Seedling survival, soil seed bank status and disturbance response of the critically endangered *Grevillea maxwellii* McGill (Proteaceae)

Final Report to Bankwest Landscope
Visa Conservation Card Trust Fund
February 2003

Anne Cochrane and Sarah Barrett
Department of Conservation and Land Management
120 Albany Highway
Albany, Western Australia 6330

Seedling survival, soil seed bank status and disturbance response of the critically endangered *Grevillea maxwellii* McGill (Proteaceae)

SUMMARY

This project investigated certain ecological and recruitment parameters of the critically endangered Western Australian shrub, *Grevillea maxwellii* McGill.

The soil stored seed reserve under 10 dead and 10 live plants was investigated by taking forty 15 x 15 x 2cm soil samples from each of two sites (Vacant Crown Land and private property). There was an irregular distribution of the soil stored seed bank, with low numbers (less than 20 seed per m^2) of highly viable seed (100% germination) were found within the soil seed bank at the VCL reserve site. At the private property, no evidence of a soil stored seed bank was found.

Three treatments (fire, raking and aqueous smoke application) and a control were used to assess the species response to disturbance at the VCL site. There was a significant germination response to burning (38 seedlings in total). Disturbance by raking and application of smoked water (as Regen 2000 @ 100ml/m^2) produced a negligible recruitment (1 and 4 seedlings respectively). No seedlings emerged within the control (no treatment) plots. Survival of seedlings was low over the nine-month monitoring period (39% survival for fire generated seedlings and no survival for seedlings generated from raking or smoke application). Plant deaths occurred between October 2002 and February 2003. It would appear that drought conditions were the main cause of seedling mortality. However, herbivore grazing also contributed to mortality in burnt plots.

One hundred and seventeen seedlings in five 5 x 5 m quadrats, estimated to be less than 2 years old, were monitored for survival over a 14-month period encompassing two summers (November 2001 to February 2003). At this site, 16.2% of seedlings survived. Initial seedling deaths in autumn 2002 appeared to be due to herbivore grazing of young growth however, subsequent recovery of apparently dead seedlings suggests that drought was also a significant factor Mortality over the summer of 2002-2003 appeared to be primarily due drought conditions as there was little evidence of grazing. It is possible that this seedling recruitment, noted in November 2001, was a product of exceptionally heavy rains in January 2000 (341mm), with more than 3 times the average rainfall recorded for that month (102mm, based on records collected between 1968 and 2001). We hypothesise that there is a possible link between seed germination and episodic rainfall events.

This report recommends further study to quantify the effect of grazing and poor moisture relations on recruitment and survival of plants, in addition to continued monitoring of seedling recruitment, growth and survival over the long term.

INTRODUCTION

Grevillea maxwellii (McGill) was first collected by James Drummond from the Pallinup area of Western Australia (34° 17' 00"S 118° 26' 00"E) in 1840. It is a small, spreading shrub to just over 1 m tall and 1.5 m across. The leaves of *G. maxwellii* are pinnate, up to 7.5 cm long, with three to six lobes, each of which are divided into three smaller lobes. Large red flowers are produced in August and September, and are borne at the end of branchlets that usually curve downwards. The flowers are often found sheltered beneath the foliage giving the plant an attractive layered appearance (Brown *et al.* 1998). Individual plants may vary in form from erect to prostrate, and foliage colour can range from ashy grey-green to dark green. Flowers may be prominent on some plants and hidden in others. *Grevillea maxwellii* is related to *G. asparagoides*, *G. batrachioides* and *G. secunda*. *G. asparagoides* and *G. batrachioides* differ in their longer pistil and saccate perianth. *G. secunda* has no glandular hairs on the upper perianth surface and no edge veins visible on the upper surface of the leaf lobes (Olde and Marriott 1995).

Grevillea maxwellii grows in low open heath in shallow brown loamy soil over granite on rocky hilltops and slopes to the Pallinup River east of the Stirling Range. Plants become rarer as the soil depth increases (Robinson & Coates 1995). They grow in association with species such as *Calothamnus quadrifidus*, *Hakea lissocarpha*, *Allocasuarina huegeliana*, *Agonis spathulata*, *Calectasia grandiflora*, *Hakea marginata*, *Hypocalymma angustifolium*, *Allocasuarina campestris*, *Xanthorrhoea platyphylla*, *Stypandra glauca*, *Melaleuca uncinata*, *Eucalyptus pleurocarpa*, *Borya sphaerocephala*, *Dryandra nivea*, *Daviesia flexuosa*, *Kunzea recurva*, *Gastrolobium spinosum* and *Phyllanthus calycinus*. The species is likely to be pollinated by nectarivorous birds (Olde & Marriott 1995), although Obbens suggested mammal pollination a possibility (Obbens 1997). Insects and bird predation of fruits is high (personal observation A. Cochrane).

