Germination and *ex situ* storage of *Hakea dohertyi* (Proteaceae) seed

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Abstract: Fresh seeds of the endangered *Hakea dohertyi* Haegi (Proteaceae) germinated at 15°C (with 12 hour light) within 14 days. At higher temperatures (20°, 25°C) seeds were slower to germinate. After 28 days only 5% of seeds germinated at 30°C, but when moved to 15°C, close to 100% of seeds germinated within 14 days. Having established optimum germination conditions, the effects of *ex situ* storage conditions and duration were examined. Storage at low moisture content appeared to have little effect on the germinability of *Hakea dohertyi* seeds and this species can be considered orthodox in that respect. Seeds stored at 4.5 or 9% moisture content, 5 or -20°C and tested after 1 and 7 years of storage achieved close to 100% germination. Issues relating to the *in situ* and *ex situ* conservation of *Hakea dohertyi* are discussed.

***Cunninghamia* (2003) 8(1): 129–132**

Introduction

There is a high degree of diversity and local endemism in the Proteaceae of the Sydney region (Myerscough et al. 2000). *Hakea dohertyi* Haegi, discovered in 1986, is one of a number of endangered endemic species occurring in the region that are threatened by land management changes (Fisher et al. 1995). It is an obligate seeding, bradysporous species mainly restricted to an 18 km^2 area around the Kowmung River in Kanangra Boyd National Park (Benson & McDougall 2000) (Figure 1). Less than 7000 individuals are found in this area and the disjunct Burragorang (Tonalli Cove) population, of less than 100 individuals could be threatened by changes in water level of the nearby Warragamba dam. Other threats to the species include very hot (>300°C) or prolonged fires (>3 min.) that kill seeds (Steenbeeke 1995), and goats may eat young plants (Benson & McDougall 2000). Juvenile and adult plants are killed by fire (Steenbeeke 1995) and this may affect the survival of such species in the event of a once-off high intensity fire that can kill both plants and canopy stored seed, or frequent low-level fires that kill juvenile plants (Myerscough et al. 2000).

Hakea seeds are generally non-dormant and begin to germinate in 15–100 days at temperatures between 15° and 28°C (Fox et al. 1987, Wrigley & Fagg 1989, Elliot & Jones 1990). Steenbeeke (1995) observed that fresh seed of *Hakea dohertyi* is very viable, demonstrating almost 100% viability in bench germination tests (temperature and light conditions were not specified). Seeds of this species stored in 'room conditions' lose viability over time; viability appears to drop after 1 year (Steenbeeke 1995) and seeds are dead after ten years in these conditions (M. Doherty pers. comm). Little else is known about the effect of storage conditions on the long-term viability of *Hakea* species *per se* (Hong et al. 1998) although the long viability of the canopy held seed (Steenbeeke 1995) indicates potential for successful long-term storage *ex situ*. This study was conducted to investigate the specific temperature requirements for germination of *Hakea dohertyi* seeds and to identify the *ex situ* storage potential of seeds of this species.

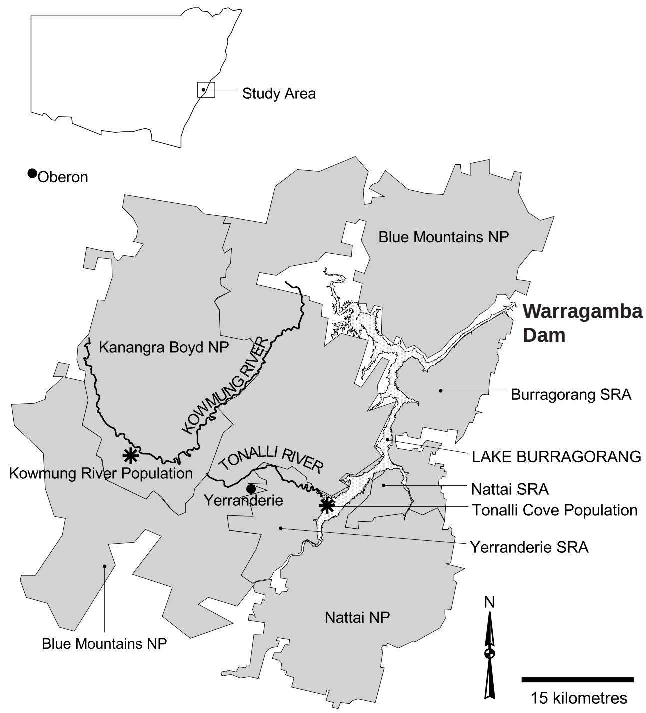

Fig. 1. Locations of *Hakea dohertyi* populations, in Warragamba catchment area, used in this study.

