RESPONSES OF FABACEAE SEED LOTS TO CONTROLLED AGEING TESTS

Brooks, S.J, and Brazier D. A. and Warren, C.
Tropical Weeds Research Centre, Department of Primary Industries
Charters Towers, 4820.

ABSTRACT

The controlled ageing test (CAT) seeks to inform the persistence of weed seeds using a short-term laboratory test. In the CAT, seed lots are exposed to an ageing environment of 45 °C and 60% humidity for up to 203 days. The main output is the point at which relative germinability drops to 50% of the germinability prior to ageing (P_{50} value). Data can be compared to other sources such as long-term burial studies or used to categorise the potential longevity of weeds without longer term data sources. Seed of 12 weeds from the Fabaceae family have been assessed once or twice in the CAT environment. There was variation within and between genera of Fabaceae, with the laboratory test suggesting 11 of the 12 weeds are more likely to form a persistent seed bank, greater than 3 years.

INTRODUCTION

In the absence of seed input, the length of weed control programs is determined by the longevity of the viable seed bank. This length influences the cost of activities and the duration of follow-up seedling control which should be considered in 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 primary experimental source of local information on weed seed persistence. This remains a common, standard, and robust way of estimating weed seed longevity (Brooks *et al*. 2023b). Buried packet trials are site specific, require tens of thousands of seed, notable land, technical resources, and take 2 to 15 years to complete. Recently a method of monitoring field seedling emergence has been reported (Brooks *et al.* 2023a). This involves secure field enclosures (seedling emergence cages) which contain intact fruit or pods on the soil surface, where seedlings are recorded and removed fortnightly. These observations run over many years, are site-specific and require more than 8000 seeds in pods (Brooks *et al.* 2023a).

For newly identified weed species there may be little known about the seed longevity, and limited seed available for long-term study. So initial decisions on management, including eradication are made in the absence of seed longevity information. The Controlled Ageing Test (CAT) is a shorter laboratory test of relative seed persistence (Brooks *et al.* 2022). Seeds are exposed to an ageing environment and removed after 1 to 203 days and germinated. The germination data is used to determine the days in the ageing environment when germination drops to 50% of an initial, unaged value (day 0), this value in days is called P_{50}. Long *et al.* (2008) found a broad correlation between field trials of weed seed longevity and P_{50} values from the CAT. They proposed that the P_{50} classify the seeds into longevity categories of transient seed banks less than 1 year (P_{50}<20), short-lived seed banks between one to three years (20<P_{50}<50) and long-lived seed banks over three years (P_{50}>50).

In ranking seed of 195 species by family, Probert *et al.* (2009) found five Fabaceae species to have one of the highest mean family P_{50} values (mean 83.5 days, range 12-185). Seed of 12 weeds from the Fabaceae family have been assessed once or twice in the CAT environment. Where available, the results are compared to results from buried packet trials, seedling emergence cages and other literature values.

MATERIALS AND METHODS

Between 2019 and 2023, a series of Controlled Ageing Tests (in batches 1-10) were conducted on many seed lots including the 12 Fabaceae seed lots listed in Table 1. Seed lots were separated from pods and stored at laboratory room temperature until used in the respective batch or batches. Seed was sorted into 24 lots of 50 for testing.

Table 1. Fabaceae weeds included in different controlled ageing test (CAT) batches**.**

Scientific name	Common name	Batch
Acaciella angustissima (Mill.) Britton & Rose	White ball acacia	3 and 7
Albizia lebbeck (L.) Benth.	Albizia, Indian siris	10
Dichrostachys cinerea subsp. *malesiana Brenan & Brummitt.*	Chinese lantern	7
Leucaena leucocephala (Lam.) de Wit	Leucaena	2 and 7
Parkinsonia aculeata L.	Parkinsonia	7 and 10
Prosopis pallida (Humb. & Bonpl. ex Willd.) Kunth	Mesquite	7
Senna alata (L.) Roxb.	Candle senna	2 and 7
Senna hirsuta (L.) H.S.Irwin & Barneby	Hairy senna	7
Senna obtusifolia (L.) H.S.Irwin & Barneby	Sicklepod	2 and 7
Senna occidentalis (L.) Link	Coffee senna	7
Vachellia farnesiana (L.) Wight & Arn.	Mimosa bush	9
Vachellia nilotica (L.) P.J.H.Hurter & Mabb.	Prickly acacia	7

