The Effect of Plant Maturity and Cooling Duration on Flowering and Plant Habits of *Pycnosorus thompsonianus* under Short Days

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ABSTRACT— ***As a potential Australian native flower species, Pycnosorus thompsonianus was selected to study the effects of chilling duration and ages at chilling on its plant growth habit and subsequent flowering in southern Queensland during September 2009 to March 2010. Seedlings of four age groups (1, 7, 14, 28 days old) were exposed to different cooling periods (0, 3, 7, 14 and 21 days) at 20/10°C under short days (11h). The species was found to have a facultative requirement for flowering in response to low temperature. Generally, the growth and flowering were enhanced in accordance with the increase of seedling ages and cooling duration under short-day condition. Further studies on the effects of chilling duration on flowering of P. thompsonianus are necessary to develop a production guideline for commercial production.***

Keywords – Short days, Cooling, Anthesis, Growth habit, Australian native species.

1. INTRODUCTION

The commercial native floriculture in Australia has commenced since the 1950's (Wild *et al.*, 2007). Since then there has been a growing interest in commercialization of wildflowers, including native daisies (Asteraceae), for local and export markets (Bunker, 1995; Sharman & Sedgley, 1988). There is, however, only limited information on bush – harvested cut flowers and foliage production in Queensland (Wild *et al.*, 2007). In addition, the industry has not addressed the strong market demand in terms of novel flowers (Joyce, 2005).

According to Parlevliet (2006), one of the strategies for new crop development in Queensland is selecting and developing native plants which are not presently utilized. Of those, there are only a few of the 1000 species of the family Asteraceae have been researched and developed for commercial purpose. Members of the Asteraceae have considerable potential for use as bedding and flowering pot-plants (Bunker, 1994, 1995; Plummer & Bell, 1995).

Chilling duration has been reported to enhance floral development (Emsweller & Borthwick, 1937; Gleichsner & Appleby, 1996; Horva´th *et al.*, 2003; King *et al.*, 1992; Michaels & Amasino, 2000; Pearson *et al.*, 1995; Samach & Coupland, 2000) and growth traits (Gleichsner & Appleby, 1996; Hsiao & Ku, 2004; Pearson *et al.*, 1995; Tanigawa *et al.*, 2009; Vince & Mason, 1954) of various plant species. Many Australian species have also been reported to flower in response to chilling (King *et al.*, 1992), in which seedling age prior to chilling influenced floral development (Cave *et al.*, 2011; Cave & Johnston, 2010; Ha & Johnston, 2013).

Pycnosorus thompsonianus Everett & Doust (Asteraceae) is one of the potential native species which is currently being developed as new flower species for local and export markets (Johnston & Joyce, 2007). 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 & Doust, 1992). So far, little information on *Pycnosorus thompsonianus* has been published (Everett & Doust, 1992; UQ, 2008). In our previous publication, the influences of day-lengths and chilling duration on flowering of *P. thompsonianus* were investigated (Ha *et al.*, 2013). To fully understand the flowering responses and growth habits of the species, this study was conducted to determine the effects of chilling periods and seedling ages at chilling on its plant habits and subsequent flowering under SDs.

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) (Johnston, 2009) on 14th September 2003. Seeds were cleaned and stored in the cold room of Queensland Seed Technology Laboratory at 5°C until required.

To avoid contamination problems during the germination process reported by previous studies on native floricultural

species (Johnston *et al.*, 2004; Mullins *et al.*, 2002), seeds were sterilized 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. Seeds were planted sequentially to provide seedlings of the appropriate ages for the experiment.

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.

Seedlings were held in a short day (SD) bay at 30/20°C before being exposed to different cooling periods. After 2 weeks, seedlings were transplanted individually to 100mm (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), 1 g L^{-1} 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), 1.3g L^{-1} Osmoform® [N:P:K 18:2.2:11 + 1.2Mg] (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

Two bays in the research greenhouse at University of Queensland Gatton nursery were used and were set at a temperature of 20/10 and $30/20^0$C (day/night) under short days of 11 hours of sunlight from 6:00am – 5:00pm; at this time the blackout curtain in each bay was 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}. The experiment started from 28 September 2009 and terminated on 1 March 2010.

