FINAL RESEARCH REPORT FOR THE AUSTRALIAN FLORA FOUNDATION RESEARCH GRANT 1993

Development, Germination and Dormancy *of Actinotus helianthi* (Flannel Flower) Seeds

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Introduction

Actinotus helianthi, the Sydney or Common Flannel Flower, has considerable horticultural potential as a cut flower, and as a rockery or container plant. It belongs to the family Apiaceae (syn. Umbelliferae), which includes many well known culinary herbs and vegetables, such as carrot, celery, parsley and dill. Flannel flowers are one of. the more common and attractive wildflowers found growing naturally in sandstone country along the east coast of NSW and southern Queensland. However, their use in cultivation is presently restricted because of erratic germination from seed. The aim of the current project was to investigate the development, germination and dormancy of flannel flower seeds in order to obtain a greater understanding of their germination requirements, and to thereby find a means of achieving better germination from seed. This will in turn help to reduce pressure, on wild populations, by increasing the production of plants in cultivation.

Specifically, the objectives of this project were:

i) to investigate whether the poor seed germination is due to low seed viability or seed dormancy;

ii) to examine ways of improving germination;

iii) and to study the seed anatomy.

Materials and Methods:

During late 1992 and early 1993, seed was collected from eight locations around NSW, in areas extending from the Royal National Park south of Sydney to South West Rocks on the North Coast (see Appendix 1). Up to three separate collections were made from each site providing

early, mid and late season collections. Experiments were then conducted using seeds from these 20 seedlots.

Seed was collected from plants when the bracts surrounding the inflorescence had begun to fall off. During this time seeds changed from green (olive) to brown in colour. Obviously empty seeds were removed from each batch of seeds prior to use in any experiments.

More specific methodology is outlined in each of the relevant sections below.

Results and Discussion

Non-germinability of seeds under conditions of adequate moisture, good aeration and suitable temperature can generally be attributed either to seed inviability or seed dormancy. Therefore, investigations were undertaken to determine which of these factors, or whether both, could explain the erratic germination of A. *helianthi* seeds.

INVESTIGATIONS INTO SEED VIABILITY AND SEED STRUCTURE

Seeds may be incapable of germinating because the embryo, the part of the seed which develops into the new plant, is non-viable or missing. Therefore initial investigations d at determining whether or not an embryo was present within the seed,and if present, whether or not it was viable.

i) Seed Viability:

Determination of embryo viability was attempted using tetrazolium staining techniques. However, this method proved unreliable, as it was difficult to interpret the staining patterns of the embryos, and the technique appeared to underestimate the proportion of viable seeds. It was therefore decided not to pursue this aspect of.the work. (Accurate interpretation of the tetrazolium technique requires considerable practical experience, and is useful only for economically important species for which a protocol has been developed -see International Seed Testing Association, 1976; Peterson, 1980).

ii) Embryolessness:

Seeds from 16 seedlots, representing 7 sites and up to 3 collection times, were dissected under a stereo (binocular) microscope to determine the percentage of seeds without embryos. Seeds were

soaked in distilled water at 20°C for 5-7 days prior to dissecting to enable easier removal of the seed covering structures. The embryo was then located by removing a small amount of endosperm at the base of the seed using fine forceps. A minimum of 5 replicates of 10 seeds from each seedlot were used. (In some seedlots, seeds had been divided into green (immature) and brown (mature) types, and in these cases the two types were tested separately.)

The results (Appendix 2)showed that a small proportion of seeds lacked embryos, although they had a well-developed endosperm and outwardly appeared normal. This phenomenon is quite common in the Apiaceae (Flemion and Waterbury, 1941; Flemion and Uhlmann, 1946) and has been found to be caused by plant bugs of the genus Lygus (Remion ,1949; Flemion *et al.*, 1949). The proportion of embryolessness found in the seedlots tested ranged from 4 to 26%, with an overall average of 12% of seeds.

iii) Undeveloped Seeds:

A small proportion the seeds investigated (ranging from 0 to 22% of seeds - see Appendix 2) also contained partially developed or disintegrated endosperm (referred to as "undeveloped" seeds). The average proportion of these undeveloped seeds in the 16 seedlots investigated was 5%.

iv) Seed Structure and Anatomy:

Seeds of several species of Apiaceae have been found to contain immature or rudimentary embryos, and this has been shown to be a cause of delayed germination in these species, with the embryos requiring further development before germination can occur (Flemion and Uhlmann, 1946; Stokes, 1952,1953; Robinson, 1954; Dale and Harrison, 1966; Jacobsen and Pressman, 1979; Atwater, 1980; Baskin and Baskin, 1984; Karssen *et al.*, 1989). Therefore, an examination of the structure of the seeds of A. *helianthi* was undertaken in order to determine whether this may be a cause of the poor germination in this species.

