Age and size of flowering *Mikania micrantha* plants raised in a controlled environment

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Summary *Mikania micrantha* is a rampant vine and target of a national cost-shared weed eradication program on mainland, Australia. A series of trials were conducted in a quarantine glasshouse to inform the surveillance activities of this serious weed. The trials aimed to determine the time taken for *M. micrantha* to grow from seed or fresh cuttings to flowering in a glasshouse. They also investigated seasonal patterns of flowering behaviour and documented plant sizes at first flowering. Distinct seasonality is reflected in the trial results and field records of mature plants from control teams. *Mikania micrantha* grows within and on the margins of tropical forests. The minimum size of plants when they first flowered is considered in relation to detecting vines in this complex tropical environment.

Keywords Mikania, eradication, maturity, tropics.

INTRODUCTION

Mikania micrantha Kunth (Mikania vine) is a rampant, smothering tropical weed, readily capable of vegetative dispersal and seed dispersal by wind, water, or machinery. It is one of the most serious weeds across tropical Asia, the Indian sub-continent, and Pacific regions (Day *et al.* 2016). *Mikania micrantha* was first discovered near Mission Beach in north Queensland and included in the nationally cost-shared 'National Tropical Weeds Eradication Program' (NTWEP). An update on the progress towards the eradication of *M. micrantha* from mainland Australia was presented by Brooks and Jeffery (2018b). *Mikania micrantha* was also discovered near Forrest Beach, Ingham, Bingil Bay and Speewah, but current active infestations are near Mission Beach and Bingil Bay.

Weed eradication programs conduct surveys to prevent the occurrence of mature plants and dispersal events, and to deplete the soil seed bank (Brooks and Setter 2014a). The rate and time at which eradication teams revisit infestations is driven by the need to detect seedlings in the juvenile life phase. Field research on mature eradication target species can be impractical (Brooks and Setter 2014a). So, trials are often limited to quarantined environments where ideal growth conditions provide information on the minimum time to, and size of, mature plants. The first flowering behaviour of *M. micrantha* was investigated by raising plants from seed and cuttings at regular intervals over three years.

GLASSHOUSE TRIAL METHODS

The pot trials were on plants raised under ideal conditions inside a quarantine glasshouse at Charters Towers. Temperatures are a minimum 20 ºC night and 25 ºC day, although with evaporative cooling the day temperature may go up to 35 deg C. *Mikania micrantha* readily propagates vegetatively, with plants readily establishing from double node cuttings (Macanawi *et al.* 2011).

Treatment timing In trial one, six pots were established per month for 12 months between the 1/8/2012 and 1/7/2013; three pots were from cuttings and three from seed. This is the only trial that raised seedlings from seed. In trial two, six pots of cuttings were established at two- or four-week intervals between 20/10/2014 and 30/1/2015. In trial three, six pots were established from cuttings fortnightly from the 10/11/2015 until the 2/2/2016.

Establishment from seed In trial one, every 30-31 days for a year, 5 seeds were germinated in each of 10 peat jiffy pots in a Thermoline® incubator for 28 days. Seeds were randomly taken from a pool of 2000+ seeds collected near Mission Beach in 2011. Seedlings were transplanted into individual tube pots (1-part vermiculite +1-part sand +2-parts peat) standing in shallow water containers in the glasshouse for 16 days. The largest 9 seedlings were distributed between 3 x 29cm pots each month. Seedlings were thinned after a further 30 days to keep only the largest seedling per pot.

From cuttings Twelve source plants were cultivated from the same seed source as is used for the 'seed raised' plants above. Stem cuttings (20) with 2 nodes were taken at the same time as the seeds (trial 1 above) were germinated. Cuttings were planted in constantly wet tube pots containing the same mix as the seed tube pots and grown for 44 days. Then 3 cuttings from these pots per block per month were planted in the larger 29 cm diameter pots. Cuttings were thinned to keep the largest plant after

30 days. This process was repeated for trials 2 and 3 at the treatment timing mentioned above.

Growing conditions Pots and soil were steam treated prior to the trial commencing. The 29 cm pots were filled with local garden soil mix (2-parts organic garden soil + 2-parts bulk clay soil + 1-part sand), with a layer of weed mat at the base. Five grams of a slow-release fertilizer was added to each pot on day 74 when the thinning was complete. All pots in trials one and two were standing in large troughs of approximately 5 cm deep tap water. Pots were added to the water pool 48 hours before the seedlings and cuttings were planted. Pots used in trial 3 were regularly watered for 2 minutes every 6 hours with 2 irrigation drippers flowing at 6 L per hour, which commenced 48 hours before the cuttings were added. Pots were spaced to mitigate any neighbour shade effects from the oldest plants, and vines were trained up 3m bamboo poles to limit interference between pots and to assist with growth measures.