Methods

Seed collection and processing

Composite collections of seeds were made from a range of trees at the small Tonalli Cove population (Lake Burragorang) in June and December 1995 and from the larger Kowmung River population in May 1996 (Figure 1). No more than 10% of the seed held on the trees was collected at any time. The seeds were used fresh or were dried at room temperature and stored in the range 7–9% moisture content (MC), measured gravimetrically using the fresh weight. Some seeds from the Kowmung River were also dried over silica gel to 4.5% MC. The seeds dried to 4.5 or 9%M were then stored at 5°C or -20°C in sealed foil packets in the NSW Seedbank at Mount Annan Botanic Garden. These storage conditions were as close to the International Seed Testing Standard as possible (Cromarty et al. 1990). The following experiments were performed only on *Hakea dohertyi* seeds from the Kowmung population because of the larger number of seeds collected from that site.

Optimum temperature for germination

Freshly collected seeds were sown onto a sheet of Whatman's No. 1 filter paper over a bleached circle of terry towelling material to maintain moisture (distilled water) close to seeds. There were 80 seeds for each treatment split between eight 70 mm petri dishes. Temperatures of 15, 25 and 30°C were supplied by growth cabinets (Lindner and May Pty Ltd) and all seeds received 12 continuous hours of light in a diurnal period. Seeds were monitored daily for germination, deemed to have occurred when 2 mm of radicle had emerged.

Seed Storage requirements

After one and seven years of storage, seeds from the four treatments (4.5 or 9% MC and 5°C or –20°C) were subjected to optimal germination conditions determined in the first experiment, viz. eight replicate dishes of ten seeds per treatment were placed randomly in a 15°C controlled temperature cabinet (12 hr light). Prior to the commencement of germination conditions, seed packets were opened and seed moisture was allowed to equilibrate in room conditions for 24 hr, to avoid imbibition injury (Ellis et al. 1985). Seed germination was recorded as before.

Table 1. Effects of storage conditions (temperature and seed moisture content [MC]) and duration of storage on germination of *Hakea dohertyi* seed (± s.e.)

Years of storage	Storage conditions 5°C		-20°C	
	4.5% MC	9% MC	4.5% MC	9% MC
First germination (mean days)				
1	13.9 ± 0.3	13.0 ± 0.0	13.2 ± 0.2	12.9 ± 0.2
7	12.2 ± 0.2	12.2 ± 0.2	12.4 ± 0.3	12.0 ± 0.0
50% germination (mean days)				
1	15.6 ± 0.4	14.7 ± 0.5	15.2 ± 0.4	15.9 ± 0.3
7	14.1 ± 0.3	14.2 ± 0.3	14.4 ± 0.2	14.2 ± 0.3
Final germination %				
1	97.5 ± 1.6	100.0 ± 0.0	100.0 ± 0.0	97.5 ± 2.5
7	98.7 ± 1.2	96.2 ± 2.6	98.7 ± 1.2	100.0 ± 0.0

Results

Climatic regime

The climate in the area is characterised by average maximum summer temperatures of between 20° and 25°C and winter minimums less than 5°C and down to -2°C (figure 2, Bureau of Meteorology 2002). Rainfall is up to 170% higher in summer than in winter, with a monthly average of between 40 and 100 mm.

Optimum temperature for germination

Germination proceeded most rapidly at 15°C (Figure 3). Maximum seed germination occurred within 14 days at this temperature, while seeds at 25°C took more than 28 days to achieve maximum germination. At 30°C germination proceeded very slowly with less than 5% of seeds germinating in the first month. When seeds were moved from 30°C to 15°C, germination proceeded relatively rapidly, with the majority of seeds germinating after two weeks at the lower temperature.

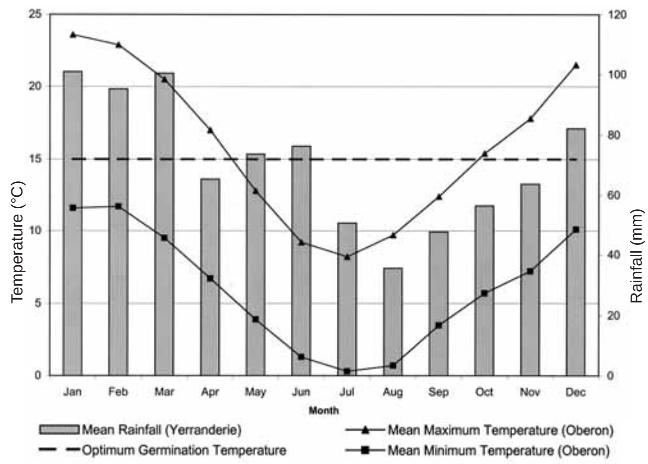

Fig. 2. Climatic averages for the closest available meteorological stations to *Hakea dohertyi* (temperature, Oberon 1991–2002; rainfall, Yerranderie 1908–1979).

