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Seed production of pink paper daisy (*Rhodanthe chlorocephala* subsp. *rosea)* and yellow strawflower *Schoenia filifolia* subsp. *subulifolia* (Asteraceae)

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Summary

Everlasting daisies provide a stunning display of flowers in Western Australia during the wildflower season. Two of these species, Pink paper daisy (*Rhodanthe chlorocephala* subsp. *rosea)* and yellow strawflower (*Schoenia filifolia* subsp. *subulifolia* have recently been brought into cultivation.

The impact of water deficit on plant growth, flowering and seed yield were examined in both glasshouse and field experiments. Adequate watering, particularly during early seedling growth, was essential for high yield. Water deficit modified the plant canopy by reducing stem number and branching which limited the sites for terminal inflorescence and seed production. The same proportion of stems produced inflorescences in well-watered and water-deficit plants and stem number contributed more to seed yield than any other component. Water deficit reduced seed weight of *R. chlorocephala* but only at the lowest level of irrigation (25% A pan). Well-watered *R. chlorocephala* produced more seeds per plant than water-deficient plants. Differences in seed number were entirely due to differences in stem number. In contrast, water deficit reduced seed number per inflorescence and seed weight in *S. filifolia.* Severe water deficit inhibited branching in *S. filifolia* and this effect was more profound than in *R. chlorocephala*. Water deficit had no consequent effect on *R. chlorocephala* seed quality measured as seed germination. This contrasted with *S. filifolia* where water deficit reduced seed viability 6 months after harvest.

Germination of seeds of both *R. chlorocephala* and *S. filifolia* were poor at harvest. Seeds were stored for at a range of temperatures (5-65°C) for 10 months. *Rhodanthe* seeds lost dormancy within two months when stored at room temperature (25°C). They withstood storage temperatures up to 55°C and after ten months at this temperature, germination was still 90%. Yellow strawflower was 100% dormant at harvest and required a long period of dry storage (3 months at 25-40°C) to overcome dormancy. Heat (65-105°C for 12 h to 13 d) was further investigated as a means of overcoming dormancy. Seeds exposed to 80°C for 11 days germinated (88%). Seeds exposed to >80°C had reduced germinability due to reduced viability. The mechanisms of dormancy were further investigated. Seedcoats of *S. filifolia* were permeable to water and dormancy was imposed by the embryo. Exogenous gibberellic acid (30 μM GA_3) alone, or in combination with KNO_3 (10 mM), broke dormancy in intact seeds. Chlormequat and paclobutrazol, which interfere with gibberellin biosynthesis, reduced germination in seeds in which dormancy had been broken by either dry storage or heat. Applied GA_3 reversed this inhibitory effect. Thus GA biosynthesis was required for the germination of dormant *S. filifolia* seeds. Dry storage or heat facilitated the transition of seeds from a dormant to a non-dormant stage by increasing the ability of seeds to synthesize endogenous gibberellins.

Introduction

Everlasting daisies (Asteraceae. Tribe Inuleae) are native to inland areas of Western Australia, a region with long droughts and high temperatures. Commonly they germinate in the autumn, and grow during the milder, wet winter, seeding before the summer drought. Many of them have attractive forms, and have been used for commercial production. *Rhodanthe chlorocephala* subsp. *rosea,* pink paper daisy (previously *Helipterum roseum*) and *Schoenia filifolia* subsp. *subulifolia*, yellow strawflower (previously *Helichrysum subulifolium*) are two species which have show promise. The species are propagated commercially from seed, often from bush picked seed. Information about their agronomy is very scant, in particular the effect of irrigation on plant growth and development, and particularly on seed production. Another problem in some species is seed dormancy, so that full germination is rarely achieved (Mott, 1972; Bunker, 1994; Plummer et al., 1995).

This project examined three aspects of seed production in the species *Rhodanthe chlorocephala* and *Schoenia filifolia,* firstly the effect of moisture stress on seed production, secondly the effect of storage temperature on seed viability, and thirdly the potential for high temperature treatments to break dormancy.

