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# Effects of temperature and burial on seed germination and persistence of the restricted invasive *Stevia ovata* in northern Queensland

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**Abstract.** *Stevia ovata* Willd. is an invasive weed that has become naturalised in northern Queensland, Australia. To aid management of current infestations, this study evaluated seed germination under a range of constant (13−48°C) and alternating (11/7 to 52/42°C) temperature regimes and quantified the potential longevity of soil seed banks. The effect of different soil types, levels of pasture cover and burial depths on seed longevity was investigated in both the dry- and wet-tropics of North Queensland. Germination of *S. ovata* occurred under a wide range of both constant (13−39°C) and alternating day/night temperatures (16/12 to 52/42°C), but optimum conditions ranged between 24 and 27°C and 24/20 and 37/31°C respectively. As temperatures declined below the optimum, an increasing proportion of seeds went into a state of enforced dormancy. In contrast, higher than optimum temperatures caused a proportion of seeds to lose viability. Differential responses in seed longevity of *S. ovata* occurred between the two experimental sites. In the wet-tropics, seed viability was <1% after 12 months and fully expired after 18 months, irrespective of burial depth. In the dry-tropics, seeds persisted for longer (nil viability after 24–42 months) and burial depth had a significant effect. Surface located seeds tended to exhibit a faster rate of decline in viability than seeds buried below ground. These findings have implications for the duration of control/eradication programs and also suggest that *S. ovata* has the potential to greatly expand its current distribution, particularly into cooler areas of Australia.

**Additional keywords:** burial depth, germination rate, innate dormancy, seed quiescence, viability.

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## Introduction

*Stevia ovata* Willd. (syn. *Stevia rhombifolia* Kunth.) of the sunflower family Asteraceae is native to subtropical and tropical America, from the highlands of Texas south to the highlands of Mexico, Ecuador, Peru, and Paraguay (King and Robinson 1987; Soejima *et al*. 2001, 2017; Watanabe *et al*. 2001; Csurhes 2008; Madan *et al*. 2010). More recently, Soejima *et al*. (2017) suggested that the genus *Stevia* originated in Mexico 7.0–7.3 million years ago. Within its native range, *S. ovata* grows at elevations of 1000–3000 m and occupies a range of habitats which are generally open and disturbed (Grashoff 1972; Soejima *et al*. 2001, 2017; Watanabe *et al*. 2001; Csurhes 2008). It was initially reported in Australia in 2007 near Ravenshoe on the Atherton Tableland in North Queensland at an elevation of ~900 m above sea level (Csurhes 2008; Sydes *et al*. 2010).

At present, *S. ovata* is limited in its distribution to the pastoral district of North Kennedy in North Queensland where it is listed as a naturalised species (Bostock and Holland 2010; Sydes *et al*. 2010). The threat posed by *S. ovata* saw it listed as a declared weed under local law by the Tablelands Regional Council (2013) and it was ranked as the sixth highest priority weed in their Local Area Pest Management Plan for 2013–2017. Other major industry advisory bodies such as the Far North Queensland Pest Advisory Forum (FNQPAF) and Wet Tropics Management Authority (WTMA) have also included *S. ovata* in their weed lists. Recently, the *Biosecurity Act 2014* listed *S. ovata* as category 3 restricted invasive

 

biosecurity matter, which prohibits the distribution or disposal of this weed.

*Stevia ovata*, commonly referred to as 'candy leaf' in Australia, is a multi-branched and straggly perennial shrub (Soejima *et al*. 2017), 40–80 cm tall with mostly opposite leaf and branch arrangement on the stem. The leaves are lanceolate (9.1–9.5 cm long; 1.9–2.1 cm wide), apex acuminate, base cuneate and margins are distinctly serrate. The stem is covered with hairs and darkens to a reddish colour at the base (Csurhes 2008). The plant's perennial habit arises from its unusual root system, which consists of multiple swollen lateral roots (tuberous) attached to the lower end of a relatively compacted stem base which bear numerous triangular shoot buds (Fig. 1; F. Bebawi, pers. obs.). Once the primary shoot system flowers and produces seed, the primary stem gradually withers and dies off by winter. This allows new shoots (ratoons) to rise from the shoot buds below ground the following spring/summer and the cycle repeats itself until all shoot buds are exhausted (F. Bebawi, unpubl. data). Thus this plant's perennial habit is achieved through a form of 'agamospermy' and increases the longevity of the plant and prolongs the occupation of any site it invades (Aarssen 2008; Booth *et al*. 2010).

The inflorescence of *S. ovata* is a type of raceme which consists of congested white florets (2–6) borne terminally on small corymbs that are arranged in loose panicles. *Stevia ovata* has the potential to produce up to 6000 seeds per plant per growing season, from an average number of 240 flowering branches per plant bearing an average number of 1285 flowers per plant and with an average number of 4.6 black seeds per flower (F. Bebawi, unpubl. data). Seeds are contained in slender black achenes (~3 mm in length) that are devoid of any persistent pappus (bristle like hairs), in contrast to *Stevia rebaudiana* (Bertoni) Bertoni, which bears persistent pappus (Kumar and Sharma 2012). Flowering occurs around May (late autumn) and seed maturation commences around June (early winter) in northern Queensland (Setter *et al*. 2016). The ability of *S. ovata* to reproduce sexually and asexually (Soejima *et al*. 2017) is a strategy shared by other plants such as wild garlic (*Allium sativum* L.).


