# Some factors that may influence the invasiveness of *Mikania micrantha* Kunth ex H.B.K. in Fiji

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**Summary** A study was undertaken to predict the tolerance and persistence of *Mikania micrantha* Kunth ex H.B.K. seed in the soil seed bank in Viti Levu, Fiji. A laboratory-controlled ageing test (LCAT) indicated that *M. micrantha* seed would likely survive in the soil seed bank for between 1 and 3 years. A second study was conducted to determine the effect of sodium chloride (NaCl), at concentrations similar to those occurring naturally in the soils of Viti Levu, Fiji, on the germination rate and root development of *M. micrantha*. Average percent germination in the controls was >93% and this occurred within 5 days of the start of incubation (30/15°C ± 1°C, 12 h photoperiod). Moderate salinity (200mM NaCl) significantly reduced germination to *c.* 60% and high salinity (300mM NaCl) down to 6%. Primary root growth was inhibited by low concentrations of NaCl (25mM). However, in such treatments, seedlings survived by producing a high density, shallow fibrous root system around their stem bases. Thus, seedling establishment could occur even in moderate levels of NaCl. This implies that *M. micrantha* can tolerate moderate salinity conditions and may be able to grow in areas where other weed species cannot grow so well. Both studies have identified components of the adaptive mechanism employed by *M. micrantha* in invading agricultural land in Viti Levu. This knowledge on seed longevity and salt tolerance could facilitate the development of management plans of this weed in Fiji.

**Keywords** Sodium chloride (NaCl), salinity, laboratory-controlled ageing test (LCAT).

## INTRODUCTION

*Mikania micrantha* Kunth ex H.B.K. (Asteraceae; mile-a-minute) is native to Central and South America (Ruas *et al*. 2000). It was introduced and established in many countries in Asia and the Pacific Islands and has become a weed of economic and environmental importance (Zhang *et al*. 2004). Several studies have reported on its biology, management and other ecological aspects but to our knowledge, there have been no studies on the effect of salinity on seed germination and seedling development. In addition, there is little known on the seed bank persistence of *M. micrantha*. In the present study, the adaptive mechanism that contributes to the invasive potential of *M. micrantha* under saline soil conditions was examined. A better understanding of germination and seedling performance under saline conditions could enhance our knowledge on the species' invasive potential and prediction of its spread and distribution in Fiji. In addition, knowledge of the persistence of *M. micrantha* seed in the soil would enable land managers and farmers to make faster and better informed decisions for management (Long *et al*. 2008). The lack of understanding of *M. micrantha* seed bank dynamics poses a major barrier in managing this weed. The complex interactions between *M. micrantha* seed bank and its soil surroundings mean that seed persistence in the field is perhaps difficult to determine or predict.

In this study, a laboratory-controlled ageing test (LCAT) was used to predict the persistence of *M. micrantha* seed in the soil seed bank in Viti Levu, Fiji. This is a first such study on *M. micrantha* seed.

## MATERIALS AND METHODS

**Source of seed** On 8 July 2008, dried mature *M. micrantha* seed was harvested from a sugarcane (*Saccharum officinarum* L.) farm at Yako village (17°51'01.2"S, 177°20'23.4"E) about 12 km south of Nadi, Fiji. The seed was collected from *M. micrantha* plants at the time of their natural dispersal, when they were dry and would be discharged from plants when agitated. The seed was transported in paper bags to Koronivia Research Station about 180 km east of Nadi where it was cleaned. The cleaned seed was air-dried in a cardboard box (to prevent wind from dispersing them) at room temperature for 3 days. The seed was packed inside two paper envelopes and sealed in a zip-lock bag and hand-carried to Brisbane, Australia. The

seed was subjected to quarantine regulations imposed by the Australian Quarantine and Inspection Service (AQIS), then stored at 15 ± 1°C and 15 ± 1% relative humidity (RH) until used in the experiments carried out in an AQIS approved laboratory at the University of Queensland.

**Laboratory-controlled ageing test** This study was conducted according to the standard protocol of The Millennium Seed Bank, Royal Botanic Gardens, Kew, UK (Davis and Probert 2004), with reference to (Long *et al*. 2008). Twelve replicates of 50 seeds per treatment were placed in individual open glass vials over a 47% RH lithium chloride (LiCl) solution (370 g $L^{-1}$ deionised $H_2O$) within an air-tight polycarbonate enclosure (NHP Electrical, Murarrie, Qld, Australia) at 20 ± 1°C for 14 days to equilibrate their water content (Davis and Probert 2004). Following equilibration, vials were transferred to a second polycarbonate enclosure at 60% RH (LiCl, 300 g $L^{-1}$) in an oven set at 45°C. A T-tec data-logger (Temperature Technology, Adelaide, Australia) was used to record the temperature and RH inside the enclosures and water was added to the enclosures whenever the RH fell by 1%. This latter enclosure formed the accelerated ageing condition used throughout this study. Two samples of 50 seeds were randomly selected at each time period from the ageing environment and surface sterilised by washing them in a 0.2% solution of sodium hypochlorite (NaOCl) for 10 min, then rinsing with several flushes of distilled water and dried at room temperature. The NaOCl solution acted as a leaching agent to remove any germination inhibitory chemicals from the seed and also surface fungi that may also inhibit germination. Germination potential was evaluated by placing three replicates of 50 seed into 90 mm diameter Petri dishes lined with two layers of Whatman No. 1 filter paper, each irrigated with 5 mL of distilled water. The dishes were sealed with Parafilm and incubated at 30/15 ± 1°C (day/night) with a 12 h photoperiod for 40 days. Seeds were considered germinated when radicles had protruded >1 mm and germination was scored daily. The percentage of normal seedlings produced was plotted against the time in the ageing environment (days).

