Survival of tropical weed species propagules after immersion in fresh, brackish and salt water

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Summary Neem (*Azadiractha indica* A.Juss.), Navua sedge (*Cyperus aromaticus* (Ridl.) Mattf. & Kük.), Hymenachne (*Hymenachne amplexicaulis* (Rudge) Nees), Bellyache bush (*Jatropha gossypifolia* L.) and Leuceana (*Leucaena leucocephala* ssp. *glabrata* (Rose) Zarate) are serious weeds in tropical north Queensland. Water movement is a significant dispersal vector for seeds of each of these species, particularly along water courses, but little is known about seed survival following immersion and movement in water. To improve knowledge of the effect of water immersion on seed viability seeds from each species were placed in fish tanks containing fresh, brackish and salt water for set periods of time before testing their viability.

Seeds of four of the species tested remained viable for the maximum test period of 98 days (14 weeks). Only Neem lost all viability after seven days in salt water and 14 days in the fresh and brackish water treatments. After 98 days in salt water, Leucaena seed retained almost half its original viability, whereas Bellyache bush and Navua sedge seed viability was reduced to approximately a third. Notably, Hymenachne seed viability remained relatively unaffected by immersion in all water types.

Although seeds can be transported in water, a suitable habitat is still required for germination and plant establishment and survival. By determining the length of time seed can remain viable after immersion in water, and whether immersion promotes, delays or inhibits viability gives an insight into the role water plays in the dispersal of these introduced weed species.

Keywords water dispersal, hydrochory, seed immersion, *Azadiractha indica, Cyperus aromaticus*, *Hymenachne amplexicualis, Jatropha gossypifolia, Leucaena leucocephala* ssp. *glabrata*

INTRODUCTION

"During the spring of last year, it occurred to me that it would be worthwhile, in relation to the distribution of plants to test how long seeds could endure immersion in sea water, and yet retain their vitality. As far as I knew, this had not been tried by botanists, who would have been far more capable of doing it than myself." Darwin (1857).

Determining the seed longevity of a weed species is a fundamental requirement for success when planning control activities (Panetta 2004, Brooks and Setter 2012). Relevant studies detailing the seed longevity of weed species in soil within Tropical North Queensland, such as for Bellyache bush exist (Bebawi *et al.* 2012) but information on their longevity in water is lacking.

Water dispersal of plant propagules (hydrochory) plays a major role in the expansion and establishment of populations of many weed species (Setter *et al.* 2008). Brooks *et al.* (2017) demonstrated this by observing a strong correlation between Siam weed (*Chromolaena odorata* (L.) R.M.King & H.Rob.) plants establishment and its spread along watercourses.

Populations of some species such as Hymenachne are closely associated with wetlands, drains, creeks, and rivers (Houston 2002), while other species such as Pond apple (*Annona glabra* L.) successfully disperse via ocean currents (Mason *et al.* 2008). Water is also one of several dispersal vectors for tree species such as Neem and as a result many infestations in north Queensland are located in creeks and river systems (Setter *et al.* 2009).

Many free floating or anchored aquatic species have developed specialist mechanisms for dispersal via water. Hygrophilla (*Hygrophilla costata* Nees) disperses successfully via stem fragments (Setter *et al.* 2017) while the anchored aquatic weed Limnocharis (*Limnocharis flava* (L.) Buchenau) produces fruit which readily dehisce and float, dropping seeds as they are transported by flowing water (Weber and Brooks 2013). Others, such as Navua sedge, grow and reproduce in areas of high rainfall that are frequently inundated with freshwater, thus facilitating dispersal (Vogler *et al.* 2015).

Within many creek/river systems there exists a natural transition from freshwater through to brackish and saltwater. Within the freshwater environs ideal conditions exist for localized seed dispersal and seedling establishment. As the water becomes more saline, either periodically or more permanently, conditions for successful recruitment decline. This is the case with Pond apple which can produce fruit in a freshwater location, deposit fruit/seed into a water body for dispersal then have the seeds transported via marine currents onto a suitable beachhead for seedling establishment (Mason *et al*. 2008).

