optimum dose for *Dianthus caryophyllus* to get compact plants with a darker colour of flowers and leaves (Banon et al. 2002) and had effectively shortened flower stalks of *Ixia* (Wulster and Ombrello, 2000), but was clearly too high for *Brunonia*. The Pac dose needs to be reduced, or growth retardants with lower degree of activity such as daminozide or cycocel might be tested for the future studies.

Brunonia treated with GA_3+ Pac did not experience a delay in flowering, had intermediate peduncle length at anthesis, plant height and width of the GA_3-treated and the Pac-treated plants. These results demonstrate that when GA_3 was combined with Pac, GA_3 partially

counteracted the severe growth inhibition effects of Pac. Gibberellins promote stem elongation, and Pac retards growth due to its inhibition effect on gibberellin biosynthesis (Gocal et al. 2001, Karaguzel et al. 2004). GA_3+ Pac-treated plants were compact and attractive due to less elongated peduncles, but the plants produced fewer inflorescences compared to untreated plants (Fig 1.1). Further research is required to determine the appropriate rate of Pac and GA_3 application for *Brunonia.*

GA_3 application promoted earlier flowering in *Calandrinia* but the GA_3-treated plants were 14 cm taller and 3 cm wider compared to the control plants (Table 1.3, Fig 1.1). Elongated plants are less desirable and are more difficult to handle, so the application of GA_3 to *Calandrinia* is not recommended. In contrast, the Pac-treated plants were compact, did not experience delay in flowering, and produced a similar number of flower buds to untreated plants, so application of 0.25 mg a.i. of Pac is considered appropriate for *Calandrinia*.

Fig 1.1. *Brunonia* (A) and *Calandrinia* (B). Left to right: untreated, GA_3, Pac , and GA_3+Pac-treated plants.

Conclusion

Both *Brunonia* and *Calandrinia* can be classified as facultative LD plants; they flowered earlier under LDs but still flowered under SDs. Application of 10 µl GA_3 at 500 mg L^{-1} can promote an earlier flowering of *Brunonia* and *Calandrinia* in SDs. However, GA_3 application to *Calandrinia* was not recommended since it resulted in elongated plants. Pac at 0.25 mg/plant was suitable to produce a compact *Calandrinia* plants, but a reduced dose is recommended for *Brunonia*, applied once the flower buds are visible to obtain plants with shorter inflorescences. GA_3+ Pac-treated *Brunonia* looks compact and attractive due to less elongated peduncles, but the plants produced fewer inflorescences compared to untreated plants. Further research is required to determine the appropriate rate of GA_3 + Pac application for *Brunonia.* Growing plants for 8 weeks in SD followed by transfer to LD is recommended for commercial production of *Brunonia* to obtain vigorous, compact and attractive plants.

Acknowledgements

This project was funded by The Australian Flora Foundation and The University of Queensland. We thank Mr. Allan Lisle for support with statistical analysis, Brigitte Pruess and Bradley Pearce for plant maintenance, Ade Damayanti and Chiqa Graciosa for their assistance with data collection.

References

Banon, S.A., Gonzalez, E.A., Cano, J.A., Franco, J.A., and Fernandez, J.A., 2002. Growth, development and colour response of potted *Dianthus caryophyllus* cv. Mondriaan following paclobutrazol treatment. Sci. Hort 94, 371-377.

Boss, P.K., Bastow, R.M., Mylne, J.S., Dean, C., 2004. Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell 16, (Supplement, s18-s31).

Cave, R.L., Johnston, M.E., 2010. Vernalization promotes flowering of a heat tolerant *Calandrinia* while long days replace vernalization for early flowering of *Brunonia*. Sci. Hort. 123, 379-384.

Chen, J., R.J. Henny, D.B. Mc Connell, D.B., Caldwell, R.D., 2003. Gibberellic acid affects growth and flowering of *Philodendron* 'Black Cardinal'. Plant Growth Regul. 41, 1-6.

Demeulemeester, M.A.C., Rademacher, W., Mierop, A.V.D., Proft, M.P.D., 1995. Influence of gibberellin biosynthesis inhibitors on stem elongation and floral initiation on *in-vitro* chicory root explants under dark and light conditions. Plant Growth Regul.17, 47-52.

Faust, J.E., Korczynski, P.C., Klein, R., 2001. Effects of paclobutrazol drench application dates on poinsettia height and flowering. Hort Tech. 11, 557-560.

Gibson, J.L., Whipker, B. E., 2001. Ornamental cabbage and kale growth responses to daminozide, paclobutrazol, and uniconazole. Hort Tech. 11, 226-230.

Gocal, G.F.W., Sheldon, C.C., Gubler, F., Moritz, T., Bagnall, D. J., MacMillan, C.P., Li, S.F. , Parish, R.W., Dennis, E.S., Weigel, D., King, R.W., 2001. GAMYB-like genes, flowering, and gibberellin signaling in *Arabidopsis*. Plant Physiol. 127, 1682-93.

Gray, M., Knight, J. (Ed)., 2001. Flora of Melbourne, Hyland House Publishing Pty. Ltd., Melbourne, pp. 8, 9, 36, 72, 324.

Harrison, D.K., Wickramasinghe, P., Johnston, M.E., Joyce, D.J., 2009. Evaluating mutation breeding methods to fast-track the domestication of two Australian native *Calandrinia* species for ornamental horticulture. Acta Hort. 829, 85-90.

Henny, R.J., Chen, J., Mellich, T.A., 2000. Flowering response of three *Spathiphyllum* cultivars to treatment with three levels of Gibberellic Acid. Proc. Fla. State Hort. Soc 114: 169-170.

Jones, M.G., Zeevart, J.A.D.,1980. Gibberellins and the photoperiodic control of stem elongation in the long-day plant *Agrostemma githago L.* Planta 149, 269 273 (1980)

Karaguzel, O., Baktir, I., Cakmakci, S., Ortacesme, V., 2004. Growth and flowering responses of *Lupinus varius* L. to paclobutrazol. Hort Sci. 39, 1659-1663.

King R.W., Moritz, T., Evans, L.T., Junttila, O., Herlt, A.J., 2001. Long-day induction of flowering in *Lolium temulentum* involves sequential increases in specific gibberellins at the shoot apex. Plant Physiol 127, 624 - 632.

Kim, S., Hertogh, A.A. Nelson, P.V., 1999. Effects of plant growth regulators applied as sprays or media drenches on forcing of Dutch-grown bleeding heart as a flowering potted plant. Hort Tech 9, 629-633.

Kozlowska, M., Zajac, M.R., Stachowiak, J. Janowska, B., 2007. Changes in carbohydrate contents of *Zantedeschia* leaves under gibberellin-stimulated flowering. Acta Physiol. Plant 29, 27-32.

MacMillan, C.P., Blundell, C.A., King, R.W., 2005. Flowering of the grass *Lolium perenne*: Effects of vernalization and long days on gibberellin biosynthesis and signalling. Plant Physiol. 138, 1794-1806.

Mishra, D.K., Mishtra, H.R., Yadava, L.P., 2005. Influence of paclobutrazol on early flowering and aesthetic value of China aster (*Callistephus chinensis* L. Nees). J. Applied Hort 7, 34-37.

Mouradov, A., Cremer, F., Coupland, G., 2002. Control of flowering time: interacting pathways as a basis for diversity. Plant Cell 14, s111-s130.

Rademacher, W., 2000. Growth retardants: effect on gibberellin biosynthesis and other metabolic pathways. Ann. Rev. Plant Physiol. Plant Mol. Biol. 51, 501-531

Ross, J., O'Neill, D., 2001. New interactions between classical plant hormones. Trends Plant Sci. 6, 2-4.

Talon, M., Zeevaart, J.A.D., 1992. Gibberellins and stem growth as related to photoperiod in *Silene armeria* L. Plant Physiol. 92, 1094-1100.

Thompson, D.I., Anderson, N.O., van Staden, J., 2005. Watsonias as container plants: using paclobutrazol for flowering and height control. S. A. J. Bot 73, 426-431.

Warner, R.M., Erwin, J. E., 2003. Effect of plant growth retardants on stem elongation of *Hibiscus* species. Hort Tech. 13, 293-296.

