Hill, James (2016) *The effects of changing climates on seed production and seed viability on tropical plant species*. PhD thesis, James Cook University.

https://doi.org/10.4225/28/5ab2f8dbcafd8

The effects of changing climates on seed production and seed viability on tropical plant species

Thesis submitted by

James Hill BSc. Honours JCU Cairns

January 2016

For the degree of Doctor of Philosophy

in the College of Science and Engineering

James Cook University

Cairns

Statement of Access

I, the undersigned, author of this work and copyright owner of this thesis, wish for this work to be embargoed until 1 December 2017, after which I grant the University a permanent non-exclusive licence to store, display or copy any or all of the thesis, in all forms of media, for use within the University, and to make the thesis freely available online to other persons or organisations.

I understand that James Cook University will make this thesis available for use within the University Library and via Australian digital Thesis network for use elsewhere.

I understand that as an unpublished work, a thesis has significant protection under the Copyright Act and I do not wish to place any further restrictions on access to this work.

……………………..	20.01.17…………….
(Signature)	(Date)

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

…………………….. 20.01.17…………………..

(Signature) (Date)

Electronic copy

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to James Cook University Library is an accurate copy of the print thesis submitted, within the limits of the technology available

……………………..	20.01.17……………….
(Signature)	(Date)

Declaration on Ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the joint *NHMRC/AVCC Statement and Guidelines on Research Practice* (1987), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The research methodology did not require clearance from the James cook University Experimentation Ethics Review Committee.

……………………. 20.01.17……………….

(Signature) (Date)

Table of Contents

Declaration 3

Electronic copy 4

Table of Contents 6

Table of figures 8

Table of Tables 10

Acknowledgements 12

Statement of the Contribution of Others 14

Contribution of others to manuscript production 15

ABSTRACT 16

Chapter 1: General Introduction 22

Justification of Research 32

Components of this thesis 36

Chapter 2. Decreased seed production associated with severe drought in a seasonal tropical forest 38

Introduction 38

Methodology 42

Discussion 63

Chapter 3. Dispersal of desiccation-sensitive seeds is not coincident with high rainfall in a seasonal tropical forest in Australia. 71

Introduction. 71

Methodology. 73

Results. 76

Discussion 79

Chapter 4. How long does it take for different seeds to dry? 89

Introduction. 89

Methods. 92

Results. 98

Discussion 110

Chapter 5. Size isn't everything for desiccation sensitive seeds. 119

Introduction 119

Methods 124

Results 130

Discussion 137

Chapter 6. Synthesis and implications of climate change on desiccation sensitive seeds 148

Implications for the future 154

Table of figures

Figure 1 Location of Smithfield Conservation Park (SCP) within the Wet Tropics World Heritage Area in North Queensland (-16.8106, 145.6813) (Map courtesy of the Wet Tropics Management Authority). 43

Figure 2 Meteorological data from the Cairns Airport situated 14km south of the study site. Figure represents the mean monthly rainfall, the mean minimum and maximum monthly temperature and the mean hours of sunlight per month. (data supplied by the Australian Bureau of Meteorology). 51

Figure 3 A comparison of the mean Walsh's seasonality index (± SE) between aseasonal tropical forest sites (n=9), seasonal tropical forest sites (n=9) and Cairns, Queensland. Data for tropical forest sites was obtained from the CTFS network (Leigh 2004). 52

Figure 4 Pattern of temporal concentration in seed release. 4a). Frequency distribution of dispersal periods within months. 4b). A polar plot with mean angles and vector. Vector angles represent mean weeks and vector lengths represent the temporal concentration. Vector lengths range from 0 to 1, 0 indicating a perfectly uniform distribution, 1 representing temporal concentration within in a single week (increments are 0.2). Closed circles represent species displaying a significant seasonal distribution in seed rain and open circles represent species displaying a uniform distribution as determined via Rayleigh's test. 54

Figure 5 The distribution of species with poor seed production across years, showing highest frequencies of poor performance during the year 2002. Frequency distribution of the numbers of species with poor seed production within a given year. 61

Figure 6 A - O. The monthly distribution of seeds dispersed from 15 rainforest plant species that produce recalcitrant seeds. Columns represent the cumulative monthly number of seeds over a four year period (2002-2005) using seed traps (see methods). The solid line represents the average rainfall (70 yrs). 80

Figure 7 Time course of seed desiccation to 1% mean (± SE) relative seed moisture content (W_r) for 24 rainforest plant species that produce desiccation sensitive seeds in north Queensland, Australia, and the fit of a negative exponential model (equation 3). The model fit is represented as a solid line for species where the fit was deemed acceptable, otherwise the fit appears as a dashed line. Species codes are as follows (A) Cryptocarya mackinnoniana, (B) Helicia australasica, (C) Endiandra leptodendron, (D) Toechima erythrocarpum, (E) Dysoxylum alliaceum, (F) Dysoxylum pettigrweanum, (G) Diploglottis diphyllostegia, (H) Syzygium kuranda, (I) Agyrodendron peralatum, (J) Endiandra bessaphila, (K) Garcinia warrenii, (L) Connarus conchocarpus, (M)

Palaquium galactoxylon, (N) Myristica globosa, (O) Cryptocarya murrayi, (P) Endiandra longipedicellata, (Q) Endiandra cowleyana, (R) Castanospora alphandi, (S) Endiandra hypotephra, (T) Cupaniopsis foveolata, (U) Chionanthus ramiflora, (V) Mischocarpus exangulatus, (W) Agyrodendron polyandrum, (X) Cupaniopsis flagelliformis.............. 101

