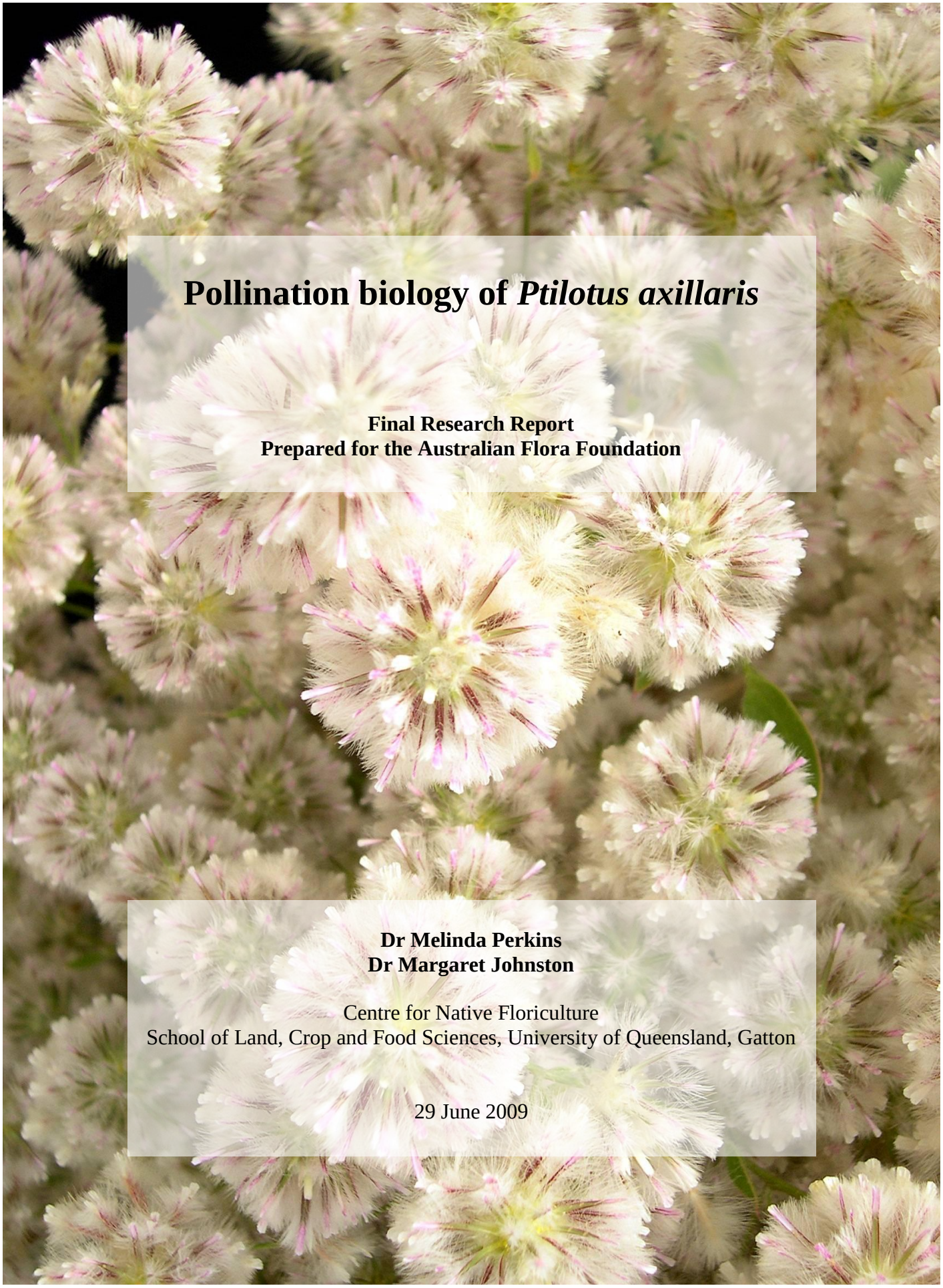

Pollination biology of *Ptilotus axillaris*

Final Research Report
Prepared for the Australian Flora Foundation

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Abstract

Ptilotus axillaris (Amaranthaceae) is a little known Australian species purported to have ornamental potential, however previous breeding attempts have had limited success. Aspects of pollination biology and flowering physiology were investigated to ensure future breeding work employs appropriate strategies for efficient seed production. Seven stages of floral development were characterised. Stages that encompassed anther development, maturation and pollen dehiscence were of longer duration in male-fertile genotypes, however the time between anthesis and flower closure was shorter (compared with male-sterile genotypes). On the basis of seed set data, no significant difference in stigma receptivity was detected during the 14 d period between anthesis and flower closure. *In vitro* pollen germination showed viable pollen was produced 0 to 2 d following anthesis, suggesting that *P. axillaris* is homogamous. Partial to complete self incompatibility was observed, with ratios of seed set from self-pollinations to that of cross-pollinations (index of self incompatibility) being 0.27 or lower. Cultivation under low temperatures (25/10°C day/night) was shown to promote floral initiation. Flower initiation was delayed by high temperatures (35/20°C day/night) but once visible buds were present, flower development was more rapid at high temperatures. However, if plants are maintained continuously under 35/20°C flowering will be suppressed. Flowering is greatly enhanced at 25/10°C, as is plant form. Plants at anthesis had significantly fewer leaves when maintained under long days (16 h photoperiod) compared with short days (11 h photoperiod), suggesting that *P. axillaris* may be a facultative long day plant.