Seed lots were subjected to a 'hydration' phase then an 'ageing' phase following a protocol of Hay *et al.* (2006). Each batch used two replicate IP67 electrical boxes, labelled A and B. Seed lots were placed in individual open glass vials, half in each of the two sealed boxes. For the hydration phase, seed lots were kept in a dark Thermoline® incubator at 20°C and 47% relative humidity with a lithium chloride solution of 320 g/L H2O for 14 days. For the ageing phase, the temperature was increased to 45°C with 60% relative humidity lithium chloride solution (370 g/L H2O). Seeds remained in the dark ageing environment for 2 to 203 days and removed at each retrieval interval and germinated (Brooks *et al.* 2022).

Batch 2 started the hydration phase on 31/01/2019, batch 3 on 3/7/2019, batch 7 on 3/3/2021, batch 9 on 24/5/2022 and batch 10 22/2/2023. For batches 2, 3 and 9 retrievals from the ageing environment were after 0, 2, 7, 14, 21, 28, 35, 42, 56, 77, 98, 126 days. The retrievals for batch 7 were after 0, 7, 14, 28, 42, 56, 77, 98, 119, 147, 175 and 203 days of ageing. The batch 10 retrievals of parkinsonia were after 0, 9, 14, 21, 28, 42, 56, 77, 98, 113, 126, 147 days of ageing.

At the conclusion of the hydration phase, a seed lot of each weed was removed from boxes A and B and germinated prior to the ageing phase (day 0, retrieval). Further retrievals from the ageing environment were on the days listed above. 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°C 12hr diurnal cycle. Germinated seeds (identified by radicle emergence) were counted and removed periodically. Ungerminated seed were scarified after approximately 28 days. Scarification was conducted by either, submergence in 98% solution of sulfuric acid for 25 minutes or nicking the outer seed coat with secateurs.

The total germination from the retrieval at ageing day 0 was used a reference value. Germination from later retrievals was calculated as a proportion of the day 0 germination per box. Where seed lots were repeat tested, proportion germinable data from 4 boxes and 2 batches was used in the regression analysis. Proportion germinable data was used to create a negative logistic regression curve (equation 2 in Long *et al.* 2008). In four cases the logistic equation did not provide a normalised fit to the data and a linear regression equation was fitted. In a further four cases neither the logistic or linear regression equations provided a normalised fit to the data and a polynomial regression equation was fitted. Curves were fitted in Genstat® 24th edition VSNi. The P_{50} values presented are the days in the ageing environment at which the germination drops to 0.5 of the unaged reference (1.0). The logistic equation provides a P_{50} value and standard error, the P_{50} values from the linear and polynomial equations was calculated (without standard error) using an equation solver in Microsoft Excel®.

RESULTS AND DISCUSSION

The fitted equations are shown for each weed in Figure 1. Individual box and batch data points are not shown. The main parameter from the CAT, the P_{50} values are discussed relative to other available experimental sources of seed longevity data.

A logistic curve was fitted to the Albizia batch 7 data. The P_{50} of 43.1 ($\pm$ 8.33) indicating shorter term persistent seed bank, and was the lowest value obtained. Mimosa bush (P_{50} = 51.9 from linear regression) was on the arbitrary border between short and long lived. These two seed lots responded differently to the other ten in the tests and are likely to require shorter control programs where seed input is prevented.

Other seed lots greatly exceeded the threshold for long lived seed, though the final values were strongly influenced by the data from the last three retrievals in batch 7. There were also differences between the replicate boxes and repeat batches that created a wider variation in relative germinability after the day 126 retrievals. Due to this variation, fewer data points and different curves fitted, minor differences between species with fitted P_{50} values between 150 and 190 may not be reflected in all field situations. The data supports a longer exposure to the ageing environment for potentially long-lived seed lots, as discussed by Probert. *et al.* (2009), who reported P_{50} values of 367 to 711 days for Australian Myrtaceae species.