**Fig. 1.** Candy leaf (*Stevia ovata*) slightly purplish swollen roots and relatively white triangular axillary buds. Photo obtained from a 185-day-old plant (F. Bebawi, unpubl. data).

With limited information available on the biology of *S. ovata*, particularly under Australian conditions, the present study focussed on quantifying aspects of its seed ecology. Seed germination and viability responses to both constant (Experiment 1a) and alternating temperatures (Experiment 1b) were investigated to better understand its potential distribution. The potential persistence of soil seed banks was also quantified by undertaking seed burial trials in both the dry-(Experiment 2a) and wet-tropics (Experiment 2b) of northern Queensland.

## Materials and methods

### *Seed collection*

Seeds used for all experiments were sourced from infestations within a 12 km radius of Ravenshoe (17°65′S, 145°51′E), which is located 123 km south-west of Cairns, northern Queensland (Fig. 2). This involved either directly plucking fresh *Stevia ovata* Willd. achenes (seed) from flower heads or collecting whole flower heads and manually threshing and cleaning the seeds afterwards. During collections, seeds were gathered from at least 50 individual plants within infestations and then stored in glass jars at room temperature until required. For experiment 1a seeds collected in May 2011 were used initially, with the experiment repeated 2 months later on 20 July 2011. For experiment 1b seeds collected in October 2011 were used initially, with the experiment repeated two months later on 16 December 2011. For experiments 2a and 2b, collections were undertaken in October 2011 and July 2011 respectively.

### *Experiment 1a – seed germination under constant temperature regimes*

This laboratory experiment was conducted at the Tropical Weeds Research Centre in Charters Towers (20°09′S, 146°26′E) on 3 May 2011 and was repeated two months later on 20 July 2011 to determine the germination temperature range of *S. ovata* seeds under constant temperature regimes.

Lots of 50 randomly selected seeds were placed in Petri dishes (9 cm diameter) containing two layers of Whatman No. 1 filter paper (Maidstone, UK) moistened with 10 mL of distilled water. Groups of four Petri dishes were then placed vertically on top of each other in plastic containers (11 × 17 × 7 cm) to help maintain a moist environment, with the lids perforated at the four corners to allow air circulation.

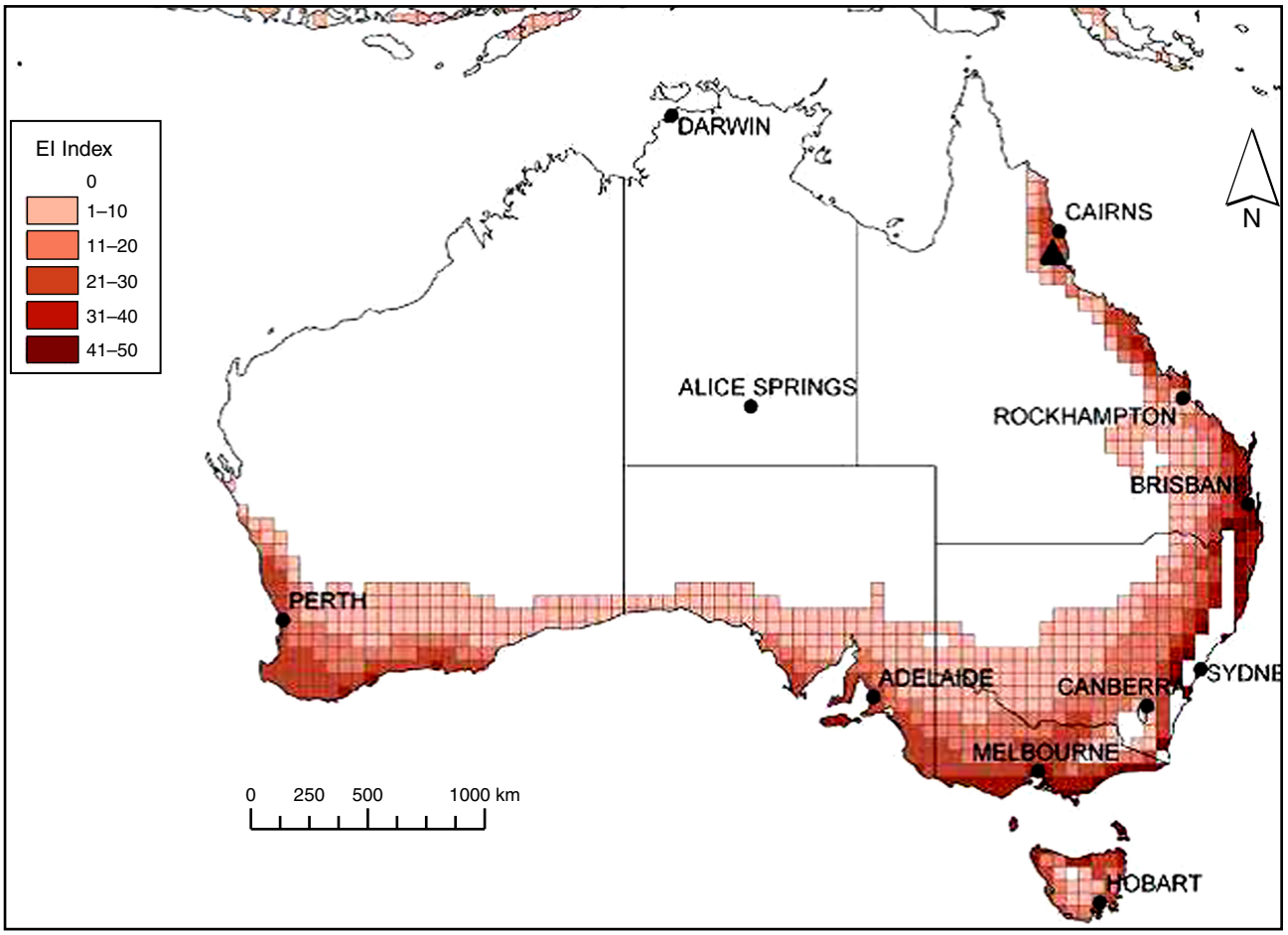


**Fig. 2.** Current (▲) and potential distribution (□□□) of *Stevia ovata* in Australia (reproduced with permission from Murphy *et al*. 2009). EI, ecoclimatic index: where 0 = unsuitable, <10 marginal, >10 suitable and >20 optimal.

