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Seed ecology of Captain Cook tree [*Cascabela thevetia* (L.) Lippold] – germination and longevity

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Abstract. *Cascabela thevetia* (L.) Lippold (Apocynaceae), commonly known as Captain Cook tree or yellow oleander, has established large infestations in riparian areas along several creeks and rivers in northern Queensland. To better understand the ecology of *C. thevetia* and the implications for its spread and management, this study reports seven experiments related to the seed ecology of its yellow and peach biotypes. We quantified its germination response to ambient (Experiment 1a and 1b), alternating and constant temperature (Experiment 2a and 2b) regimes and exposure to different light conditions (Experiment 3). Seed longevity under two soil types, two levels of pasture cover and three burial depths was also determined (Experiment 4a and 4b).

Both loose seeds and seeds still within pods (kernels) of the two biotypes of *C. thevetia* were able to germinate in all months of the year in northern Queensland, irrespective of the large differences in monthly ambient temperatures experienced at the Charters Towers study site. Both biotypes also germinated across a wide range of alternating day/night temperatures from 16/12°C to 47/37°C and constant temperatures from 17°C to 44.0°C. Germination of the two biotypes was significantly greater (4-fold) and faster (7 days earlier) under shade than under natural light conditions. Over all biotypes, soil types, levels of pasture cover and burial depths, no seeds of *C. thevetia* remained viable after 2 years: longevity was much less in many circumstances.

The results demonstrate that *C. thevetia* seeds can germinate over a wide temperature range, whereas the ability of seed to remain viable at low temperatures highlights the potential for expansion of its current potential distribution towards southern latitudes of the Australian continent. Across all experimental conditions, the yellow biotype displayed superior seed germination and viability traits compared with the peach biotype. Seed banks of the peach and yellow biotypes of *C. thevetia* are short-lived (2 years), which may be exploited when developing management strategies to reduce its impacts.

Additional keywords: biotypes, burial depth, temperature requirements, viability, yellow oleander.

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Introduction

Cascabela thevetia (L.) Lippold [syn. *Thevetia peruviana* (Pers.) K. Schum] is native to tropical America but has become naturalised in Australia, Africa and in several other countries and islands where it commonly exists as two biotypes based on flower colour: peach and yellow (Alvarado-Cárdenas and Ochoterena 2007; Bebawi *et al*. 2014; Alvarado-Cárdenas *et al*. 2017). In North Queensland, *C. thevetia* is most commonly known as 'Captain Cook tree' whereas in southern Queensland it tends to be called 'yellow oleander' (S. M. Csurhes, unpubl. data).

Cascabela thevetia is a popular ornamental plant in domestic gardens and amenity situations, but over this time it has 'jumped the garden fence' and established itself in small or large populations scattered over an extensive area, across coastal and sub-coastal Queensland (Csurhes and Edwards 1998; Bebawi *et al*. 2014). Batianoff and Butler (2002) listed *C. thevetia* as one of the 200 invasive naturalised plants in south-east Queensland and Grice and Martin (2005) considered it a significant threat to biodiversity in tropical rangelands of Australia. The white-flowered biotype is not naturalised in Queensland but is featured on various gardening internet sites. It is uncertain whether this biotype exists in Queensland (S. M. Csurhes, unpubl. data).

Cascabela thevetia is a restricted invasive plant (Category 3) under the *Biosecurity Act 2014* in the State of Queensland in riparian and woodland areas (Queensland Government 2016). Every part of the plant is also poisonous, particularly its seeds (Everist 1974; de Padua *et al*. 1999; Randall 2002). Although *C. thevetia* is endowed with multiple reproductive strategies (sexual and asexual), it reproduces mainly from seed. Sexually, it is very prolific as its fruits are multi-seeded containing up to four seeds, an evolutionary advantage which makes it a good colonist (Huenneke and Vitousek 1990; Sakai *et al*. 2001; Faiz Bebawi, unpubl. data). It can also reproduce vegetatively from stem and root cuttings. Highly disturbed areas like creek banks and creek beds promote aggressive root suckering once lateral roots of *C. thevetia* are exposed to light conditions (Faiz Bebawi, pers. obs.). These multiple attributes of sexual and asexual reproduction not only have implications on the management of *C. thevetia* but also on its evolution (Baker 1974).

An incomplete understanding of the invaders' biology, community relationships and the ecological impacts of invasion often impedes weed management (Mullett 2001). The National Weeds Program of Australia emphasised the need for assessment of reproductive attributes of target species before eradication is attempted, otherwise control resources can be wasted in unsuccessful attempts at eradication (Csurhes and Edwards 1998). To respond to this need, several ecological studies were initiated by Biosecurity Queensland (Department of Agriculture and Fisheries) to improve ecological knowledge related to management of *C. thevetia* (Bebawi *et al*. 2014, 2015*a*, 2016*b*).

The objectives of the present study were to determine the germination requirements and seed longevity of the peach and yellow biotypes of *C. thevetia*, which are important attributes relevant to its management in the dry tropics of North Queensland.

Materials and methods

Seven experiments were conducted at the Tropical Weeds Research Centre in Charters Towers, northern Queensland (20°09′S, 146°26′E). Long-term mean annual rainfall for Charters Towers is 658 mm with 54% of this occurring during the summer months (December–February) (BOM 2012). The mean summer maximum daily temperature is 37.6 ± 0.4°C and in winter (June–August) is 28.2 ± 0.7°C. The mean lowest daily temperature in summer is 29.9 ± 0.5°C and in winter (June–August) is 22.3 ± 0.6°C.

For all experiments, seeds were sourced from the same locations, but the date of collection may have varied. Ripe fruits of the peach biotype were collected 40 km NE of Charters Towers (Will Creek, Mingela; 19°49′S, 146°33′E; elevation 250 m above sea level). Collections of fruits of the yellow biotype were undertaken from an infestation located in South Townsville next to a copper refinery (10°32′S, 146°84′E; elevation 17 m above sea level), 130 km NE of Charters Towers.

