CSIRO PUBLISHING

The Rangeland Journal, 2012, **34**, 429–438
http://dx.doi.org/10.1071/RJ12051

Persistence of bellyache bush (*Jatropha gossypifolia* L.) soil seed banks

Faiz F. Bebawi[A,C], *Shane D. Campbell*[A] and *Robert J. Mayer*[B]

[A]Biosecurity Queensland, Department of Agriculture, Fisheries and Forestry, Tropical Weeds Research Centre, PO Box 187, Charters Towers, Qld 4820, Australia.
[B]Agri-Science Queensland, Department of Agriculture, Fisheries and Forestry, PO Box 1085, Townsville, Qld 4810, Australia.
[C]Corresponding author. Email: Faiz.Bebawi@daff.qld.gov.au

Abstract. Bellyache bush (*Jatropha gossypifolia* L.) is an invasive shrub that adversely impacts agricultural and natural systems of northern Australia. While several techniques are available to control bellyache bush, depletion of soil seed banks is central to its management. A 10-year study determined the persistence of intact and ant-discarded bellyache bush seeds buried in shade cloth packets at six depths (ranging from 0 to 40 cm) under both natural rainfall and rainfall-excluded conditions. A second study monitored changes in seedling emergence over time, to provide an indication of the natural rate of seed bank depletion at two sites (rocky and heavy clay) following the physical removal of all bellyache bush plants.

Persistence of seed in the burial trial varied depending on seed type, rainfall conditions and burial depth. No viable seeds of bellyache bush remained after 72 months irrespective of seed type under natural rainfall conditions. When rainfall was excluded seeds persisted for much longer, with a small portion (0.4%) of ant-discarded seeds still viable after 120 months. Seed persistence was prolonged (>96 months to decline to <1% viability) at all burial depths under rainfall-excluded conditions. In contrast, under natural rainfall, surface located seeds took twice as long (70 months) to decline to 1% viability compared with buried seeds (35 months). No seedling emergence was observed after 58 months and 36 months at the rocky and heavy clay soil sites, respectively. These results suggest that the required duration of control programs on bellyache bush may vary due to the effect of biotic and abiotic factors on persistence of soil seed banks.

Additional keywords: burial conditions, seedling emergence, seed longevity.

Received 2 August 2012, accepted 13 November 2012, published online 17 December 2012

Introduction

Bellyache bush (*Jatropha gossypifolia* L.) is an invasive shrub in northern Australia (Csurhes 1999; Bebawi *et al*. 2007; Randall *et al*. 2009; Smith 2011). It is native to South America and the drier islands of the Caribbean (Heard *et al*. 2002) and was introduced to northern Australia in 1888, most probably for medicinal and ornamental purposes (Everist 1974; Parsons and Cuthbertson 2001). It has also naturalised in many other countries, including Mozambique, Zambia, Chad, Cameroon, Indonesia, Singapore, New Guinea and New Caledonia (Bebawi *et al*. 2007).

Bellyache bush is spread predominantly by seed (Bebawi and Campbell 2002*a*), although it can regenerate readily from vegetative material, such as garden cuttings or plants dislodged during floods (Parsons and Cuthbertson 2001; Bebawi *et al*. 2007). Bellyache bush produces capsules containing three seeds and mature plants can produce up to 12 000 seeds/year (Bebawi and Campbell 2002*a*). Soil seed banks up to 9.3 M seeds/100 m^2 have been recorded in northern Australia for seeds at 0–40-cm soil depth beneath the canopy of a densely-infested bellyache bush site, with most seed found on or within 5 cm of the soil surface (Bebawi and Campbell 2002*b*).

Seed dispersal occurs initially via dehiscent capsules capable of catapulting seeds as far as 13 m (Bebawi and Campbell 2002*a*). Once on the ground, bellyache bush seeds are susceptible to localised dispersal by ants. For example, in northern Australia, meat ants (*Iridomyrmex spadius* Schattuck) can disperse seeds >40 m from the parent plant (Bebawi and Campbell 2002*a*). Other ant species (e.g. *Pogonomyrmex cunicularius* Mayr) are reported to spread 84% of seeds of other *Jatropha* species in Argentina (Aranda-Rickert and Fracchia 2011). Water, humans and some animals, such as the great bowerbird (*Chlamydera nuchalis* Jardine & Selby), are potential long-distance dispersers of bellyache bush seeds (Ashley 1995; Smith 1995; Bebawi *et al*. 2007). These dispersal mechanisms contribute to bellyache bush's range extension, particularly in years of exceptionally wet seasons (Bebawi and Campbell 2004).

Dense infestations of bellyache bush can cause major economic and environmental impacts, primarily through the exclusion of pasture grasses and overall reduction in biodiversity

(Bebawi *et al.* 2007; Randall *et al.* 2009). Though there are several effective control techniques for bellyache bush plants, significant seedling recruitment usually occurs following the application of control measures (Bebawi *et al.* 2011). This is particularly the case for those treatments, such as mechanical control and burning, that disturb the herbaceous layer (Bebawi and Campbell 2002*b*, 2002*c*; Bebawi *et al.* 2004, 2011). To achieve more complete control, seedling establishment must be managed until the soil seed bank is exhausted and reinfestation from external sources (e.g. neighbouring infestations) must be minimised (Bebawi *et al.* 2011). Weed seed bank dynamics should be considered in the weed management planning process (Panetta and Groves 1990; Cardina and Sparrow 1996; Campbell and Grice 2000). In particular, information on the persistence of soil seed banks can be used to estimate the likely duration and cost of control/eradication programs (Cunningham *et al.* 2003; Myers and Bazely 2003; Setterfield *et al.* 2004).

Limited data are available on the germination and viability of bellyache bush seed. Fresh bellyache bush seeds tend to have high viability, but low germinability (Bebawi and Campbell 2004), although in Australia some ants (e.g. meat ants) appear to increase the germinability of seeds through the effects of their foraging (Bebawi and Campbell 2004). In terms of the persistence of seeds in the soil seed bank a preliminary trial undertaken in the Northern Territory found that less than 36% of bellyache bush seed could be recovered 6 months after burial at depths of 0, 1, 5, and 10 cm (Pitt 1992). Greatest numbers of remaining viable seed were recovered from 0 and 10 cm (Miller and Pitt 1992; Pitt 1992).

This paper reports the findings of two long-term field studies on the persistence of bellyache bush seed banks. A seed burial trial quantified the effects of rainfall conditions and burial depth on the persistence of both intact and ant-discarded seeds. A second trial used measurements of seedling emergence over time to indicate the rate of seed bank depletion at two field sites (rocky and heavy clay) following physical removal of all bellyache bush plants.

Materials and methods

Seed burial

Site description

The seed burial experiment was established in open woodland on a cattle property (19°54′S, 146°13′E) 23 km NNW of Charters Towers. The soil comprised a sandy A horizon of varying thickness (0–5 cm depth) over a sandy loam to sandy clay B horizon (5–40 cm depth). Bellyache bush seeds were buried within these two soil horizons. All understorey vegetation was physically removed before commencement of the experiment and regular weeding was undertaken thereafter.

