Issues and solutions for researching weed eradication target species

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Summary Species biology drives the frequency, duration and extent of survey and control activities in weed eradication programs. Researching the key biological characters can be difficult when plants occur at limited locations and are controlled immediately by field crews who are dedicated to preventing reproduction. Within the National Four Tropical Weeds Eradication Program and the former National Siam Weed Eradication Program, key information needed by the eradication teams has been obtained through a combination of field, glasshouse and laboratory studies without jeopardising the eradication objective. Information gained on seed longevity, age to reproductive maturity, dispersal and control options has been used to direct survey and control activities. Planned and opportunistic data collections will continue to provide biological information to refine eradication activities.

Keywords Miconia, clidemia, limnocharis, mikania, chromolaena, seed banks, phenology.

INTRODUCTION

The current National Four Tropical Weeds Eradication Program (NFTWEP) commenced field operations in 2003 (Erbacher *et al.* 2008). The species targeted are: *Clidemia hirta* (L.) D.Don; *Limnocharis flava* (L.) Buchenau.; *Miconia calvescens* DC; *Miconia nervosa* (Sm.) Triana; *Miconia racemosa* (Aubl.) DC; and *Mikania micrantha* Kunth. The former National Siam Weed Eradication Program (NSWEP) targeted *Chromolaena odorata* (L.) R.M.King & H.Rob and ran between 1997 and 2012 (Jeffery 2012). The multi-stemmed scrambling shrub *C. odorata* and the rampant vine *M. micrantha* are in the Asteraceae family. *Limnocharis flava* is an anchored aquatic herb from the Limnocharitaceae or Alismataceae (Weber and Brooks 2013). The remaining species are in the Melastomataceae family and are bird dispersed shade-tolerant shrubs, except for *M. calvescens* which grows to a small tree. All species are native to tropical and subtropical areas of Central and South America and pose serious threats to tropical environments, agriculture and tourism.

Seed longevity, dispersal mechanisms and time to, and season of maturity are aspects of species biology that guide eradication survey and control activities (Jeffery 2012). This paper presents an overview of research that has been integrated with field operations, presented to provide insights for researchers involved in weed eradication programs.

THE NEED FOR SEED

Obtaining suitable quantities of viable seed may well be the bane of the eradication researcher, for this is a pre-requisite for many other trials. Ideally seed would be collected from multiple parent plants in different populations, to ensure some genetic diversity but this is usually impossible. Field crews remove all plants and potentially reproductive material when encountered in the field. So any fruit for research is sporadically collected, at whatever stage it is found from a few plants, within the local incursion. Seed was also harvested from cultivated *M. racemosa*, *C. hirta* and *L. flava* plants in locked enclosed shade tunnels or quarantine glasshouses. However, the only local collection of *M. nervosa* seed was from 99 berries from six field plants found in December 2012. For the wind dispersed Asteraceae and the slower maturing *M. calvescens* (4+ years) we were reliant on field collected seed.

Germinate it When the program commenced there were no known published germination tests for any of the NFTWEP species. Thankfully, all species except for *L. flava* readily germinate in Petri dishes under a standard tropical 30/20°C, 12/12 h, day/night incubator regime. More than 80% of fresh and stored Melastome seed routinely germinates. Methods for germinating *L. flava* in centrifuge tubes are discussed in Weber and Brooks (2013); germination can be variable but overall viability is usually consistent.

Count, photograph and store it Seed numbers per fruit provides base-line information for population and dispersal modelling, particularly where researchers are unable to collect reproductive data from multiple mature plants. For example, local *C. hirta* fruit contains an average of 801 seeds per fruit, but there is variation between samples from different areas (Breaden *et al*. 2012). To extract seed from soil seed banks it has to be identifiable, so new seeds are photographed and measured. Air dried and sorted seed retains viability for future tests, for years in a fridge at 3°C.

HOW LONG DOES THE SEED LAST?

Bury seed Once sufficient viable seed has been obtained, the highest research priority is to investigate longevity. There are various sources of seed longevity information for the target species (Brooks and Setter 2012), with long-term buried seed packet being the more definitive trials. Packets of seed are regularly retrieved and remaining seed sorted and germinated. Retrieval schedules are usually six monthly for the first two years, then annually for 7 to 16 years with a few extras in case any buried seed remains viable. Long-term experiments are planned with options to space out the last samples over two years should the need arise. The buried packet trials involving Melastome species (Table 1) have up to 24 retrieval times and use 15,000 seeds per species; access to a secure, uniform location for trial duration is needed.