Four age groups of seedlings (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 30/20°C with 7 single replicate plants allocated for each treatment. The following parameters were recorded: number of days to first visible floral bud (FVFB) and anthesis; number of branches at FVFB, number of inflorescences per plant at week 6, 12 and 23; percentage of flowering plants; and the dried weight of shoot and inflorescences at week 23. A completely randomized design was used. Data obtained were subjected to analysis of variance using the General Linear Model procedure in Minitab® (Release 15, ; Minitab Inc., PA, USA) statistical package with least significant differences (LSD) calculated at 5% level of significance.

3. RESULTS

3.1. Effects of chilling duration and ages on floral development of *Pycnosorus thompsonianus*

There were a number of plants which did not flower in all chilling and age treatments. Generally, number of non-flowering plants decreased in accordance with the increases in chilling duration or the age of plants prior to chilling (Table 3.1).

Table 3.1. Number and percentage of non-flowering plants of *P. thompsonianus*

Treatment	Total plants	Percentage of non-flowering plants (%)
Chilling duration		
0 day	28	71.4
3 days	25	64.0
7 days	27	70.4
14 days	28	32.1
21 days	28	14.3
Age groups		
1 day old	31	67.7
1 week old	35	62.9
2 weeks old	35	42.9
4 weeks old	35	28.6

Since there were a large number of plants in all treatments that remained vegetative and the time to first VB and anthesis varied largely among plants within a treatment, there was no significant difference in time to first VB and anthesis among plants of different chilling and age levels (Table 3.2).

No plant had inflorescence buds at week 6 and there were very few inflorescences until week 12, when plants chilled for 21 days had significantly more inflorescences/plant (2.43) compared to other chilling treatments (0.3 - 1.2 inflorescences/plant) ($P < 0.001$) (Table 3.2).

Table 3.2. Effects of ages and chilling duration on floral development of *P. thompsonianus*

Treatment	Days to first VB	Days to anthesis	Inflorescences per plant at week 12	Inflorescences per plant at week 23	Inflorescence dried weight per plant (gram)
Chilling duration					
0 day	90.74	94.29	0.29 (a)	1.46 (a)	0.056 (a)
3 days	73.32	83.64	0.50 (a)	2.32 (a)	0.082 (ab)
7 days	87.73	97.73	0.39 (a)	1.36 (a)	0.033 (a)
14 days	85.87	97.60	1.15 (a)	4.43 (ab)	0.114 (b)
21 days	73.23	87.53	2.43 (b)	7.36 (b)	0.158 (b)
P-value	n.s.	n.s.	***	**	*
Age					
1 day	79.65	91.51	0.66	1.49 (a)	0.031 (a)
1 week	82.02	93.63	0.66	1.71 (a)	0.030 (a)
2 weeks	82.12	88.32	1.43	3.97 (ab)	0.102 (a)
4 weeks	84.91	95.18	1.06	6.37 (b)	0.191 (b)
P-value	n.s.	n.s.	n.s.	*	***
Chilling*Age	n.s.	n.s.	n.s.	n.s.	n.s.

*Experiment was terminated after 161 days from 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.*

At 23 weeks, plants that were chilled for 21 days had 7.36 inflorescences per plant, significantly higher ($P < 0.01$) than those of control, 3 and 7 day chilling treatments with 1.5, 2.3 and 1.4 inflorescences/plant, respectively, but similar to plants that was chilled for 14 days (4.4 inflorescences/plant). As expected, inflorescence dry weights of 14 and 21 days chilled plants were 0.11 and 0.16g, respectively, significantly heavier ($P < 0.05$) than those of control (0.06g) and 7 day chilled plant (0.03g), while similar to those of plant which were chilled for 3 days (0.08g)) (Table 3.2).

