Estimating tropical weed seed longevity with a laboratory test

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Summary Longevity of weed seeds in the soil drives the cost and duration of weed control activities. Traditional methods for estimating weed seed longevity, such as repeated field soil sampling and buried packet trials can take many years and substantial resources to complete. A laboratory process, a Controlled Ageing Test (CAT) exposes seeds to an 'ageing environment' of 45 ºC temperatures and 60% humidity. Data from this test is used to sort species into relatively transient, short lived or long-lived categories of weed seed longevity. This paper reports on examples from a series of trials that seek to correlate the data from CAT batches with longevity data from buried packet trials.

Keywords controlled ageing test, soil seedbank, weed seed persistence, tropics.

INTRODUCTION

In the absence of seed input, the longevity of weed seed in the soil will drive the length of weed control activities. This length influences the cost of activities and follow-up seedling control should be considered in landholder weed management plans. There are several sources of information that can be used to determine the likely persistence of weed seeds in the soil. The five main sources are buried packet trials, soil samples collected within infestations, controlled age testing, field control records and native range studies; each method has its own pros and cons (Brooks and Setter 2012). The burying and retrieval of seed packet trials has, for many years been the main and sometimes only formal source of local information on weed seed persistence. This remains a common, standard, and robust way of estimating weed seed longevity (reference section has 8 examples). Buried packet trials require seed, land and technical resources and take 1 to 15 years to complete. For newly identified weed species there may be little known about the seed longevity, limited seed available for long-term study and lack of a climatically suitable long-term site. So initial decisions on management, including eradication are made in the absence of seed longevity information.

Hay *et al.* (2006) and Probert *et al.* (2009) use a procedure to estimate the longevity of seed lots held in storage. Where seeds are exposed to an ageing environment and removed after 1 to 125 days and germinated under standard incubator conditions. The germination data is used to determine the number of days in the ageing environment when germination drops below 50% of an initial, unaged value (day 0), this value in days is called P_{50}. A study by Long *et al.* (2008) found a broad correlation between field trials of weed seed longevity and P_{50} values from the Controlled Ageing Test (CAT). The CAT has also been used to rank seed of species in the same plant families by relative persistence (Probert *et al.* 2009).

Selected data from a series of controlled ageing tests is reported and compared to the results of local buried seed packet trials. Some weed species without local burial trials are included as their results are applicable to local management efforts.

MATERIALS AND METHODS

Between 2018 and 2021, a series of CATs were conducted in seven batches containing seed lots of 1 to 13 species. A subset of the test results is reported in this paper (Table 1).

Seed Seed was collected at the date and location in Table 1, then carefully separated from fruit or pods and stored at laboratory room temperature until used in the CAT batch or batches. Unless otherwise noted, seed was sorted into 24 lots of 50 for testing. *Ziziphus mauritiana* Lam. was tested using 48 lots of 30 intact endocarps (kernels). Half the kernels were kept 'intact', with the seeds kept within the kernels for the hydration and ageing phases. Seeds were then removed from the intact kernels for germination at each retrieval time. Seeds were removed (cracked) from the other 24 kernels before exposure to the hydration and ageing phases. Seed totals for each lot of 24 kernels was recorded. *Cascabela thevetia* (L.) Lippold was sorted into 22 lots of 20 kernels and were kept in the kernels for the hydration and ageing phases. At each retrieval time seeds were removed from 2 x 20 kernels and counted prior to germination.

Experimental conditions Seed lots were subjected to a 'hydration' phase then an 'ageing' phase following a protocol of Hay *et al.* (2006). Each CAT batch used two replicate IP67 electrical boxes (labelled A and B). Seed lots were placed in individual open glass vials, plastic jars or centrifuge tubes, half in each of the sealed boxes. For the hydration phase, lots were exposed to a 47% relative humidity lithium chloride solution (320 g/L H_2O) in a dark 20ºC Thermoline® incubator for 14 days.

For the ageing phase, the temperature was increased to 45ºC with 60% relative humidity (lithium chloride at 370 g/L H_2O). Seeds remained in the dark ageing environment for 2 to 203 days and removed at each retrieval interval and germinated. Test conditions were checked with Onset® Hobo® temperature and humidity loggers and the lithium chloride solution was adjusted as necessary. Unless otherwise stated a seed container was removed from each box in the ageing environment on days 0, 2, 7, 14, 21, 28, 35, 42, 56, 77, 98, 126. This was the standard retrieval schedule. In batch 4, the final two retrievals for *Tecoma stans* L. Kunth were retrieved at days 91 and 98 due to no germination in the previous retrieval. In batch 6, there was no day 126 retrieval. The retrievals for batch 7 were on days 0, 7, 14, 28, 42, 56, 77, 98, 119, 147, 175 and 203. Batch 2 started on the 31/1/2019, batch 3 on the 3/7/2019, batch 4 on the 21/1/2020, batch 5 on the 3/6/2020, batch 6 on the 18/1/2021 and batch 7 on the 3/3/2021.

