

Seed viability of native grasses is important when revegetating native wildlife habitat

Sean M. Bellairs and Melina J. Caswell

Research Institute for the Environment and Livelihoods and School of Environment,
Charles Darwin University, Darwin, NT 0909, Australia
Email: sean.bellairs@cdu.edu.au

Abstract

Native grasses are a dynamic and essential component of the majority of terrestrial ecosystems in the Northern Territory. Restoring native grasses in disturbed environments is important for providing faunal habitat, reducing surface erosion and resisting weed invasion. However, establishing native grasses has been problematic in many regions of Australia due to seed viability issues. We investigated 48 seed lots of 29 Northern Territory native grass species to determine whether seed quality was an issue for establishment of tropical native grasses. Seed lots were largely collected by commercial seed suppliers, rather than by research staff, so the samples reflect seed lots that could be sourced for revegetation projects. The seed purity, proportions of filled seeds, visually viable seeds and metabolically active seeds were assessed. Viability responses to storage were investigated in 15 seed lots. The proportion of florets that contained a seed (caryopsis) ranged from 10–97% (average 62%) and between 0–79% of the florets contained metabolically active seeds (average 36%). Two seed lots had viability of 0–10% and 12 of the 48 seed lots had less than 30% seeds that were metabolically active and potentially viable. Thus, seed quality limits establishment of tropical native grasses from sown seeds in the Northern Territory. When using native grasses to establish native habitat it is important to assess the quality of the seeds and use a sufficient quantity of seeds for effective establishment of these grasses. Seeds of many species will maintain viability for several years if stored in cool dry conditions. Seed for revegetation projects can therefore be collected and stored over several years.

Introduction

Native grasses are a feature of the vegetation communities of the Northern Territory. Spear grasses (*Heteropogon* and *Sorghum* spp.) and spinifex (*Triodia* spp.) are particularly dominant grasses in tropical and arid vegetation communities. In tropical communities there is typically a considerable diversity of other native grass species present. Native grasses provide a range of valuable ecological functions including:

- providing food for granivorous mammals and birds;
 - providing habitat for native fauna;
 - resisting invasion by introduced weed and improved pasture species; and
 - assisting with control of surface erosion.
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When re-establishing native vegetation communities during revegetation activities, the establishment of native grasses is often problematic. Active establishment of native grasses as part of revegetation activities generally relies on sown seeds, and those seeds need to be of good quality. In many regions of Australia seed biology issues relating to seed viability and seed dormancy often limit establishment of sown native grasses. We suggest this is also the case in the tropics of the Northern Territory.

The seed of a grass is termed a caryopsis and this is typically enclosed within two sheathing covering layers (the palea and lemma) to create a floret and several florets are enclosed within two glumes (Fig. 1). The seed of a native grass may be dispersed either

as a bare caryopsis or enclosed within the floret. Seed lots of native grasses generally contain florets, other inflorescence material such as the stigma and stamens (Fig. 2), and sometimes vegetative material, as well as the caryopses (Fig. 3). There may be a low proportion of viable seeds in a seed lot if the seed lot contains a high proportion of vegetative material, a high proportion of empty florets, or a high proportion of damaged or dead caryopses. A simple indicator of poor seed fill is to lightly press on the sides of the florets to feel the caryopsis. Alternatively, the floret



Fig. 1. Mature 'seeds' of Cockatoo Grass (*Alloteropsis semialata*) showing the intact spikelets (middle) and the actual seeds (caryopses) after extraction from the covering structures (top). The bottom spikelet has been opened to remove the caryopsis and the remains of the outer glumes and the inner lemmas covering the two florets can be seen. Opening or cutting the spikelet to check for a filled caryopsis is an easy initial test for checking the proportion of spikelets that contain viable seeds.



Fig. 2. Cockatoo Grass (*Alloteropsis semialata*) (above) and Giant Spear Grass (*Heteropogon triticeus*) (below) flowering. The Cockatoo Grass flowers have orange and yellow stamens and purple feather-like stigmas. The Giant Spear Grass has purple stamens and long brown awns protrude from the apex of the inflorescence.

can be cut in half to visually inspect the caryopsis. Sometimes caryopses may be of normal size and appearance but still be dead. To test viability in this case, a sample of the caryopses can be treated with tetrazolium chloride, a dye that will turn red if the tissues of the caryopsis are metabolically active (Merritt 2006).