For Experiments 1 (a,b), 2 (a,b) and 3, fruits were collected on 8 August 2007. For the seed longevity trials, ripe fruits of the peach and yellow biotypes for the initial experiment (4a) were collected on 8 and 15 August 2008, respectively. Fruits of the peach biotype for the repeat experiment (4b) were collected on 12 October 2010. All fruits were brought back to the Tropical Weeds Research Centre in Charters Towers and placed in jackets of aluminium mosquito gauze (1 m^2) in a glasshouse under dry conditions until required. Experiments 1b, 4a and 4b used seeds still contained in the endocarp (hereafter referred to as pods), after the leathery rind (ectocarp and mesocarp) was physically removed. All other experiments used seeds. This entailed cracking open the fruit using sharp garden secateurs to extract intact seeds from them. Ripe fruits of *C. thevetia* are indehiscent, i.e. do not naturally split open unless exposed to soil moisture or forced open by wild life.

Experiment 1a – seed germination under natural temperature conditions

The experiment commenced on 7 September 2007 and was a 2×12 factorial replicated four times using a split-plot design. Factor A was the two biotypes (peach and yellow) assigned to the main plots and factor B was 12, monthly sowing periods (January through to December) assigned to the subplots.

Experimental units were plastic pots (50 cm diameter × 40 cm depth) that were filled with river loam soil and placed out in the open air on bare soil. Some chemical and physical properties of the river loam soil are given in Table 1.

Treatments were implemented by sowing 50 seeds into individual pots at the beginning of each month (from September 2007) for 12 months, with the experiment repeated the following year. At the beginning of each month a random sample of ripe pods was obtained from the original pod pool and cracked open to obtain seed for planting. Pots were watered daily to field capacity.

Seed germination and viability were initially determined from four sub-samples of 50 seeds of the seed pool before sowing to ensure that seed was of good quality and vigour. Germinated seeds were removed from each pot as they emerged. Un-germinated seeds in pots were exhumed after 4 weeks from sowing and tested for viability.

Viability of un-germinated seeds was tested using the tetrazolium method (Moore 1985) by placing them in Petri dishes filled with 10 mL of 1% tetrazolium chloride for 5 days. Seeds that were pink, when cut longitudinally with a sharp scalpel, were considered to be viable. Germination percentages were calculated on the basis of total viable seed numbers.

Maximum and minimum daily ambient temperatures for Charters Towers over the duration of the experiment were obtained from the Bureau of Meteorology (BOM 2012).

Experiment 1b – germination from intact pods

This experiment was initiated on 3 December 2007 and terminated on 24 December 2008. It was similar in design to Experiment 1a except that it determined the effects of date of planting on germination of 50 freshly harvested pods which form the natural means of seed dispersal (Fig. 1). As the number

Table 1. Chemical and physical components of the river loam used in the studies

NO_3-N, nitrate nitrogen; K, potassium; P, phosphorus; NH_4-N, ammonium nitrogen; N: nitrogen

Chemical analysis		Physical analysis	
pH	7.9	Coarse sand (%)	76
Salinity (EC dS/m)	0.01	Fine sand (%)	19
P (mg/kg)	4.0	Silt (%)	3
K (meq/100 g)	0.2	Clay (%)	2
NO_3-N (mg/kg)	3.0	Total carbon (%)	0.11
NH_4-N (mg/kg)	3.0		

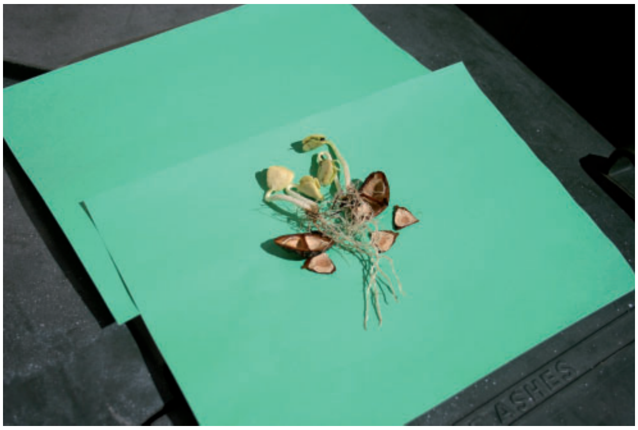

Fig. 1. Germinated pod of *C. thevetia* showing four carpels and four germinated seedlings emerging.

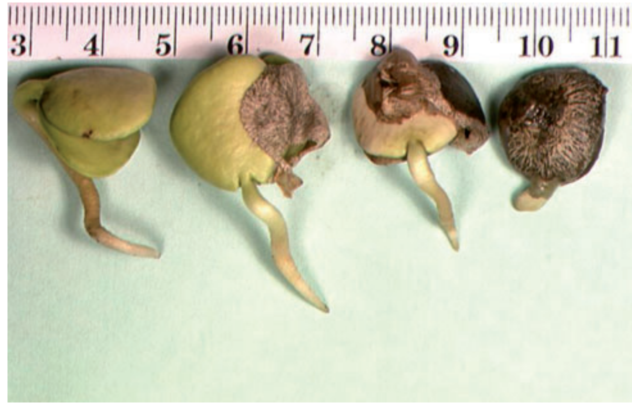

Fig. 2. Germinating seeds of *C. thevetia* showing a pair of green cotyledons and growth of the radicle.

of seeds per pod would have varied between pods (1–4 seeds per pod), pod germination was assessed as positive if only one seed germinated from each pod. Another difference from Experiment 1a was that germination was monitored on average for 8 weeks. Un-germinated pods were retrieved and seed content tested for viability using the procedure described in Experiment 1a. Un-germinated pods were considered viable if only one seed within the pod tested positive to staining. Germination from pods was calculated as a percent of the total pods planted, i.e. 50 pods.

