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Does lignum rely on a soil seed bank? Germination and reproductive phenology of *Muehlenbeckia florulenta* (Polygonaceae)

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Abstract. Tangled lignum ('lignum') is a dioecious, multi-stemmed woody shrub that is common in flood-prone areas of inland Australia, including the Murray–Darling Basin. It is often leafless during dry periods, but maintains vegetative growth by stem layering, and responds rapidly to rainfall or flooding by production of shoots, leaves and flowers. This study considers the viability of lignum seeds (contained in achenes) under various conditions of temperature, light, moisture and storage or burial. The seeds are not innately dormant, and germinate within 14 days under ideal conditions. From 66 to 86% of fresh and dry-stored seeds germinate in fluctuating temperatures (15°C/5°C, 24°C/10°C, 31°C/15°C), and optimally at 24°C/10°C, given moisture and light. They also germinate in water (56% success), and remain buoyant for 5–25 days. Germination is inhibited by constant temperatures of 12 and 24°C (4.0–4.8% success) and continuous darkness (6.0–56.0% success), but increases on return to light. Seed viability is depressed by 10% after 70-day dry storage and by 48% after 92-day burial in soil over winter. In one year's (2002) observations of a population on the River Murray floodplain near Morgan, South Australia, winter- and spring-seeding plants produced viable seeds 14–30 days after anthesis, and although rainfall in winter (July) produced a pulse of seedlings, none became established. Achenes were shed soon after maturation, but soil samples revealed very few germinable seeds. It therefore appears that the seeds do not persist for long on the mother plant or in the soil. The persistence of lignum in environments prone to erratic droughts and floods appears to depend mainly on its capacity to tolerate drought, maintain vegetative growth and respond quickly to watering.

Introduction

The floodplains of large, lowland rivers are dynamic environments governed by climate, soils and hydrology. In dry regions, floodplains are subject to intensive flow regulation and land use, and widespread degradation is indicated by changes in the range, abundance and diversity of plants (e.g. Margules and Partners Pty Ltd 1990; Bren 1992; van Splunder *et al.* 1995). Resource managers are well aware that the survival and growth of riparian plants depend on water (cf. Armstrong *et al*. 1994; Kozlowski 1997), but they may overlook the need to monitor regeneration (e.g. McCosker 1998; Howell and Benson 2000; Walker 2002). In many species, surprisingly little is known of the conditions needed to promote germination and seedling establishment, although the fate of these vulnerable stages may influence the composition and 'health' of entire communities (e.g. Cavers and Harper 1967; Howe and Smallwood 1982; Pettit and Froend 2001). Many floristic communities, including those in riparian areas, are maintained by seed banks (e.g. Thompson and Grime 1979; Araki and Washitani 2000), although species that predominate in a seed bank may not necessarily dominate the above-ground vegetation at any given time (Grime 2001). In some species, the seeds remain viable for long periods, and may be capable of dormancy (Thompson 1987). They may also show a light requirement for germination, prolonged germination, sensitivity to temperature fluctuations and temperature-induced secondary dormancy (Thompson and Grime 1979; Grime *et al*. 1981; Araki and Washitani 2000). Seeds that remain viable in the soil for two or more germination seasons following dispersal are a 'persistent' seed bank (Baskin and Baskin 1998). This is typical of ruderal plants, for example, in environments prone to disturbance. The likelihood that a species will form a persistent seed bank may be inferred, albeit with limited confidence, from the morphology and physiology of the seeds (e.g. Hölzel and Otte 2004).

Tangled lignum (*Muehlenbeckia florulenta* (Meissn.)), a member of the Polygonaceae, could be expected to rely on a persistent soil seed bank. It is a common shrub in flood-prone areas such as the Lake Eyre and Murray–Darling

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Basins in inland Australia, and prevalent on the floodplain of the River Murray in South Australia. Along the Murray it occurs in mono-dominant stands, or as an understorey to eucalypts, in areas with a flooding frequency of 3 in 10 years (Craig *et al*. 1991). It has a woody, multi-stemmed habit and frequently a dry, leafless appearance, but the stems alone are capable of maintaining high levels of photosynthetic activity (C. Chong. unpubl. data). Lignum is drought-tolerant and regenerates from a persistent rootstock that is at least 2–3 m deep (Craig *et al*. 1991). Where there is abundant soil moisture, the shrubs have a dense, rounded form rising up to 3 m or more; in drier areas they are comparatively sparse and stunted. Rainfall and flooding promote vegetative growth and rapid production of shoots, leaves and flowers (Campbell 1973; Pressland and Keenan 1985; Roberts and Marston 2000). Lignum is dioecious (Laubengayer 1937), like its congeners, with male flowers larger than those of females (5.6 and 4.1 mm in diameter, respectively: C. Chong, unpubl. data). Most published information about the species is of a taxonomic nature (e.g. Chorney 1986; Ronse Decraene *et al*. 2000), but anecdotal reports suggest that the achene (fruit) is dispersed by floodwaters and the seeds germinate in wet mud (Campbell 1973). The phenology of flowering and fruit production, pollination biology and requirements for germination and seedling establishment are unknown.