Keywords

Ptilotus axillaris, Mat mulla mulla, Amaranthaceae, self-incompatibility, pollen viability, stigma receptivity, floral development, flowering physiology, temperature

1 Introduction

The development of new plant varieties through targeted breeding programs requires a fundamental understanding of the reproductive biology and flowering physiology for the species of interest. For many ornamental plant species, extensive knowledge already exists. However, for many of the lesser-known Australian native plant species currently being developed for their ornamental potential as cut flowers/foliage, potted colour or landscape plants, very little published information is available.

Ptilotus axillaris (Amaranthaceae), commonly known as Mat Mulla Mulla, is a prostrate herbaceous annual endemic to the central and western regions of Western Australia (Western Australian Herbarium 1998-). It has received recent interest for its potential as a hanging basket product, as it forms an attractive trailing mass of large, soft-textured white/pink inflorescences (Figure 1) over a long flowering period (April to November).

Figure 1. *P. axillaris* potted specimen (a), showing trailing plant form and floriferous nature; and variation in inflorescence colour from pale pink (b) to dark pink (c).

Under cultivation, seed set resulting from open-pollination is observed to be very low. This may be an indication of herkogamy, dichogamy, self-incompatibility (SI) and/or

absence of pollen vector (Dafni 1992). Also, high rates of male-sterility have been observed for this species, with approximately half of all genotypes under cultivation in our nursery exhibiting reduced anthers devoid of pollen. Controlled-pollination provides greater success, but is a laborious exercise given the ovary is unilocular (producing a single seed).

The primary aim of this study was to provide a basic knowledge of the pollination biology of *P. axillaris* to ensure future breeding work employs appropriate strategies for efficient seed production. Firstly, the stages of floral development were characterised, including measurement of their timing and duration. This was followed by an i*n vitro* pollen germination study to determine when pollen viability is greatest. Seed set arising from controlled pollinations was used to profile stigma receptivity over time, and also to determine the level of self-incompatibility.

Manipulation of flowering through environmental control was also investigated, as it is of great benefit to a breeding program to have year-round flower production. In this instance, the effects of temperature and daylength on time to flowering, number of visible buds and number of inflorescences were examined for both cutting and seed propagated plants.

2 Research methodology

The following experiments were conducted between July 2007 and November 2008 at the Plant Nursery Unit of the University of Queensland, Gatton Campus (latitude 27° 33' S, longitude 152° 20' E). Unless otherwise stipulated, plants were cultivated under greenhouse conditions (mean light intensity 548 $\mu mol.m^{-2}s^{-1}$ photosynthetically active radiation) and at the time of the experiment were maintained in 180 mm pots of a standard potting medium composed of composted pine bark medium supplemented with: 2 gL^{-1} Osmocote Plus 8-9 month, N15-P4-K7.5 Mg1.8; 1 gL^{-1} Osmocote Exact 3-4 month, N16-P5-K9.2 Mg1.8; 2 gL^{-1} Nutricote 7 month, N16-P4.4-K8.3 (Yates, Padstow, Australia); 1.3 g L^{-1} Osmoform 4 month, N18-P2.2-K11 Mg1.2; 1.3 gL^{-1} Osmocote

Coated Iron, Fe28-S17; 1.2 gL^{-1} Dolomite, Ca14-Mg8 (Flinders Trading Pty. Ltd., Strathpine, Qld, Australia); and 1.2 gL^{-1} SaturAid (Debco, Tyabb, Vic, Australia). All nutrient additives were manufactured by Scotts Australia, Baulkham Hills, NSW, Australia unless stated otherwise. Plants received drip irrigation as needed.

2.1 Stages of floral development

The floral development of *P. axillaris* was empirically grouped into seven stages according to morphology: (1) tepal tips visible but not pigmented; (2) tepal tips pigmented and flower length >10 mm; (3) trichomes extending >30° from tepal surface; (4) anthesis, pollen dehiscence, stamens parallel to pistil; (5) stamens reposed against tepals, anthers brown with little or no visible pollen, stigmatic papillae extended; (6) flower closed; and (7) tepals brown, flower readily detached from inflorescence (Figure 2).

