

From Seeds to Success: Propagation of *Gahnia trifida* for Ecological Restoration.

Jenny Guerin, Dan Duval, Thai Te.

South Australian Seed Conservation Centre

Botanic Gardens and State Herbarium

Abstract

Gahnia trifida is a tussock forming sedge endemic to wetlands, creek lines, and swamp margins in southern Australia. Along with several other members of the Cyperaceae family, *G. trifida* is often excluded from restoration projects due to difficulties with obtaining viable seed collections and complex seed dormancy. This study aimed to identify crucial steps required for the successful propagation of *G. trifida*. Initial observations highlighted the importance of timely seed collection from multiple individuals and populations, with peak viability observed during summer (December to February). Laboratory investigations found that scarification treatments, including heat shock, acid treatment, nicking, and seed excision, were effective methods for promoting germination. Germination of excised seeds reached 100%, indicating that dormancy in *G. trifida* is primarily imposed by the testa. In addition, warm incubator temperature regimes were found to improve germination rates. Nursery methods were developed based on these laboratory results, enabling the successful production of thousands of *G. trifida* plants for current restoration projects in South Australia. Hundreds of plants have now successfully established in Stipiturus Conservation Park, enriching the biodiversity of this nationally listed, threatened ecological Fleurieu Swamp community. This study highlights the positive impact of developing propagation methods for sedges and their utilisation in the restoration of degraded habitats.

Introduction

Loss of habitat is one of the major threats to species survival and is a primary driver for wildlife extinction. Plant communities form the foundation for a host of organisms within ecosystems, and a fundamental aim of ecological restoration and landscape rehabilitation is to restore the functional plant species that were present prior to habitat degradation (Standards Reference Group 2018). However, there remain many plant species that are not utilized in restoration programs due to a lack of knowledge about seed ecology and propagation methods.

Sedges have important ecological roles within their habitat, often dominating creek margins, damp woodlands and swamps and contribute to retaining soil moisture, nutrient cycling, water filtration and soil stability (Barrett 2013). Sedges are important larval host plants for many species of butterflies, dragonflies and other insects as well as providing nesting and shelter sites for birds, mammals, and reptiles. For this project *Gahnia trifida* Labill. was required for habitat restoration, specifically for the endangered Southern Emu-wren (*Stipituris malachurus intermedius*). *G. trifida* forms part of the low dense vegetation cover to one metre above ground that the emu-wren requires for protection. It is particularly important in burnt swamp habitat as *G. trifida* re-sprouts and grows rapidly to provide shelter in the initial years post-fire (Jason Van Weenen, pers. comm., January 2024). It is also the main larval food host plant for the threatened Golden-haired Sedge-skipper (*Hesperilla chrysotricha*). Interestingly, the introduction of propagated *G. trifida* plants to a constructed urban wetland at the Adelaide Botanic Gardens now supports the only population of Varied Sedge-skipper (*Hesperilla donnysa*) on the Adelaide Plains.

While sedge species may dominate certain vegetation associations, propagules may not be available for restoration programs due to a lack of understanding about nut (seed) viability and germination requirements. One of the main limitations of propagating plants from seeds has been the inability to collect viable seeds from wild populations. In many species development occurs over more than one season and non-viable seed remains attached to the spikelets resulting in collections that are not viable (Kodym, Turner and Delpratt, In situ seed development and in vitro regeneration of three difficult-to-propagate Lepidosperma species (Cyperaceae). 2010).

In South Australia *G. trifida* is a tussock forming sedge found growing in damp areas such as creek lines and swamp margins in near coastal and inland regions, mainly in

freshwater springs, swamps and watercourses. It grows to > 1m high with erect panicles, 20-40 cm long; and brown spikelets, 4-5 mm long, in dense, oblong, spike-like clusters. The nuts are described as obovoid-oblong, bluntly trigonous, blackish, and covered with longitudinal rows of a transparent shiny fine reticulate-papillose layer (State Herbarium of South Australia, 2021).

Little is known about dormancy alleviation and practical nursery techniques for propagating *G. trifida* for large scale restoration. The seeds of *G. trifida* have a thick testa (seed coat), and a small, capitate, embryo and exhibit complex dormancy, similar to other Cyperaceae (Turner 2013, Martin 1946). These traits have been observed in other sedge species such as *Lepidosperma*, and *Eleocharis* (Panaia, et al. 2009, Turner 2013). There are very few reports of successful germination techniques for *Gahnia* species although one exception is *G. sieberiana* seeds that germinated in response to a combination of heat and smoke treatments (Thomas, Morris and Auld 2003). *G. filum* appears to lack dormancy, exhibiting a high level of germination without treatment in laboratory conditions (South Australian Seed Conservation Centre 2015). Other *Gahnia* species occurring in SA have more complex dormancy mechanisms. Considerable effort has gone into propagating *G. radula* using in vitro techniques due to difficulties in sourcing viable seeds and achieving germination (Kodym, Clarke, et al. 2014). In vitro techniques were also developed for difficult to propagate species of *Lepidosperma* (Kodym, Turner and Delpratt 2010). Further understanding about seed development and dormancy in difficult to propagate Cyperaceae species will lead to improved landscape restoration through broad scale plantings or via enhanced direct seeding methods.