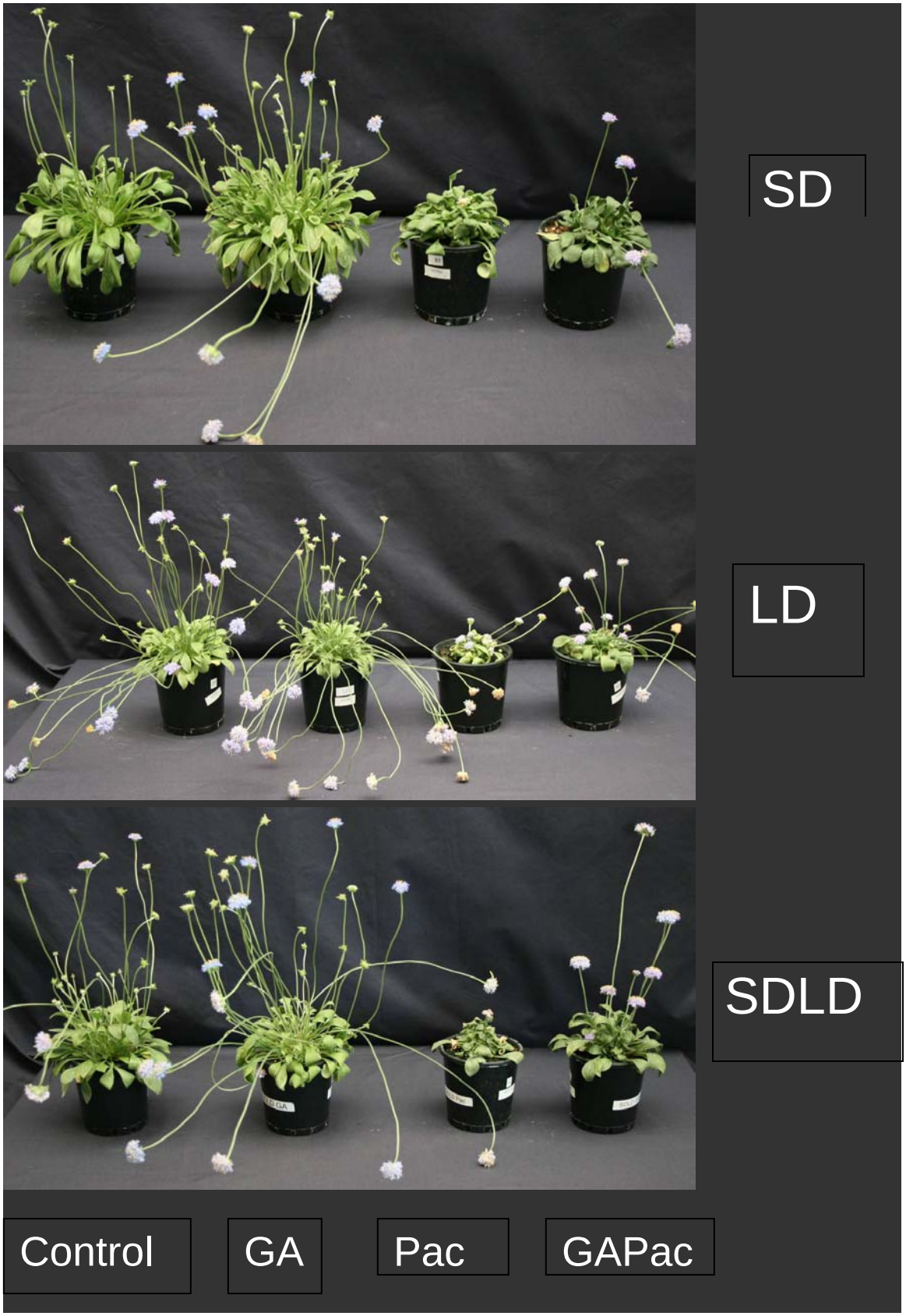
Wilfret, G.J., Barrett, J.E.,1994. Effect of growth regulators on growth and flower development of potted azaleas. Proc. Fla. State Hort. Soc 107, 175-177.

Wilson, R.N., Heckman, J.W., Somerville, C. R., 1992. Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol. 100, 403-408.

Wu, K., Li, L., Gage, D.A., Zeevart, J.A.D., 1996. Molecular cloning and photoperiod-regulated expression of Gibberellin 20-oxidase from the long day plant Spinach. Plant Physiol 11, 547-554.

Wulster, G.J., Ombrello, T. M., 2000. Control of height and flowering of *Ixia* hybrids as container plants. Hort Sci. 35, 1087-8.

Zeevaart, J.A.D., 2006. Florigen coming of age after 70 years. Plant Cell 18, 1783-1789.


SD
LD
SDLD
Control
GA
Pac
GAPac

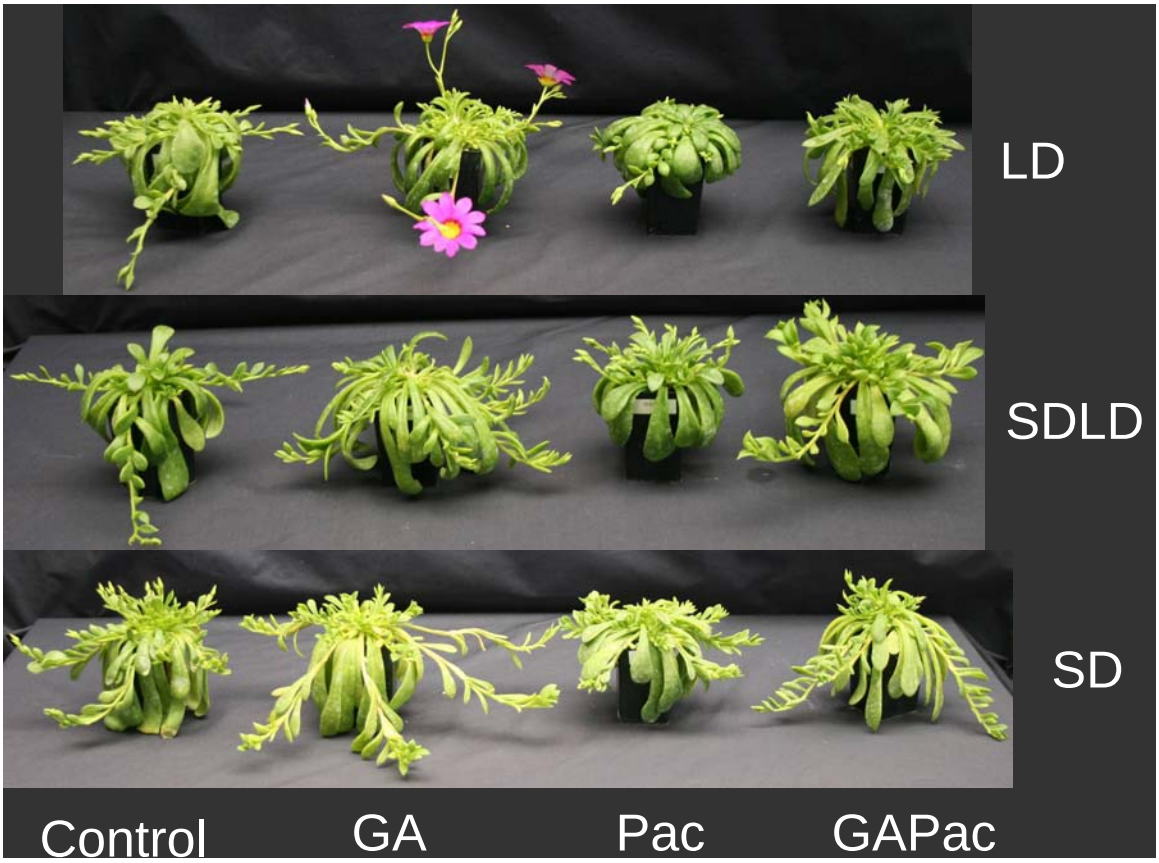


Figure 1.2: Plants of *Brunonia* and *Calandrinia* from each daylength and plant growth regulator treatment.

Section 2: The Effect of Daylength and Temperature on Flowering of *Pycnosorus thompsonianus*

M.T. Ha [A], S. Krisantini [B] , M. E. Johnston [AC]

[A] The University of Queensland, School of Agriculture and Food Sciences, Gatton, Australia 4343

[B] Bogor Agricultural University, Department of Agronomy and Horticulture, Bogor, Indonesia 16680

[C] Corresponding author; email: m.johnston@uq.edu.au

Abstract

The flowering responses of *Pycnosorus thompsonianus*, to daylength and temperature were investigated to study the flowering regulation of this potential ornamental plant at The University of Queensland Gatton, southern Queensland, Australia. Plants were cooled at 20/10°C or kept at 30/20°C for 21 or 42 days under short day (SD), long day (LD), or short day for six weeks before transfer to long day (SDLD).

LDs promoted earlier flowering and plants under LDs flowered regardless of temperature regimes. Cool temperatures and cooling periods were required for flowering of plants under SDs, but this was not important for plants under LDs and SDLDs. Plants under SDs without cooling only produced 3 inflorescences per plant whereas plants which received 21 or 42 days of cooling had 19. Forty-two percent of the SD plants under 30/20°C remained vegetative after a 20 week growing period. Extending the cooling period from 21 to 42 days induced earlier flowering of plants in all daylengths but did not increase number of inflorescences per plant.

