

Responses of *Limnocharis flava* and *Mikania micrantha* seeds to saline treatments.

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Summary An experiment was conducted to investigate the potential for seeds of two tropical weeds to germinate or retain viability in saline environments. The germination and viability of *Limnocharis flava* and *Mikania micrantha* seeds were recorded after exposure to river and seawater and various concentrations of sodium chloride solutions. Both weeds are targets of a tropical weed eradication program and the survey areas overlap with saline habitats. After exposure to the solutions seeds responses were categorised as healthy germination, unhealthy germination, germinated and died, non-viable and viable. The results can be used to inform weed habitat suitability in coastal areas as the healthy germination of seeds is limited by salt concentrations above 150 mM (NaCl) or 9.3 ppt.

Keywords eradication, germination, tropics, salinity.

INTRODUCTION

Limnocharis flava (L.) Buchenau and *Mikania micrantha* Kunth are targets of the National Tropical Weeds Eradication Program (NTWEP) (Brooks and Jeffery 2018, Brooks *et al.* 2022). Both weeds occur along water courses in coastal tropical locations and have the potential to compete with crops on salt affected land (Macanawi *et al.* 2018, Putra *et al.* 2023). There are records of *M. micrantha* impacting soil, native vegetation and animals in saline and intertidal native mangrove forest communities (Biswas *et al.* 2018, and references therein).

Locally, *L. flava* and *M. micrantha* infestations and surveillance buffers overlap with fresh, brackish and sea water. On-ground surveys by NTWEP field crews around known infestations are the most expensive component of NTWEP operations (Jeffery and Brooks 2016). Determining seeds germination tolerances to salinity will assist in minimizing operational exposure in coastal systems, which are the natural habitat of saltwater crocodiles (*Crocodylus porosus* Schneider, 1801 (Crocodylidae)). Refining habitat suitability using information on the fate of seeds and tolerances to increasing salinity can be used to inform the extent of searches in coastal areas.

In addition to standard sodium chloride solutions this experiment investigated seeds germination and viability survival in sea water and a mixture of sea and fresh river water. Water samples were included to assist in the field implications of the salt solution responses.

MATERIALS AND METHODS

Germination test solutions were prepared with 25 to 300 m Mol of sodium chloride (NaCl molecular weight 58.44 g mol⁻¹) in 400 ml of distilled water (Table 1). There was also a distilled water reference treatment. To provide reference points to field situations two solutions of sea and river water (50% each by volume) and 100% seawater were additional treatments (Table 1). The conductivity and salinity of the two field water solutions were matched with masses of NaCl, creating 206 and 404 mM solutions. These additional salt solutions were 50 and 100% seawater equivalent salt solution treatments. Conductivity and salinity of all solutions were recorded with a multi parameter laboratory water meter calibrated at 25° C. The experiment was conducted in 2011.

Table 1. Saline water solutions and water type treatments applied to *Limnocharis flava* and *Mikania micrantha* seeds.

Treatments	NaCl (M mol)	Salinity (ppt)	Conductivity (mS)
Distilled water	0	0.00	0.00
Salt solution	25	1.54	2.97
Salt solution	50	3.07	5.69
Salt solution	100	6.12	10.83
Salt solution	150	9.27	15.90
Salt solution	250	15.37	25.30
Salt solution	300	18.46	29.90
50% Sea, river water		12.68	21.20
Salt solution ~ 50% sea	206	12.67	21.20
100% sea		24.91	39.20

Salt solution ~100% sea	404	24.90	39.20
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Seedlots and Germination *Limnocharis flava* seeds were collected from plants cultivated in a quarantine glasshouse in early 2011. Immersion in centrifuge tubes is the usual way to germinate *L. flava* seeds (Webber and Brooks 2013). Ten seeds were germinated in 15 x 1.5 ml centrifuge tubes per treatment. Fifteen tubes per treatment were filled with each of the solutions in Table 1 and *L. flava* seeds were exposed to the treatments in the tubes for four weeks. *Limnocharis flava* data per tube was summed to three replicates of 50 seeds per treatment.