Measurements Plant leader length was recorded when seedlings were transferred to pots and at thinning. Weekly checks of flowering bud presence or absence were conducted. Monthly data of maximum plant height (longest leader length), stem diameter at ground level and number of distinct leaders were conducted but the interim growth data is not shown. Height data was not collected once the leaders reached 4 m tall and became inter-twined at the top of the glasshouse. Length of flowering leader (4 m or less), date of first flowers and basal diameter at flowering were recorded. Plants were destructively harvested four weeks after flowering was observed. There was no attempt made to record the time of seed viability as plants are insect pollinated (Day *et al*. 2016). Additional growth data, wet and dry biomass (stem, leaf and flower) at harvesting and flower production data was collected but is not reported here. Soil and all biomass samples were disposed of by deep burial on site after baking at 80+^{o}C for 48+ hours.

GLASSHOUSE TRIAL RESULTS

Trial 1 All the plants that flowered were from the first six treatments established between the 1/8/12 and 31/12/12 at a minimum of 130 days (Table 1). Flowering was observed between 29/4/13 and 21/6/13. Sixteen flowering plants had reached 4 m tall and the roof of the glasshouse, the remaining 4 were at least 3 m tall. The flowering plants tended to have larger diameters than the non-flowering plants in the same cohort. However, diameter was related to age and no threshold size for flowering was evident in the data.

There were fewer flowering plants from seed source, and they tended to be smaller but still at least 3 m tall. The plants from cuttings were taller when thinned at day 74, but the seed raised plants had similar heights when flowering (Table 1). The sample of flowering plants was less than expected in trial 1 and statistical comparisons of seed and cutting plant sources were not conducted.

Plants from treatment 7 established on 30/1/13, were between 92 and 156 cm tall with diameters less than 0.36 cm by late April 2013. Plants from treatment 8 (established 28/2/13,) were under 1 m tall and not thinned when most of the earlier plants started flowering in mid-May. Plants established in treatments 9 to 12 (1/4/13, 1/5/13, 31/5/13 and 1/7/13) did not flower until April 2014.

Plants that did not flower between late-April and June 2013 continued to grow and commenced flowering in April 2014, when the vines had become intertwined and per plant data was not collected at harvest time.

Table 1. Establishment dates and first flowering times and sizes of *Mikania micrantha* raised in trial 1 and flowering in 2013. Maximum number of plants flowering is 3.

N	Establish date	Plant source	Number flowering	First flowering date	Days to flowering	Height range (cm)	Mean stem diameter (cm)
1	1/8/2012	cuttings	1	17/5/13	289	400	1.05
		seed	1	10/5/13	282	400	1.29
2	31/8/12	cuttings	3	17/5/13	259-280	400	1.15
		seed	2	17/5/13	259	350-400	1.04
3	2/10/2012	cuttings	3	10/5/13	220-227	350-400	1.10
		seed	1	10/5/13	220	400	0.85
4	31/10/2012	cuttings	3	29/4/13	180-198	400	0.78
		seed	1	21/6/13	233	400	0.84
5	30/11/2012	cutting	1	21/6/13	203	400	1.08
		seed	1	17/5/13	168	400	0.71
6	31/12/2012	cuttings	2	10/5/13	130-165	300-400	0.85
		seed	1	17/5/13	137	300	0.64

Trial 2 Growth and establishment in trial 2 was poorer than the other trials and led to the decision to use drippers in trial 3. One of six plants established on 20/10/2014 flowered after 203 days on the 11/5/15. Four of 6 plants established on the 19/11/14 flowered 159 to 176 days later starting on the 27/4/14. One of the 6 plants established on 19/12/14 flowered after 146 days on 14/5/15. None of the plants established on the 2/1/15, 16/1/15 or 30/1/15 flowered. Heights at flowering ranged between 167 and 350 cm. Plants that survived and did not flower were harvested at an age of 300 days.

Trial 3 Growth and flowering behaviour was more consistent in this trial (Table 2), and all plants survived to flowering or destruction. Across the first 5 fortnightly establishment dates the plants flowered at an earlier age and smaller size (Table 2). The minimum was for plants established on the 5/1/16 which all flowered after 112 days on the 26/4/16 and less than 3.2 m tall. Subsequent treatments established on the 19/1/16 (6 plants) and 2/2/16 (2 plants) flowered late May and early June and had larger heights and diameters than treatment 5 (5/1/16). There were 14 cuttings struck in 2016 that flowered in the same year. Plants that did not flower in April to June 2016 were destructively harvested at an age of 300 days.

Table 2. Establishment dates and first flowering times and sizes of *Mikania micrantha* raised in trial 3 from cuttings and flowering in 2016. Maximum number of flowering plants is 6.