Seed bank densities Three sites where field soil has been sampled, sieved for seed and any intact seed germinated are listed in Table 1; this is a very labour intensive method. The *M. calvescens* site has been difficult to access due to tree trunks suspended above the sample area after cyclone Yasi. Until 2007 the data reflected a low and variable seed bank and the ten retrieval times were insufficient for this species.

The *C. hirta* field sample area had a high initial seed bank density, although seeds from the individual cores and totals from plots can still vary substantially within a metre or two. This small area had over 20 large mature plants on discovery, which created a high seed bank density (and a useful research site), but is not typical of the entire infestation. Viable seed and seedlings are still being recorded at the *L. flava* seed bank sample site, 11 years after the last recorded seed input (Weber and Brooks 2013). The annual amount of the seed retrieved varies considerably. The period of seedling emergence at this site has not yet been matched at other infestations. Ultimately the *L. flava* and *C. hirta* seed sample areas may end up being outlying cases around which biological and management assumptions are made. Hence, there is some risk in applying the findings to the entire incursion. However, this risk is unavoidable given the resources needed to process field samples for many years, a preference for a high initial seed density and the limited available sites.

HOW FAR DO WE NEED TO SEARCH?

It is impossible to measure the seed dispersal of target species in the field. However, search areas are based on potential dispersal buffers

Float and drop seed *Clidemia hirta, M. calvescens*, *M. micrantha*, *L. flava* and *C. odorata* seeds have been immersed in fresh and salt water. The buoyancy of seed of all target species and *L. flava* fruit has also been tested. *Miconia calvescens*, *M. micrantha*, *L. flava* and *C. odorata* seed have all been germinated in Petri dishes with increasing levels of salinity. Individual seeds from the Asteraceae species have also been measured, timed dropping from two metres to determine terminal velocity and germinated. These trials will provide baseline information for dispersal and habitat suitability to refine survey buffers.

Table 1. Overview of current soil seed-bank persistence trials and retrieval schedules.

Trial type	Species	Location	Establish date or first field sample	Next scheduled retrieval	Next sample number
Buried packet	*C. hirta*	South Johnstone	Oct 2010	Oct 2014	6 of 24
Buried packet	*M. calvescens*	South Johnstone	Oct 2010	Oct 2014	6 of 24
Buried packet	*M. micrantha*	South Johnstone	Oct 2011	Oct 2014	5 of 12
Buried packet	*M. nervosa*	South Johnstone	Oct 2013	Oct 2014	2 of 24
Buried packet with soil and cover factors	*C. odorata*	Charters Towers	Dec 2009	Dec 2014	7 of 11
Buried packet with immersion factors	*L. flava*	Charters Towers (glasshouse)	Feb 2012	Feb 2015	3 of 20
Field seed bank density	*L. flava*	Feluga	August 2003	Aug 2014	11
Field seed bank density	*M. calvescens*	El Arish	Sept 2004	NA	6 of 10
Field seed bank density	*C. hirta*	Julatten	June 2012	Sept 2014	5 of 24

Frugivore dispersal It is not possible to retain Melastome fruit on field plants to record frugivore behaviour. However, the use of analogous fruit size categories in combination with data on fruit types, net bird movements and gut passage times were used to model the potential dispersal of *M. calvescens* (Murphy *et al.* 2010) and *C. hirta* (Breaden *et al*. 2012).

WHEN DOES IT MATURE?

The length of the juvenile phase and the timing of fruit production of each species are key biological drivers of eradication team's survey activities. Reproductive escapes also contribute to the soil seed bank, and are reported annually to funding providers. Seeding plants are used to generate search buffers based on dispersal potential.

Retained plants The regular tagging of *L. flava* seedlings in the field was combined with glasshouse observations to determine the shortest and mean times to seed production to determine resurvey frequency (Brooks *et al*. 2008). Since the survey frequency was lowered to every 4 weeks in 2007 based on the research findings, there have only been four possible cases (two confirmed) of seed production in over 500 field visits to known infestations (S Brooks, unpublished data).