The seeds which were found to be empty or embryoless in the study outlined above accounted for only a relatively small proportion of the non-germinability found (maximum 34% - see Appendix 2). However, the embryos in those seeds in which an embryo was found were generally extremely small in size, and located in a basal position within the seed. Anatomy work was therefore undertaken using freeze microtomy techniques Gerlach, 1984) and the sections examined under the light microscope, to further investigate the structure of flannel flower seeds. This procedure

involved fixing the seeds in alcohol (70% ethanol) and embedding in 25% (w/v) gelatin prior to sectioning on a freeze microtome. The sections obtained showed that the seed (strictly speaking a fruit or mericarp) consisted almost entirely of endosperm, enclosing a small embryo and surrounded by a thin testa (seed coat) and a pericarp (fruit coat) (Figure 1). The tiny size and rudimentary nature of the embryo therefore may well be a contributing factor in the poor germination shown by this species. Many species in the Apiaceae family respond to stratification treatments (cold moist storage) during which the embryo increases in size (Stokes, 1952; Baskin and Baskin, 1984; Ojala, 1985). This treatment may be beneficial also for seeds of A. *helianthi*, even though it grows naturally in a warm temperate environment

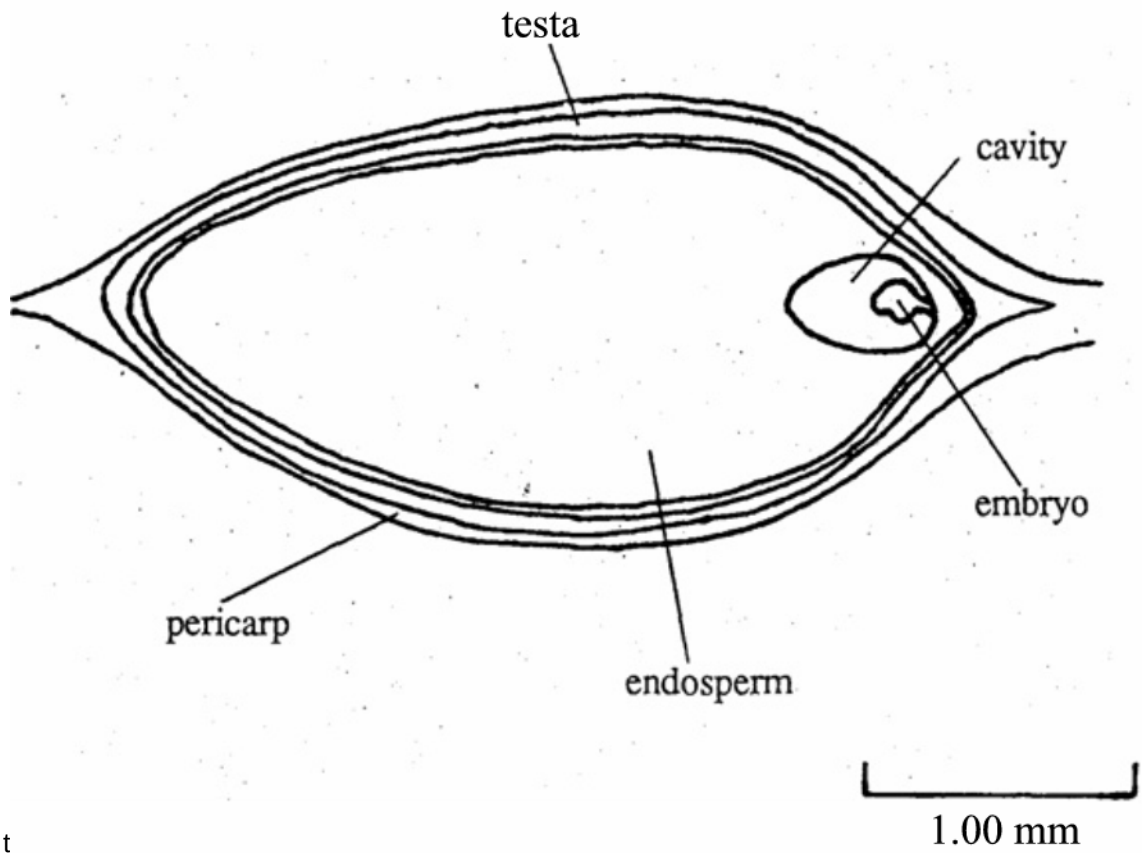

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Figure 1**:** Schematic drawing of a longitudinal cross section of a flannel flower seed.

