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SOIL SEED BANK LONGEVITY INFORMATION FOR WEED ERADICATION TARGET SPECIES

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ABSTRACT

The longevity of seed in the soil is a key determinant of the cost and length of weed eradication programs. Soil seed bank information and ongoing research have input into the planning and reporting of two nationally cost shared weed eradication programs based in tropical north Queensland. These eradication programs are targeting serious weeds such as Chromoleana odorata, Mikania micrantha, Miconia calvescens, Clidemia hirta and Limnocharis flava. Various methods are available for estimating soil seed persistence. Field methods to estimate the total and germinable soil seed densities include seed packet burial trials, extracting seed from field soil samples, germinating seed in field soil samples and observations from native range seed bank studies. Interrogating field control records can also indicate the length of the control and monitoring periods needed to exhaust the seed bank. Recently, laboratory tests which rapidly age seed have provided an additional indicator of relative seed persistence. Each method has its advantages, drawbacks and logistical constraints.

Keywords: Seed banks, eradication, seed packet burial, Clidemia, Miconia, Chromolaena

INTRODUCTION

Eradication or the elimination of all vegetative individuals of a weed species and exhaustion of its seed bank is the well-intentioned goal of many weed control programs, but can be difficult to attain. When all infestations and all the plants within the infestation are located and effectively treated, so that infestations are managed to prevent fresh seed input, the duration of the program is determined by the persistence of seed in the soil.

Two tropical weed eradication programs are targeting serious tropical weeds such as Siam weed (*Chromoleana odorata* (L.) King & Robinson), clidemia (*Clidemia hirta* L. D Don), limnocharis (*Limnocharis flava* Buchenau), miconia (*Miconia calvescens* DC.) and mikania vine (*Mikania micrantha* Kunth), principally in north Queensland. The Siam

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weed eradication program commenced in 1994 when this serious weed was discovered on mainland Australia. The 'Four Tropical Weeds' Eradication Program (targeting clidemia, miconia, mikania vine and limnocharis) commenced operations in late 2003 (Erbacher *et al.,* 2008). Single infestations of the shrubs *Miconia racemosa* (Aubl.) DC. and *Miconia nervosa* (J.E. Smith) Triana. were identified in north Queensland in 2002 and 2004 respectively, and are included in eradication program as they co-occur with *M. calvescens* infestations.

Both eradication programs receive funding via national cost share arrangements from the Federal, Queensland and other state governments potentially affected by the weeds. The programs are required to estimate a timeframe and resources required for eradication, and knowledge of seed persistence is an important component of such estimates. Ongoing research into seed persistence also has input into activities of and reporting for the two programs. Information on soil seed bank persistence may also help inform decisions about when infestations are declared eradicated. This paper summarises sources of seed bank information, what is known about the main target species and what are some of the constraints in investigating seed persistence. Since much of this research in ongoing, an overview of trials is presented.

Sources of Seed Bank Information

Information on soil seed bank persistence can be sought from the invasive and native ranges across of the targeted species.

Repeated collection of soil samples from within infestations is a common method for estimating rates of seed bank depletion. This process is suited to eradication programs where field crews aim to prevent fresh seed production by controlling plants. Ideally, the sampled areas should contain a high initial seed density and they need to be accessible and locatable for the duration of the sampling, which may take many years. Such studies are enhanced by data on the initial weed population and ongoing seedling emergence. Methods to estimate the total seed bank include physical (including sieving) or chemical extraction of seed from field soil samples. Soil samples can also be collected from infestations over time and kept under favourable conditions, such as in a glasshouse, to determine the germinable seed bank.

Seed can also be buried in permeable packets filled with field soil and the packets retrieved over time to test seed viability. These trials eliminate the factors of seed immigration and emigration, so depletion of a set number of seeds can be investigated (Van Mourik *et al.,* 2005). Depletion can occur through seed death or germination. Germination can hasten the decline, particularly in packets exposed to light on the soil surface. Biotic agents such as soil fauna, bacteria and

fungi may cause seed depletion, but the packets may concentrate seed, making fungal attack more likely (Van Mourik *et al.,* 2005). Seed is germinated at the commencement of packet trials to provide data on initial viability and germinability.