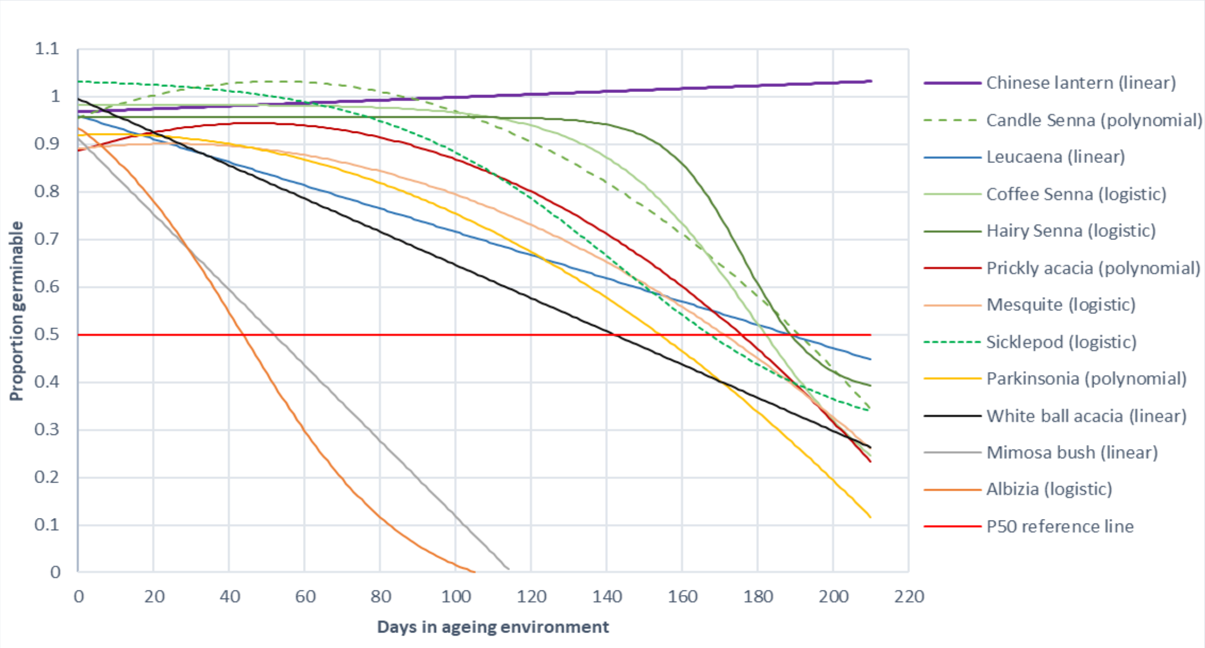

Figure 1. Regression curves of proportion of seed germinable over days in the ageing environment. Curves are ranked by P_{50} values.

White ball acacia is Prohibited Matter and being actively controlled at locations throughout eastern Queensland. The values for white ball acacia (P_{50} = 142.0 from linear equation) and sicklepod (P_{50} = 142.3 $\pm$23.3, logistic equation) were similar and at the lower end of the remaining persistent seed lots. Though they would still require long term control operations. Sicklepod is currently in buried packet trials at wet and dry tropical sites, and has shown greater, long-lived persistence in the wet tropics.

Sicklepod was one for four *Senna* sp. that were subjects of controlled aged testing. The others returned similar values hairy senna (P_{50} = 170.0 $\pm$7.85), coffee senna (P_{50} = 178.5 $\pm$32.1) from logistic equations and candle senna (P_{50} = 191.0 (polynomial)). Except for a P_{50} value of 75.7 for coffee senna (Probert *et al*. 2009), no other longevity data has been identified. Field operations may need to continue for more than five years for *Senna* sp.

