The effects of burial depth and water stress on Melastome weed seeds

Simon J. Brooks[1], Rose K. Easton[2] and Kirsty L. Gough[1]
[1] Tropical Weeds Research Centre, Department of Agriculture and Fisheries, PO Box 187, Charters Towers, Queensland 4820, Australia
[2] School of Earth and Environmental Sciences, University of Queensland, St Lucia, Queensland 4702, Australia
(simon.brooks@daf.qld.gov.au)

Summary Two seed trials were conducted to provide insight into the conditions under which high priority tropical rainforest weeds from the family Melastomataceae can establish. Seeds of *Clidemia hirta*, *Miconia calvescens*, *Miconia nervosa* and *Miconia racemosa* were placed at depths of 0, 2, 5, 9, 14 and 20 mm in small containers and incubated. Emergence declined with depth, with no emergence from 9 mm depth or greater. Seeds of the same species were also subjected to moisture stress in incubated Petri dishes, via exposure to water potentials (stress) between 0 and −1 MPa. Despite their different native and invasive home ranges seeds of the *Miconia* species germinated at relatively low water potentials between 0 and −0.33 MPa. In comparison, *Clidemia hirta* seed germinated under higher moisture stress at −0.5 and −0.67 MPa. These weeds form persistent soil seed banks which may need managing for decades. Management actions and potential impacts can be informed by understanding the factors influencing their establishment from seed.

Keywords Eradication, polyethylene glycol, rainforest.

INTRODUCTION

Eradication programs are seeking to manage the soil seed bank by preventing additions and running down the viable seed to extinction. Yet the processes governing seed germination and seedling emergence are rarely documented for species invading tropical rainforest, though some are implied from native and invasive geographical extents. Estimates of the potential distribution and impacts of these species can also be based on their native and invasive ranges. However, the Melastomataceae species used in these trials have different native and introduced geographical ranges (Table 1). The infestations of *Miconia* species are the targets of a nationally cost-shared 'National Tropical Weeds Eradication Program' (NTWEP) (Jeffery and Brooks 2016). *Clidemia hirta* was included in the NTWEP until the discovery of the second local infestation in 2015 (Brooks *et al.* 2016).

Table 1. Local infestations, broad native and introduced ranges and seed lot sources of species tested.

Species and life form	Australian extent and discovery*	Other invasive range	Native range**	Seed sources used in trials
Clidemia hirta (L.) D. Don (shrub)	2 infestations Julatten (2001), Misty Mountain (2015)	Many tropical and subtropical countries and islands	Widespread, Central and South America and the Caribbean, 23° N to 27° S	Fresh glasshouse May 2016 (originally Julatten), field Misty Mountain July 2015
Miconia calvescens DC. (small tree)	Multiple infestations in north Qld and NSW (1997–2014)	Widespread mostly on tropical islands	Common across Central and eastern South America, 17° N to 25° S	Field, Mossman area (July 2009)
Miconia nervosa (J.E Smith) Triana (shrub)	1 infestation Whyanbeel (2004)	None known outside South America	Widespread Central and South America. 18° N and 18° S	Field, Whyanbeel (December 2012)
Miconia racemosa (Aubl.) (shrub)	1 infestation Kuranda (2001)	None known	Limited, Caribbean and north west coast of South America 19° N to 2° S	Shade house plants from Kuranda (February 2013)

*Information from Jeffery and Brooks (2016), Brooks *et al.* (2016), references there-in and unpublished.
**Native range information is inferred from maps of herbarium specimens held by the Missouri and New York Botanical Gardens.

Past seed research Seeds of all species are less than 250 µm long and experiments on the same Melastome seed lots (Table 1) have shown that seeds only start to germinate after two or three weeks, and may take eight weeks to cease germinating (S. Brooks unpubl. data). Although slow, 95 to 100 % of the viable seed germinates, so there is little evidence of physiological dormancy under ideal incubator conditions. Buried seed packet trials on these four species are now three to seven years old, both the buried packet trials and local field control records indicate that buried seeds can persist in the soil for many years (Brooks and Setter 2014).

To explain the occurrence of persistent but non-dormant seeds, studies have suggested that small seeded tropical pioneer and early successional species have 'risk averse' seeds (Daws *et al*. 2008, Silveira *et al.* 2013). Where seeds only germinate when conditions are suitable for an extended period and the chance of survival is high. Such species have small seed reserves to draw on and with superficial surface roots seedlings are also thought to be susceptible to short periods of drought (Pearson *et al*. 2002 (citing Engelbrecht *et al.* 2001)). The *Miconia* species are targeted for eradication, and progress towards their eradication is made when absence records accrue annually under climatically suitable conditions. The local tropical climate is presumably suitable for germination during wetter periods of most years, however the germination requirements of each of these species have not been documented. As moisture levels have an influence on the germination and survival of seedlings, a trial to investigate the effects of declining water potentials (increasing moisture stress) on seed germination was conducted.