This study was undertaken to develop an understanding of the seed biology of *G. trifida* with the aim of improving propagation techniques. The first part was to examine seed viability from different collections to determine what characters could be used in the field to improve collection strategies. Secondly, different treatments were applied to overcome dormancy and improve germination rates under different seasonal conditions. Finally, the study aimed to adapt these methods in a nursery setting to provide seedlings for ongoing restoration activities in the Stipiturus Conservation Park (CP), Deep Creek National Park (NP) and Field River Valley Project adjacent Glenthorne (NP).

Methods

Seed collection and viability testing.

Seeds used for experiments were collected from the Fleurieu Peninsula region; Deep Creek NP, Morialta CP and a peat bog on private property near Ashbourne. Seeds were cleaned by rubbing through a stack of sieves (sizes in μm 1700,1400, 1000, 500) and aspirated using a vacuum separator. Seeds were dried in a controlled environment room set to 15% RH at 15 °C for 2 months and then heat sealed in foil packets and stored at -20 °C until use. Seeds removed from storage were allowed to rehydrate to ambient conditions for at least 24 hours before use.

Seed viability was determined using a cut test of 20 seeds, examined under a dissecting microscope, and via X-ray (Faxitron) analysis of 50 seeds. Viability was assigned based on visual assessment using the criteria that seeds were fully developed and filled with healthy, cream coloured endosperm, within minimal air gap between endosperm and integument, and free from insect damage. Seed viability of collections before aspiration typically had between 5-10% viable seeds. After processing, the final seed viability was estimated; Ashbourne (80%), Deep Creek NP (55%), Morialta CP (100%).

Laboratory Germination Experiments.

Preliminary laboratory experiments.

Initial investigations were conducted to test a range of different experimental treatments to identify treatments that stimulated germination for further investigation.

Effects of incubator temperatures: stratification, winter, and spring/autumn conditions.

Heat shock was applied (100°C for 7 min) to 50 seeds, which were then soaked in gibberellic acid 250 mg/L for 24 hours. Seeds were placed onto 1% (w/v) agar in glass Petri dishes and sealed with plastic wrap to prevent drying. The seeds were placed in incubators with the following temperature regimes:

Winter conditions: 15°C 20 h (3am→11pm); 5°C 4 h (11pm→3am) / 10 h light (8am→6pm); 14 h dark (6pm→8am).

Spring/autumn conditions: 22°C 12 h (8am→8pm); 10°C 12 h (8pm→8am) / 12 h light (8am→8pm); 12 h dark (8pm→8am).

1. Dishes were placed in a 5 °C fridge in the dark for 6 weeks then placed in the spring/autumn incubator.
2. Dishes were placed in the winter incubator for 6 weeks then placed in the spring/autumn incubator.
3. Dishes were placed in the winter incubator.
4. Dishes were placed in the spring/autumn incubator.

From these experiments the highest germination was observed from dishes placed in the spring/autumn incubator. This incubator was used for all subsequent experiments.

Effect of different scarification treatments.

Seeds collected from Morialta CP were subjected to the following treatments:

1. Seeds excised from the seed coat with a scalpel, under a dissecting microscope.
2. Seeds nicked over the radicle with a scalpel.
3. Boiling water poured over seeds then allowed to cool.
4. Soaked in Sulphuric Acid (18.4 M) for 60 min, removed and washed thoroughly with water.

For this experiment one set of 25 seeds was tested for excised seeds (treatment 1) due to the low success rate of excising seeds without damage. Four replicates of 25 seeds were used for treatments 2 and 3, and four replicates of 45 seeds was used for treatment 4. Following pre-treatments, seeds were placed in glass Petri dishes containing moist sterile sand overlaid with filter paper. Dishes were sealed with plastic wrap to prevent drying. Replicates were placed in an incubator set to spring/autumn conditions. Germination was defined as the emergence of the radicle and was assessed weekly upon radicle emergence until no further germination was observed. Sand was moistened as required throughout the experiment.

Long leaching experiment.

Batches of 50 seeds collected from Ashbourne were soaked in water for 3 weeks and 6 weeks, water changed twice per week. After leaching seeds were plated onto Petri

dishes with filter paper over damp cotton pads and sealed with cling wrap. Experiments were checked monthly over 5-months.