In this study, the main goal was to determine whether lignum has a persistent soil seed bank (*sensu* Baskin and Baskin 1998). Germination requirements were determined by investigating the effects of temperature, light and moisture on seeds in the laboratory, and the effects of storage and burial on the viability and germination responses of seeds in the field. Observations of the reproductive phenology and seed bank of a natural population are also described.

Materials and methods

Study site

Field studies were conducted on the River Murray floodplain at Scotts Creek (34°05.8′S, 139°40.4′E), 10 km south of Morgan, South Australia. The region experiences cool winters and hot, dry summers. Annual rainfall is 303 mm, with a slight winter–spring peak, and average daily temperatures are 14.2–31.3°C in January and 3.9–15.7°C in July (data for Morgan, 1989–2001, Bureau of Meteorology, Adelaide).

The study area is a small (<1 km^2) part of the Brenda Park–Scotts Creek wetland complex (Jensen *et al*. 1999; Fig. 1). It is bounded to the east by the Murray and to the south and west by Scotts Creek, and extends northward as Brenda Park. Cattle and sheep grazed in the area from 1905 to 1985, but the main large grazers now are kangaroos (*Macropus fuliginosus*). Soils are 2-m deep, grey cracking clays overlying sand. The water table is from 3.0 to 7.0 m Australian height datum (AHD), and 1–4 m below the soil surface (Henderson *et al*. 2001). Groundwater salinity is 0.92–40.4 mS cm^{-1}, and highest near the margins of Brenda Park Lagoon. Historically, the area experienced flooding once in every 2 years, but river regulation has reduced this to once in every 3 years (Jensen *et al*. 1999).

Lignum (0.6 to >3 m high) is most abundant along the river banks and in the eastern area of the floodplain where soil and groundwater

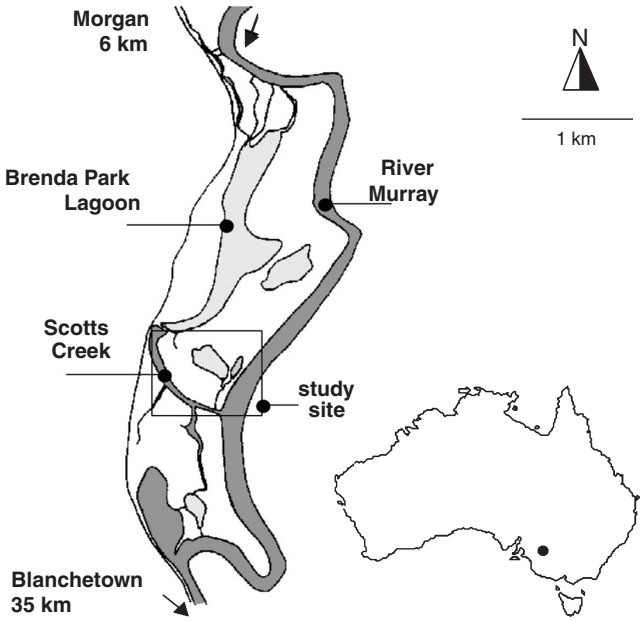

Fig. 1. The River Murray and floodplain at Scotts Creek, near Morgan, South Australia, showing the location of the study site. Light shading indicates temporary wetlands and dark shading permanent wetlands.

salinities are low (0.45 and 3.5 mS cm^{-1}, respectively) and the water table is within 1–2 m of the soil surface (Henderson *et al*. 2001). Grasses dominate in low-lying lagoon areas and *Nitraria billardieri* (nitre bush) occurs on higher ground. Stands of *Eucalyptus camaldulensis* (river red gum), *E. largiflorens* (black box) and *Acacia stenophylla* (river cooba) occur in riparian areas. Other species include *Muehlenbeckia horrida* (spiny lignum), *Enchylaena tomentosa* (ruby saltbush), *Myoporum platycarpum* (mallee sandalwood), *Persicaria* spp. (slender knotweeds) and unidentified samphires.

Germination tests for seed viability, germination and dormancy

Mature achenes, rich brown in colour and enclosed in yellowing perianths, were collected from female plants at Scotts Creek between May and June 2002, and subjected to light and temperature treatments described below. For each treatment, replicate groups of achenes were placed in Petri dishes containing Whatman 41 filter paper moistened with reverse osmosis (RO) water and incubated in growth cabinets under controlled temperature and light (cool fluorescent: 250 µmol m^{-2} s^{-1}). Dishes incubated in light were wrapped in cling film to prevent water loss, and those incubated in darkness were wrapped in aluminium foil to exclude light. The dishes were randomly re-positioned on several occasions during the course of each treatment, and the filter papers were remoistened if required. Germination, indicated by protrusion of the radicle, was recorded daily for treatments incubated in light. Tests were concluded when no germination had been observed for 5 days, and seeds incubated in darkness were then exposed for inspection. The viability of ungerminated seeds was determined with 1% 2,3,5 triphenyltetrazolium chloride (hereafter 'tetrazolium'), which stains actively respiring tissue red. Cumulative percentage germination data were fitted to a Gompertz model by least-squares methods (Gan *et al*. 1996), to estimate time to 50% (T_{50}) and 80% (T_{80}) of final percentage germination.