Daylength was more effective than temperatures for promoting earlier flowering and for increasing the flower production. This is the first study about flowering responses of the Australian native species *Pycnosorus thompsonianus* under different growing environment and the only published study on members of the Asteraceae to determine the amount of chilling required by young seedlings to order to promote flowering.

Keywords: Anthesis; Australian species; Cooling; Ornamental; Photoperiod; Vernalisation; Visible bud stage.

Introduction

Pycnosorus thompsonianus (Asteraceae) is an Australian native annual with green to silver grey narrow leaves. It has small bright yellow egg-shaped flower heads with erect peduncles. *Pycnosorus* normally flowers in spring and summer. *P. thompsonianus* occurs in semi-arid areas and often flower on mass on floodplains after winter rainfall (Everett and Doust 1992).

Daylength and temperature have been reported to affect floral initiation and flower development of members of the Asteraceae family, including the short day plant chrysanthemum (Kofranek 1980). Several Australian species including *Bracteantha bracteata (*syn. *Helichrysum bracteatum)* and *Rhodanthe chlorocephala* subsp. *rosea* (syn. *Helipterum roseum)* (Sharman and Sedgley, 1988; Sharman et al. 1989) response to long days, *Lawrencella davenportii* and *L. rosea* response to short days (Bunker, 1995), whereas *Brachycome halophila* was reported to be day neutral even though it failed to reach anthesis under 8h SDs at 25/25°C (Bunker, 1995) during the 84 day experimental period. Response to temperature is less clear but Mott and McComb (1975) reported that *Schoenia cassiniana (*syn, *Helichrysum cassinianum)* and *Helipterum craspedioides* required 30 days at 15 to 20°C to flower and constant temperature of 25°C inhibited floral initiation of *Rhodanthe chlorocephala* subsp. *rosea (*syn. *Helipterum roseum)* (Sharman et al. 1989a, b and 1990). *Ozothamnus diosmifolius (*syn. *Helichrysum diosmifolium)* flowering was blocked under high temperatures (26/18°C) under LDs and SDs. Plants only flowered under LDs under moderate temperatures (20/12 and 23/15°C), while under low temperatures (17/9°C) plants flowered in both LDs and SDs (Halevy et al. 2001). A summary of the reported daylength and temperature responses of all Australian members of the Asteraceae is reported in Appendix 1. Another factor that may be importance is light intensity. Halevy et al. (2001) reported high solar radiation under LDs greatly increase the number of flowering stems of *Ozothamnus diosmifolius*.

Many other studies have reported a range of diverse flowering responses of Australian species to temperature, daylength and light intensity including *Acacia* (Sedgley 1985),

Chamelaucium (Shillo et al 1984; Dawson and King 1993, *Anigozanthos* (Motum and Goodwin 1987), *Eucalyptus* (Moncur 1992), *Pimelea* (King et al. 1992; King et al. 1995; Seaton and Plummer 2004), *Boronia* and *Hypocalymma* (Day et al. 1994), *Hardenbergia* (King 1998), *Crowea*, *Lechenaultia* and *Verticordia* (King et al. 2008), *Brunonia* and *Calandrinia* (Cave et al 2010ab; Wahyuni et al. 2011 in press).

The diversity of flowering responses reported means that each species requires investigation of their environmental responses if they are to be used as potted colour. In addition it is important to understand when the seedling is able to perceive the flowering signal and to determine how much is required. This study reports the effect of daylength, temperature regimes and their durations on flowering of *Pycnosorus*.

Materials and methods

Plant materials

All seeds used were collected at Wallen Station in south western Queensland (GPS: 27˚57'748"S; 148˚ 00'834"E) on 14th September 2003. Seeds were cleaned and stored in the Queensland Seed Technology laboratory cold room at 5°C until required.

For the initial experiment *Pycnosorus* seeds were sterilised with 2 g L^{-1} chlorine sown into 9-cm diameter plastic Petri dishes containing 10 g L^{-1} Agar with 0, 50 and 100 mg L^{-1} GA_3. Petri dishes were sealed with parafilm to avoid seed desiccation prior to placement in an air conditioned room at 25^0C and 16 h photoperiod for 3 days. Seeds for other experiments were surface sterilised and germinated on filter paper soaked with 50 mg L^{-1} GA for 24 hours before transfer to Petri dishes without GA.

Seeds were then planted into 100-cell trays containing propagation medium of peat (TM Marketing Pty Ltd., Torrens Park, SA, Australia), perlite (Chillagoe Perlite, Mareeba, QLD, Australia) and vermiculite (Peter Bacon Enterprises, Rocklea, QLD, Australia) of 1:6:3 with 2 g L^{-1} Basacote® Mini 3 month [N:P:K = 13:6:16] (Compo do Brazil S.A, Brazil).

Seedlings were held for 11 days for Experiment 1 and 21 days for Experiment 3 in a short day bay at 30/20°C before transplanting to individual 100 mm (0.5 L) diameter plastic pots

containing growth media of 100% composted pine bark (Basset Barks Pty Ltd., Glasshouse Mountains, QLD, Australia) with 2 g L^{-1} Osmocote® plus 8-9 month (NPK: 15 - 3.9 - 9.1 plus 1.5Mg and TE) Osmocote® plus 3-4 month [N:P:K 16:5:9.2 + 1.8 Mg and TE], 2 g L^{-1} Nutricote® [N:P:K 16:4.4:8.3] (Chisso-Asahi Fertilizer Co.,Ltd. Tokyo, Japan), 1.3 g L^{-1} Osmoform® [N:P:K 18:2.2:11 + 1.2 Mg] (Scotts Australia, Baulkham Hills, NSW, Australia), 1.3 g L^{-1} Coated iron [Fe:S 28:17], 1.2 gL-1 Dolomite® [Ca:Mg 14:8] (Yates, Australia) and 1.2 g L^{-1} Saturaid® (Debco, Melbourne, Australia).

Treatments

The study consisted of three separate experiments conducted at different times. Four bays in the research greenhouse at University of Queensland Gatton nursery were used and were set at a temperature of 20/10 and 30/20°C (day/night, 11 h cycle, 6am-5pm), each with long day (LD) or short day (SD), or 6 weeks SD then LD (SDLD). The SD was 11 hours of sunlight from 6 am-5pm at which time the blackout curtain in each bay closed. The LD was 16 hours (11 hours sunlight + 5 hours incandescent light). Five hours night break for the long day treatment was provided with 100W incandescent lamp; <4.5 µmol.$m^{-2}$$sec^{-1}$ (Sylvania, Indonesia) from 9pm to 2am. Humidity and temperature sensors (Vaisala®, Finland) were used to record the temperature and humidity in each bay every 15 minutes. The light intensity ranged from 300 to 600 µmol.$m^{-2}$$sec^{-1}$.

In the first preliminary experiment plants were placed in two different constant temperatures, 20/10°C or 30/20°C each under three different daylengths: SDs, LDs, or 6 weeks under SDs then transferred to LDs. There were 12 plants for each daylength and temperature treatment derived from seed treated with 0 (replicates 1-4), 50 (replicates 5-8) or 100 (replicates 9-12) mg L^{-1} GA_3.

In the second experiment four age groups of seedlings, i.e. 1, 7, 14 and 28 days old were exposed to different cooling periods under SDs: 0 (without cooling), 3, 7, 14 and 21 days prior to transfer to transfer to 30/20°C with seven plants allocated for each treatment.

In the third experiment plants were exposed to either 21 or 42 days cooling period under SDs, LDs, or SDLDs prior to transfer to 30/20°C at the same daylengths with ten plants per treatment.

Plants were observed every two days and the number of days to first visible floral bud (FVFB), number of branches at visible buds and anthesis was recorded. The number of inflorescences per plant was recorded at week 8, 12, 16 (Experiment 1 only) and 23 (Experiment 2 only). A completely randomized design was used within each three daylength (LD, SD and SDLD). Data obtained were subjected to analysis of variance using the GLM procedure in Minitab® version 15.

Results

Experiment 1: The effects of temperature and day length on growth and flowering of Pycnosorus.

The GA seed treatment had no effect on flowering. Reproductive growth in long days (LDs) commenced earlier than in short days (SDs) (Table 2.1 and 2.2). Plants under LDs reached FVFB at 27-40 days (Table 2.2) and all plants under warm (30/20°C) LDs had FVFB at week 4 (Table 2.1). Plants under SDs reached FVFB at 52-75 days (Table 2.2). Forty-two percent of the plants under warm SD did not flower until experiments were terminated at week 16 (Table 2.1).