Figure 2. Stages of floral development in *Ptilotus axillaris*: (1) tepal tips visible but not pigmented; (2) Tepal tips pigmented and flower length >10 mm; (3) trichomes extending >30° from tepal surface; (4) anthesis, pollen dehiscence, stamens parallel to pistil; (5) stamens reposed against tepals, anthers brown with little or no visible pollen, stigmatic papillae extended; (6) flower closed; (7) tepals brown, flower readily detached from inflorescence.

Timing of floral development stages 2 to 7 was determined through daily observation of 30 flowers (ten flowers from each of three inflorescences) from each of six genotypes.

Three genotypes were "male fertile" and three were "male sterile", the latter exhibiting reduced anthers with no visible pollen. Data for each stage were expressed as time from anthesis.

2.2 Pollen viability

Flowers from three genotypes were harvested at 0, 1, 2 and 3 d after anthesis (n=5) and immediately placed under high humidity in a 90 mm Petri dish lined with moist filter paper to pre-hydrate the pollen grains. After 2 h, anthers were excised from the flowers and placed in 10 μL droplets of culture medium (1 anther per droplet) on the inner surface of a 90 mm Petri dish lid. The droplet was gently stirred with a flame-sterilised dissecting needle to disperse the pollen and the anther removed. The culture medium was an aqueous solution of 40% (w/v) sucrose, containing 0.1% (w/v) boric acid and 0.3% (w/v) calcium nitrate. Lids were inverted over Petri dish bases containing ~2 mL culture medium. The resultant cultures were a modification of the hanging drop technique described by Shivanna and Rangaswamy (1992). They were sealed in a plastic bag and incubated for 24 h at 35°C. Cultures were again stirred to evenly disperse the pollen grains and observed under a stereo light microscope (x40) for pollen tube growth. Counts of total number of pollen grains and number of germinated pollen grains, per field of view (FOV), were obtained for five FOV per replicate. Pollen germination rate (%) was calculated for each treatment.

2.3 Stigma receptivity

Three male-sterile gentoypes were selected as the maternal parents for this trial. One other genotype, which had previously exhibited the highest pollen viability, was selected as the pollen donor. All plants were maintained in 140 mm pots and drip irrigated as needed throughout the duration of the trial. Hand-pollination of 10 to 20 flowers was conducted at 0, 2, 4, 6, 8, 10, 12 and 14 d after anthesis using pollen harvested from flowers at stage 4 of their floral development. All pollinations were conducted within a 2 d period. Identification of the hand-pollinated flowers was achieved by clipping one or more tepals and tagging the inflorescence. Uncontrolled pollination was prevented by securing custom-made bags (70 x 60 mm) of micro-perforated polypropylene over each inflorescence, prior to anthesis of the flowers. The bags were kept on the inflorescence

until the flowers reached stage 7 of their floral development (approximately 10 weeks after pollination), at which time they were harvested and examined for seed. The rate of seed set was recorded for each treatment.

2.4 Self-incompatibility

Controlled pollinations were conducted on stage-5 flowers between 9 am and 11 am, within a 2 d period. Twenty self-pollinations and 20 cross-pollinations were conducted on each of four male-fertile genotypes using freshly-harvested pollen from stage-4 flowers. The pollen donor for the cross-pollinations was any one of the other three genotypes. A further 20 flowers (stage-5) from each genotype were marked as unpollinated controls. All flowers used in the trial were identified by clipping one tepal and tagging the inflorescence. Flowers were harvested upon reaching stage 7 and examined for seed. The rate of seed set was recorded for each treatment. SI was assessed by calculating the index of self-incompatibility (ISI), a term developed by Zapata and Arroyo (Zapata and Arroyo 1978) to represent the ratio of fruit set from self-pollination to the fruit set from controlled-pollination. Mean daily minimum and maximum temperature during the trial were and °C, respectively.

2.5 Environmental effects on flowering of cutting propagated plants

P.axillaris cuttings with two internodes were dipped in 2 gL^{-1} IBA, planted in cell trays with propagation medium consisting of equal parts of peat, perlite and vermiculite plus 2 gL^{-1} Osmocote Exact Mini 3-4 month N16-P3.5-K9.1 Mg1.2 (Scotts Australia, Baulkham Hills, NSW, Australia) and placed under intermittent mist with bottom heating at 27 ± 2.9°C air temperature; ambient day length (12.1 ± 0.8 h) and light (146 ± 3 µmol m^{-2} s^{-1}). Three weeks later, rooted cuttings were transplanted into 50 mm tubes using the propagation medium and placed in a greenhouse. Five weeks later, the plants were transplanted into 140 mm pots of standard potting medium.

Plants were graded within replicates and randomly allocated to one of four temperature controlled greenhouses with set points at 25/10 or 35/20°C (day/night) operating on a 12 h cycle (6:00-18:00 h daily) under an 11 (SD) or 16 (LD) h photoperiod. Variation in temperature from the set point was ± 2.0°C. The SD treatment (6:00-17:00 h daily) was

provided by ambient light (380 ± 44 µmol m^{-2} s^{-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} s^{-1}) supplied by 100 W incandescent lamps (Osram Sylvania, Danvers, MA, USA). Plants were hand watered as required.