Seed collection

Seeds used in this study were either intact (carunculate) or ant-discarded (seeds whose caruncles and exotegmen were eaten by meat ants and ultimately discarded on their middens with other nest waste, as described by Bebawi and Campbell (2002*a*). Intact seeds were obtained from capsules harvested in February 2001 from plants located at Southern Cross Creek (20°01′S, 146°01′E), 26 km NNW of Charters Towers. Capsules were placed in jackets of aluminium mosquito gauze (1 m^2) to dry for a week in a dry glasshouse and dehisce (capsules shattered) spontaneously. Dehisced capsules were initially passed through a 5-mm aluminium sieve to remove capsule debris and later through a 3-mm sieve to collect clean intact bellyache bush seeds. Ant-discarded seeds were collected from meat ant (*I. spadius*) nests at the same site. They were cleaned from midden material using the same sieving process as for intact seeds. Damaged, empty seed shells and dark brown seeds (previous year's harvested seeds) were removed and only grey-brown seeds (current year's harvested seeds) were used in this study. A preliminary investigation showed that ants discard bellyache bush seeds on their middens on the soil surface about 4 weeks after they have harvested them. Consequently, intact seeds in this study were harvested about 4 weeks before ant-discarded seeds were collected to ensure both seed lots were of a similar age.

From both the intact and ant-discarded seed lots, 144 sub-samples of 40 seeds were randomly selected and placed in bags of 70% shade rating shade cloth ($4 \times 4 \times 0.5$ cm; ~1.1×2.4 mm mesh size) to simplify seed retrieval while maximising soil/seed contact.

Trial design

The experiment established on 19 March 2001 was a $2 \times 2 \times 6 \times 13$ factorial replicated three times. There were two rainfall conditions (natural and rainfall-excluded), two seed types (intact or ant-discarded), six burial depths/conditions (0, 5, 10, 20 and 40 cm and 0 cm mulch-covered) and 13 retrieval times (0, 3, 6, 9, 12, 24, 36, 48, 72, 84, 96, 108 and 120 months).

Three blocks each comprising two 2.4×2.0-m plots were established, with adjacent plots separated by a 1.5-m buffer. Within blocks one plot was randomly selected to receive natural rainfall and the other to have rainfall excluded. Shelters were erected to exclude rainfall. Railway sleepers were placed around the plot to a height of 23 cm and a roof of polycarbonate material was attached to the sleepers with hinges, leaving a 10-cm gap between sleepers and roof. This design minimised temperature differences between inside and outside of the shelters, and allowed light to diffuse through but excluded rainfall.

Cylindrical PVC (Polyvinyl Chloride) pipes (11 cm diameter × 50 cm height) were used for the seed burial. PVC pipes were perforated at the base for drainage; four holes were also drilled on the sides of the PVC pipes at each depth of 5, 10, 20 and 40 cm to allow for further drainage as well as the insertion of thermocouples (1.5 mm diameter) to record soil temperature. Blotting paper was placed at the base and the sides of the pipes to prevent soil loss but allow free drainage.

Pipes were systematically filled on site using ~6.5 kg of topsoil, with a bag of intact and a bag of ant-discarded seeds placed side by side at depths of 0, 5, 10, 20 and 40 cm. Gypsum blocks were also placed at each burial depth to help determine available soil moisture.

In each of the six plots, 12 of the PVC pipes were placed 15 cm apart in a centrally located 50 cm deep trench, which was then backfilled. Bags of seeds for the 0-cm depth mulched treatment were placed on the soil surface in rectangular PVC open-ended containers (200 × 10 × 5 cm) beside the buried PVC pipes. Bags were then covered with 5 cm of leaf mulch.

Germination and viability test protocol

One large cylinder and one small PVC container (0-cm mulched treatment) from each of the six plots were retrieved at each of the 13 predetermined times. Germination and viability of seeds were tested at the Tropical Weeds Research Centre (Charters Towers, Queensland).

Bags containing buried seeds were washed under tap water to remove attached soil particles. Seeds were then removed from the bags and placed in water in 29.5-mL plastic containers. Floating empty shells from germinated seeds and ungerminated seeds which sank were counted. Ungerminated seeds were placed at 0.5-cm depth in perforated PVC end caps (11 cm diameter × 2.5 cm height) filled with 300 g (dry weight) of fine river sand. The sand was watered to approximate field capacity. When water had drained freely for 2 days through the perforated base, the caps were placed in a controlled-environment glasshouse set at a 12/12-h temperature of 30/20 ± 1°C. The caps were watered daily.

Germination was recorded by counting emerged seedlings on a daily basis and removing them afterwards. When no seedlings had emerged for 7 consecutive days, the soil was washed through a 3-mm aluminium mesh sieve to retrieve non-germinated seeds. Viability of non-germinated seeds was tested using the tetrazolium method (Moore 1985). Seeds were placed for 5 days in Petri dishes filled with 10 mL of 1% triphenyl tetrazolium chloride and kept in complete darkness at 30°C. Seeds were then cut longitudinally with a sharp scalpel and those that showed pink embryos were considered viable.

Soil temperature measurements and calculation of Growing Degree Days

Soil temperatures were measured on an hourly basis under both natural rainfall and rainfall-excluded conditions at the six seed burial depths. Type K steel encased thermocouples were placed through the holes into the PVC pipe and hooked to a data logger (Data Electronics Pty Ltd, Brisbane, Qld) charged using a solar panel, with data downloaded monthly.

Soil temperatures were converted to GDD (Growing Degree Days) by averaging daily maximum and daily minimum temperatures for each day, subtracting an assumed minimum temperature required for growth to proceed and then summing over days (Wilen *et al*. 1996; Ball *et al*. 2004; Growing-degree days 2012). When the average daily temperature is equal to or less than the base temperature, no degree days are accumulated. From our experience, the base temperature at which growth generally starts for woody plants in the vicinity of Charters Towers is ~22°C, which usually occurs at the onset of spring in September.

Rainfall and available soil moisture

Records of monthly rainfall for 2001–10 at the experimental site were obtained from the Australian Bureau of Meteorology (BOM 2012). Available soil moisture under both natural rainfall and rainfall-excluded conditions at the six seed burial depths was calculated monthly using gypsum soil blocks (sensors) and the measuring instrument, Model KS-D1 Moisture Tester (Delmhorst Instrument Co, Towaco, NJ, USA), which records the electrical resistance of the gypsum. These readings provided an indication of the moisture available to the seeds and were converted to available soil moisture using calibration curves.

Data analysis

Predicted times to 50 and 1% viability were calculated by applying 'best-fit' exponential decay models to each treatment replicate. Time to 0% viability was not calculated due to the presence of a small number of seeds remaining viable in some treatments at the end of the experiment. Statistical analysis of the results using ANOVA was undertaken with Fisher's protected l.s.d. tests used to identify differences between treatments. Soil GDD and available soil moisture data were analysed similarly (GENSTAT 8.1 Committee 2005).