Grevillea maxwellii was declared as Rare Flora in September 1994 and was ranked Critically Endangered (CR) in September 1995. It currently meets World Conservation Union (IUCN 1994) Red List Category 'CR' under criteria B1+2ce due to the species being known from one location, and a continuing decline in both the area of habitat and the number of plants. The main threats are drought, weeds and inappropriate fire regimes.

MATERIALS AND METHODS

Soil Seed Bank

Investigations into the density of soil-stored seed of *G. maxwellii* were undertaken in November 2001 at two of the known populations (Vacant Crown Land (VCL) Barrs Rd. and on the private property "Yarramunda"). Soil samples (15 cm x 15 cm x 20 mm) were collected from beneath the canopy of twenty randomly selected plants per population and adjacent to either living or dead adult plants. Two samples were taken from around each plant, giving a total of 40 samples per population. These soil samples were air dried and passed through a series of different sieves. Gravel and larger rocks and litter were fractioned off, removed and soil aggregations broken up until two fractions remained above and below the normal size range of *G. maxwellii* seeds. The fraction below the size range was discarded; the remaining fraction was examined for *G. maxwellii* seeds. A laboratory assessment of the level and rate of germination for seeds retrieved from the soil-stored seed bank was made using

protocols previously proven to be the most successful (A. Cochrane unpublished data). Seeds were pre-treated with smoked water for 24 hours, manually scarified with a sharp scalpel knife and placed on a 1% w/v agar solution containing 25mg/ L^{-1} of gibberellic acid (as GA_3) in covered Petrie dishes. Seeds were incubated at 15°C with 12 hours of alternating light and darkness.

Seedling survival

Five 5m x 5m plots were established at "Yarramunda" property (private property of Peter and Jane Crossing) in November 2001 amongst recent seedling recruitment. It was possible these seedlings had germinated in response to unusually high summer rainfall the previous year (Figure 1). Plots contained either one or two live or dead adults plants or plants were recorded as being immediately adjacent to the plots (ie within 1-2m of plot boundary). Seedling numbers ranged from 10 in Plot 2 to 34 in Plot 1. Each seedling was numbered and marked with a metal tent peg and measured. The growth stage of each seedling (whether seedling or juvenile leaves were present) was recorded.

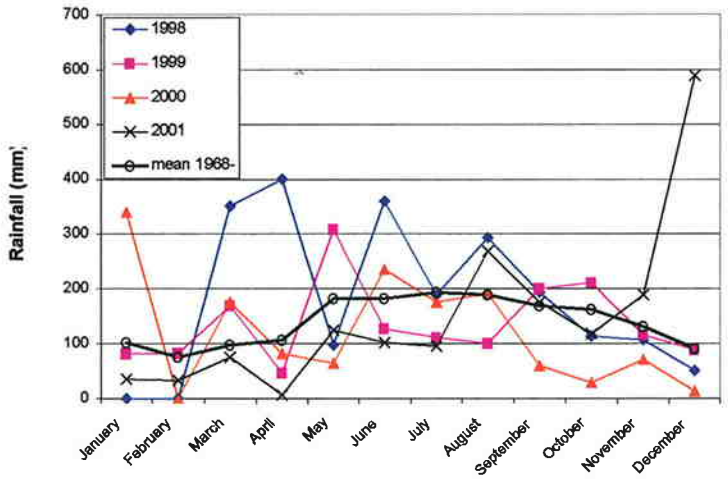

Figure 1. Monthly rainfall (mm) for "Yarramunda" property 1998-2001 and mean rainfall (mm) for the period 1968-2001.

The role of disturbance in stimulating germination of the soil stored seed reserve

Experimental plots were established on VCL at Barrs Rd. in May 2001 to determine the effect of disturbance (smoke treatment, raking and fire) on the population dynamics of *G. maxwellii*. Each plot was centred on a single plant of *G. maxwellii*. Five live and five dead plants were used in each of a control and two treatments (smoke application and hand raking); five dead plants were used for the fire treatment. A 2m x 2m area was delineated around each of the 35 plants as the experimental plots. The canopy size (width of the canopy at the widest point and the width of the

canopy at 90° to the widest point) of each plant in the 35 experimental plots was measured prior to treatment (Table 1).

Table 1. Canopy width measurements of dead and live plants from 35 experimental plots prior to treatment.

