The Effect of Photoperiod and Temperature on Flowering of *Pycnosorus Thompsonianus*

Tuan M. Ha[1,3], Santi Krisantini[2] and Margaret E. Johnston[3]

[1] Thai Nguyen University of Agriculture and Forestry, Faculty of Agronomy, Thai Nguyen city, Vietnam 23000.

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

[3] The University of Queensland, School of Agriculture and Food Sciences, Gatton Campus, Gatton, Australia 4343.

ABSTRACT: ***The flowering responses of Pycnosorus thompsonianus to day-lengths and temperatures were investigated to study the floral regulation of this potential ornamental plant in southern Queensland, Australia. Plants of different treatments 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 transferring to long day (SDLD) in environment-controlled greenhouse bays during 2009 - 2010. LDs promoted earlier flowering and plants under LDs flowered regardless of temperature regimes. Cool temperatures and cooling periods were required for efficient flowering of plants under SDs, but were not important for plants under LDs and SDLDs. Forty-two percent of the plants under warm (30/20°C) SD remained vegetative after a 16-week growing period. Cooling for 21 days was sufficient for plant growth and floral development of the species. Daylength was more effective than temperatures for promoting earlier flowering and for increasing flower production.***

Keywords - Daylength, Vernalisation, Cooling, Anthesis, Australian native species, Visible bud stage

1. INTRODUCTION

Pycnosorus thompsonianus (Asteraceae) is an Australian native annual with green to silver grey narrow leaves which has potential as flowering potted plants or bedding plants. It has attractive small bright yellow egg-shaped flower heads with erect peduncles. The species normally flowers in spring and summer. It occurs in semi-arid areas and often flowers on mass on floodplains after winter rainfall (Everett and Doust 1992).

Photoperiods and temperatures have been reported to affect floral initiation and flower development of members of the Asteraceae family. In several Australian species, including *Bracteantha bracteata (*syn. *Helichrysum bracteatum)* (Sharman and Sedgley, 1988) and *Rhodanthe chlorocephala* subsp. *rosea* (syn, *Helipterum roseum)* (Sharman *et al* 1989), flower in response to long days (LDs), *Lawrencella davenportii* and *L. rosea* flower in response to short days (SDs) (Bunker 1995), whereas *Brachycome halophile* was described as day-neutral even though it failed to reach anthesis under 8h SDs at day and night temperatures of 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).

Flowering of *Ozothamnus diosmifolius (*syn. *Helichrysum diosmifolium)* 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). The diverse flowering responses of Australian species to temperature, photoperiod and light intensity have been reported, 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).

The diversity of flowering responses reported means that each species requires investigation of their environmental responses for commercial production. However, no study has been reported on the flowering characteristics and flowering responses of *P. thompsonianus* to different growing environments.

The objective of this research was to determine the influence of photoperiods, temperature regimes and cooling durations on flowering of *P. thompsonianus*. This information is important to develop new floriculture species for commercialisation.

2. MATERIALS AND METHODS

2.1. Plant materials and growing media

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.

Seeds were sterilised with 2 g L^{-1} chlorine sown into 9-cm diameter plastic Petri dishes containing 10g L^{-1} Agar with 50 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 until germination.

Germinated 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) with 2 g L^{-1} Basacote® Mini 3 month [N:P:K = 13:6:16] (Compo do Brazil S.A, Brazil) in greenhouse bays according to scheduled treatments. This date was set as the start of the experiment.

After 2 weeks, seedlings were transplanted into individual 100 mm (0.5L) diameter plastic pots. The media used was 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 1.5 Mg 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 g L^{-1} Dolomite® [Ca:Mg 14:8] (Yates, Australia) and 1.2 g L^{-1} Saturaid® (Debco, Melbourne, Australia).

2.2. Treatments, Data Collection and Analysis

Experiments were conducted in a research greenhouse at the University of Queensland Gatton nursery (27°34'S, 152°20'E). The study consisted of two separate experiments conducted at different times. The first experiment studied the effects of photoperiods (daylengths) and temperatures on growth and flowering of *P. thompsonianus*, conducted during 3 July - 6 November 2009. The second experiment on the effect of cooling durations on its flowering was conducted during 9 February – 9 July 2010, and repeated between 9 March and 8 August 2010.