Seedling emergence

Site descriptions

To gain an understanding of the natural rundown of bellyache bush soil seed banks, monitoring of seedling emergence was conducted on two grazing properties north of Charters Towers. The two sites selected (referred to as rocky and heavy clay) initially contained monocultures of bellyache bush averaging 140 000 ± 4000 plants/100 m^2. While measurements were not taken, visually the rocky site appeared to have a greater portion of large reproductive plants. Infestations were typical of the disturbed habitats in which bellyache bush is often found growing in north Queensland and, according to local landholders, had established ~20 and 45 years earlier at the clay and rocky site, respectively. Cattle were excluded from both sites for the duration of the study.

The rocky site (19°53′S, 146°14′E) had a red duplex soil (Northcote 1974) with a pH of 6.6 and averaging 36% clay, 11% silt, 34% fine sand and 19% coarse sand. Basalt rocks, from 50 to 125 000 cm^3 in size, were spread throughout the site. Herbaceous vegetation consisted of a few grasses (60% cover), including giant speargrass [*Heteropogon triticeus* (R.Br.) Stapf], dark wiregrass (*Aristida calycina* R.Br.), buffel grass (*Cenchrus ciliaris* L.), forest blue grass [*Bothriochloa ewartiana* (Domin) C.E.Hubb.] and native oatgrass [*Themeda avenacea* (F.Muell.) Maiden & Betche]. The shrub stratum (30% cover) was dominated by bellyache bush, currant bush (*Carissa ovata* R.Br.), African boxthorn (*Lycium ferocissimum* Miers) and white currant bush [*Flueggea virosa* (F.Muell.) G.L.Webster], while a mixture of narrow-leaved iron bark (*Eucalyptus crebra* F.Muell.) and whitewood [*Atalaya hemiglauca* (F.Muell.) F.Muell. ex. Benth.] dominated the tree stratum (10% cover).

The heavy clay site (20°01′S, 146°11′E) had a cracking clay soil (Northcote 1974) with a pH of 7.7 and averaging 43% clay, 14% silt, 24% fine sand and 19% coarse sand. Herbaceous vegetation (70% cover) consisted of native couch [*Brachyachne convergens* (F.Muell) Stapf], golden beardgrass (*Chrysopogon fallax* S.T.Blake) and Indian couch [*Bothriochloa pertusa* (L.) A.Camus]. The shrub stratum (20% cover) was dominated by bellyache bush, chinee apple (*Ziziphus mauritiana* L.) and false sandalwood (*Eremophila mitchellii* Benth.), while a mixture of carbeen (*Eucalyptus tessellaris* F.Muell.), bauhinia [*Lysiphyllum cunninghamii* (Benth.) de Wit] and emu apple (*Owenia acidula* F.Muell.) dominated the tree stratum (10% cover).

Site design and measurements

At each site, a uniform 25-m^2 area of dense bellyache bush was selected and six randomly located permanent quadrats (50 × 50 cm) established. Each area was surrounded by a 15-m buffer zone kept free of bellyache bush to minimise the risk of seed entering the plots from nearby bellyache bush plants. The size of the buffer was greater than the known ballistic dispersal range of seeds and the likelihood of seed movement by ants was considered negligible given the absence of nests in the immediate area.

In December 2000, all bellyache bush seedlings in all quadrats were counted and removed. All other bellyache bush plants in the 25-m^2 areas were also manually removed to prevent new seed being added to the seed bank. Once a month thereafter, bellyache bush seedlings that had emerged in the quadrats were counted and removed. Preliminary testing indicated that although some seedlings emerged and died between recording periods, they were still easily identifiable, as their stems remained intact. Seedling removal of all species (including bellyache bush) was also performed over the rest of the 25-m^2 areas on a monthly basis to ensure that the level of competition imposed remained relatively consistent over time.

The study ended after 6 years of monitoring. By this time no bellyache bush seedlings had emerged in quadrats for at least the previous 15 months. For each site, monthly seedling emergence data was then summed to produce cumulative seedling emergence graphs expressed as a percentage of total seedling emergence.

Records of rainfall between 2000 and 2006 were obtained from a nearby weather station (19°56′S, 146°11′E). Soil moisture content was determined monthly for the soil profile (0–5 cm depth). Three soil samples from each site were collected and immediately stored in waterproof glass jars, transferred to the laboratory and weighed. The samples were then oven-dried at 100°C for 48 h and reweighed. The gravimetric soil moisture content was expressed as the percentage of oven dry soil weight.

Results

Seed burial

Environmental conditions

Annual rainfall received at the site, under natural conditions, was below average for the first 6 years of the experiment (2001–06) and above average for the last 4 (2007–10) (Fig. 1). During the drier years, between one and five rainfall events of 25 mm or more fell annually, while during the wetter years this increased to 11–15 events annually (Fig. 1).

Rain shelters were highly effective in limiting moisture availability, with average moisture content under natural rainfall conditions 6 times higher (0.46%) than under rainfall-excluded treatments (0.08%) over the life of the experiment. Significant differences ($P < 0.01$) also occurred between burial depths for soil moisture content under natural rainfall conditions, which averaged 0.5% in the sub-soil at 5–20-cm depths compared with 0.4% at the soil surface at 0-cm depth (Fig. 2).

Despite the presence of structures over the rainfall-excluded plots, soil temperatures at the ground surface were not significantly different ($P > 0.05$) between natural and rainfall-excluded plots over the life of the experiment. However, significant interactions ($P < 0.01$) between rainfall conditions and burial depth were detected for cumulative GDD. Cumulative GDD were, on average, 14% greater under rainfall-excluded conditions compared with natural conditions. Cumulative GDD were significantly greater in the top soil profile compared with sub-soil profiles (Fig. 3). For example, cumulative GDD at the 0 cm (without mulch) depth averaged 151 and 213 thermal units under natural and rainfall-excluded conditions compared with 135 and 148 thermal units at 40-cm depth, respectively. There was a significant negative correlation ($r = -0.67$) between burial depth and cumulative GDD.

Effects of seed type, rainfall condition and burial depth on seed persistence

The two seed types (intact and ant-discarded) used in this trial differed significantly ($P < 0.05$) in terms of their initial weight and moisture content but not their viability or

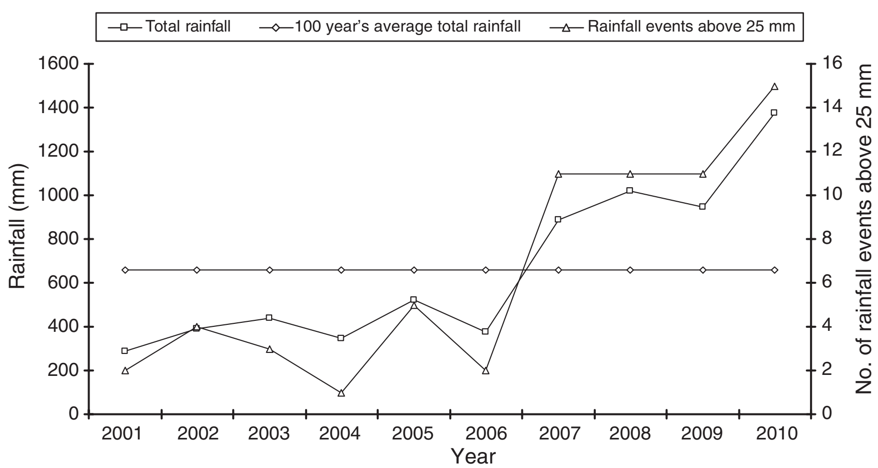

Fig. 1. Annual rainfall between 2001 and 2010 and the total number of rainfall events that exceeded 25 mm, along with the long-term mean (grey bars).