Initial plant height, width and number of shoots were recorded on 13 April 2007 when plants were placed into the research greenhouse bays. Number of days to the first visible bud and to anthesis and total number of visible buds was recorded weekly or fortnightly until 8 June 2007 (56 days after transfer to the greenhouse). Plants were pruned on 22 June to the rim of the pot removing all inflorescences and fertilised with 2 gL^{-1} Osmocote Exact 3-4 month N16-P5-K9.2 Mg1.8. Data was collected on the number of visible buds and number of inflorescences at anthesis every 2 weeks for 10 weeks. A completely randomized design was used within each greenhouse environment. Data were collected on 8 replicate plants. Data were analysed using the one-way analysis procedure in Minitab 15.

2.6 Environmental effects on flowering of seed propagated plants

P. axillaris seeds were collected from plants grown at The University of Queensland (UQ) Gatton nursery in 2007. The seeds were sown on 18 April 2008. Seeds were surface-sterilized in a solution of 2 gL^{-1} chlorine plus a drop of wetting agent (Agral; ICI crop care, Australia) for 10 minutes and triple rinsed with deionised water and seeds were treated with GA_3 at 100 mgL^{-1} for 24 h and prior to sowing. Seeds were sown into tubes (0.125 L) containing propagation medium (described in Section 2.5) and placed under intermittent mist with under bench heating at 27 ± 2.9 °C air temperature; ambient daylength (12.1 ± 0.8 h) and light (146 ± 3 µmol m^{-2} s^{-1}) were used. Two to three days later, seedlings were transferred to a 35/20°C (day/night) greenhouse bay. The plants were transplanted into 100 mm pots (0.5 L) of standard potting medium on 22 May 2008. Plants were hand watered as required.

On 7 May 2008, three week old seedlings were graded within a replicate and randomly allocated to one of four temperature controlled greenhouses (described in Section 2.5).

After 8 weeks, plants were maintained in the existing environment or transferred to another temperature or daylength environment in all possible combinations of temperature and daylengths (16 treatment combinations; see Figures 5 & 6). They were maintained in this new environment for 4 weeks.

Plants were observed every 2 d and the number of days to visible inflorescence buds and anthesis and the numbers of leaves at the visible bud stage and at anthesis were recorded. At the end of the experiment (12 weeks) the number of inflorescence buds and inflorescences at anthesis were recorded and the total number of inflorescences calculated. A completely randomized design was used within each greenhouse environment. Data was collected on four replicate plants. Data were subjected to analysis of variance using the GLM procedure in SAS version 9.1 (SAS Institute Inc., NC, USA).

3 Results and discussion

3.1 Stages of floral development

Given that *P. axillaris* is a gynomonecious species, it was important to include both male-sterile and male-fertile genotypes in the current study. Gynomonecism has been reported to occur in other Amaranthaceae species (Sohmer 1972; Stewart and Barlow 1976). High rates of male sterility ranging from 37 to 70% have been reported for populations of *P. obovatus*, with male-sterile genotypes exhibiting anthers that were "obviously shriveled or commonly absent" (Stewart and Barlow 1976). In *P. axillaris*, two anthers are consistently present, but appear reduced in size and devoid of pollen in male-sterile genotypes.

Differences in the timing of floral development have now been found between male-sterile and male-fertile genotypes (Table 1). Overall, *P. axillaris* flowers remain open for a mean period of 19.5 ± 1.5 d. This suggests that there is a large window of opportunity for pollination, provided the stigma is receptive during this time. However, male fertile genotypes remain open for a significantly shorter period (16.4 ± 1.7 d) than male sterile genotypes (22.5 ± 2.4 d). Contamination with self-pollen does not appear to be a

contributing factor, even given the close proximity of the anthers to the stigma and the homogamous nature of species (refer to Section 3.3). As later results show, availability of viable pollen is limited to the first 2 d following anthesis (Section 3.2), and controlled self-pollination results in flower closure within 24 h (Section 2.4). However, the floral development data show no instances of flower closure occurring within the first 6 d following anthesis.

The time between Stage 3 and anthesis was longer in male-fertile genotypes (11.7 ± 1.2) than in male-sterile genotypes (8.5 ± 0.9). Also, Stage 4 was more extended in male-fertile (3.2 ± 0.3) than male-sterile (1.3 ± 0.1) genotypes. These stages encompass anther development, maturation and pollen dehiscence and it stands to reason that they are of shorter duration in male-sterile genotypes.

Table 1. Timing of floral development in *P. axillaris*, relative to time of anthesis. Different letters within the same column denote significant difference (P<0.05).