Individual trays were placed into 10 temperature compartments within a Multi-Temperature Incubator (Model: LMMT-10, Linder and May, Northgate, Qld). Seeds received 12 h of darkness and 12 h of light, with temperatures within each compartment measured on an hourly basis using type K steel encased thermocouples connected to a data logger (Data Electronics Pty Ltd, Brisbane, Qld). Across the 10 temperature compartments, seeds were exposed to a temperature range of 13–48°C (Table 1).

Germinated seeds from each Petri dish were counted and removed daily and if necessary distilled water was added to keep the filter paper moist. The Petri dishes' positions within the plastic containers were also re-randomised daily. Germination was considered to have ceased in a dish when no seeds germinated for two weeks. Un-germinated *S. ovata* seeds were initially checked for 'enforced dormancy' (i.e. seed quiescence) by testing whether they would germinate when placed in an incubator set at alternating day/night temperatures of 30/20°C. This temperature regime was selected because it promoted the highest germination of its sister species *Stevia rebaudiana* (Bertoni) Bertoni (Macchia *et al*. 2007). Any remaining seeds were then checked for viability using the tetrazolium method (Moore 1985).

**Table 1. Day and night temperatures associated with each of the 10 compartments of the Linder and May multi-temperature incubator when set at constant and/or alternating temperature regimes**

| Compartment no. | Temperature (°C) for 12 h light/12 h dark | | | | |
|---|---|---|---|---|---|
| | Constant | | Alternating | | |
| | Day | Night | Day | Night | Average |
| 1 | 13 | 13 | 11 | 7 | 9.0 |
| 2 | 17 | 17 | 16 | 12 | 14.0 |
| 3 | 20 | 20 | 20 | 16 | 18.0 |
| 4 | 24 | 24 | 24 | 20 | 22.0 |
| 5 | 27 | 27 | 29 | 24 | 26.5 |
| 6 | 31 | 31 | 33 | 28 | 30.5 |
| 7 | 35 | 35 | 37 | 31 | 34.0 |
| 8 | 39 | 39 | 41 | 35 | 38.0 |
| 9 | 43 | 43 | 47 | 39 | 43.0 |
| 10 | 48 | 48 | 52 | 42 | 47.0 |

### *Experiment 1b – seed germination under alternating day/night temperature regimes*

This laboratory experiment was similar to experiment 1a in all details except the seeds were exposed to alternating day and night temperature regimes. The 10 temperature compartments

delivered a temperature range of 11–52°C during the day and 7–42°C during the night in progressional order (see Table 1). The initial experiment commenced on 24 October 2011 and was repeated 2 months later on 16 December 2011.

### Experiment 2a – seed persistence in the dry-tropics

#### Site description

The experimental site (38 × 36 m) was located at the Tropical Weeds Research Centre, Charters Towers, North Queensland (20°09′S, 146°26′E; elevation 318 m). The site was secured with a rabbit and kangaroo exclusion fence. It had been previously cleared of shrubs and trees, but would have originally comprised an open-woodland. The ground cover vegetation is listed in Table 2.

Long-term mean annual rainfall for Charters Towers is 658 mm with 54% of this occurring during the summer months (December–February) (BOM 2016). The mean summer maximum daily temperature is 37.6 ± 0.4°C and in winter (June–August) is 28.2 ± 0.7°C. Rainfall and ambient temperature at the field site were measured using an on-site automatic weather station (Campbell Scientific, Logan, UT, USA). Annual rainfall recorded at the site between 2012 and 2015 was generally lower than the long-term mean for Charters Towers (658 mm), totalling 832, 506, 550, and 490 mm per annum respectively.

#### Experimental design

This experiment comprised a multi-factor, split plot design with four replicate blocks. Factors consisted of two soil types (alluvial river loam and clay), two levels of pasture cover (pasture present or pasture excluded), four seed burial depths (0, 2.5, 10 and 20 cm) and nine retrieval times. For *S. ovata*, experimental units were 50 randomly selected seeds, placed in 180 µm woven stainless wire mesh bags (7 × 7 cm). Burial of *S. ovata* seeds commenced in June 2012 with retrievals at 3, 6, 12, 18, 24, 30, 36, 42 and 60 months afterwards or until no viable seeds were recorded for two consecutive retrievals. A full description of the overall design and implementation of treatments of a larger experiment, of which this was a component, is provided in the methodology section of Bebawi *et al*. (2015).

#### Germination and viability testing

Germination and viability testing was undertaken on 64 lots of 50 seeds at commencement of the trial to determine initial germinability and viability, and then on any seeds removed from packets during designated retrievals. Seeds were placed in Petri dishes (9 cm diameter) containing two layers of Whatman No. 1 filter paper moistened with 10 mL of distilled water and then subjected to the same germination and viability testing procedures described for Experiment 1a.