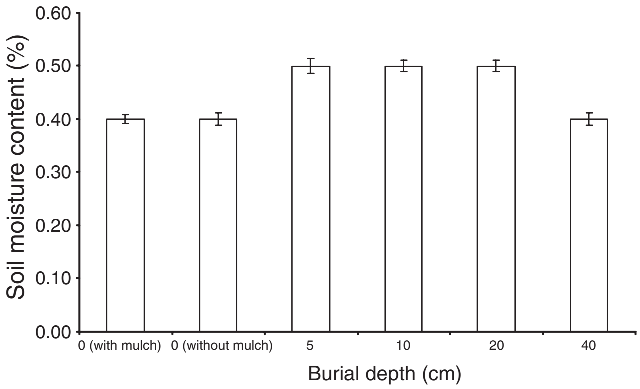

Fig. 2. Soil moisture content (%) as affected by burial depth under natural conditions over all years, and seed types. Vertical bars indicate the s.e. of the means.

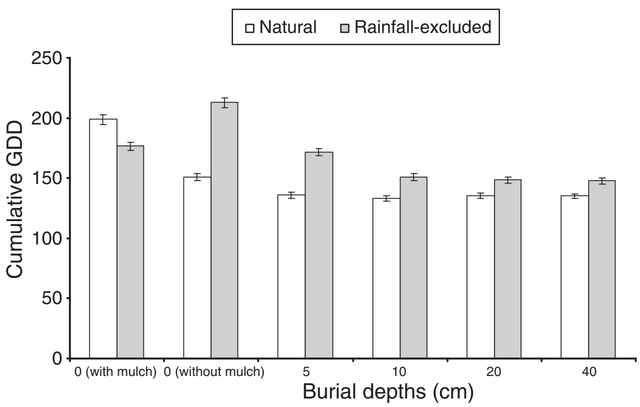

Fig. 3. Cumulative Growing Degree Days (GDD) as affected by burial depth under natural and rainfall-excluded conditions. Vertical bars indicate the s.e. of the means.

germinability ($P > 0.05$). Intact seeds weighed 49.5 ± 1.3 mg and had a moisture content of $2.26 \pm 0.02\%$. Ant-discarded seeds were heavier (58.4 ± 2.3 mg) but their moisture content was lower ($1.05 \pm 0.04\%$) than that of intact seeds. Seed viability averaged $81\% \pm 0.9\%$ across both seed types, with $52\% \pm 2.2\%$ of viable seeds dormant and the remaining $48\% \pm 2.2\%$ germinable.

Persistence of seed lots varied depending on seed type, rainfall conditions and burial depths (Fig. 4). Regression analysis of the time taken for seed viability to decline to 50 and 1% identified significant interactions between seed type $\times$ rainfall condition, rainfall condition $\times$ burial depth and seed type $\times$ burial depth ($P < 0.05$).

Viability of intact and ant-discarded seeds decreased rapidly under natural rainfall conditions, taking on average only 6 months to decline to 50%. In contrast, when rainfall was excluded ant-discarded seeds persisted for longer, averaging 40 months for viability to decline to 50% compared with 8 months for intact seeds. Similar patterns occurred with seed viability decline to 1%, but the times were much longer. Intact and ant-discarded treatments averaged 47 months under natural rainfall conditions, whereas under rainfall-excluded conditions it took 88 and 118 months for intact and ant-discarded seeds to decline to 1% viability, respectively. No viable seeds of bellyache bush remained after 72 months irrespective of seed type under natural rainfall conditions. When rainfall was excluded, a small portion of viable ant-discarded seeds (<1%) was still present at the end of the trial (i.e. after 120 months).

Seeds persisted for longer at all burial depths under rainfall-excluded conditions compared with those that received natural rainfall (Fig. 5*a*), averaging 103 and 47 months for viability to decline to 1%, respectively. Despite some variability, seed persistence was prolonged (>96 months to decline to 1% viability) at all burial depths under rainfall-excluded conditions. Under natural rainfall there were highly significant differences between the two surface located seed lots (with and without mulch) and those that were buried at 5, 10, 20 and 40 cm (Fig. 5*a*). Surface located seeds took on average 70 months to decline to 1% viability while buried seeds took on average 35 months.

Both seed types generally persisted for longer if located on the soil surface than if buried. Ant-discarded seed persisted longer than intact seed overall burial depths, although at 0 (both mulched and non-mulched) and 40 cm below ground persistence did not differ significantly ($P > 0.05$) (Fig. 5*b*).

Seedling emergence

Rainfall and soil moisture

The study period was characterised by a series of dry years, with annual rainfall averaging only 393 mm, 40% less than the long-term mean of 657 mm/year (Willcocks and Young 1991). Nevertheless, rainfall events considered sufficient to promote seedling emergence (greater than 25 mm) occurred in all years (Fig. 6), similar to conditions in the seed burial trial, which was located within 6 km of both sites (Fig. 1). While rainfall was generally highest in summer, rapidly declining during autumn to lowest in winter (Fig. 7), soil moisture demonstrated a slower rate of decline, particularly during the autumn period. On average, soil moisture tended to be higher at the heavy clay site compared with the rocky site, particularly during the December–June period (Fig. 7).

Cumulative seedling emergence

In total, an average of 348 ± 26 and 113 ± 17 bellyache bush seedlings/m^2 emerged at the rocky and heavy clay soil sites, respectively.

Seedling emergence occurred primarily during the wet season periods (November–March). It was most prolific in the first 12 months and declined rapidly thereafter, particularly at the heavy clay soil site. The average time taken to reach 50% of total seedling emergence was 1 and 11 months at the heavy clay and rocky sites, respectively (Fig. 6).

No new seedlings were recorded after 36 months at the heavy clay soil site or 58 months at the rocky site (Fig. 6).