Genotype	Timing of floral stage* (days)				
	Stage 2	Stage 3	Stage 5	Stage 6	Stage 7
Male fertile genotypes	-20.3 ± 2.2 a	-11.7 ± 1.2 a	3.2 ± 0.3 a	16.4 ± 1.7 a	56.3 ± 9.4 a
Male sterile genotypes	-15.8 ± 1.7 a	-8.5 ± 0.9 b	1.3 ± 0.1 b	22.5 ± 2.4 b	68.0 ± 8.9 a
All genotypes	-18.1 ± 1.4	-10.1 ± 0.8	2.3 ± 0.2	19.5 ± 1.5	63.5 ± 6.5

**Commencement of Stage 4 = Day 0*

3.2 Pollen viability

Pollen tube growth (Figure 3) was observed in all but the Day 3 treatment (Figure 4). This is consistent with the observation that pollen is discoloured and the stamens reposed against the tepals by this time (i.e. Stage 5). Mean pollen germination rates were low (<7%) and inconsistent between replicates, resulting in no significant differences being detected. Values reported in literature for other species are typically in the range of 20 to 80% (Horsley *et al.* 2007; Mercado *et al.* 1994; Rihova *et al.* 1996; Rosell *et al.* 1999; Yan 2001). Attempts to develop a method that resulted in higher pollen germination rates, including manipulation of culture medium composition (sucrose, calcium nitrate

and agar concentrations) and incubation time/temperature combinations, were unsuccessful (data not presented).

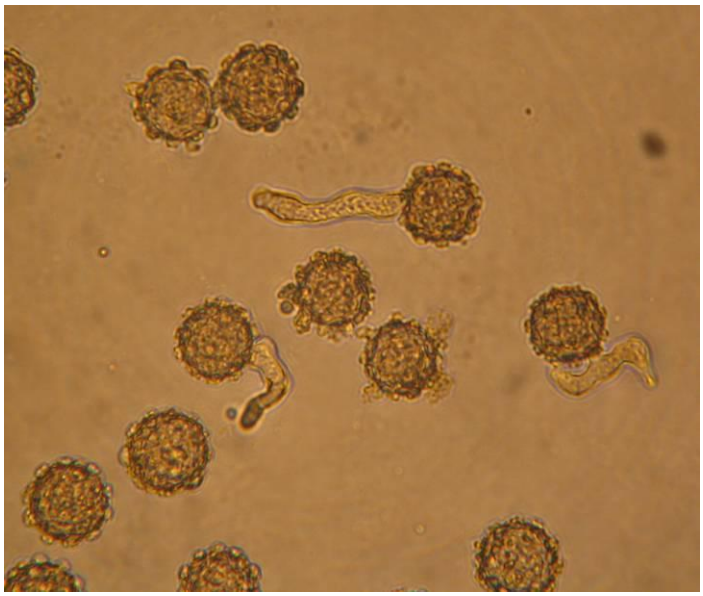

Figure 3. Pollen tube growth observed after 24 h *in vitro* culture at 35°C (x40 magnification).

Rupturing of pollen grains *in vitro* was prevalent and may have been the main reason for the poor pollen germination rates. During preliminary trials, rupturing of up to 100% of pollen grains was observed in some treatments. Pre-hydration of pollen grains in a high humidity environment had a marked effect on reducing pollen grain rupture rates, with rates of <29% observed in the current trial. Shivanna and Heslop-Harrison (Shivanna and Heslopharrison 1981) suggest that at the time of dispersal, the vegetative cell membranes of the partly dehydrated pollen grain are largely dissociated and do not form an osmotic barrier, but that normal properties are recovered during controlled hydration which would normally take place on the stigma.

The findings from the current trial indicate that pollen should be harvested within 48 h of anthesis to ensure maximum seed set from subsequent controlled pollinations. If significant differences in pollen viability are to be detected within this 48 h period, the methodology will need to be optimized further to improve germination rates and/or the trial repeated with greater replication.