Based on these initial findings, additional experiments were conducted to further investigate the effects of different treatments on rates of germination.

Experiment 1: Effect of leaching, gibberellic acid, and heat shock treatments on germination.

Seeds collected from Deep Creek NP were subjected to the following treatments:

1. Control (no treatment).
2. Leaching in water for 5 d.
3. Leaching in gibberellic acid (500 mg/L) for 5 d.
4. Heat Shock 100°C for 5 min.
5. Heat Shock 100°C for 5 min, followed by leaching in water for 5 d.
6. Heat Shock 100°C for 5 min, followed by leaching in gibberellic acid (500 mg/L) for 5 d.

For this experiment four replicates of 25 seeds were used. Following pre-treatments, seeds were placed in glass Petri dishes containing moist sterile sand overlaid with filter paper. Dishes were sealed with plastic wrap to prevent drying. Replicates were placed in an incubator set to spring/autumn conditions. Germination was defined as the emergence of the radicle and was assessed weekly upon radicle emergence until no further germination was observed. Sand was moistened as required throughout the experiment.

Experiment 2: Effect of heat shock temperature and duration on germination.

Seeds collected from Deep Creek NP were used for assessing heat shock treatments. A factorial experiment was conducted to investigate the effects of dry-heat temperatures (80, 90, 100, and 110 °C) and exposure durations (2, 10, and 30 minutes) on germination. These treatments represent the range of heat shock temperatures and

exposure periods that topsoil-stored seeds might be exposed to during a fire event. Heat was applied by placing seeds, in a single layer, in glass Petri dishes and quickly placing in a fan-forced laboratory convection oven set at the required temperature for the specified time.

For this experiment 30 seeds were used for each treatment. Seeds were allowed to cool after heating and placed onto 1% (w/v) agar in glass Petri dishes. Plates were sealed with plastic wrap to prevent drying. Plates were placed in an incubator set to spring/autumn conditions. Germination was defined as the emergence of the radicle and was assessed weekly upon radicle emergence until no further germination was observed.

Experiment 3: Germination response of different Gahnia species to gibberellic acid, smoke water and heat shock (results shown in Fig 4.).

Seeds from collections of different *Gahnia* species (stored at -18°C in 2016) were withdrawn from long term storage for this experiment. Viability was assessed for each collection *G. ancistrophylla* (85%), *G. deusta* (76%), *G. lanigera* (56%), *G. sieberiana* (86%) and *G. trifida* (77%).

The following treatments were applied.

1. Control (no treatment).
2. Soaked in gibberellic acid (500 mg/L) for 24 h.
3. Soaked in smoke water (10%(v/v)) for 24 h.
4. Heat Shock 90°C for 15 min.
5. Heat Shock 90°C for 15 min, followed by soaking gibberellic acid (500 mg/L) plus smoke water (10% (v/v)) for 24 h.

For this experiment 50 seeds were used for each treatment. Seeds were placed onto 1% (w/v) agar in glass Petri dishes and sealed with plastic wrap to prevent drying. Plates were placed in an incubator set to spring/autumn conditions. Germination was defined as the emergence of the radicle and was assessed weekly upon radicle emergence until no further germination was observed.

Nursery Experiments.

Seeds collected from Ashbourne were untreated (control), or subjected to heat shock at 100 °C for 5 min. Treatments were set up as follows:

1. Control.
2. Control with bog method.
3. Heat shock.
4. Heat shock with bog method.

Treated seeds were transferred to 10 cm round pots with native potting mix supplied by BioGro. The experiment was set up using four replicate pots with 50 seeds per treatment. Pots that were assigned to the bog treatment were placed in shallow dishes (~5cm) that were filled daily throughout the experiment. All pots were irrigated daily for 10 min with overhead misting. Pots were set up in July 2020 and scored every 4 weeks for 9 months.

Results

Seed collection and viability testing.

The collection of viable seeds is crucial for the success of sexual propagation. The timing of seed collection was a critical factor in obtaining collections with useful quantities of viable seeds. Table 1 shows the viability of seed collections made in different seasons that were processed at the South Australian Seed Conservation Centre between 2007 and 2023. Collections with the highest viability were made in summer months from December to February, whereas collections during other seasons had markedly lower viability. Viable seeds were healthy and filled as determined by x-ray and/or cut tests (Fig.1). There were very few populations sampled that were found to produce viable seeds at all, and within these populations only some individuals (~10%) produced viable seeds.

Mature, viable seeds appeared dark and shiny and hung by a filament from the spike-like inflorescence (Fig. 1). This feature was a reliable indicator of ripeness and good seed fill. Observations over several years showed that these seeds consistently had high viability. Seeds retained within the spikelets after viable seeds had shed were generally larger, pale, and unfilled. This has also been reported in *Lepidosperma scabrum* (Turner 2013).