Seed storage requirements

The tested storage conditions and duration appeared to have little effect on seed germination of *Hakea dohertyi* (Table 1). The number of days to first germination appeared to have been slightly slower when compared with data from the optimisation experiment (not statistically tested), but 50% germination was achieved within 16 days, and final germination percentages were at or near maximum.

Discussion

This work confirms the Steenbeeke (1995) observation that canopy stored seed of *Hakea dohertyi* has high viability. The seed germination pattern of *Hakea dohertyi* under different temperature regimes is similar to other woody Grevilleoideae species including *Hakea* (Fox et al. 1987, Lamont & Groom 1998). The high germinability of seeds at 15°C, compared with the other temperatures, shows that this temperature is within the optimum range for laboratory germination, and indicates that seeds are most likely to germinate *in situ* during times of moderate temperature when moisture is available for germination and seedling establishment. This 'temperature sensing' strategy is typical of many species from the Mediteranean climatic regions of Australia (Bell 1999). Steenbeeke (1995) predicted that most germination would occur in the cooler months from May to August but observed that seeds are dropped at all times of the year, due to follicle opening as a result of secondary thickening (this species is not strongly bradysporous as it holds some but not all of its seeds on the plant for extended periods). Although rainfall in the area where *Hakea dohertyi* grows is generally lower during winter and early spring than in summer and autumn

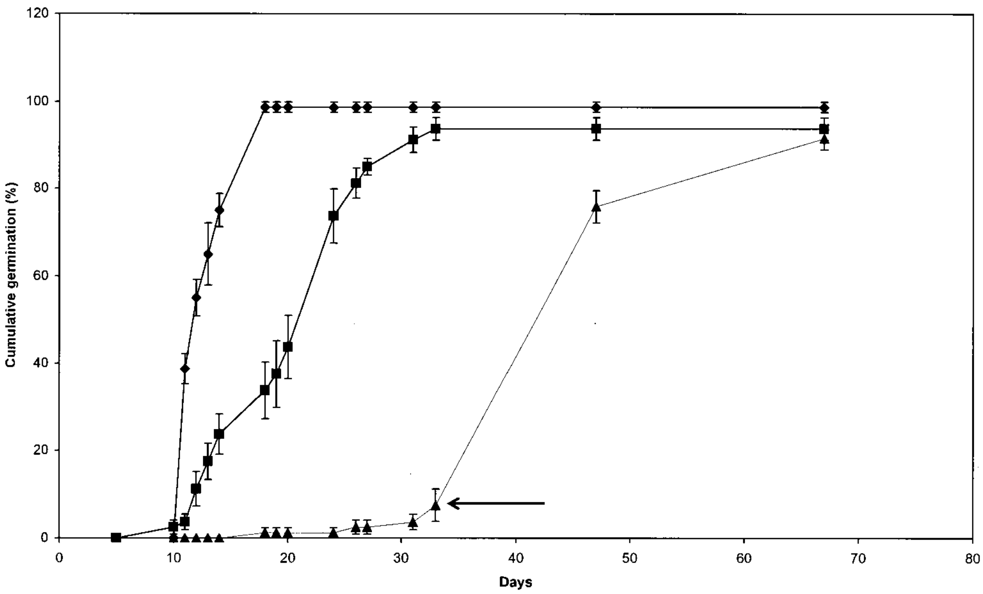

Fig. 3. Effect of temperature on germination of freshly collected seeds of *Hakea dohertyi*. ◆ 15°C, ■ 25°C, ▲ 30°C. Arrow indicates that seeds in the 30°C treatment were moved to 15°C on day 34. Vertical bars represent standard errors of the means.

(Figure 2), average rainfall even in the driest month (August) exceeds 35 mm. As a result, given lower evaporation rates than in summer, sufficient moisture may be available for germination. It is less likely that dehisced seeds of *Hakea dohertyi*, would germinate during hot summer conditions (>25°C) when they may quickly lose viability, be predated or rot in wet or humid conditions.