Mikania micrantha seeds were collected from a new infestation at Mission Beach in June 2011. Three replicates of 50 seeds per treatment placed in petri dishes on filter paper over inverted watch glasses. Dishes were treated with 10 ml of each of the solutions in Table 1 and incubated for three weeks.

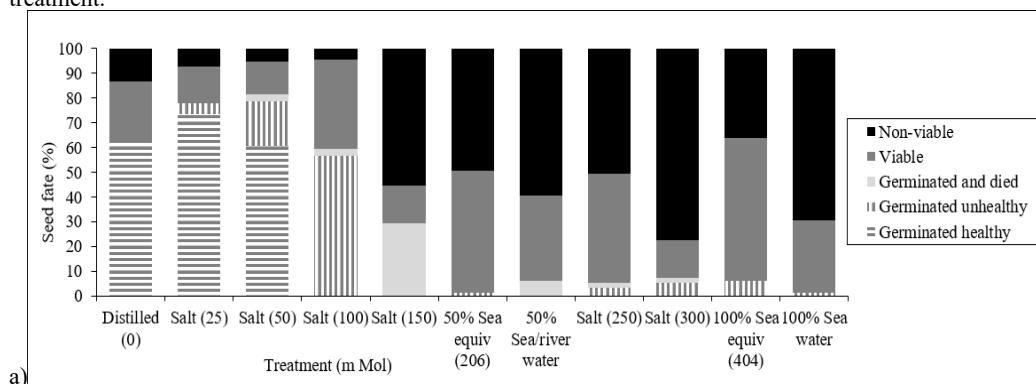
Petri dishes and centrifuge tubes were incubated in a Thermoline incubator set at 20/30°C, 12/12 hour night/day cycle. After incubation seed fate was classified into the following categories: healthy germination, unhealthy germination with a poor or brown radicle growth, and germinated and died. Ungerminated seeds were categorised into physically solid (viable) or collapsed when pressed with forceps (not viable).

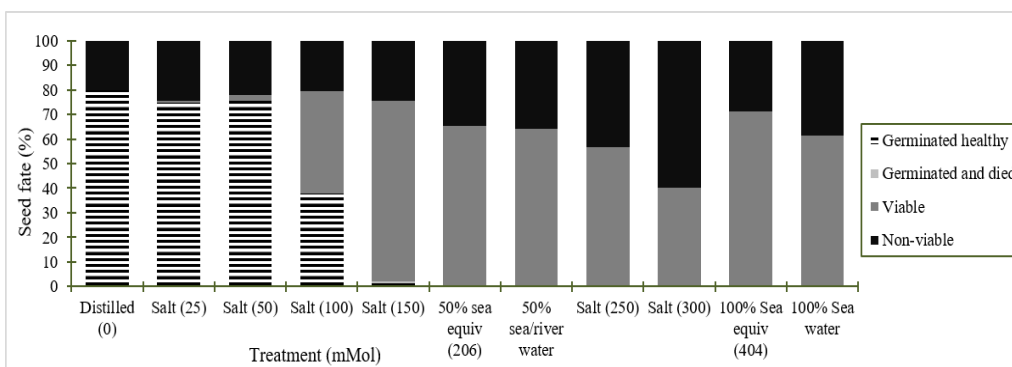
Data from both weed seed lots were summarised as the percentage fate of each germination or viability categories over three replicates of each treatment. Additional log-linear regression models (Poisson distribution and log-link function) of germination categories (healthy + unhealthy, and healthy + unhealthy + died) for each replicate were fitted against the concentration of salt solutions mM (NaCl) in Genstat 24th Edition. Separate regression analyses were run for each weed against the salt solutions in Table 1.

RESULTS AND DISCUSSION

Viable or germinable seeds were recorded for both weeds across all the treatments and the percentage fate is shown in Figure 1. The set time over which the treatments are imposed may have interacted with the speed of the effects on seed lots and resulted in some variation in germination and viability outcomes, particularly at higher salinity solutions.