There was no significant difference for inflorescences/plant between plants of all age levels at week 12, ranging from 0.66 to 1.43. However by 23 weeks, plants that received chilling for 21 days had 6.4 inflorescences/plant significantly higher ($P < 0.05$) than those of 1 day and 1 week old seedlings with 1.5 and 1.7 inflorescences/plant, respectively, but not significantly higher to plants chilled at 2 week old with 4.0 inflorescences/plant (Table 3.2).

In addition, plants chilled at 4 week old had 0.19g/plant inflorescence dry weight, significantly higher ($P < 0.001$) than those chilled at 1 day, or at 1 and 2 week old, which were 0.03g; 0.03g and 0.10g, respectively (Table 3.2).

3.2. Effects of chilling duration and ages on plant growth habit of *P. thompsonianus*

Chilling for 3 days delayed time to branching by 4 - 5 days compared to the control and 14 and 21 day chilling with 22, 22 and 21 days, respectively ($P < 0.001$). However chilling for 3 days was similar to chilling for 7 day (24 days). In addition, plants that were chilled as 1 day old seedlings did not branch for 27 days, which was 5 - 6 days later than other age groups ($P < 0.001$) (Table 3.3).

There was a significant interaction between chilling duration and age regarding time to branching where plants chilled for 3 days showed a decreasing time to branching when they were older at chilling, whereas those chilled for 14

and 21 days were fairly stable with time to branching unaffected by chilling (P < 0.001).

Table 3.3. Influences of chilling duration and ages on plant growth habit of *P. thompsonianus*

Treatment	Days to branching	Plant height at week 6 (cm)	Plant width at week 6 (cm)	Plant height at week 12 (cm)	Plant width at week 12 (cm)	Dried weight of shoots (gram)
Chilling duration						
0 day	21.75 (a)	11.35	13.61	17.78 (a)	28.83 (a)	4.858 (a)
3 days	25.65 (b)	10.96	13.62	18.00 (a)	26.48 (a)	3.898 (b)
7 days	24.19 (ab)	10.75	13.21	18.53 (a)	30.30 (b)	4.432 (ab)
14 days	22.00 (a)	11.86	15.45	22.40 (ab)	30.87 (b)	5.506 (a)
21 days	20.57 (a)	11.91	15.08	25.25 (b)	32.21 (b)	4.836 (a)
P-value	***	n.s.	n.s.	***	*	***
Age						
1 day	27.07 (a)	11.73	14.00	21.15	31.11 (a)	3.760 (a)
1 week	21.23 (b)	10.87	14.20	20.84	30.59 (a)	3.223 (a)
2 weeks	22.40 (b)	11.85	14.78	21.42	30.70 (a)	5.680 (b)
4 weeks	20.63 (b)	11.00	13.80	18.15	26.55 (b)	6.162 (b)
P-value	***	n.s.	n.s.	n.s.	***	***
Chilling*Age	***	*	n.s.	*	**	***

*Experiment was terminated after 161 days from planting. Values followed by different letters within a column are significantly different according to Tukey test and t-test. n.s.: not significant, *P<0.05, **P<0.01, ***P<0.001.*

Plant forms at week 6 after planting were not influenced by chilling duration and seedling ages prior to chilling. At week 12, 21 days of chilling increased plant height (25.3cm) compared to 0, 3 and 7 day chilling treatments at 17-18cm, while plants that were chilled for 14 days were intermediate (22.4cm) (P < 0.001). Moreover, plant widths were similar among plants received 7, 14 and 21 day chilling with 30 - 32cm, which was significantly wider (P < 0.05) than those of control (28.8cm) and 3 day chilling (26.5cm) (Table 3.3).

Plant heights were similar among plant age groups at week 12, ranging from 18 to 21cm. However, plant width was significantly smaller (P < 0.001) in 4 week old plants. There was an interaction between chilling duration and plant age prior to chilling treatment; the longer duration of chilling and younger plants produced wider canopies (P < 0.01) (Table 4.6). Plants that were chilled for 3 days showed an increasing plant height with age at chilling, while those chilled as 21 days showed a decline in plant height with age (P < 0.05).