Discussion

Seed bank persistence

The seed burial and seedling emergence studies have quantified the persistence of bellyache bush soil seed banks. Overall, seed persistence was highly variable in response to moisture

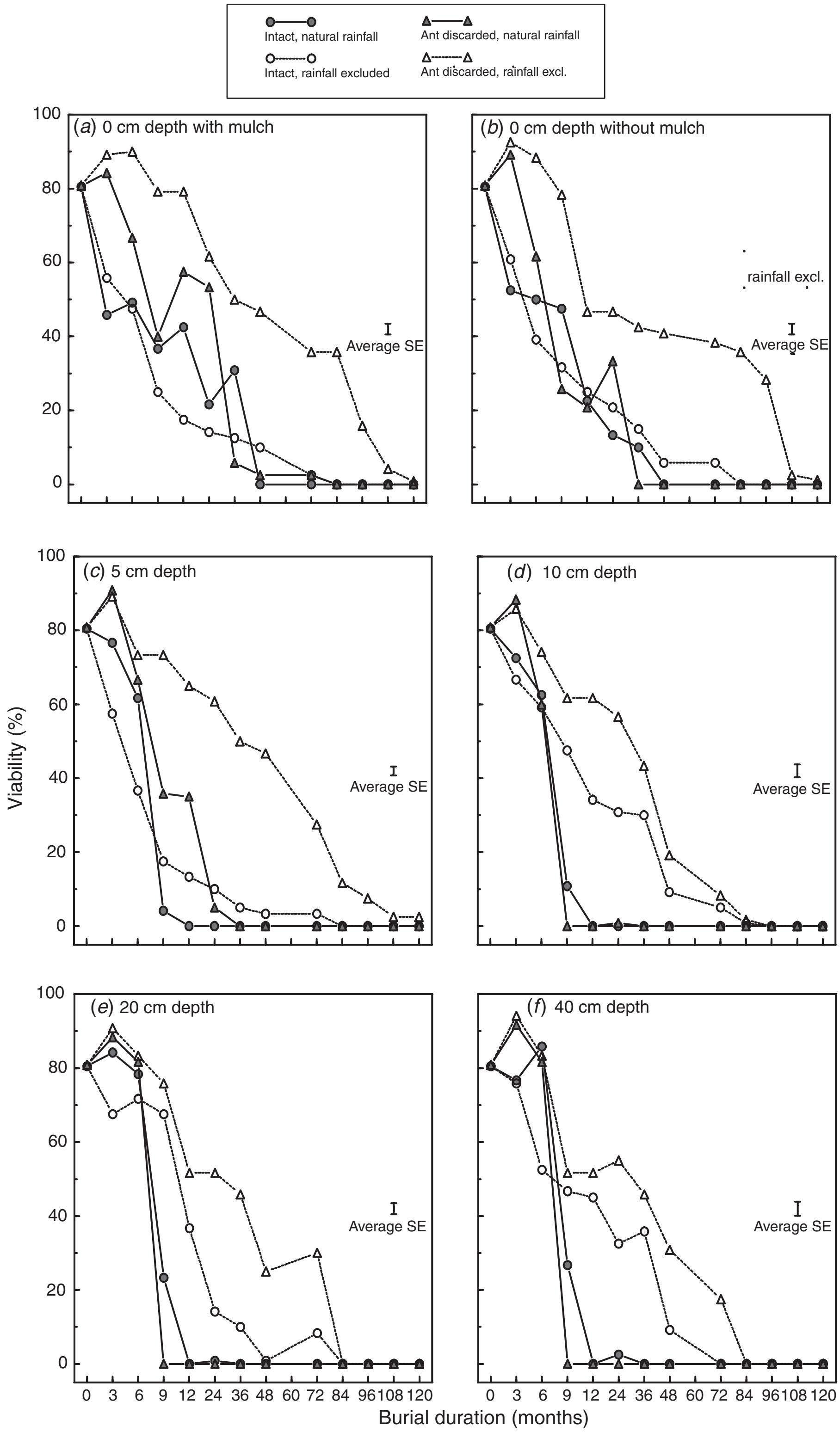

Fig. 4. (*a–f*) Viability response of '• intact seed under natural rainfall', '○ intact seed with rain excluded', '▲ ant-discarded seed under natural rainfall' and 'Δ ant-discarded seed with rain excluded', left on the soil (bare or under mulch) or buried at various depths. The time-scale changes from 3- to 12-month intervals after the first year. The average standard errors shown are for all non-zero means.

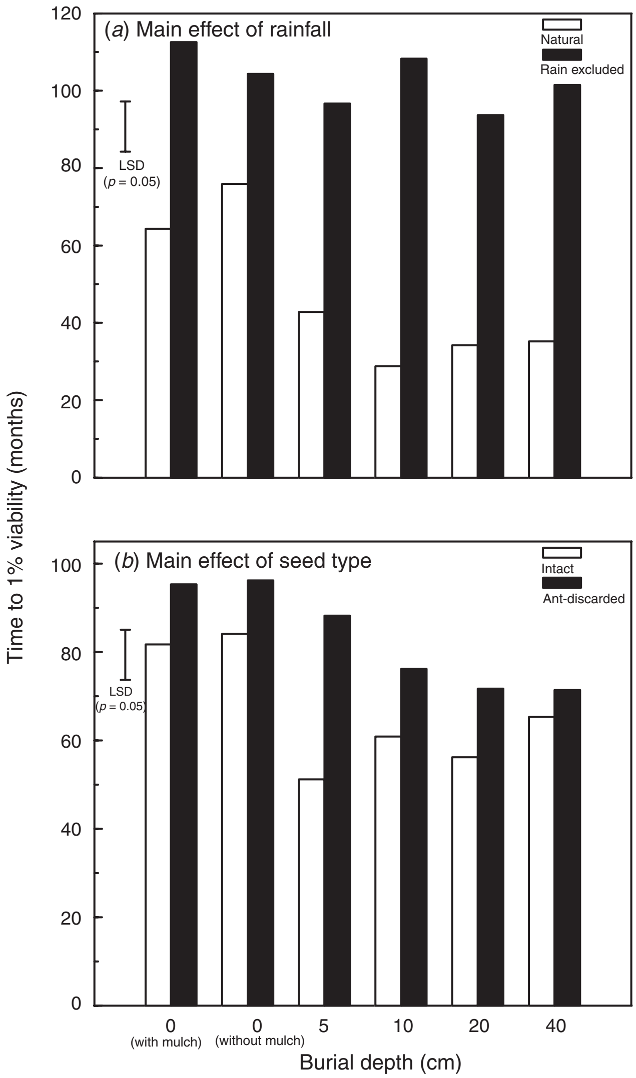

Fig. 5. Estimated time to 1% viability of bellyache bush seeds (*a*) as affected by burial depth and natural and rainfall-excluded conditions over all seed types and (*b*) as affected by burial depth and seed types over all conditions. Vertical bars indicate the s.e. of the means.

availability, burial depth and seed type (i.e. intact or ant-discarded). Based on loss of viability in the burial trial and seedling emergence patterns at both the rocky and heavy clay soil sites, 3–4 years appeared to be the shortest duration that seed persisted when exposed to natural rainfall conditions. In contrast, the seed burial trial demonstrated that a small proportion of seed could remain viable for over 10 years under dry conditions.