SEED GERMINATION EXPERIMENTS

In order to determine some of the basic germination requirements of flannel flower seeds, a number of seed germination experiments were conducted under controlled conditions in the laboratory. These experiments incorporated investigations into the following five aspects of germination:

i) the effects of temperature and light;
ii) the effects of storage;
iii) the effects of the seed coverings;
iv) the effects of gibberellic acid; and
v) the effects of seed source and time of collection on seed germination.

Materials and Methods

In all germination experiments, seeds were germinated in 9 cm glass petri dishes on Ekwip U70 filter papers, trimmed to fit and moistened with distilled water until just saturated (approximately 10 ml). All germination experiments were carried out in constant temperature cabinets with a 12 hour photoperiod provided by NEC Biolux 18 Watt flourescent tubes, at 20°C unless otherwise stated. Individual treatments consisted of four replicates of 50 seeds each, and all dishes were sealed with Parafilm to prevent water loss through evaporation. Radicle protrusion of at least 2 mm was taken as the criterion for germination. Additions or alterations to this methodology are outlined in each of the sections below.

i) Temperature/Light Experiments:

A favourable temperature is vital for the germination of all seeds, with the range of temperatures allowing some germination lying between 5 and 45°C in most species Hartmann and Kester, 1983). Light is a pre-requisite for germination in several species; in others complete darkness is necessary. However, for most species, light either increases existing germination or has no effect. In many species an interaction occurs between light and temperature (Evenari 1956; Mayer and Poljakoff-Mayber, 1989), with light often being necessary for germination only at higher temperatures. This is the case with seeds of celery (Pressman *et al.,* 1988; Thomas *et al.,* 1975) and lettuce (Heydecker and Joshua, 1976).

To determine the effects of light and temperature on germination, seeds were germinated at a range of constant temperatures from 10° to 35°C, at 5° intervals, in both the light and in darkness. Light was excluded from dark treatments by wrapping the petri dishes in alfoil and germination

was checked using a green safe light. The experiment was undertaken using both fresh (3 week old) and stored (10 month old) seed collected from Hawks Nest in November 1992.

The optimum temperature for maximum germination was found to be 15°C for both fresh (data not shown) and stored (Figure 2) seed, but germination commenced earlier at 20°C (data not shown). There was little difference between light and dark treatments at any temperature for fresh seed, and overall germination percentages were much lower, with the highest germination obtained (17.5%) being for seed germinated in the dark at 15°C. The results obtained for stored seed showed germination to be promoted by light only at the higher temperatures of 20 and 25°C. This is similar to the situation in lettuce and celery seed, which exhibit thermodormancy (Evenari, 1956; Thomas *et al.*, 1975; Heydecker and Joshua, 1977; Thomas, 1983; Pressman *et al.*, 1988).

ii) Dry Storage Experiments:

It is commonly noted in the literature on propagation of A. *helianthi* that 'fresh' seed-gives good germination (Rogers, 1975; Elliot and Jones, 1982; Blombery, 1984). Hence the initial trials aimed to examine the effect of seed age, along with time and place of collection, on dormancy.