Table 2.1. Percentage of *Pycnosorus* plants with visible flower buds under different daylength and temperature regimes

Daylength	Temperature	Percentage of plants with visible flower buds (week)						
		3	4	6	8	10	12	16
LD								
	20/10°C	17	42	67	100	100	100	100
	30/20°C	67	100	100	100	100	100	100
SD								
	20/10°C	0	0	8	91	100	100	100
	30/20°C	0	0	8	16	33	42	58
SDLD								
	20/10°C	0	0	25	91	100	100	100
	30/20°C	0	0	8	33	100	100	100

Table 2.2. Days to first visible bud, to anthesis and number of inflorescences per plant under different daylengths and temperature regimes [1)]

Daylength (DL)	Temperatures (T)	Days to first VB	Days to Anthesis	VB to Anthesis (days)	Number of inflorescences/plant at week		
					8	12	16
LD							
	20/10°C	40.2 b	57.9 b	17.8 b	18.1 b	37.6 e	47.8 c
	30/20°C	27.5 a	39.7 a	12.2 c	19.1 b	44.2 f	75.2 e
SD							
	20/10°C	52.6 c	77.5 cd	24.9 a	5.1 a	19.6 b	38.1 b
	30/20°C	75.4 f [2)]	84.2 de [3)]	7.4 d [3)]	1.5 a	7.8 a	15.0 a
SDLD							
	20/10°C	56.0 d	80.8 d	24.8 a	3.8 a	26.6 c	42.5 bc
	30/20°C	63.5 e	74.1 c	10.6 c	1.0 a	31.0 d	68.8 d
DL		**	**	ns	**	**	**
T		*	*	**	ns	ns	*
DL x T		**	**	*	ns	ns	**

Notes:

1) Values followed by different letters within a column are significantly different according to Tukey test and simple t-test. n.s.: not significant, *P<0.05, **P<0.01, ***P<0.001.

2) Only 58 % of the plants initiated floral buds when the experiment was terminated at 16 weeks
3) Only 50% of the plants had reached anthesis when the experiment was terminated at 16 weeks

Flower buds appeared earlier under LDs (Table 2.1 and 22) and the plants had more inflorescences under warm temperatures (30/20°C) than under cool temperatures of 20/10°C at week 16 (Table 2). In contrast, plants under SDs flowered earlier and had more inflorescences per plant under low temperatures (Table 2.1 and 2.2). All plants under warm SDLDs had FVFBs four weeks after transferred to LDs (SD was applied up to week 6) whereas only 33% of plants kept under warm SDs had FVFBs at the same time (Table 2.1).

***Experiment 2: Effects of cooling duration and plant ages on floral development of* Pycnosorus.**

The results of the first experiment demonstrated that cooling is important for *Pycnosorus* flowering grown under SDs. A further study was then conducted to determine the optimum cooling periods for flowering under SDs, and whether or not seedling age prior to cooling affect flowering responses.

Similar to the results on the first experiment, there were plants that did not flower under the SDs. Generally, number of flowering plants increased with the age of plants prior to cooling, or with the increases in cooling duration (Table 2.3). Twenty-days old seedlings had more flowering plants than the younger age groups (Table 2.3) and more inflorescences per plant at week 12 and 23 (Table 2.3). The longest cooling period of 21 days had more flowering plants (Table 2.3) and had more inflorescences per plant at week 23 compared to those had shorter cooling periods (Table 2.3).

Table 2.3. Effects of age and cooling duration on floral development of *Pycnosorus* under short days.

Treatment	Percentage of flowering plants (%)	Days to first VB	Days to anthesis	Number of inflorescences/plant at week	
				12	23
Plant Age (days)					
1	33.3	79.6	91.5	0.7	1.5 a
7	37.1	82.0	93.6	0.7	1.7 a
14	57.1	82.1	88.3	1.4	3.9 ab
28	71.4	84.9	95.2	1.0	6.4 b
Cooling duration (days)					
0	28.6	90.7	94.3	0.3 a	1.5 a
3	36.0	73.3	83.6	0.5 a	2.3 a
7	29.6	87.7	97.7	0.4 a	1.3 a
14	67.9	85.8	97.6	1.1 a	4.4 ab
21	85.7	73.2	87.5	2.4 b	7.4 b
Plant Age	-	ns	ns	ns	*
Cooling duration	-	ns	ns	***	**
Plant Age * Cooling duration	-	ns	ns	ns.	ns

Note: Experiment was terminated at week 23 after planting. Values followed by different letters within a column are significantly different according to Tukey test and simple t-test. n.s.: not significant, *P<0.05, **P<0.01, ***P<0.001.

None of the plants flowered up to week 11. Plants cooled for 21 days had visible bud at 73 days, whereas plants received cooling of less than 21 days did not have visible buds until 85 to 90 days (Table 2.3). However, there were no significant differences in the time to first VB and time to anthesis among plants of age levels and different cooling periods (Table

2.3). This might be related to the large number of plants across all treatments that remained vegetative during the course of the experiment even though the experiment was extended to 23 weeks. In addition, there was variation in the time to FVFB and to anthesis among plants within a treatment. *Pycnosorus* were competent to perceive chilling as one day old seedlings, but cooling for seedlings at 4 week old stage was found to be the most effective for growth and flowering.

This experiment has confirmed the results of the first experiment on the requirement of cooling for *Pycnosorus* flowering grown under SDs, and that the longest period of cooling (21 days) was the most effective to promote development and flower production of *Pycnosorus* grown under SDs. However, the plants cooled for 21 days under SDs in this experiment only produced two inflorescences at week 12 and seven inflorescences per plant at week 23. In addition, 14 % of the plants received 21 days cooling remained vegetative till the end of experiment. A further study was then conducted to determine whether or not extension of cooling periods from 21 to 42 days would enhance flowering of *Pycnosorus* grown under different day lengths: SDs, LDs, SDLDs.

Experiment 3: The Effect of Cooling Periods under Different Day Lengths on *Pycnosorus* Flowering

Extending the cooling period from 21 to 42 days promoted earlier flowering and anthesis in plants under SDs (Table 2.4). All plants under LDs had visible floral buds at 25-30 days (Table 2.4) with 8 branches per plant (Table 2.5). Under SDs 70 % of the plants were still vegetative at week 12 (data not shown). Plants under SDs had 19 branches per plant at the first visible floral bud (Table 2.5). All plants under SD flowered in this experiment. Daylength had the greatest effect on flowering while the extension of the cooling period did not increase number of inflorescences per plant at week 12 and 16, and did not significantly affect flower development in all daylengths (Tables 2.4 and 2.5).

Table 2.4. Effects of cooling duration on floral development of *Pycnosorus* at different daylengths

Cooling Period (C)	Daylength (D)	Days to first visible bud (VB)	Days to anthesis	VB to anthesis (days)
3 weeks				
	LD	25.0 a	39.0 a	14.0
	SD	66.0 f	84.0 f	17.7
	SDLD*)	41.8 c	56.2 c	14.4
6 weeks				
	LD	30.7 b	48.9 b	18.2
	SD	55.8 e	74.3 e	18.5
	SDLD*)	48.5 d	62.9 d	14.2
Cooling Period		ns	ns	ns
Daylength		**	**	ns
C x D		*	**	ns

*) Six weeks of SDs followed by LDs

Table 2.5. The effect of cooling period on number of branches at visible bud stage and number of inflorescences/plant at different daylengths

	Number of Branches at first VB	Number of inflorescences/ plant at week	
		12	16
Cooling Period			
3 weeks	12.7	15.3	32.9
6 weeks	14.0	16.2	35.8
Daylengths			
LD	8.3 a	28.5 c	54.9 c
SD	19.5 c	4.1 a	13.4 a
SDLD	13.6 b	14.6 b	34.7 b
Cooling Period	ns	ns	ns
Daylengths	**	**	**
Cooling Period X Daylength	ns	ns	ns

Plant morphology

Plants grown under LDs elongated as they became floral compared to those grown under SDs (Fig 1).

Figure 1. Sixteen-week-old *Pycnosorus thompsonianus* plants under SDs (left) and LDs (right). Plants grown under LDs (right) flowered earlier and were more elongated than plants under SDs.

Discussion

LDs reduced the time to the FVFB and to anthesis (Table 2 and 4) and increased number of inflorescences per plant compared to SDs. However, flowering occurred under SDs, suggesting that *Pycnosorus* is a quantitative LD plant. This is similar to the reports for *Bracteantha bracteata (*syn. *Helichrysum bracteatum)* and *Rhodanthe chlorocephala* subsp. *rosea* (syn. *Helipterum roseum)* (Sharman and Sedgley, 1988; Sharman *et al* 1989a,b). Enhanced flowering of Australian native species *Calandrinia* and *Brunonia* under LDs have been reported (Cave and Johnston, 2010). This is the first study about the flowering responses of another Australian native *Pycnosorus thompsonianus* to daylengths. Daylength interacted with temperatures in affecting time to the first visible floral buds and to anthesis (Table 2.2 and 2.4). Plants under warm LDs flowered earlier (Table 2.1) and

produced more inflorescences per plant (Table 2.2) than plants under cool LDs, whereas the effects of temperatures on plants under SDs were the opposite (Table 2.1 and 2.2). Once floral initiation has occurred warm temperature accelerates floral development.