The following treatments were imposed:

Group 1 was tested within 14 days of collection (11 May 2002). Six replicate samples of 50 achenes were incubated under each

of three different temperature regimes (15°C/5°C, 24°C/12°C, 31°C/15°C) under a 12/12 h light/dark cycle. These regimes represent average day/night temperatures in winter, spring and summer, respectively, at Blanchetown, ~35 km from Scotts Creek (Bureau of Meteorology data).

Group 2 was dried in paper bags in the dark for 21 days after collection (13–20 June 2002), then transferred to paper envelopes in the dark at 5°C for a further 49 days. Five replicate samples of 50 achenes were incubated under the three alternating temperature regimes described for Group 1.

Group 3 was as for Group 2, except that after drying for 21 days the achenes were stored for 49 days at 24°C. Five replicate samples of 50 achenes were incubated under the three temperature regimes, as for Groups 1 and 2.

Group 4 comprised achenes collected in autumn (11 May 2002) and stored in paper bags at room temperature in the dark, for 21 days. On 2 June, 50 randomly chosen achenes were enclosed in each of 21 polyester mesh envelopes (50 × 50 mm, mesh 0.5 mm) and buried in soil at 20-mm depth under litter, near the mother plants. Soil temperatures during the period of burial were monitored with five Optic StowAway™ loggers. The envelopes were exhumed on 2 September (92 days, or approximately the duration of winter) and opened in darkness under a safe green light. The achenes were rinsed and checked for evidence of germination.

An additional sample of achenes was tested to determine the effects of longer storage on germinability. This was Group 5, from plants on the Murray floodplain at Ninkle Nook, near Renmark (coll. M. A. Siebentritt, The University of Adelaide, December 2000). The seeds had been stored dry at room temperature, in the dark, for 21 months, and were incubated under each of the temperature regimes described for Groups 1–3.

Buoyancy

To examine the likelihood that lignum seeds are dispersed by water ('hydrochory'), six replicates of 25 achenes were floated in 200 mL RO water in clear cylindrical tubs (50 × 90 mm diameter) and exposed to a 12/12 h, 24°C/10°C regime. Percentage buoyancy and germination of seeds were recorded daily until all were non-buoyant. Again, the viability of ungerminated seeds was assessed with tetrazolium.

Burial experiment

Pilot tests indicated that lignum seeds needed sustained moisture for germination. In subsequent work, germination was tested at different depths of burial in soil, using a siphon device (after Araki *et al*. 1998) to maintain a constant water table (hence saturated soil) in 300 × 240 × 130 mm deep plastic containers. The containers were supplied with water via a 13-mm-diameter polyethylene tube from an 11-L reservoir with a 450-mm acrylic riser as an air vent. Checks on gravimetric water content confirmed that saturation was maintained at all depths. The soil used was clay from Scotts Creek, after it had been sieved (2 mm), spread on trays in a glasshouse under natural light and temperature conditions and kept saturated for 3 weeks to germinate any seeds.

Experiments were conducted in a greenhouse under natural light and air-temperature conditions (11.6–29.8°C). Achenes (10 per depth treatment) were sown into each of six replicate containers and partitioned with plastic fly-wire mesh to ensure that the achenes were not displaced. Both pre-germinated and dried achenes were sown. The former were incubated on moistened filter paper in Petri dishes for 4 days at 24/10°C, as in the germination tests described above, and those with 1–2-mm radicles were planted. Pre-germinated and dry achenes were planted at depths of 0 (soil surface), 10 and 40 mm. Soil temperatures at planting depths were monitored at 15-min intervals with five Optic StowAway™ loggers in randomly selected containers. Seedling emergence (i.e. cotyledons above soil surface) and survival were recorded daily for 21 days. Achenes and seedlings were then exhumed by the method described below, and viability was assessed by using tetrazolium.

Soil seed bank sampling

The presence and spatial distribution of seeds were determined from surface soil samples collected along three transects (500–700 m long, 150 m apart, parallel to the river channel) through lignum habitat at Scotts Creek. Eight quadrats (5 × 5 m) were located randomly along each transect, and 20 soil cores (50 mm diameter, 50 mm deep) were augured in each quadrat. Of these 20 cores, 10 were from directly under lignum plants and 10 were from open ground (>1 m from any plant). The samples were mixed, returned to the laboratory and dried to a constant weight at 30°C in foil containers (190 × 90 × 50 mm deep). Seeds were extracted following Malone (1967): a 500-g soil sample was agitated for 2 min in a 1-L aqueous solution of 50 g sodium hexametaphosphate, 25 g sodium bicarbonate and 125 g magnesium sulphate. Floating debris was decanted, rinsed and retained on a 250-µm sieve; this was repeated three or more times before drying at 30°C to facilitate seed recovery. Trials beforehand confirmed that the seeds would germinate after extraction and drying at 30°C. Seed viability was assessed with tetrazolium.

