Seed germination in the rare shrub *Grevillea kennedyana* (Proteaceae)

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Germination of fresh seeds of the rare arid zone shrub *Grevillea kennedyana* F. Muell. (family Proteaceae) is most successful at 10°C with light, and 15°C with or without light. The seeds were non-dormant and did not require further germination enhancing substances, such as smoke or fire to achieve > 80% success. These results do not support the role of fire in the management strategies suggested in the Recovery Plan for this species. *Grevillea kennedyana* seeds are orthodox and can be dried at 15°C and 15% RH and stored at 4 and -18°C for a short period. In relating the results of this trial to the germination requirements of *Grevillea kennedyana* in the field, it appears that successful germination will require low temperatures (i.e. < 15°C in winter) at the time of seasonal rainfall. Further studies are required to determine the cause of the poor seedling recruitment observed in the field.

Introduction

Grevillea kennedyana F. Muell. (Proteaceae) is a rare shrub species found only in a small number of populations in the far north-west of New South Wales (in Sturt National Park) and the south-western corner of Queensland, on the edge of the arid zone. It is a shrub growing to about 1.5 m high, with many erect branches and narrow grey leaves. The flowers are brilliant orange-red giving rise to the common name Flame Spider Flower. *Grevillea kennedyana* is listed as Vulnerable under Schedule 2 (NSW *Threatened Species Conservation Act 1995*) and coded nationally as 2VCa i.e. it is vulnerable with a natural habitat smaller than 100 km and considered adequately conserved (Briggs & Leigh 1996).

In Sturt National Park propagation and spread of *Grevillea kennedyana* plants within populations is by rhizomes, rather than by seed germination. No seedlings have been observed though seed is produced on the plants. The aim of this study was to investigate the viability of fresh and short-term, stored seed, and the optimal temperature and light requirements for germination of the species under controlled environmental conditions. From this we hope to to understand whether the species is capable of sexual reproduction and if so, what environmental cues are required for seed germination.

Most seed-propagated species have specific requirements that allow establishment in the field at the optimal time of year (Mott & Groves 1981). Successful seed germination requires several factors or environmental conditions such as temperature, moisture and light to be met simultaneously or sequentially. *Grevillea kennedyana* occurs in the arid region of Australia where rainfall is low and unreliable (climate data, Appendix 1). Many desert shrubs exhibit physical and physiological seed dormancies to ensure survival under these conditions (Baskin & Baskin 1998).

Methods

Collection and drying

Seeds from three populations of *Grevillea kennedyana* were collected in Sturt National Park on 11 November 2000. These collections were from population 1 (Three Sisters Hills), population 2 (Onepah) and population 3 (Olive Downs Escarpment). The seeds were collected by picking the whole, fully sized fruit (follicle) by hand. Many of the fruits on the plants had already split, and dropped their seed, others were still too small, with less developed, immature-looking seeds. These fruits were left on the plants. Heavy rain was experienced subsequent to collection and the harvested seeds may have been affected by fungal growth.

At Mount Annan Botanic Garden, the follicles were removed from the paper collection bags, spread on trays and placed into a dehumidifying room at 15°C and 15% relative humidity (RH). They were left to equilibrate under these conditions. Sufficient drying of the follicles was determined by repeated weighing until the mass did not further decrease. Ten seeds from Three Sisters Hills population were used to determine the moisture content. These were weighed after drying, placed into an oven (103°C for 17 hours) and re-weighed. The moisture content (%) was then calculated based on the dry weight. The number of follicles, and mature and immature seeds collected from each of the three populations were counted.

Seed germination — temperature and light requirements

After drying, 400 seeds from Three Sisters Hills (pop. 1) were separated from the main collection and grouped into eight lots of 50. To determine the optimal temperature requirements, four controlled environment growth cabinets (Lindner & May Pty Ltd) were set up at constant temperatures of 10°C, 15°C, 20°C and 25°C. Half of the seeds were exposed to 12 hours light and 12 hours dark (2 x NEC cool white 18W fluorescent tubes), while the other half were wrapped in aluminium foil for the duration of the trial to simulate dark conditions i.e. seeds buried under the soil surface.

Seeds were placed into 70 mm glass petri dishes containing agar substrate (8 g/L agar in de-ionised water) to a depth of half the dish. Agar rather than filter paper was chosen as the growth medium as it holds adequate moisture for the duration of the trial. Also, agar being translucent, allowed easy assessment of germination from both the top and the bottom of the dishes. Ten seeds were placed into each dish and each treatment was based on five replicate dishes. The experiment was set up on 10

January 2001 and completed on 21 February 2001. Seed germination was recorded daily during the early part of the trial and every second day after three weeks with seeds from the dark treatments being assessed under green, filtered light. Germination was recorded once the radicle reached 3–4 mm in length. After the first week, significant fungal growth had occurred on the seeds as well as a leaching of brown substances from the seeds into the agar. All the seeds were transferred onto new agar plates and a fungicide added (Thiram, 1 g/L in water).