Hakea dohertyi is listed as an Endangered Species under the NSW *Threatened Species Conservation Act* 1995 and an understanding of its fire ecology is essential for *in situ* management. The last recorded fire affecting *Hakea dohertyi* populations in the region was in 1957, but little is known about its effects. Steenbeeke (1995) observed that some plants appeared not to have been affected by this fire and that across populations there was an uneven spread of age classes resulting from recruitment from dehisced seed in the absence of fire. *Hakea* species differ in the degree to which they retain seed in the canopy (Lamont & Groom 1998), but the survival of seeds within the fruit following fire is greater as fruit size increases (Bradstock et al. 1994). Steenbeeke (1995) found that seeds of *Hakea dohertyi* are killed when fruit are subjected 300°C for only 1 min making canopy-held seed of this species very vulnerable to fire. While there is potential for seedling recruitment from spontaneous leakage of bradysporous seed, young plants of such species may be predated (Bradstock 1985 quoted from Myerscough et al. 2000) and are vulnerable to fire (Steenbeeke 1995). The greatest potential threat to populations of *Hakea dohertyi*, and other bradysporous obligate-seeding species, is local extinction from a series of frequent fires, when fire intervals are less than the juvenile period (Gill & Bradstock 1995). In *Hakea dohertyi* the juvenile period is not less than 5 years (Steenbeeke 1995). Recruitment of seedlings after any fire is unpredictable due to variability and unpredictability of post-fire weather conditions.

Given that there is adequate moisture, temperature appears to be the main factor controlling germination of *Hakea dohertyi* seeds. This species appears typical of many other bradysporous Grevilleoideae in that their seeds do not display physical or physiological dormancy (Myerscough et al. 2000) and, as with other species with canopy-stored seed, probably have only a small or no soil-seedbank (Baskin & Baskin 1998). Canopy stored seed of some species such as the South African *Protea compacta* may exhibit dormancy on release from the infructescence (Brown 1993, Dixon et al. 1995)(such dormancy was previously not recognised by authors such as Bell et al. (1993), possibly because of the age of the seed, which was no longer dormant, or some other unrecognised factor). There is a possibility that the seeds used in this experiment have been smoke-primed *in situ* or that the seed is no longer dormant due to the length of time it has been on the plant. We propose that smoke priming may take place in many species in the Sydney region that are not directly affected by fire, due to the levels of dormancy-breaking substances contained in the air following major fire events in and around the region fulfilling this function. Confirmation of this proposal is required by monitoring fire events and stored-seed germination responses, although canopy stored seed is less likely to be as affected as soil seed bank stored species (Lamont & Groom 1998). It would also be useful to investigate the effects of various combinations of heat shock and smoke on a wider range of Proteaceae species of the Sydney region as these factors have been shown to greatly affect germination patterns of seeds of some species (Edwards & Whelan 1995, Kenny 2000, Morris 2000).

Hakea dohertyi has orthodox seed storage behaviour, that is, by the definition of Roberts (1973), it can withstand desiccation and can therefore be stored *ex situ*. This storage behaviour is an advantage for long-term storage of seeds for conservation or other purposes. Storage of seeds in a desiccated condition appeared to have only a small effect on the speed of germination, when compared with fresh seed, and did not decrease viability of seed, as distinct from storage under room conditions. While the results indicate a slightly decreased speed of germination after 7 years, we are cognizant of the inherent difficulties when conducting longitudinal studies, even when using essentially identical treatments (Morrison & Morris 2000). Taking comparison differences into account, it is still clear that seed of this species remains viable after at least seven years of storage at a range of moisture contents and temperatures. That viability did not drop dramatically from the time of collection to the 7-year test indicates that this species may well remain viable for many more years under quite simple storage conditions. Indeed, Steenbeeke observed more than 80% viability of 15-year-old canopy-stored seeds of *Hakea dohertyi* indicating the potential for very long *ex situ* storage periods. Seeds may be dried to between 4.5 and 9% moisture montent, and stored in a domestic fridge (~5°C) or freezer (~-20°C), preferably the latter as this will give the best storage life in the long term. Although not specifically tested for *Hakea dohertyi*, our experience with many other species is that the low seed moisture levels must be maintained over time. It is obvious that moisture content within the range tested would be adequate, but for long-term storage, the moisture content should be 5 ± 1% (Cromarty et al. 1990). Maintenance of moisture content is most easily achieved by storage in a hermetically sealed container or, as in this case, in vacuum heat-sealed foil packets routinely used at the NSW Seedbank.

While it is possible to store *Hakea dohertyi* seeds, *ex situ* seed populations cannot substitute for natural populations. Translocation of threatened species is not a highly successful conservation strategy (ANPC 1997, Hogbin 2002). Seed-banking of endangered species is only a minor conservation measure, in terms of maintaining diversity of natural populations. Rather, it gives us the opportunity to research, display and educate on the importance of *in situ* conservation. Fortunately, at this time, there is little evidence that this species is greatly threatened by current *in situ* management practices but an intense or frequent fire regime, would be seriously detrimental to the survival of *Hakea dohertyi*.

Acknowledgements

We wish to acknowledge Australian Water Technologies, as well as members of staff at Mount Annan Botanic Garden, particularly Patricia Meagher and Joanne Tyler. Also, Greg Steenbeeke for generous help with information on this species.

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Manuscript accepted 16 June 2003