Results

Germination responses to light and temperature

From 63.7 to 86.0% of freshly matured seeds incubated in light germinated successfully in the three alternating temperature regimes (Group 1). Percentage total germination varied between regimes (ANOVA: $F_{2,15} = 25.5, P < 0.0001$), and peaked at 24°C/10°C (Tukey's honestly significant difference (HSD), $\alpha = 0.01$) (Fig. 2*a*). Responses within temperature treatments were rapid and synchronous. Times to first radicle emergence were 7 days in the cooler (15°C/5°C) regime and 3 days in both the 24°C/10°C and 31°C/15°C regimes. Germination occurred most rapidly at 24°C/10°C, reflected in low values of T_{50} (4.07 days) and T_{80} (4.98 days). The average viability of seeds under this regime was little different from that of fresh seeds (86 cf. 90%, respectively).

Thermal pre-treatment (Groups 2 and 3) affected germination. For seeds stored at 5°C and 24°C for 49 days before testing, the time to onset of germination was comparable to that of fresh seeds, but there were differences in the final percentage germination (ANOVA: $F_{2,45} = 7.88$, $P = 0.001$).

Dry storage at 5°C enhanced viability (76%), and also promoted germination in the coldest regime. Thus, seeds stored at 5°C and incubated at 15/5°C produced 70% germination, similar to those incubated at 24°C/10°C (76%) (ANOVA: $F_{1,8} = 1.11$, $P = 0.32$) (Table 1).

Dry-stored seeds showed slightly higher germination rates than fresh seeds, indicated by T_{50} and T_{80} (Table 1), but the increases were <2 days. Seeds subjected to field burial (Group 4), and those from the population at Ninkle Nook, did not show this effect. The Ninkle Nook samples germinated at comparable rates when incubated at 31°C/15°C ($T_{50} = 4.12$ days, $T_{80} = 6.02$ days) and