Poor seed viability can be due to site factors affecting the grass plant, such as adverse seasonal growing conditions, habitat features that do not suit the species, or damage by fungi or insects. Some species are genetically disposed to poor seed production (Jacobs 1973). When collecting the seeds, seed maturity and collecting techniques can affect viability. After collection, storage conditions (including temperature, humidity, fungi and insects) affect the rate of deterioration of seed quality (Merritt 2006).

We investigated 48 seed lots of 29 native grass species to determine whether seed quality was an issue for the establishment of native grasses in the Top End of the Northern Territory. The proportions of filled seeds, seeds of normal appearance and metabolically active seeds were assessed. Viability responses to seed storage in an air conditioned room were investigated by repeated testing of 15 of the seed lots.

Materials and Methods

Seeds were mainly supplied by Greening Australia NT and by Kakadu Native Plant Supplies, with a list of desired species sent to their collectors. Seeds were collected in the Jabiru, Darwin and Katherine regions. Four seed lots (*Alloteropsis semialata* Lot 3; *Eriachne ciliata* Lot 3, *Eriachne schultzeiana* Lot 2 and *Thaumastochloa major* Lot 1) were collected with assistance from Charles Darwin University (CDU) staff and/or students. Seeds were collected between April 2005 and May 2011. The date or month of collection provided by the seed collector or seed supply company and the date of testing were recorded.



Fig. 3. Seeds and associated structures of Giant Speargrass (*Heteropogon triticens*) (top left), Kangaroo Grass (*Themeda triandra*) (top right), Love Grass (*Eragrostis spartinooides*) (bottom left) and Wanderrie grass (*Eriachne schultzeiana*) (bottom right). The small brown seeds or caryopses extracted from the covering structures are shown for Love Grass and Wanderrie Grass.

Stored seeds were accepted but we requested that new seed lots were sent to CDU as soon as possible after collection and cleaning of florets from vegetative material.

Most seed lots were tested within two months of arrival at CDU. Four of the 48 seed lots were not tested until 9–10 months after arrival and five seed lots were tested 14–16 months after being received (*Aristida inaequiglumis* Lot 2, *Eulalia aurea*, *Heteropogon contortus*, *Sorghum plumosum*, *S. timorensis*). If sufficient seeds were available, seed lots were resampled and retested after one or two years of storage.

Seed purity refers to the weight of undamaged florets and caryopses as a proportion of the total weight of the seed lot. Seed purity was assessed by removing all vegetative material, chaff and obviously damaged florets from undamaged florets and caryopses for small samples. Larger seed lots were sub-sampled using halving techniques prior to assessing purity of four sub-samples and the purity result is the average of those four sub-samples.

Seed fill and cut tests were conducted using four replicates of 25 florets. Seed fill data denoted the percentage of florets that contained a caryopsis within them when the florets were opened. For the cut test, the caryopsis was removed from the floret and inspected under a dissecting microscope, where the percentage of florets with visually viable caryopses were counted. Unfilled florets, shrivelled, discoloured or damaged caryopses and caryopses that had a missing embryo were assessed as not viable. Possibly viable seeds that were only slightly smaller or slightly discoloured were counted as viable and included in the tetrazolium assessment below. *Eragrostis spartinooides* did not have seed fill of florets assessed as the seeds disperse as caryopses and don't retain the outer floret structures.

For those seeds that were visually assessed to be viable or possibly viable, 2,3,5 triphenyl tetrazolium chloride (TTZ) was used to determine any metabolic activity. This colourless solution becomes red in response to metabolic activity in the tissue. Four replicates of 25 seeds were preconditioned by placing them in water at room temperature for 24 hours. The covering structures were removed or pierced away from the embryo to ensure water uptake without causing damage to the embryo. After imbibition, the seeds were cut through the embryo (or close to the embryo if cutting caused damage) except for *Eragrostis spartinooides* seeds, which were too small to cut. Half of each seed was then placed into 1% TTZ solution in a glass vial covered with aluminium foil to keep the incubating seed in darkness. The vials were placed in an incubator at 30°C for 24 hours, after which the seeds were removed and inspected under a dissecting microscope. Seeds with deep red-stained embryos and storage tissues were considered viable. Seeds that were unstained were not viable. Seeds with the embryo stained pale pink, or mottled staining of the storage tissues, were considered possibly viable. This resulted in a minimum and maximum proportion of viable seeds as assessed by TTZ.

