



Assessing the storage potential of Australian rainforest seeds: a decision-making key to aid rapid conservation

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Abstract

Seed banking of rainforest species is hindered by lack of knowledge as to which species are tolerant of desiccation and freezing. We assessed 313 Australian rainforest species for seed banking suitability by comparing the germination percentage of fresh seeds to seeds dried at 15% RH and seeds stored at -20°C after drying. We then compared desiccation responses to environmental, habit, fruit and seed characteristics to identify the most useful predictors of desiccation sensitivity. Of 162 species with $\geq 50\%$ initial germination, 22% were sensitive to desiccation, 64% were tolerant and 10% were partially tolerant; the responses of 4% were uncertain. Of 107 desiccation tolerant species tested for response to freezing, 24% were freezing sensitive or short-lived in storage at -20°C . Median values for fresh seed moisture content (SMC), oven dry weight (DW) and the likelihood of desiccation sensitivity ($P_{D,S}$) were significantly greater for desiccation sensitive than desiccation tolerant seeds. Ninety-four to 97% of seeds with $\text{SMC} < 29\%$, $\text{DW} < 20\text{ mg}$ or $P_{D,S} < 0.01$ were desiccation tolerant. Ordinal logistic regression of desiccation response against environmental, habit, fruit and seed characteristics indicated that the likelihood of desiccation sensitivity was significantly increased by a tree habit, fleshy fruit, increasing fresh SMC and increasing $P_{D,S}$. The responses observed in this study were combined with earlier studies to develop a simple decision key to aid prediction of desiccation responses in untested rainforest species.

Keywords Desiccation sensitivity · Desiccation tolerance · Ex situ conservation · Freezing sensitivity · Seed banking · Seed storage behaviour

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Introduction

Rainforests are highly diverse plant communities (Corlett and Primack 2008; Sommerville et al. 2018) that provide habitat for a wide range of fauna, essential goods such as food, medicines, and clean water, and essential services such as maintenance of soil fertility, regulation of air quality and sequestration of carbon (Nahuelhual et al. 2007; Golden et al. 2012; Brandt et al. 2014; Alamgir et al. 2016; Delgado-Aguilar et al. 2017). The capacity to provide such services has been found to be greatest in areas of highest diversity (Brandt et al. 2014) yet rainforest diversity around the world continues to decline under pressure from logging and clearing for agriculture and mining (Corlett and Primack 2008; FAO 2015; Sommerville et al. 2018). Additional pressures from drought, fire, disease and climate change are driving some species towards extinction (Costion et al. 2015; Makinson 2018; Sommerville et al. 2019; Amigo 2020; Fensham et al. 2020; Halofsky et al. 2020). There is a need to act quickly to conserve rainforest diversity in ex situ collections, both to guard against the extinction of individual species and to provide a source of material for restoring rainforest habitats.

Seedbanking is the most efficient and cost-effective method for conserving seed-bearing species ex situ (Offord and Meagher 2009) and protocols for conserving seed from relatively dry habitats have been well-honed over the past few decades. Species in these habitats tend to produce seeds with characteristics essential to surviving storage in a seedbank (Tweddle et al. 2003; Wyse and Dickie 2017), i.e. tolerance of drying to low moisture content and tolerance of storage at cold temperatures (Roberts 1973). In contrast, these characteristics are considered to be lacking in the seeds of many rainforest species (Vázquez-Yanes and Orozco-Segovia 1993). Tweddle et al. (2003), for example, found that 47% of 178 tree and shrub species from tropical-subtropical evergreen rainforest were sensitive to the drying required for seedbanking. Similar results were obtained by Hamilton et al. (2013) for Australian rainforests (43% of 69 woody species), while Lan et al. (2014) found the proportion to be much higher in tropical rainforest in Southern China (68% of 41 wild woody species). In contrast, herbaceous species often have desiccation tolerant seeds and recent modelling by Wyse and Dickie (2017) indicated that the proportion of species with desiccation sensitive seeds in evergreen rainforest may be as low as 18.5% when herbaceous species are included.

The expectation of high levels of desiccation sensitivity in rainforest habitats has meant that the bulk of global seed banking efforts to date have been directed toward dryland species. The Millennium Seedbank in the Royal Botanic Gardens, Kew, for example, now holds seed collections for more than 36,975 species from 189 countries and territories, the majority of which were collected from dry habitats (Liu et al. 2018). The ongoing destruction of rainforests around the globe indicate that it is essential to begin directing more effort towards seed banking species from wetter environments. This, in turn, requires a concerted effort to determine which species may be stored using conventional methods and which may not.

In 1996, Hong and Ellis published a reliable protocol for determining seed storage behaviour based on testing germination following drying to progressively lower moisture content and storage at progressively lower temperatures. Accurate determination of the response to drying and storage using this protocol demands thousands of seeds which the authors acknowledged are not always available for wild species. Several options for adapting the protocol were proposed for situations where seeds were limited; however, for rainforest species, application of even the modified protocols is complicated by the difficulty

of collecting seeds in sufficient number for complete testing (particularly for species with very large seeds), and the great length of time it can take to complete a single germination experiment (up to 2 years, in some cases; Sommerville et al. 2018).

A protocol reducing the number of seeds required to 100 was developed by Pritchard et al. (2004a, b). This method is useful for gaining an indication of response to desiccation to low moisture content but doesn't give an indication of whether a species might tolerate drying to a lesser extent, nor whether the desiccation tolerant species are likely to tolerate cold storage. The method is also still subject to the vagaries of seed germination, and the requirement to hold a control sample of seeds in moist storage can be difficult to meet due to the tendency of rainforest seeds to lose viability (Mattana et al. 2019), become contaminated with fungi (Baskin and Baskin 2014) or to begin germinating (Berjak and Pammenter 2008) under moist storage conditions. A surrogate method for distinguishing desiccation sensitive from desiccation tolerant species, without performing germination tests, would greatly aid the conservation of such species.

A variety of morphological and ecological differences have been observed between desiccation sensitive and desiccation tolerant seeds that might be useful in this regard. For example, 99% of seeds with physical dormancy (i.e. a water-impermeable seed coat) have been found to be tolerant of both desiccation and cold storage (Tweddle et al. 2003). The average moisture content (at maturity) and dry weight of desiccation sensitive seeds have been found to be significantly greater than that of desiccation tolerant seeds (Hong and Ellis 1996; Daws et al. 2005; Hamilton et al. 2013), while seeds with a dry weight less than 10 mg were found, in one study, to be uniformly desiccation tolerant (Hamilton et al. 2013). The average seed coat ratio (the ratio of the mass of the dried seed coat to the whole seed) of desiccation sensitive seeds has been found to be significantly lower than desiccation tolerant seeds (Chen et al. 2020), while seeds with green embryos have generally been found to be desiccation sensitive (Hamilton et al. 2013). In terms of ecology, desiccation sensitivity has been found more frequently in species growing in moist habitats than arid habitats (Tweddle et al. 2003; Wyse and Dickie 2017) and has been found more likely to occur in seeds dispersed during the wet season than the dry season (Daws et al. 2005). All of these observations are useful to understanding seed ecology and the potential for desiccation sensitivity; however, overlap in the values of some traits, and notable exceptions in others, has limited their individual usefulness in predicting the desiccation response of untested species.

Several attempts have been made to develop models for predicting desiccation sensitivity using two or more seed traits. One method proposed by Hong and Ellis (1996) utilised dry seed weight and seed moisture content at shedding. This method accurately predicted desiccation tolerance for small seeds with relatively low moisture content, and desiccation sensitivity for large seeds with high moisture content, but could not distinguish between desiccation responses for seeds in the middle of the range for both characters. Similar results were obtained using this model by Ellis et al. (2007) and Lima et al. (2014). Daws et al. (2006) developed a model utilising dry seed weight and seed coat ratio to derive a value for the probability of desiccation sensitivity (P_{D-S}). This method was able to predict desiccation sensitivity with 85% accuracy for seeds that met particular criteria (i.e. $P_{D-S} > 0.5$; Daws et al. 2006; Lan et al. 2014). The method is useful for identifying some species that could be ruled out for standard seedbanking but does not distinguish between desiccation sensitive and desiccation tolerant seeds with P_{D-S} -values below 0.5. Pelissari et al. (2018) produced a revision of this model that incorporated the water content of the embryo and endosperm at shedding and found the model correctly classified both

desiccation tolerance and desiccation sensitivity in 46 of 50 tropical tree species. This method seems promising but has not yet been validated for species with a non-tree habit.

Recent modelling based on storage behaviour data for a large number of species has also provided useful information on indicators of desiccation response. Wyse and Dickie (2018) analysed data for 17,539 species held in the Kew Seed Information Database and determined that the most useful predictor of desiccation tolerance for a given species was the known response of other species in the same genus. The authors also found seed mass to be a significant predictor of desiccation sensitivity; however, this factor had a relative influence of only 2.7% in the model and so may be more difficult to apply directly. The model provided a great step forward in predicting seed storage behaviour for untested species; however, the data analysed were of necessity biased towards dryland species, and there are many genera occurring in rainforest for which no species have yet been tested (> 790 genera in the South Pacific region alone; Sommerville et al. 2018). In addition, there are some notable examples of genera which contain both desiccation sensitive and desiccation tolerant species (e.g. *Acer* and *Araucaria*; Hong and Ellis 1996). A step-by-step decision-making tool incorporating all of the above factors would aid the efforts of the seed banking community to conserve rainforest species from previously untested genera.

To derive some clarity from this complexity, we aimed to test the desiccation and freezing tolerance of 313 Australian plant species occurring in a variety of rainforest types. We compared desiccation responses to seed moisture content, seed weight, seed coat ratio and P_{D-S} to see if patterns observed in previous studies applied to this set of species. We analysed desiccation response in relation to a range of environmental, fruit, and seed characteristics to identify the most useful predictors of desiccation tolerance or sensitivity. Finally, we combined the results of this study with the findings of previous authors to develop a decision-making tool to facilitate more rapid screening of rainforest species for seedbanking.

Materials and methods

Collection

Fresh fruits for the majority of species tested were collected from wild populations in littoral, subtropical, temperate or dry rainforests in New South Wales, Australia (Fig. 1). The fruits collected consisted of a mixture of fleshy indehiscent, fleshy dehiscent, dry indehiscent and dry dehiscent types (Fig. 2). Where population density allowed, mature whole fruits were sampled from multiple individuals, but collections were generally only possible from a small number of individuals. For 17 species, seeds were collected from 1 to 3 individuals cultivated at the Australian Botanic Garden, Mount Annan; ten of these species were originally sourced from a known wild provenance; Online Resource 1). Fruits were collected directly from the plant or, if inaccessible on the plant itself, freshly fallen fruits (i.e. showing no obvious signs of deterioration) were collected from the ground beneath the plant. For species with asynchronous ripening, mature individual fruits were selected from the fruit available on the day of collection. For species with fleshy fruits or suspected high seed moisture content, whole fruits were placed in plastic bags and held in a portable electric fridge at 10 °C for up to ten days during transport to the Australian PlantBank. Dry-fruited species were placed in calico bags and held at ambient temperature during



Fig. 1 Seeds assessed for desiccation sensitivity were collected from a variety of rainforest habitats on the east coast of NSW, Australia

transport. Herbarium specimens, with accompanying data on habitat and location, were collected for each species and lodged at the National Herbarium of NSW.

Processing

The processing of fleshy fruited species commenced as soon as possible after material arrived at PlantBank; however, in some cases, material was stored at 15 °C for several days before cleaning. The total period of storage from collection to initial germination testing was 14 days or less. Seeds of dry-fruited species were generally extracted by hand. Seeds of fleshy fruited species were extracted by hand or by macerating the fruit and separating seed from pulp by flotation. For species where it was difficult to separate seed from flesh (e.g. *Fontainea* spp), the fruits were first macerated then agitated in a solution of 10 ml L⁻¹ enzyme (Everzym Liquid, Ever SRL, Pramaggiore, Italy) for up to 48 h to facilitate flesh removal.