Four bays in the research greenhouse were used and were set temperature of 20/10 or 30/20°C (day/night), each with long day (LD) or short day (SD), or 6 weeks under SD then transferred to LD (SDLD). There were twelve single replicate plants for each photoperiod and temperature treatment. Variation of actual temperature from the set points was ± 2.0°C. The SD was 11 hours of sunlight from 06:00am to 05:00pm at which time the blackout curtain in each bay was closed. The LD was 16 hours (11-hour sunlight + 5-hour incandescent light). Five-hour night break for the LD treatment was provided with 100W incandescent lamps; <4.5 µmol.m^{-2}sec^{-1} (Sylvania, Indonesia) from 9:00pm to 2:00am. 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 second experiment, plants were exposed to either 21 or 42 days of cooling period under SD, LD, or SDLD prior to transfer to 30/20°C at the same photoperiods with ten plants per treatment.

Plants were observed every day. Scoring was conducted on the number of flowering plants for each treatment, the number of days from the transplanting date to the first visible floral bud (FVFB), days from the first flower initiation to anthesis, and number of branches at visible bud stage. The number of inflorescences per plant was recorded at weeks 8, 12 and 16. A completely randomized design was used within each photoperiod (LD, SD and SDLD). Data obtained were subjected to analysis of variance using the General Linear Model procedure in Minitab® version 15.

3. RESULTS

Experiment 1: The effects of temperatures and photoperiods on growth and flowering of *P. thompsonianus*

The plants under LD flowered earlier than plants in SD (Tables 1 and 2). Plants under LD reached visible bud stage at 27-40 days (Table 2) and all plants under warm (30/20°C) LD had visible flower buds at week 4 (Table 1). Plants under SD reached visible bud stage at 52-75 days (Table 2). Forty-two percent of the plants under warm SD did not flower until experiments were terminated at week 16 (Table 1).

Under LDs, flower buds appeared earlier (Table 1 and 2) and the plants had more inflorescences under warm temperature (30/20° C) than under cool temperature of 20/10°C at week 16 (Table 2). In contrast, plants under SD

flowered earlier and had more inflorescences per plant under low temperatures (Table 1 and 2). All plants under warm SDLDs had visible floral buds four weeks after transferred to LDs (SD was applied up to week 6), whereas only 33% of plants kept under warm SD had visible flower buds at the same time (Table 1).

Photoperiods interacted with temperatures in affecting flowering of *P. thompsonianus* (Table 2). Under LDs plants flowered faster and had more inflorescences under warm LDs than cool LDs, while under SDs, cool temperatures reduced time to flower initiation and more inflorescences were observed (Table 2).Under SDLDs, cool temperatures reduced the time to the first visible bud, but delayed subsequent floral development, so that the number of inflorescences was lower when the experiment was terminated at 16 weeks (Table 2).

Table 1. Percentage of plants with visible flower buds under different day-lengths and temperature regimes

Daylength	Temperatures	Percentage of plants with visible flower buds (week from transplanting)						
		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. Days to first visible bud, to anthesis and number of inflorescences per plant under different day-lengths and temperature regimes [1)]

Daylength (DL)	Temperature (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	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.

Experiment 2: The Effect of Cooling Duration under Different Photoperiods on Flowering of *P. thompsonianus*

Chilling duration did not influence flowering and number of branches and inflorescences in *P. thompsonianus* (Table 3, 4). However, it interacted with photoperiod in affecting time to FVFB and anthesis (Table 3). Extending from 21 to 42 days of cooling under SD, time to flowering was shortened by 10 days. In contrast, the number of days from

transplanting to FVFB and anthesis were extended to nearly 6 and 10 days, respectively, under LD (Table 3).

Daylength had strong influence on reproductive growth and plant habit. LDs reduced time to flowering (Table 3), reduced branch number, but increased number of inflorescences (Table 4) probably because plants under LDs had elongated growth (Figure 1).