Information gained through seed longevity studies in water can be used in conjunction with known water flow rates and directions to estimate the amount of seed dispersal from an infestation, as well as likely ‘destinations’ for deposition of viable seed (Mason *et al.* 2008).

In this study we test the effect of immersion of seed in water on the viability of seed from five species known to have water as a dispersal vector in tropical Queensland.

MATERIALS AND METHODS

Treatments: Sixteen fish tanks were filled with one of three water treatments: sea water, creek water, 50/50 sea and creek water mix (brackish). Water samples were collected from the ocean and a mountain fed creek in the Wet Tropics. An additional four tanks were not filled with water but used as an ‘air’ comparison with fewer retrieval times as it was anticipated that little decline in seed viability would occur over the 14-week trial period. Samples of 50 seeds were randomly selected from a field-collected bulk seed pool of each species and sown into 130 µm nylon sieve mesh bags then placed into the tanks. Retrieval times were: 2, 4, 7, 14, 28, 42, 70 and 98 days for each water treatments. Bags were randomly selected for each species from each tank at each retrieval time.

Water Quality: The water/air tanks were housed in a temperature-controlled environment set at 20ºC with abundant external ambient light. The water remained at a constant pH 10 and salinity was measured at 0, 15 and 34 ppm for the fresh, brackish, and saltwater treatments respectively. Aeration was provided to mimic natural water conditions and assist with water /seed interaction over time.

Germination and Viability testing: At each retrieval time, seeds were placed in a Petri dish on top of a distilled water moistened Whatman no.5 filter paper, over an inverted watch glass which was on top of another sheet of filter paper. The filter papers were kept moistened during incubation at 20ºC with 12 hrs of darkness alternating with 30ºC and 12 hrs of light. Records of germination (radicle emergence of 2 mm or more) was made every 3–7 days for the duration of the trial and germinates removed. Monitoring concluded when no further germination was recorded for two consecutive weeks. Bellyache bush seed was checked for viability using the tetrazolium method (Moore 1985). Due to the small size of the seeds of the other test species, any that failed to germinate were subjected to tests of rigidity with forceps to assess viability (Borza *et.al.* 2007).

Tetrazolium testing was used to determine the viability of large ungerminated seeds. Seeds were placed into Petri dishes containing a tetrazolium chloride solution. The dishes were then wrapped in foil and incubated at 25ºC for 24 hrs. Viable seeds were those that showed red staining on the embryo and those that failed to stain were deemed non-viable. (Mao *et al.* 2019).

Statistical Analysis: Mean seed viability and the standard error of the mean (SE) for each species in each treatment and retrieval time was calculated using Genstat (VSN International 2017) and presented graphically.

RESULTS

Neem seed lost all viability after seven days in salt water and 14 days in the fresh and brackish water treatments. Seeds of Hymenachne, Navua sedge, Bellyache bush and Leuceana remained viable for the maximum test period of 98 days (14 weeks). After 98 days in salt water, Leucaena seed retained almost half its original viability, whereas Bellyache bush and Navua sedge seed viability was reduced to about a third (Figure 1). It is notable that Hymenachne seed viability remained relatively unaffected by immersion in any of the water types.

DISCUSSION

This research demonstrated that seeds of all species except Neem could survive for at least 98 days in fresh, brackish and saline water (Figure 1). Seeds in the tanks either germinated in the water, died, or entered/maintained dormancy that was broken upon retrieval and viability testing, which changed conditions. High levels of salt generally either inhibit seed germination by producing low osmotic potential preventing water uptake or the salt is toxic to seeds and can kill a significant portion of immersed seeds (Guia *et al.* 2010, Vincente *et al.* 2020).