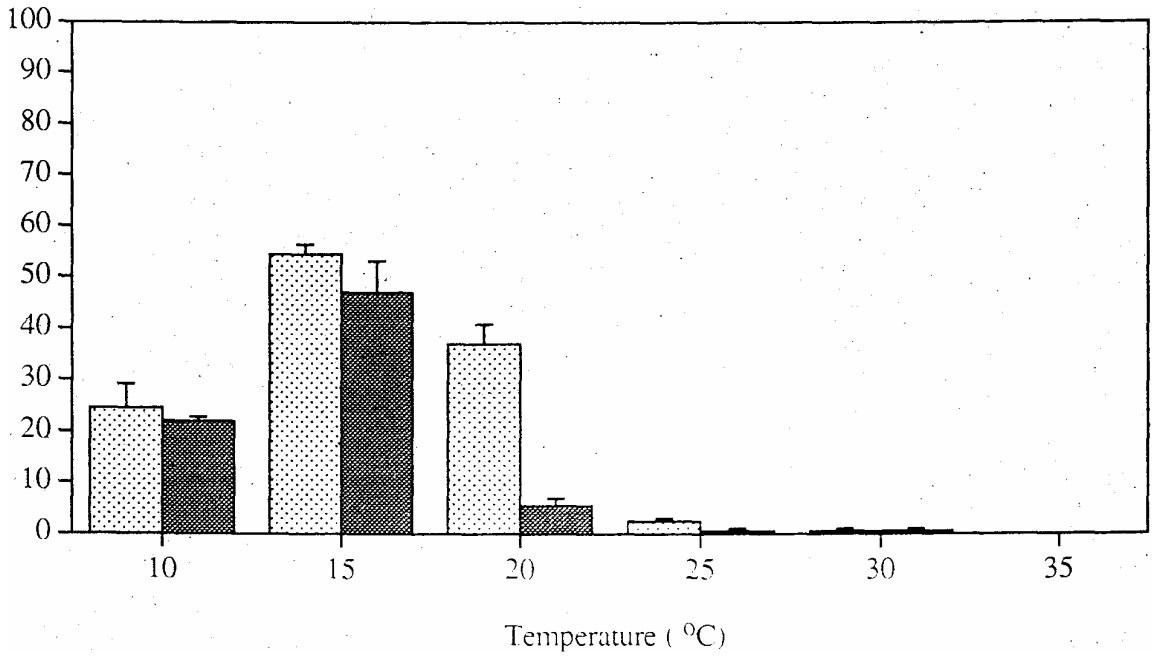

Figure 2: Germination of *A. heliaianthi* seed at different temperatures in the light (light columns) and in the dark (dark columns). Seed was collected at Hawks Nest in November 1992. Error bars shown are standard errors (mean).