A sub-sample of fresh seeds was set aside for germination testing and determination of seed moisture content. The remainder were transferred to a drying room maintained at approximately 15 °C and 15% relative humidity (RH). Equilibration to the drying environment was determined by periodically measuring the equilibrated RH of a sub-sample of seeds using a Rotronic® eRH meter (Model HP23-AW-A) with a Rotronic® water activity probe (Model HC2-AW; Pryde Measurement, Ingleburn NSW). Seeds were considered dry when the RH matched that of the drying room or when there was no further change in two successive measurements. A sub-sample of dried seeds was set aside for germination testing and determination of seed moisture content. The remainder were vacuum-sealed into laminated foil bags and placed into storage at -20 °C for at least one month before



Fig. 2 A selection of the rainforest fruits collected representing fleshy and dry, dehiscent and indehiscent fruit types: **a** *Archidendron hendersonii*; **b** *Cryptocarya laevigata*; **c** *Sloanea woollsii*; **d** *Argirodendron trifoliolatum*; **e** *Emmenosperma alphitonioides*; **f** *Davidsonia pruriens*; **g** *Pittosporum angustifolium*; **h** *Cynanchum elegans*; and **i** *Dysosylum fraserianum*

thawing and germination testing. For some species, seed collections already stored in vacuum-sealed packets at the Australian PlantBank were used to infer tolerance of drying and freezing, without comparison to fresh seeds, if they showed a high germination percentage after freezer storage (see Online Resource 1). In those cases, seed moisture content was tested on the thawed seeds and was assumed to approximate the moisture content at the time of packaging.

Determination of moisture content and dry weight

Seed moisture content (SMC) and oven dry weight (DW) were determined for whole fresh seeds and for seeds that had equilibrated to the drying environment. To determine SMC, seeds were weighed, dried for 17.5 ± 0.5 h at 103°C (following ISTA 2007), cooled to

room temperature over silica gel in an airtight container, then re-weighed. Seeds extracted from fleshy fruit (either by hand or using the maceration technique) were blotted dry with paper towelling prior to initial weighing.

For most species, average SMC and DW were calculated from 10 replicates of individual, randomly selected, seeds. For small-seeded species, SMC and DW were determined from 3 replicates of 5 to 100 seeds, depending on seed size. SMC was calculated as recommended by ISTA (2007).

Determination of seed coat ratio and P_{D-S}

Seed coat ratio (SCR) was determined by extracting the embryo and endosperm from ten individual seeds (for large-seeded species) or three replicates of 10 seeds (for smaller-seeded species). The separated embryo/endosperm and covering structures (endocarp and testa) from each seed were dried for 17.5 ± 0.5 h at 103°C , cooled to room temperature over silica gel in an airtight container, then re-weighed. SCR was calculated as the mean ratio of the weight of the dried covering structures to the weight of the whole dried seed (Grubb and Burslem, 1998; Pritchard et al. 2004a, b). The likelihood of desiccation sensitivity (P_{D-S}) was calculated using the formula proposed by Daws et al. (2006):

$$P_{D-S} = \frac{e^{3.269 - 9.974a + 2.156b}}{1 + e^{3.269 - 9.974a + 2.156b}}$$

where a is SCR and b is \log_{10} (seed dry weight) in g.

Germination testing

Germination tests were attempted for a total of 313 species representing a variety of plant forms (herbs, climbers, shrubs, shrub/trees and trees) from a range of rainforest habitats. To assess the impact of storage, the germination percentage of fresh seeds was compared to that of seeds dried at 15% RH and $15\text{--}20^\circ\text{C}$, and seeds stored at -20°C after drying. For a few species, germination following drying at higher relative humidity was also assessed. The germination conditions applied to each species (incubation temperature, substrate and pre-treatments) were selected based on those reported for previous germination tests where such information was available. This information was derived from an in-house database maintained by The Royal Botanic Gardens and Domain Trust, the online Kew Seed Information Database (Royal Botanic Gardens Kew 2020) and Floyd (2008).

In the absence of previous germination data, a substrate successfully used for germination of related species was selected and the species' distribution was used to determine the incubation temperature: species collected south of Sydney in New South Wales were germinated at 20°C ; all others were germinated at 25°C , with the exception of 13 species germinated in a glasshouse which were subjected to variable temperatures governed by the season they were sown (Online Resource 1). A 12-h photoperiod was maintained for all laboratory experiments; the photoperiod for glasshouse experiments ranged from 10 h in winter to 14 h in summer (<https://www.timeanddate.com/sun/australia/sydney>, accessed 9 Dec 2020). Where possible, woody endocarps were cracked in a vice or removed entirely prior to germination testing.

Tests conducted on agar consisted of three to five replicates of ten seeds per species. For large seeds, and those that were rapidly contaminated with fungi when sown on agar, 30–50 seeds were sown into individual cells of a plastic germination tray containing a 1:1 mix of steam-sterilised sharp sand and perlite. The germination trays were watered to run-off at the time of sowing then covered with a plastic hood. A reservoir of water was maintained beneath each germination tray for the duration of the experiment to assist in maintaining high relative humidity.

Seeds were considered to have germinated when 2 mm of the radicle had emerged (for seeds sown on agar) or the hypocotyl had emerged (for seeds sown in sand-perlite mix). Observations of germination were made weekly, initially, then less frequently if germination was prolonged and sporadic. Experiments were terminated when all seeds had germinated, or when no further germination had been observed in the preceding month. Seeds sown in seed-raising mix were left indefinitely so long as they appeared viable. At the conclusion of each experiment, any seeds that failed to germinate were dissected to assess their viability; the final germination percentage was adjusted for any empty seeds identified.

For germination experiments performed using three to five replicates in Petri dishes, differences in germination among fresh, dried and freezer-stored seeds were analysed using a non-parametric one-way analysis of variance (Kruskal and Wallis 1952). For germination experiments performed by sowing seeds into individual cells of seed-raising mix, differences among treatments were analysed using a 2×2 contingency table with chi-squared statistics calculated using the ‘ $N-1$ ’ method recommended by Campbell (2007).

Seed storage behaviour

Seed storage behaviour was assessed for seeds that had a fresh germination percentage of 50% or higher. In cases where fresh seed was not available, or the germination percentage was higher after drying, this criterion was applied to the germination percentage after drying. Seeds were described as ‘desiccation sensitive’ if the germination after drying was less than 5% and a post-germination cut test indicated any non-germinating seeds were no longer viable. Seeds were classified as ‘desiccation tolerant’ if germination after drying was not significantly less than germination when fresh or ‘partially desiccation tolerant’ if germination after drying was significantly reduced and any non-germinating seeds were no longer viable. Seeds were classified as ‘freezing tolerant’ if germination after freezing was not significantly different to germination after drying, and ‘freezing sensitive or short-lived’ if germination after freezing was significantly less than germination after drying. Based on these assessments, and the seed storage categories described by Hong and Ellis (1996) and Royal Botanic Gardens Kew (2020), seeds were categorised as ‘recalcitrant or intermediate’ (R/I) if sensitive to desiccation at 15% RH, ‘intermediate’ (I) if partially desiccation tolerant or freezing sensitive/short-lived, and ‘probably orthodox’ (O_p) if found to be tolerant of both drying and freezing.

Assessment of desiccation response by plant habit and habitat

The habit of each species, and the range of habitat types occupied, were determined by reference to Harden et al. (2006, 2007) and the online databases ‘Australian Tropical Rainforest Plants’ (Zich et al. 2018) and ‘PlantNET’ (RBG&DT 2020). Species were grouped into five habit categories (herb, climber, shrub, shrub/tree or tree) and four habitat categories: wet rainforest only (including tropical, subtropical, warm temperate and cool temperate

rainforest); dry rainforest only (including dry rainforest, monsoon rainforest and vine thickets); wet and dry rainforest (including any combination of wet and dry rainforest types); and mixed habitats (including any combination of rainforest and non-rainforest habitats). Species occurring in littoral rainforest were placed in the ‘wet and dry’ category since that habitat has characteristics of both rainforest types (Floyd 1990; Harden et al. 2006). Differences in the proportion of desiccation sensitive species among habit and habitat categories were analysed using 2×2 contingency tables with chi-squared statistics calculated using the ‘ $N-1$ ’ method recommended by Campbell (2007). Differences in the proportion of desiccation sensitive species among habit and habitat categories were also analysed for a larger dataset incorporating species from this study and 59 Australian rainforest species assessed by Hamilton et al. (2013).

Assessment of desiccation response by seed characteristics

Differences in fresh SMC, DW, SCR and $P_{(D-S)}$ among species classified as desiccation tolerant, partially desiccation tolerant or desiccation sensitive were compared using a non-parametric one-way analysis of variance (Kruskal and Wallis 1952) for this dataset and for a larger Australian dataset incorporating 59 species assessed by Hamilton et al. 2013. For each variable, ‘cut-off’ values above or below which the majority of seeds were desiccation sensitive (or desiccation tolerant) were noted.

The Australian dataset was then combined with data on 48 species from tropical rainforest in Southern China (Lan et al. 2014) and 104 species from tropical semi-deciduous rainforest in Panama (Daws et al. 2006). Differences in fresh SMC, DW and $P_{(D-S)}$ between desiccation sensitive and tolerant seeds were again assessed using a non-parametric one-way analysis of variance (Kruskal and Wallis 1952) and values marking a point above or below which the majority of seeds were either desiccation sensitive or tolerant were noted. The full range of values for each seed characteristic was then subdivided into 4–6 categories (each containing at least 45 species) and plotted against the relative proportions of desiccation sensitive and tolerant seeds. For this purpose, the 17 partially desiccation tolerant species from this study were included in the desiccation tolerant group on the assumption they will be storable with a slightly higher moisture content. $P_{(D-S)}$ values were used as provided in the datasets compiled by Lan et al. (2014) and Daws et al. (2006) but were calculated from the values for seed dry weight and seed coat ratio presented in Hamilton et al. (2013).

Modelling of desiccation responses

Logistic regression was used to assess the relative influence of latitude, climatic variables, habitat, habit, and fruit and seed characteristics on the likelihood of desiccation sensitivity. Data on latitude and longitude for each collection location were obtained using a hand-held GPS unit at the time of seed collection. Data on mean annual rainfall, temperature, and maximum temperature for each collection location were derived from the ANUClimate collection datasets compiled by Xu et al. (2014a, b, c) and described in Xu and Hutchison (2010).

Ordinal logistic regression with a logit link function was first used to fit a full model (Model 1) linking the desiccation response (sensitive, partially tolerant or tolerant) of 156 species from the present study to latitude, climate (mean rainfall, temperature, and maximum temperature at the collection location), plant characteristics [habit, habitat range, fruit

type (fleshy or dry) and fruit dehiscence type (dehiscent or indehiscent)] and DW. Predictor variables that were not associated with the outcome variable at a statistically significant level were removed from the model one by one (least significant variables first) until a reduced model was obtained where all the variables remaining were statistically significant ($P < 0.05$). Pearson and Deviance goodness-of-fit tests were applied to check the fit of the final model. A second OLR model combining significant predictors from Model 1 with fresh SMC and P_{D-S} was then fitted to a subset of 75 species for which those data were available (Model 2).

The 17 ‘partially desiccation tolerant’ species were then excluded and Binary Logistic Regression was used to analyse a third dataset assessing response to desiccation against habit, habitat, fresh SMC, DW, SCR and P_{D-S} for 139 species from this study and an additional 59 Australian species assessed by Hamilton et al. (2013). For this analysis, a logit link function was employed and the Hosmer–Lemeshow goodness-of-fit test was used to check model fit. All analyses were conducted in Minitab® v16.2.4 (Minitab Inc. Pennsylvania).