Materials and methods

Moisture stress and seed yield

Glasshouse trial

The experiment was conducted from September 1994 to February 1995 with photoperiods of 12 to 14 h and maximum and minimum temperatures of 30ºC and 18ºC, respectively. Seed of *R. chlorocephala* and *S. filifolia* were sown in 2.5 L pots containing 1800 g sterilised potting mix and thinned to two seedlings per pot. Seeds of *S. filifolia* were incubated at 20/15°C day/night for three weeks, since they require low temperatures for germination (Plummer et al., 1995). Pots were then transferred to the glasshouse. For the first three weeks after planting, all pots were maintained at a soil water content of 0.42 g water per g dry soil to allow seeds to germinate. Thereafter plants were subjected to water deficits.

High, medium and low water regimes were used. For high water supply, soil was rewatered daily to -2.2 kPa or 0.375 g g^{-1}, for medium and low water supply, soil was rewatered to -10 kPa (0.243 g g^{-1}) or -100 kPa (0.205 g g^{-1}), respectively. There were three replicates per treatment arranged in randomized complete blocks. Plants were harvested at weekly intervals from 78-113 days after planting and stem numbers were determined. Branching was assessed by counting the numbers of primary, secondary, tertiary and quaternary stems per plant.

Field trial

A field experiment was conducted from August to December 1995. Plot size was 10 m^2. Seed of *R. chlorocephala* and *S. filifolia* were sown with a 50 cm row spacing and thinned 30 days after emergence to a plant spacing of 5 cm within the row. Weeds were manually controlled. Irrigation was applied by sprinklers and all plots received irrigation equal to 100% A pan replacement until treatments began at 45 days after planting. Irrigation was applied daily. The irrigation regimes were 100% class A pan evaporation replacement, 50% replacement and 25% replacement. The experimental design was a split plot with three replications. The main plots were the three irrigation regimes. The two species of everlasting daisies, *R. chlorocephala* and *S. filifolia* were subplots. Plants were harvested at 112 days after planting. Branching was explored through the numbers of primary, secondary, tertiary and quaternary stems per plant. Stem numbers, inflorescence numbers, seed yield and its components were recorded.

Seed storage

Seeds from the field experiment were stored at ambient temperature for three months before transfer to storage temperatures of 5, 15, 25, 30, 40, 55 or 65°C. Germinability of *R. chlorocephala* was monitored at 2-monthly intervals. Viability and germinability of *S. filifolia* were determined at monthly intervals. Seed viability was assessed by the tetrazolium test (Moore, 1973) on four replicates of 25 seeds. Seed germinability was assessed as follows:- Three replicates of 25 seeds were placed on Whatman No. 1 filter paper resting on several sponge pieces soaked in deionised water mixed with fungicide (Propamocarb, 600 g/L active ingredients, 2% v/v) in sealed plastic dishes and incubated at 20°C for 35 days. Germination was defined as radicle emergence and expressed as the percentage of all seeds.

Data were analysed as a three way factorial (water regimes; storage temperatures and storage periods). Analysis of variance of all experiments were performed by SuperANOVA, Abacus Concepts Inc. Mean comparisons were made using Fisher's Protected least significant difference (LSD at P=0.05).

Effect of heat treatments on dormancy

Newly harvested seed of *S. filifolia* were 100% dormant. Dormant seeds were exposed to 65, 80, 95 or 105°C from 12 h to 2 weeks. Germinability and viability were determined.

Results

Moisture stress and seed yield

Glasshouse experiment

Reduced water supply decreased branching in both species but *R. chlorocephala* was less affected than *S. filifolia* (Table 1). In *R. chlorocephala*, water deficit did not substantially affect the pattern of branching since plants in all treatments produced similar proportions of secondary, tertiary and quaternary stems. The impact was on stem numbers, as low water supply reduced stem numbers to about one third of plants receiving high water supply (Figure 1). In contrast, water deficit affected both the pattern and quantity of branching in *S. filifolia.* Only well-watered *S. filifolia* produced primary and secondary stems whereas low water supply almost eliminated branching.