Seed burial depth could also influence the establishment and control of Melastome seedlings. The emergence of *Miconia argentea* (Sw.) DC seedlings dropped dramatically from 0 to 2.5 and 5 mm and was not recorded at soil depths of 10 mm or deeper; further, *M. argentea* did not germinate under dark conditions (Pearson *et al*. 2002). A second trial was conducted to determine the effect of burial depth on the emergence of seedlings.

METHODS

Seed germination Seed sources and ages are listed (Table 1). The irregular availability of field seed collections is a limitation of researching eradication target species (Brooks and Setter 2016). Since collection, the seed has was air dried, removed from fruit and stored in a refrigerator at 3°C. Each experimental unit (Petri dish or aluminium tray) was labelled, placed on larger trays which were enclosed in a clear plastic bag and placed in a Thermoline® incubator running a 30/20°C, 12h/12h, day/night temperature regime for the duration of the trials. Trials commenced in September 2016.

Water stress experimental treatments A laboratory trial was conducted on the same seed lots (Table 1), except the *C. hirta* seed was sourced from Misty Mountains. This trial investigated the effect of moisture stress on seed germination. Petri dishes with 50 seeds of each seed lot were treated with a poly-ethylene glycol (PEG) solution. The equation of Michel (1983) was used to determine the amount of PEG 8000 (Fisher Scientific) needed to create solutions with water potentials of −0.08, −0.17, −0.33, −0.5, −0.67, −0.83 and −1 MPa. Each treatment × seed lot combination was replicated three times. Each dish contained 2 × Whatman No 1, 90 mm filter papers and was placed in plastic bags and incubated for eight weeks. Germination (production of a radicle) was recorded every 2 to 7 days for 12 weeks with the germinated seeds removed and counted, dishes and topped up with water as required. Un-germinated seeds were subjected to physical tests of viability.

Depth experimental treatments Melastome seeds were placed at soil depths of 0, 2, 5, 9, 14 and 20 mm and seedling emergence recorded. In preparation for the trial, several kilograms of a locally obtained (commercial) predominantly clay (+ organic/sand) soil mix was passed through a 1 mm sieve and baked for 120 hours at 70°C. Three 250 mL samples of this soil were weighed, placed in nursery tube pots with a small base lining of weed mat, weighed again and immersed in water overnight. After immersion the tubes were removed from the water, covered with a plastic bag and allowed to drain for three days, and reweighed. The weights were used to calculate the soils bulk density (dry weight of a known volume), the field capacity and the wet weight of a known volume. The volume and weight of soil when saturated was used to maintain the weights of the experimental units (aluminium trays) near field capacity for the duration of the experiment and to prevent waterlogging the different volumes of soil.

The trial was conducted in small round flat-bottomed aluminium trays (weigh boats, generic brand from Proscitech Townsville), 75 mm diameter and 32 mm deep. Each depth and seed lot treatment was replicated three times in separate trays. The base of each tray was covered with 10 mm of soil. A 50 mm diameter filter paper was placed in the middle on the base layer of soil and one lot of 50 seeds were placed on the middle of the filter paper. To create the 2, 5, 9, 14 and 20 mm depth treatments, prepared dry soil was carefully added on top of each filter paper and seed layer. Each tray was weighed with dry soil and bought

up to the field capacity weight with distilled water. During the trial the trays were watered to avoid disturbing the soil above the seed and to keep trays close to the target wet weight. The trial ran for 58 weeks during which the trays were allowed to dry out between weeks 13 and 18, before being re-wet. Seedling emergence was recorded every 2 to 7 days and seedlings removed.

Statistics Regression equations were fitted to the untransformed germination or total emergence data for each treatment using Genstat® 16th edition VSN International Ltd. A Poisson distribution with a log link function fitted the emergence depth data, however the mean data is shown in the results. Logistic non-linear regression curves were fitted to mean germination data for each seed lot in the water stress trial.

RESULTS

Water stress experiment The most marked comparison between the two genera represented is at −0.5 MPa where 0% of the *M. calvescens* and *M. nervosa* germinated, 1.3% (2 seeds) of the *M. racemosa* germinated and 81.3% of the *C. hirta* germinated (Figure 1). With 41.3% germination at −0.67 MPa *C. hirta* seed is more tolerant of moisture stress conditions than the three *Miconia* species. Despite their different native and invasive ranges the three *Miconia* species showed a similar moisture stress threshold with no germination lower than −0.5 MPa. The viability of the *M. calvescens* seed may have been influenced by the age of the seed.

Depth experiment Seedling emergence declined from unburied seed to a depth of 5 mm and no seedlings emerged from the 9, 14 or 20 mm treatments (Figure 2). There was some variation in the 2 and 5 mm data due to emergence near cracks that appeared in the clay soil. The variability of emergence of the

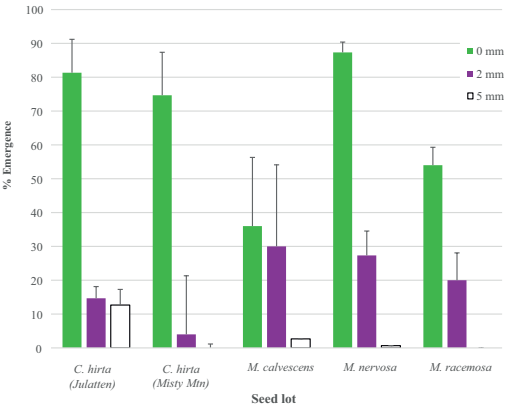

Figure 2. Mean emergence of different Melastome seed lots (+ SE) from burial under 0, 2 and 5 mm of soil. 9, 14 and 20 mm results not shown, all = 0.