Results

Seed viability of the grass species was variable, with *Aristida inaequiglumis* Lot 2 and *Themeda triandra* Lot 1 having less than 2–6% of viable florets, whereas *Heteropogon triticeus* Lot 1 and *Eragrostis spartinooides* had 79% and 90–100% viable seeds respectively (Table 1). Average viability of all grasses was moderate (37–42%).

Table 1. Viability of native grass seed lots. Each row is a separate seed lot. Age is the approximate time in weeks from collection to testing. Purity is the proportion by weight of caryopses plus florets that contain caryopses relative to the seed lot weight. Seed fill is the proportion of florets containing a caryopsis. Cut test is the proportion of florets containing a visually viable caryopsis. The last columns are the minimum and maximum viability of florets after tetrazolium (TTZ) testing as a proportion of florets (or caryopses if caryopses are shed from the florets).

Species	Age (weeks)	Purity (%)	Seed fill (%)	Cut test (%)	TTZ min (%)	TTZ max (%)
Cockatoo Grass <i>Alloteropsis semialata</i> (R.Br.) Hitchc.	5	98	53	35	25	33
	38	94	71	56	26	36
	4	-	61	-	20	41
Kerosene Grass <i>Aristida bolathera</i> Domin	34	35	97	94	65	76
Feathertop Threawn <i>Aristida inaequiglumis</i> Domin	52	66	44	44	24	35
	91	60	45	37	0	2
Golden Beard Grass <i>Chrysopogon fallax</i> S.T.Blake	58	68	85	68	34	46
	39	88	66	56	50	53
Ribbon Grass <i>Chrysopogon latifolius</i> S.T. Blake	45	91	52	24	14	18
Silky Heads <i>Cymbopogon bombycinus</i> (R.Br.) Domin	38	59	70	58	56	56
Queensland Bluegrass <i>Dichanthium sericeum</i> (R.Br.) A.Camus	40	86	43	34	30	31
	144	79	43	41	25	29
Hare's Foot Grass <i>Ectrosia leporina</i> R.Br.	39	55	55	49	49	49
Love Grass <i>Eragrostis spartinooides</i> Steudel	56	8	n/a	100	90	100
Wanderrie Grass <i>Eriachne agrostidea</i> F.Muell.	10	50	59	49	49	49
	18	56	65	41	34	37
Longawn Wanderrie Grass <i>Eriachne armittii</i> F.Muell. ex Benth.	20	32	67	46	37	41
	41	51	52	37	33	35
Wanderrie Grass <i>Eriachne avenacea</i> R.Br.	94	80	64	60	59	59
Wanderrie Grass) <i>Eriachne burkittii</i> Jansen	112	100	78	39	23	33
	11	78	49	20	13	14
Slender Wanderrie Grass <i>Eriachne ciliata</i> R.Br.	12	32	60	26	16	18
	9	42	89	53	48	49
Pan Wanderrie Grass <i>Eriachne glauca</i> R.Br.	31	100	74	42	19	26