Plants that were chilled for 3 days had a significantly lower (P < 0.001) shoot dry weight (3.9g) lower compared to the control, and plants chilled at 14 and 21 days but similar to plants chilled for 7 days (4.4g). Plants that were 2 and 4 weeks old prior to chilling had a similar shoot weight, significantly higher than plants which were chilled as 1 day and 1 week old seedlings (Table 3.3).

Among the chilling periods from 3 to 21 days, there was an interaction between age and chilling duration; longer duration of chilling and older plants prior to chilling gave heavier shoot weight (P < 0.001) (Table 3.3).

Plants that received chilling as 4 week old seedlings had a higher (P < 0.001) ratio of inflorescence weight to shoot weight (3.0%) compared to plants chilled as 2 and 1 week and 1 day old seedlings with 1.8%, 0.9% and 0.8%, respectively. Total top-plant weights of 4 and 2 week old plants of 6.353 and 5.782g, respectively were significantly heavier (P < 0.001) than those of 1 day and 1 week old seedlings with top weights of 3.79 and 3.25g, respectively (Figure 3.1).

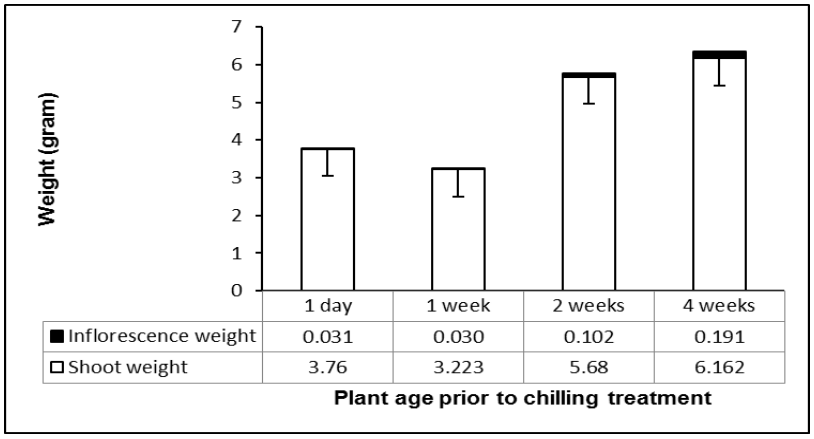

Figure 3.1. Effects of plant ages prior to chilling treatment on shoot and inflorescence weight of *P. thompsonianus. Bars indicate S.E. for shoot weight. n = 35.*

4. DISCUSSIONS

4.1. Effects of chilling duration on flowering

Plants of *P. thompsonianus* flowered without chilling and hence the species have a facultative requirement for low temperature (Finnegan *et al.*, 1998; McDonald & Kwong, 2005; Michaels & Amasino, 2000).

There were many plants that did not initiate inflorescences in all treatments. Interestingly, the percentages of flowering plants reduced gradually with the increase of chilling duration (Table 3.1). Additionally, the final inflorescence number for plants chilled for 21 days was higher than that of control plants and plants received 3 and 7 day chilling. These results imply that although longer durations of chilling generally gave higher number of inflorescences, 21 days of chilling might not be sufficient for this species since 14.2% plants still remained vegetative at 23 weeks when the experiment was terminated (Table 3.1). Further studies with longer duration of chilling are therefore necessary.

4.2. Effects of plant maturity prior to chilling on flowering

P. thompsonianus was competent to perceive chilling as one day old seedlings and they did flower. This suggests a short juvenile phase of these species. Cave & Johnston (2010) stated that the short juvenility stage indicates an ephemeral trait, and 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).

The oldest group (4 weeks old prior to chilling) had the highest inflorescences number and weight at 23 weeks. These are consistent with the study results of Markowski & Ryka (1981) and Townsend (1982) in which the older plants prior to cold induction showed higher floral production. According to Cave & Johnston (2010), the increased floral production in older plant group might be due to the longer periods for branching and development.