Table 1. Effect of water regimes on the secondary, tertiary and quartenary stem numbers per plant of *Rhodanthe chlorocephala* and *Schoenia filifolia. Schoenia* did not produce any tertiary or quartenary stems. The total includes a primary stem; ns = not significant.

Water regime	*Rhodanthe*				*Schoenia*	
	2'	3'	4'	Total	2'	Total
Low	5.2	5.5	1.3	13.0	0.2	1.2
Medium	6.9	8.3	3.0	19.2	2.1	3.0
High	14.1	14.4	2.4	31.9	4.5	5.5
LSD(p= 0.05)	1.1	2.6	ns	2.9	1.1	2.9

Figure 1. Influence of water supply on stem and inflorescence production in *Rhodanthe chlorocephala* subsp. *rosea* 94 days after planting in pots. From left to right: high (-2.2 kPa), medium (-10 kPa) and low (-100 kPa) water supply.

Field experiment

Reduced irrigation decreased branching (Table 2). Stem numbers of *R. chlorocephala* and *S. filifolia* showed a similar response to irrigation. Plants watered at 25, 50 or 100% A pan evaporation produced 11, 14 or 21 stems per plant. The effect of irrigation on seed yield was greater than on branching. Plants irrigated at 25% A pan produced only one third of the seed of those irrigated at 100% A pan.

Table 2. Vegetative growth (stems per plant) and seed yield (g m^{-2}) of *Rhodanthe chlorocephala* and *Schoenia filifolia* grown under different irrigation regimes

Irrigation (% of A pan)	*Rhodanthe*		*Schoenia*	
	Stems per plant	Seed yield (g m^{-2})	Stems per plant	Seed yield (g m^{-2})
25	13.0	34.3	8.7	16.2
50	14.3	48.4	13.7	31.3
100	18.5	79.9	22.3	57.5
LSD (p=0.05)	4.4	18.1	4.4	18.1

Seed storage trial

Preharvest irrigation had no effect on *R. chlorocephala* seed germination during the first 3 months of storage. Subsequent postharvest storage temperature had a small effect on germinability. Storage temperatures of 15-55°C did not affect germinability of *R. chlorocephala* as seed still had high germinability (94-97%) after 3 months. The highest germination was obtained from seed stored at 30ºC (97%). Only extreme temperatures (65°C and 5°C) were detrimental to seed germination and these decreased germination slightly, to about 90% (Figure 2).

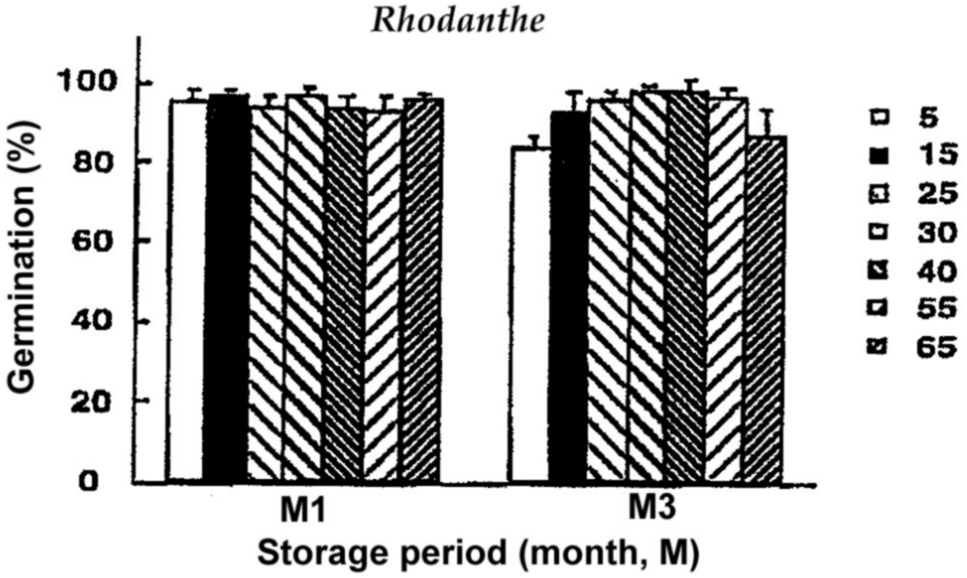

Figure 2. Effect of storage temperatures on germinability of *Rhodanthe chlorocephala*. Seeds were stored at ambient temperature for 3 months after harvest before being subjected to these storage temperatures.