Cooling is important for flowering of plants under SDs and flowering was inhibited under warm SDs (Table 2.2). Twenty-one days of cooling at the start of SDs was sufficient; continuous cooling was not required for flowering under SDs, indicated by the longer time to flower (Table 2.2) compared to plants exposed to only 21 or 42 days of cooling followed by warm temperatures in the third experiment (Table 2.4).

Extended cooling period from 21 to 42 days under SDs promoted time to FVFB by 11 days (Table 2.4), but did not increase number of inflorescences per plant (Table 2.5). The promotion of earlier visible floral buds and anthesis and higher flower production following a short-term cooling period followed by warm temperatures has been previously reported in qualup bell (*Phymelea physodes*), a Western Australia native species (Seaton and Plummer, 2004). Therefore subjecting plants to a lower temperature pulse might be a useful method for scheduling flowering for the potted plant trade.

The inhibition of flowering under warm SDs was further confirmed by the decreased percentage of plants that flowered as the period of cooling under SDs shortened (Table 2.3). Warm SDs promoted vegetative growth of plants, resulting in significantly more branches per plant at FVFB stage, i.e. 19 branches in SDs in contrast to 8 under LDs (Table 2.5). Temperature regimes at the onset of SDs was important for flower initiation of the SD plant *Chrysanthemum* (Asteraceae); high temperatures during the first 42 days at SDs significantly delayed floret initiation and differentiation (Cockshull et al, 1994).

There have been a number of studies reporting the importance of vernalisation for flowering. The temperatures required for vernalisation vary with plant species, e.g. 5°C for radish (Yoo, 1977), 15° C for *Centradenia* (Friis and Christensen, 1989) and below 15°C in *Heliotrope* (Park and Pearson, 2000). The temperature ranges used in this experiment (20/10°C) were effective to induce flowering in *Pycnosorus.*

Plants under LDs flowered regardless of temperature regimes, suggesting that LDs could replace cooling requirement of *Pycnosorus*. Similar results were reported by Cave and Johnston (2010) for *Brunonia*.

Number of inflorescences per plant in the second experiment (Table 2.3) were by far fewer than in the plants under cool SDs in the first experiment (Table 2.2), probably because seedlings in the second experiment were exposed to cool temperatures when very young (1 day to 4 weeks old) and they received little chilling (0 to 21 days). To optimise flowering 21 days of chilling (20/10˚C) needs to be applied when seedlings are about 28 days old. A question that remains is whether returning the seedlings to warm temperatures (30/20˚C) after the short chilling periods used in Experiment 2 resulted in devernalisation and explains the lower flower numbers recorded in this experiment.

Daylength is more important than temperatures for *Pycnosorus* flowering. Plants under LDs and SDLDs consistently flowered earlier with more number of inflorescences per plant compared to SDs at the same time (Table 2.1, 2.2, 2.4 and 2.5). All plants under warm SDLD flowered at week 10, i.e. 4 weeks after transfer from SDs to LDs, whereas 67% plants remained vegetative under warm SD (Table 2.1).

The duration of flower development was affected by the interaction between daylength and temperatures (Table 2.2). Generally flower rate development was more rapid under warm temperature (30/20°C) compared to cool temperatures (20/10°C), but under SDs, and warm temperatures flowering was delayed. Commercially it is recommended that plants be grown under cool temperatures (20/10˚C) and SDs for 6 weeks to promote vegetative growth before transfer to LDs to promote flowering.

Conclusion

LDs promoted earlier flowering and increased the number of inflorescence compared to SDs or SDLDs, but flowering occurred under SDs, suggesting that *Pycnosorus* is a quantitative LD plant. Plants under LDs flowered well regardless of temperature regimes, but cool temperatures was required for flowering of plants under SD; 40% of plants under constant 30/20°C SD failed to initiate floral buds. *Pycnosorus* were competent to perceive chilling as one day old seedlings, but cooling at the 4 week old stage was most effective for flowering. Cooling period to induce flowering under SDs should not be less than 21 days. Extending the cooling period from 21 to 42 days induced earlier flowering but did not increase number of inflorescences in all daylengths.

Acknowledgements

This project was funded by The Australian Flora Foundation and The University of Queensland. We thank Mr. Allan Lisle for support with statistical analysis and Mrs Vishu Wickramasinghe for data collection and plant maintenance.

References

Bunker K.V., 1995. Year-round production of Australian daisies (Asteraceae) as flowering pot plants. Sci. Hort. 61 (1-2), 101-113.

Cave R.L., Johnston M.E., 2010. Vernalization promotes flowering of a heat tolerant *Calandrinia* while long days replace vernalization for early flowering of *Brunonia*. Sci. Hort. 123 (3), 379-384.

Day S., Loveys B.R., Aspinall, D., 1994. Manipulation of flowering and vegetative growth of Brown Boronia (*Boronia megastigma* Nees.) and White Myrtle (*Hypocalymma angustifolium* Endl.) using plant growth regulators. Sci. Hort. 56, 309-320.

Dawson, I.A., King, R.W., 1993. Effect of environment and applied chemicals on the flowering and form of Geraldton Wax (*Chamelaucium uncinatum* Schauer). Sci Hort. 54, 233-246.

Everett J., Doust, A.N.L., 1992. New species and a new combination in *Pycnosorus* (Asteraceae: Gnaphalieae). Telopia 5 (1), 39-43.

Friis, K., Christensen, O.V., 1989. Flowering of *Centradenia inaequilateralis* `Cascade' as influenced by temperature and photoperiod. Sci. Hort. 41, 125-130.

Halevy, A.H., Shlomo, E., Shvartz, M., 2001. Environmental factors affecting flowering of rice flower (*Ozothamnus diosmifolius* Vent.). Sci. Hort. 87, 303-309.

King , R.W., Dawson, I.A., Speer, S.S., 1992. Control of growth and flowering in two Western Australian species of *Pimelea*. Aust. J. Bot. 40 (3), 377-388

King, R.W., Pate, J.S., Johnston, J., 1996. Ecotypic differences in the flowering of *Pimelea ferruginea* (Thymelaceae) in response to cool temperatures. Aust. J. Bot. 44 (1), 47-55.

King, R.W., 1998. Dual Control of Flower Initiation and Development by Temperature and Photoperiod in *Hardenbergia violacea*. Aust. J. Bot. 46, 65-74.

Kofranek, A.M ., 1980. Cut Chrysanthemums. In *Introduction to Floriculture* (Ed Larsen, RA) pp. 14-33. Academic Press Inc., New York.

Motum, G.J., Goodwin, P.B., 1987. The control of flowering in kangaroo paw (*Anigozanthos* spp.). Sci. Hort. 32 (1-2), 123-133.

Mott J.J., McComb A.J ., 1975. Role of photoperiod and temperature in controlling phenology of three annual species from an arid region of Western Australia. J. Ecol. 63 (2), 633-641.

Moncur, M.W., 1992. Effect of low temperature on flora induction of *Eucalyptus lansdowneana* F. Muell & J. Brown subsp. *lansdowneana*. Aust. J. Bot. 40, 157-67.

Pearson S., Parker A., Hadley P., Kitchener H.M., 1995. The effect of photoperiod and temperature on reproductive development of Cape Daisy (*Osteospermum jucundum* cv 'Pink Whirls'). Sci. Hort. 62, 225-235.

Sedgley M., 1985. Some effects of temperature and light on floral initiation and development of *Acacia pycnantha*. Aust. J. Plant Physiol. 12, 109-118.

Seaton, K.A., Plummer J.A., 2004. Observations on environmental control of flowering of Qualup bell (*Pimelea physodes*). Aust. J. Exp. Agric. 44, 821- 826.

Sharman K.V., Sedgley M., 1988. Floral initiation and development in *Helipterum roseum* (Hook.) Benth. and *Helichrysum bracteatum* (Vent.) Andrews (Asteraceae). Aust. J. Bot. 35 (5), 575-587.

Sharman K.V., Sedgley M., Aspinall D., 1989. Effects of photoperiod, temperature and plant age on floral initiation and inflorescence quality in the Australian native Daisies *Helipterum roseum* and *Helichrysum bracteatum* in relation to cut-flower production. J. Hort. Sci. 64 (3), 351-359.

Wahyuni ,S., Krisantini, S. , Johnston, M.E., in press. Plant Growth Regulators and Flowering of *Brunonia* and *Calandrinia* sp. Sci. Hort. (In press)

Yeh D.M., Atherton J.G., Craigon J., 1997. Manipulation of flowering in cineraria. III. Cardinal temperatures and thermal times for vernalization. J. Amer, Soc. Hort. Sci. 72, 379-387.