	Canopy Width (cm) *Mean (range)*	*Canopy Width 90° (cm)* *Mean (range)*
Dead plants (n=15)	116.5 ± 6.5 (67-160)	92.7 ± 6.3 (44-134)
Live plants (n=20)	92.0 ± 6.3 (59-150)	77.5 ± 6.6 (46-138)
All plants (n=35)	106.0 ± 5.2 (59-160)	86.2 ± 4.7 (46-138)

On May 20th 2002 between 10.30am and 12 noon, five experimental plots were subjected to a moderately hot autumn burn for roughly five minutes per plot. Prior to ignition an area of approximately 1m around each plot was raked free of litter and plants to minimise the encroachment of fire on the surrounding bushland. Approximately six ignition points were lit per plot. The air temperature on the day was between 18 and 18.5°C with only a light breeze that ranged between 10-16kph coming from the NW to NNW. Relative humidity ranged between 46% as the first plot was ignited to 35% by the ignition of the last plot. Each plot was assessed to have sustained between 95-99% burn with flame heights varying between 0.5-0.7m (plot 1) and up to 1.5m high (plot 3). Each fire was left to die down for approximately one hour before personnel left the site. Two District staff, two volunteers and one research staff were on site at the time with one light fire vehicle on standby. Ten plots (5 dead and 5 live) were treated with an aqueous smoke solution (Regen 2000) at 100ml/m^2. Each of these smoke treated plots was pre-treated with a soil wettener (Wettasoil^{HG} by Garden King) at 5ml/m^2 to ensure even penetration of the smoke solution.

Fire applied to dead plants of G. maxwellii *on May 20, 2002*

Burn plot immediately after fire was applied, May 20, 2002

Ten plots (5 dead and 5 live) were hand raked to bare mineral soil using a rake hoe. Controls were left untreated.

Monitoring period

Monitoring of seedling survival and growth in plots at "Yarramunda" occurred over a 14 month period between 30/11/2001 and 05/02/2003. Monitoring of recruitment after treatment at Barrs Rd. Reserve occurred over a 7.5-month period between 15/08/2002 and 05/02/2003.

Monitoring seedling survival at "Yarramunda", 11 October 2001

RESULTS

Soil Seed bank

A total of 17 whole seeds were recovered from the 40 soil samples from VCL at Barrs Rd. (Table 2). Three seed were found beneath live plants; 14 seed was found beneath one dead plant, attesting to the fact that the soil stored seed reserve is not distributed evenly within the habitat of *G. maxwellii*. No seed was retrieved from beneath plants at "Yarramunda". A few samples contained seed fragments and shrivelled seed attesting to a proportion of the seed crop being either predated and/or aborted.

Table 2: Total number of whole seed of G. maxwellii *retrieved from forty 15 x 15 x 2cm soil samples taken from adjacent to 10 live and 10 dead adult plants at VCL Barrs Rd. Seed viability determined by laboratory germination trials under optimal conditions.*

Total no. of seed	17
Mean no. seed in 15cmx15cm sample	0.425
Seed viability	100%

Full germination was attained for all retrieved soil-stored seed. First germination commenced at 10 days with final germination achieved after 44 days (Figure 2).

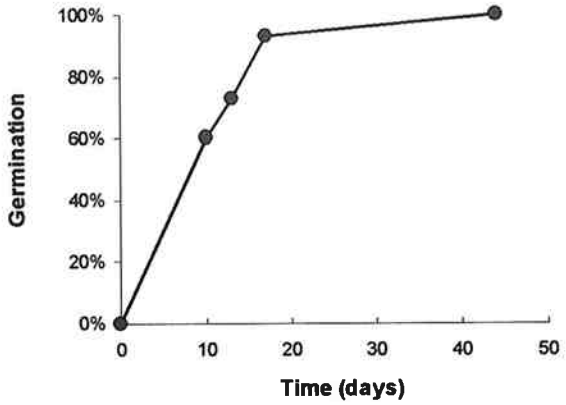

Figure 2. Percent germination over time in days of seed retrieved from soil seed bank of Grevillea maxwellii *at VCL, Barrs Rd.*

Seedling survival

Seedling survival at "Yarramunda" was monitored for 14 months from 30/11/2001 to 05/02/2003. Percentage survival after the first summer was 53% in April 2002 and this had decreased to 16.2% by the end of the study in February 2003 (Table 3). Grazing of seedlings was noted on 30.6% of seedlings in the five monitoring plots but this did not necessarily affect subsequent seedling survival as several seedlings considered to be dead due to grazing had recovered by August 2002. Deaths in the second summer (2002-2003) were attributed primarily to drought, as there was little evidence of grazing. The mean height increment for the 19 surviving seedlings over the 14-month period was 2.7 cm (Table 4); at least 42 % of these individuals had been grazed the previous year. Four of the surviving seedlings showed negative growth over this period, three of these had been grazed while the fourth had been physically damaged.