Experiment 2a – seed germination under alternating temperature regimes

This laboratory experiment commenced on 27 June 2008 to determine the germination temperature range of seeds of the yellow and peach biotypes of *C. thevetia*. Lots of 50 freshly collected seeds were placed on moist extra-thick absorbent premium wipe towels in plastic trays (11 cm × 17 cm × 7 cm). Wipe towels were kept moist during the course of the experiment by spraying with distilled water whenever required. Tray lids were perforated at the four corners to allow air circulation. For each biotype, four trays (replicates) were placed in each of 10 temperature compartments (Multi-Temperature Incubator Model: LMMT-10, Linder and May, Northgate, Qld, Australia) delivering alternating regimes of light and temperature on a 12-h day and 12-h night cycle. Temperatures within each compartment were measured on an hourly basis using type K steel encased thermocouples connected to a data logger (Data Electronics Pty Ltd, Brisbane, Qld, Australia). Across the 10 temperature compartments, seeds were exposed to a temperature range of 11°C–52°C during the day and 6°C–40°C during the night: actual day/night temperatures in each compartment were 11/6°C, 16/12°C, 21/16°C, 25/19°C, 29/23°C, 33/27°C, 37/30°C, 42/33°C, 47/36°C and 52/40°C.

Germinated seeds (Fig. 2) from each tray were counted and removed daily. The position of the trays was also re-randomised daily within each chamber to minimise heat exposure and light bias. Germination was considered to have ceased when no seeds germinated for 4 weeks. Seed viability was determined as described in Experiment 1a.

Experiment 2b – seed germination under constant temperature regimes

This experiment commenced on 4 June 2009 and was similar to Experiment 2a in all details except the seeds were exposed to a constant temperature range. The actual temperature recorded in each compartment were 13°C,17°C, 20°C, 24°C, 27°C, 31°C, 35°C, 39°C, 44°C, and 49°C.

Experiment 3 – effect of shade

A 2 × 2 factorial experiment replicated four times was conducted between December 2007 and February 2008 to determine the effects of shade on germination of the two biotypes of *C. thevetia* (peach and yellow). The experiment incorporated a split-plot design with four replications. There were two light conditions (100% or 30% of natural light) assigned to the main-plots, and the two biotypes of *C. thevetia* (peach and yellow) were assigned to the subplots. Full details of method are given in Bebawi *et al*. (2014).

Lots of 50 freshly collected seeds of *C. thevetia* were sown in plastic pots (50 cm diameter × 40 cm depth) filled with river loam soil similar to that used in Experiment 1. Germinated seeds from each pot were counted and removed daily. Germination was considered to have ceased when no seeds germinated for 4 weeks. Pots were watered daily to field capacity and treatments were randomised within each block. Monthly temperatures in the natural light and shaded treatments were recorded about mid-day using a Cole–Parmer scope and laser sighting infrared thermometer, which was pointed at a piece of A4 white paper held up in the air and the reading recorded.

Experiment 4a (initial) and b (repeat) – seed longevity

The seed longevity experiments on *C. thevetia* were part of a larger study established in 2008 to test the longevity of seeds of several weed species. It comprised a multi-factor, split-plot design to expose seed lots to two soil types (alluvial river loam and clay), two levels of pasture cover (pasture present or pasture excluded), and four burial depths (0, 2.5, 10 and 20 cm). Each treatment combination was replicated four times (blocks) and the experimental design allowed for pre-determined retrievals of seed lots to be undertaken up to nine times in order to monitor changes in viability over time. A full description of the design and implementation of treatments is given by Bebawi *et al.* (2015*b*). For *C. thevetia* an additional factor was incorporated into Experiment 4a with seeds of the two biotypes included for testing. In Experiment 4b, only the peach biotype was included after both biotypes demonstrated similar longevity patterns in Experiment 4a.

Six-hundred and forty sub-samples of 25 pods were randomly selected from the bulk collections mentioned previously for both biotypes in Experiment 4a but only for the peach biotype in Experiment 4b. Each sub-sample was then placed in poly-net bags (4 cm × 4 cm × 0.5 cm; 1.1 mm × 2.4 mm mesh size) to allow seed retrieval while maximising soil/seed contact. With pods of *C. thevetia* containing between 1 and 4 seeds, 25 pods were chosen to give an average of 50 seeds per seed lot.

Experiment 4a commenced in January 2008 with retrievals designated to occur 3, 6, 12, 18, 24, 36, 48, 60 and 72 months after burial or until no viable seeds were recorded for two consecutive retrievals. The 3- and 6-month treatments did not end up being taken, with the first retrieval made 12 months after burial. Experiment 4b commenced in November 2010, with retrievals designated to occur 3, 6, 9, 12, 18, 24, 36, 48 and 72 months after burial or until no viable seeds were recorded for two consecutive retrievals. Rainfall and ambient temperature at the field site were measured using an on-site automatic weather station (Campbell Scientific, Logan, UT, USA).

After being retrieved, bags containing buried seeds were washed to remove attached soil particles. To determine 'viability' (germinated plus dormant seeds), the un-germinated pods were removed from the bags, washed in water, cracked open in the laboratory and all healthy seeds were placed on moist wipe towels in plastic trays (11 cm × 17 cm × 7 cm). Wipe towels were kept moist during the course of the experiment by spraying with distilled water whenever required. Tray lids were perforated at the four corners to allow air circulation. Trays containing seed were then placed in a germination cabinet set at 30°C/20°C for 12 h day/12 h night respectively. Germinating seeds (identified by radicle emergence) were counted and removed daily for 14 days (Fig. 2). Seeds that did not germinate were checked for viability as described in Experiment 1a.