Fig. 1. Seed viability in *G. trifida*. a) Ripe seeds hanging from filaments *Gahnia trifida*; b) Dark viable seeds and c) Cut test of seed collection with 80% viability, nonviable seeds indicated by red line. (Images from Plants of South Australia website (South Australian Seed Conservation Centre 2015)).

Table 1. Viability of *G. trifida* seeds collected from South Australian Herbarium Regions during different seasons.

Season	Collection Date	Herb Region	% Viability
Summer	01-Feb-08	SE	100
Summer	25-Jan-23	SL	100
Summer	25-Feb-16	SL	85
Summer	04-Dec-19	SL	80
Summer	18-Feb-16	SE	55
Summer	16-Feb-22	KI	52
Summer	22-Jan-23	SL	47
Summer	06-Dec-19	SL	46
Summer	01-Feb-22	SL	38
Spring	08-Aug-17	SL	10
Spring	01-Aug-19	SL	8
Spring	01-Sep-18	SL	1
Autumn	02-Apr-22	SL	0

Laboratory Germination Experiments.

Dormancy and germination of G. trifida: preliminary laboratory experiments.

Seeds from the family Cyperaceae, including *G. trifida* seeds, are endospermic containing small, capitate embryos. Germination in freshly harvested seeds was delayed by more than 30 days, demonstrating the presence of seed dormancy (Baskin and Baskin 2004).

Preliminary trials in the laboratory showed that incubation temperature was an important factor in determining germination response. In an early experiment, germination was increased (14%) in spring/autumn conditions compared to 5% in winter conditions after heat shock (100 °C for 7 min) and gibberellic acid ((250 mg/L) 24 h) treatment. Stratification for 6 weeks in winter conditions prior to spring/autumn

conditions resulted in 9% germination, and cold stratification at 5° C for 6 weeks did not increase germination above 0%. Extended leaching times of leaching in water for 3 weeks and 6 weeks were also trialled and resulted in 0% germination.

Seeds excised from freshly collected nutlets had 100% germination, with no other treatment, incubated spring/autumn conditions. However, the process of seed excision is arduous, seeds are easily damaged, and it is not a useful technique for restoration which requires large quantities of seeds. The observation that excised seeds germinated readily, suggested that dormancy was imposed by the hard testa surrounding the seeds. Alternative scarification methods also produced a positive germination response; specifically, nicking the seed coat (12%), partially dissolving the seed coat with concentrated sulfuric acid (60 minutes) (7%), and boiling water treatment (2%). The dry heat treatment was the easiest method to use for large quantities of seeds, and other methods yielded no significant improvement in germination compared to heat shock. The results from these preliminary experiments were used to design further experiments, testing the effects of heat shock.

Experiment 1: Effect of leaching, gibberellic acid, and heat shock treatments on germination.

G. trifida seeds were subjected to several treatments, including leaching in water, leaching in gibberellic acid, heat shock treatment, and combinations of heat shock and leaching. The highest germination rate of 32% for this experiment was observed following a heat shock treatment at 100 °C for 10 minutes (Fig. 2). Leaching in water or gibberellic acid (500mg/L) for 5 days did not enhance germination above the control. Surprisingly, leaching after heat shock treatment resulted in decreased germination compared to heat shock alone.