Figure 1. The germination or viability fate of *Limnocharis flava* (a) and *Mikania micrantha* (b) seeds in salt solutions (mM NaCl) and 50 and 100% sea water mixes. Data expressed as a percentage of 150 seeds per treatment.





b)

Limnocharis More than 60% of *L. flava* seeds germination were classified as healthy in distilled water, 25 and 50 mM salt solutions (Figure 1a). The highest unhealthy germination (56.6%) was recorded in the 100 mM solution. The highest germinated and died fate (29.3%) was recorded in the 150 mM solution. In salt solutions of 206 mM and above, 1.3 to 7.3% of seed had germinated unhealthy or germinated and died. The difference between the points and fitted lines in Figure 2a reflect the greater germinated and died seed fates in single points of 150 and 300 mM solutions.

The non-viable percentage of *L. flava* seed was below 13% in the distilled, 25, 50 and 100 mM solutions, and above 36%, but variable at 150 mM and above. A variable percentage of viable seeds were found in all treatments, and a longer treatment time may have altered this outcome with more time for seeds to attempt germination. Similarly, *L. flava* seeds that germinated unhealthy may have also died or been rendered non-viable with a longer exposure time.

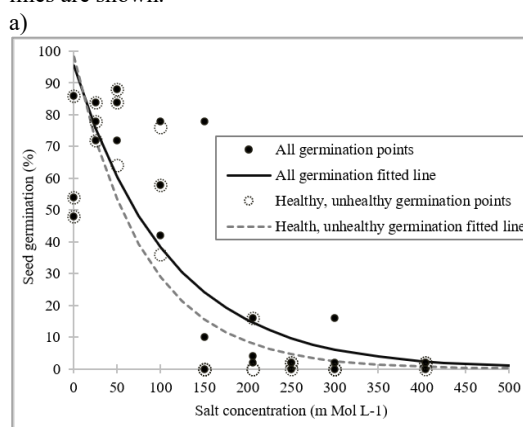
There were small differences between the *L. flava* seed germination fates in 50/50% sea/river treatment and the 206 mM salt solution equivalent treatment (Figure 1a). *Limnocharis flava* seeds retained a higher level of viability (relative to non-viable) in the 404 mM solutions than the other highly saline 300mM and 100% seawater solutions. However, it is not possible to determine if this is a response to the highest concentration of sodium chloride or reflects the seed responses over the exposure time frame.

Purta *et al.* (2023) recorded a large and significant decline in *L. flava* seedling heights, leaf sizes, and weights when grown in 50 and 75 ppm NaCl solutions. The inhibition of *L. flava* seedling growth was also greater in the 75 ppm salt treatments and 10 cm water compared to 5 and 0 cm immersion treatment combinations (Purta *et al.* 2023). Their most saline environment tested (75 ppm) is well below the lowest concentration

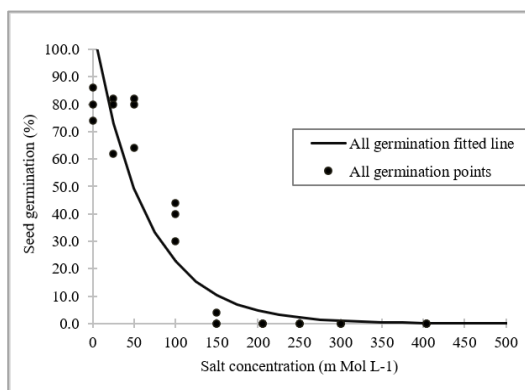
of the 25 mM, 1.54 ppt salt solution tested in the current trial. Although the current trial recorded up to 9% 'unhealthy' and 'died' germination above 150 mM (9.27 ppt) it seems unlikely that seedlings would establish when immersed in saline water well above 75 ppm.

Limnocharis flava also reproduces via vegetative pups but these are on flowering stalks of established plants (Webber and Brooks 2013). The combination of low germination and a lower chance of establishing healthy plants limits the potential for reproduction via seed or vegetatively under moderately saline conditions.