A polynomial equation was fitted to the Parkinsonia data from batches 7 and 10, (P_{50}= 154.1). Long *et al.* (2006) reported a P_{50} value of 122 for parkinsonia seed, which was scarified prior to hydration. Field trials of buried parkinsonia found persistent seed banks (4+ years) were formed in wetter habitats (van Klinken *et al.* 2008). Seedlings are still emerging three years after placement in the seedling emergence cages (Brooks *et al.* 2023a) and emergence is heavily concentrated in bare plots. Trials are consistent in indicating long-lived seed persistence beyond four years.

A polynomial equation was fitted to the mesquite data from batch 7 and P_{50} = 171.2. The categorisation as a long-lived seed is reflected in the emergence of mesquite continued for 7.2 years in the seeding emergence cages (Brooks *et al.*

2023a). When buried in a packet trial, the relative seed viability was less than 5% after 1 year and declined further after 1.5, 2 and 3 years to be exhausted after 4 years (Brooks *et al.* 2023 b). The lower persistence of the mesquite seed in the buried packet trial is inconsistent with data from the seedling emergence cages and CAT.

The relative germinability of prickly acacia started to decline after 150 days in the ageing environment a P_{50} = 175.7 days, was calculated from a polynomial equation. Prickly acacia was the first seed lot to be buried in packets in September 2008, and viable seed was obtained from all treatments at the final retrieval in 2021 (Brooks *et al.* 2023b). A repeat trial of prickly acacia seed longevity commenced in March 2014 and is expected to run for 15 or more years, all depths (except surface packets) had 3-15% mean viable seed after 10 years (D. Brazier, unpublished data). The longevity of prickly acacia seed is further evidenced by seedling emergence (to date) 8.6 years after placement in the seedling emergence cages (Brooks et al. 2023a).

A linear equation was fitted to two batches of leucaena seed in the CAT and P_{50} = 188.3, with higher germinability in batch 7 than 2. This indicates a long-lived seed bank, which is also reflected in the data of Probert *et al.* (2009) (P_{50} = 75.2). Campbell *et al.* (2019) mentioned the retrieval of a small percentage of buried seed after 8 years. Brooks *et al.* (2023a) reported leucaena seedling emergence for at least 5.7 years after pods were placed in field cages. Three trials show leucaena forms a long-lived seed bank which likely extends beyond eight years when buried.

Two collections of Chinese lantern from adjacent infestations in Townsville were tested separately in batch 7. The seed lots behaved similarly in the ageing environment and a linear equation from the combined data was fitted. No P_{50} value could be calculated, and this seed was very tolerant of the ageing environment. Chinese lantern was the only weed not to show any consistent decline over 203 days in the ageing environment. A longer CAT would be required to calculate a P_{50} value.

SUMMARY

Three of the twelve Fabaceae weeds tested showed different responses to the ageing environment. The relative germinability of Albizia and Mimosa bush dropped far more quickly that the other seed lots tested and may reflect a requirement for shorter term control programs. While the Chinese lantern germinability did not drop over 203 days of ageing. The relative germinability of the remaining nine seed lots, including four *Senna* species, all halved after 142 to 191 days of ageing. Differences between P_{50} values in this study and those of Long *et al.* (2008) and Probert *et al.* (2009) may reflect the use of seed that was scarified prior to hydration.

Studies are seeking to correlate CAT data with buried packet seed longevity data (Long *et al.* 2008, Brooks *et al.* 2022). So, it is encouraging to report CAT results that are broadly consistent with local experimental data sources such as local buried packet and seedling emergence trials. Although there was some inconsistency between the mesquite CAT, buried packet trial and the seedling emergence data. The CAT provides a data point to cross reference new or unstudied weeds seed persistence with other trials.

A laboratory trial such as such the CAT, and field trials such as buried packet and seedling emergence usually provide complementary indications of weed seed longevity. Under local conditions the duration of emergence and plant absence from weed control records, may also provide land managers with interim guidance as to the length and progress of control programs, where seed production is prevented.

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