In the repeat trial, at the 3-month retrieval the number of seeds that germinated in the bags while buried in the field was also recorded by counting emerged seedlings. However, this was not possible in later retrievals due to disintegration of emerged seedlings.

Data analysis

Genstat was used for all statistical analyses (Genstat 8.1 Committee 2005) and Fisher's least significant differences test was used to determine differences between treatments whenever analysis showed treatment effects to be statistically significant. Split-plot ANOVA were carried out according to the actual statistical designs described previously. All statistical analyses of seed germination and viability, and pod germination and viability were undertaken on arcsine-transformed data, which were later back-transformed for display. Regression analysis was used to relate seed germination and viability, and pod germination and viability to ambient temperature or incubation temperatures. In all ANOVA and regression analyses the statistical assumptions of normality and equality of variance were checked by inspecting the patterns in the residuals in data values from the fitted models.

Results

Experiment 1a – seed germination under natural temperature conditions

Although seed germination of both biotypes was recorded in every month, a significant biotype × month interaction ($P < 0.01$) occurred across the 2 years of the study (Fig. 3). Monthly variation between biotypes was greatest during the April–June period. Nevertheless, for both biotypes seed germination was generally low (18–31%) between January and March, before increasing between April and July. It then remained relatively high (61.5–80.5%) until October, after which it steadily declined to a minimum of 15% in December. There was a significant negative correlation ($r = -0.57$, $P < 0.05$) between ambient temperature and seed germination over all biotypes, months and years.

Experiment 1b – germination from pods under natural temperature conditions

Significant interactions ($P < 0.01$) in germination from pods were detected between biotypes and months (Fig. 4). Germination was recorded in all months during the study period but it was highly variable between months, particularly for the peach biotype (Fig. 4). Minimum germination from pods of both biotypes occurred in November, averaging 6% and 24% for the peach and yellow biotype, respectively. Maximum germination from pods averaged 96% for both biotypes, but occurred at the onset of spring (September) for the peach biotype compared with the end of autumn (May) for the yellow biotype. In contrast to seed germination, there was no significant correlation between germination from pods and ambient temperatures ($P > 0.05$).

Experiment 2a – seed germination and viability at alternating temperature regimes

Significant interactions ($P < 0.05$) in seed germination (Fig. 5*a*) and in seed viability (Fig. 5*b*) were detected between biotypes and alternating temperature regimes. Nil germination occurred for both biotypes at the coolest (11°C /6°C) and warmest (52°C/40°C) alternating temperature regimes (Fig. 5*a*). In between, germination of both biotypes initially rose steadily with increasing temperatures until an optimum temperature regime was reached. Thereafter, further temperature increases resulted in a steady decline in germination. The peach biotype exhibited maximum germination (average of 55%) across a broad alternating temperature range, from 25°C/19°C to 37°C/30°C. The optimum temperature range for germination of the yellow biotype was higher than the peach biotype, with maximum germination (average of 78%) obtained at 37°C/30°C and 42°C/33°C.

Seed viability demonstrated a comparable response to seed germination, except at the cooler temperature regimes of 11°C/6°C, 16°C/12°C and 21°C/16°C (Fig. 5*b*). Exposure to 11°C/6°C, resulted in 18.5% and 14.5% of seeds of the peach and yellow biotype remaining viable respectively, but none of these seeds germinated. At 16°C/12°C and 21°C/16°C, 7% and 78% of

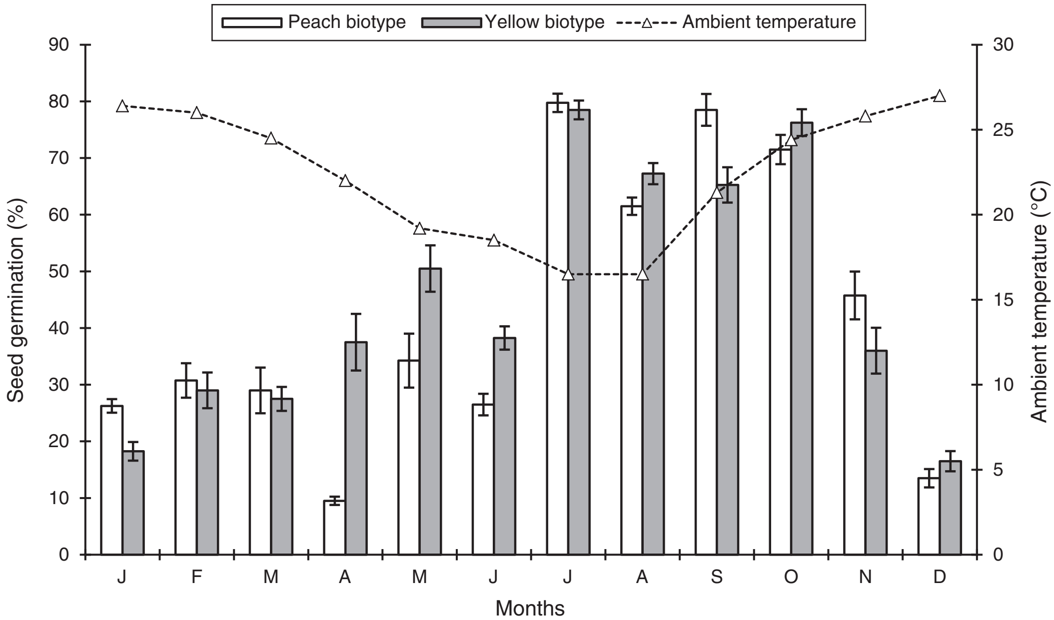

Fig. 3. Seed germination response of the peach and yellow biotypes of *C. thevetia* as affected by planting date in outdoor pots averaged over 2 years associated with mean monthly ambient temperatures. Vertical bars indicate the s.e of the means.