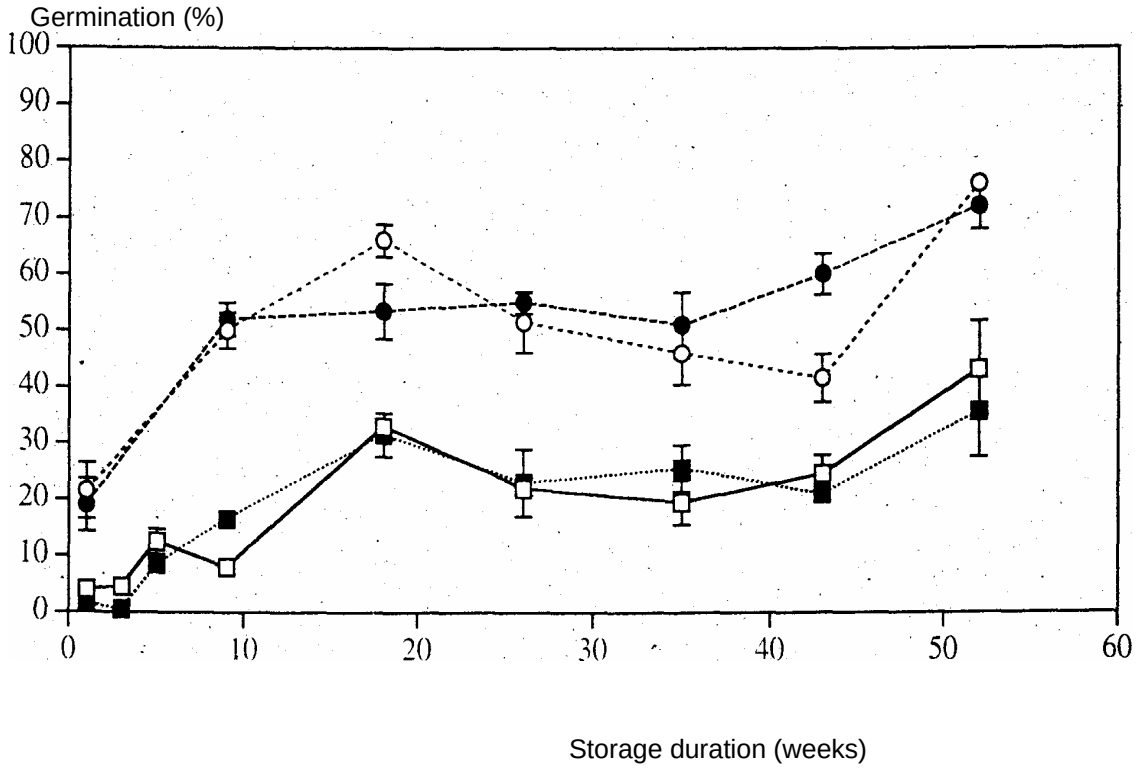

Figure 3: Change in final germination percentage of A.*helianthi.* seed with duration of dry storage at 21º C. Error bars shown are standard errors (mean). Seed was collected at Hawks Nest (squares) or at Stony Range Flora Reserve, Dee Why (circles), in November 1992. Open symbols indicate green (immature) seed; filled symbols indicate brown (mature) seed.

For storage experiments, seeds were kept at 21°C in airtight glass jars containing silica gel (to absorb excess moisture), and samples of seed were taken for germination initially fresh seed not more than 3 weeks old), and then at monthly or bimonthly intervals. Several seedlots were used, collected from eight different sites along the NSW coast from Sydney to South West Rocks, and at two or more separate times during the season. At collection seeds were separated into green (immature) or brown (mature) seed.

In contrast to the original expectations, germination shown by fresh seed was almost invariably poor. Although the actual germination percentages obtained varied with seedlot (collection site and time of collection), germination rarely reached more than 30%, and was usually lower than 10%, for fresh seed. A notable exception was seed from South West Rocks, where fresh seed gave germination percentages as high as 65%. Seed source appeared to be an important factor in

determining the degree of dormancy, with seed from some sites giving consistently higher germination than that from others (see Section v).

Significant improvements in final germination percentages, however, were observed within 2-4 months of dry storage (Figure 3), and by 12 months in storage, germination in most seedlots had at least trebled. This indicates the need for after-ripening of the seed, possibly to allow maturation of the embryo or breakdown and neutralisation of inhibitors. After-ripening is a common phenomenon in seeds of grasses and cereals (Crocker and Barton, 1957; Whalley, 1987), as well as in many other plant families (Crocker and Barton, 1957; Stokes, 1965), and has also been observed in some members of the Apiaceae (Robinson, 1954; Dale and Harrison, 1966; Dale, 1974; Baskin and Baskin, 1975, 1990).

iii) Seed Covering Experiments:

In many species exhibiting dormancy, the seed coverings play an important role in the control of germination. This may be because the pericarp and/or testa limit water entry or gas exchange, or contain inhibitors to germination, or in some cases prevent the escape of inhibitors from the endosperm or embryo itself (Bradbeer, 1988). Seed germination inhibitors have been located in several Apiaceae (Chaturvedi and Muralia, 1975). To investigate the role, if any, the seed coverings play in the dormancy of flannel flower, experiments were conducted to determine the effect on germination of the removal of these coverings.