Development of a key to desiccation response

A step-by-step key to assigning the most likely desiccation response to untested species was developed using the most informative characteristics from this study combined with characteristics identified as strongly associated with desiccation tolerance or sensitivity in previous studies, i.e. dry weight and fresh seed moisture content (Hong and Ellis 1996; Ellis et al. 2007), habit and physical dormancy (Tweddle et al. 2003), P_{D-S} (Daws et al. 2006) and genus (Wyse and Dickie 2018). To test the utility of the key, we assumed no prior data on genus was available (Step 1) then applied Steps 2 to 6 in consecutive order to the 156 species from this study for which the desiccation response had been determined. Species assigned to a desiccation response were removed from the pool of species at each step (regardless of whether the assignment was correct or incorrect) and the subsequent step was applied to the species remaining till no further species could be assigned.

Results

Germination

Of the 313 species tested, a germination response $\geq 50\%$ was achieved for 162 species from 63 families (Online Resource 1); the remaining species germinated too poorly to enable reliable assessment of storage behaviour. For 22 species with germination $\geq 50\%$, pre-treatments or germination conditions differed between successive experiments due to attempts to control contamination or improve germination, or due to experimental error. These differences were ignored for the purpose of assigning storage behaviour if the seed germinated as well, or better, under the altered conditions as they did under the original conditions. Where the initial germination test was conducted on water agar and subsequent tests were on agar containing 250 ppm GA_3 , any reduction in germination in the latter was assumed to be due to the storage treatment given that GA_3 is a germination promoter and has not been observed to be toxic in this lab at the concentration used.

Of the 93 species for which fresh seeds were sown on agar, at least 35% appeared to be dormant based on the time to 50% of final germination exceeding 30 days (Online Resource

1; seeds sown in propagation mix could not be evaluated for dormancy as it was not possible to observe the timing of radicle emergence). Drying appeared to induce dormancy for *Diospyros pentamera* (germination significantly reduced after drying ($P < 0.05$) but seeds still viable) and to break dormancy for *Hymenosporum flavum* and two accessions of *Rhodomyrtus psidioides* (germination faster or significantly greater after drying ($P < 0.05$); Online Resource 1). Freezing appeared to break residual or drying-induced dormancy for *Diplocyclos palmatus*, *Entolasia marginata*, *Ficus virens*, *Helmholtzia glaberrima* and for one of two accessions of *Neoachmandra cunninghamii* (germination significantly greater after freezer storage ($P < 0.05$) than after drying; Online Resource 1).

Response to drying

Of the 162 species with a germination response $\geq 50\%$, 36 (22%) were desiccation sensitive, 103 (64%) were desiccation tolerant, and 17 (10%) were partially desiccation tolerant. The behaviour of six species (4%) was uncertain. Desiccation sensitivity was found in 20 families and was common in Lauraceae (4 of 5 species) and Sapindaceae (4 of 6 species; Table 1). Desiccation sensitive seeds tended to have larger dimensions than desiccation tolerant seeds and were more likely to possess such features as a woody endocarp, green embryo, or a thin seed coat surrounding a large embryo (Figs. 3 and 4). The response to desiccation was consistent among species for 14 of the 18 genera in which two or more species were studied (*Acronychia*, *Argyrodendron*, *Cordyline*, *Dysoxylum*, *Ficus*, *Fountainia*, *Flindersia*, *Parsonsia*, *Pittosporum*, *Psychotria*, *Rhodamnia*, *Senna*, *Solanum* and *Vesselowskyia*; Table 1). For the remaining four genera (*Ceratopetalum*, *Cryptocarya*, *Diospyros* and *Alphitonia*), the desiccation response was variable among species. The response to desiccation was consistent among genera within 12 of the 26 families for which two or more genera were studied; responses were variable among genera in 14 families.

Response to freezing

A total of 105 fully or partially desiccation tolerant species were tested for their tolerance of freezing: 73 species (70%) were tolerant of short-term storage (1 to 36 months) at $-20\text{ }^{\circ}\text{C}$; 27 species (26%) were either sensitive to freezing or short-lived in storage (Table 1, Online Resource 1). The response of 5 species was uncertain. Freezing sensitive/short-lived species were found in 14 families and were most common in the Myrtaceae (4 of the 8 species tested).

Assessment of desiccation response by plant habit and habitat

Tree species produced a significantly higher proportion of desiccation sensitive seeds (42%) than herbs (7%), climbers (17%), shrubs (5%) or shrub/trees (17%) ($P = 0.006$, $n = 156$; Fig. 5a). The relationship between habit and desiccation sensitivity remained when the dataset from the present paper was combined with 59 species from the earlier Australian study (Hamilton et al. 2013; Fig. 5b).

Species with a habitat range restricted to wet rainforests, or occurring in both wet and dry rainforests, had a higher proportion of desiccation sensitive species (30 and 25%, respectively) than species restricted to dry rainforest or with a distribution extending to non-rainforest habitat (0 and 11%, respectively; Fig. 6a); however, the differences between

Table 1 Storage behaviour of rainforest species collected from New South Wales, Australia

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	$P_{D,s}$	Response to drying	Response to freezing	SSB
Acanthaceae	<i>Harnieria hygrophiloides</i>	Shrub	TDRf, STRf, WTRf, DRf, VT, O	0.0017	0.24	0.016	15% RH Tolerant	- 20 °C Tolerant	Op
Anacardiaceae	<i>Euroschinus falcata</i>	Tree	STRf, DRf, Lrf, O	0.0143	0.60	0.011	Tolerant	Tolerant	Op
Annonaceae	<i>Metogyne stenopetala</i>	Shrub/tree	STRf, Lrf	0.1551	0.21	0.376	Pt tolerant	Sens/SL? ^g	I
Apocynaceae	<i>Astonia constricta</i>	Shrub/tree	DRf, Lrf, VT	0.0010	ND ^b	-	Tolerant	Tolerant	Op
	<i>Cynanchum elegans</i>	Climber	DRf, VT	0.0050	0.60	0.004	Tolerant	Tolerant	Op
	<i>Marsdenia rostrata</i>	Climber	TRf, STRf, WTRf, CTRf, Lrf, DRf	0.0165	0.63	0.001	Tolerant	Tolerant	Op
	<i>Melodinus australis</i>	Climber	TRf, STRf, WTRf, Lrf, DRf	0.0296	ND ^c	-	Tolerant	Sens/SL	I
	<i>Parsonsia fulva</i>	Climber	STRf, WTRf, CTRf	0.0100	ND ^b	-	Tolerant	Tolerant	Op
	<i>Parsonsia induplicata</i>	Climber	STRf, WTRf, CTRf	0.0038	ND ^b	-	Tolerant	Tolerant	Op
	<i>Tabernaemontana pandacaqui</i>	Shrub	TRf, MF, STRf, Lrf, WTRf	0.0453	0.14	0.268	Tolerant	ND ^c	ND
	<i>Tylophora benthamii</i>	Climber	TRf, MF, STRf, DRf, Lrf	0.0128	0.46	0.005	Tolerant	ND ^c	ND
Araceae	<i>Gymnostachys anceps</i>	Herb	TRf, Rf, O	0.1096	ND ^c	-	Sensitive	NA	R/I
	<i>Pothos longipes</i>	Climber	TRf, STRf, WTRf	0.0351	0.06	0.380	Sensitive	NA	R/I
Araliaceae	<i>Hydrocotyle pedicellosa</i>	Herb	TRf, Rf margins	0.0002	ND ^a	-	Tolerant	Tolerant	Op
	<i>Polyscias murrayi</i>	Tree	TRf, STRf, WTRf, Lrf	0.0019	0.38	0.002	Pt tolerant	Tolerant	I
Areaceae	<i>Linospadix monostachyos</i>	Shrub	STRf, WTRf, CTRf	0.0722	0.19	0.254	Sensitive	NA	R/I
Argophyllaceae	<i>Argophyllum nullumense</i>	Shrub/tree	STRf, WTRf	0.00005	ND ^a	-	Tolerant	Tolerant	Op
Asparagaceae	<i>Cordylone rubra</i>	Shrub	STRf, WTRf, Lrf	0.0127	0.31	0.021	Tolerant	Sens/SL? ^g	ND
	<i>Cordylone</i> sp. Mt Banda Banda	Shrub	CTRf	0.0128	0.32	0.019	Tolerant	Sens/SL	I
Asteliaceae	<i>Neostelia spectabilis</i>	Herb	CTRf	0.0002	-	-	Tolerant	Tolerant	Op
Asteraceae	<i>Sigesbeckia orientalis</i>	Herb	TRf, O	0.0008	0.51	0.002	Tolerant	Tolerant	Op
	<i>Vittadinia tenuissima</i>	Herb	Rf understory	ND ^c	ND ^b	-	Tolerant	Sens/SL	I
Atherospermat- aceae	<i>Doryphora sassafras</i>	Tree	STRf, WTRf, CTRf	0.0170	0.18	0.085	Tolerant	Sens/SL	I

Table 1 (continued)

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	P_{D-S}	Response to drying	Response to freezing	SSB
Bignoniaceae	<i>Pandorea pandorana</i>	Climber	WTRf, CTRf, GRf, LRf, DRf, VT	0.0038	–	–	Tolerant	Tolerant	Op
Boraginaceae	<i>Ehretia acuminata</i>	Tree	TDRf, DRf, STRf, WTRf	0.0131	0.83	0.000	Pt tolerant	Sens/SL	I
Capparaceae	<i>Apophyllum anomalum</i>	Shrub	VT	0.0279	0.45	0.010	Pt tolerant	Sens/SL	I
Celastraceae	<i>Celastrus subspicatus</i>	Climber	Warm Rf	0.0264	0.19	0.120	Tolerant	Tolerant	Op
	<i>Denhamia silvestris</i>	Shrub	DRf, VT, O	0.0078	0.37	0.008	Tolerant	Sens	I
	<i>Elaeodendron australe</i> var. <i>australe</i>	Shrub/tree	STRf, LRf, DRf, VT	0.2545	0.90	0.001	Tolerant ^l	Tolerant	ND
Commelinaceae	<i>Pollia crispata</i>	Herb	TRf, other Rf, Rf margins	0.0007	ND ^a	–	Tolerant	Tolerant	Op
Convolvulaceae	<i>Calyptegia marginata</i>	Climber	STRf, WTRf, LRf and margins	0.0123	ND ^c	ND	Tolerant	Tolerant	Op
Cucurbitaceae	<i>Diplocyclos palmatus</i>	Climber	TRf, MF, STRf, LRf, DRf, VT	0.0085	0.48	0.005	Tolerant	Tolerant	Op
	<i>Neoaechmanandra cunninghamii</i>	Climber	STRf, WTRf, DRf	0.0022	0.53	0.001	Tolerant	Tolerant	Op
Cunoniaceae	<i>Ackama paniculosa</i>	Tree	STRf, WTRf, CTRf	0.0001	ND ^a	–	Tolerant	Tolerant	Op
	<i>Ceratopetalum apetalum</i>	Tree	WTRf, CTRf, STRf	0.0205	0.61	0.002	Pt tolerant	ND	I
	<i>Ceratopetalum gummiferum</i>	Shrub/tree	Rf margins, O	0.0105	–	–	Tolerant	Sens/SL ^{2g}	I
	<i>Davidsonia pruriens</i>	Tree	TRf	0.1156	0.19	0.028	Sensitive	NA	R/I
	<i>Karrabina benthamiana</i>	Tree	STRf, WTRf	0.0006	ND ^b	–	Tolerant	Tolerant	Op
	<i>Vesselowskya rubifolia</i>	Shrub/tree	CTRf	0.0036	ND ^b	–	Tolerant	Tolerant	Op
	<i>Vesselowskya venusta</i>	Shrub/tree	CTRf	0.0001	ND ^b	–	Tolerant	Tolerant	Op
Cyperaceae	<i>Cyperus gracilis</i>	Herb	STRf, O	0.0020	–	–	Tolerant	Tolerant	Op
Dilleniaceae	<i>Hibbertia scandens</i>	Climber	TRf, STRf, WTRf, CTRf, LRf, O	0.0052	0.47	0.006	Tolerant	ND	ND
Dioscoreaceae	<i>Dioscorea transversa</i>	Climber	TRf, STRf, WTRf, DRf, LRf, O	0.0103	0.30	0.023	Tolerant	Tolerant	Op
Ebenaceae	<i>Diospyros australis</i>	Shrub/tree	TRf, STRf, LRf, DRf, VT	0.1713	ND ^c	–	Sensitive	NA	R/I
	<i>Diospyros fasciculosa</i>	Tree	TDRf, STRf, LRf, DRf	0.1339	0.12	0.582	Pt tolerant	Tolerant	I