Yoo, K.C., 1977. Studies on the physiology of bolting and Flowering in *Raphanus sativus* L. J. Korean Soc. Hort. Sci.18, 157-161.

Section 3: The Effect of Low Temperature on Flowering of *Rhodanthe floribunda*

Abstract

Introduction

Rhodanthe floribunda (DC) Wilson (syn. *Helipterum floribundum*) commonly called the white sunray or white paper daisy is found in semi-arid areas of Qld, NSW, SA, WA and NT (Barker et al. 2002). It is a floriferous and attractive plant with potential as potted colour species. Bunker (1995) reported that *R. floribunda* was a facultative LD plant. An earlier study by Roberts (2005) showed that low minimum temperatures (below 10˚C) experienced in April, May and June plantings in south east Queensland were found to reduce the time to the first visible bud (Roberts 2005). The aim of the following study was to quantify the amount of chilling required and to determine whether the seedling age at chilling influenced flowering.

Materials and methods

Plant materials

All seeds used were collected at Wallen Station in south western Queensland (GPS: 27˚57'748"S; 148˚ 00'834"E) on 14th September 2003. Seeds were cleaned and stored in the Queensland Seed technology laboratory cold room at 5°C until required.

Rhodanthe seeds were surface sterilised and germinated on agar plates $1gL^{-1}$ with 50 mg L^{-1} GA_3 for 1 week. Seeds were planted sequentially to provide seedlings of the appropriate ages for the experiment.

Seeds were then planted into 100-cell trays containing propagation medium of peat (TM Marketing Pty Ltd., Torrens Park, SA, Australia), perlite (Chillagoe Perlite, Mareeba, QLD,

Australia) and vermiculite (Peter Bacon Enterprises, Rocklea, QLD, Australia) of 1:6:3 with 2 g L^{-1} Basacote® Mini 3 month [N:P:K = 13:6:16] (Compo do Brazil S.A, Brazil).

Seedlings were held for in a short day bay at 30/20 °C before transplanting to individual 100 mm (0.5 L) diameter plastic pots containing growth media of 100% composted pine bark (Basset Barks Pty Ltd., Glasshouse Mountains, QLD, Australia) with 2 g L^{-1} Osmocote® plus 8-9 month (NPK: 15 - 3.9 - 9.1 plus 1.5Mg and TE) Osmocote® plus 3-4 month [N:P:K 16:5:9.2 + 1.8 Mg and TE], 2 g L^{-1} Nutricote® [N:P:K 16:4.4:8.3] (Chisso-Asahi Fertilizer Co.,Ltd. Tokyo, Japan), 1.3 g L^{-1} Osmoform® [N:P:K 18:2.2:11 + 1.2 Mg] (Scotts Australia, Baulkham Hills, NSW, Australia), 1.3 g L^{-1} Coated iron [Fe:S 28:17], 1.2 gL-1 Dolomite® [Ca:Mg 14:8] (Yates, Australia) and 1.2 g L^{-1} Saturaid® (Debco, Melbourne, Australia).

Treatments

Two bays in the research greenhouse at University of Queensland Gatton nursery were used and were set at a temperature of 20/10 and 30/20°C (day/night, 11 h cycle, 6am-5pm), each with short day (SD). The SD was 11 hours of sunlight from 6 am-5pm at which time the blackout curtain in each bay closed. Humidity and temperature sensors (Vaisala®, Finland) were used to record the temperature and humidity in each bay every 15 minutes. The light intensity of the greenhouse bay was 380 ± 44 µmol m^{-2} s^{-1}.

In the experiment four age groups of seedlings, i.e. 1, 7, 14 and 28 days old were exposed to different cooling periods at 20/10°C under SDs: 0 (without cooling), 3, 7, 14 and 21 days prior to transfer to transfer to 30/20°C with 10 plants allocated for each treatment. Plants were observed every two days and the number of days to first visible floral bud (FVFB) and anthesis, and the number of branches at FVFB was recorded. The number of inflorescences per plant was recorded at week 6, 12 and 23. A completely randomized design was used. Data obtained were subjected to analysis of variance using the GLM procedure in Minitab® version 15.

Results

Effects of ages and chilling duration on floral development of Rhodanthe

Seedlings that were chilled for 7 to 21 days reached the FVFB stage in 42 - 47 days, significantly earlier ($P < 0.05$) than the control (54 days) and those were chilled for 3 days (62 days) (Table 3.1).

Chilling for 21 days significantly reduced ($P < 0.05$) the time to anthesis (60 days) compared to the control (67 days) and chilling for 3 days which greatly delayed time to anthesis (81 days) (Table 3.1).

Plants that received the chilling treatment as 1 day old seedlings reached the FVFB in 55 days, significantly more ($P < 0.05$) than 1 week old seedlings (47 days) but similar to 2 and 4 week old seedlings. However, time to anthesis of 4 weeks old (62 days), 2 week old (67 days) and 1 week old seedlings (65 days) were significantly shorter ($P < 0.05$) than that of 1 day old seedlings (74) (Table 3.1).

In addition, 5% plants chilled for 0, 3 and 7 days did not reach the VB stage by the end of experiment (23 weeks from planting); and 12.5%, 17.5% and 2.5% plants initiated flower buds but did not reach anthesis, respectively.

Plants chilled for 3 days had an average of 0.2 inflorescences per plant, significantly lower ($P < 0.05$) than that of plants chilled for 21 days (1.3) but similar to other treatments at week 6. At week 12, plants that received 21 day chilling had the highest number of inflorescences/plant (52.3), significantly greater ($P < 0.01$) than the 14 day cold treated plants (32.8 inflorescences/plant) which was significantly higher than 3 day chilled plants (13.8) but similar to control (18.9) and plants receiving 7 day chilling. However at week 23 when the experiment ended, non-chilled plants, and 3 and 7 day cold induced plants had similar number with 32, 34 and 38 inflorescences/plant, respectively; while plants that were chilled for 21 days had the higher number ($P < 0.05$) (69 inflorescences/plant) which was higher but not significantly higher than and 14 day chilled plants which had 50 inflorescences/plant (Table 3.1).

When averaged over chilling duration plants from each age group had a similar number of inflorescences at week 6 after planting with 0.5 - 0.8 inflorescence/plant. At week 12, plants that were chilled as 4 week old seedlings had the highest number ($P < 0.001$) of inflorescences/plant (46), while those chilled as 1 day seedlings had 15 inflorescences/plant had significantly fewer than those chilled as 2 week old seedlings (31) but similar to those chilled as 1 week old seedlings (24). Similar results were obtained at the end of the experiment (23 weeks) with plants chilled as 4 week old seedlings having 83 inflorescences/plant followed by plants chilled as 2 week old seedlings (53), while there were no significant difference between plants chilled as 1 day old seedlings (18) and 1 week old seedling (24) (Table 3.1).

Table 3.1. Effects of ages and chilling duration on floral development of *Rhodanthe*.

Treatment	Days to first visible bud (VB)	Days to anthesis	Inflorescences per plant at week 6	Inflorescences per plant at week 12	Inflorescences per plant at week 23	Top dry weight per plant (gram)
Chilling duration						
0 day	54.52 (a)	67.13 (b)	0.6 (ab)	18.9 (ab)	31.82 (a)	0.115 (a)
3 days	61.73 (b)	80.84 (c)	0.2 (a)	13.8 (a)	34.40 (a)	0.106 (a)
7 days	47.01 (c)	62.85 (ab)	0.7 (ab)	29.1 (ab)	38.39 (a)	0.130 (a)
14 days	47.48 (c)	64.78 (ab)	0.8 (ab)	32.8 (b)	50.16 (ab)	0.159 (ab)
21 days	42.60 (c)	59.63 (a)	1.3 (b)	52.3 (c)	68.52 (b)	0.224 (b)
P-value	*	*	*	***	*	*
Age						
1 day	55.23 (a)	74.11 (c)	0.7 (a)	14.9 (a)	18.33 (a)	0.041 (a)
1 week	47.42 (b)	64.92 (ab)	0.7 (a)	24.1 (ab)	24.44 (a)	0.060 (ab)
2 weeks	49.69 (ab)	67.32 (b)	0.5 (a)	30.9 (b)	52.48 (b)	0.146 (b)
4 weeks	50.34 (ab)	61.83 (a)	0.8 (a)	47.5 (c)	83.38 (c)	0.340 (c)
P-value	*	*	n.s.	***	***	***
Chilling* Age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Experiment was terminated after 23 weeks from planting. Flowers were dried at 60^0C for 24h. Values followed by different letters within a column are significantly different according to Tukey test and simple t-test. n.s.: not significant, * P<0.05, **P<0.01, ***P<0.001.