**Salt stress** The effect of salinity on germination and seedling growth of *M. micrantha* was evaluated by placing three replicates of 50 seed into 90 mm diameter Petri dishes lined with two layers of Whatman No. 1 filter paper, each moistened with 5 mL NaCl (Ajax Finechem Pty Ltd, Australia) solution (either 0, 25, 50, 100, 150, 200, 250 or 300mM) and incubated for 7 days (30/15 ± 1°C, day/night, with a 12 h photoperiod). Minitab 15 was used for statistical analysis. The range of NaCl concentrations used in this experiment reflect NaCl levels that exist in tropical soils (Chauhan and Johnson 2008). Petri dishes were sealed with Parafilm™ (Perchiney Plastic Packaging Company, Chicago) then placed inside transparent zip-lock plastic bags to minimise evaporative water loss. Seed was considered to be germinated when the radicles had protruded >1 mm. The length of seedling was measured on the 7th day of incubation as most *M. micrantha* seedlings had germinated by this time of incubation (results not presented).

## RESULTS AND DISCUSSION

**Laboratory-controlled ageing** This test was designed to compare seed longevity parameters, within and between genera and species (Hay *et al*. 2006, Long *et al*. 2008). Correlations between seed longevity (determined by the LCAT) and soil seed bank persistence have recently been demonstrated (Long *et al*. 2008). They suggested that plants with seeds that show a $P_{50}$ (i.e. time for the viability to fall to 50%) of <20 days have a field persistence of <1 year and are referred to as having transient seed banks while those that show a $P_{50}$ of 20 to 50 days have a field persistence of between 1 to 3 years and are referred to as plants with short-lived seed banks. Species that show $P_{50}$ values of >50 days have an extended field persistence of >3 years and are referred to as having long-lived seed banks (Long *et al*. 2008).

*Mikania micrantha* had a $P_{50}$ of 48 days (Figure 1), which from the above discussion, suggests a field persistence of between 1 to 3 years. However, this result does not agree with Brooks *et al*. (2008) who conducted similar trials and suggested that *M. micrantha* seed may persist for about 7 years in the soil seed bank. The variation in seed longevity may be attributed to the fact that the seed came from plants grown under different environmental conditions, such as soil moisture and air temperature (Long *et al*. 2009), which are known to modify a number of seed characteristics.

**Salt stress** Germination of *M. micrantha* seeds differed significantly ($F_{1,8}$ = 124.83, P <0.001) between the various NaCl concentrations tested. Germination generally decreased with an increase in NaCl concentration, with only 6% of seeds germinating at the highest concentration of 300mM compared with seeds in the control of >93% (Figure 2). Over 60% of seeds could still germinate a NaCl concentration of 200mM.

Even though some *M. micrantha* seed germinated at the two highest NaCl concentrations (250 and 300mM), the radicles of the germinated seedlings were discoloured and shorter than for those germinating on

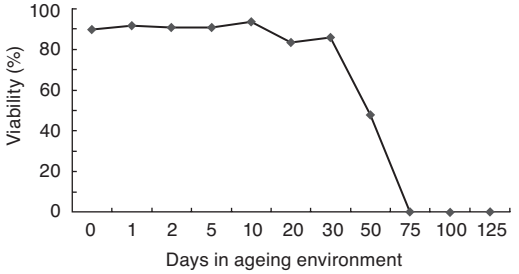


**Figure 1.** Viability of *M. micrantha* seed after laboratory controlled ageing at 60% RH and 45°C. Germination data are expressed as the mean cumulative normal germination of two replicates of 50 seeds from each treatment during a 40 day incubation period at 30/15°C (day/night, with 12 h photoperiod).