Table 1 (continued)

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	P_{D-S}	Response to drying	Response to freezing	SSB
Elaeocarpaceae	<i>Diospyros mabacea</i> E	Tree	STRf	0.1701	0.36	0.145	Sensitive	NA	R/I
	<i>Diospyros pentamera</i>	Tree	STRf, DRf, LRF	0.1396	–	–	Tolerant	Tolerant	Op
	<i>Elaeocarpus obovatus</i>	Tree	STRf, DRf, LRF	0.1360	0.85	0.001	Sensitive	NA	R/I
Escalloniaceae	<i>Sloanea australis</i>	Tree	STRf, WTRf	0.0491	0.36	0.042	Sensitive ^l	NA	R/I
	<i>Anopterus maclayanus</i>	Shrub/tree	STRf, WTRf, CTRf	0.0195	0.26	0.070	Tolerant	Tolerant	Op
	<i>Acalypha capillipes</i>	Shrub	STRf, DRf	0.0029	0.33	0.005	Tolerant	Sens/SL	I
Euphorbiaceae	<i>Baloghia inophylla</i>	Tree	TDRf, STRf, LRF, DRf	0.0540	0.36	0.063	Tolerant	Sens/SL	I
	<i>Fontainea australis</i> V	Shrub/tree	STRf	0.9581	0.66	0.040	Pt tolerant	ND ^c	I
	<i>Fontainea oraria</i> CE, E	Shrub/tree	LRF	0.7992	0.76	0.011	Pt tolerant	ND ^c	I
Fabaceae	<i>Archidendron hendersonii</i> V	Tree	TRf, TDRf, STRf, LRF, DRf	0.3455	0.09	0.810	Sensitive	NA	R/I
	<i>Callerya megasperma</i>	Climber	STRf, LRF	8.8250	0.05	1.010	Tolerant	ND ^c	ND
	<i>Desmodium acanthocladium</i> V	Shrub	STRf, DRf	0.0197	0.10	0.103	Tolerant	Tolerant ^f	Op
Gesneriaceae	<i>Mucuna gigantea</i>	Climber	TRf, MF, STRf, LRF	2.2627	0.28	0.770	Tolerant	Tolerant	Op
	<i>Pararchidendron pruinosum</i>	Shrub/tree	TRf, STRf, LRF, WTRf, DRf	0.0271	0.51	0.010	Tolerant	Tolerant	Op
	<i>Senna acclinis</i>	Shrub	Margins STRf, LRF, DRf	0.0124	0.74	0.000	Tolerant	Tolerant	Op
Hemerocallidaceae	<i>Senna barronfieldii</i>	Shrub	STRf, WTRf, CTRf	0.0115	0.31	0.020	Tolerant	ND	ND
	<i>Fieldia australis</i>	Climber	WTRf, CTRf	0.00004	ND ^a	–	Tolerant	Tolerant	Op
	<i>Geitonoplesium cymosum</i>	Climber	TRf, STRf, WTRf, LRF, DRf, VT	0.0115	0.27	0.029	Tolerant	ND ^c	ND
Lamiaceae	<i>Callicarpa pedunculata</i>	Shrub/tree	TRf, STRf, O	0.0008	0.82	0.000	Tolerant	Sens/SL	I
	<i>Clerodendrum floribundum</i>	Shrub/tree	LRF	0.0274	0.65	0.004	Pt tolerant	ND	I
	<i>Gmelina leichhardtii</i>	Tree	STRf, LRF	0.4291	0.94	0.008	Tolerant	Sensitive	I
Lauraceae	<i>Plectranthus nitidus</i> E	Herb	TRf	0.0002	ND ^a	–	Tolerant	Tolerant	Op
	<i>Cinnamomum oliveri</i>	Tree	TRf, TDRf, STRf, WTRf	0.2245	0.06	0.780	Sensitive	NA	R/I
	<i>Cryptocarya laevigata</i>	Tree	STRf	0.9955	0.32	0.515	Sensitive ^{d,l}	NA	I ^k
	<i>Cryptocarya micronetra</i>	Tree	STRf, WTRf, DRf	0.5387	0.51	0.081	Pt tolerant ^k	ND ^c	I

Table 1 (continued)

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	P_{D-S}	Response to drying	Response to freezing	SSB
	<i>Cryptocarya rigida</i>	Tree	STRf, WTRf, LRF	0.4684	0.09	0.839	Sensitive	NA	R/I
	<i>Endiandra introixa</i>	Tree	WTRf	38.900	0.42	0.899	Sensitive	NA	R/I
	<i>Litsea reticulata</i>	Tree	STRf, WTRf	0.2420	0.05	0.808	Sensitive? ^g	ND ^c	I
	<i>Neolitsea dealbata</i>	Shrub/tree	TRf, STRf, WTRf, DRf, O	0.1700	0.14	0.554	Sensitive	NA	R/I
Linderniaceae	<i>Artanema fimbriatum</i>	Shrub	TRf, STRf	0.0001	ND ^a	–	Tolerant	Tolerant	Op
Lomandraceae	<i>Lomandra spicata</i>	Herb	Most types Rf	0.0228	ND ^b	–	Pt tolerant	Tolerant	I
Malvaceae	<i>Abutilon oxycarpum</i>	Shrub	STRf, DRf, VT	0.0012	0.41	0.001	Tolerant	Tolerant	Op
	<i>Argyrodendron actinophyllum</i>	Tree	STRf, WTRf, DRf	0.4125	0.77	0.014	Sensitive	NA	R/I
	<i>Argyrodendron trifoliolatum</i>	Tree	TRf, STRf	0.1952	0.56	0.036	Sensitive	NA	R/I
	<i>Brachychiton acerifolius</i>	Tree	TRf, TDRf, STRf, DRf	0.2473	0.49	0.050	Tolerant	Tolerant	Op
	<i>Corchorus cunninghamii</i> E	Herb	STRf, WSF	0.0014	0.58	0.000	Tolerant	Tolerant	Op
	<i>Sterculia quadrifida</i>	Tree	TRf, TDRf, MF, LRF, DRf, VT	0.6072	0.35	0.361	Sensitive	NA	R/I
Melastomataceae	<i>Melastoma affine</i>	Shrub	LRF, O	0.00006	ND ^a	–	Tolerant	Sens/SL	I
Meliaceae	<i>Dysoxylum mollissimum</i>	Tree	TRf, STRf, LRF	0.0906	0.13	0.446	Sensitive	NA	R/I
	<i>Dysoxylum rufum</i>	Tree	TRf, STRf, DRf	0.1928	0.09	0.702	Sensitive	NA	R/I
Menispermaceae	<i>Carronia multiselepala</i>	Climber	STRf	0.3953	ND ^c	–	Sensitive	NA	R/I
	<i>Stephania japonica</i> var. <i>discolor</i>	Climber	TDRf, MF, STRf, WTRf, DRf, LRF	0.0129	0.59	0.001	Tolerant	Sens/SL	Op
Monimiaceae	<i>Wilkiea huegeliana</i>	Shrub/tree	STRf, LRF, WTRf, DRf	0.5300	0.34	0.348	Sensitive	NA	R/I
Moraceae	<i>Ficus fraseri</i>	Shrub/tree	TRf, TDRf, STRf, LRF, DRf	0.0005	ND ^a	–	Tolerant	Tolerant	Op
	<i>Ficus hemeana</i>	Tree	TRf, MF, STRf, LRF, DRf	0.0006	ND ^a	–	Tolerant	Tolerant	Op
	<i>Ficus virens</i>	Tree	STRf	0.0003	ND ^a	–	Tolerant	Tolerant	Op
	<i>Ficus waikinsiana</i>	Tree	STRf, WTRf, LRF	0.0013	–	–	Tolerant	Tolerant	Op

Table 1 (continued)

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	P_{D-S}	Response to drying	Response to freezing	SSB
	<i>Maclura cochinchinensis</i>	Climber	TRf, TDRf, STRf, WTRf, LRF, DRf	0.0095	0.32	0.015	Sensitive	NA	R/I
Myrtaceae	<i>Archirhodomyrtus beckleri</i>	Shrub/tree	TRf margins, STRf, WTRf, O	0.0011	0.87	0.000	Tolerant	Sensitive	I
	<i>Astromyrtus dulcis</i>	Shrub	DRf, O	0.0021	0.76	0.000	Tolerant ¹	Sens/SL	I
	<i>Backhousia subargentea</i> E	Tree	DRf	ND ^c	ND ^c	–	Tolerant	ND ^c	ND
	<i>Gossia fragrantissima</i> E	Shrub/tree	STRf, DRf	0.0138	0.50	0.004	Tolerant	Tolerant	Op
	<i>Lenwebbia prominens</i>	Shrub/tree	STRf, WTRf	0.0172	0.97	0.000	Tolerant	Tolerant	Op
	<i>Ptilotostigma glabrum</i>	Shrub/tree	STRf, WTRf, LRF, O	0.0554	0.12	0.355	Sensitive	NA	R/I
	<i>Rhodamnia maidentana</i>	Shrub	STRf	0.0314	0.90	0.000	Tolerant	Sens/SL	I
	<i>Rhodamnia rubescens</i> CE	Shrub/tree	STRf, WTRf, LRF, DRf	0.0036	0.96	0.000	Tolerant	Tolerant	Op
	<i>Rhodomyrtus psidioides</i> CE	Shrub/tree	STRf, LRF, Rf margins	0.0051	–	–	Tolerant	Sensitive	I
	<i>Syzygium unipunctatum</i>	Tree	TRf	0.4917	–	–	Sensitive	NA	R
Nyctaginaceae	<i>Uromyrtus australis</i> E	Shrub/tree	WTRf	0.0134	0.94	0.000	Tolerant	Sens/SL	I
	<i>Pisonia umbellifera</i>	Shrub/tree	TRf, STRf, LRF	0.0505	0.16	0.271	Tolerant	Tolerant	Op
Onagraceae	<i>Ludwigia octovalvis</i>	Herb	TRf, Rf, VT, O	0.0001	ND ^a	–	Tolerant	Tolerant	Op
Paracryphiaceae	<i>Quintinia verdonii</i>	Tree	STRf, WTRf	0.00003	ND ^a	–	Tolerant	Tolerant	Op
Pennantiaceae	<i>Pennantia cunninghamii</i>	Tree	TRf, TDRf, STRf, WTRf	0.1681	ND ^c	–	Sensitive	NA	R/I
Phylodraceae	<i>Helmholtzia glaberrima</i>	Herb	Rf gullies	0.0001	ND ^a	–	Tolerant	Tolerant	Op
Phyllanthaceae	<i>Cleistanthus cunninghamii</i>	Shrub/tree	STRf, LRF, DRf	0.0177	0.51	0.003	Tolerant	Tolerant	Op
Piperaceae	<i>Piper hederaceum</i> var. <i>hederaceum</i>	Climber	TRf, STRf, WTRf, LRF	0.0200	0.26	0.052	Sensitive ¹	NA	R/I
Pittosporaceae	<i>Hymenosporum flavum</i>	Shrub/tree	TRf, STRf, WTRf, DRf, O	0.0039	0.57	0.005	Tolerant	Tolerant	Op
	<i>Pittosporum angustifolium</i>	Shrub/tree	VT	0.0140	0.67	0.004	Tolerant	Tolerant	Op
	<i>Pittosporum lancifolium</i>	Shrub/tree	STRf, DRf	0.0084	0.37	0.008	Tolerant	Tolerant	Op
	<i>Pittosporum multiflorum</i>	Shrub	Most types Rf	0.0122	0.23	0.044	Tolerant	Sensitive	I
Poaceae	<i>Entolasia marginata</i>	Herb	TRf, STRf, O	0.0011	ND	–	Tolerant	Tolerant	Op