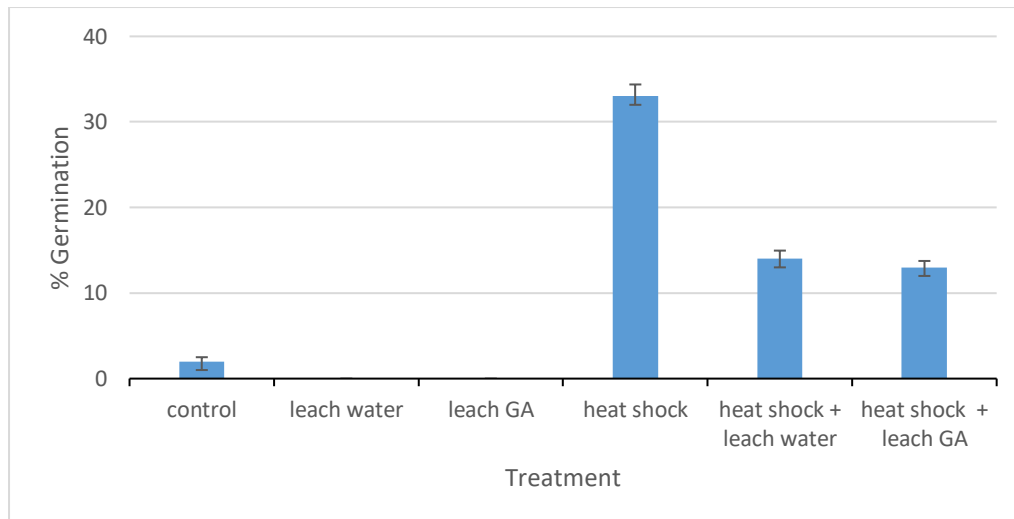


Fig. 2. Experiment 1. Average germination (% \pm s.e.) for *G. trifida* seeds after different treatments. Leach water (soaked in water for 5 d); leach GA (gibberellic acid (500 mg/L) for 5 d); heat shock (100 °C oven for 10 min); heat shock + leach water (100 °C oven for 10 min, then leach in water 5 d); heat shock + GA (100 °C oven for 10 min, then leach in gibberellic acid (500 mg/L) for 5 d). Seeds were incubated in spring/autumn conditions. Four replicates of 25 seeds each, experiment duration = 9 months.

Experiment 2: Investigation of heat shock: duration and temperature limits.

Results from Experiment 1 showed that dry heat applied for 10 minutes at 100 °C was effective in alleviating dormancy in approximately one-third of the *G. trifida* seeds tested. The effectiveness of various heat shock treatments was further investigated in Experiment 2, by varying both the duration and temperature of dry heat (Fig. 3). The temperature range required for heat shock to promote germination was between 80 °C and 110 °C. The highest germination (30%) in this experiment was recorded after 10 min exposure at 100 °C. A 2-minute exposure was insufficient to promote a response at 80 °C, but germination increased to 10% at 90 °C, 13% at 100 °C, and 20% at 110 °C. At 90 °C, germination was highest (23%) after 30 minutes. At 110 °C, prolonged exposure (10 and 30 minutes) exceeded the thermal tolerance of the seeds, resulting in 0% germination. In summary, high-temperature heat pulse treatment is effective, but control is required to apply sufficient heat without causing excessive temperatures or durations that reduce viability. The effective temperature range is comparable to

conditions that seeds buried close to the soil surface are exposed to when a fire front passes (Read, et al. 2021, Williams, et al. 2004).

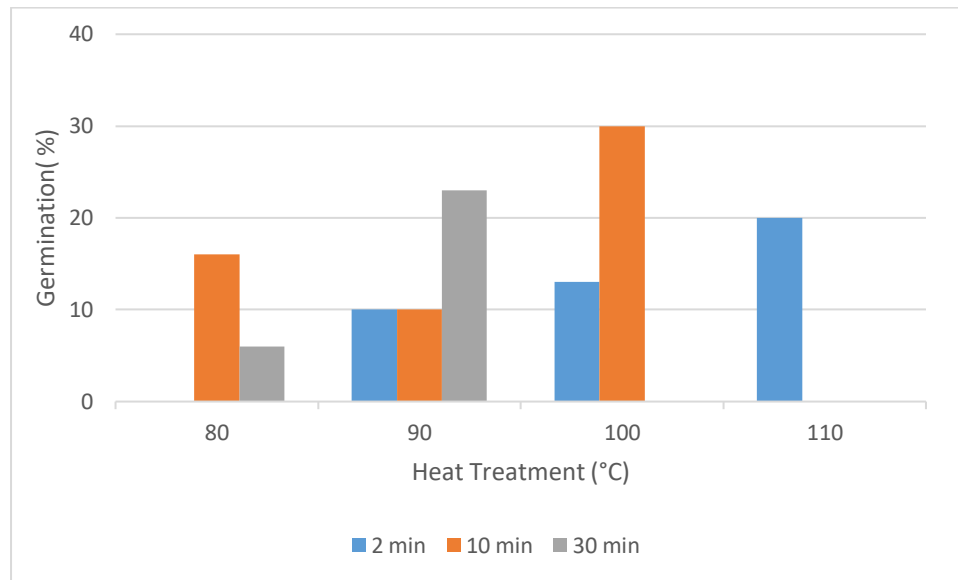


Fig. 3. Experiment 2. Effect of temperature and duration of heat treatments on germination of *G. trifida* seeds. Seeds were heated in an oven set at 80, 90, 100 and 110 °C for 2, 10 or 30 min. Seeds were incubated in spring/autumn conditions. For each treatment one plate of 30 seeds, experiment duration = 6 months

Experiment3: Response of five Gahnia species to combinations of heat, smoke water, and gibberellic acid.