Both eradication programs maintain databases which record field visit information (including plant absence). To report on eradication progress each infestation or management area is allocated to a 'control' status if any plants were present at any time during the year. If no plants were found in a year then a 'monitoring' status is allocated. The time spent in the control phase provides an indication of the persistence of viable seeds over a variety of infestations. The length of the monitoring phase provides a buffer period between the control phase and declaring eradication, during which seedlings could emerge from the seed bank. Interrogating field control records can indicate the length of these phases across multiple infestations. Seed persistence can vary between populations and locations (Panetta, 2004).

Recently, a procedure undertaken in a controlled laboratory environment has provided an additional indicator of weed seed persistence (Long *et al*. 2008). The controlled aging test (CAT) determines number of days it takes for the viability of seeds to decline to 50% (P_{50}) when exposed to 65% humidity and 45^{o}C. The following seed persistence terms, P_{50} values and equivalent periods of field persistence are adapted from Long *et al.* (2008):

- Transient seed banks, P_{50}<20, field persistence <1 year,
- Short term seed banks, P_{50} = 20 to 50, field persistence 1 to 3 years,
- Long lived seed banks, P_{50} = >50 field persistence 3+ years.

Investigating the Seed Longevity of Eradication Target Species Siam Weed

Witkowski and Wilson (2001) studied increasingly older Siam weed infestations in KwaZulu-Natal and found a higher seed bank density in sunny than shaded micro-sites. In a glasshouse trial, they found that less than 9% of seeds (surface and buried at 1 cm) were viable, but failed to germinate in 12 months. Overall they concluded that; although most seeds are short lived, some buried seeds formed a persistent seed bank.

Siam weed was first discovered in 1994 near Bingil Bay on the wet tropical coast of north Queensland. It has since been found in other coastal areas of north Queensland, including the Johnstone and Tully River catchments and near Mossman. Siam weed has also been found in the drier inland Tableland areas of the upper Herbert River and west of Townsville. There have been several local investigations of soil seed bank persistence of Siam weed on the wet tropical coast. Soil samples were collected from one of the first Siam weed locations (Bingil

Bay) identified in 1994 and germinated in a glasshouse. Seedling emergence equivalent to a seed density of 9050 seeds/m^2 was recorded (M. Setter, unpublished data). Samples collected and germinated from the same area seven years later revealed 99.9% fewer seeds (12.5 seeds/m^2); during this time seed input was believed to have been prevented. No seed germinated from samples collected 12 years after discovery. In 2000, a trial in which permeable packets were filled with 50 locally obtained Siam Weed seeds and buried at 0, 2 and 10 cm was established near South Johnstone (M. Setter pers comm. 2008). In the first four years of the trial the surface-situated seed showed the greatest run down, after five years of annual retrievals 1.5% of seeds (across all depths) was viable and no viable seed was found after seven years when the final sample was taken.

There has also been interest in Siam weed seed longevity in the drier tropical Tableland and Townsville areas. As the source of these infestations is not confirmed, there may be genetic or environmental differences to the wet tropics infestations that could influence seed longevity. From 2003 annual soil samples were collected from an infestation in the Upper Herbert catchment and seedling recruitment was recorded on transects in this infestation. No seedlings emerged from the 5th year of sampling and no seedlings were noted after four years of monitoring the field transects. A buried seed longevity trial was established at Charters Towers in December 2008. Seed from a Townsville infestation was buried in fine nylon mesh packets placed at 0, 3 and 13 cm within a holed pipe (container) filled with soil. The top of the containers was covered with 30% shade cloth to contain the packets and lower the effect of sunlight on the nylon surface packets. The trial includes four soil types with grassed or bare ground sub-plots. Sufficient containers were buried to allow for six monthly retrievals for two years, annual retrievals between two and seven years and three extra retrievals if required after seven years.