Based on a dichotomous key developed by Thompson *et al.* (1997) that classifies seed longevity into three categories [transient (viable $\leq$1 year), short-term persistent (viable 1–5 year) and long-term persistent (viable $\geq$5 year)], current results suggest that bellyache bush has a short- to long-term persistent seed bank depending on prevailing conditions. Other members of the family Euphorbiaceae also have short- to long-term persistent seed banks as well. For example, seeds of the false caper (*Euphorbia terracina* L.) in Western Australia (Brown and Bettink 2011) and leafy spurge (*Euphorbia esula* L.) in the USA have remained viable in the soil for up to 5 and 8 years, respectively (Northern Prairie Wildlife Research Centre 2006).

Effect of soil moisture, burial depth and seed type on seed persistence

The large difference in persistence of bellyache bush seeds between natural rainfall and rainfall-excluded treatments reflects the variability that may occur in rangeland environments. Under average to above-average rainfall conditions, seed germination will be a major contributor to rundown of bellyache bush seed banks. In contrast, germination opportunities will be greatly reduced during prolonged dry periods, resulting in increased persistence of soil seed reserves. Similarly, Vivian-Smith and Panetta (2009) found that longevity of *Lantana camara* L. seed banks was reduced under high soil moisture conditions. In their study, *L. camara* seeds were predicted to persist for 11 years under natural rainfall conditions, compared with only 3 years for seed lots that received supplementary irrigation to keep soil moisture levels close to field capacity.

Differences in seedling emergence patterns between soil types in the seedling emergence trial and in viability between burial depths under natural rainfall conditions in the seed burial study also appear to be at least partially related to soil moisture availability. Seedling emergence ceased earlier at the heavy clay soil site, which tended to have higher soil moisture than the rocky site and hence more favourable conditions for germination to occur. Similarly, seed persistence was shorter for seeds buried below ground than those located on the surface where soil moisture tended to be lower. There are numerous other factors that could influence depletion of seed banks at different depths in the soil profile, including aeration, light availability, temperature and hard-seededness (Roberts and Feast 1972; Popay and Thompson 1979; Holmes and Newton 2004). In comparison to moisture availability, however, temperature and associated GDD did not appear to be a major influence on persistence of seed of bellyache bush in the burial experiment.

The reason for the greater cumulative seedling emergence at the rocky site compared with the heavy clay site is not clear given that they had similar densities of bellyache bush, although the infestation was older and may have contained a greater proportion of mature reproductive plants. The loss of seeds from the heavy clay soil site before they could germinate may also have been greater than the rocky site. The cracking nature of the heavy clay soil would be conducive to loss of seeds through deep burial and the surface lacked protective areas compared with the rocky site that had a scattering of small to large rocks where the seeds could gain some protection. Nevertheless, with no evidence of a relationship between seed bank density and persistence found in the literature, we expressed emergence over time as a percentage of the total emergence to allow for a standardised comparison.

The longer persistence of ant-dispersed seeds than intact seeds under prolonged dry conditions during the seed burial trial is difficult to explain and warrants further investigation. Both seed types exhibited a similar level of viability and germinability at the start of the study, but ant-discarded seeds tended to be larger and

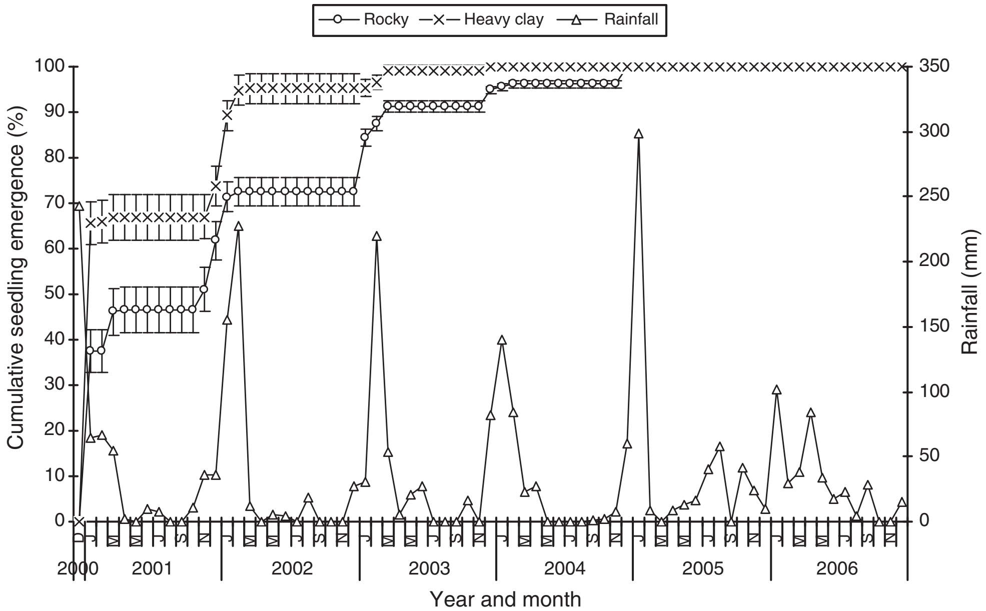

Fig. 6. Monthly rainfall and cumulative seedling emergence at the rocky and heavy clay sites. Vertical bars indicate the s.e. of the means.

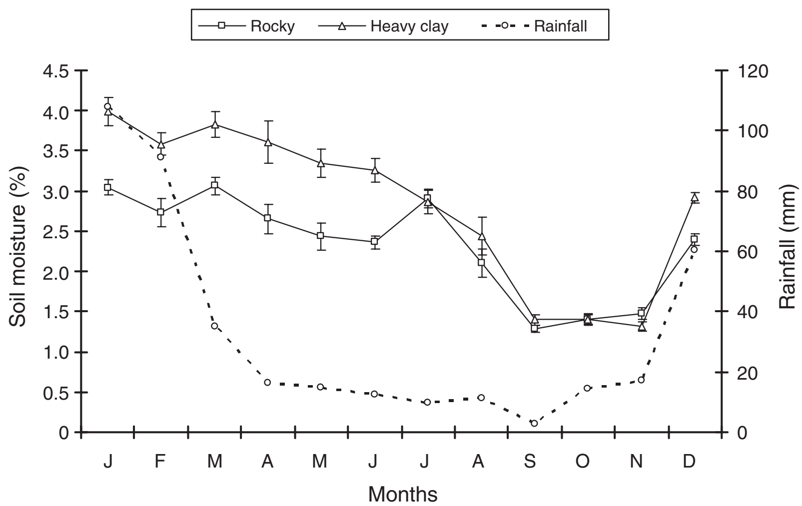

Fig. 7. Average monthly rainfall and monthly soil moisture content at the rocky and heavy clay site over all years. Vertical bars indicate the s.e. of the means.

have lower moisture content. Meat ants appeared to be selectively choosing larger seeds and perhaps these seeds have attributes that prolong their viability. It could also be that the ants did not discard seeds that they could use and these may have been the more germinable or more damaged ones. Studies on other plants have demonstrated correlations of seed size and morphology with seed persistence (Bekker *et al*. 1998; Ghersa and Martinez 2000; Moles and Westoby 2006; Traba *et al*. 2006). While not the case in the present study, previous research found that meat ants tended to promote the germination of bellyache bush seeds by removing the caruncle and scarifying the outer seed coat. In one study, 100% germination was recorded for ant-discarded seeds, compared with only 8% germination for intact seeds (Bebawi and Campbell 2004).