Germination A reference sample of each seed lot was removed from boxes A and B and germinated prior to the ageing phase (day 0). Each retrieved seed lot was placed in a 90mm petri dish, on top of moistened filter paper and an inverted watch glass. All petri dishes were kept moist with distilled water and germinated in a Thermoline® incubator running at 30/20 ºC 12hr diurnal cycle, except for the *Senecio madagascarensis* Poir. which was germinated at 23/17 ºC 12hr diurnal cycle reflecting its cooler distribution. Germinated seeds (identified by radicle emergence) were counted and removed periodically. Ungerminated seed of the Fabaceae species was scarified after approximately 28 days. Scarification was conducted by either, submergence in 98.08% solution of sulfuric acid for 25 minutes or knicking the outer seed coat with secateurs.

Table 1. Species, collection details, CAT batch, P_{50} value and category. (+) indicates the regression line did not drop below the 50% in the duration of the test. * P50 category as defined by Long *et al.* (2008).

Weed species	Collection location, month and year	CAT batch	P_{50} value (day)	P_{50} category*
Acaciella angustissima	Calcium 2019	3	111	Long-lived
Andropogon gayanus	Mareeba 2018	2	16	Transient
Cascabela thevetia	South Townsville 08/2020	6	25	Short-lived
Calotropis procera	Upper Burdekin River 12/2019	4	109	Long-lived
Calotropis procera (repeat)	Upper Burdekin River 12/2019	5	89	Long-lived
Cryptostegia grandiflora	Charters Towers 12/2019	4	125	Long-lived
Cyperus aromaticus	Innisfail 2018	3	82	Long-lived
Leucaena leucocephala	Charters Towers 06/2018	2	69	Long-lived
Leucaena leucocephala (repeat)	Charters Towers 06/2018	7	203+	Long-lived
Parkinsonia aculeata	Upper Burdekin River 12/2020	7	127	Long-lived
Senecio madagascarensis	Herberton 07/2018	4	19	Transient
Senna alata	Townsville 07/2019	3	126+	Long-lived
Senna alata (repeat)	Townsville 07/2019	7	190	Long-lived
Stevia ovata	Ravenshoe 10/2018	2	16	Transient
Tecoma stans	Charters Towers 07/2019	4	30	Short-lived
Ziziphus mauritiana (cracked)	Charters Towers 11/2019	4c	40	Short-lived
Ziziphus mauritiana (intact)	Charters Towers 11/2019	4i	20	Short-lived

Data analysis The total germination from the retrieval at day 0 was used a reference value. Subsequent germination was calculated as a proportion of the day 0 germination per box. Proportion data from the A and B boxes was used to create a negative logistic regression curve (equation 2 in Long *et al.* 2008) in Genstat® 19th, 21st edition VSNi®. The P_{50} value in Table 1 is nearest whole day determined from the regression line. Where seed lots were repeat tested, data from 4 boxes and 2 batches was used to create the regression line, this combined value is mentioned below. The P_{50} categories (Table 1) classified the seeds into longevity categories as defined by Long *et al.* (2008), with transient seed banks less than 1 year (P_{50}<20), to be short-lived seed banks of one to three years (20<P_{50}<50) and long-lived seed banks over three years (P_{50}>50).

COMPARISON WITH BURIAL TRIALS

Apocynaceae The seed of *Cryptostegia grandiflora (*Roxb.) R. Br. in Table 1 indicated a long-lived seed based on a fall in the germination on the last (day 126) of retrievals. This is different to the field trial reported by Bebawi *et al*. (2003), who reported 0%

viable after 3 years, which would fit in the short-lived category. They also recorded a drop below 50 % viability between 11 and 20 years of cool, dry storage. The CAT can be applied to seeds held in cold storage (Probert *et al.* 2009) and generally less persistent seeds are correlated with a drop below 50% viable in under 20 years of storage. The other species in this family tested was *C. thevetia***.** There was more consistency between the CAT data in Table 1 (short-lived) and the study of Bebawi *et al.* (2016) who found a small proportion of seed viable after 12 months burial and none after two years.

Asclepiadaceae There are large differences between the CAT data reported for *Calotropis procera* Aiton (W.T Aiton) in Long *et al.* (2008) (P_{50} = 28.7) and Probert *et al.* (2009) (P_{50} = 18.5 days) and the data in Table 1. After storage, the value for batch 5 was lower than 4 (Table 1). However, in both boxes in both batches the germination data from the first nine retrievals (day 56) was above 90% and was classified as long-lived. Bebawi *et al.* (2015) described the results of the buried packet trials as fitting in the short-lived category (0% after 18 months) and consistent with the findings of Long *et al.* (2008). Despite running the same seed source of *C. procera* in two CAT batches our results were inconsistent with the other published studies.

Asteraceae The germination of *S. madagascarensis* seed dropped quickly in the CAT (Table 1) and it could be classified as transient, although close to the short-lived category. Through the extrapolation of data, *S. madagarscarensis* seed was suspected to be longer lived (3-5 years, maybe more, Sindel, (2009)), though longer-term field data sources have not been identified. A short seed life would aid the management of the isolated northern incursion. The second Asteraceae, *Stevia ovata* Willd. also showed a transient seed bank (Table 1). In buried packet trials in the wet tropics this species was exhausted in 18 months and after 36 months in the dry tropics (Bebawi *et al.* 2018a). The CAT data appears to be an under-estimate of the field seed longevity, as *S. ovata* burial trials show a short-lived seed life.