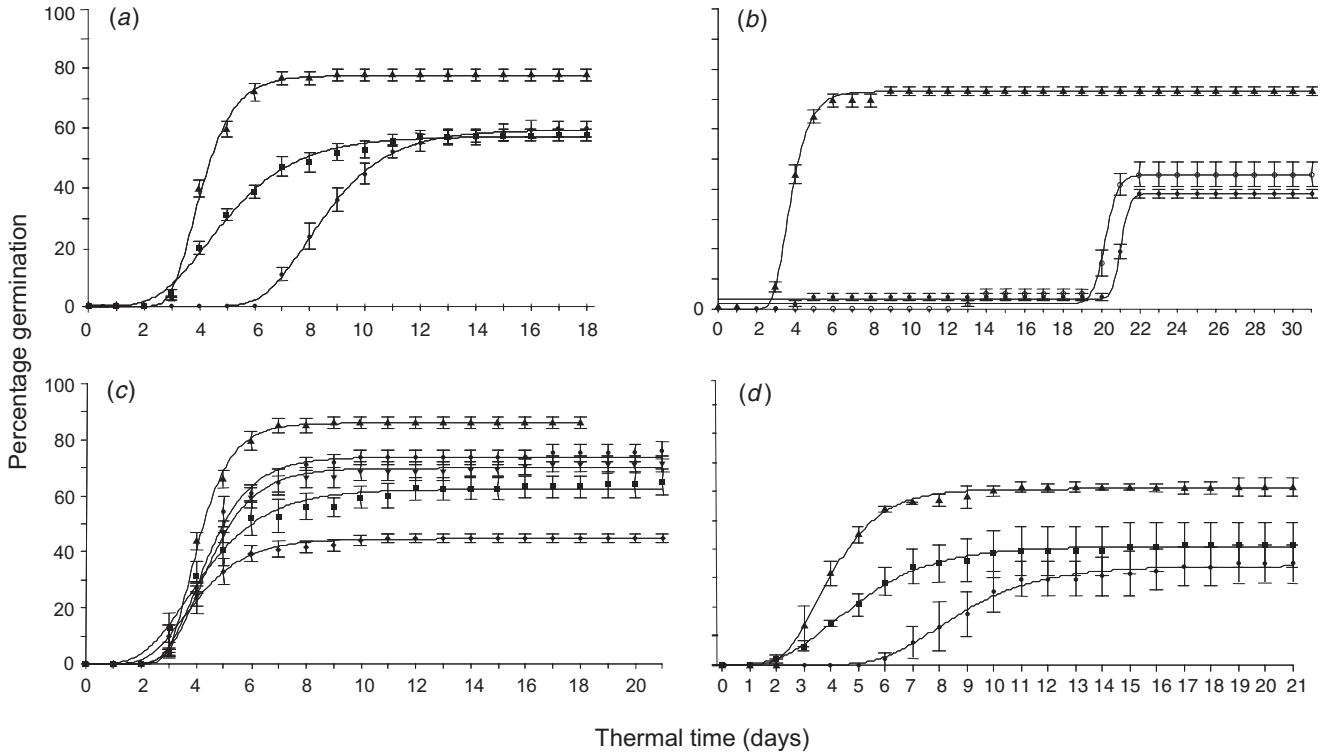

Fig. 2. Mean cumulative percentage germination of *Muehlenbeckia florulenta* seeds subjected to various treatments. Seeds are from Scotts Creek unless stated otherwise. Bars indicate ± s.e. Fitted lines are Gompertz models (in each case, $r^2 > 0.99$). (*a*) Fresh seeds incubated in light (12/12 h) at 24°C/10°C (▲), 15°C/5°C (●) and 31°C/15°C (■). Each group contained six replicates of 50 seeds. (*b*) Fresh seeds incubated in light (12/12 h) at 12°C (■), 24°C (●) and 24°C/12°C (▲). On day 18, seeds incubated at 12°C and 24°C were transferred to the 24°C/12°C regime. Each group contained five replicates of 25 seeds. (*c*) Seeds incubated in light (12/12 h) at 24°C/10°C. The seeds were fresh (▲), dry-stored at 24°C (●), dry-stored at 5°C (▼) or buried in the field (◆). In addition, seeds from a population at Ninkle Nook (■) were incubated under the same conditions. Each group contained five replicates of 25 seeds. (*d*) Seeds buried in the field at Scotts Creek for 92 days and subsequently incubated in light (12/12 h) at 24°C/10°C (▲), 15°C/5°C (●) and 31°C/15°C (■). Each group contained five replicates of 25 seeds.

Table 1. Summary of germination treatments

Lignum seeds were obtained from the River Murray floodplain at Scotts Creek (Groups 1–4) and Ninkle Nook (Group 5), and incubated under five temperature regimes and two light regimes. 'Seed age' refers to the physiological ages of seeds at the commencement of the treatments; 'sample size' indicates the number of Petri dishes and seeds per dish, respectively, in each treatment. See Table 2 for further details

Group number, treatment (seed age)	Temperature (°C)	Light/dark (h)	Sample size
1. Fresh (14 days)	15/5	12/12	6 × 50
2. Dry, dark storage at 5°C (10 weeks)	24/10	0/24	5 × 25
3. Dry, dark storage at 24°C (10 weeks)	31/15		5 × 25
4. Buried at field site (13 weeks)	12		7 × 50
5. Dry, dark storage (21 months)	24		5 × 25

24°C/10°C ($T_{50} = 4.32$ days, $T_{80} = 6.32$ days) (Table 1). Maximum average percentage germination was 65% under the 24°C/10°C regime.

Seeds were sensitive to fluctuating temperatures. Germination was suppressed at constant temperatures of 12 and 24°C, compared with the response at 24°C/12°C (ANOVA: $F_{2,12} = 769.6$, $P < 0.0001$; Tukey's HSD, $\alpha = 0.01$). After 18 days of incubation at 12 and 24°C, mean germination was 4.8 and 4.0%, respectively. When these seeds were removed to 24°C/12°C, germination occurred within 2 days (Fig. 2*b*). Seeds from the Ninkle Nook population showed similar sensitivity (C. Chong, unpubl. data).

A strict light requirement for germination was not apparent in seeds freshly matured (14 days old) or recovered from storage or field burial (10–13 weeks old)

(Fig. 2*c*). Mean germination of fresh seeds in darkness varied from 13% (15°C/5°C) to 46% (24°C/10°C). For stored seed, average germination ranged from 6.0% (dry stored at 5°C, 15°C/5°C incubation) to 56% (Ninkle Nook population, 24°C/10°C incubation). For both populations, germination in darkness was inhibited most in the 15°C/5°C regime (Table 2). On removal to light, seeds from all dark treatments germinated within 5 days. Fresh seeds incubated in darkness retained high viability (85–94%).

Field burial

Seeds were buried for 13 weeks in soil at Scotts Creek to assess germination after exposure to seasonal conditions. The time to radicle emergence in field-buried seeds (3 days) was similar to that in fresh and dry-stored seeds (see also T_{50}, T_{80} data: Table 1). The final germination attained by field-buried seeds, however, was much less. Maximum average total germination was 45% at 24°C/10°C (Fig. 2*d*), or 52% of the total germination of fresh seeds. Thus, there were significant differences between the viability of field-buried seeds and fresh and dry-stored seeds (ANOVA: $F_{4,63} = 43.65$, $P < 0.0001$). Tetrazolium tests indicated that all viable seeds had germinated within the 21-day incubation period (Table 1).

No *in situ* germination was detected on exhumation of the field-buried seeds. During the experiment (June to September 2002), buried seeds were exposed to widely varying diurnal temperatures, although there was little variation in temperatures at the surface and 20-mm depth. The minimum (-4.76 ± 0.02°C) was recorded on 30 June and the maximum (38.2 ± 0.03°C) on 2 September. The amplitudes of diurnal fluctuations and maximum daily temperatures were slightly damped in July (mid-winter).

Winter 2002 was unusually dry: rainfall at Blanchetown was 12.4 mm in June, 18.6 mm in July and 10.0 mm in August, compared with 105-year averages of 26.4, 25.3 and 26.0 mm, respectively (annual average 271 mm) (Bureau of Meteorology data). A pulse of emergence was observed on 7 July 2002, when soil moisture content was 0.124 ± 0.003 g g^{-1} (mean ± s.e.). The emergent seedlings were clustered in high densities directly under mature plants, but most had died when revisited on 21 July.