Field data, field samples and a buried seed trial indicate that Siam weed develops a long lived seed bank, with most viable seed exhausted between four and eight years.

Miconia

Meyer (2010) reported on the density of Miconia seeds germinated from soil samples collected on Raiatea in 1992, 1993, 1995 and 2008. From 4500 seeds/m^2 in 1993 there was a rapid decline in germinable seed density to below 1000 seeds/m^2 in 1995; however, seeds germinated in the 2008 samples. The emergence of seedlings 16 years after the removal of locally reproductive plants indicates a very persistent seed bank. Meyer (2010) also reports soil seed densities approaching 50 000 seed/m^2 under Tahitian infestations.

Miconia has been identified at 57 locations in Queensland and New South Wales, though naturalisation has only been recorded at 28 locations (Brooks and Jeffery, 2010). Most naturalised infestations are in a control phase, some for at least 10 years. For example, following the removal of mature miconia from a small infestation near Julatten in 2001, local seedling recruitment has been observed in 2011 (K. Erbacher, Pers. Comm., 2011). Additional seed input is unlikely as field crews search hundreds of hectares annually around this location for another eradication target without finding any mature miconia (Brooks *et al*., 2009). Control records indicate that *M. nervosa* and *M. racemosa* have developed persistent seed banks (Brooks *et al.,* 2009), but no further seed studies have been undertaken and no studies of seed persistence have been identified in their native ranges.

At a large 'El Arish' miconia infestation, sampling (20 surface cores each from 1 m^2 plots replicated across six blocks) and sieving of the soil samples was conducted annually between 2004 and 2008. Samples can be taken five more times at greater than yearly intervals. There has been no decline in the number of seeds extracted (via sieving) from samples collected between 2004 and 2008, though the viability of the extracted seeds is being assessed; the seeds extracted equate to a total field density of less than 2000 seeds/m^2 (S. Brooks unpublished data). A few seedlings emerged in the sampled area annually from 2005 to 2010. The closest mature trees were removed in September 2004.

A buried seed packet trial was established in October 2010 in the area used for the wet tropics Siam weed buried packet trial at South Johnstone. Fifty miconia seeds were placed into 180 µm stainless steel mesh packets. Packets were placed a holed container filled with soil at the soil surface and buried 3 and 10 cm below the soil surface. Containers will be removed every six months for two years and annually between 2 and 16 years. The trial design allows for seven additional retrievals after 16 years if viable seed remains and the retrieval schedule can be altered during the trial. The containers were covered with 30% shade cloth to contain the packets and lower the effect of sunlight on the surface packets until grasses shaded the containers.

Clidemia

Mendes-Rodrigues *et al.* (2008) assessed the viability of four clidemia seed samples collected near Uberlandia (Brazil), two fresh collections, one sample stored under laboratory conditions for two years and one buried in permeable packets in local soil for two years. Germination from all samples was similar and high (range 87.7 to 94.2%); 93.1% of seed germinated after being recovered from the packets. In the same area of Brazil, Pereira-Diniz and Ranal (2006)

found small numbers of germinable clidemia seed at various soil depths from the surface to 30-35 cm in microhabitats of a gallery forest; concluding that species found in the deeper samples formed a persistent seed bank. In the Bragantina area of Brazil, Vieira and Proctor (2007) found clidemia seed made considerable contributions to the total soil seed bank and seed rain in a primary forest and in 5, 10 and 20 year old secondary forest plots, although clidemia was not recorded in censuses of nearby plants. Medeiros (2004) reported evidence of a widespread and persistent seed bank, when clidemia seedlings emerged in plots on Hawaii with limited seed input. Further anecdotal reports indicate that clidemia develops a seed bank that persists for at least 3 years (Medeiros, 2004) and seedling emergence may occur up to 10 years after the removal of mature plants (Smith, 1992). Overseas field observations indicate that clidemia can quickly develop a persistent seed bank in a variety of vegetation types, even when the frequency of mature plants is low.