### Experiment 2b – seed persistence in the wet-tropics

#### Site description

The experiment was conducted at the Centre for Wet Tropics Agriculture, South Johnstone (17°36′S, 145°59′E; elevation 15 m). The soil at the site was a red volcanic loam and the vegetation comprised a ground layer dominated by introduced pasture species (Table 2), with no shrubs or trees present in the immediate area.

Long-term mean annual rainfall for South Johnstone is 3289 mm with 41% of this occurring during the summer months (December–February) (BOM 2016). The mean summer maximum daily temperature is 31.0 ± 0.1°C and in winter (June–August) is 24.5 ± 0.3°C. Rainfall during the experiment was collected from a weather station located on site. Annual rainfall recorded at the site from 2012 until 2014 was similar to the long-term mean for South Johnstone (3289 mm), averaging 3200, 3237 and 3243 mm respectively.

#### Experimental design

A split plot experiment was implemented in November 2011, with seven retrieval times allocated to main plots, three burial depths to sub plots and each treatment replicated four times.

Lots of 50 randomly selected seeds were placed in 180 µm woven stainless wire mesh bags (7 × 7 cm) and then buried at depths of 0, 3 and 10 cm inside 28, 90 mm diameter PVC pipes, similar to Experiment 2a and as described in Bebawi *et al*. (2015). The pipes were filled with the same soil as the plot area and covered with 30% shade cloth over the top to prevent movement of the surface located seed packets. In a 2.5 × 3.0 m area at the experimental site, four blocks each containing seven of the PVC pipes were established, with the pipes buried at the required depths and spaced 50 cm apart. One pipe from each block was retrieved at 12, 18, 24, 36, 48, 60 and 72 months after burial or until no viable seeds were recorded for two consecutive retrievals.

**Table 2. List of ground cover vegetation within the study site at Charters Towers (dry tropics) and South Johnstone (wet tropics)**

| Dry tropics (Charters Towers) | | Wet tropics (South Johnstone) | |
|---|---|---|---|
| Common name | Scientific name | Common name | Scientific name |
| Buffel grass | *Pennisetum ciliare* (L.) Link. | Signal grass | *Brachiaria decumbens* Stapf. |
| Budda pea | *Aeschynomene indica* L. | Creeping signal grass | *Brachiaria humidicola* (Rendle) Schweick. |
| Dark wiregrass | *Aristida calycina* R.Br. | – | – |
| Feathertop Rhodes grass | *Chloris virgata* Sw. | – | – |
| Indian couch | *Bothriochloa pertusa* (L.) A.Camus | – | – |
| Purpletop chloris | *Chloris inflata* Link. | – | – |
| Red Natal grass | *Melinis repens* (Willd.) Zizka | – | – |
| Sabi grass | *Urochloa mosambicensis* (Hack.) Dandy | – | – |
| Siratro | *Macroptilium atropurpureum* (DC.) Urb. | – | – |

### *Germination and viability testing*

Germination and viability testing was undertaken on 12 lots of 50 seeds at commencement of the trial to determine initial germinability and viability, and then on any seeds removed from packets during designated retrievals. A similar germination testing procedure to that described in Experiment 1a was undertaken, except that seed lots were placed on moist Whatman No. 1 filter paper over an inverted watch glass in the 9 cm Petri dishes and germination was monitored every 3–7 days until no germination had been recorded in two consecutive assessments. Viability tests were undertaken on un-germinated seeds by visually inspecting the embryos to see if they remained potentially viable (white and firm) or were dead (dark and soft).

### *Data analysis*

All statistical analyses were performed using GENSTAT 16 (VSN International, Hemel Hempstead, UK), with Fisher's protected least significant differences (l.s.d.) test used to determine differences between treatment means whenever analysis showed treatment effects to be statistically significant ($P < 0.05$). For datasets with a binomial distribution, analysis was undertaken on arcsine-transformed values, and treatment means were later back-transformed for display.

For the constant (1a) and alternating temperature experiments (1b), one-way analysis of variance (ANOVA) was undertaken to distinguish differences in germination, germination rate and viability between the 10 temperature regimes that were tested. Germination was calculated as a percentage of the number of seeds that germinated out of the original 50 seeds in response to imposed treatments. Viability included those seeds that germinated in the Petri dishes (both initially and then once exposed to optimum temperature conditions) plus those that tested positive using the tetrazolium method. To compare germination rates, a germination rate index was calculated (Maguire 1962). This involved dividing the number of germinated seeds obtained at each daily counting in the standard germination test by the number of days seeds had been in the Petri dish. The values obtained at each count were then summed at the end of the germination test to obtain the germination rate index.

For the seed persistence trials, viability data was analysed using a simple split-plot analysis of variance for Experiment 2b, but more complex multiple split-plot analysis of variance for Experiment 2a, as dictated by the experimental design.

## Results

### *Experiment 1a – seed germination and viability under constant temperature regimes*

#### *Germination*

Significant differences ($P < 0.001$) in germination occurred between constant temperature regimes (Fig. 3). Maximum germination (average of 71%) was recorded at constant temperatures of 24–27°C (Fig. 3). The level of germination then reduced gradually with declining temperatures but sharply with increasing temperatures. At the lowest constant temperature regime of 13°C, 30% germination was still recorded. In contrast, at 39°C germination was <1%, with nil germination recorded at temperatures of 43°C or higher.