Treatments were administered to five species of *Gahnia* to assess the germination response of different species within the genus. Seed viability was assessed before the experiment: *G. ancistrophylla* (85%), *G. deusta* (76%), *G. lanigera* (56%), *G. sieberiana* (86%) and *G. trifida* (77%). The germination response varied between species, and, in general, was low for all treatments (Fig. 4). The exception was *Gahnia sieberiana*, which exhibited markedly higher germination in all treatments with the highest at nearly 80% in response to smoke water treatment. Smoke water or gibberellic acid had little to no effect on the other species tested. The other species showed the highest response to either heat shock or a combination of heat shock followed by the application of smoke

water and gibberellic acid. These results highlight the diversity of responses among species within the genus to germination enhancing treatments.

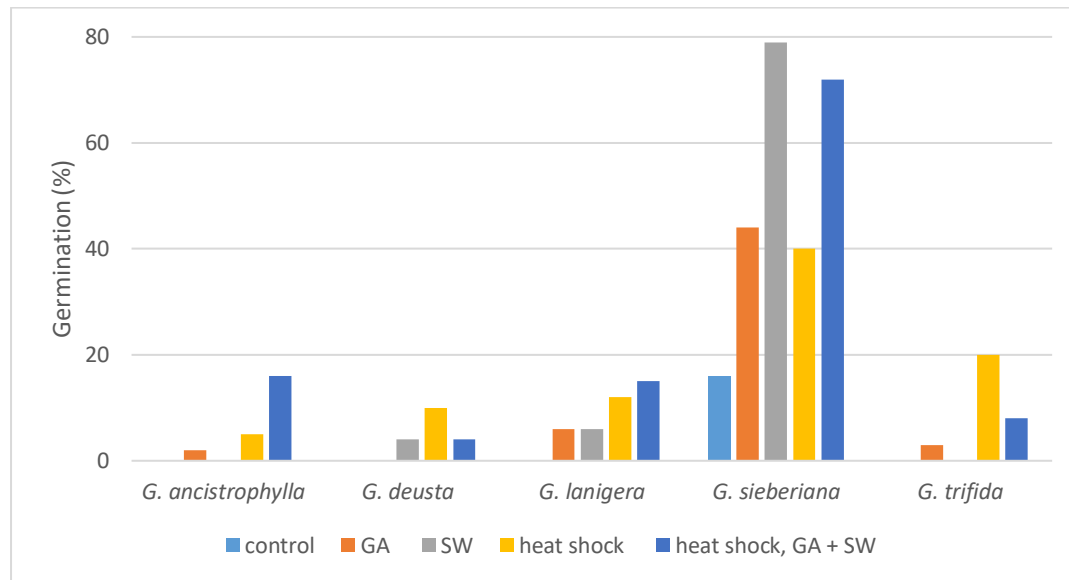


Fig. 4. Experiment 3. Germination response of different *Gahnia* species to treatment with GA (gibberellic acid (500 mg/L 24 h)); SW (smoke water (10% (v/v)) 24 h); heat shock (90 °C 15 min); heat shock, GA+ SW (90 °C 15 min, then gibberellic acid (500 mg/L) + smoke water (10% (v/v/)) for 24 h). Seeds were incubated in spring/autumn conditions. For each treatment one plate of 50 seeds, experiment duration = 5 months.

Nursery Experiments

Effect of heat shock on seedling emergence in nursery pots.

A method to raise seedlings in the nursery was trialled based on the results of laboratory experiments. The findings were similar as heat shock increased seedling emergence compared to untreated seeds grown in potting soil (Fig.5.). Coincidentally, a bog method was tested by placing pots in trays of water. There was no significant difference between the bog method and daily irrigation for treated seeds sown in nursery conditions. Seeds were sown in July and started to emerge in November. Laboratory experiments indicated higher seed germination rates in warmer conditions, and this trend was reflected in the nursery, where seedlings also emerged in warmer

temperatures. The highest germination rate was 13% using a dry heat treatment with daily irrigation.