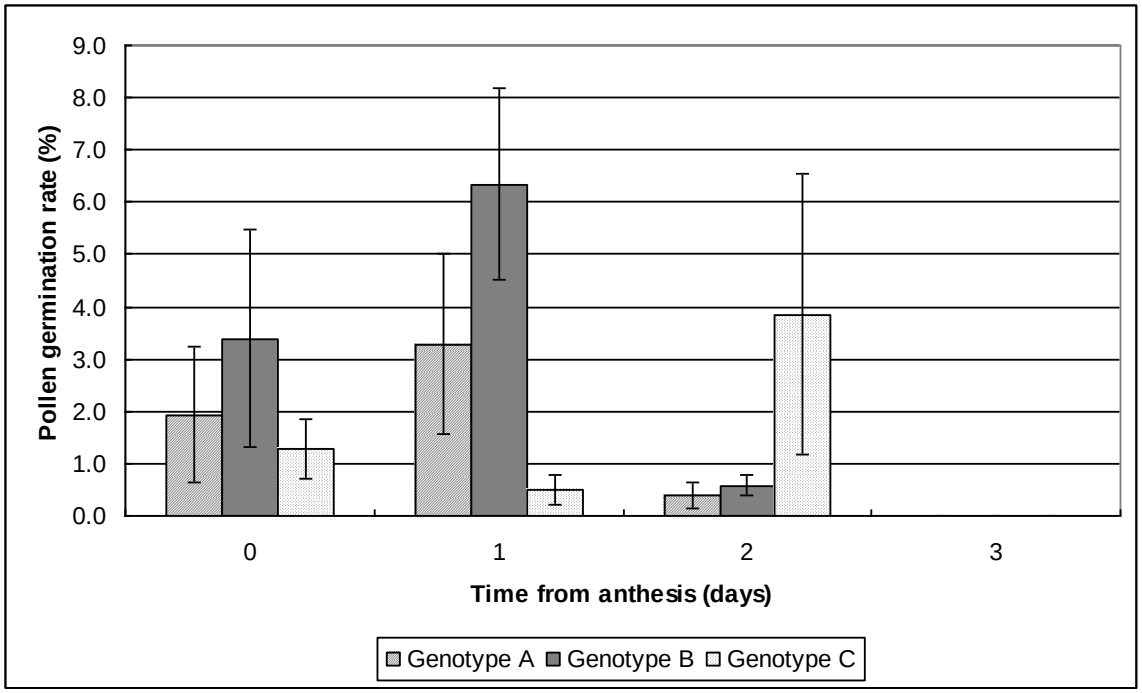

Figure 4. Effect of flower age on *in vitro* pollen germination rates for three *Ptilotus axillaris* genotypes. Error bars indicate ± standard error of the mean.

3.3 Stigma receptivity

The pollination times used in this experiment were limited by the duration for which the flowers remained open. Flower closure (Stage 6) was mostly evident in Day 12 and Day 14 treatments, with mean closure rates of 21% and 61%, respectively. This is in disagreement with the floral development data presented in Section 3.1, which shows flowers of male-sterile genotypes remain open for 22.5 ± 2.4 d. Seasonal variation may be the cause, as the current experiment was conducted during late spring whereas the previous experiment was conducted during late winter and early spring. Mean daily minimum and maximum temperatures were 12.4 and 29.5°C during the floral development trial, compared with 17.1 and 36.0°C during the stigma receptivity trial. This is supported by later results which show floral development is more rapid under high temperatures (Section 3.6).

Initially it was assumed that development of the stigmatic papillae signaled the onset of stigma receptivity, as is reported to be the case for other species (Buide and Guitian 2002;

Kalinganire *et al.* 2000; Masierowska and Stpiczynska 2005; Yi *et al.* 2006). However, this study shows that the stigma is receptive from the time of anthesis (onset of Stage 4) through until the flower closes (Stage 6). No significant difference in seed set was observed for pollinations conducted at different times within this period, but a trend of decreasing mean seed set rate with increasing time from anthesis was observed.

These results indicate that *P. axillaris* is a homogamous species i.e. pollen availability and stigma receptivity occur simultaneously. During the stage at which this occurs (Stage 4), the stigma is closely surrounded by the anthers. With no temporal or spatial separation of the male and female functions, it is possible that a self-incompatibility mechanism may be responsible for the observed low rates of natural seed set. This was tested in the following experiment.

Table 2. Rate of seed set (%) in flowers pollinated at different times after anthesis.

Time of pollination (days after anthesis)	Genotype of maternal parent			Mean ± SE
	D	E	F	
0	75	55	40	56 ± 10
2	71	30	20	40 ± 15
4	75	0	20	31 ± 22
6	73	11	25	36 ± 19
8	57	11	10	26 ± 16
10	73	25	0	32 ± 21
12	33	6	15	18 ± 8
14	50	20	0	23 ± 15

3.4 Self-incompatibility

For all genotypes, cross-pollination resulted in higher seed set rates than self-pollination (Table 3). In the case of two genotypes, self-pollination did not produce any seed at all. All self- and cross-pollinated flowers were observed to be closed on the day following pollination, indicating that the deposition of pollen on the stigma was successful. The unpollinated control flowers produced no seed, indicating that uncontrolled self-pollination or cross-pollination via other vectors (e.g. insects or wind) did not occur.

This was to be expected, since seed set had not previously been observed in these plants under greenhouse conditions. However, it was important to confirm this observation for the trial, since the pollinated flowers were not emasculated to prevent self-pollination or bagged to prevent access by pollen vectors, and any uncontrolled pollination would have confounded the findings.

Table 3. Percentage seed set and index of self-incompatibility (ISI) resulting from the controlled pollination of flowers from four *P. axillaris* genotypes.