Clidemia is known only at one location in Australia. This infestation was discovered and first controlled in 2001, with seedling recruits from an active seed bank recorded in 2011 (K. Erbacher Pers Comm., 2011). Within the core area of highest recruitment there has been limited fresh seed produced since 2004 and probably back to 2001 (Brooks *et al.,* 2009). Clidemia seed was included in the buried packet trial established with miconia seed in October 2010 and using the same methodology described above. This trial will complement field population run down data and provide information about seed persistence at recently discovered patches.

Mikania vine

It was generally thought that Mikania seeds would persist for around seven years (Brooks *et al.,* 2008); however, few published studies have been identified. Buried seed trials have not been conducted locally as seed is rarely encountered in the field and is destroyed immediately when found. A few samples have been collected in the past 13 years but their viability has been low (1 to 18%). There is also a reluctance to cultivate specimens under quarantine conditions, given the seeds are wind dispersed and known distribution in Australia is limited to 15 infestations around three locations. With few new discoveries and over half of the infestations in a monitoring phase, the eradication of this weed is going well (Brooks *et al.* 2008). Recently, Macanawai *et al.* (2010) conducted a controlled aging test (CAT) on Mikania vine seed from Fiji and found it fitted into the short term persistence category, with P_{50} = 48 days.

Limnocharis

Ortiz Domínguez and González (2001) collected soil seed bank samples from nine rice paddy areas near Calabozo, Venezuela. Some

of their samples were germinated in trays in a humid laboratory environment and some where chemically separated to determine the total seed bank. They reported that limnocharis formed a large proportion of total seeds present (55.7% of total weeds) in the chemically separated samples but a lower proportion of germinable weeds present (12% of total weeds). Ismail and Phaik-Kong (2004) did not record limnocharis plants at one of their study sites in Malaysian rice paddies, although limnocharis accounted for 4.1% of the seed bank. Across several sites limnocharis also made a significant contribution (1.9 to 16%) to the total seed bank at soil depths to 15 cm. Both the studies above indicate a greater presence of limnocharis seed in the seed bank than is evident in rice field populations.

Between 2001 and 2010, 15 naturalised populations of limnocharis (occurring in unconfined habitats such as drains and creeks) were detected on the north east coast of tropical Queensland. A further 13 infestations were plantings confined in urban water features (Brooks *et al*., 2008). A quick transition to monitoring status has been recorded amongst 11 confined infestations and three unconfined infestations.

The number of seeds extracted (sieved) from 40 soil samples collected at a limnocharis infestation in a perennial spring fed stream (near Feluga) in 2003 and annually from 2005 to 2010 is shown in Table 1. Despite no confirmed records of seed input since November 2003, viable seed was found in one of the 2010 soil samples. The silt level at the site varies and there has been year to year variation in the number of seeds. Thus it remains to be seen whether the decline in 2010 is maintained in later samples. Although the number of plants removed during control activities has declined between 2005 and 2010 (Table-1), viable seed remains at this site and indicates that limnocharis forms a long lived soil seed bank. This site is constantly wet and limnocharis depletion may be faster with annual dry periods. The quarantine risks in conducting a buried seed trial in a flowing water setting are too large, but a glasshouse immersion trial with varied periods of immersion is being planned. A germination test has been determined for limnocharis seed, but the results can be inconsistent.

Comments on the Practicalities of Estimating Seed Bank Persistence

The eradication target species present such entrenched problems across much of their invasive ranges that the focus of management is often on suppression and eradication is usually not an objective on any large scale. Studies of seed persistence seem less of an imperative for suppression campaigns, particularly for environmental weeds. However, some studies have been identified where target

weeds, such as limnocharis and mikania vine, occur in agricultural situations.