Table 1 (continued)

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	P_{D-S}	Response to drying	Response to freezing	SSB
Proteaceae	<i>Panicum bisulcatum</i>	Herb	STRf, O	0.0006	ND	–	Tolerant ^g	Sens/SL	Op
	<i>Eidothea hardeniana</i>	Tree	WTRf	9.8089	0.84	0.050	Pt tolerant	ND ^c	I
	<i>Helicia ferruginea</i> V	Shrub/tree	STRf, WTRf	0.1842	0.62	0.010	Sensitive	NA	R/I
	<i>Hicksbeachia pinnatifolia</i> V	Tree	STRf	1.7564	0.52	0.202	Sensitive	NA	R/I
	<i>Lomatia arborescens</i>	Shrub/tree	STRf, WTRf, CTRf	0.0128	0.39	0.085	Tolerant	Tolerant	Op
Putranjivaceae	<i>Orites excelsus</i>	Tree	STRf, WTRf, CTRf	0.0188	0.30	0.039	Tolerant	Tolerant	Op
	<i>Drypetes deplanchei</i>	Tree	TDRf, MF, STRf, LRF, DRf, VT	0.2709	0.44	0.085	Tolerant	Tolerant	Op
		Tree	MF, DRf, O	0.0128	0.74	0.000	Tolerant	Tolerant	Op
Rhamnaceae	<i>Alphitonia excelsa</i>	Tree	TRf, STRf, Rf regrowth	0.0215	0.69	0.001	Pt tolerant	Sens/SL	I
	<i>Emmenosperma alphitonioides</i>	Tree	TRf, TDRf, STRf, WTRf, DRf	0.0381	0.54	0.006	Pt tolerant	Tolerant	I
Rousseaceae	<i>Abrophyllum ornans</i>	Shrub/tree	TRf, STRf, WTRf	0.0001	ND ^a	–	Tolerant	Tolerant	Op
	<i>Curtisia viburnea</i>	Shrub/tree	STRf, WTRf	0.00004	ND ^a	–	Tolerant	Tolerant	Op
Rubiaceae	<i>Gynochthodes jasminoides</i>	Climber	TRf, MF, STRf, WTRf, CTRf, LRF, DRf, VT	0.0044	0.69	0.000	Pt tolerant	Sens/SL	I
	<i>Psychotria daphnoides</i>	Shrub	STRf, DRf, VT	0.0111	0.33	0.014	Tolerant? ^g	Tolerant? ^g	ND
Rutaceae	<i>Psychotria loniceroides</i>	Shrub/tree	STRf, WTRf, LRF, DRf	0.0064	0.21	0.030	Tolerant	Tolerant	Op
	<i>Psychotria simmondsiana</i>	Shrub/tree	STRf, WTRf, CTRf	0.0081	0.21	0.039	Tolerant	Sens/SL ^f	I
	<i>Randia moorei</i> E	Shrub	STRf, LRF	0.0027	0.28	0.005	Tolerant	Tolerant	Op
	<i>Acradenia euodiformis</i>	Tree	STRf, WTRf, CTRf	0.0115	0.15	0.090	Tolerant	Sens/SL	I
	<i>Acronychia imperforata</i>	Shrub/tree	LRF, DRf, MF	0.0109	0.56	0.001	Tolerant	ND ^c	ND
Flindersiaceae	<i>Acronychia oblongifolia</i>	Shrub/tree	STRf, WTRf, DRf, LRF, VT	0.0052	–	–	Tolerant	Tolerant	Op
	<i>Clausena smyvellicana</i> E	Tree	LRF	0.0543	0.03	0.550	Sensitive ^l	NA	R/I
	<i>Flindersia pimenteliana</i>	Tree	TRf	0.0381	–	–	Tolerant ^l	Tolerant	Op
	<i>Flindersia schottiana</i>	Tree	STRf, LRF	0.0600	0.29	0.093	Tolerant	Sens/SL ^h	I

Table 1 (continued)

Family	Species	Habit	Habitat range	Dry wgt (g)	SCR	P_{D-S}	Response to drying	Response to freezing	SSB
	<i>Geigeria salicifolia</i>	Tree	TRf, MF, STRf, WTRf, LRF, DRf, VT	0.0278	0.82	0.000	Tolerant	Tolerant	Op
	<i>Micromelum minutum</i>	Tree	TRf, TDRf, LRF, DRf	0.0308	0.09	0.309	Sensitive ^l	NA	R?
Sapindaceae	<i>Alectryon subdentatus</i>	Shrub/tree	DRf, VT	0.1202	0.41	0.070	Pt tolerant	ND ^c	I
	<i>Castanospora alphanatii</i>	Tree	TRf, STRf	1.6727	0.14	0.917	Sensitive	NA	R/I
	<i>Cupaniopsis flagelliformis</i>	Tree	TRf, TDRf, STRf, DRf	0.1845	0.11	0.852	Sensitive	NA	R/I
	<i>Diploglottis australis</i>	Tree	STRf, WTRf, DRf	0.1288	0.12	0.530	Sensitive	NA	R/I
	<i>Jegera pseudorhus</i>	Tree	STRf, LRF, DRf	0.0744	ND ^c	–	Pt tolerant	ND ^c	I
	<i>Lepiderema pulchella</i> V	Tree	STRf	0.0568	ND ^c	–	Sensitive	NA	R/I
	<i>Mischarytera lautereriana</i>	Tree	TRf, STRf	0.5400	0.09	0.861	Sensitive	NA	R/I
	<i>Mischocarpus australis</i>	Tree	STRf	0.0518	0.19	0.211	Sensitive ^{yg}	ND ^c	ND
	<i>Sarcopteryx stipata</i>	Tree	STRf, WTRf, LRF	0.1149	0.17	0.386	Sensitive ^{yg}	ND ^c	ND
Sapotaceae	<i>Planchonella australis</i>	Tree	STRf, LRF, DRf	1.7648	0.83	0.012	Sensitive ^d	NA	I ^k
Simaroubaceae	<i>Ailanthus triphysa</i>	Tree	STRf, LRF, DRf	0.0315	0.21	0.161	Tolerant	ND ^c	ND
	<i>Quassia bidwillii</i>	Shrub	STRf, O				Tolerant	Tolerant	Op
	<i>Quassia</i> sp. Mount Nardi	Shrub/tree	STRf, WTRf	0.2912	0.62	0.017	Tolerant ^l	Sens/SL	I
Solanaceae	<i>Nicotiana forsteri</i>	Herb	STRf, VT, MF, O	0.0001	ND ^a	–	Tolerant	Tolerant	Op
	<i>Solanum nemophilum</i>	Shrub	VT margins, O	0.0011	0.47	0.001	Tolerant	Tolerant	Op
	<i>Solanum vicinum</i>	Shrub	WTRf, CTRf, margins of STRf	0.0007	ND ^a	–	Tolerant	Tolerant	Op
Thymelaeaceae	<i>Wikstroemia indica</i>	Shrub	STRf, LRF, DRf, O	0.0156	0.34	0.021	Tolerant?	Tolerant? ^l	ND
Urticaceae	<i>Boehmeria macrophylla</i>	Shrub	STRf	0.0001	ND ^a	–	Tolerant	Tolerant	Op
	<i>Dendrocnide excelsa</i>	Tree	STRf, DRf	0.0005	ND ^a	–	Tolerant	Tolerant	Op
Vitaceae	<i>Cissus antarctica</i>	Climber	TRf, STRf, WTRf, LRF, DRf	0.0651	ND ^b	–	Tolerant? ^g	ND ^c	ND
	<i>Tetrastigma nitens</i>	Climber	TRf, STRf, LRF, DRf	0.0890	0.35	0.086	Tolerant	Tolerant	Op

Table 1 (continued)

Species names followed by **CE**, **E**, or **V** are listed as Critically Endangered, Endangered or Vulnerable, respectively, under State or Federal legislation. Habitat range includes the full range of rainforest and other habitats occupied by each species. Habitat classifications follow Harden et al. (2006, 2007) and Zich et al. (2018) for trees, shrubs & climbers and RBG&DT (2020), Zich et al. (2018) or collection habitat description for herbs. *Rf* rainforest, *CT* cool temperate, *D* dry, *L* littoral, *MF* monsoon forest, *ST* subtropical, *T* tropical, *WT* warm temperate, *VT* vine thicket, *O* other, including one or more of the following non-rainforest habitats: wet sclerophyll forest, sclerophyll forest, open forest, woodland, scrub or grassland. Seed dry weight (Dry wgt), seed coat ratio (SCR), and the likelihood of desiccation sensitivity ($P_{D,S}$) represent the mean values for 10 individual seeds, or three replicates of 10–100 seeds, depending on seed size. Seed response to desiccation at 15% relative humidity was classified as: 'Tolerant' if there was no significant reduction in germination compared to fresh seeds; 'Pt tolerant' (partially tolerant) if treatment resulted in a significant decline in germination; 'Sensitive' if treatment reduced germination to 5% or less. Seed response to freezing at -20°C was classified as: 'Tolerant' if no significant reduction in germination compared to dried seeds; 'Pt tolerant' if treatment resulted in a significant decline in germination; or 'Sensitive' if treatment reduced germination to 5% or less. Freezing tolerance was classified as 'Sens/SL' (sensitive or short-lived) if germination was significantly reduced and seeds had been held for an extended time in the drying room before freezing or had been held in the freezer for longer than 6 months. Seed storage behaviour (SSB) was classified according to the Kew Seed Information Database (Royal Botanic Gardens Kew 2020) as: O_p (probably orthodox) if seeds tolerated both drying and freezing; R/I (recalcitrant or intermediate) if seeds were sensitive to drying at 15% RH; and I (intermediate) if seeds were only partially tolerant of drying or were tolerant of drying but sensitive to freezing or short-lived in storage. Genera highlighted in bold text are those for which no storage behaviour data had previously been published. *ND* not determined, *NA* not applicable. See Online Resource 1 for detailed germination and storage data

^aSeed too small to test SCR

^bEmbryo difficult to separate from testa

^cInsufficient seed for test

^dSubsequent tests showed this species was tolerant of partial drying

^eData derived from Royal Botanic Gardens Kew Seed Information Database (2018)

^fTest for freezing tolerance conducted on a different accession to test for desiccation tolerance

^gUngerminated seeds appeared viable on termination of test so drying/freezing may have induced dormancy

^hSeed may have been mechanically damaged by vacuum sealing rather than freezing

ⁱWide variation among replicates may have prevented detection of a significant difference

^jGermination reduced, but not significantly

^kBased on response to partial drying

^lResponse identical to that reported by Hamilton et al. (2013)

habitat categories were not significant ($P=0.099$, $n=156$). This pattern was repeated in the combined dataset (Fig. 6b).

Assessment of desiccation response by seed characteristics

The values for fresh SMC, DW, SCR and P_{D-S} for each desiccation response category overlapped in range but, in all cases, the median values for desiccation sensitive seeds were significantly different to desiccation tolerant or partially tolerant seeds (Fig. 7).

Fresh SMC ranged from 9.7 to 85.6% for desiccation sensitive seeds, 3.7 to 63.6% for desiccation tolerant seeds and 7.2 to 62.7% for partially desiccation tolerant seeds. The median fresh SMC of desiccation sensitive seeds was significantly greater than both desiccation tolerant ($P=0.000$, $n=79$) and partially desiccation tolerant seeds ($P=0.001$, $n=46$; Fig. 7a). Low fresh SMC was a good indicator of desiccation tolerance; of 39 species with a fresh SMC < 29%, only 1 was desiccation sensitive compared to 50% of the 60 species with a fresh SMC $\geq 29\%$.