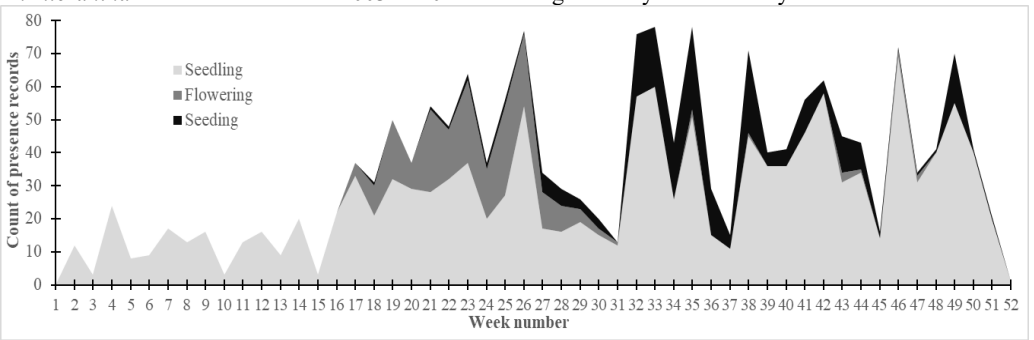
N	Establish date	Number flowering	First flowering date	Days to flowering	Height range (cm)	Mean diameter (cm)
1	10/11/15	3	2/5/16	174-211	400	1.13
2	24/11/15	5	18/4/16	146-161	400	0.87
3	8/12/15	6	18/4/16	132-143	320-400	0.74
4	22/12/15	6	18/4/16	118-126	236-400	0.63
5	5/1/16	6	26/4/16	112	150-320	0.52
6	19/1/16	6	30/5/16	132-146	250-380	0.62
7	2/2/16	2	4/6/16	123-129	200-380	0.66

FIELD INFORMATION

Field data Presence data collected by the NTWEP (Brooks and Jeffery 2018b) in the survey and control on *M. micrantha* was summarized to record the frequency of seedling, flowering and seeding events. There was no separation of the initial control records for new infestations (which often contain mature plants) and revisit records for known infestations (predominantly seedlings). Presence records (n=1774) between 2003 and 2021 were classified into reproductive status and week of the year (1 to 52). Each point and date may have more than one plant type present.

The field data clearly shows the flowering plants being observed from late April (week 17 onwards) (Figure 1), with most flowering observations in May and June, leading to the observation of seeding plants from week 27 onwards. The retention of seed on plants may account for the prolonged time of seed observations. Occasional later season flowering behaviour is observed in the field; however, no glasshouse plants commenced flowering after July in each calendar year.

Figure 1. Count of field presence records with seedlings, flowering or seeding plants, aggregated from all *M. micrantha* infestations between 2003 to 2021 and categorized by week of the year.



Vegetation types To provide context to the glasshouse trial data, information on the types of vegetation present in the *M. micrantha* search area in the Mission Beach area (~281 ha - K. Erbacher pers comm.) was extracted from the Regional Ecosystem database (Queensland Herbarium 2021). Regional ecosystems are classified by a numerical code of bioregion, land zone and vegetation community. The biodiversity classification of 'endangered' is also noted. The predominant remnant vegetation types in the Mission Beach search area include 'Mesophyll to notophyll vine forest' (7.12.1a), 'open areas in vine forests, dominated by sprawling vines, from cyclone damaged forest' (7.12.40a), 'Simple mesophyll vine forest' (7.8.1d, endangered), 'Mesophyll vine forest' (7.3.10a, endangered) and '*Melaleuca leucadendra* L. (L) open forest and woodland (7.3.25a). These descriptions show that the Mission Beach search area contains forest types typified by native vines. The area includes a variety of complex tropical forest types including cyclone disturbed forest (Brooks and Jeffery 2018a). *Mikania micrantha* can also grow in cleared 'non remnant' vegetation, such as grazing land. The *M. micrantha* patches treated by Brooks and Setter (2014b) at Mission Beach were growing horizontally amongst tropical grasses.

DISCUSSION

A NTWEP field team, mostly dedicated to *M. micrantha* control, are searching for juvenile green vines amongst tropical rainforests with native vines and woodland vegetation. Most plants flowering in the glasshouse trials were several metres tall and at least 1.5 m when growing with support. Whilst the diameters are slender, the plants are likely to be of a reasonable size when first flowering.

Under field conditions it could be expected that in moist well-lit habitats a subset of plants could establish and reproduce in the same calendar year. Across three trials, initial flowering was observed between the 18th of April and the 6th of July. This is consistent with the field data and demonstrates that first flowering behaviour is seasonally driven. Flowering behaviour was determined by time of the year rather than plant size.

Most plants commenced flowering in May or June at ages between 112 and 280 days, depending on their time of establishment. The latest establishment date for a plant flowering in the same year was the 2nd of February when flower buds recorded 112 days later. Under controlled conditions, there were cohorts where not all plants flowered, and a range of plant sizes at which some did.

The field data and information from the glasshouse trials was broadly consistent. February to May is a particularly important time to find and control *M. micrantha* prior to seed production. The timing of surveys is more critical than the interval between surveys. Ideally surveys would provide two opportunities to detect plants before they produce seed. However, in some years, late tropical wet season rainfall can limit access to coastal infestations until April or May (K. Erbacher pers comm.). In these years only a single search and control visit would be possible prior to potential seed production.

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