Table-1. Summary of bank seed samples collected from a Limnocharis infestation in north Queensland and approximate number of plants controlled from November 2003.

Data	2003	2005	2006	2007	2008	2009	2010
Seeds extracted from 40 samples	623	358	1252	489	530	323	8
% of samples with seeds	70.7	77.5	82	55.5	67.5	47.5	2.5
Average seeds per sample	15.2	9.0	31.3	12.2	13.3	8.1	0.4
Average seed viability (%)		64.7	54.4	59.5	80.7	57.9	100
Number of plants controlled*	397	1037	426	416	99	22	23

* 593 plants were controlled in 2004 but no soil samples were collected.

Seed bank dynamics are also studied in the neo-tropical native ranges of the target species, and several studies serve to reinforce the greater occurrence in seed bank samples than in the vegetative phase. Native range seed bank studies typically report on different habitats, vegetation assemblages or soil depths on a range of species, but over limited time frames.

When effective survey and control activities prevent seed production then field records can also provide be an indication, over a range of infestations, of the time frame over which plants establish from the seed. However, such data can be influenced by variation in past visit frequencies, data gaps or inaccuracies and changing the scale of the reporting units.

A number of buried packet trials have been established on the target species. Such trials can investigate treatments such as soil depth, different soil types and altered moisture regimes. Buried packet trials can be labour intensive to establish, but they are easy to maintain. Packet trials require sufficient flexible retrieval times and hardy permeable materials to cover the possible duration of seed persistence. The buried seed trials have been conducted on land owned by government research centres, as the monitoring and containment of target species seed is essential to the eradication programs. The piped burial containers have been used to reduce root growth into packets, making them easier to extract, and they could be relocated if the need arose. The containers also create and maintain consistent burial depths. However, increases in soil moisture in the base of the containers may also increase seed depletion rates (F. Bebawi, Pers. Comm., 2009).

Seed batches used in the recent Siam weed, miconia and clidemia buried packet trials have been set aside for a CAT. Running the CAT on the same seed lots as those used in the buried seed trial will

complement the results of field studies and assist in validating the CAT on tropical weeds. Importantly the CAT provides a standardised laboratory methodology to quickly obtain an indication of relative persistence; although there is already evidence of long lived seed banks from field data on all the target species.

Seed bank determination from field samples can be via seed extraction, typically by sieving in these cases to estimate of the total seed bank. Sieving soil samples is labour intensive and some year to year variation may result from different levels of diligence in processing the samples, particularly where minute seeds are involved. Alternatively, larger field samples are often germinated under consistent, quarantine conditions to assess the 'germinable' seed bank over time. The seed densities obtained from germinating samples may be less than the total seed bank if a proportion of the seed bank remains dormant.

For each of the eradication target species information from field populations, field soil samples and laboratory studies will continue to be collated to add to the baseline estimates of seed persistence provided by the buried packet trials. While there is already field evidence of long lived seed banks in all the target species, more precise information on seed bank persistence will help to refine estimates of the length of the eradication programs.

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REFERENCES CITED

Brooks, S.J. and M. Jeffery. 2010. Status of *Miconia calvescens* and the eradication program in Australia. *In* L.L.Loope, J.Y. Meyer, B.D. Hardesty and C.W. Smith. (eds.). Proc International Miconia Conference, Keanae, Maui, Hawaii. Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. http://www.hear.org/conferences/miconia2009/proceedings/.

Brooks, S.J., F.D. Panetta and K.E. Galway. 2008. Progress towards the eradication of mikania vine (*Mikania micrantha*) and limnocharis (*Limnocharis flava*) in northern Australia. Invasive Plant Sci. Manage. 1: 296-303.

Brooks, S.J., F. D. Panetta, and T. A. Sydes. 2009. Progress towards the eradication of three melastome shrub species from northern Australian rainforests. Plant Prot. Quart. 24(2): 72-8.