Table 3: Number of seedlings and percent survival of seedlings of G. maxwellii *in five 5 x 5m plots monitored at "Yarramunda" over the 14 month period from 30/11/2001 to 05/02/2003*

Plot No.	No. of seedlings present at monitoring					% survival
	30/11/2001	10/04/2002	15/08/2002	11/10/2002	05/02/2003	
1	34	19	17	17	7	20.5
2	10	2	2	2	2	20
3	16	11	11	11	2	12.5
4	25	7	7	7	2	8
5	32	28	19	18	6	18.7
Total	**117**	**67**	**56**	**55**	**19**	**16.2**

Table 4. Mean height increments of seedlings of G. maxwellii *in five 5 x 5m plots monitored at "Yarramunda" over the 14 month period from 30/11/2001 to 05/02/2003*

Plot No.	Mean height increment (cm)
1	4.4
2	2.7
3	6.7
4	-0.6
5	0.5
Overall mean (N=19)	**2.7 ± 0.8**

The role of disturbance in stimulating germination of the soil stored seed reserve

Seedlings were first recorded on 15/08/2002 in the burn plots, three months after treatment (Table 5). No recruitment was recorded in the other three experimental plots at this point. Further monitoring occurred on 11/10/2002 on which date further seedlings were noted, in particular in the burn plots. Four seedlings were recorded in smoke plots and one seedling in a rake plot. A number of seedlings were noted just adjacent to the burnt plots, indicating a transferred effect of the heat (or smoke). No grazing was noted on any seedlings at Barrs Rd. VCL in October 2002. Final monitoring on 05/02/2003 a 65% overall reduction in total seedling numbers since the previous monitoring in October 2002. No seedlings in raked or smoked plots had survived. Considerable grazing (86% of surviving seedlings) had occurred in burnt plots but was not apparent in the dead seedlings in the raked and smoked plots.

Table 5. Total number of seedlings of G. maxwellii *emerging within five 2m x 2m plot for live and dead plants in each treatment at Barrs Rd. Reserve between 15/08/2002 and 02/05/2003*

Treatment	Date of monitoring					
	15/08/2002		11/10/2002		02/05/2003	
	Live	Dead	Live	Dead	Live	Dead
Control	0	0	0	0	0	0
Smoke	0	0	3	1	0	0
Rake	1	0	0	0	0	0
Burn		22		38		15
Total	**23**		**42**		**15**	

DISCUSSION

This current research has shown that *Grevillea maxwellii* maintains a small, but patchy, soil-stored seed reserve in at least one of the sites studied and that this seed bank is highly viable. We can offer no explanation as to why there was no soil-stored seed reserve found at the "Yarramunda" site. The stimulation of the seed reserve is maximised with the application of fire, and seedling survival is likely to be linked to herbivore grazing and summer drought.

Edwards and Whelan (1995) also reported the presence of a small soil-stored seed bank under the canopy of *Grevillea barkylana,* with mean seed densities estimated at 10.9, 14.1 and 4.3 seeds per m^2 for three sites in New South Wales. No seeds were retrieved from outside the canopy area of the plants studied. These seed densities are smaller than those reported during this present study (<20 seed per m^2).

The soil-stored seed reserve of species in the genus *Grevillea* is known to respond to one or more fire-related cues (Kenny 2000; Morris 2000). At Barrs Rd. VCL, fire certainly provided the most convincing stimulus for seedling emergence with a significant increase in germination in burnt plots over the control, smoke or raked plots. Heat associated with fires is an important mechanism for releasing dormancy in many species (Fenner 1992) and the role of heat in the induction of seed germination of many Western Australia fire-following plant species is well documented (for example see Bell *et al.*1993; Bell 1998). Heat acts to fracture the seed coat of hard-seeded and other species (Bewley and Black 1994).

The application of smoke in either aqueous or aerosol form has also been shown to increase stimulation of germination in species from Mediterranean fire-prone environments (for example see Brown and van Staden 1997; Keeley and Fotheringham 1998; Roche *et al* 1998), although whilst reviewing recent literature on germination in Australian species, Bell (1998) noted that a number of studies reported that smoke-induced seed germination was less successful than heat in the induction of germination. Our results also show that smoke treatment did not stimulate germination of the soil-stored seed to such a degree as heat from fire, and only four seedlings emerged in plots treated with aqueous smoke over the nine months of monitoring. None of these survived summer drought conditions.