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Species	Age (weeks)	Purity (%)	Seed fill (%)	Cut test (%)	TTZ min (%)	TTZ max (%)
Northern Wanderie Grass <i>Eriachne obtusa</i> R.Br.	16 39 15	100 72 82	51 53 62	41 36 49	25 41 38	32 44 42
Wanderie Grass <i>Eriachne schultzeana</i> F.Muell.	63 4	60 -	83 80	81 80	15 24	24 50
Wanderie Grass <i>Eriachne trisetata</i> Nees ex Steud.	56 95	30 17	42 40	40 27	27 26	34 27
Silky Browntop <i>Eulalia aurea</i> (Bory) Kunth	94	67	23	13	10	12
Black Speargrass <i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. & Schult.	92	36	73	65	27	36
Giant Speargrass <i>Heteropogon triticeus</i> (R.Br.) Stapf	- 91 40 21	100 27 52 70	91 89 75 62	83 75 29 38	79 70 20 30	79 72 26 36
Fire Grass <i>Schizachyrium fragile</i> (R.Br.) A.Camus	50	68	70	59	53	56
Pigeon Grass <i>Setaria apiculata</i> (Scribn. & Merr.) K.Schum.	126	100	87	73	53	64
Darwin Canegrass <i>Sorghum intrans</i> F.Muell. ex Benth.	92	55	69	61	59	60
Plume Sorghum <i>Sorghum plumosum</i> (R.Br.) P.Beauv.	92	46	57	56	51	52
Black Soil Canegrass <i>Sorghum timorense</i> (Kunth) Buse	41	68	89	83	62	66
<i>Thaumastochloa major</i> S.T.Blake	4 18	47 76	65 80	48 69	41 69	47 69
Kangaroo Grass <i>Themeda triandra</i> Forssk.	37 45 29	9 24 13	10 57 72	6 40 51	0 40 46	6 40 49
Curly Spinifex <i>Triodia bitextura</i> Lazarides	40	65	21	20	17	18
Mean of all seed lots	47.7	60.7	62.6	49.6	36.7	41.8
Minimum	4	8	10	6	0	2
Maximum	144	100	97	100	90	100

Seed purity was highly variable, ranging from 8–100%, but purity is dependent on the level of cleaning. The *Eragrostis* sp. seed batch had only 8% pure seeds but the seeds were tiny and numerous – the 17.8g seed lot still contained 24,200 seeds. Seed purity can also be highly variable between seed lots of a species; *Heteropogon triticeus* Lot 1 contained 100% pure seed whereas Lot 2 contained only 27% pure seeds.

Once chaff, vegetative material and damaged florets had been discarded, seed viability was largely dependent on the seed fill of the florets. On average, 37% of florets did not contain seeds. However, the proportion of filled seeds could be much lower – 90% of the florets of *Themeda triandra* Lot 1 did not contain caryopses and 79% of *Triodia bitextura* florets were empty. In contrast, other seed lots contained a high proportion of filled seeds – 72% of florets of *Themeda triandra* Lot 3 and 97% of the florets of *Aristida holathera* contained caryopses.

Closer inspection of the seeds was important for assessing viability as some filled seeds were not viable. Across all the seed lots, 62.6% of florets contained a caryopsis but only 49.6% of the caryopses were of normal appearance and contained an undamaged embryo. For the two seed lots of *Eriachne burkittii*, closer visual inspection determined that less than half of the filled florets contained viable caryopses. In contrast for the *Eriachne schultziiana* florets, almost all of caryopses present appeared viable after microscopic inspection.

Some caryopses that appeared normal and viable when visually inspected were not viable as they were not metabolically active when tested with TTZ. For some species, such as *Eriachne schultziiana*, visual inspection substantially overestimated the number of viable seeds, with 81% of seeds appearing viable in Lot 1 when inspected, but only 24% having any metabolic activity. For other species, such as *Cymbopogon bombycinus*, all the seeds that visually appeared to be viable were also metabolically active.

For the seed lots that were retested after at least one year of storage in the CDU laboratory, the decline in viability averaged 6% per year or a loss of 12% of viable seeds per year (Fig. 4). For some species, such as *Eriachne schultziiana*, there was substantial reduction in viability after 1–2 years but for others, such as *Eragrostis spartinooides*, there was little decline in viability. The *Eragrostis spartinooides* seed lot investigated in this study maintained viability and germination levels after 4.5 years storage and hence some native grass seed lots are able to be stored for quite some time. *Eriachne obtusa* and *Chrysopogon fallax* seed lots were able to be stored for two years with similar levels of viability to the initial assessments, however, after 3–4 years storage viability levels were low. *Eriachne schultziiana* and *Heteropogon triticeus* were able to be stored for 1–1.5 years, however, after further storage viability levels were low. *Alloteropsis semialata* seeds lost viability after 2 years storage with very low viability after 3 years.