Dormancy was present in both *R. chlorocephala* and *S. filifolia* seed. The domancy intensity varied among plant species and water regimes. One month old *R. chlorocephala* seeds from plants grown at 25%, 50% and 100% A pan had 19, 21 and 37% dormant seed, respectively. However, dormancy was released by 2 months after harvest in most seed stored at room temperature. More than 90% of *S. filifolia* seeds were dormant before being placed in various storage temperatures, 3 months after harvest. The rate of dormancy release in *S. filifolia* seed was temperature dependent (Figure 3). After 3 months storage, at 5 or 15°C seed attained 53% and 63% germinability, respectively. Seed stored at high temperature (65°C) had high germination (80%) after two months storage. However at 65°C, seed did not completely lose dormancy and one month later, germination declined to 60% due to reduced viability. Seeds stored at 25, 30 or 40°C had approximately 90% germination after 3 months storage at these temperatures.

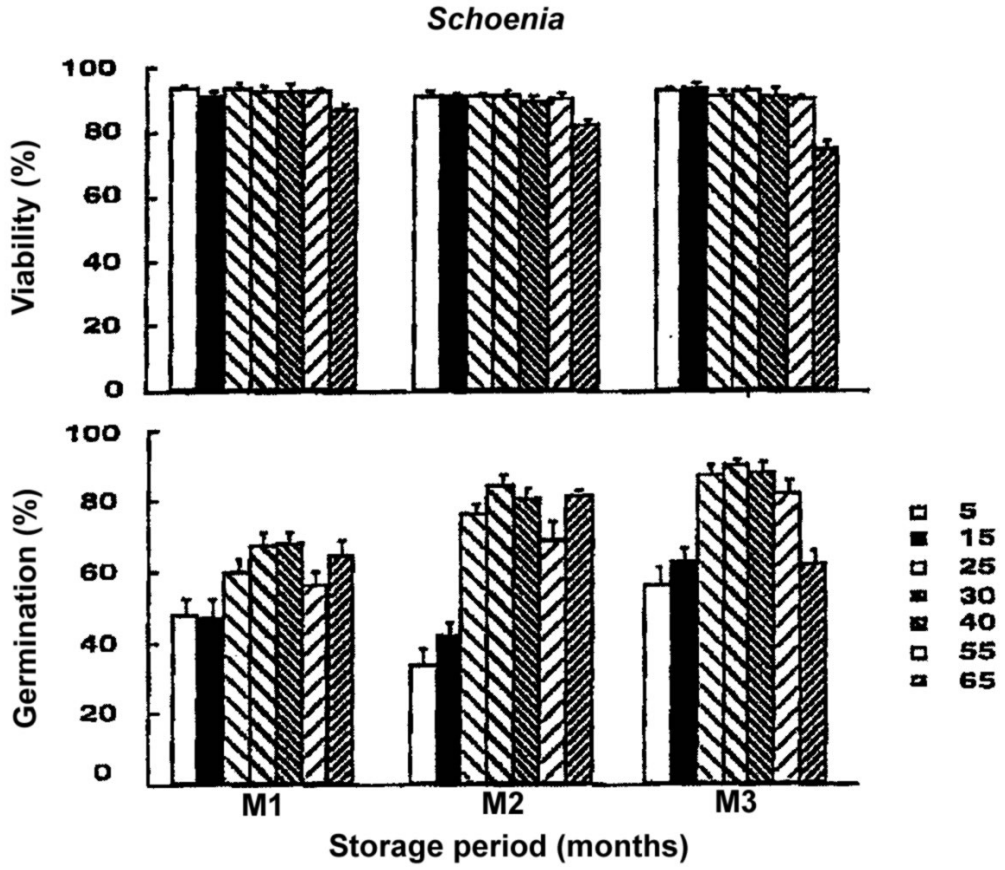

Figure 3. Effect of storage temperatures on viability and germinability of *Schoenia filifolia* seed after 3 months storage. Seeds were stored at ambient temperature for 3 months after harvest before being subjected to these storage temperatures.