Genotype	Seed set (%)			ISI
	Control*	Self	Cross	
G	0.0	0.0	68.4	0.00
H	0.0	12.5	52.4	0.24
I	0.0	15.0	55.0	0.27
J	0.0	0.0	80.0	0.00
Mean ± SE	0.0 ± 0.0	6.9 ± 4.0	64.0 ± 6.4	0.13

**not pollinated*

The low ISI values indicate partial to complete SI within this species (Zapata and Arroyo 1978). SI is a major disadvantage for flower breeders when development of inbred lines for hybrid production is a breeding objective (Anderson 2005). For *P. axillaris*, the occurrence of individuals expressing partial SI means that hybrid production may be possible, provided the fertility of the inbred progeny can be maintained to avoid inbreeding depression (Anderson 2005).

3.5 Environmental effects on flowering of cutting propagated plants

At the commencement of the trial there were no differences between plants for initial height, width or number of branches. A small number of visible buds were obvious after 7 d and the number of visible buds increased over time. After 28 d, the increase in visible bud number was exponential. There were no inflorescences at anthesis until 42 d after potting (Table 4).

High temperatures 35/20°C significantly reduced the number of visible buds under both long and short days (2.6 and 2.3 fold, respectively) but increased the number of

inflorescences at anthesis, suggesting that high temperatures increased the rate of floral development (Table 5). Similarly after pruning, high temperatures reduced the number of visible buds (2.5 fold) and increased the number of inflorescences at anthesis, especially for plants held under high temperatures and 16 h daylength (Table 5).

The results suggest that high temperatures 35/20°C suppress the development of new buds but increase the rate of development of initiated buds. Long daylengths (16 h) did not increase the number of visible buds but may together with 35/20 °C promote the rate of floral development. A second more comprehensive experiment was completed in 2008.

Table 4. Mean number of visible buds and inflorescences at anthesis for *Ptilotus axillaris* averaged over greenhouse environments.

Time (d)	Mean number of visible buds*	Mean number of inflorescences at anthesis*
Days after potting		
7	1.9 a	0.0 a
21	15.8 ab	0.0 a
28	22.7 b	0.0 a
42	43.5 c	5.5 b
56	76.4 d	18.4 c
Days after pruning		
14	2.8 a	0.0 a
28	29.3 b	0.1 a
42	70.8 c	4.6 a
56	90.5 c	16.8 b
70	75.5 c	44.5 c

*Different letters indicate significant differences at p=0.05 within a column.

Table 5. Mean number of visible buds and inflorescences at anthesis for *Ptilotus axillaris* averaged over days after potting and pruning.

Environment (temperature/daylength)	Mean number of visible buds*	Mean number of inflorescences at anthesis*
Days after potting		
25/10°C: 11 h	47.5 b	0.6 a
35/20°C: 11 h	18.0 a	7.2 b
25/10°C: 16 h	45.8 b	1.1 a
35/20°C: 16 h	20.4 a	9.2 b
Days after pruning		
25/10°C: 11 h	76.5 b	5.2 a
35/20°C: 11 h	29.7 a	14.2 ab
25/10°C: 16 h	83.1 b	9.2 a
35/20°C: 16 h	32.4 a	24.2 b

*Different letters indicate significant differences at p=0.05 within a column.

3.6 Environmental effects on flowering of seed propagated plants

All plants reached the visible bud stage during the 12 week experiment duration. High temperatures 35/20°C significantly delayed the appearance of the first visible bud and anthesis and increased leaf number at first visible bud not significantly at anthesis (Table 6). Long days (16 h) reduced but not significantly time to anthesis by 6 d and significantly reduced leaf number at anthesis suggesting that *P. axillaris* is a facultative long day plant. Plants held under long days (16 h) for 8 weeks had 149 leaves while those under short days had 185 leaves (data not shown).

Table 6. Effect of temperature on mean time to first visible inflorescence bud and anthesis and leaf number at these stages.

Day/night temperature (°C)	Time to first visible bud (d)*	Time to anthesis (d)*	Leaf number at first visible bud*	Leaf number at anthesis*
25/10	23.3 a	71.1 a	10.6 a	157.2 a
35/20	28.8 b	77.6 b	16.8 b	177.7 a

Different letters indicate significant differences at p=0.05 within a column.

Daylength did not influence the number of visible buds, inflorescences at anthesis or total number of inflorescences. Plants held under 25/10°C for 8 weeks had significantly more visible buds, inflorescences at anthesis and total numbers of inflorescences (Table 7). However, when plants held at 25/10°C with visible buds were transferred to 35/20°C buds developed rapidly giving significantly more inflorescences at anthesis. Initial temperature had a greater influence on flowering than the transfer temperature (Table 7).

Table 7. Mean number of visible buds and inflorescences at anthesis for *Ptilotus axillaris* after the initial 8 week temperature treatment and 4 weeks after transfer to different temperature treatment.

Day/night temperature (°C)	Mean number of visible buds*	Mean number of inflorescences at anthesis*	Total number inflorescences*
8 weeks			
25/10°C	79.1 a	26.5 a	105.5 a
35/20°C	53.9 b	6.4 b	60.4 b
4 weeks after transfer			
35/20°C → 25/10°C	81.9 a	5.8 a	87.7 a
25/10°C → 35/20°C	51.1 b	27.1 b	78.3 a

*Different letters indicate significant differences at p=0.05 within a temperature regime for 8 and 4 weeks.