DW ranged from 0.01 to 38.9 g for desiccation sensitive seeds, < 0.0001 to 8.8 g for desiccation tolerant seeds, and 0.002 to 9.8 g for partially desiccation tolerant seeds. The median DW of desiccation sensitive seeds was significantly greater than both desiccation tolerant ($P=0.000$, $n=137$) and partially desiccation tolerant seeds ($P=0.033$, $n=52$; Fig. 7b). Very light-weight seeds were most likely to be desiccation tolerant. Of the 84 species with a seed dry weight < 0.02 g, only 1 was desiccation sensitive; the remainder were fully (95%) or partially (4%) desiccation tolerant. In contrast, of the 74 species with seed dry weight ≥ 0.02 g, 47% were desiccation sensitive.

Separation of the embryo and testa was not possible for very small seeds, nor for seeds in which the embryo/endosperm was firmly attached to the testa or in which the testa was folded into the embryo. SCR and P_{D-S} were therefore determined for 109 species ranging in dry weight from 0.0008 to 38.9 g. SCR ranged from 0.03 to 0.83 for desiccation sensitive seeds, 0.05 to 0.97 for desiccation tolerant seeds and 0.12 to 0.84 for partially desiccation tolerant seeds. The median SCR for desiccation sensitive seeds was significantly lower than both desiccation tolerant ($P=0.000$, $n=89$) and partially desiccation tolerant seeds ($P=0.000$, $n=43$, Fig. 7c) while the median P_{D-S} was significantly higher than both desiccation tolerant ($P=0.000$, $n=89$) and partially desiccation tolerant seeds ($P=0.000$, $n=43$; Fig. 7d). Low P_{D-S} values were useful for predicting desiccation response; of 39 species with $P_{D-S} < 0.01$, all were either desiccation tolerant (82%) or partially tolerant (18%). The value used by Daws et al. (2006) to predict desiccation sensitivity ($P_{D-S} > 0.5$) correctly predicted desiccation sensitivity for 12 of 16 species (75%). When results were categorised by plant habit, $P_{D-S} > 0.5$ correctly predicted desiccation sensitivity for 10 of 12 tree species (83%). A lower value of $P_{D-S} > 0.3$ correctly predicted desiccation sensitivity for 13 of 15 tree species (87%).

Seed characteristics as predictors of desiccation response

Similar relationships between desiccation response and fresh SMC, DW and P_{D-S} were found in the dataset combining seed characteristics from 348 rainforest species in Australia, Panama and Southern China. The median values for fresh SMC, DW and P_{D-S} of desiccation sensitive seeds were all significantly greater than for desiccation tolerant seeds ($P=0.000$ in all cases; $n=286$, 347 and 299, respectively), while the median value for SCR

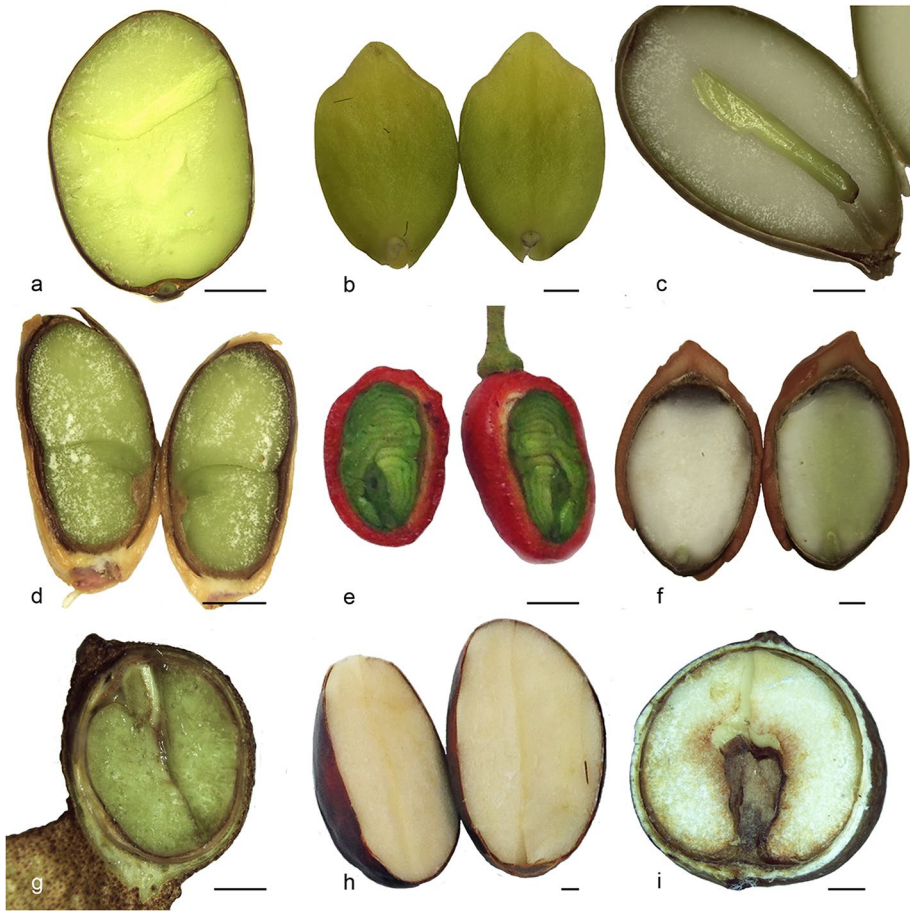


Fig. 3 A selection of seeds found sensitive to drying to equilibration with 15% relative humidity: **a** *Harpulia pendula*; **b** *Helicia ferruginea*; **c** *Pennantia cunninghamii*; **d** *Lepiderema pulchella*; **e** *Micromelon minutum*; **f** *Sloanea woollsii*; **g** *Argyrodendron trifoliolatum*; **h** *Cupaniopsis flagelliformis*; and **i** *Cryptocarya foetida*. Scale bar = 2mm

was significantly less ($P=0.000$, $n=299$). Seeds with a fresh SMC $<20\%$ were desiccation tolerant (Fig. 8a) while 98% of species with DW <0.02 g were fully or partially desiccation tolerant (Fig. 8b). A P_{D-S} value >0.5 correctly predicted desiccation sensitivity for 78 of 93 species (84%) in the combined dataset, and for 56 of 64 tree species (88%). Of the 15 species that had a $P_{D-S} >0.5$ but were desiccation tolerant, seven were from the Fabaceae, a family that has a high proportion of species with physically dormant and orthodox seeds. The lower value of $P_{D-S} >0.3$ correctly predicted desiccation sensitivity for 79% of all species, or 86% of trees, in that range. A P_{D-S} value <0.01 correctly predicted desiccation tolerance for all 70 species in that range (Fig. 8c).

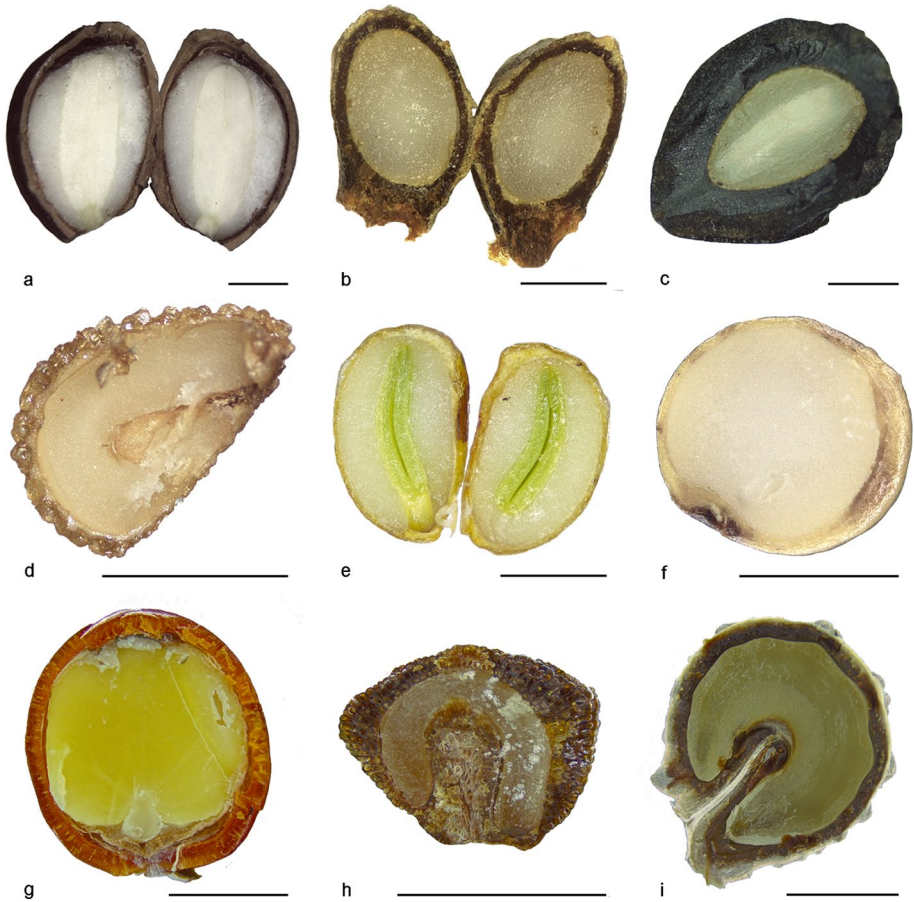


Fig. 4 A selection of seeds found tolerant of drying to equilibration with 15% relative humidity: **a** *Akania bidwillii*; **b** *Hibbertia scandens*; **c** *Geijera salicifolia*; **d** *Rhodomyrtus psidioides*; **e** *Denhamia sylvestris*; **f** *Pittosporum multiflorum*; **g** *Emmenosperma alphonoides*; **h** *Archirhodomyrtus beckleri*; and **i** *Stephania japonica* var. *discolor*. Scale bar = 2mm

Modelling of responses

Ordinal logistic regression (OLR) of desiccation response for 156 Australian species against latitude, rainfall, mean and maximum temperature, habit, habitat category, fruit characteristics and seed dry weight (Model 1) identified two significant predictors—habit (tree, $P=0.004$) and fruit type (fleshy, $P<0.001$). With odds ratios of 5.3 and 4.8, respectively, these two factors significantly increased the likelihood of desiccation sensitive seeds compared to plants with other habits or dry fruit. Pearson and Deviance goodness-of-fit tests indicated the model fitted the data well ($P=0.645$ and 0.452, respectively; $n=156$), with 70.2% concordance between the response variable and predicted probabilities. OLR of a smaller dataset combining habit and fleshiness with fresh SMC and P_{D-S} identified both fresh SMC and P_{D-S} as the only significant predictors ($P=0.001$ and 0.009, respectively; $n=75$). Note that this dataset excluded many small and low weight seeds for which fresh SMC and P_{D-S} were not determined. Odds ratios of 1.04 and 32.8, respectively,

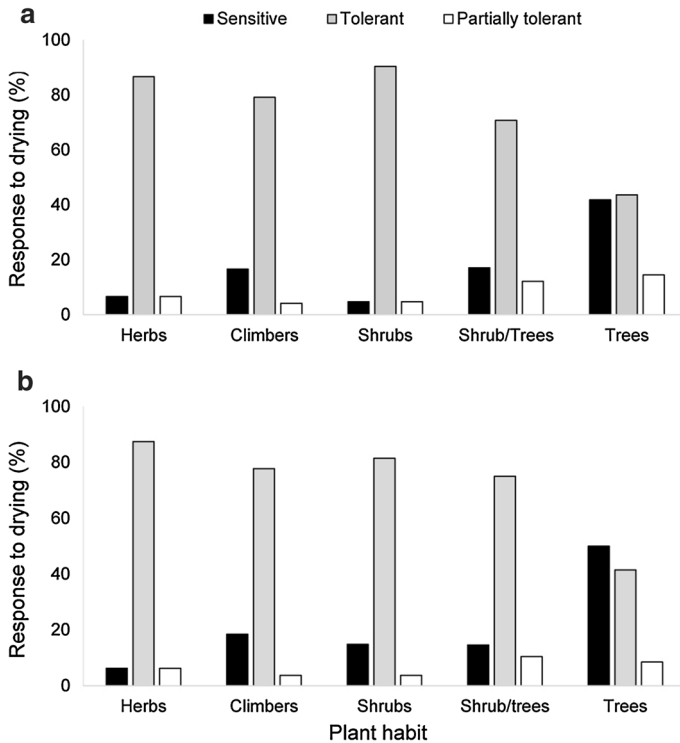


Fig. 5 The relative proportions of desiccation sensitive, tolerant and partially tolerant seeds among Australian rainforest species with habits described as herb, climber, shrub, shrub/tree or tree for: **a** species from this study only ($n = 12, 23, 22, 35$ and 52 , respectively); and **b** species from this study combined with those published in Hamilton et al. (2013; $n = 16, 27, 27, 48$ and 94 , respectively)

indicated a small increase in the likelihood of desiccation sensitivity with each percentage point increase in SMC and a large increase in the likelihood of desiccation sensitivity with an increase in P_{D-S} . Both Pearson and Deviance goodness-of-fit tests indicated the model fitted the data well ($P = 0.733$ and 0.991 , respectively) with 84% concordance between the response variable and predicted probabilities.