At Barrs Rd. VCL, at least 75% of recently dead and 86% of live seedlings exhibited signs of grazing, although the prime factor that contributed to low seedling survival after recruitment arising from experimental manipulation is not clear. Herbivore grazing or drought conditions or a combination of these factors may have been responsible. The burnt plots may have been preferentially grazed due to their open nature and the increase in nutrient loads after fire, but insufficient numbers of seedlings preclude statistical analysis.

G. maxwellii is able to regenerate from seed in the absence of any noticeable disturbance as indicated by seedling emergence at "Yarramunda". It is possible that the recently noted germination of seed may have been linked to episodic rainfall events. Rainfall data collected on site at "Yarramunda" for the period between 1968 and 2001 (attached as Appendix 1) shows the mean January rainfall at 102mm. In January 2000, 341mm of rain was recorded. Germination of seed of many native species is geared towards avoiding moisture stress. Unseasonally heavy rains may trigger recruitment outside the normal season of germination. In addition, large episodic rainfall events are likely to be associated with temperature changes that may have triggered germination.

Between November 2001 and April 2002, loss of seedlings at "Yarramunda" was mainly attributed to herbivore grazing (presumably kangaroos). However, drought is also likely to have been a significant factor in seedling mortality as several apparently dead and grazed seedlings recovered following winter rains. Little to no grazing was

noted between May 2002 and February 2003. Overall seedling numbers remained reasonably stable between May 2002 and October 2002 with 82% survival during that period. The major decline in seedling numbers occurred between October 2002 and February 2003 and seedling death during this time is attributed to drought conditions.

RECOMMENDATIONS

Further research is recommended to i) to quantitatively determine the effect of grazing on recruitment and survival of plants by means of herbivore exclusion cages erected around *G. maxwellii* seedlings; ii) to establish the effect of poor moisture relations on recruitment and survival of plants by watering of seedlings of *G. maxwellii* over the summer months, in particular when drought conditions prevail. Ongoing monitoring of seedling regeneration and seedling growth and survival should be considered a priority.

REFERENCES

Bell, D.T., Plummer, J.A. and Taylor, S.K. 1993. Seed Germination Ecology in Southwestern Western Australia. *The Botanical Review* **59**, 1, 25-73.

Bell, D.T. 1998. The process of germination in Australian species. *Australian Journal of Botany* **47,** 4, 475-517.

Bewley, D.J. and Black M. 1994. *Seeds. Physiology of Development and Germination.* Plenum Press, New York.

Brown, N.A.C. and van Staden J. 1997. Smoke as a germination cue: a review. *Plant Growth Regulation* **22**, 115-124.

Brown, A., Thomson-Dans, C. and Marchant, N. (Eds). 1998. *Western Australia's Threatened Flora*. Department of Conservation and Land Management, Western Australia.

Edwards, W. and Whelan R. 1995. The size, distribution and germination requirements of the soil-stored seed-bank of *Grevillea barklayana* (Proteaceae). *Australian Journal of Ecology* **20**, 548-555.

Fenner, M., 1992. *Seeds. The Ecology of Regeneration in Plant Communities.* Cab International, Oxon.

International Union for the Conservation of Nature 1994. *IUCN Red List Categories.* IUCN Species Survival Commission, Gland, Switzerland.

Keeley, J. E. and Fotheringham C. J. 1998. Smoke-induced seed germination in California chaparral. *Ecology* **79,** 7, 2320-2336.

Kenny, B. 2000. Influence of multiple fire-related germination cues on three Sydney Grevillea (Proteaceae) species. *Austral Ecology* **25**, 664-669.

McGillivray D.J. 1993. *Grevillea, Proteaceae. A taxonomic revision.* Melbourne University Press, Carlton.

Morris, E. C. 2000. Germination response of seven east Australian Grevillea species (Proteaceae) to smoke, heat exposure and scarification. *Australian Journal of Botany* **48**, 179-189.

Obbens, F. 1997. *Monitoring and preliminary weed control on populations of critically endangered flora.* Unpublished report to the Department of Conservation and Land Management, Western Australia.

Olde, P.M. and Marriott, N.R. 1995. *The Grevillea Book Vol 2.* Kangaroo Press, Kenthurst NSW.

Robinson & Coates 1995. *Declared Rare and Poorly Known Flora in the Albany District. Wildlife Management Program No. 20.* Department of Conservation and Land Management, Western Australia.

Roche, S., Dixon, K.W. and Pate, J.S. 1998. For everything a season: Smoke-induced seed germination and seedling recruitment in a Western Australian *Banksia* woodland. *Australian Journal of Ecology* **23**, 111-120.