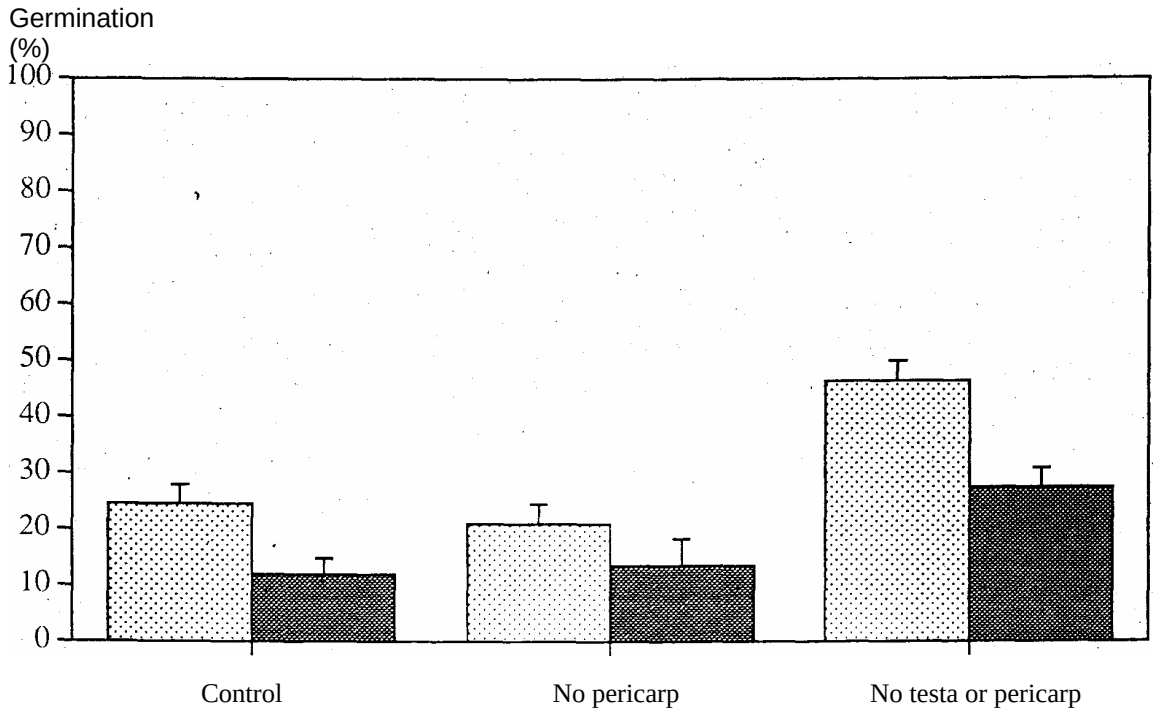

Figure 4: Germination of A. *helianthi* seeds with or without seed coverings- Seed was collected at Hawks Nest (light columns) or Allambie Heights (dark columns) in November 1992. Error bars shown are standard errors (mean).

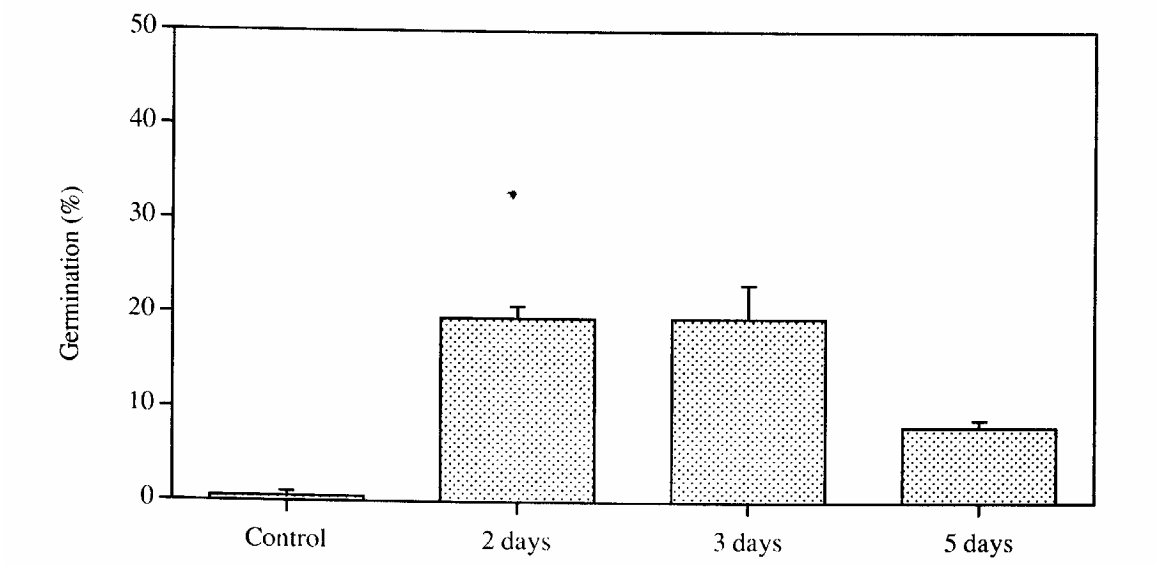

Figure 5: Germination *of A. helianthi* seed with the seed coverings (pericarp and testa) removed 2, 3 and 5 days after imbibition. Seed, was collected at Garigal National Park (Killarney Heights) in March 1993. Error bars shown are standard errors (mean).

Germination of intact (control) seeds was compared to that of seeds where either the pericarp (fruit coat), or both the pericarp and testa (seed coat), had been removed. Seeds were imbibed for 4 to 5 days prior to removal of seed coverings to facilitate removal and minimize damage to seeds. Four different seedlots were used in these experiments, representing, three collection sites. For three of these seedlots, both fresh (5-6 weeks old) and stored (10 month old) seed were used.