Comparison of populations

Seed from the two other populations was placed in the most suitable growth cabinet environment (15°C with light) for assessment. Fifty seeds of the three seed collections were sown at 15°C with 12 hours light on 24 January 2001 and the trial concluded on 21 February 2001. Further germination or dormancy-breaking methods were not required as the seed had a high initial germination percentage.

Storage

Seeds were dried at 15°C and 15% RH and then packaged into vacuum-sealed laminated foil envelopes. Fifty seeds were stored in the freezer (-18°C ± 2°C), 50 were stored in the coolroom (4°C ± 2°C) and 50 remained in the air-conditioned laboratory at room temperature (20 ± 2°C). These were stored from 21 December 2000 until 9 January 2001. The seeds from each treatment were then removed from the foil envelopes and the moisture content was allowed to equilibrate at room temperature in the laboratory. They were sown in petri dishes as described above at 15°C with light to determine the effects of short-term storage.

Seedling establishment

Several days after germination, seeds were placed into 50 mm pots containing two parts sand and one part coir (v:v) with slow-release fertiliser added. These plants were subsequently placed into a temperature controlled greenhouse (21°C ± 4°C) and their growth observed for two months.

Data analysis

Percentage germination or mean days to germination were calculated for each petri dish (replicate unit). The treatment response was determined by averaging the replicate values within each treatment. Statistical analysis could not be conducted on the results of the first experiment as the temperature and light regimes could not be replicated. Germination results are therefore tabled and shown as a figure to indicate trends. An analysis of variance was conducted on the final germination values from the storage experiment.

Fig. 1. Seeds of *Grevillea kennedyana* approximately 9 mm long.

Results

Collection

There were two seeds in each follicle although in many instances only one seed was fully developed. Mature seeds were approximately 9 mm in length and dark brown in colour, with a pale coloured apex (Fig. 1). The immature seeds were distinctly shrunken in appearance. In the field and upon drying, the seeds were released from the follicle and simply dropped to the ground, as they do not possess a substantial wing. The moisture content of the seed after drying at 15% relative humidity and 15°C was 6.2%. A mature dried seed weighed approximately 38 mg.

Germination — temperature and light requirements

Germination of seed occurred in a range of light and temperature conditions (Table 1 & Fig. 2). The mean number of days to first radicle emergence was lowest at 15 and 20°C and germination was generally faster in light than in the dark at all temperatures. The germination percentage after 15 days in the controlled environment cabinets was greatest at 15°C in light. Germination was greater in the light than in the dark at 10 and 15°C but not at higher temperatures at this time. Final germination percentages were highest at 15°C (light and dark) and at 10 °C in the light (Fig. 2). Germination was slowest and lowest at 25°C.

Table 1. Grevillea kennedyana seed germination response to temperature and light.

	Incubation temperature							
	10°C		15°C		20°C		25°C	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Mean days to first germination	13.8	20.0	7.6	12.6	7.4	19.4	14.6	26.2
Germination (%) at 15 days	34	2	72	46	36	18	4	2
Final germination (%)	74	46	78	80	46	50	30	14

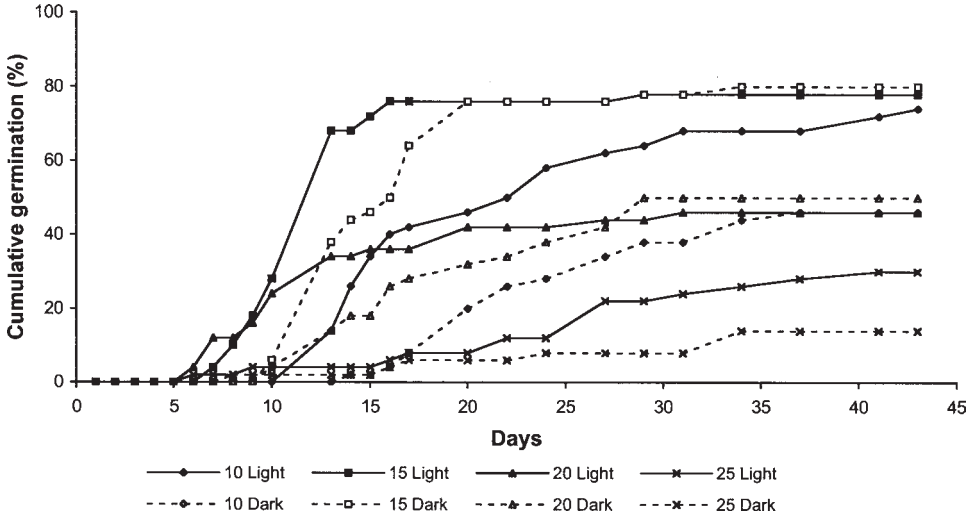

Fig. 2. Germination of *Grevillea kennedyana* seeds in four temperature and light regimes.