Constant temperature regimes also significantly affected the rate of germination (Fig. 4). Like maximum germination percentage, the maximum germination rate was most rapid between 24 and 27°C and decreased as temperatures declined or increased beyond the optimum range.

#### *Viability*

Seed viability was highest (73%) at constant temperatures of 24–27°C (Fig. 3). As temperatures declined from 27°C, an initial reduction in the viability of seeds was recorded at 20°C, but it then stabilised and remained relatively constant,

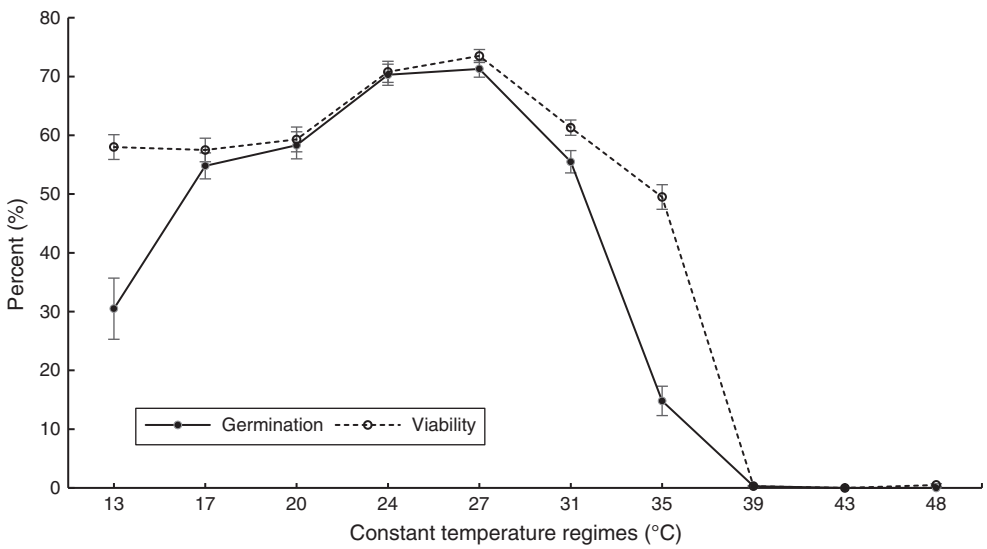


**Fig. 3.** The effect of constant temperature regimes on seed germination and seed viability of *Stevia ovata*, averaged over initial and repeat experiments. Vertical bars indicate the s.e. of the means.

averaging 58% even at the lowest temperature regime (13°C). As temperatures increased above 27°C seed viability displayed a similar but more gradual decline than seed germination, with <1% viability recorded at temperatures ≥39°C. The differential responses between seed germination and seed viability at suboptimal temperatures were due to dormancy mechanisms (Fig. 3). At optimal temperatures (24−27°C), 98% germination of viable seeds occurred, with only 2% exhibiting innate dormancy. With both declining and increasing temperatures away from the optimum range, the level of enforced dormancy (i.e. seed quiescence) increased. For example, at 13 and 35°C only 46 and 37% of viable seeds germinated, respectively. The majority (96%) of the remaining viable seeds readily germinated at completion of the trial once they were exposed to more favourable temperature conditions (30/20°C).

### *Experiment 1b – seed germination and viability under alternating day/night temperature regimes*

#### *Germination*

Significant differences ($P < 0.001$) in seed germination percentage (Fig. 5) were detected between alternating

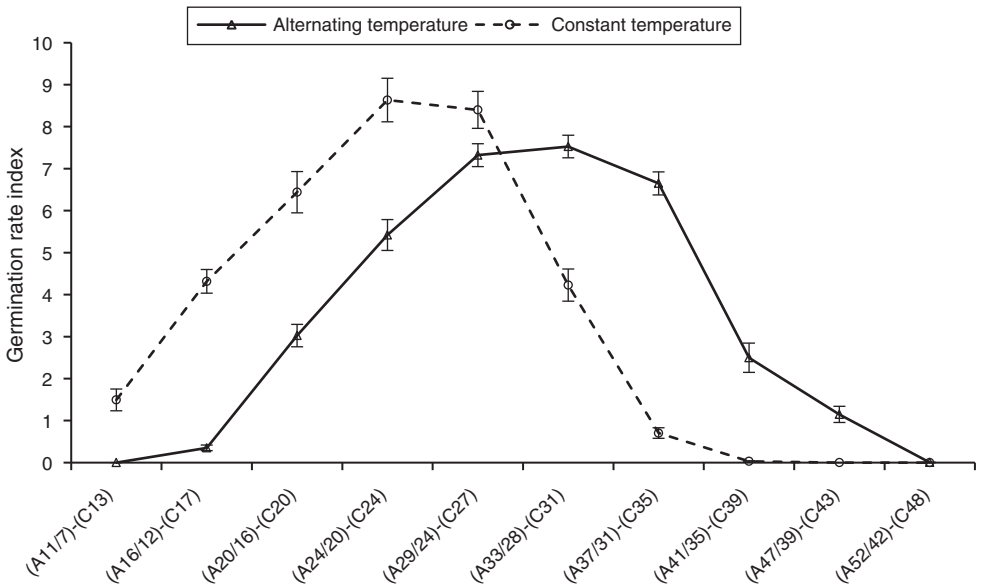


**Fig. 4.** Germination rate index for *Stevia ovata* associated with constant and alternating temperature regimes, averaged over initial and repeat experiments. Vertical bars indicate the s.e. of the means.