Effect of heat treatment on dormancy

Seeds of *S. filifolia* exposed to 65°C for 14 days did not attain full germinability (Table 3). Storage at 95°C for 72 h killed more than half the seeds but almost all of the remaining viable seeds were released from dormancy. Increased exposure time at this temperature progressively reduced germinability due to reduced viability. Seeds exposed to 80°C for 7 days maintained their viability at more than 90% but germinability did not exceed 30%. Extended exposure at 80°C till 11 days decreased viability to 87% but increased germinability to 78%.

Table 3. Effect of heat and exposure time on viability and germinability of *Schoenia filifolia* dormant seeds.

Temperature (°C)	Exposure time	Viability (%) (± SE)	Germinability (%) at days after incubation at 20°C.	
			7 days	35 days
65°C	12h	93 ± 1.4	0	1 ± 1.4
	3 days	87 ± 3.6	0	5 ± 2.7
	5 days	95 ± 1.9	0	8 ± 3.3
	7 days	91 ±5.4	0	15 ± 4.9
	14 days	95 ± 1.0	10±4.1	18±2.6
80°C	12h	90±2.8	0	5 ± 2.7
	3 days	91 ± 4.8	0	26 ± 16.7
	5 days	93 ± 3.0	0	33 ± 6.8
	7 days	94± 3.7	0	24±8.3
	11 days	87 ± 3.4	69 ± 2.6	78 ± 5.0
	13 days	87 ± 2.5	72 ± 7.3	76 ± 7.5
95°C	12 h	78 ± 1.6	0	30 ± 14.7
	3 days	41 ± 1.4	0	49±9.4
	5 days	27 ± 2.4	0	0
	7 days	0	0	0
105°C	12 h	75 ± 3.5	0	5 ± 4.2o
	1 day	0	0	0

Discussion

In both glasshouse and field experiments it was evident that water deficit reduced the number of stems per plant and that this was a primary component of seed yield. Water deficit halved stem numbers per plant in both experiments. The impact of water deficit on branching depended on the growth stages experiencing water deficit, in turn, the differences in plant growth and development between the two species was reflected in their response to water deficit. *R. chlorocephala* grew much more quickly than *S. filifolia*. In the glasshouse experiment water deficit was imposed 21 days after planting at which time secondary stems of *R. chlorocephala* had already been

determined. Consequently, *R. chlorocephala* was less affected by water deficit than *S. filifolia.* This contrasts with the field experiment where *R. chlorocephala* and *S. filifolia* responded to irrigation in a similar manner due to the later imposition of the treatment. The sensitivity of early vegetative growth of *R. chlorocephala* to water deficit was reported by Crofts (1994), who found that leaf number, stem number and stem length were all reduced by water deficit. In other Australian daisies water deficit reduces the number of nodes to the first flower in *Schoenia cassiniana* but has no effect on node number in *Helipterum craspediodes* (Mott et al., 1974, 1975). Water deficit also affects vegetative growth in *Chamelaucium uncinatum* and this response has been used to improve the quality of wax flower stems used for cut flowers (Akilan et al., 1994).

Water deficit reduced seed yield by restricting branching in both *R. chlarocephala* and *S. filifolia.* Work on other daisies, *Schoenia cassiniana* and *Helipterum craspediodes*, showed that water deficit reduces seed yield through decreases in flower number, seed number and seed weight (Mott et al, 1975). In sunflower (*Helianthus annus*, Asteraceae), which only has one flowerhead per plant, the effect of water deficit was on seed number per head and seed weight. Seed yield is most sensitive to water deficit at flowering (Lazim, 1985).