4.3. Effects of chilling duration on plant growth habit

Seedlings initiated branches in 20 - 25 days after transplanting under both 20/10 and 30/20°C (Table 3.3). This might reflect the ephemeral nature of this species (Houle, 2002). Neither continuous 30/20˚C nor 21 days of chilling influence branching, but plants chilled for 3 days showed a delayed branch initiation of several days compared to other treatments. This might suggest an immediate effect of an abrupt change in temperatures as reported by Sun et al. (2008), resulted in a delayed growth rate.

Unlike other members of the Asteracae, *P. thompsonianus* initiates branches at base, elongating to 35cm high (Harden, 1992). In the current experiment, plants of *P. thompsonianus* elongated with flowering, while non-flowering plants remained more compact. The higher number of inflorescences in plants that were chilled for 21 days (Table 3.2) meant a higher numbers of flowering stems leading to taller and wider plants. Vince & Mason (1954) state that chrysanthemum requires a period of cold treatment for normal stem extension and rapid floral development. Plants will fail to elongate under constant hot temperature. In this study, plants under constant 30/20°C were smaller than those under 21 day chilling because they were less floral.

According to Hsiao & Ku (2004), a prolonged period of vernalization led to reduced growth parameters of garlic. In

this experiment, however, the duration of chilling, might not have been sufficient for floral development of *P. thompsonianus*. Thus, there was no difference in shoot weight except for 3 day chilled plants with slightly lower weight than other groups. Chen *et al.* (1982) found that heat adaptability of tomato increases with age, whereas the seedlings at cotyledon stage are most sensitive to temperature treatment. In this experiment, 14.3% of 1-day old plants died after being moved from 20/10 to 30/20°C whereas 10.71% and 3.75% of seedlings chilled for 3 and 7 days also died, suggesting an effect of abrupt temperature change on young seedlings.

4.4. Effects of plant maturity on plant growth habit

There have been many studies on the influence of plant ages and vernalization on flowering of ornamental species, which show that older plants are usually more perceptive to chilling, which leads to more efficient floral development (Cave & Johnston, 2010; Goodwin *et al.*, 1995; Wellensiek, 1958; Wellensiek & Hakkaart, 1955). However, little research has been carried out regarding effects of plant maturity prior to chilling on plant growth habit. Tanigawa et al. (2009) found an increased stem length and leaf number in five weeks old plants of chrysanthemum cultivars, while two cultivars out of ten did not show this response to vernalization (2.5°C for 6 weeks). These authors did not present data on the final inflorescence and shoot weights. In this study, plants chilled at 4 weeks old showed the widest canopy in *P. thompsonianus* (Table 3.3). Cave & Johnston (2010) argued that the more effective floral development and vegetative growth obtained in older plants could be explained by a longer period of branching and development.

4.4. Interaction between chilling duration and plant ages

There was a significant interaction between chilling duration and ages for *P. thompsonianus* where longer period of chilling and younger seedling prior to chilling induced wider canopies. Moreover, under 21 day chilling treatment, younger plants gave taller heights. However, the final shoot dry weights of older plants were heavier than those of young plants (Table 3.3). These results suggest that plant growth habit can be modified by level of chilling and chilling time. Nonetheless, the results of floral development showed that longer chilling period coupled chilling treatment at older age of seedlings induced better flowering of *P. thompsonianus*.

5. CONCLUSION

P. thompsonianus has a facultative requirement to low temperature. With regard to chilling duration, though 3-week chilling treatment resulted in higher inflorescence number, weight and stem length than those of non-chilled plants, the chilling duration might not have been sufficient for complete floral development under SDs since there was a high proportion of plants (14.3%) remained vegetative.

Generally, the oldest plant group (4 weeks old) prior to chilling treatment reached higher values of growth and development parameters.

Further research with longer duration of chilling are recommended to further investigate the floral development of *P. thompsonianus*.

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