Binary Logistic Regression (BLR) of desiccation response (desiccation tolerant or sensitive only) against habit, habitat, dry weight, fresh SMC, SCR and P_{D-S} for Australian species from the present study and Hamilton et al. (2013) also identified fresh SMC and P_{D-S} as the only significant predictors ($P < 0.001$ in both cases; $n = 118$). Odds ratios of 0.93 and 0.00, respectively, in this case suggested fresh SMC was the more influential variable, with an increase in fresh SMC reducing the likelihood of desiccation tolerance. The Hosmer–Lemeshow goodness-of-fit test indicated the model fitted the data well ($P = 0.074$), with 92% concordance between the response variable and predicted probabilities. Note that 3 palms were excluded from this analysis as that habit category was not present in both data sets.

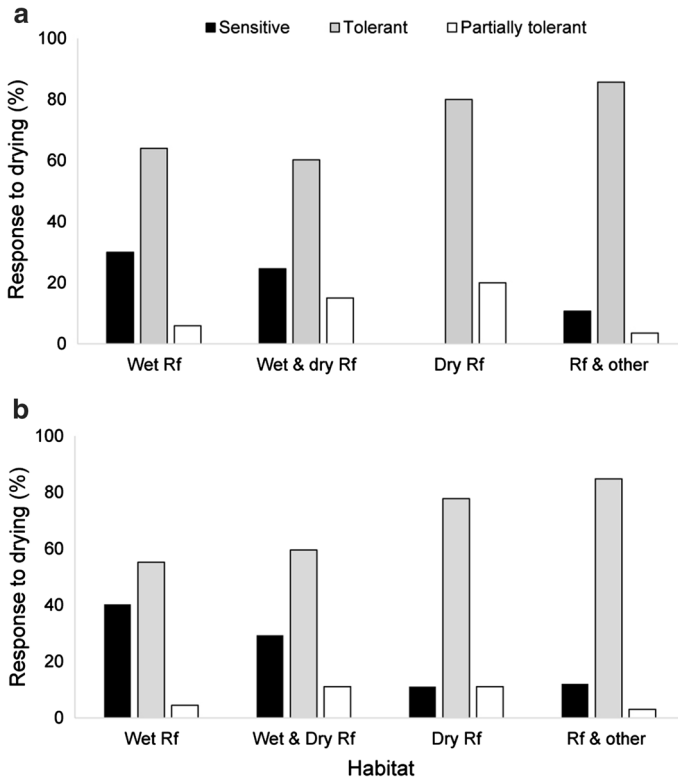


Fig. 6 The relative proportions of desiccation sensitive, tolerant and partially tolerant seeds among Australian plant species occurring in wet rainforest only, both wet and dry rainforest, dry rainforest only, or a combination of rainforest and non-rainforest habitats for: **a** species from this study only ($n=50, 73, 5$ and 28 , respectively); and **b** species from this study combined with those published in Hamilton et al. (2013; $n=67, 99, 9$ and 33 , respectively)

Key to desiccation response

The decision key developed to aid determination of the most likely response to desiccation consisted of seven consecutive steps incorporating an assessment of: the known response of other species in the genus; seed coat permeability; seed dry weight; fresh seed moisture content; P_{D-S} ; the presence or absence of a woody endocarp; and embryo colour (Table 2). Of the 156 species used to test the utility of the key, 141 were able to be assigned to a desiccation response without germination testing; 127 of those (90%) were assigned correctly. Of the 100 species assigned a ‘Desiccation Tolerant’ response, 98% had been shown in this study to be fully (92 spp.) or partially (6 spp.) tolerant of desiccation to 3–7% moisture content. Of the 15 species not accounted for, ten lacked data on dry weight, fresh seed moisture content or P_{D-S} .

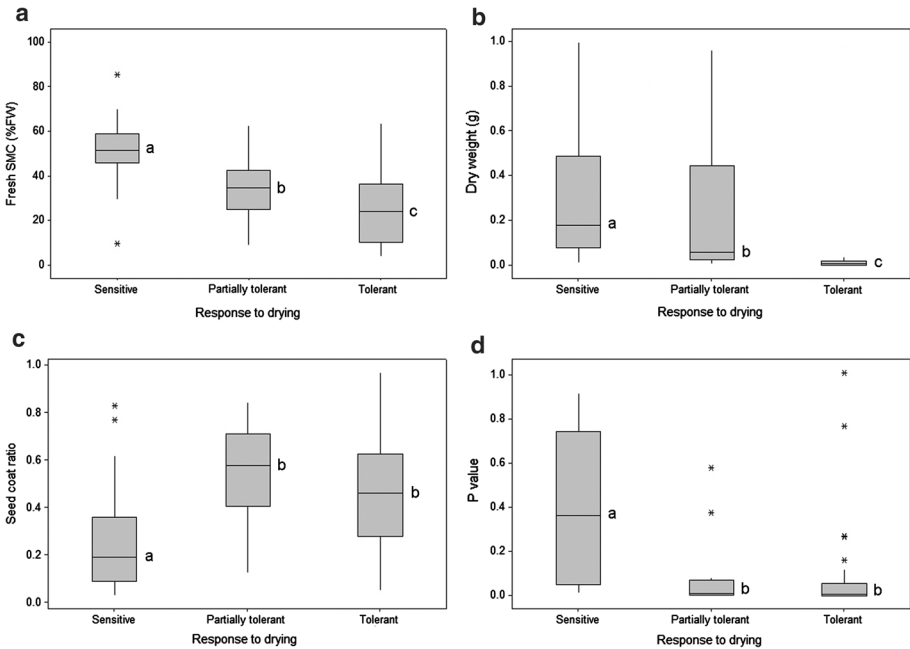


Fig. 7 The response of seeds to drying in relation to: **a** fresh seed moisture content (% fresh weight basis); **b** seed dry weight; **c** seed coat ratio; and **d** P_{D-S} (or ‘P value’), a figure representing the likelihood of desiccation sensitivity based on seed coat ratio and dry weight. Different superscripts within each chart represent significant differences ($P \leq 0.001$) in median values among the desiccation response categories. Data were analysed using pairwise non-parametric one-way analysis of variance (Kruskal and Wallis, 1952) in Minitab v16

Discussion

The need to preserve rainforest species in ex situ collections has become critical to mitigate on-going losses in diversity through habitat loss, disease and a changing climate. In eastern Australia, prolonged drought and catastrophic fires have recently added to these pressures with the 2019–20 bush fire season burning 54% of the World Heritage listed Gondwana Rainforests in New South Wales (NSW DPIE 2020). Seed banking may be a useful technique to apply in this context but information on the suitability of seed for banking has been lacking for many rainforest species. The work presented here provides insight into the seed storage behaviour of 162 native Australian rainforest species from 136 genera and 63 families. For most of the species tested, and 55 of the genera, such data had not previously been published (Table 1; Sommerville et al. 2018). Given that the known storage behaviour of species within a genus is a useful predictor for untested species in that genus (Wyse and Dickie 2018), the results may potentially be extrapolated to many other species and provide a strong step forward in our efforts to conserve rainforest flora. For eight genera in the Myrtaceae, the work also provides the urgent preliminary research needed to conserve species on the brink of extinction due to Myrtle Rust.

There remain many genera of tropical and subtropical rainforests, however, for which no data on storage behaviour are available (Sommerville et al. 2018). For species in those genera, the key to desiccation response presented here (Table 2) will provide a useful aid to

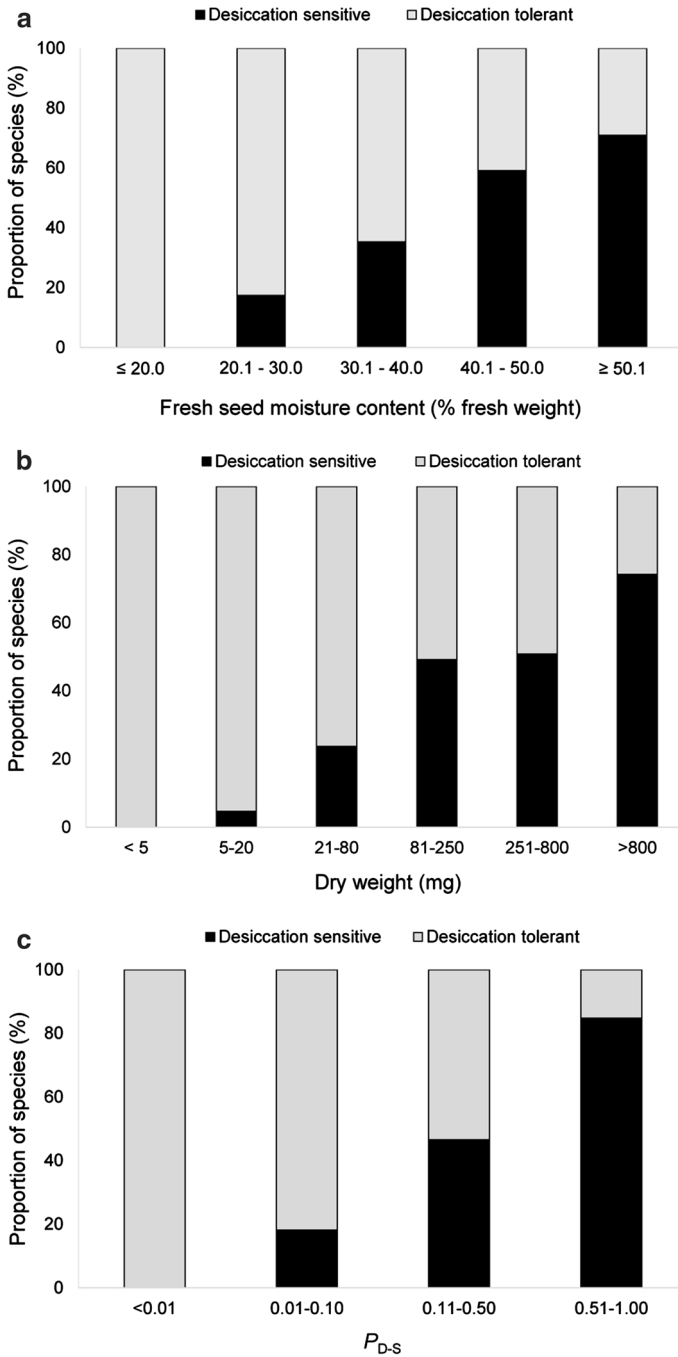


Fig. 8 The relative proportions of desiccation sensitive and desiccation tolerant seeds in relation to **a** fresh seed moisture content ($n=289$); **b** seed dry weight ($n=364$) and **c** P_{D-S} (a figure representing the likelihood of desiccation sensitivity based on seed coat ratio and dry weight; $n=313$) for rainforest species occurring in Australia, China and Panama

quickly identifying those species likely to be suitable for standard seed banking and those likely to require alternative conservation measures. As in previous studies, the relationships observed here between storage behaviour and fresh seed moisture content, dry weight and P_{D-S} only allowed reliable prediction of desiccation response at one or both ends of the range for each variable (e.g. Daws et al. 2006; Ellis et al. 2007; Lima et al. (2014); the difficulty in predicting storage responses for species falling within the middle of those ranges remained. While other characteristics, such as tree habit and fleshy fruit, were shown to significantly increase the likelihood of desiccation sensitivity, these still did not make reliable predictors for the purpose of seed banking. The key resolves these problems by combining the most useful range of values for the best predictors into a step-by-step process for assigning a desiccation response.