Seed buoyancy

Fresh seeds remained buoyant for at least 5 days. Subsequent loss of buoyancy occurred with the onset and progression of germination, rapid separation of cotyledons from the achene coat and root extension. After 24 days, when none of the seeds remained afloat, the average germination was 46.0 ± 2.9% (mean ± s.e.). Tetrazolium tests confirmed that all viable seeds had germinated.

Burial

Seeds buried in (saturated) soil experienced similar temperatures (16.8–26.1°C), with little diurnal variation (4.6 ± 1.4°C; mean ± s.e.). Overall, they showed low rates of post-germinative emergence and seedling survival. Times to first emergence of cotyledons in pre-germinated seedlings were 3 days at the surface (10-mm burial) and 6 days at a greater depth (40-mm burial). Seeds sown at 10-mm and 40-mm depths exhibited especially low rates of final emergence (3.3–15%).

Planting depth also had an inhibitory effect (Wilcoxon test, χ^2 approximation: 26.21, d.f. = 2, $P < 0.0001$), such that emergence from 40 mm was <12% (Table 3). Three weeks after initial emergence, there were significant losses: seeds sown at the surface, 10-mm and 40-mm depths experienced 43, 50 and 72% mortality, respectively (Table 3). Few 'dry-sown' seeds (seeds stored dry, but sown in saturated soil) germinated successfully: only three (5.0 ± 2.2%; mean ± s.e.) germinated after 14 days, and the remainder were non-viable, containing brown, collapsed embryos.

Soil seed bank sampling

The 24 sample plots (5×5 m^2 each) contained variable lignum cover. Most achenes were recovered directly under seeding plants, in densities of 1–31 achenes per sampled soil area (26–789 achenes m^{-2}). However, tetrazolium tests and microscopy indicated that only 1–3 seeds (by extrapolation, 26–76 seeds m^{-2}) in nine plots were viable.

Discussion

Tangled lignum is a common shrub in flood-prone areas of inland Australia, including the floodplain of the River Murray in South Australia. This study describes the responses of lignum seeds to temperature, light and moisture, and the moderating effects of the age of seeds and the storage/burial environment. These data, and observations of the phenology of a population on the Murray floodplain, are used to elucidate the germination biology of the species and the role of the soil seed bank in regeneration.

Germination characteristics

The high rate of germination (66–86% success) in fluctuating temperatures, and corresponding low values for T_{50} (4.00–8.99 days) and T_{80} (4.98–11.42 days), indicate that the seeds of lignum are not dormant *sensu* Baskin and Baskin (1998). The rapid, discrete germination response suggests that, in nature, most seeds would germinate soon after dispersal, and few would remain in the soil seed bank. The abilities of lignum seeds to remain buoyant (5–24 days) and germinate in water support the likelihood that seeds are dispersed by water ('hydrochory').

Lignum seeds do not exhibit 'conditional dormancy' (cf. Washitani and Masuda 1990; Araki and Washitani 2000;

Table 2. Responses of lignum seeds to temperature, light and storage conditions

Seeds from five sources (Groups 1–5, Table 1) were incubated under three alternating temperature regimes and two light regimes, namely (*a*) 12/12 h light/dark and (*b*) continuous darkness. Data are mean (s.e.) percentage germination after incubation (A) and after removal to 24°C/10°C, 12/12 h light for 5 days (B), and the remaining percentage of viable, ungerminated seeds (C). T_{50} and T_{80} are days taken by 50% and 80% of total germinants, respectively. Numbers of seeds per group are shown in Table 1