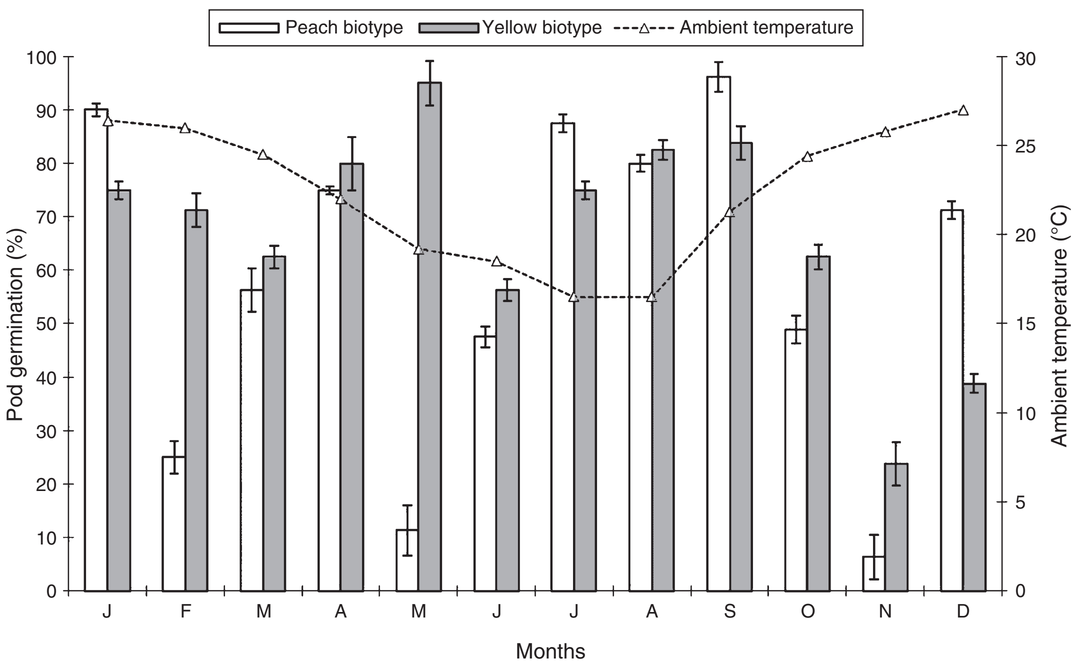

Fig. 4. Pod germination response of the peach and yellow biotypes of *C. thevetia* as affected by planting date associated with monthly ambient temperatures. Vertical bars indicate the s.e. of the means.

viable seeds of the peach biotype and 43% and 91% of the yellow biotype germinated, respectively. In contrast, 100% germination of viable seeds of both biotypes occurred for all other temperature regimes, except the highest one (52°C/40°C), which recorded nil viability at the end of the study (Fig. 5*b*).

Experiment 2b – seed germination and viability at constant temperature regimes

Significant differences ($P < 0.001$) in germination and seed viability occurred between constant temperature regimes (Fig. 6), but not between biotypes ($P > 0.05$) and there was no significant interaction ($P > 0.05$). No seeds germinated at the lowest temperature of 13°C, with germination continuing to remain low (≤5%) until temperatures exceeded 24°C. Germination then increased rapidly to a peak of 51% at 27°C, before declining steadily to less than 2% at 39°C (Fig. 6). Once the temperature reached 49°C nil germination was recorded.

Seed viability also peaked at 27°C but it exhibited a more gradual increase and subsequent decline with increasing temperatures than that recorded for seed germination (Fig. 6). Furthermore, despite not germinating, a small percentage of seeds were viable at the lowest (13°C) and highest temperature regimes (49°C). As for the alternating temperature experiment, under suboptimal temperatures, a larger proportion of viable seeds remained in enforced dormancy, with germinability increasing closer to the optimal temperature regime. When exposed to 27°C, 97% of all viable seeds germinated. In contrast, less than 10% of viable seeds germinated at temperatures of 17°C and below, or 39°C and above (Fig. 6).

Experiment 3 – effect of shade

Temperatures recorded in the shaded treatments averaged 29.9°C, compared with 35.3°C under natural light. Significant differences ($P < 0.01$) in germination response were detected between light conditions, but not between biotypes ($P > 0.05$) (Fig. 7). There was also no significant light condition × biotype interaction ($P > 0.05$). Germination of the two biotypes was 4-fold greater under shade than under natural light conditions. Germination also occurred more quickly, commencing on average 7 days faster under shaded conditions compared with natural light.

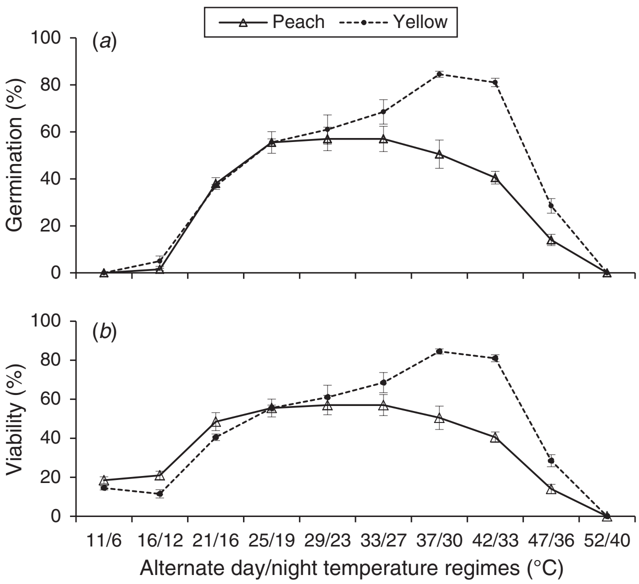

Fig. 5. (*a*) Seed germination and (*b*) seed viability response of the peach and yellow biotypes of *C. thevetia* as affected by a range of alternating day/night temperatures. Vertical bars indicate the s.e. of the means.