The fate of the seeds within the tanks was determined by factors such as the ability of the seed coat to remain intact, thus preventing the initiation of germination or degradation of the seed and/or tolerance to the toxic effects of salt on the embryo within the seed. For each species the effect of salt water on seed viability was generally not significant compared to that of brackish or fresh water following 98 days of immersion (Figure 1). Irrespective of whether the tested species are aquatic specialists or

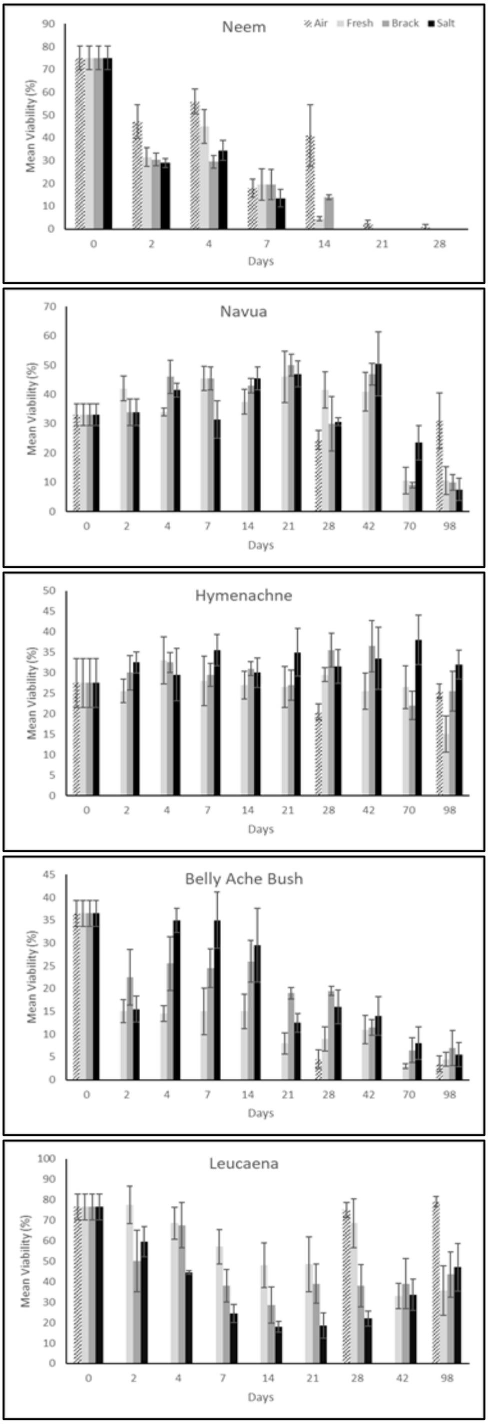

Figure 1: Effect of water treatments on seed viability of Neem, Navua sedge, Hymenachne, Belly Ache Bush and Leucaena. Vertical lines indicate ± 1 SE.

not they retained a significant level of viability for a considerable time. This is similar to the observations of Brooks *et al.* (2017) for Siam weed seeds after 18 weeks of immersion in similar water treatments to those used in this study

This survival of seed of each species would allow both short and long-distance water dispersal, dependent on other factors such as current flows and buoyancy of seed. Even if seeds were only buoyant for short periods, suspended in the water column or located on the stream bed they could be moved downstream by the flow of water or as bed load within the stream.

Together with viability, other factors will determine how long and how far seed may be distributed by water. For example, De Jager *et al.* (2019) found that large seeds generally disperse longer distances than smaller seeds in lowland streams, and that stream vegetation also greatly influenced dispersal distance. The results of Mason *et al.* (2008) showed how modelling the water dispersal of seeds and mapping the locations of deposition allowed for targeted management programs.

Although the duration of seed buoyancy was not quantified in this study, buoyancy along with seed survival when immersed in water is an important aspect when considering seed dispersal by water and developing effective management programs.

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