Erbacher, K., T. A. Sydes, K. E. Galway, and S. J. Brooks. 2008. The national four tropical weeds eradication program: a case study for future weed eradication projects in the wet tropics. *In* R.D. Van Klinken, V.A. Osten, F.D. Panetta and J.C. Scanlan. (eds). Proc of the 16th Australian Weeds Conference' (Qld Weeds Society, Brisbane). pp. 430-2.

Ismail, B. S. and K. Phaik-Hong. 2004. A study of weed populations and their buried seeds in the soil of MARDI Research Station and at Farmers' Rice Fields in Sungai Burung, Selangor, Malaysia. Pertanika J. Trop. Agric. Sci. 27(2): 113– 120.

Long, R.L., F. D. Panetta, K. J. Steadman, R. Probert, R. M. Bekker, S. Brooks, and S. W. Adkins. 2008. Seed persistence in the field may be predicted by laboratory-controlled aging. Weed Sci. 56: 523-8.

Macanawai, A. R., M. D. Day, T. Tumaneng-Diete, and S. W. Adkins. 2010. Some factors that may influence the invasiveness of *Mikania micrantha* Kunth. ex. H.B.K. in Fiji. *In* S.M. Zydenbos. (ed.). Proc. 17th Australasian Weeds Conf.. Christchurch, New Zealand. New Zealand Plant Protection Society, pp. 95-8.

Medeiros. A.C. 2004. Phenology, reproductive potential, seed dispersal and predation and seedling establishment of three invasive plant species in a Hawaiian rain forest. Ph.D. Thesis, Department of Zoology, University of Hawaii at Manoa, Honolulu.

Mendes-Rodrigues, C., M. Ranal, and P. E. Oliveira. 2008. Could seed dormancy and polyembryony explain the success of *Clidemia hirta*? IX Simposio Nacional Cerrado, II Simposio Internacional Savanas Tropicais. (ParlaMundi, Brasilia).

Meyer, J. Y. 2010. The *Miconia* saga: 20 years of study and control in French Polynesia (1988-2008) *In* L. L. Loope, J.Y. Meyer, B.D. Hardesty and C.W. Smith. (eds.). Proc. International Miconia Conf., Keanae, Maui, Hawaii. Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa.http://www.hear.org/conferences/miconia2009/proceedings/.

Ortiz Domínguez, A. and L. González. 2001. Preliminary study of weed seed banks of certain soils of rice areas of Calabozo, Guárico. *Agronomía Tropical* (Maracay), 51/4, 501-517. In Spanish with English summary.

Panetta, F.D. 2004. Seed banks: the bane of the weed eradicator. *In* B.M. Sindel and S.B. Johnson. Proc. 14th Australian Weeds

Conf., Weed Society of New South Wales, Wagga Wagga, New South Wales, Australia. pp. 523–526.

Pereira-Diniz and M. Ranal. 2006. Germinable soil seed bank of a gallery forest in razilian Cerrado. Plant Ecol. 183: 337–348.

Smith, C.W. 1992. Distribution, status, phenology, rate of spread and management of *Clidemia* in Hawai'i. *In* C.W. Smith, C.P. Stone, and J.T. Tunison. (eds.) *Alien plant invasions in native ecosystems in Hawaii*. (University of Hawaii. Honolulu). pp 241-253.

Van Mourik, T.A., T. J. Stomph, and A. J. Murdoch. 2005. Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks. J. Appl. Ecol. 42: 299–305.

Witkowski, E. T. F. and M. Wilson. 2001. Changes in density, biomass, seed production and soil seed banks of the non-native invasive plant, *Chromolaena odorata,* along a 15 year chronosequence. Plant Ecol. 152: 13–27.

Vieira, I.C.G. and J. Proctor. 2007. Mechanisms of plant regeneration during succession after shifting cultivation in eastern Amazonia. Plant Ecol. 192: 303-315.