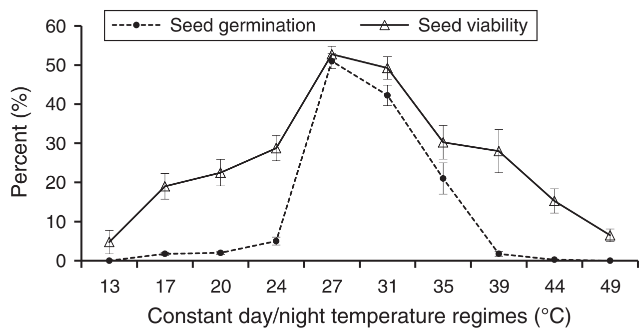

Fig. 6. Seed germination and seed viability response of *C. thevetia* across both biotypes at 10 constant day/night temperatures. Vertical bars indicate the s.e. of the means.

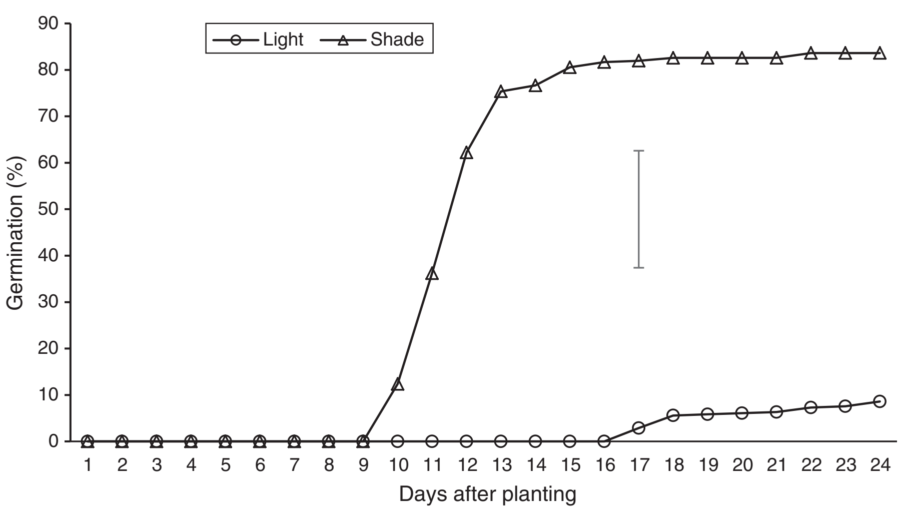

Fig. 7. Cumulative germination over time (days after planting) as affected by natural light and artificial shade across all biotypes and planting densities of *C. thevetia*. Vertical bar indicates the l.s.d. at $P = 0.05$.

Experiment 4 – seed longevity

Rainfall

Annual rainfall recorded at the site between 2008 and 2012 was consistently greater than the long-term mean for Charters Towers (658 mm), totalling 1365, 1125, 1323, 1037 and 832 mm per annum, respectively (Table 2).

Despite commencing at different times, Experiments 4a and 4b were exposed to similar amounts of rainfall over their respective 2-year duration (2490 and 2357 mm), but there were seasonal differences. In Experiment 4a, seed lots received extremely high rainfall (over 400 mm each month) during the first 2 months of burial (January–February 2008). This was followed by below average monthly rainfall until November, except for a very wet July period where 169 mm was recorded. The following wet season produced well above average rainfall and again during January and February monthly rainfall exceeded 400 mm. Thereafter, a typical pattern for dry-tropical areas occurred until the experiment finished, although monthly rainfall was below the long-term mean in all months except March.

Although seed lots in Experiment 4b were not exposed to as high a rainfall as Experiment 4a during the first 2 months, they received well above average rainfall for the first 7 months that they were buried (October 2010–April 2011). This was followed by typical dry season conditions with rainfall slightly below the long-term mean in most months. The next wet season again produced above average rainfall, with 3 months (December, February and March) recording in excess of 200 mm. Rainfall then tapered off during autumn and winter and was still below average when the experiment finished in December 2012. The only exception was the July period where 135 mm was recorded, much greater than the long-term average of only 17 mm.

Experiment 4a – initial seed longevity

At the time of burial, the yellow biotype exhibited significantly higher ($P < 0.05$) viability (expressed as a percent of total seed number) than the peach biotype, averaging $80 \pm 2\%$ and $58 \pm 3\%$, respectively (Fig. 8). Viable seeds of both biotypes were highly germinable (100%) with nil dormancy recorded. Following burial, <1% of viable seeds remained after 12 months, irrespective of biotype, soil type, level of pasture cover or burial depth. At 18 months after burial, nil viability was recorded across all treatments.

Table 2. Monthly rainfall (mm) recorded at the experimental site between 2008 and 2012, plus monthly averages (mm) over 100 years reported by BOM (2012)

Month	Years 2008	BOM 2009	2010	2011	2012	100-year averages
Jan.	447	440	172	167	93	137
Feb.	480	458	248	216	218	130
Mar.	10	19	111	216	268	103
Apr.	5	93	41	78	9	43
May	6	23	8	22	42	24
June	2	5	9	31	12	26
July	169	0	5	7	135	17
Aug.	0	1	82	0	0	13
Sept.	16	1	63	0	15	15
Oct.	3	0	74	16	3	22
Nov.	155	20	240	22	14	41
Dec.	72	65	270	263	22	87
Total	1365	1125	1323	1037	832	659

Experiment 4b – repeat seed longevity for the peach biotype

Only the peach biotype was tested in the repeat experiment, with retrievals undertaken more regularly given the short longevity of *C. thevetia* in Experiment 4a. Initial seed viability (percent of total seed number) and germinability of viable seeds averaged $54 \pm 3\%$ and 100% (percent of total viable seeds), respectively. After burial, a significant ($P < 0.001$) burial depth × burial time interaction occurred (Fig. 9). Seed viability declined rapidly for seeds buried at 2.5- and 10-cm depths (Fig. 9) and was totally exhausted at the 6- and 3-month retrievals, respectively. In contrast, surface located seed lots exhibited a steadier rate of decline in viability and took until the 24-month retrieval to record nil viability. The pattern of decline in viability coincided with high germination of seeds in the field in the first 3 months after burial (Fig. 10). On average, 38% of seeds germinated within 3 months if buried, significantly more ($P < 0.05$) than surface-located seeds, which averaged 23% (Fig. 11).