In a similar field trial, *C. hirta* seedlings were regularly tagged and followed to flowering. This field data combined with glasshouse fruiting data showed *C. hirta* could produce mature fruit in 365 days (Graham and Setter 2007). In field trials of *C. hirta* and *L. flava*, there was little indication of seasonality in the flowering behaviour, hence seed could be produced at any time of the year (Brooks *et al.* 2008, Breaden *et al.* 2012) and surveys are needed throughout the year.

By retaining some *M. calvescens* plants *in situ* instead of destroying them immediately, several years of reproductive observations were collated by enclosing panicles in fine mesh bags (Murphy and Brooks 2010). This trial showed that fruit production was concentrated mid year which is different to observations of flowering behaviour overseas (Murphy and Brooks 2010). Measurements from *M. calvescens* growth plots pushed the annual survey frequency out to 18–24 months (K Galway, pers. comm. 2007).

Where plants are retained in the field, sites have to be accessible for monitoring at regular intervals in any season. Access issues prevented the establishment of *M. nervosa* field growth plots. With the exception of one *M. calvescens* trial, all field retained plants have been removed upon flowering, with any fruit observations conducted weekly under controlled conditions.

Pot trials Pot trials have been established in which seedlings are raised at monthly intervals for a year and grown under ideal watering conditions until they flower. These trials have confirmed that the Asteraceae species first commence flowering in late April or early May, and take a minimum of around four months to mature. Plants younger than four months commence flowering at the same time, the following year. Surveys concentrated between February and June aim to prevent seed production. This pattern is consistent with field records.

Field crew data An essential component of decisions about mature plant occurrences is that the size, date, location and observations of reproductive behaviour are systematically recorded by field crews and entered in a database. Monthly records of flowering and seeding have been maintained for all species to verify the seasonality patterns. There is no single size at which Melastome species are mature, but all field records are cross referenced with data from the other sources to determine if field locations need to be buffered for searching. In the case of *M. nervosa*, field crew data on mature plant sizes is the only source of mature plant data.

WHAT ELSE HAPPENS IN THE FIELD?

Control measures Eradication programs rely on implementing effective control measures. There are currently no significant issues in treating the NFT-WEP species, but undertaking trials can be problematic when plants are rare. For example, stakeholders enquired about alternative *M. micrantha* herbicides and it took three years until a sufficient quantity of accessible immature vines was located to conduct a small field trial.

Opportunities All field trials have resulted from field crews locating new infestations either before or shortly after control activities. This has led to the collection of diameters and heights on over 1000 *M. calvescens* at a new field site in September 2004, with this information since used in several models (see Murphy and Brooks 2010). Population, vegetative, bud and fruiting data was also obtained from a newly discovered *L. flava* infestation in 2008 (Weber and Brooks 2013).

Spread prevention Field research areas are usually small, well known to crews and clearly marked to limit accidental removal during control activities. It is also essential that researchers conduct the same spread prevention measures as field crews (Bocking *et al.* 2008) and procedures are in place to prevent

the spread of reproductive material from glasshouses, laboratories, or in transit.

OVERALL

Researchers working on the target species have successfully developed approaches to investigate most of the relevant biological attributes of the target species. Information on soil seed bank longevity is important for determining the potential duration of an eradication program, but can be difficult to determine due to a lack of seed. This has been overcome by obtaining fruits from reproductive plants encountered and controlled in the field or cultivating herbaceous plants under quarantine conditions. After developing consistent germination tests, research including buried seed longevity trials, estimating viable seed densities from field samples and assessment of fruit and seeds dispersal abilities have also been conducted.

Through field data collection by operational staff, the retention of field plots or growing plants under controlled conditions, information on growth rates, age to maturity, size at maturity and seasonal fruit production has been compiled and used to inform search activities. Programs budgets are built around the cumulative survey area for each species. This is a function of search buffers, based on seeding plant occurrences and dispersal potential and the survey frequency, based on age to maturity and seasonal trends.

Despite the limitations posed by researching rare weeds, a variety of techniques has provided a scientific understanding of the target species to underpin decisions about the programs search and control activities.

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