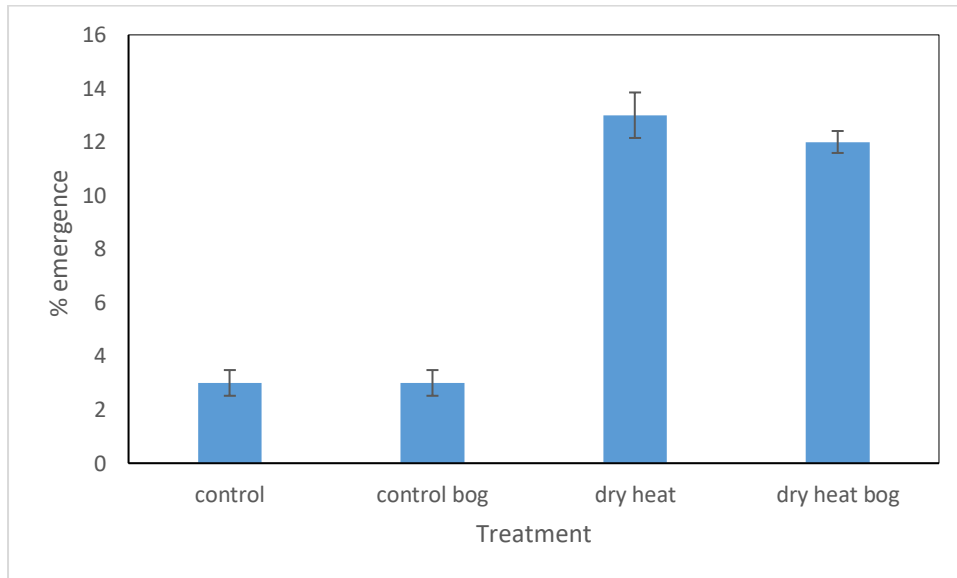


Fig. 5. Effect of heat shock (100 °C for 10 min) on seedling emergence in nursery pots. Bog method pots were placed in trays of water, other pots were placed on the bench. All pots were irrigated for 10 min per day. For each treatment four replicates of 50 seeds each, experiment duration = 9 months.

After testing germination in plant pots, a trial method was tested for growing large numbers of seedlings. Seeds were treated with heat shock (100 °C for 10 min) and sown into seedling trays with native potting mix at a rate of ~ 1000 seeds per tray and irrigated for 10 min per day over several months.

Seedling emergence was first observed after 4 months when seeds sown in early spring (September). Seeds continued to germinate for several months after initial emergence was observed. Young seedlings were pricked out and potted into tube-stock as they emerged and grew rapidly, with good vigour (Fig. 6) and strong root systems. There was minimal loss of plants after transferring from trays to tube-stock pots.



Fig. 6. Seedlings growing in tube-stock pots in the Adelaide Botanic Garden Nursery.

Utilisation of G. trifida in the restoration of the Fleurieu Swamp Threatened Ecological Community in Stipiturus Conservation Park.

Hundreds of seedlings were planted into marginal zones around peat bog swamp in Stipiturus CP in June 2020. Survivorship after 3 years was high (80 to 90%) when planted into soils with the adequate moisture and hydrological regimes, and kangaroo grazing was managed in the first 2 years. Survival was highest when larger robust plants were translocated according to Luke Price, Hills & Fleurieu Landscapes Regional Ecologist (pers. comm., December, 2023). A seed collection from translocated plants in January 2023 had > 55% viability, demonstrating that plants had successfully established with capacity for ongoing recruitment. These plants can be used as a sustainable seed source for future projects.

Since this method was developed over 5000 plants have been supplied to restoration programs in South Australia, including Hills and Fleurieu Landscapes Board restoration of Stipiturus Conservation Park and Green Adelaide Landscapes Board Field River project, National Parks and Wildlife restoration at Deep Creek NP.



Fig. 7. *G. trifida* translocation site at Stipiturus CP in January 2024, photo Dan Duval.

Discussion

The collection of viable seeds is a prerequisite for sexual propagation and collecting ripe seeds was essential for ensuring good viability. However, obtaining viable seeds from *G. trifida* was also contingent on other factors, such as population size, adequate rainfall and a healthy, intact habitat. For example, small numbers of plants found near Stipiturus CP, along degraded roadsides did not produce viable seeds regardless of the season. Survey of several populations was required to find those that produced viable seeds. Even so, in populations where viable seeds were collected, only a portion of plants produced large quantities of viable seeds. These populations had factors in common such as large numbers of plants and sufficient soil moisture. A reliable indicator for seed collection is the presence of dark, shiny, nuts dangling from spike like clusters by filaments. This developmental stage is likely to enhance the dispersal of ripe *Gahnia* seeds. Some force, such as wind or animal disturbance, is required to break the filaments. This mechanism is likely to achieve momentum to disperse over a wider area compared to passive seed release.

Seeds of *G. trifida* germinated sporadically over several months both in the laboratory and the nursery. The laboratory conditions involving long term incubation in moist conditions promoted fungal growth on the seeds which may have reduced the final germination results. The removal of seed coats effectively eliminated dormancy, suggesting that dormancy is likely imposed by the seed coat. The other most effective treatments to promote germination involved scarification of the seed coat. The scarification methods tested resulted in relatively small increase in germination (~30%). Of the methods tested heat shock was the most effective and easiest to apply, especially for large numbers of seeds. Despite the low germination recorded in laboratory (32%) and nursery trials (13%), thousands of plants were produced using heat shock and sowing directly into potting soil. Although viable seeds are not always easy to locate, healthy plant populations can produce an abundance of viable seeds. Seedlings keep emerging for several months, so over an extended period, there are sufficient seedlings available for ongoing nursery propagation. Although a high % of seeds may remain dormant, the technique is effective and applicable for nurseries to produce seedlings for broad scale restoration.