As expected, top dried weight of plants that were chilled for 21 days (0.224g) was significantly higher ($P < 0.05$) than that those chilled 0, 3 and 7 days with 0.115, 0.106 and 0.130g/plant respectively, but similar to plants chilled for 14 days (1.159g/plant) (Table 3.1). Moreover, chilling was more effective for older plants. Plants that were chilled as 4 week old seedlings showed the highest ($P < 0.001$) top dry weight (0.340g), followed by 2 week old seedlings (0.146g) which was higher than 1 day old seedlings (0.041g), but similar to that of 1 week old seedling (0.060g) (Table 3.1).

There were no interaction between chilling duration and plant age in relation to floral development parameters of *R. floribunda* (Table 3.1).

Thermal time

Chilling for 21 days gave the lowest number of growing degree days (GDD) for time to FVFB (419) and anthesis (623), while 3 day chilling period induced the highest figures with 744 and 979 degree days respectively (Table 3.2).

Table 3.2. Growing degree days (GDD) for time to first visible floral bud and anthesis of *Rhodanthe.*

Chilling duration	GDD for time to FVFB	GDD for time to anthesis
0 day	670.1	825.14
3 days	743.8	978.66
7 days	542.8	737.53
14 days	513.6	726.25
21 days	418.6	627.95

Note: Average age of seedlings was 11 days (for all age treatments) at transplanting. n = 40.

Discussion

Effects of chilling duration on flowering

Plants of *R. floribunda* flowered without chilling and hence have a facultative requirement for low temperature (Finnegan et al. 1998; Michaels and Amasino 2000; McDonald and

Kwong 2005). Five percent of plants remained vegetative in the non-chilled control and those plants chilled for 3 and 7 days, and 12.5%, 17.5% and 2.5% plants, respectively, did not reach anthesis; while all plants that received 14 and 21 day chilling flowered, reaching FVFB in shorter time than control and plants chilled for 3 days. These results are in agreement with Gleichsner and Appleby (1996) who found that longer chilling duration (to a limit) reduces the time to flowering of ripgut brome (*Bromus diandrus*). Pearson *et al.* (1995) also found that at least 2 week duration of cold at 12^0C accelerated floral development of Cape daisy *Osteospermum jucundum* cv 'Pink Whirls'. The results presented in this study further confirmed the role of low temperature in promoting early flowering of *R. floribunda* reported by Roberts (2005).

Chilling for 3 days resulted the longest time to FVFB and anthesis (Table 3.1), suggesting that a short duration of chilling might not be enough to induce a stable floral induction stage as is reported in many vernalization studies (Michaels and Amasino 2000; McDonald and Kwong 2005; Taiz and Zaiger 2006) where the common temperature range of 0 - 7^0C were used as vernalization treatment, while chilling temperature ($20/10^0$C) used in this study was not in the range reported.

McDonald and Kwong (2005) state that plants can be devernalized under hot temperature following a short period of vernalization (usually less than five days). Further, the devernalizing effect of hot temperature decreases in accordance with the increase of vernalization duration (Michaels and Amasino 2000; Taiz and Zaiger 2006), thus plants might not be devernalized if they have achieved a saturated and stable status. Some authors suggested that devernalization can be prevented by placing plants that has just been vernalized into a 'neutral' temperature (around 15^0C) for several days (Yeh et al. 1997; Hopkins and Hüner 2009; Cave and Johnston 2010). Further, Sun et al. (2008) showed that reduction of photosynthesis and stomatal conductance of chrysanthemum resulted from sudden change of temperature from $23/18^0$C to $33/28^0$C (D/N). In this study, plants that were transferred to $30/20^0$C after 3 day chilling were younger than other plant groups under 7, 14 and 21 days, thus, they might have been more susceptible to this abrupt change of temperature.

In addition to the effect on flower development, chilling influenced total inflorescence number. Plants that received chilling as 21 day old seedlings had more inflorescences and a higher inflorescence weight than the control plants and plants chilled for 3 and 7 days (Table 3.1). This result is consistent with the results reported by several authors who concluded that a certain period of low temperature is needed to promote flower development; shorter durations do not influence flowering (Pearson et al. 1995; Horva´th et al. 2003) or flowering development is less (King et al. 1992; Michaels and Amasino 2000).

Effects of plant maturity prior to chilling on flowering

Plants of *R. floribunda* were competent to perceive chilling as one day old seedlings and they did flower. This suggests a short juvenile phase of these species. Cave and Johnston (2010) stated that the short juvenility stage maybe an ephemeral trait. The capacity to promote flowering by exposing plants to chilling can be utilized for commercial production by shortening production time. In other ornamental species such as cineraria, plants were not be able to perceive chilling stimulus for floral development until the plants reach 6 - 7 leaves (cv. 'Cindy Blue') or 7 - 8 leaves (cv. 'Cindy Dark Red') (Yeh & Atherton 1997).

Although there was not clear difference for *R. floribunda* with regards to time to FVFB and inflorescence number at week 6 among age groups, the number of days to anthesis and inflorescence numbers at 12 and 23 weeks indicated that older plants prior to chilling, showed more floral development (Table 3.1). In addition, top dry weight was higher for older plants. These are consistent with the study results of Markowski and Ryka (1981) and Townsend (1982) in which the older plants prior to cold induction showed higher floral production. According to Cave and Johnston (2010), the increased floral production in older plant group might be due to the longer periods for branching and development.

Thermal time

The number of GDD required for flowering can be used as a benchmark to predict time to floral development in commercial production of an ornamental crop (Huang et al. 1999; Lee et al. 2008). The results of this study for *R. floribunda* suggests that for a rapid and efficient flowering, chilling treatment of 3 weeks at $20/10^{0}$C should be included as time to

FVFB and anthesis could be reduced to 419 and 628 GDD, respectively, under SD (11h daylength).

Acknowledgements

This project was funded by The Australian Flora Foundation and The University of Queensland. We thank Mr. Allan Lisle for support with statistical analysis.

References

Bunker K.V., 1995. Year-round production of Australian daisies (Asteraceae) as flowering pot plants. Sci. Hort. 61 (1-2), 101-113.

Cave R.L., Johnston M.E., 2010. Vernalization promotes flowering of a heat tolerant *Calandrinia* while long days replace vernalization for early flowering of *Brunonia*. Sci. Hort. 123 (3), 379-384.

Finnegan, E.J., Genger, R.K., Kovac, K., Peacock, W.J., Dennis, E.S. 1998, DNA methylation and the promotion of flowering by vernalization, Proc. Nat. Acad. Sciences USA., 95(10), 5824-9.

Gleichsner, J.A., Appleby, A.P. 1996, Effects of vernalization on flowering in ripgut brome (*Bromus diandrus*). Weed Sci. 44(1), 57-62.

Hopkins, W.G., Hüner, N.P.A. (Eds) 2004, *Introduction to Plant Physiology*, 3rd Edn, John Wiley & Sons, Inc., London.

Horva´th, E., Szalai, G., Janda, T., Pa´ldi, E., Ra´cz, I., La´sztity, D. 2003, Effect of vernalisation and 5-azacytidine on the methylation level of DNA in wheat (*Triticum aestivum L.*, cv. Martonva´sa´r 15)', Plant Sci. 165, 689 - 92.

Huang, N., Funnell, K.A., MacKay, B.R. 1999, Vernalization and growing degree-day requirements for flowering of *Thalictrum delavayi* ‘Hewitt's double', HortSci. 34(1), 59-61.

King, R.W., Dawson, I.A., Speer, S.S., 1992. Control of growth and flowering in two Western Australian species of *Pimelea*. Aust. J. Bot. 40 (3), 377-388

King, R.W., Pate, J.S., Johnston, J., 1996. Ecotypic differences in the flowering of *Pimelea ferruginea* (Thymelaceae) in response to cool temperatures. Aust. J. Bot 44 (1), 47-55.

King, R.W., 1998. Dual control of flower initiation and development by temperature and photoperiod in *Hardenbergia violacea*. Aust. J. Bot., 46, 65-74.

Kofranek, A.M ., 1980. Cut Chrysanthemums. In *Introduction to Floriculture* (Ed Larsen, RA) pp. 14-33. Academic Press Inc., New York.

Lee, K.K., Johnston, M.E., Harrison, D.K.,Williams, R.R. 2008, Evaluation of key horticultural traits for *Ptilotus nobilis* in sub-tropical regions, Sci. Hort.118(3), 236-41.

Markowski, A., Ryka, C. 1981, Effect of age of plants and other factors during vernalization on generative development of winter rape (*Brassica-napus*-Oleifera) under controlled growth conditions, *Bulletin de l'Academie Polonaise des Sciences Serie des Sciences Biologiques*, 29(9-10), 415-22# Abstract only.

McDonald, M.B., Kwong, FY (eds) 2005, *Flower Seeds Biology and Technology*, CABI publishing, Oxfordshire, UK.