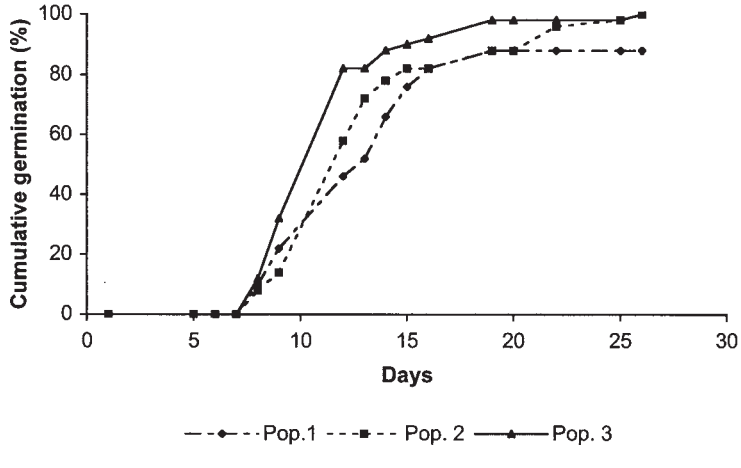

Fig. 3. Germination of *Grevillea kennedyana* seeds from three distinct populations (Pop. 1: Three Sisters Hill; Pop. 2: Onepah; Pop. 3: Olive Downs Escarpment) at 15°C with light.

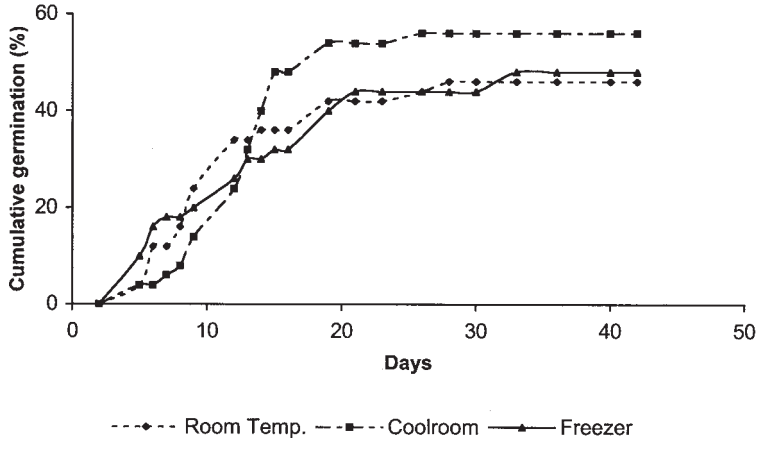

Fig. 4. Germination of *Grevillea kennedyana* seeds in 15°C and light after storage at room temperature, 4°C and -18°C.

Comparison of populations

There were no detectable differences in germination rate and final germination values between the three populations (Figure 3).

Storage

Seeds could be stored at 4°C (refrigerator temperature) and -18°C (freezer temperature) without loss of viability, when compared with seeds stored at room temperature in the short term, for up to 40 days (Fig. 4). Final germination percentages for seed stored at 4°C and -18°C were not significantly different ($P > 0.05$) from those stored at room temperature or from each other.

Seedling establishment

Initially, many of the seedlings exhibited chlorotic cotyledons when placed in the greenhouse. In most cases the seedlings outgrew this chlorosis, producing healthy green leaves. Where chlorosis was severe, the seedlings often died. This phenomenon requires further investigation.

Discussion

Forty-day-old seeds of *Grevillea kennedyana* exhibited a very high level of viability when incubated at the optimum temperature (15°C) indicating a species with non-dormant seeds, at least while they are 'fresh'. This suggests that the conditions for germination in the wild may not be optimal at the time of seed fall, in summer, when minimum mean temperatures are in excess of 15°C, and remain so until the next autumn. However some seed germination might be expected, especially if soil moisture is adequate, as seeds can germinate at higher temperatures. For large-scale regeneration seeds would need to persist in the soil seedbank for some months. The longevity of seeds in the soil seedbank needs to be investigated.