Implications for management

The present studies suggest that after initial treatment of a bellyache bush infestation, ongoing control will be needed for ~4–5 years under average rainfall conditions for control of seedlings. This is consistent with results from a management trial that investigated primary and secondary control options for bellyache bush (Bebawi *et al*. 2011). After 4 years of the most effective treatment, annual foliar spraying of bellyache bush with a selective herbicide towards the end of the wet season, no new bellyache bush seedlings were found. Under dry conditions, the duration of control programs may need to be considerably longer than 4 years with seed persistence extended, mainly due to less opportunities for germination to occur.

With seed banks in the seed burial trial generally displaying an exponential decline under natural rainfall conditions, seedling regrowth and the resources required to control it would be greatest in the first few years and decline rapidly thereafter. This was the case in the seedling emergence trial, where half of all seedlings recorded emerged within the first 1 and 11 months at the heavy clay and rocky sites, respectively. Nevertheless, given the large seed banks that bellyache bush can develop, particularly under dense infestations, survival of only a small proportion of seeds means there may still be a relatively large viable seed bank. Previous studies have reported 9.3 M bellyache bush seeds per 100 m^2 in the soil seed bank (Bebawi and Campbell 2002*b*) under a dense infestation. Even at a 1% survival rate there would be 9.3 viable seeds per m^2, providing opportunities for recruitment to occur in the absence of any control activities.

For bellyache bush, the biggest challenge to depleting soil seed reserves is preventing replenishment through further seed input. This is most likely to occur from plants that survive control efforts, new seedlings reaching reproductive maturity before implementation of follow-up control activities or seed being brought in from external sources (e.g. contaminated machinery/equipment and/or during floods from upstream infestations) (Randall *et al*. 2009).

Under favourable rainfall conditions, seed production (Bebawi *et al*. 2005*a*, 2005*b*) and seedling emergence of bellyache bush could be expected to occur throughout most months of the year in tropical environments. These seedlings could also reach reproductive maturity quickly and start replenishing the seed bank if not controlled. In a pot trial where plants where regularly watered, new plants flowered and produced fruits within 55 and 65 days, respectively (Bebawi *et al*. 2005*b*). In a field study in north Queensland, the time to first flowering averaged 74 days in cleared areas, 294 days in rocky sites and 454 days in grazed pastures (Bebawi *et al*. 2005*b*). These findings suggest that while annual control activities such as those reported during a large-scale management trial (Bebawi *et al*. 2011) may be appropriate under some situations, if highly favourable growth conditions prevail (such as high rainfall and limited or no pasture composition), more frequent follow-up control programs would be needed to avoid replenishment of the seed bank.

Given its phenological and reproductive attributes, eradication of bellyache bush is most likely feasible for small infestations, with a reduction in size and density of infestations a more realistic goal for land managers dealing with extensive areas. Nevertheless, management of soil seed reserves will still need to be considered to achieve this outcome.

Acknowledgements

We gratefully acknowledge the Robert von Wald, Bob Taylor and Michael Penna families for allowing us to undertake research on their properties. We also thank the former Department of Employment, Economic Development and Innovation for providing financial support to the project. We are grateful to Mrs B. Madigan and Dr D. Panetta for reviewing the manuscript and to the staff of the Tropical Weeds Research Centre for their assistance with implementation of treatments.

References

Aranda-Rickert, A., and Fracchia, S. (2011). *Pogonomyrmex cunicularius* as the keystone disperser of elaiosome-bearing *Jatropha excisa* seeds in semi-arid Argentina. *Entomologia Experimentalis et Applicata* **139**, 91–102. doi:10.1111/j.1570-7458.2011.01111.x

Ashley, M. (1995). 'The Biology and Management of *Jatropha gossypifolia*.' Technical Bulletin No. 234. (Department of Primary Industry and Fisheries: Darwin, NT.)

Ball, D. A., Frost, S. M., and Gitelman, A. I. (2004). Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days. *Weed Science* **52**, 518–524. doi:10.1614/WS-03-067

Bebawi, F. F., and Campbell, S. D. (2002*a*). Seed dispersal of a myrmecochorous weed, bellyache bush (*Jatropha gossypifolia*) in riparian landscapes of northern Queensland. *In*: 'Proceedings of the Royal Society of Queensland, Landscape Health in Queensland Symposium'. (Eds A. J. Franks, J. Playford and A. Shapcott.) pp. 98–102. (The Royal Society of Queensland: Brisbane.)

Bebawi, F. F., and Campbell, S. D. (2002*b*). Effects of fire on germination and viability of bellyache bush (*Jatropha gossypifolia*). *Australian Journal of Experimental Agriculture* **42**, 1063–1069. doi:10.1071/EA01125

Bebawi, F. F., and Campbell, S. D. (2002*c*). Impact of fire on bellyache bush (*Jatropha gossypifolia*) plant mortality and seedling recruitment. *Tropical Grasslands* **36**, 129–137.

Bebawi, F. F., and Campbell, S. D. (2004). Interactions between meat ants (*Iridomyrmex spadius*) and bellyache bush (*Jatropha gossypifolia*). *Australian Journal of Experimental Agriculture* **44**, 1157–1164. doi:10.1071/EA03194

Bebawi, F., Vitelli, J., Campbell, S., and Madigan, M. (2004). Integrated control methods for bellyache bush (*Jatropha gossypifolia* L.) in northern Queensland: preliminary results. *In*: 'Proceedings of the 14th Australian Weed Conference'. (Eds B. M. Sindel and S. B. Johnson.) pp. 150–152. (Weed Society of New South Wales: Sydney.)

Bebawi, F. F., Mayer, R. J., and Campbell, S. D. (2005*a*). Phenology of bellyache bush (*Jatropha gossypifolia* L.) in northern Queensland. *Plant Protection Quarterly* **20**, 46–51.

Bebawi, F. F., Mayer, R. J., and Campbell, S. D. (2005*b*). Flowering and capsule production of bellyache bush (*Jatropha gossypifolia* L.) in northern Queensland. *Plant Protection Quarterly* **20**, 129–132.

Bebawi, F. F., Vitelli, J. S., Campbell, S. D., Vogler, W. D., Lockett, C. J., Grace, B. S., Lukitsch, B., and Heard, T. A. (2007). The biology of Australian weeds 47. *Jatropha gossypifolia* L. *Plant Protection Quarterly* **22**, 42–58.

Bebawi, F. F., Vitelli, J. S., Campbell, S. D., and Mayer, R. J. (2011). Impact of control strategies on bellyache bush (*Jatropha gossypifolia* L.) mortality, seedling recruitment, population dynamics, pasture yield and cost analysis. *The Rangeland Journal* **33**, 277–286. doi:10.1071/RJ10038

Bekker, R. M., Bakker, J. P., Grandin, U., Kalamees, R., Milberg, P., Poschold, P., Thompson, K., and Willems, J. H. (1998). Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology* **12**, 834–842. doi:10.1046/j.1365-2435.1998.00252.x

BOM (2012). Mean annual rainfall and maximum daily temperature ranges. Available at: www.bom.gov.au (accessed 19 October 2012).