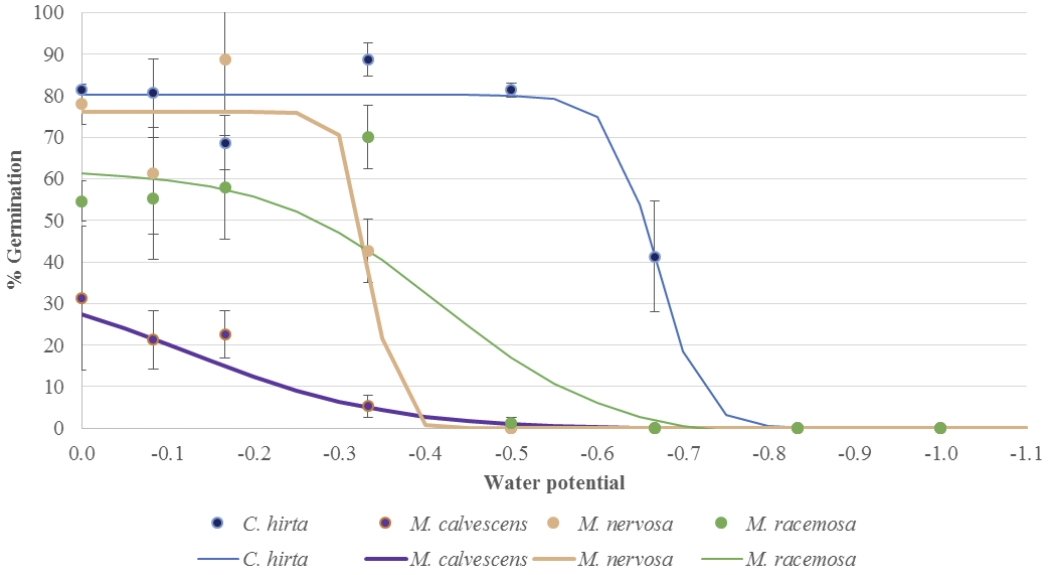

Figure 1. Mean germination of Melastome seed lots (± SE) exposed to levels of decreasing water potential. Points are species means of three replicates for each treatment. Lines are logistic curves plotted from parameters (not shown) estimated in Genstat.

M. calvescens seed was thought to be as a result of the age of the seed.

DISCUSSION

The depth experiment had similar results to those of Pearson *et al.* (2002) and showed that small seeded Melastomes will only emerge if seed is near the soil surface. This is consistent with studies (Pearson *et al*. 2002, Silveira *et al.* 2013) that have identified litter gaps, a light requirement and shallow depth as conditions under which species with small seed reserves will establish. Similarly, seed that is incorporated into the soil profile is unlikely to germinate but some will remain viable and create a persistent buried soil seed bank. If there are events that erode or expose soils with buried seed, such as uprooted trees or flood water, then seedlings could emerge and require control. Following these events the emergence of *Miconia* seedlings after a long period of plant absence could influence the progress of the eradication program.

As the species appear to lack any physiological dormancy, they are unlikely to respond to germination stimulants. However, there may be physical or chemical methods that deplete the emergent portion of the seed bank depth in the top centimetre of the soil. Seed bank depletion treatments applied to the top soil layer may assist in reducing the length and cost of long term control operations and the risk of plants escaping detection and producing fruit. Methods such as flame weeding could be applied to small dense areas of recruitment or around isolated mature plants expediting the transition to a state where plants are continually absent. Further research on the depth profile in the soil seed banks of the *Miconia* species is being planned. A test of light and dark germination requirements may assist in explaining the results of the depth trial.

Eco-physiological data can be used to refine or corroborate climatic parameters used to predict the potential distributions of invasive species (Kriticos *et al.* 2005). The three melastome shrub species have limited local distributions on which to base estimates of potential distribution, impacts and cost-share apportionment. Information to predict the invasive potential of *M. racemosa* is limited by narrow climatic ranges and such information could greatly under-estimate its potential distribution. Despite their different native and invasive ranges, the three *Miconia* species showed a similar threshold of moisture stress and higher moisture requirements for germination than *C. hirta*. A greater tolerance of lower moisture levels could assist in explaining the wider native range and indicate a greater potential invasive extent of *C. hirta*. The moisture trial was an important step in defining the germination requirements of each species. This trial will be combined with current temperature gradient studies to create a fuller picture of each species germination requirements.

The combination of these two trials with age to maturity trials (Brooks and Setter 2014) should provide a deeper understanding of field emergence patterns under local conditions. This information helps to build the biological profile, including potential impacts, for those species with limited known invasive ranges and local distributions while improving the management of these high priority invasive tropical Melastomes.

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