Studies on other *Grevillea* species has emphasised the role of fire in promoting germination, in terms of seed responses to smoke, heat and scarification. *Grevillea barklyana* (Vaughton 1998), *Grevillea buxifolia* subsp. *buxifolia*, *Grevillea diffusa* subsp. *filipendula*, *Grevillea juniperina*, *Grevillea linearifolia*, *Grevillea mucronulata*, *Grevillea sericea* and *Grevillea speciosa* (Morris 2000) are coastal *Grevillea* species which often show dormancy that is broken by smoke (Kenny 2000; Morris 2000). All had low initial germination results that improved with smoke and/or heat treatments. At this stage we do not see any reason to investigate the use of smoke stimulation on seeds of *Grevillea kennedyana*, as suggested in the Recovery Plan (NSW National Parks & Wildlife Service 2000). This species does not occur in a fire prone area (the last documented fire was in 1975) and does not experience the frequent fire regimes affecting coastal *Grevillea* species. If secondary dormancy is observed at a later time then smoke could be investigated along with other dormancy breaking treatments.

Seeds of *Grevillea kennedyana* can tolerate desiccation and be stored for at least short periods at low temperatures without loss of viability — they can be considered orthodox. This is typical of small seeds and those from arid environments (Wieland 1995). Storage allows a low cost effective method for short term ex situ conservation and provides material for further examination without having to disturb natural populations regularly. Repeated germination tests over several years are required to determine seed viability in storage over the longer term.

Germination may require a given amount of rainfall at a suitable temperature but seedling survival may require more significant rainfall. This combination of factors is particularly important in arid environments (Baskin & Baskin 1998). The germination results documented here were carried out with an adequate water supply — further work would need to be done to determine the limiting volume of water necessary for germination.

The absence of seedlings in the field may be due to grazing. *Grevillea kennedyana* seedlings may be vulnerable to grazing, particularly by rabbits (Duncan 1992) but though rabbit numbers have decreased significantly since the spread of the calici virus, there is still no evidence of seedling establishment (NSW NPWS 2000). Site monitoring may help to determine if seedlings are emerging and being grazed, or whether there are other causes for the lack of seedling recruitment.

Seed predation by insects, birds and mammals could also be an issue. Research carried out on the tropical *Grevillea pteridifolia* showed significant predation of seeds by ants (Majer & Lamont 1985), this could also be a contributing factor with *Grevillea. kennedyana*.

Seedlings attempting to establish in summer may be hampered by lack of soil moisture or extreme high temperatures. The severe chlorosis on the cotyledons of some of the cultivated seedlings may be a factor in seedling survival in the field. Seedling survival *per se* through to adulthood requires further investigation. Edaphic factors such as soil pH, moisture as well as climate and predatory behaviour should be considered along with soil seedbank persistence. The seedlings appear quite variable in leaf form; studies into the genetic variability of this species within and between populations may also be worthwhile to determine the amount of variability within and between populations. This could provide information on the extent of clonality in this species and whether recruitment of seedlings is taking place.

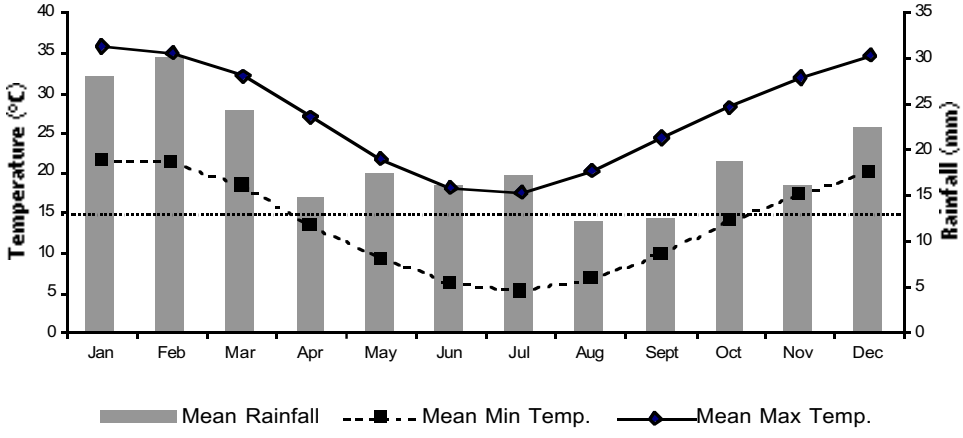

Fig. 5. Climatic averages for Tibooburra (1886–2000 Bureau of Meteorology 2001), showing optimal germination temperature 15°C (dotted line).

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