With regards to soil type, a more rapid rate of decline in viability occurred in the clay soil compared with the river loam soil. Viability averaged 11% and 15% in the clay and river loam

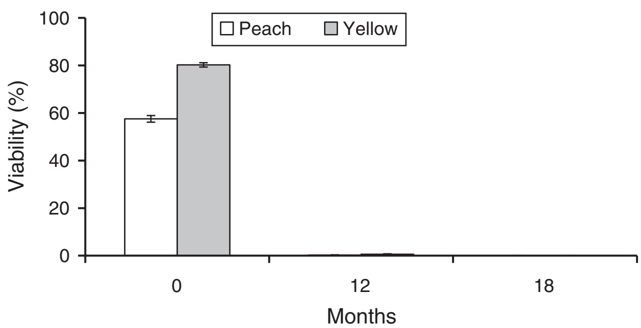

Fig. 8. Viability of the peach and yellow biotypes (initial Experiment 4a) of *C. thevetia* seeds as affected by burial duration over all soil types, pasture cover and burial depths. Vertical bars indicate the standard error of the means.

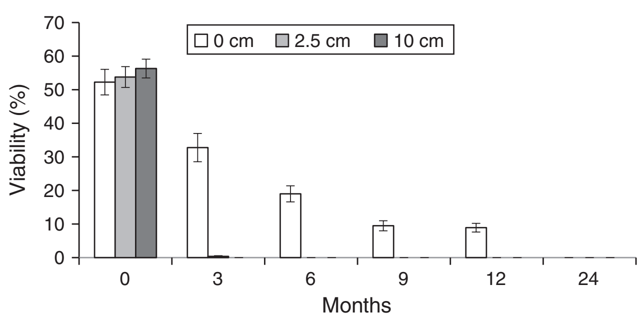

Fig. 9. Seed viability of the peach biotype (repeat Experiment 4b) of *C. thevetia* as affected by burial depth and burial duration over all soil and pasture cover types. Vertical bars indicate the s.e. of the means.

Fig. 10. Seedlings of *C. thevetia* germinating in retrieved bags of buried seed lots. Yellow bags were placed on the soil surface whereas the red and blue were buried at 2.5 cm and 10 cm respectively.

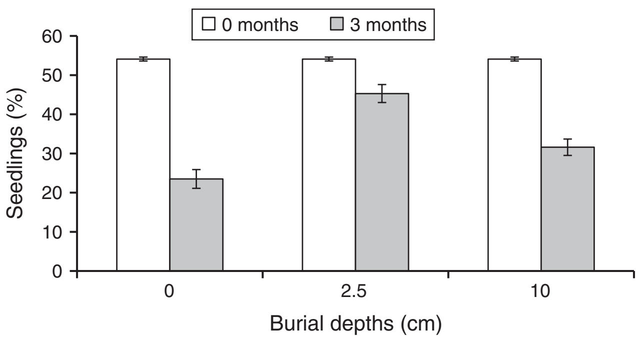

Fig. 11. The proportion (%) of seedlings of the peach biotype that emerged at 0 months (Control) and 3 months after burial at different depths. Vertical bars indicate the s.e. of the means.

soil respectively over all burial durations, levels of vegetation cover, and burial depths.

Discussion

Ripe seeds of both the peach and yellow biotypes of *C. thevetia* exhibited high germinability and an ability to germinate over a wide range of temperatures. Shaded conditions were conducive to greater seed germination compared with natural light. In the field, soil seedbanks appear to be completely exhausted within 2 years, and more rapidly under many conditions (e.g. buried seeds).

Seed germination and viability

Although only one provenance of each biotype was tested, the yellow biotype tended to exhibit greater seed viability than the peach biotype, but germinability was similar. Freshly collected ripe seeds of both biotypes would readily germinate if exposed to favourable temperature and moisture conditions.

Under prevailing temperature conditions at Charters Towers and with abundant soil moisture, seed germination occurred in all months of the year, although there were distinct highs and lows and some variation between biotypes. Germination (particularly loose seeds) tended to be lowest during summer and greatest from late winter to mid-spring when temperatures were not quite as hot. The pattern of germination was less pronounced if seeds were still contained in pods. Whether the pods buffered the temperatures for the seeds or the assessment criterion of one germination event per pod contributed to this finding warrants further investigation. We do not believe that a potential loss of vigour and viability over time of the seeds and pods used for monthly sowings contributed to the variation that occurred, as relatively high germination was still being recorded in some months towards the end of the trials, particularly Experiment 1a.

Overall, *C. thevetia* demonstrates a broad temperature tolerance, but there are some distinct differences between biotypes. The results from the alternating temperature experiment (2a) tend to suggest that the yellow biotype may be more suited to tropical conditions for germination and establishment, whereas the peach biotype preferred slightly cooler alternating temperatures more aligned to sub-tropical conditions. However, it is important to note that under a constant temperature regime no significant differences were recorded between the two biotypes, and at this stage it is unclear why these differential responses between alternating and constant temperature regimes occurred.