Discussion

Generally, native grass seed lots newly received from seed suppliers in the Darwin region of the Northern Territory had reasonable levels of seed viability, similar to those for Australian native grass seed lots generally. Viability of four native grasses used for minesite rehabilitation in Western Australia was generally lower, ranging from 19–39% (Dixon 1997) and three seed lots of *Themeda australis* from New South Wales ranged from 52–68% (Nolan *et al.* 1997). Farley *et al.* (2013) assessed viability of 13 native grass

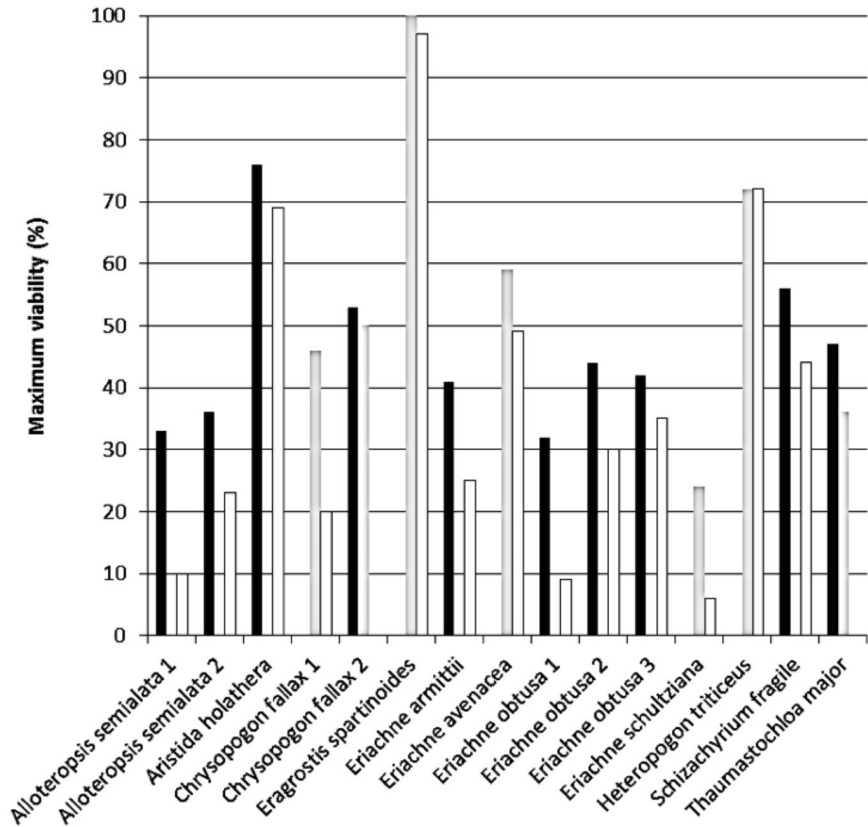


Fig. 4. Changes in grass seed viability over time (age since collection) of seed lots stored in sealed containers in an air conditioned room. Values are maximum viability of filled seeds that stained with tetrazolium chloride, a dye that turns tissue red if it is metabolically active. Where more than one seed lot was tested, the seed lot number is after the species name. Black: less than 1 year old; grey: 1–2 years old; white: 2 years old.

species that were hand collected and received at the seed laboratory within seven days of collection. Two species had higher than 80% viability and three species had less than 30% viability. The studies above all had viability tested using TTZ similarly to this study.

Commercial seed lots of exotic grasses used for sowing pastures and revegetation, which have been bred to have high seed production, often exceed 90% pure seeds (McCormick *et al.* 2009) and viability may exceed 85–90% (Cole and Johnston 2006; McKays Grass Seeds 2014; Grass Seed Online 2014) depending on the species. Tropical perennial grass cultivars from northern NSW purity ranged from 68–95% (Lodge and McCormick 2010). As purity and viability is lower for native grasses it is important to take this into account when determining seed application rates. To achieve the same number of

viable seeds as a seed lot with 90% purity and 90% viability, native grass seed lots with an average of 60% purity and 40% viability need a 3.375 times higher seed application rate (assuming seed sizes are the same). There is a tendency to apply less native grass seed because it is more expensive, but to achieve the same viable seed application rate, relatively more seeds need to be applied.