Figure 2. The germination fate of (a) *Limnocharis flava* and (b) *Mikania micrantha* seeds in salt solutions (NaCl, m mol L⁻¹). Three replicate points (50 seeds each) per salt solution and fitted lines are shown.



b)



Mikania Healthy germination of *M. micrantha* seeds declined from 75.3% to 1.3% between 50 and 150 mM NaCl solutions. No *M. micrantha* seeds were classified with ‘unhealthy germination’. A more rapid response to increasing salinity could account for the lack of germinated unhealthy *M. micrantha* seeds. Only 0.7 % unhealthy and died germination (1 seed each) was recorded in salt 150 and 300 mM, and 50% sea and 100% seawater treatments. The resultant small differences in the point data and imperceptible difference in the fitted lines meant only the ‘all germination’ data was plotted in Figure 2b.

In the distilled water treatment, 20% of *M. micrantha* seeds were viable. The percentage of viable seeds was higher in all the salt treatments, with a maximum 60% in the 300 mM salt solution.

There were only small differences between the *M. micrantha* seed germination fates in 50% sea/river, and 100% seawater treatments and their salt solution equivalent treatments (206 and 404 mM) (Figure 1 b).

Healthy germination of *M. micrantha* seed was limited to solutions less than 150 mM. Higher germination of Fijian sourced *M. micrantha* seed was recorded at 150 and 250 mM by Macanawi *et al.* (2018), over a 7-day treatment exposure time. Though they noted shorter and discoloured radicles at 250 and 300 mM. Also, that *M. micrantha* may still have a competitive advantage over other weeds and salt affected crops in coastal areas of Viti Levu (Macanawi *et al.* 2018). Potential impacts across cropping and environmental lands, including mangrove forests (Biswas *et al.* 2018) warrant caution when interpreting the results from the current seed germination trial. *Mikania micrantha* readily reproduces from vegetative cuttings but establishment from cuttings under saline

conditions was not studied and could be an area of future research.

SUMMARY AND IMPLICATIONS

Healthy seed germination was limited to salt concentrations below 150 mM for both *L. flava* and *M. micrantha* seed lots. The establishment of healthy plants from seed in saline areas over 150mM (9.3 ppt, Table 1) seems less likely.

The trial provided data on the fate of seeds compared the salinity of 50 and 100% sea water with matched salinity and conductivity readings of sodium chloride solutions. This provides reference points for applying seed responses to standard saline solution tests to field samples. Similarities between the responses of these two weeds to sea water solutions and 204 and 406 mM salt solutions allow for some broad comparisons from lab results to field situations. Particularly as the sea water salinity can vary temporally and spatially. Seawater sampling for a recent weed seeds experiment recorded a much higher salinity level (36.8 ppt) than the 24.9 ppt in Table 1.

Viable or germinable seeds were recorded for *L. flava* and *M. micrantha* weeds across all the salinity treatments. Remaining viable under increasingly saline conditions may enable some seeds of both weeds to persist through conditions that are unfavourable to healthy germination and then seedling establishment.

This experiment complemented unpublished dispersal information from seed buoyancy and immersion trials in fresh river water, seawater, and a 50/50 mix of both water types. *Chromolaena odorata* (L.) R.M.King & H.Rob. is in the same Eupatorieae tribe of Asteraceae as *M. micrantha*. The occurrence of viable seeds of *L. flava* and *C. odorata*, (but not *M. micrantha*) is consistent with other trials where seeds remain viable for up to 126 days immersed in sea, river and 50/50 mixes of water (authors unpublished data). Immersion in different water types is less of a barrier to dispersal. This trial shows that a percentage of seeds can remain viable in saline conditions but may need to reach a lower salinity environment to germinate and establish as seedlings.

Both *L. flava* and *M. micrantha* are unlikely to successfully germinate in saline environments. Salt is a barrier to weed establishment and places limits on habitat suitability. In combination with aquatic dispersal data, seed responses to salinity will assist in developing habitat suitability maps and refining NTWEP surveillance methodology for improving eradication efficiency and

minimizing operational exposure in coastal systems to *C. porosus*.

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