Brown, K., and Bettink, K. (2011). Management notes (for the Swan NRM Region). Available at: http://florabase.dec.wa.gov.au (accessed 17 October 2012).

Campbell, S. D., and Grice, A. C. (2000). Weed biology – a foundation for weed management. *Tropical Grasslands* **34**, 271–279.

Cardina, J., and Sparrow, D. H. (1996). A comparison of methods to predict weed seedling populations from the soil seed banks. *Weed Science* **44**, 46–51.

Csurhes, S. M. (1999). 'Bellyache bush (*Jatropha gossypifolia*) in Queensland.' – Pest Status Review Series – Land protection. (Queensland Department of Natural Resources: Brisbane.)

Cunningham, D. C., Wolde-ndorp, G., Burgess, M. B., and Barry, S. C. (2003). 'Prioritising Sleeper Weeds for Eradication: Selection of Species Based on Potential Impacts on Agriculture and Feasibility of Eradication.' (Bureau of Resource Sciences: Canberra.)

Everist, S. L. (1974). 'Poisonous Plants of Australia.' (Angus and Robertson: Sydney.)

Genstat 8.1 Committee. (2005). 'Genstat Release 8.1. Reference Manual.' (VSN International Ltd.: Hemel Hempstead, UK.)

Ghersa, C. M., and Martinez, M. (2000). Ecological correlates of weed seed size and persistence in the soil under different tilling systems: implications for weed management. *Field Crops Research* **67**, 141–148. doi:10.1016/S0378-4290(00)00089-7

Growing-degree days. (2012). Wikipedia. Available at: http://en.wikipedia.org/wiki/Growing-degreeday (accessed 5 October 2012).

Heard, T. A., Chan, R. R., and Segura, R. (2002). Prospects for the biological control of bellyache bush, *Jatropha gossypifolia*. *In*: 'Proceedings of the 13th Australian Weed Conference'. (Eds H. Spafford Jacob, J. Dodd and J. H. Moore.) pp. 366–369. (Plant Protection Society of Western Australia: Perth.)

Holmes, P. M., and Newton, R. J. (2004). Patterns of seed persistence in South African fynbos. *Plant Ecology* **172**, 143–158. doi:10.1023/B:VEGE.0000026035.73496.34

Miller, I. L., and Pitt, J. L. (1992). 'Is *Jatropha gossypifolia* the next *Mimosa pigra*?' Transcript of BARC Seminar 26 June 1992. (Berimah Agricultural Research Centre, Department of Primary Industries and Fisheries: Darwin, NT.)

Moles, A. T., and Westoby, M. (2006). Seed size and plant strategy across the whole life cycle. *Oikos* **113**, 91–105. doi:10.1111/j.0030-1299.2006.14194.x

Moore, R. P. (1985). 'Handbook on Tetrazolium Testing.' (International Seed Testing Association: Zurich.)

Myers, J. H., and Bazely, D. R. (2003). 'Ecology and Control of Introduced Plants.' (Cambridge University Press: Cambridge, UK.)

Northcote, K. H. (1974). 'A Factual Key for the Recognition of Australian Soils.' 4th edn. (Rellim Technical Publications: Adelaide.)

Northern Prairie Wildlife Research Centre. (2006). An assessment of exotic plant species of Rocky Mountain National Park – *Euphorbia esula* L.– Leafy spurge (Euphorbiaceae). Available at: www.npwrc.usgs.gov/resource/plants/explant/euphesul.htm (accessed 15 January 2008).

Panetta, F. D., and Groves, R. H. (1990). Weed management and revegetation programs. *Proceedings of the Ecological Society of Australia* **16**, 545–549.

Parsons, W. T., and Cuthbertson, E. G. (2001). 'Noxious Weeds of Australia.' 2nd edn. (CSIRO Publishing: Melbourne.)

Pitt, J. L. (1992). Willeroo Station – Control of bellyache bush (*Jatropha gossypifolia*). Progress Report 1981–1995, Department of Primary Industries and Fisheries, Weeds Branch. Technical Annual Report – 1991/92, Darwin, Northern Territory.

Popay, A. I., and Thompson, A. (1979). Some aspects of the biology of *Carduus nutans* (L.) in New Zealand pastures. *In*: 'Proceedings 7th Asian-Pacific Weed Science Society Conference'. (Eds R. W. Medd and B. A. Auld.) pp. 343–346. (Asian-Pacific Weed Science Society: Sydney.)

Randall, A., Campbell, S., Vogler, W., Bebawi, F., and Madigan, B. (2009). 'Bellyache Bush (*Jatropha gossypifolia*) Management Manual – Current Control Options and Management Case Studies from Across Australia.' (Queensland Primary Industries and Fisheries, Department of Employment, Economic Development and Innovation, Queensland Government: Brisbane.)

Roberts, H. A., and Feast, P. M. (1972). Fate of seeds of some annual weeds in different depths of cultivated and undisturbed soil. *Weed Research* **12**, 316–324. doi:10.1111/j.1365-3180.1972.tb01226.x

Setterfield, S. A., Bellairs, S., Douglas, M. M., and Calnan, T. (2004). Seed bank dynamics of two exotic grass species in Australia's northern Savannas. *In*: 'Proceedings of the 14th Australian Weeds Conference'. (Eds B. M. Sindel and S. B. Johnson.) pp. 555–557. (Weed Society of New South Wales: Sydney.)

Smith, N. M. (1995). 'Weeds of Natural Ecosystems: A Field Guide to Environmental Weeds of the Northern Territory.' (Environment Centre: Darwin, NT.)

Smith, N. M. (2011). 'Weeds of Northern Australia: A Field Guide.' (Environment Centre: Darwin, NT.)

Thompson, K., Bakker, J. P., and Bekker, R. M. (1997). 'Soil Seed Banks of NW Europe: Methodology, Density and Longevity.' (Cambridge University Press: Cambridge, UK.)

Traba, J., Azcarate, F. M., and Peco, B. (2006). The fate of seeds in Mediterranean soil seed banks in relation to their traits. *Journal of Vegetation Science* **17**, 5–10. doi:10.1111/j.1654-1103.2006.tb02417.x

Vivian-Smith, G., and Panetta, F. D. (2009). Lantana (*Lantana camara*) seed bank dynamics: seedling emergence and seed survival. *Invasive Plant Science and Management* **2**, 141–150. doi:10.1614/IPSM-08-130.1

Wilen, C. A., Holt, J. S., and McCloskey, W. B. (1996). Predicting yellow nutsedge (*Cyperus esculentus*) emergence using degree-day models. *Weed Science* **44**, 821–829.

Willcocks, J., and Young, P. (1991). 'Queensland's Rainfall History.' (Queensland Department of Primary Industries: Brisbane.)