Environmental factors that plants experience during development and maturation, including daylength, temperature, light quality and altitude, affect subsequent seed germinability (Gutterman, 1992). The results from these experiments showed that water deficit had a critical role in limiting vegetative development of both species as increasing irrigation increased branching. Preharvest irrigation, however, had no effects on germinability of *R. chlorocephala* determined 3-6 months after harvest. Growth of peanut seedlings is reduced by water deficit (Ketring, 1991) but not peanut seed viability (Ramamoorthy et al., 1996). In contrast, in carrot (*Daucus carota*) increasing irrigation decreases germination especially in the later-developing seed (Steiner et al., 1990).

Storage temperatures had a pronounced effect on seed germinability and viability of both *R. chlorocephala* and *S. filifolia.* Temperature in non dormant seeds determines the rate of germination (Robert, 1988) and in *R. chlorocephala* and *S. filifolia* the optimum temperature is 15/20°C (Plummer et al., 1995). Temperature also affects the

rate of dormancy loss and temperature, together with seed moisture content, determines the rate of deterioration of many seeds (Robert, 1988). *R. chlorocephala* seeds were able to tolerate a large range of storage temperatures, 15-55°C from 3-6 months after harvest, and maintain their germinability above 90%. The high storage potential of *R. chlorocephala* seeds has also been reported by Peishi (1995) who found that seed stored at 7 to 38°C and a seed moisture content of 12-15% for 2 years, has germinability exceeding 80%.

Generally, seed possesses its greatest viability at physiological maturity and from this point on the seed gradually deteriorates and eventually dies (Justice et al., 1979). However seed deterioration can be retarded by suitable storage conditions. The guide used by Harrington (1960, 1972) is that each 1% reduction in seed moisture content or each 5°C reduction in temperature doubles the storage life of seed, and the effect of these two parameters is additive. On this basis one would expect that *R. chlorocephala* seeds should be stored at low temperatures to extend their longevity. However storage of seed at 5°C had an adverse effect on seed germinability. This is probably because a change in seed moisture content at the lower storage temperatures had occurred. Seed moisture content increased from 0.049 g g^{-1} before storage to 0.067 and 0.080 g g^{-1} dw after one month storage at 5°C and 15°C respectively (unpublished results). In fact, our work suggests that *R. chlorocephala* seed are very capable of enduring high storage temperatures and could be stored at room temperature, provided the moisture content of the seed is maintained at a low level.

The loss of dormancy in *S. filifolia* after seed harvest was a function of temperature. The warmer the storage temperature, the greater the loss of dormancy. Storage at high temperature hastens loss of dormancy in the seeds of other winter annuals such as *Polygonum persicaria* (Bouwmeeter et al., 1993), *Helipterum craspedioides* and *Helipterum cassinianum* (Mott, 1972). Seeds of *S. filifolia* attained 80% germinability after 2 months of storage at 65°C. However storage at this temperature for another month reduced germination to 60% because of reduced viability. Maximum seed germination (approximately 90%) was reached after 3 months storage at 25, 30 or 40°C. After dormancy is released, continued storage at high temperature would be expected to adversely affect seed longevity and accelerate seed deterioration.

Short term exposure to temperatures up to 95°C, also overcame seed dormancy in *S. filifolia.* Maximum germinability was obtained when seed was exposed to 80°C for 11 days. Temperature thus has a crucial role in breaking dormancy in *S. filifolia,* however, the mechanism involved is unknown. Dormancy does not appear to involve a hard seed coat, as germination is not improved by scarification (Bunker, 1994). However, removing the seed coat improves seed geminability by 35% in seeds stored at 5°C, but not in seeds stored at 38°C, which have already broken dormancy (Peishi, 1995). In *S. filifolia*, after some storage, dormancy can be overcome by applying gibberellic acid and/or potassium nitrate (Rogers, 1996).

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