The number of inflorescences was significantly lower under 35/20°C than 25/10°C but transfer from 35/20°C after 8 weeks to 25/10°C greatly increased number of visible buds (Table 7). In contrast, plants grown at 25/10°C for 8 weeks then transferred to 35/20°C for 4 weeks had a similar number of inflorescences but significantly more inflorescences at anthesis. However it is likely that had the experiment been continued and the plants maintained at 35/20°C for longer than 4 weeks, flowering would be suppressed by these high temperatures (Figures 5 & 6). This suppression of floral growth at high temperatures is evident in Figure 6, where the apical shoot growth is appears quite vegetative. Plant quality in terms of compactness was improved at 25/10°C.

Figure 5. *Ptilotus axillaris* under high temperature (35/20 ºC).

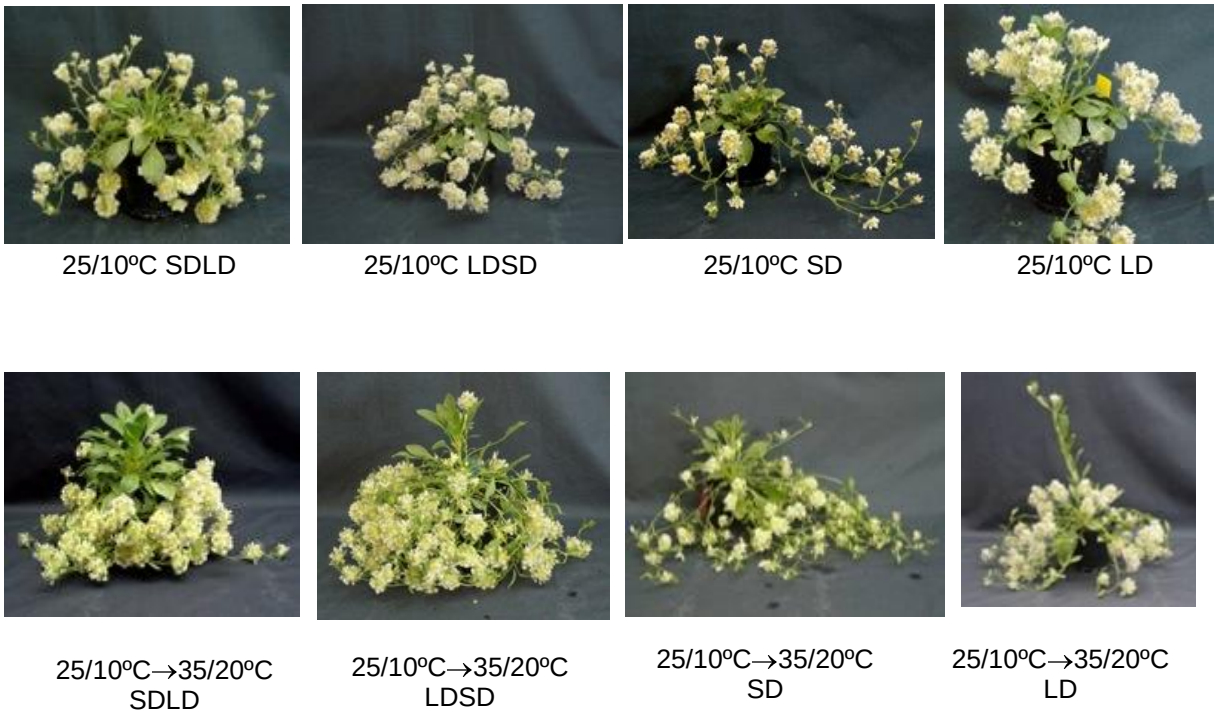

Figure 6. *Ptilotus axillaris* under low temperature (25/10ºC).

These results would suggest that *P. axillaris* is similar to *P. nobilis* which also initiates flowers early (Orzek *et al.* 2009) suggesting that sucrose or other carbohydrates are likely to be the flowering signal. This will inhibit efficient vegetative propagation.

4 Conclusions

P. axillaris is an out-crossing species, given that it exhibits gynomonecism and partial to complete self-incompatibility. Development of inbred lines for hybrid development may be possible, provided parents are carefully selected on the basis of self-compatibility and high fertility. Controlled pollinations may be conducted on stigmas at any time between anthesis and flower closure, using pollen harvested from flowers within 2 d of anthesis. Seed set rates of up to 75% were possible when stigmas were pollinated on the day of anthesis, with stigma receptivity appearing to decline after this time. However this was not statistically significant. Temperature can be used to manipulate flowering in *P. axillaris*. Cultivation of plants under low temperatures may provide year-round flowering and hence improve the efficiency of future breeding programs. It is also proposed that *P. axillaris* is a facultative long day plant.

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