Group	15°C/5°C					24°C/10°C					31°C/15°C				
	A	B	C	T_{50}	T_{80}	A	B	C	T_{50}	T_{80}	A	B	C	T_{50}	T_{80}
(*a*) 12/12 h light															
1	66.0 (3.0)	68.3 (2.8)	10.0 (1.6)	8.50	10.3	86.0 (2.1)	86.0 (2.1)	0.3 (0.3)	4.07	4.98	63.7 (2.2)	73.0 (7.9)	4.7 (3.0)	4.95	7.00
2	48.8 (4.6)	53.6 (4.5)	1.6 (1.0)	8.99	11.4	71.2 (2.9)	71.2 (2.9)	0.8 (0.8)	4.48	5.80	40.8 (4.6)	49.6 (7.2)	0.8 (0.8)	5.86	8.70
3	70.4 (3.5)	74.4 (3.9)	1.6 (1.0)	8.70	11.1	76.0 (3.1)	76.0 (3.1)	0.0 (0.0)	4.43	5.75	56.0 (6.8)	61.6 (11.5)	0.8 (0.8)	5.11	7.41
4	25.6 (4.8)	27.2 (4.6)	0.0 (0.0)	8.74	11.2	44.8 (1.5)	44.8 (1.5)	0.0 (0.0)	3.99	5.43	30.4 (5.6)	30.4 (5.6)	0.0 (0.0)	4.91	7.26
5	44.8 (3.2)	48.8 (4.3)	6.4 (3.5)	7.99	9.47	64.8 (4.6)	64.8 (4.6)	0.0 (0.0)	4.32	6.32	48.8 (3.2)	52.0 (2.5)	2.4 (1.0)	4.12	6.02
(*b*) Continuous darkness															
1	13.3 (1.7)	74.3 (3.3)	12.0 (3.3)			46.3 (1.6)	70.3 (4.6)	24.0 (6.7)			31.0 (2.2)	60.3 (3.7)	24.7 (3.7)		
2	6.0 (2.0)	41.6 (7.1)	4.8 (2.0)			34.0 (6.1)	48.8 (5.3)	0.8 (0.8)			15.0 (1.5)	40.0 (5.2)	2.4 (2.4)		
3	8.8 (3.2)	56.8 (8.5)	6.4 (2.7)			34.4 (3.5)	62.4 (7.4)	1.0 (0.3)			19.2 (3.2)	61.6 (11.5)	3.2 (1.5)		
4	8.0 (1.8)	25.6 (4.3)	0.0 (0.0)			14.4 (3.5)	21.6 (4.8)	0.0 (0.0)			16.8 (3.2)	26.4 (2.7)	0.0 (0.0)		
5	11.2 (9.0)	40.0 (9.0)	17.6 (5.7)			56.0 (3.8)	57.6 (4.1)	0.0 (0.0)			40.8 (4.6)	39.2 (4.6)	0.0 (0.0)		

Table 3. Emergence of buried, pre-germinated lignum seeds
Three groups of 10 seeds were germinated in Petri dishes then planted at three depths. Data are mean percentages (s.e.) of emergent seedlings after 8 and 21 days. In contrast, the same number of seeds sown direct into the soil yielded only three germinants

Depth (mm)	Day 8	Day 21
0	100.0 (0.0)	56.7 (4.9)
10	30.0 (4.5)	15.0 (5.6)
40	11.7 (5.4)	3.33 (2.1)

Baskin and Baskin 2002). Rather, germination occurs readily on return to light after incubation in the dark, at various temperatures and under various storage conditions. Lignum thereby differs from its congener *M. australis*, a climbing vine endemic to New Zealand. In *M. australis*, the seeds exhibit a delayed, gradual germination response over ~90 days, through summer, and may remain on the mother plant for several months before dispersal (Burrows 1996). In *M. florulenta*, the time between ripening and dispersal of the seed is brief (14 days) in both winter- and spring-seeding plants, and there is no indication of an aerial seed bank. Lignum seeds appear to be rapidly released and germinate opportunistically, regardless of season, provided that moisture, temperature and light conditions are met.

The sensitivity of lignum seeds to temperature and light in the laboratory suggests that there are constraints to germination in the natural environment. Maximal germination of fresh and stored seeds occurred in the variable 24°C/10°C regime, whereas there was minimal germination at constant temperatures of 24 and 12°C ($4.0 \pm 1.26\%$, $4.8 \pm 1.5\%$, respectively; mean ± s.e.). Regardless of thermal pre-treatment or the age of achenes, the time to germination under cold incubation (6–7 days at 5°C) was twice that in the warmer regimes (3 days). Thus, low temperatures may suppress germination in natural populations (diel temperatures at Scotts Creek in winter 2002 varied from −4 to 31°C). After 70-day storage at 5°C, the germinability of seeds at fluctuating cold temperatures (15°C/5°C) was enhanced. This suggests that after a cold winter the minimum temperature for germination may be lowered, and the remaining viable seeds may germinate regardless of the temperature. Thermal sensitivity is typical of many herbaceous wetland plants, and is associated with the ability to capitalise on 'windows of opportunity' for seedling establishment (Thompson *et al*. 1977).

The relatively low survival and emergence of lignum seeds buried at 10–40-mm depth suggest that the soil surface is a more favourable micro-environment for germination. Nearly all dry-sown seeds failed to germinate, indicating that a period of high moisture is a prerequisite (cf. Araki *et al*. 1998; Hamman *et al*. 2002). Given the requirement for fluctuating temperatures (even under high light), germination probably was inhibited by the low amplitude of diurnal fluctuations (mean 4.6°C) below the surface.

The viability of lignum seeds depends on the thermal storage environment as well as physiological age. Seeds in dry, cold storage (5°C) retained most germinability across all thermal regimes, perhaps because cool temperatures promote favourable levels of relative humidity, and hence seed longevity (Baskin and Baskin 1998). Viability declined from 86 to 76% after 70-day dry storage, and the effect was exacerbated in seeds buried in soil over winter (48% viability after 92 days). None of the buried seeds germinated *in situ*. Variable diel temperatures in winter (–4 to 31°C) may have provided opportunities for germination, but there would have been too little moisture most of the time, owing to low seasonal rainfall. The only exception was in July, when prolific seedlings emerged in response to 18 mm of rainfall. The significance of seed age in consideration of quantitative germination tests is illustrated by the relative viability of dry-stored (76%) and field-buried seeds (45%). Indeed, lignum seeds stored in paper bags at room temperature have been found to be viable after 15 years (R. Neumann, The University of Adelaide, unpubl. data 2000).