Bignoniaceae An unpublished field trial near Moura in central Queensland found *T. stans* had short lived to transient seed bank when buried (W Vogler pers comm). The results from the CAT indicate a short seed life, which may be a slight over-estimate of buried seed longevity.

Cyperaceae Viable seed of *Cyperus aromaticus* (Ridley) Mattf. & Kük was found in packets that had been buried for 15 years in soil at South Johnstone (M Setter and J Vitelli unpubl. data). The CAT data in Table 1 supports the field test results that this species forms a long-lived seed bank, although the CAT data may be an underestimate of buried seed persistence in the wet tropics.

Fabaceae Probert *et al.* (2009) reported CAT results that showed species in this family form long-lived seed banks. So, CAT batch 7 (Fabaceae seed lots) was aged for up to 203 days and all seed lots were found to fit the persistent category (Table 1). Long *et al.* (2006) reported a P_{50} value of 122 for *Parkinsonia aculeata* L. seed, which was scarified prior to ageing. This is like the value in Table 1, which was obtained for seed scarified after ageing. Field trials of buried *P. aculeata* found persistent seed banks (4+ years) were formed in wetter habitats (van Klinken *et al.* 2008). Buried packet trials and the CAT data are consistent in indicating long-lived seed persistence beyond 3 years. Data from a *Leucaena leucocephala* (Lam.) de Wit. seed longevity trial was summarized in Campbell *et al.* (2019). They reported viable buried seed was still present (>4%) after eight years. This indicates a long-lived seed bank, which is also reflected in the data of Probert *et al.* (2009) (P_{50} = 75.2) and Table 1 (batch 2). The CAT data for batch 2 was influenced by values in both boxes below 50% at days 75 and 126. In CAT batch 7, there was a more consistent decline to 60% at day 203, although the combined line plateaued at 62%. All trials show *L. leucocephala* forms a long-lived seed bank which may extend well beyond three years.

There was little data available on the weeds *Senna alata* (L.) Roxb. and *Acaciella angustissima* (Mill.) Brit. & Rose. *Senna alata* has been noted as an emerging weed in north Queensland and showed no decline below P_{50} in batch 3. Seed from the same collection was used in batch 7 and this only approached P_{50} after 190 days (the combined line plotted at 196 days). The predicted seed life for *S. alata* may be well beyond 3 years and it would be expected to form a long-lived seed bank. *Acaciella angustissima* is being controlled at several north Queensland infestations and the data in Table 1 indicates this species also has a long-lived seed.

Poaceae Buried trials show *Andropogon gayanus* Kunth. forms a very short-lived seed bank with viability dropping to below 10% after 6 months, 1% after 12 months and 0% after 30 months (Bebawi *et al.* 2018b). The results in Table 1 fit into the transient category. It is possible some buried seed (10-20 cm) would be short lived, but both data sources indicate low overall viability beyond 12 months.

Rhamnaceae The study by Bebawi *et al*. (2016) found seed from buried 'intact' *Z. mauritiana* kernels was exhausted quickly (6-18 months) and surface kernels reached 0% viable in 24 months. The intact treatment in Table 1 reached P_{50} in 20 days, which is consistent with a short-lived seed bank and the field trials. The P_{50} for the seed removed from the kernels was 40 days, although this is higher than the intact kernels, it is still classified as short-lived.

DISCUSSION

Much of the CAT data from most of the tropical weed species tested was broadly consistent with the published data from buried packet trials. The greatest differences occurred when the CAT data on *C. procera* and *C. grandiflora* was compared to buried packet trials and other sources. There were also inconsistencies in the CAT results for *L. leucocephala*. The CAT remains a useful tool to broadly categorize seed longevity where time, seed and field sites are in short supply, such as with new incursions. However, until the source of some of these differences is better understood it could be misleading to use the CAT data as a sole source of longevity information.

There can be useful, predictive trends in relative seed persistence amongst some plant families and within short lived genera (Probert et al. 2009). Ultimately the CAT data applies to the source population and a single value will not capture variation between populations (Long *et al.* 2008). The overall mean seed longevity data from buried packet trials often reflects the combination of depth, ground cover and soil factors, which are not factors covered by the laboratory test.

The three tropical species that were classified as transient based on the CAT data in Table 1, have been found to be short-lived in field trials. The transient range described by Long *et al.* (2008), may better reflect later field data if the P_{50} was less than 16. More testing, ranking and correlation analysis may provide the scope to refine the zero-to-three-year categories into more defined or overlapping zones. Further analysis may also help to categorize P_{50} values for very long-lived species that are found to be viable after five or ten years of field burial.

This series of experiments is continuing to compare the results from the CAT with field longevity trials and refine the predictability of this shorter laboratory-based test. This could be a useful and efficient tool to inform weed control programs where little seed longevity information is available. However, the CAT may not be a consistent predictor for all tropical seed lots and is best interpreted in conjunction with other field or trial data.

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