Michaels, S.D., Amasino, R.M. 2000, Memories of winter: vernalization and the competence to flower, Plant Cell and Environ. 23(11), 1145-53.

Pearson S., Parker A., Hadley P., Kitchener H.M., 1995. The effect of photoperiod and temperature on reproductive development of Cape Daisy (*Osteospermum jucundum* cv 'Pink Whirls'). Sci. Hort 62, 225-235.

Roberts, J. M., 2005. The effect of planting date and daylengthon flowering and growth habit of Rhodanthe floribunda. Honours Thesis, The University of Queensland Gatton Campus, Gatton, Queensland Australia.

Seaton, K.A., Plummer J.A., 2004. Observations on environmental control of flowering of Qualup bell (*Pimelea physodes*). Aust. J. Exp. Agric. 44, 821- 826.

Sun, X.Z, Meng, C.S., Wang, X.F., 2008, Effects of high temperature stress on photosynthesis and chlorophyll fluorescence of cut flower chrysanthemum (*Dendranthema grandiflora* 'Jinba'), *Yingyong Shengtai Xuebao*, 19(10), 2149-54.

Taiz, L., Zeiger, E., 2006. *Plant Physiology*, 4th edn, Sinauer Associates, Inc., Massachusetts.

Townsend, C.E. 1982, Influence of seedling age and duration of vernalization on flowering of Cicer milkvetch, *Crop Sci.* 22(6), 1242-5.

Sharman K.V., Sedgley M., 1988. Floral initiation and development in *Helipterum roseum* (Hook.) Benth. and *Helichrysum bracteatum* (Vent.) Andrews (Asteraceae). Aust. J. Bot. 35 (5), 575-587.

Sharman K.V., Sedgley M., Aspinall D., 1989. Effects of photoperiod, temperature and plant age on floral initiation and inflorescence quality in the Australian native Daisies *Helipterum roseum* and *Helichrysum bracteatum* in relation to cut-flower production. J. Hort. Sci. 64 (3), 351-359.

Wahyuni ,S., Krisantini, S. , Johnston, M.E., Plant growth regulators and flowering of *Brunonia* and *Calandrinia* sp. Sci. Hort (In press)

Yeh D.M. and Atherton J.G., 1997. Manipulation of flowering in cineraria. 11. Juvenility. J. Hort. Sci 72, 55-66.

Yeh D.M., Atherton J.G., Craigon J., 1997. Manipulation of flowering in cineraria. III. Cardinal temperatures and thermal times for vernalization. J. Amer, Soc. Hort. Sci. 72, 379-387.

Yeh, D.M., Atherton, J.G., Craigon, J. 1997., Manipulation of flowering in cineraria .IV. Devernalization', J. Hort. Sci. and Biotech. 72(4), 545-51.

Appendix 1: Summary of reported daylength and temperature responses of Australian Asteraceae species.

Species	**Syn**.	**Days to floral initiation (FI) or visible first floral bud (FVFB)**	**Days to anthesis**	**Temperature regime**	**Daylength response**	**Reference**
Bracteantha bracteata	*Helichrysum bracteatum*	66 (FI)	121	28.8 and 16˚C (Max and Min.)	Facultative LDP	Sharman and Sedgley 1988
Bracteantha bracteata	*Helichrysum bracteatum*	38-90 (FVFB) glasshouse 69-154 (field0	63-121 (glasshouse) 93-207(field)	28.9 and 15.2 (glasshouse) 25.8 and 8.7 (field) (Max and Min)	Facultative LDP	Sharman and Sedgley 1989a
Bracteantha bracteata	*Helichrysum bracteatum*	34-73 (FVFB)	59-96	20˚C	Facultative LDP	Sharman and Sedgley 1989b
Rhodanthe chlorocephala subsp. *rosea*	*Helipterum roseum*	39 (FI)	66	28.8 and 16˚C (Max and Min.)	Facultative LDP	Sharman and Sedgley 1988
Rhodanthe chlorocephala subsp. *rosea*	*Helipterum roseum*	43-78 (FVFB) glasshouse 36-91 (field)	66-123 (glasshouse) 61-169 (field)	28.9 and 15.2 (glasshouse) 25.8 and 8.7 (field) (Max and Min)	Facultative LDP	Sharman and Sedgley 1989a
Rhodanthe chlorocephala subsp. *rosea*	*Helipterum roseum*	20-61 (FVFB)	39-86	20˚C Flowering inhibited at constant 25˚C, 12 h photoperiod and 250 Wm^{-2}		Sharman and Sedgley 1989b and Sharman et al. 1990

Brachycome halophila		Growth cabinet 25/25˚C (FVFB), in 54 (8h) SDs and 45 (16h) LDs 24 days at 25/15˚C (glasshouse) 18 days at 23/10˚C (field)	not in SD 56 LDs, at 25/25˚C 42 days at 25/15˚C 51 days at 23/10˚C	25/25˚C 25/15˚C 23/10˚C Daylength in glasshouse and field was 11 h and increasing	Day neutral	Bunker 1995
Brachycome iberidifolia		Growth cabinet 25/25˚C (FVFB), in 88 SDs and 52-56 (12 or 16h) LDs, 53 days at 25/15˚C (glasshouse) 54 days at 23/10˚C (field)	not in SD 70-76 LDs at 25/25˚C 70 days at 25/15˚C 79 days at 23/10˚C	As above	Facultative LDP	Bunker 1995
Chrysocephalum apiculatum	*Helichrysum apiculatum*	Growth cabinet 25/25˚C (FVFB), in 26 in 12 h SDs and 62 16h LDs	Not in SDs (8h) 52 in 12h SDs 73LDs at 25/25˚C	As above	Facultative LDP	Bunker 1995
Lawrencella davenportii	*Helichrysum davenportii*	Growth cabinet 25/25˚C (FVFB), in 48 (8h) SDs and 73 (16h) LDs	74 SDs 89 LDs at 25/25˚C	As above	Facultative SDP	Bunker 1995
Lawrencella rosea	*Helichrysum lindleyi*	Growth cabinet 25/25˚C (FVFB), in 36 (8h)SDs and 47 (16h) LDs 13 days at 25/15˚C (glasshouse) 14 days at 23/10˚C (field)	68 in SD 78 LDs at 25/25˚C 37 days at 25/15˚C 38 days at 23/10˚C	As above	Facultative SDP	Bunker 1995
Rhodanthe floribunda	*Helipterum floribundum*	Growth cabinet 25/25˚C (FVFB), in 73 (8h)SDs 56 (12h) and 31(16h) LDs 62 days at 25/15˚C (glasshouse) 48 days at 23/10˚C (field)	Not in SD (8 or 12h) 57 LDs at 25/25˚C 78 days at 25/15˚C 79 days at 23/10˚C	As above	Facultative LDP	Bunker 1995

Rhodanthe manglesii	*Helipterum manglesii*	Growth cabinet 25/25˚C (FVFB), in 78 (8h)SDs 50 (12h) and 49(16h) LDs 51 days at 25/15˚C (glasshouse) 47 days at 23/10˚C (field)	104 SD (8h) 89LDs at 25/25˚C 77days at 25/15˚C 68 days at 23/10˚C	As above	Facultative LDP	Bunker 1995 ;
Schoenia cassiniana	*Helichrysum cassinianum*			15-20°C for 30 days	Facultative LDP	Mott and McComb 1975
Schoenia cassiniana	*Helichrysum cassinianum*	Growth cabinet 25/25˚C (FVFB), in 80 (8h), SDs 68 (12h) and 70 (16h) LDs 25 days at 25/15˚C (glasshouse) 40 days at 23/10˚C (field)	106 SD (8h) 85LDs at 25/25˚C 50days at 25/15˚C 70 days at 23/10˚C	25/25˚C 25/15˚C 23/10˚C Daylength in glasshouse and field was 11 h and increasing	Facultative LDP	Bunker 1995
Schoenia filifolia subsp. *fififolia*		Growth cabinet 25/25˚C (FVFB), in 73 (8h), SDs 32 (12h) and 44 (16h) LDs 28 days at 25/15˚C (glasshouse) 47 days at 23/10˚C (field)	112 SD (8h) 60 (12h) and 58 (16h) LDs at 25/25˚C 55days at 25/15C 66 days at 23/10˚C	As above	Facultative LDP	Bunker 1995
Helipterum craspedioides	*Myriocephalus morrisonianus*			15-20°C for 30 days	Facultative LDP	Mott and McComb 1975
Ozothamnus diosmifolius	*Helichrysum diosmifolium*		143 days (17/9°C) 108 days (23/15°C, LD)	Under 20/12 and 23/15°C	Absolute LDP and did not flower under SD (10h)	Halevy, Shlomo and Shvartz (2001)

				Under 17/9°C Under 26/18°C	Plants flowered in both SD and LD Plants did not flower under any photoperiod	