Purity of native grass seed can be increased, but this increases the cost of the seeds. Some seeds are also likely to be lost when carrying out further cleaning and sieving processes undertaken to increase purity. Another issue for cleaning the seeds of some species, is that structures such as long awns are damaged or lost and these may be important to help the seed orient itself correctly in the soil (Loch *et al.* 2004). Often the most economic option for native grasses is to apply seeds with a relatively high proportion of chaff rather than cleaning the seed lot to a high purity. It is important though, that the seed lot does not contain undesirable species, or insects or pathogens that will cause deterioration of the seed lot. In addition, for long-term storage, reducing the volume of non-seed material is important to reduce storage space.

Viability varies considerably between species and between seed lots of the same species. It is affected by the characteristics of the species, the seasonal growing conditions, the habitat where the plants were growing, maturity when harvested and by seed pathogens and granivores before or after storage (Merritt 2006). Wells *et al.* (2000) found that the optimum harvest window for *Triodia* spp. in the Kimberley region is just 5–7 days and if harvested before or after this, a substantial reduction in viability occurs. They also found a much higher quantity and quality of seed produced in more favourable locations and seasons. To control these factors, it is desirable to either test seed lots or use several seed lots collected from different sites.

Some native grass species have very broad ranges of viability but it is important that grass seed lots are regionally sourced. Obtaining seeds sourced from plants in the region is important to ensure the establishing plants are adapted to conditions that occur in the area where they are sown. For example, a trial in Brisbane investigated the performance of a seed lot of *Heteropogon contortus* sourced from Victoria compared to several seed lots sourced from Queensland. Plants of local and regional provenance seed lots grew and flowered whereas the seeds from Victoria produced robust plants that never flowered. The Victorian sourced plants likely required specific temperature and day length conditions to trigger flowering that did not occur in Brisbane (SMB unpubl.). Similar provenance effects on flowering are observed in *Themeda triandra* (Evans and Knox 1969).

Seed fill is an important first indication of seed quality. It can be tested when the seed lot is being collected by checking a selection of florets for filled seeds, either by pressing the side of the floret or by cutting them open to observe the caryopsis. If the grasses at a site have poor seed fill then collection can shift to a site with higher seed fill. The species characteristics also need to be considered. In the *Triodia* spp. studied by Jacobs (1973)

for example, seed production was restricted to just one or two seeds per spikelet with each spikelet containing six to eight florets. Even if grown in a glasshouse with sufficient water and nutrients, a maximum of three seeds per eight or more florets were produced, so even under optimum conditions many florets in these *Triodia* species remain empty.

The cut test could also be carried out in the field using a hand lens for species with larger seeds, but it requires familiarity with the grass seed to detect filled but abnormal seeds. In contrast, the tetrazolium test requires more time (two days), specific facilities and expertise, therefore is generally carried out by commercial seed testing laboratories. The cost can be a deterrent, but for larger revegetation projects the test is worthwhile when compared to the costs associated with repeating sowing activities due to inadequate amounts of viable grass seeds.

For larger projects, seed collection over several years and storage of seed lots is an option for obtaining sufficient seeds. The seeds used in this study were dried and stored in cool dry conditions in an air conditioned laboratory. Keeping the seed lots cool and dry is important as increasing the temperature by 5°C or increasing seed water content by 1% can double the rate at which seed viability is lost (Merritt 2006). Keeping tropical grass seeds at 30°C or higher temperatures and exposed to humid conditions greatly increases seed mortality (McIvor & Reid 2011) and could result in death of all seeds of some species within a year. Under low humidity conditions in a room that was air conditioned in summer, Silcock *et al.* (1990) in Charleville, Queensland, found 15 of the 20 native grass species tested could have seeds stored for at least three years with little deterioration. Twelve of the species could be stored for more than five years.

Native grasses are important for the structural integrity of native vegetation communities in the Northern Territory and it is important that they are included – along with trees, shrubs and forbs – when restoring habitat for wildlife. Many bird species are granivorous and dependent on native grass seeds. However, if native grasses are to be established in revegetation, it is necessary to use sufficient seeds of high quality and these seeds need to be stored to preserve viability. Seeding rates need to take purity, seed fill and viability into account. Some simple tests will give an indication of viability, but for large projects laboratory testing of viability using tetrazolium or germination testing is desirable.

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