The results from two of the seedlots investigated are shown in Figure 4. As shown in this figure, removal of the pericarp had little or no effect on the final germination percentages obtained, but removal of both the pericarp and the testa significantly improved germination. Rates of germination, although not shown, were also markedly improved by removal of the testa. This indicates that the testa itself plays some role in the control of dormancy in this species, possibly through the restriction of gas exchange, or through the prevention of escape of some inhibitor contained in the embryo or endosperm (Bradbeer, 1988). Further research needs to be carried out to determine the exact nature of the block to germination located in the testa. However, it appears the effect of removing the testa is lessened the longer it remains on the seed after imbibition (Figure 5).

iv) Gibberellic Acid:

Gibberellic acid is effective in overcoming dormancy in the seeds of many species, including some umbelliferous species, particularly carrots and celery (Thomas *et al.,* 1972; Palevitch and Thomas, 1974; Biddington and Thomas, 1978; Thomas,. 1983; Thomas and Sambrooks, 1985; Gott and Thomas, 1986). Therefore it was decided to investigate the effect of this plant growth regulator on the seeds of A. *helianthi.*

Five different seedlots were tested using GA3 at a concentration of 400 ppm, with the seeds being soaked for 3-4 days before transfer to water. However, as there was only a small positive germination response shown by two out of the five seedlots tested, it was decided not to continue these experiments. Nicking the seeds to facilitate entry of the GA3 molecule may possibly have improved the germination response.

v) Seed Source and Time of Collection:

The site of collection appeared to have an important bearing on the subsequent germinability of flannel flower seeds, and although the actual germination percentage obtained varied considerably with each seedlot, some general conclusions can be drawn. The results for fresh seed

are shown in Table 1. Pour different collection sites within a radius of only several kilometres within the Sydney area yielded some good germinating seedlots (20-40% germination for fresh seed) and some very poor germinating seed lots (less than 10% even after several months dry storage). Seeds collected from South West Rocks (the most northerly collection point) were generally non-dormant, whereas seeds collected further south at Hawks Nest exhibited considerable dormancy, and seed from plants located at Macmasters Beach somewhat further south again on the Central Coast germinated quite freely, indicating that longitude was not necessarily a determining factor in the level of dormancy shown. Thus some sites (notably South West Rocks and Macmasters Beach), gave consistently better seed in terms of the germinability of fresh seed than other sites, but this difference did not seem to be related to differences soil fertility or other obvious environmental conditions. It may be related to inherent genetic differences between the populations of plants.

Generally speaking, seed collected late in the season (February/March) gave quite poor germination when compared to seed collected from the same site earlier in the same season, and this is probably related to the umbel order (later order umbels giving more dormant/less viable seed , and less seed per umbel). Differences in the dormancy and viability of seeds harvested from different umbel orders have been found for carrots and celery (Hawthorn *et al:*, 1962; Thomas *et al.*, 1978; Thomas *et al.*, 1979; Gray and Steckel, 1983; Jacobsohn and Globerson, 1980). The late harvested seeds of A. *helianthi* did not respond well to after-ripening, in contrast to the seeds from earlier umbels. Lower viability may be the causal factor.

There appeared to be little consistent difference detectable between the germination behaviour of immature (green) or mature (brown) seed, whether separated at or after collection.

Table 1**.** Germination of fresh (0-3 week old) seed at 20°C

Seedlot	Germination (%)		
	Green	Brown	Mixed
SR005+	21.5	19.0	-
SR006+	40.0	37.5	-
SR007	-	-	9.0
HN002*	4.0	1.5	-
HN003*	1.0	0	-

HN004*	3.0	4.5	-
MB004+	25.0	35.0	-
MB 005	. -	-	55.0
ALL004	-	-	0
ALL005	-	-	2.5
ALL006	-	-	1.5
KH001*	0.5	0	
KH002*	2.5	18.5	.
KH003	-	-	3.0
KH004	-	-	0
DP004	-	-	2.0
DP005	-	-	4.0
DP006	-	-	9.0
SWR002	-	-	65.0
RNP003	-	-	9.5