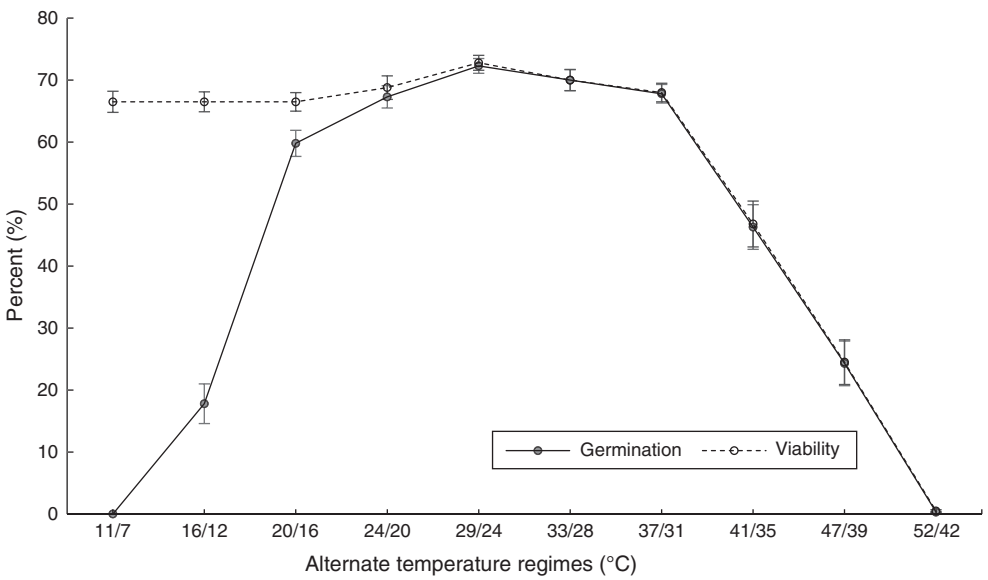


**Fig. 5.** The effect of alternating temperature regimes on seed germination and seed viability of *Stevia ovata*, averaged over initial and repeat experiments. Vertical bars indicate the s.e. of the means.

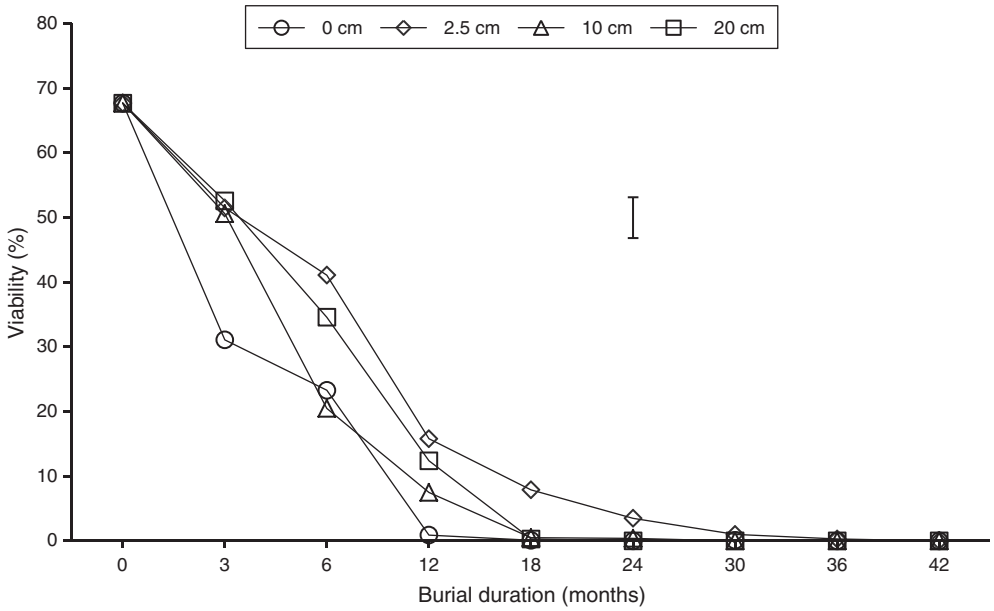


**Fig. 6.** The effect of burial depth and burial duration on mean seed viability of *Stevia ovata* in the dry tropics across all soil types and levels of vegetation cover. Vertical bar indicates the l.s.d. at $P = 0.05$.

temperature regimes. Nil germination occurred at the coolest (11°C/7°C) temperature. Germination then rose rapidly with increasing temperatures before peaking (average of 69%) across a broad optimum temperature range, from 24/20 to 37/31°C. A linear decline in germination followed as temperatures increased further, resulting in less than 1% germination at 52/42°C. The rate of germination of *S. ovata* under alternating temperatures produced a similar pattern compared with that for constant temperatures (Fig. 4), but the maximum germination rate was less than that under constant temperature conditions. Also, when compared on an average daily temperature basis, generally higher average temperatures (compared with constant temperatures) were required to promote maximum germination rates under an alternating temperature regime (between 29/24 and 33/28°C).

#### *Viability*

Seed viability demonstrated a comparable response to seed germination (Fig. 5), except below the lower end of the optimum temperature range for germination (i.e. <24/20°C). While germination was reduced at these lower temperatures, viability was not affected. No significant difference ($P > 0.05$) in viability was recorded for alternating temperature regimes between 11/7°C and 37/31°C (inclusive), with viability averaging 68.5%. As for constant temperatures, the differential response between germination and viability (Fig. 5) at lower temperatures was due to enforced dormancy. At 11/7°C, 100% of viable seeds remained dormant, yet 99% of them readily germinated when they were exposed to favourable temperatures (30/20°C) at the end of the trial. In contrast, the similar linear decline exhibited for both seed germination and seed viability as temperatures increased above the optimum range (i.e. >37/31°C), indicates that loss of seed viability was the contributing factor, with no evidence of enforced dormancy.