Table 3. Effects of cooling duration on floral development of *P. thompsonianus* at different day-lengths

Cooling Period (C)	Daylength (D)	Days to first 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

*Notes: *) Six weeks of SD followed by LD.*

Table 4. The effect of cooling period on number of branches at visible bud stage and number of inflorescences/plant at different day-lengths

	Number of branches at first VB stage	Number of inflorescences/ plant at week	
		8	12
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 (C)	ns	ns	ns
Daylengths (D)	**	**	**
C x D	ns	ns	ns

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.

4. DISCUSSIONS

LDs reduced the time to the FVFB, to anthesis (Table 2, 3 and 4) and increased number of inflorescences per plant as compared to SDs (Table 2 and 4). However, flowering occurred under SDs, suggesting that *Pycnosorus* is a quantitative LD plants. 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* 1989. 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 species, *Pycnosorus thompsonianus,* to daylength.

Daylength interacted with temperatures in affecting time to the FVFB and to anthesis (Table 2 and 3). Plants under warm LDs flowered earlier (Table 1) and produced more inflorescences per plant (Table 2) than those under cool LDs, whereas the effects of temperatures on flowering of plants under SDs were the opposite (Table 1 and 2). Once floral initiation has occurred, warm temperature accelerated floral development. This is consistent with the study results of Mott and McComb 1975 on Australian daisies such as *Helichrysum cassinianum* (syn. *Schoenia cassiniana*) and *Helipterum craspedioides*. Although high temperature at 35°C retards flowering of Asteraceae members as reported by Schwabe (1950, cited in Tanigawa et al. 2009), the temperature range in this study (30/20°C) was not at the aforementioned stress level.

The plants under SDs were slower to reach the FVFB stage than those under LDs (Table 2) and had more branches at the FVFB stage (Table 4). These results indicated that the juvenile phase of plants under SDs was extended. Even though extending the juvenile phase will increase production time, plants with increased foliage growth will have improved plant habit and ornamental value. The SDLD regime provided the best compromise, allowing the plants to reach a certain size under SDs before being transferred to inductive environment (LDs) to initiate flowering.

Cooling is important for flowering of plants under SDs and flowering was inhibited under warm SDs (Table 2). Twenty-one days of cooling would be sufficient for plant growth and flowering as further cooling of 42 days did not induce significant increase of growth and development parameters (Table 3 and 4). 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). Cockshull et al. (1994) also asserted that temperature regimes at the onset of SDs were 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. Therefore, subjecting plants to a lower temperature pulse under SDs might be a useful method for scheduling flowering for the potted plant trade.

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), below 15°C in *Heliotrope* (Park and Pearson, 2000) and 5-20°C for Australian native species (King et al. 1992). The temperature range used in this experiment (20/10°C) was effective to induce flowering in *Pycnosorus thompsonianus.*

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

Daylength is more important than temperatures for flowering of *P. thompsonianus*. Plants under LD and SDLD consistently had more inflorescences compared to plants under SDs at the same time (Table 2, 3 and 4). 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 1).

The duration of flower development was affected by the interaction between daylength and temperatures (Table 2). Generally, flower development rate was more rapid under warm temperature (30/20°C) compared to cool temperatures (20/10°C), but under SD and warm temperature, flowering was delayed. Commercially, under SDs, it is recommended that plants should be grown under cool temperatures (20/10˚C) for several weeks to promote vegetative growth before transfer to LDs to promote flowering.

5. CONCLUSION

This study showed that *Pycnosorus thompsonianus* is a quantitative LD plants; flowering occurs in both LDs and SDs, but the process is faster under LDs. Photoperiod is more important than temperatures for flowering of *P. thompsonianus*. Plants under LDs flowered well regardless of temperature regimes, but cool temperatures was required for floral development of plants under SDs; 42% of plants under constant 30/20°C SD failed to initiate floral buds. There

were more branches and improved plant habit was under SDs and cooling for 21 days was sufficient for enhanced flowering under SD for this species.