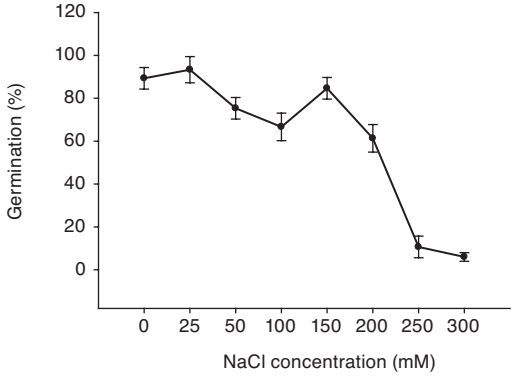


**Figure 2.** Effect of sodium chloride on the germination of *M. micrantha* seeds. The seeds were incubated for 7 days (30/15°C; day/night with a 12 h photoperiod). Vertical bars represent two standard errors of the mean. Each mean is for three replicates of 50 seeds.

the lower NaCl concentrations. This suggests that in the field, seed from this particular collection may well germinate but probably would not develop into healthy seedlings. The relatively high tolerance to high concentrations of NaCl suggests that *M. micrantha* may have a competitive edge over other weeds in invading terrestrial areas where such concentrations of salt are to be found in the soil. As an example, the seed germination of other Asteraceae species at 150mM NaCl are: *Synedrella nodiflora* (L.) Gaertn. – *c.* 10% (Chauhan and Johnson 2008), *Tridax procumbens* L. – *c.* 5% and *Chromolaena odorata* (L.) King and H.E Robins – *c.* 60% (Chauhan and Johnson 2008).

**Seedling growth** Seedling growth (in this case, as measured by total seedling length increase) was significantly different ($F_{1,7}$ = 851.61, P <0.001) between the various NaCl concentrations tested. The length of *M. micrantha* seedlings decreased with an increase in NaCl concentration (Figure 3). This trend is similar to that seen in the study conducted on *Bidens pilosa* L. (where radicle length was measured; Reddy and Singh 1992). In the present study radicle growth was completely inhibited at 25mM or more NaCl.

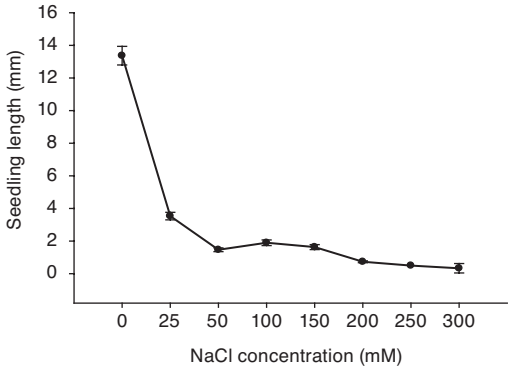


**Figure 3.** Effect of sodium chloride on growth of *M. micrantha* seedlings. Seeds were incubated for 7 days (30/15°C, day/night, with a 12 h photoperiod). Vertical bars represent two standard errors of the mean. Each mean is for three replicates of 50 seeds.

**Primary root growth** While radicle development was prevented at NaCl concentrations of 25mM or more, *M. micrantha* seedlings survived by producing a high density, shallow angled fibrous root system around their stem bases. The density of the fibrous root network decreased as the concentration of NaCl increased (visual observation). The production of a fibrous root system, at a shallow angle in saline conditions can be viewed as an adaptive mechanism for *M. micrantha.* This kind of survival mechanism has been reported to occur in many plant species growing under phosphorus deprivation (Williamson *et al*. 2001). The availability of phosphate in the soil almost invariably decreases with soil depth (Williamson *et al*. 2001) so the ability to produce shallow roots is an advantage to a plant. In the present study, when the primary root growth was restricted by NaCl, the production of a shallow, fibrous root system would allow seedlings to increase their nutrient and water acquisition, and also aid their anchorage to the soil (Wang *et al*. 2008). The reduction in primary root growth probably helps *M. micrantha* seedlings slow the uptake and transport

of NaCl to their shoots. This also suggests that *M. micrantha* possesses an adaptive mechanism to avoid drawing up large quantities of salt from deep in the soil, which may become deleterious to the young developing seedlings. Furthermore, this strategy can also be described as a compensatory adaptive mechanism to osmotic water stress and nutrient deficiency stress in saline conditions where the root system reacted by enlarging its water and ion-absorbing root surface (Muller and Schmidt 2004). The fibrous and shallow root systems are probably important adaptive features of *M. micrantha* seedlings to NaCl that confer a competitive advantage in their invasion and colonisation of saline areas and other abiotic stress areas. It can be concluded therefore, that *M. micrantha* responds to low availability of nutrients such as those seen in saline soils by adjusting its root morphology and architecture to acquire adequate nutrients under the abiotic stress conditions. This may suggest that moderate saline soils would not be a threat to *M. micrantha* invasive potential as it would be to many other plant species, including weeds. Increase in land salinisation, as caused by the entry of sea water during cyclones in cyclone-prone regions like the Pacific Islands, would restrict development of many crops and weed species but perhaps allow for the development and invasiveness of alien plant species such as *M. micrantha*. In summary, this knowledge on salt tolerance and seed longevity has given some insight into the biology of *M. micrantha* seed and will facilitate development of management strategies for this weed in Fiji.

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