### *Experiment 2a – seed persistence in the dry-tropics*

#### *Seed viability*

Initial seed viability (percent of total seed number) and germinability (percent of viable seeds) averaged $68 \pm 0.4\%$ and $97 \pm 0.3\%$, respectively, at 30/20°C. Following burial, a significant burial depth × burial duration interaction ($P < 0.01$) occurred (Fig. 6). All burial depths displayed an almost linear rate of decline over the first 12 months of burial, but it was most rapid if seeds were located on the soil surface and slowest if they were buried 2 cm below ground. Nil viability was recorded after 24 months for seeds located on the soil surface or 20 cm below ground. However, it took until 30 and 42 months before viable seed reserves were depleted at 10 and 2 cm depths respectively. Neither soil type nor the level of pasture cover had a significant influence ($P > 0.05$) on the seed persistence of *S. ovata* in this experiment.

### *Experiment 2b – seed persistence in the wet-tropics*

#### *Seed viability*

At the time of burial, seed viability (percent of total seed number) averaged $75 \pm 1.6\%$. As in Experiment 2a, burial duration significantly ($P < 0.001$) affected seed viability (Table 3). However, unlike Experiment 2a, seed viability expired across all burial depths after 18 months and even after 12 months viability was <1%. Also in contrast to Experiment 2a, there was no significant ($P > 0.05$) effect of burial depth on viability of *S. ovata*, with average viability rates of 24, 26, and 25% recorded at depths of 0, 3, and 10 cm respectively (Table 3).

## Discussion

*Stevia ovata* seeds remain viable and germinate across a broad temperature range, but under suboptimal temperatures a proportion either lose viability or exhibit enforced dormancy

**Table 3. Viability of *Stevia ovata* (candy leaf) seeds associated with burial depth and duration in the wet tropics**

Means followed by the same letter are not significantly different at $P = 0.05$

| Burial depth (cm) | Burial duration (months) | | |
|---|---|---|---|
| | 0 | 12 | 18 |
| | | Viability (%) | |
| 0 | 72 | 1 | 0 |
| 3 | 76 | 2 | 0 |
| 10 | 76 | 0 | 0 |
| Mean | 75a | 1b | 0b |

until more favourable temperatures prevail. The longevity of viable soil seed banks varies depending on prevailing environmental conditions, with soil seed reserves essentially exhausted by 12 months – only ~1% left then in the wet-tropics of northern Queensland but not until between 24 and 42 months in the dry-tropics, depending on soil depth.

### *Seed germination and viability*

The seed lots of *S. ovata* that were used across the various experiments exhibited above average viability (68 to 75%) and high germinability (97 to 99%). Seeds germinated across a wide range of constant (13−39°C) and alternating temperatures (16/12 up to 52/42°C), but optimum temperatures for germination were more restricted being between 24 and 27°C for constant temperature and between 24/20 to 37/31°C for alternating temperatures. Seed viability and germinability and the rate of germination were also generally highest or fastest at these temperatures. *Stevia rebaudiana* has similar optimum temperature requirements (Carneiro 1990) and *Parthenium hysterophorus*, another invasive herbaceous weed in northern Queensland and also a member of Asteraceae, also germinates under a wide range of temperatures (10−36°C) (Williams and Groves 1980).

As temperatures increased above the optimum range, seed germination of *S. ovata* declined, mainly because an increasing proportion of seeds lost viability. This decline in both germination and viability was more rapid under increasing constant temperatures than at comparable alternating temperatures. High temperature (40°C) was also reported to reduce total germination of *S. rebaudiana* (Tanaka 1985). The differential response in germination between high alternating and constant temperature regimes is also similar to that reported for *S. rebaudiana* (Macchia *et al*. 2007).

As temperatures declined below the optimum range, *S. ovata* viability either remained relatively constant (alternating temperatures) or declined slightly (constant temperatures). In contrast, germination was greatly reduced particularly under an alternating temperature regime. *Stevia rebaudiana* also demonstrated reduced germination (<50%) under low temperature conditions (Miyazaki and Wantenabe 1974; Madan *et al*. 2010).

The ability of *S. ovata* to maintain seed viability but go into a state of enforced dormancy (i.e. seed quiescence) under unfavourably cool temperatures, shows the great versatility of this species in terms of a survival strategy under variable temperature conditions that generally prevail in its invasive range. Seeds of other species such as *Coreopsis lanceolata* (Asteraceae) have exhibited similar behaviour when exposed to cold temperatures (Banovetz and Scheiner 1994). This seed trait enables seeds to remain temporarily inactive and consequently protected until more favourable conditions occur (Baskin and Baskin 2001; Kos and Poschlod 2007).

### *Seed longevity*

Based on the dichotomous key of Thompson *et al*. (1997), the current trial results suggest *S. ovata* has a short-term persistent seed bank (1–5 years). However, despite falling into this category, seed bank persistence of *S. ovata* may vary markedly depending on prevailing environmental conditions. Seeds buried in the wet-tropics (South Johnstone) persisted for ~12 months whereas those buried in the dry-tropics (Charters Towers) persisted for 3.5 years.