* Seed separated at collection

+ Seed separated after collection

Conclusions

Poor seed germination in *Actinotus helianthi* can be attributed to a combination of lowered viability due to embryoless and undeveloped seeds, and to seed dormancy. However, in the majority of cases, seed dormancy appears to be the overriding cause of non-germinability of freshly collected seed. The seeds of A. *helianthi* appear to undergo after-ripening in dry storage at 21°C, with the optimum duration of storage lying anywhere between 2 and 12 months. Further work to investigate the effects of temperature, light, and humidity on after-ripening are currently underway. The optimum temperature for germination of A. *helianthi* is between 15 and 20°C, with some germination occurring at temperatures between 10° and 30°C. Light promotes germination only at higher temperatures. The seed coat (testa) appears to play an important role in the control of dormancy in this species, but the exact nature of this role has yet to be elucidated.

In the wild, germination of flannel flower seeds would probably occur mostly during the autumn months when temperatures are lower, when more moisture is available for seedling growth, and

after the seeds have had time to after-ripen following their dispersal during late spring and summer.

Publications Arising from Project

To date, part of the findings of this project have been presented at the National Workshop for Australian Native Flowers, held in Queensland in February this year. This presentation was in the form of a poster, and included a paper published in the workshop proceedings (see enclosed copy). This project will form part of the requirements for a MScAgr at Sydney University, and it is hoped that further publications will be made at a later date, after submission of the thesis.

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Appendix 1

Sites and times of seed collection during the 1992/93 flowering season.

Location	Seedlot	Collection Date
Stony Range Flora Reserve, Dee Why	SR005	2.11.92
	SR006	.3.1.93
	SR007	17.3.93
Mungo Brush Rd, Hawks Nest	HN002	4.11.92
	HN003	16.12.92
	HN004	20.1.93
Bouddi National Park, Macmasters Beach	MB004	5.11.92
	MB005	20.1.93
Manly Dam Reserve, Allambie Heights	ALL004	14.11.92
	ALL005	17.1.93
	ALL006	17.3.93

Garigal National Park, Killamey Heights	KH001	24.11.92
	KH002	7'. 1.93
	KH003	25.2.93
	KH004	28.3.93
Garigal National Park, Forestville	DP004	29.11.92
	DP005	7.1.93
	DP006	25.2.93
Royal National Park, Mainbar	RNP003	15.1.93
Trial Bay Goal, South West Rocks	SWR002	21.1.93

Appendix 2

Percentage of seeds lacking an embryo (embryoless).or containing endosperm only partially developed or distintegrated (undeveloped). Values are given as means ± standard error (mean), (g) indicates green (immature) seed and (b) indicates brown (mature) seed.

Seedlot	Embryoless (%)	Undeveloped (%)	Total (%)
ALL004	0	10 ±0	10±0
ALL005	8 ±4.9	8 ±4.9	16 ±7.5
DP004	2±2	12 ±3.7	14 ± 2.4
DP005	2±2	8 ±3.7	10 ±4.5
HN002(g)	0	10 ±4.5	10 ±4.5
HN002(b)	2±2	18 ±8.6	20 ±10.5
HN003(g)	0	12 ±5.8	12 ±5.8
HN003(b)	0.	6 ±2.4	6 ± 2.4
HN004(g)	10 ±3.2	10 ±3.2	20 ± 3.2
HN004(b)	16 ±6.8	12 ±5.8	28 ±7.3
KHOO1(g)	0	4 ±2.4	4 ±2.4
KH001(b)	0	14 ±6	14±6
KH002(g)	0	10 ± 4.5	10 ±4.5
KH002(b)	2 ±2	20 ± 8.9	22 ± 8.6
KH003	4±4	12±5.8	16 ±5.1
KH004	12 ±3.7	10 ± 3.2	22 ±5.8
MB004	0	12 ±4.9	12 ± 4.9

SR005(g)	2±2 .	26 ±6.8	28 ± 6.6
SR005(b)	4 ±2.4	18 ±3.7	22 ±5.8
SR006(g)	2 ±2 '	10 ±4.5	12 ± 3.7
SR006(b)	12±4.9	10 ±3.2	22 ±5.8
SR007	22 ±5.8	4 ±2.4	26 ± 6.0
SWR002	20 ±5.5	14 ±4	34 ± 2.4
Range	0-22	4-26	4-34
Mean	5.2 ±1.4	11.7 ±1.1	17.0 ±1.3