Seed bank dynamics

Samples from Scotts Creek provided no evidence that lignum maintains a soil seed bank. The few viable seeds found in areas of dense lignum cover, the limited longevity of buried seeds, and the apparent lack of innate dormancy, a strict light requirement and extended germination period all suggest that seeds do not live beyond the second germination season after dispersal, and are not 'persistent' *sensu* Baskin and Baskin (1998).

Although the Polygonaceae vary widely in their germination requirements, at least 32 species in six genera have persistent seed banks. These include *Polygonum sieboldii*, *Rumex acetosa* and *R. conglomeratus*, in which germination is enhanced by moist chilling, and *P. longisetum*, *P. japonicum* and *P. perfoliatum*, where it is obligatory (Washitani and Masuda 1990). In *Emex australis* and *E. spinosa* germination is optimal at 30°C/20°C (Baskin and Baskin 1998) and in *M. hastulata* it peaks after brief exposure to 100°C (Muñoz and Fuentes 1989). Mechanisms of seed dormancy and seasonal reproduction in some floodplain *Persicaria* and *Rumex* species are quite unlike those of *M. florulenta* (cf. Cavers and Harper 1967). In *R. crispus* and *R. palustris*, for example, there is prolonged attachment of the achene to the mother plant, low germination rates after dispersal in late autumn and perianth-imposed mechanical dormancy that delays germination until spring (Voesenek and Blom 1992; Voesenek *et al*. 1992). In *M. florulenta*, the perianth deteriorates within 3 weeks in moist conditions and has little effect on germination or mechanical dormancy.

Seeds with small, compact morphology, a smooth coat and exacting requirements for germination are predisposed to develop a persistent seed bank (Thompson 1987). The mature, trigonous achene of lignum, however, is large (3–5 mm) and enclosed in a dry perianth. Radicle emergence within 3 days (under optimal conditions) suggests that the embryo is fully developed during the brief period of maturation on the mother plant (Ronse Decraene *et al*. 2000). These attributes, the ability to germinate over a range of temperatures and the rapid loss of viability during burial, do not favour the persistence of ungerminated seeds in the soil (cf. Thompson and Grime 1979). A small proportion of viable seeds might remain, but at Scotts Creek they apparently have a minor role in regeneration of the population.

Methods of sampling potentially could misrepresent the numbers of viable lignum seeds in the soil (cf. Bigwood and Inouye 1988; Brock *et al*. 1994). Ideally, sampling should occur after germination but before dispersal, in the same year (Baskin and Baskin 1998); however, in this study the optimal sampling time was obscured by the opportunistic nature of seed dispersal. Spatial clumping of male or female (achene-bearing) genets could bias the apparent distribution and abundance of plants and seed (e.g. McLellan *et al*. 1997; Dale 1999), although aggregated samples of numerous small soil cores were used to counter this problem. Finally, the soil-separation technique recovered non-viable as well as viable seeds, whereas germination tests alone would not have detected the non-viable component of the seed crop. More intensive, larger-scale sampling is needed to estimate true seed density.

Reproductive phenology and growth responses

In autumn- and spring-flowering lignum at Scotts Creek, successive periods of anthesis, fruit set and maturation and achene primary dispersal were remarkably rapid. The flowers are small, but synchronous anthesis in males and females and *en masse* displays would enhance their attraction for pollinators. Floral counts (C. Chong, unpubl. data) included one record of 856 flowers per tertiary branch on a large (2.5 m tall) female plant, indicating more than 9600 flowers on the plant. Another, smaller (2 m tall) plant carried an estimated 530 achenes. Thus, there are prolific achenes even under the dry conditions that prevailed in this study.

The phenology of reproduction in the Scotts Creek population was variable throughout 2002. Although plants near the riverbank flowered in April, those further inland did not flower before September. This asynchrony may have arisen because the riverbank plants had access to permanent river water, whereas the others were constrained by available surface moisture. This is supported by hydrogen isotope analyses at Scotts Creek (R. Neumann, The University of Adelaide, unpubl. data 2000), showing that the river is a primary water source for lignum plants within 6 m of the riverbank.

It is possible that the distinctive reproductive phenologies of the riverbank and floodplain plants have a genetic origin, and the polymorphic, octoploid character of *M. florulenta* (J. G. Conran, The University of Adelaide, pers. comm., June 2002) suggests that the species has considerable capacity for adaptive responses to changeable conditions (cf. Conner *et al*. 1996; Diggle *et al*. 1998; Bell and Sultan 1999; Fischer and van Kluenen 2001). This is reflected, for example, in its abundance in diverse water and nutrient regimes (Goodrick 1984; Blanch *et al*. 1999; Roberts and Marston 2000), and its growth responses to different soil-moisture regimes (Campbell 1973). These responses may entail changes in morphology or biomass allocation to optimise growth (cf. Sultan *et al*. 1998), or vegetative reproduction to maintain genets (cf. Peterson and Jones 1997).

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