Germination was low or nil at the coolest end of both alternate and constant day/night temperature regimes as well at the warmest end of the constant day/night temperature regime. However, a substantial percentage of seeds remained viable. Only the highest alternating temperature range of 52°C/40°C resulted in all seeds losing viability. The seed viability resilience demonstrated by *C. thevetia* to cold and warm temperatures tested in this study is of great survival value. It may also indicate that a substantial proportion of its seed population enters a state of 'enforced dormancy' due to unsuitable temperatures as described by Harper (1977). This seed trait enables seeds to remain temporarily inactive and consequently protected until more favourable conditions occur.

The greater seed germination that occurred under shade conditions (52%) compared with natural light (12%) warrants further consideration. It is most likely associated with the lower and more favourable ambient temperatures that occurred for germination under the shade (29.9°C) compared with that under natural light (35.3°C). A laboratory-based experiment using germination cabinets would help distinguish between the effects of temperature and light on germination of *C. thevetia*.

Seed longevity

Both biotypes of *C. thevetia* exhibited similar seed bank longevity in the present study with no viable seeds remaining after 2 years' burial, irrespective of soil type, level of pasture cover and burial depth. According to the classification system developed by Thompson *et al*. (1997) for NW Europe, *C. thevetia* falls into the category of having a short-term persistent seed bank (1–5 years). Some other rangeland weeds in northern Australia that display a similar trait include calotrope [*Calotropis procera* (Aiton) W.T. Aiton] (Bebawi *et al*. 2015*b*), chinee apple (*Ziziphus mauritiana* Lam.) (Bebawi *et al*. 2016*a*) and rubber vine (*Cryptostegia grandiflora* R. Br.) (Grice 1996; Bebawi *et al*. 2003). In contrast, lantana (*Lantana camara* L.) (Vivian-Smith and Panetta 2009) and parthenium (*Parthenium hysterophorus* L.) (Navie *et al*. 1996) are two rangeland weeds with a long-lived seed bank, with seeds found to be viable for as long as 11 and 7 years, respectively.

Prevailing environmental conditions can play a significant role in how long the soil seed bank will last for, with soil moisture a key factor. Under drier conditions seed longevity can be prolonged, often due to seeds having less opportunity to germinate (Bebawi *et al*. 2003; Vivian-Smith and Panetta 2009; Bebawi *et al*. 2012). Bellyache bush (*Jatropha gossypiifolia*) is a good example. Under favourable moisture conditions, the seed bank could be exhausted within 5 years putting it into the category of a short-term persistent seed bank (1–5 years). If dry conditions prevail seeds could remain viable for 10 years or more, which would define it as a long-term persistent seed bank (>5 years) (Bebawi *et al*. 2012).

The present study on *C. thevetia* was undertaken during a run of above average rainfall years and in the repeat experiment it was estimated that 77% of viable seeds germinated in the field during the first 3 months if buried below ground. Throughout the various trials *C. thevetia* did demonstrate relatively high germinability under a range of temperature regimes, which suggests that if it received favourable rainfall conditions it would germinate. If prolonged dry conditions were to prevail we anticipate that seed banks may last longer, similar to the response of weeds such as bellyache bush and lantana (Vivian-Smith and Panetta 2009; Bebawi *et al*. 2012), due to fewer opportunities for germination to occur. This is based on the proviso that the seeds do not naturally decay and lose viability due to other factors such as perhaps pathogens and/or predators (Simpson *et al*. 1989). A recent trial on chinee apple found that its relatively short seed longevity was not solely due to mass germination but a proportion of seeds lost viability, possibly due to the effects of pathogens (Bebawi *et al*. 2016*a*).

Ecological and management implications

The ability of *C. thevetia* to germinate across a wide temperature range suggests it is a threat to both tropical and sub-tropical regions of Australia, which is reflected in current herbarium distribution records for this species (AVH 2017). Both biotypes can readily germinate under sub-tropical temperatures. The yellow biotype can germinate at higher alternating temperature regimes indicating that it may be better adapted to hotter regions than the peach biotype. The peach biotype could still readily germinate in cooler parts of the tropics, during autumn to spring rather than summer, or by growing in favourable habitats such as riparian areas where the native trees would provide a level of shading that should reduce temperatures sufficiently for better germination conditions to occur, similar to Experiment 3. Several infestations of the peach biotype exist in northern Queensland where they are mainly found growing along riparian areas. At one of these sites (Mingela; north Queensland, 19°81′S, 146°56′E), midday soil surface temperatures during summer were found to be as much as 20°C lower in a riparian habitat than out in the open (Bebawi *et al*. 2015*a*).

The yellow biotype has several other attributes that tend to indicate that it could be more invasive than the peach biotype. The present study found that it generally produced a greater proportion of viable seeds and earlier studies suggest that following germination young plants of the yellow biotype can grow faster, reach reproductive maturity quicker and also produce more fruits and seeds (Bebawi *et al*. 2014). Consequently, priority attention should be given to areas infested with monocultures of the yellow biotype rather than those infested with the peach biotype.

The relatively short seed longevity of both biotypes is advantageous from a management perspective. Control and eradication programs are more likely to succeed if the seed bank is short lived (Campbell and Grice 2000; Dodd and Randall 2002; Panetta 2004; Panetta and Timmins 2004). Therefore, if land managers are diligent for the first 2–3 years following implementation of effective control activities, they should have little further seedling establishment provided that soil seed reserves are not replenished by plants escaping control or through dispersal (e.g. water) from external sources. *Cascabela thevetia* takes almost a year under optimum growth conditions (Bebawi *et al*. 2014) to produce fruit, so annual surveillance and control activities should be sufficient to allow treatment of new plants before they have a chance to set seed and replenish soil seed reserves (Bebawi *et al*. 2015*a*).

Conflicts of interest

The authors declare no conflicts of interest.

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