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7. REFERENCES

[1] Bunker K.V., "Year-round production of Australian daisies (Asteraceae) as flowering pot plants". Sci. Hort. 61, 101-113, 1995.
[2] Cave R.L., Johnston M.E., "Vernalization promotes flowering of a heat tolerant Calandrinia while long days replace vernalization for early flowering of Brunonia". Sci. Hort 123, 379-384, 2010.
[3] Day S., Loveys B.R., Aspinall, D., "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, 1994.
[4] Dawson, I.A., King, R.W., "Effect of environment and applied chemicals on the flowering and form of Geraldton Wax (Chamelaucium uncinatum Schauer)". Sci Hort 54, 233-246, 1993.
[5] Everett J., Doust, A.N.L., "New species and a new combination in Pycnosorus (Asteraceae: Gnaphalieae)". Telopia 5, 39-43, 1992.
[6] Friis, K., Christensen, O.V., "Flowering of Centradenia inaequilateralis `Cascade' as influenced by temperature and photoperiod". Sci. Hort. 41, 125-130, 1989.
[7] Halevy, A.H., Shlomo, E., Shvartz, M., "Environmental factors affecting flowering of rice flower (Ozothamnus diosmifolius Vent.)". Sci. Hort 87, 303-309, 2001.
[8] King, R.W., Dawson, I.A., Speer, S.S., "Control of growth and flowering in two Western Australian species of Pimelea". Aust. J. Bot. 40, 377-388, 1992.
[9] King, R.W., Pate, J.S., Johnston, J., "Ecotypic differences in the flowering of Pimelea ferruginea (Thymelaceae) in response to cool temperatures". Aust. J. Bot 44, 47 – 55, 1996.
[10] King, R.W., "Dual Control of Flower Initiation and Development by Temperature and Photoperiod in Hardenbergia violacea". Aust. J. Bot., 46, 65-74, 1998.
[11] Motum, G.J., Goodwin, P.B., "The control of flowering in kangaroo paw (Anigozanthos spp.)". Sci Hort 32,, 123-133, 1987.
[12] Mott J.J., McComb A.J ., "Role of photoperiod and temperature in controlling phenology of three annual species from an arid region of Western Australia". J. Ecol. 63, 633-641, 1975.
[13] Moncur, M.W., "Effect of low temperature on flora induction of Eucalyptus lansdowneana F. Muell & J. Brown subsp. Lansdowneana". Aust. J. Bot. 40, 157 – 167, 1992.
[14] Pearson S., Parker A., Hadley P., Kitchener H.M., "The effect of photoperiod and temperature on reproductive development of Cape Daisy (Osteospermum jucundum cv 'Pink Whirls')". Sci. Hort 62, 225-235, 1995.
[15] Putterill, J., Laurie, R., and Macknight, R., "It is time to flower: the genetic control of flowering time". BioEssays 26: 363-373, 2004.
[16] Sedgley M., "Some effects of temperature and light on floral initiation and development of Acacia pycnantha". Aust J. Plant Physiol. 12, 109-118, 1985.
[17] Seaton, K.A., Plummer J.A., "Observations on environmental control of flowering of Qualup bell (Pimelea physodes)". Aust. J. Exp. Agric. 44, 821- 826, 2004.
[18] Sharman K.V., "Sedgley M., Floral initiation and development in Helipterum roseum (Hook.) Benth. and Helichrysum bracteatum (Vent.) Andrews (Asteraceae)". Aust. J. Bot. 35, 575-587, 1988.
[19] Sharman K.V., Sedgley M., Aspinall D., "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,, 351-359, 1989.
[20] Tanigawa T., Kunitake T., Matsuno T., Yamada A., "Effects of cutting time and low-temperature treatment of rooted cuttings on stem elongation and leaf number in cultivars of Chrysanthemum morifolium Ramat." Journal of the Japanese Society for Horticultural Science 78.2: 218-223, 2009.
[21] Wahyuni, S., Krisantini, S., Johnston, M.E., "Plant Growth Regulators and Flowering of Brunonia and Calandrinia sp.", Sci. Hort. 128, 141-145, 2011.
[22] Yeh D.M., Atherton J.G., Craigon J., "Manipulation of flowering in cineraria. III. Cardinal temperatures and thermal times for vernalization". J. Amer, Soc. Hort. Sci. 72, 379-387, 1997.
[23] Yoo, K.C., "Studies on the physiology of bolting and Flowering in Raphanus sativus L." J. Korean Soc. Hort. Sci.18, 157-161, 1977.