The most plausible explanation for this variation is that rainfall conditions were more favourable for germination of *S. ovata* in the wet-tropics than the dry-tropics and that this would have depleted soil seed reserves. Mature seeds of *S. ovata* appear to have high germinability and during the current study, rainfall at the site in the wet-tropics was six times higher than rainfall at the site in the dry-tropics. Besides the total amount of rainfall being greater, the frequency and duration of rainfall events would also have been higher and provided more opportunities for seeds to germinate. Several studies have demonstrated faster depletion of viable soil seed reserves of weeds under more favourable soil moisture conditions (Bebawi *et al*. 2003; Vivian-Smith and Panetta 2009; Bebawi *et al*. 2012). For example, Vivian-Smith and Panetta (2009) modelled seed survival projections for *Lantana camara* and found that seed persistence was greatest for buried seeds under natural rainfall (11 years) than under more favourable soil moisture conditions provided through supplementary irrigation conditions (3 years).

Burial depth was another factor that caused differential responses in seed persistence between the wet- and dry-tropics. In the wet-tropics, burial depth did not influence seed bank longevity, but in the dry-tropics seeds of *S. ovata* generally persisted for longer if buried below the soil surface. However, the first retrieval undertaken in the wet-tropics occurred after 12 months burial and it is plausible that differences in burial depth may have been detected if shorter time frame retrieval intervals had occurred within the first 12 months similar to the dry-tropics.

The greater seed persistence observed in seed buried below the soil surface in the dry-tropics has been reported for some other members of the sunflower family. Wijayratne and Pyke (2012) found that burial increased seed longevity of two *Artemisia tridentata* (Asteraceae) subspecies. They reported that after 24 months, seeds buried at least 3 cm below the soil surface retained 30–40% viability whereas viability of seeds on the surface and under litter declined to 0 and <11% respectively. By comparison, *S. ovata* seeds buried at 2.5 cm below the soil surface in the dry-tropics (across all soil types and vegetation cover) retained on average 44% viability compared with 6% for seeds left on the soil surface. Buried seeds of *P. hysterophorus* also lasted much longer than seed on the

soil surface (Navie *et al*. 1999; WONS 2003; Nguyen *et al*. 2010). Although in these instances seed persistence was shorter for seeds located on the soil surface, there are many recent examples for weeds in the dry-tropics of Australia where the opposite has occurred (Bebawi *et al*. 2012, 2015, 2016). This is generally attributed to the fact that the soil surface dries out quickest after rainfall and therefore soil moisture conditions are less favourable for germination to occur compared with seeds buried below ground, resulting in the seeds remaining in a state of enforced dormancy.

The results from the alternating and constant temperature experiments suggest that temperature may possibly be a contributing factor to the differential responses between burial depths and between sites. In the hot conditions of the dry-tropics, surface temperatures may exceed the critical levels that negatively affect the viability of *S. ovata* seeds, with those buried below ground subjected to cooler conditions below the critical range. Temperatures at the site in the wet-tropics were cooler than those at the site in the dry-tropics and were combined with more favourable soil moisture conditions. Under these conditions, germination and seed bank depletion are faster than in the dry-tropics.

### *Ecology and management implications*

*Stevia ovata* is currently restricted to one area in northern Queensland but its ability to maintain viability and germinate across a wide range of temperatures highlights its potential to establish across much greater areas in Australia (Fig. 2). This is consistent with earlier modelling that used the climatic conditions in the native range of *S. ovata* to predict its potential distribution in Australia (Murphy *et al*. 2009). This study concluded that under current climatic conditions *S. ovata* appears to have the potential to invade almost the entire eastern and southern coasts of Australia, including Tasmania. Results presented on germination and viability responses to temperature regimes imposed in the present study supports the predictions made by Murphy *et al*. (2009). At temperatures >39°C, seeds of *S. ovata* lost viability but at cooler temperatures viability was relatively unaffected, with seeds forced into a state of enforced dormancy under suboptimal germination conditions. In the more southerly range of the potential distribution for *S. ovata*, this strategy would protect soil seed reserves during the cooler months of the year and allow germination and establishment to occur following rainfall during warmer months. The implications for the existing infestation near Ravenshoe is that there is considerable potential for spread over a much larger area under current climatic conditions. As such existing efforts to control *S. ovata* at the local level should continue, but consideration should also be given to dispersal methods and what initiatives could be implemented to minimise spread into favourable habitats further south. An important management action would be to maintain high traffic areas (e.g. roadsides, camping grounds) free of *S. ovata*.

The findings from the seed longevity trials in the present study provide land managers with information that can be incorporated into control/eradication programs for *S. ovata*. Seed longevity is an important attribute that often influences whether weed eradication/control programs will be successful or not (Campbell and Grice 2000; Dodd *et al*. 2015). Likelihood of success increases if soil seed banks are short-lived and if new seedlings that emerge following control activities take a long time to reach reproductive maturity (Dodd *et al*. 2015). Under the high rainfall conditions where *S. ovata* is currently located within Australia, seed banks should be exhausted within two years in the absence of replenishment from internal and/or external sources. However, during drier periods or if in the future new outbreaks are found in areas with a drier climate, longevity could be extended several years, as occurred at the dry-tropics site (3.5 years) in the present study.

In terms of age to reproductive maturity, young plants of *S. ovata* have demonstrated the potential to flower within 3 months (Setter *et al*. 2016), but synchronised flowering means that in North Queensland it does not occur until around May (late autumn) with seed maturation commencing around June (early winter). At a minimum, land managers should focus survey and control efforts during autumn in order to control plants before they have the opportunity to set seed and replenish soil seed reserves.

## Conflict of interest

The authors declare no conflicts of interest.

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