For many species, the most likely desiccation response may be determined within 48 h without germination testing, enabling more rapid identification of seeds that are suitable for banking. As the determination of desiccation response by direct testing can take anywhere from a couple of months to a couple of years, the key therefore has the potential to greatly reduce the time and effort required to determine which rainforest seeds are suitable for banking. At this stage, however, it cannot be used to assess whether a species has orthodox storage behaviour (i.e. with longevity improved by both drying and cold storage) unless other species in the genus have previously been assessed (Table 2, Step 1). Further work is needed to find seed characteristics that could be used to identify species that are freezing sensitive without germination testing; therefore, for the moment at least, tests to confirm tolerance of cold storage will still be required for species from previously unstudied genera.

Wyse and Dickie's (2018) finding that the storage behaviour of species in a genus is a useful predictor of storage behaviour for untested species in that genus was supported to a large extent by the results of this study. We found the response to desiccation to be consistent for 14 of the 18 genera in which two or more species were studied (*Acronychia*, *Argyrodendron*, *Cordyline*, *Dysoxylum*, *Ficus*, *Fontainea*, *Flindersia*, *Parsonsia*, *Pittosporum*, *Psychotria*, *Rhodamnia*, *Senna*, *Solanum* and *Vesselowskyia*; Table 1). For the remaining genera (*Ceratopetalum*, *Cryptocarya*, *Diospyros* and *Alphitonia*), the desiccation response was variable among species.

Differences in desiccation response among species in the same genus can sometimes be related to differences in fruit or seed structure. Species in the genus *Syzygium*, for example, generally produce fleshy fruits and desiccation sensitive seeds (Royal Botanic Gardens Kew 2020). *Syzygium anisatum*, however, has a dry fruit and, though the embryo is similar in structure to some other *Syzygium* species (Craven and Biffen 2005), it is tolerant of drying (unpublished data). In three species of *Derris*—all tropical lianes—investigated by Jayasuriya et al. (2012), one species with an impermeable seed coat (*D. scandens*) was desiccation tolerant, but one of the two species with a permeable seed coat (*D. trifoliata*) was desiccation sensitive. Differences in fruit and seed structure may also be a good indication of differences in desiccation response among genera in families that have predominately orthodox seeds. Species in the Fabaceae and Malvaceae, for example, generally produce hard-coated impermeable seeds and > 90% of the species tested so far have been found to be orthodox in their storage behaviour (Dickie and Pritchard 2002). Three genera from those families investigated in this study (*Archidendron* in Fabaceae, and *Argyrodendron* and *Sterculia* in Malvaceae) had species with permeable seed coats that were desiccation sensitive. Fruit and seed structure may therefore be combined with phylogeny to provide a useful indicator of seed storage behaviour.

Table 2 Decision key for rapid assessment of the desiccation tolerance of rainforest seeds

Step	Action	Outcome
1	Check for information on storage behaviour for other species in the genus	Storage behaviour in the genus is consistently orthodox and the untested species has fruit and seed similar in appearance to previously tested species Likely DT Storage behaviour in the genus is consistently recalcitrant and the untested species has fruit and seed similar in appearance to previously tested species Likely DS
2	Dissect seed with a scalpel and examine seed coat	Storage behaviour in the genus is inconsistent or no species have previously been tested Go to Step 2 Seed coat hard, difficult to dissect Go to Step 3 Seed coat soft, easy to dissect Go to Step 4
3	Conduct an imbibition test	Seed coat impermeable Likely DT Seed coat permeable Go to Step 4
4	Determine oven dry weight and fresh seed moisture content (fresh weight basis)	Dry weight < 20 mg OR moisture content < 20% Likely DT Dry weight ≥ 20 mg AND seed moisture content ≥ 50% Likely DS
5	Determine seed coat ratio and calculate $P_{D,S}$ (following Daws et al. 2006)	Dry weight ≥ 20 mg AND moisture content 20% to < 50% Go to Step 5 $P_{D,S} < 0.01$ Likely DT $P_{D,S} > 0.3$ Likely DS $P_{D,S} \geq 0.01$ and < 0.3 Go to Step 6
6	Inspect structure of dispersal unit and seed embryo	Seed encased in a woody endocarp Likely DS or PrDT Embryo green in colour Likely DS or PrDT Neither of the above Go to Step 7
7	Conduct seed storage behaviour experiments following Hong and Ellis (1996), Pritchard et al. (2004a, b) or this manuscript	

DT desiccation tolerant (tolerant of desiccation to 3–7% moisture content), *PrDT* partially desiccation tolerant (degree of tolerance variable among species), *DS* desiccation sensitive

However, for the genera *Ceratopetalum*, *Diospyros*, *Cryptocarya* and *Alphitonia*, there were no easily discernible differences in fruit or seed structure that reflected differing responses to desiccation. The four species in *Diospyros*, for example, ranged from desiccation tolerant to desiccation sensitive, though all four species produce fleshy fruits and seeds with a similar structure. Likewise, in the *Cryptocarya* genus, the three species tested ranged from desiccation sensitive to partially desiccation tolerant though all three produced seeds with a thin seed coat surrounded by a woody endocarp. These results suggest that genus is best used as a predictor for storage response of an untested species when several species with similar seed structure in that genus have been tested and have shown a consistent response.

The comparatively low proportion of desiccation sensitive species found in this study (22%) suggests that many of the seed-producing rainforest species in temperate and subtropical zones in Australia may potentially be conserved by some form of seed banking. Earlier studies of rainforest flora that have focussed on woody species found the proportion of desiccation sensitive species to be considerably higher (Daws et al. 2005; Hamilton et al. 2013; Lan et al. 2014). However, modelling by Wyse and Dickie (2017) predicted the proportion of desiccation sensitivity for subtropical to tropical moist broadleaf forest to be 18.5% if herbaceous species were included, a value much closer to that observed in this study. Given the great number of species occurring in these habitats (Sommerville et al. 2018), this represents the potential to conserve a significant amount of the world's rainforest flora. The high proportion of rainforest trees showing desiccation sensitivity, however, is a concern as a wide diversity of tree species—and the habitats they create—is a distinguishing feature of subtropical and tropical rainforests (Keppel et al. 2010; Tng et al. 2016; Ibanez et al. 2017).

Wyse and Dickie (2017) also found that seeds of up to 92% of the world's plant species may be stored using conventional methods. In the model used, however, desiccation tolerant and intermediate species were combined for the analysis on the basis of Tweddle et al.'s (2003) comment that intermediate seeds may be considered effectively desiccation tolerant in ecology, and on the basis that the limited number of intermediate species in the dataset had little impact on the outcome. While we adopted the same approach here when comparing characteristics of desiccation sensitive and desiccation tolerant seeds from Australia, China and Panama (17 partially desiccation tolerant seeds were included in the desiccation tolerant subset), we note that seeds with intermediate storage behaviour are not usually amenable to conventional seed banking (i.e. desiccation to 3–7% moisture content and storage at –18 to –20 °C). The proportion of intermediate species available for modelling is likely to increase as more data from moist tropical-subtropical habitats becomes available, particularly from island habitats where dispersal over long distances may have selected against desiccation sensitive species.

Research on the native flora of Hawaii, for example, indicated that only 3% of species tested were truly desiccation sensitive (Yoshinaga and Walters 2003) and many species tolerant of desiccation have since been found to be 'temperature-intermediate', i.e. sensitive to storage at –18 °C (Chau et al. 2019). Similarly, of the 105 desiccation tolerant species tested for freezing response in this study, 26% were found to be either freezing sensitive or short-lived in storage at –18 to –20 °C. These results suggest that standard seed banking temperatures may not be appropriate for seeds from rainforest habitat. Chau et al. (2019) came to a similar conclusion following paired studies of Hawaiian seeds stored at 5 °C and –18 °C in which they found species from ten genera had greater longevity in storage at 5 °C than at –18 °C. An investigation of more appropriate storage temperatures is now needed for the Australian species found to be sensitive to freezing, and further testing

is required to determine whether any species initially appearing tolerant of freezing after 1–2 months are in fact short-lived at that temperature.

The above results, combined with knowledge of an increasing number of orthodox species now known to be short-lived in storage (e.g. Mondoni et al. 2011; Merritt et al. 2014; Satyanti et al. 2018), suggest that the global proportion of angiosperms suitable for long-term conservation at -18 to -20 °C may be somewhat lower than previously predicted. Urgent work is now required to identify other species that are likely to be short-lived at standard seed banking temperatures and to determine the storage conditions necessary to maximise their longevity. An assessment of thermal profiles using differential scanning calorimetry may aid in both respects (Mira et al. 2019).

A limitation of the present study is that, for most species, only a single accession was tested. Differences in response to drying and storage can occur among collections of a species from different provenances and among collections from different years (Dalziell et al. 2019). These differences in response can be influenced by the relative maturity of the seed at the time of collection (Hay and Smith 2003) and the quality of the seed as a result of maternal environment and genetic make-up (Delouche 1980; Roach and Wulff 1987). However, the desiccation response was consistent for eight species common to this study and that of Hamilton et al. (2013) suggesting that the majority of observations in this study are likely to be repeatable.

Relatively poor germination of fresh seeds of 151 species prevented a direct assessment of their storage behaviour. The poor response may have been due to inappropriate conditions for germination; however, poor, slow and erratic germination of rainforest seeds has been observed previously under laboratory conditions (Mattana et al. 2019), in nursery situations (Floyd 2008; Zich et al. 2018) and in field experiments (Doust et al. 2006). In fact, Baskin and Baskin (2014) reported that 51% of more than 6700 non-pioneer tree species in tropical-subtropical evergreen rainforests had some form of dormancy. This difficulty points to the need for research into dormancy and optimum germination conditions for rainforest species while supporting the need for methods to distinguish between bankable and non-bankable seeds without germination testing.

The data and the key presented here provide a step forward for those attempting to conserve rainforest species through seed banking in Australia and in other countries with rainforest habitats. Both the data and the key may also prove useful to those studying rainforest ecology and restoration, as the identification of desiccation response can: (a) provide an explanation for the failure of some species to re-establish naturally from seed in secondary rainforests or restoration sites (Goosem et al. 2016); (b) provide a guide to seeds that it may be better to bury when direct sowing, rather than broadcast, to prevent desiccation (see Doust et al. 2006); and (c) provide an indication of the time that may be required for emergence of seeds that are desiccation tolerant but dormant (Online Resource 1). While we expect that the key may need to be refined as more data comes to hand, this first iteration has significantly increased the speed with which storage behaviour may be assessed for a wide range of rainforest species.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10531-021-02244-1>.

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Data availability All data relating to germination and storage experiments are summarised in the supplementary material accompanying this manuscript. Raw data can be made available on request.

Declarations

Conflict of interest The authors declare no conflicts of interest or competing interests.

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