Observations of *G. trifida* and *G. halmaturina* seedlings emerging after the 2020 bushfires on Kangaroo Island (KI) suggest that fire can stimulate the germination of *Gahnia* species. Heat treatment has been identified as a cue for breaking seed coat imposed dormancy. Heat shock was also found to increase germination of to 80% in *Lepidosperma scabra* seeds after 2 years burial (Turner 2013).

This study facilitated the propagation of thousands of *G. trifida* plants, highlighting the role of seed biology studies in enhancing restoration practices. The success of transplanting was demonstrated by high survivorship and production of viable seeds within 2.5 years. Lessons learned in the field included important considerations such as site selection with correct hydrology and fencing to protect from herbivores. The accumulated knowledge from this and previous studies has the potential to streamline the selection of practical methods suitable for related species.

Poor understanding of seed biology leads to the exclusion of key plant species in restoration efforts that provide critical habitat to native species, many of which may be threatened or restricted to specific ecosystems. This knowledge gap hampers the integration of an array of plant species in restoration resulting in an incomplete species mix for the targeted vegetation community. This not only compromises the overall integrity of the restored habitat but also limits the support for species dependent on these specific habitats. The methods identified in this study for successful propagation of *G. trifida* may be applicable to other species within Cyperaceae. These efforts enhanced the ecological value of restoration projects by reintroducing a key species with multiple benefits in maintaining soil moisture levels, providing a host for insect larvae, and shelter for animals.

References

- Australia, State Herbarium of South. n.d. *Electronic Flora of South Australia*. Accessed December 20, 2023. <http://www.flora.sa.gov.au/census.shtml>.
- Barrett, RL. 2013. "Ecological importance of sedges: a survey of the Australasian Cyperaceae genus *Lepidosperma*." *Annals of Botany* 111: 499-529.
- Baskin, JM, and C Baskin. 2004. "A classification system for seed dormancy." *Seed Science Research* 14: 1-16.
- Kodym, A, I Clarke, A Aponte, S Turner, E Bunn, and J Delprott. 2014. "Large-scale micropropagation of the Australian key species *Gahnia Radula* (Cyperaceae) and its return to revegetation sites." *Australian Journal of Botany* 62: 417-427.
- Kodym, A, S Turner, and J Delpratt. 2010. "In situ seed development and in vitro regeneration of three difficult-to-propagate *Lepidosperma* species (Cyperaceae)." (*Australian Journal of Botany*) 58: 107-114.
- Martin, AC. 1946. "The Comparative Internal Morphology of Seeds." 36: 513-660.
- Panaia, M, E Bunn, SR Turner, and J McComb. 2009. "Incubation Temperature critical to successful stimulation of in vitro zygotic embryo growth in four Australian Native Cyperaceae species." *Plant Cell, Tissue and Organ Culture* 97: 197-202.
- Read, J, J Guerin, D Duval, and K Moseby. 2021. "Charred and chewed chalkies: Effects of fire and herbivory on the reintroduction of an endangered wattle." *Ecological Management & Restoration* 22: 35-43.
- South Australian Seed Conservation Centre. 2015. *Gahnia filum*.
https://syzygium.xyz/saplants/Cyperaceae/Gahnia/Gahnia_filum.html.
- Standards Reference Group, Society for Ecological Restoration Australasia (SERA). 2018. *ational Restoration Standards 2nd Edition*. September. Accessed 2023.
<https://www.seraustralasia.com/standards/National%20Restoration%20Standards%202nd%20Edition.pdf>.
- Thomas, PB, EC Morris, and TD Auld. 2003. "Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region." *Austral Ecology* 28: 674-683.
- Turner, SR. 2013. "Seed Ecology of *Lepidosperma scabrum* (Cyperaceae), a dryland sedge from Western Australia with physiological seed dormancy." *Australian Journal of Botany* 61: 643-653.
- Williams, PR, RA Congdon, AC Grice, and PJ Clarke. 2004. "Soil Temperature and depth of legume germination during early and late dry season fires in a tropical eucalypt savanna of north-eastern Australia." *Austral Ecology* 29: 258-263.

Acknowledgments

Funding for this work was provided by the Australian Flora Foundation. Thanks to Matt Coulter at the Mt Lofty Botanic Gardens nursery for germination trials on heated mats and Forktree Nursery for growing and potting on plants for restoration projects supported by Hills and Fleurieu Landscape Board, Green Adelaide Landscape Board, and National Parks and Wildlife. Seed collections from Morialta CP were assisted by Jack Casley-Smith, Matt Endecott, and Jason Van Weenan (Green Adelaide).