Figure 8 The relationship between the estimated time constant (T or t0.3679) (ln) associated with seed desiccation and the mean dry seed mass (ln) of, A) twenty-four rainforest plant species; and, B) the eight species that recorded a significant positive correlation within species between T or t0.3679 and dry seed mass, from a seasonal tropical forest, Queensland, Australia.. 106

Figure 9(A-X). The relationship between seed dry mass and the time constant (T or t0.368) within 24 desiccation sensitive seeded rainforest plant species. The plotted regression equations represent those species where the relationship was significant. Species codes as in Figure7. 112

Figure 10 Seed mortality curves for 16 rain forest species that produce desiccation sensitive seeds in north Queensland, Australia. The y-axis represents the proportion of seeds germinating and the x-axis is the mean seed relative water content, Wr. Each point is based on germination in a seedlot of 20 seeds subjected to a range of desiccation treatments (Wr = 1.0, 0.8, 0.6, 0.4 and 0.2; see text for details)............... 135

Figure 11 A significant negative relationship between log mean monthly rainfall at seed shedding, ln(R_M), and SCR. This was the only significant relationship found in bivariate regressions between seed traits among 16 species producing desiccation sensitive seeds in north Queensland, Australia.. 139

Figure 12. Plot of species in seed trait space defined by two principal components. Labels and arrows show the trait variables that exhibited eigenvector scores > |0.30|. The two significant (eigenvalues >1) principal components explain 65% of the overall variance in the species-trait correlation matrix. 146

Table of Tables

Table 1 The timing of seed release (weeks), for 50 plant species within a seasonal tropical forest in the Smithfield Conservation Park, Queensland. Also present is the number of seeds collected for each species and the number of years where seeds were collected. 56

Table 2 Observed and expected frequencies of the combined number of species recording lowest seed rain in each of the four study years and the number of species that failed to produce seeds in one of the four study years. Also shown is the log-likelihood G – test for significant deviation between observed and expected frequencies and the post-hoc Freeman-Tukey deviates identifying years where significant deviations occurred. Positive values for the Freeman-Tukey deviates indicate greater than expected numbers of species, negative values fewer than expected (** significant at $P < 0.01$). 60

Table 3 Total annual rainfall and rainfall during “wet” and “dry” seasons for Cairns Airport, 10 km SE of Smithfield Conservation Park (SCP) Cairns over four years of study (2002-2005). Also shown are the 70 year mean values. Z-scores are the results of the comparisons of rainfall recorded in each season in each year against the frequency distribution of rainfall in wet or dry seasons generated from the 70 year dataset, and Walsh’s seasonality Index (W.I.) for each study year. 62

Table 4 The mean monthly angle of dispersal and associated vector length for 15 species recorded between January 2002 - December 2005 from 72 x .5 m2 seed traps within a 2ha study site in SCP. 77

Table 5 The temporal pattern of seed release was determined by recording the presence or absence of recently dispersed fruits and seeds between December 2005 and February 2007 78

Table 6. The fruit type, dispersal syndrome ((+) assisted dispersal; (-) non-assisted dispersal), desiccation sensitivity ((+) sensitive to desiccation; (-) not sensitive to desiccation), trapping method, season of dispersal, and germination success of control and dehydrated treatments of seed-lots from 32 tropical forest plant species from Cairns, Australia. 86

Table 7. Results of fitting a simple negative exponential decay model to the seed desiccation plots (Wr versus t) and the time to 63.21% relative seed moisture loss (T or $t_{0.368}$) for 24 species of rain forest trees from north Queensland, Australia. The constant variance and predicted residual error sums of squares (PRESS) were used to evaluate the strength of the relationship (see Methods). 103

Table 8. Results of fitting either a double negative exponential, or two-piece linear models to the ten species that did not conform to the single negative exponential model. Also shown are the results of the F-tests to determine significance of increase in explanatory power of more complex model. 107

Table 9 The relationship between the time constant ($t_{0.368}$) and mean dry seed mass for 24 plant species located within the seasonal tropical forests of Cairns, Australia. The Boferroni correction for multiple tests was used to identify Significant relationships were identified using the Bonferroni correction for multiple tests (i.e. sig. $P > 0.0026$) and are highlighted in bold. 113

Table 10 Traits of sixteen rain forest plant species that produce desiccation sensitive seeds. All are from a lowland tropical rain forest community in north Queensland. Traits are: Wr50 (seed relative water content at which 50% of a cohort of seeds become unviable); TG (time to germination in fully hydrated seeds); MD (seed dry mass); t0.368 (time constant for exponential decline of Wr under the desiccation treatment); SCR (ratio of seed coat and/or endocarp to embryo); and WM (the specific relative water content as defined in eqn 1). Mean values, n = 20 (see text for further details). (Species authorities are in accordance with Bostock and Holland 2010). 132

Table 11 Bivariate relationships between seed traits of sixteen tropical tree species that produce desiccation sensitive seeds within a tropical forest community, Cairns, Australia. The only significant relationship (SCR × R_M) is indicated in bold. R_M is mean monthly rainfall in the month of seed dispersal; W_{r50} (seed relative water content at which 50% of a cohort of seeds become unviable); T_G (time to germination in fully hydrated seeds); M_D (seed dry mass); $t_{0.368}$ (time constant for exponential decline of W_r under the desiccation treatment); SCR (ratio of seed coat and/or endocarp to embryo); and W_M (the specific relative water content as defined in eqn 1). Bonferroni corrected $P = 0.002$ 142

Table 12. Principal component scores of seed traits and mean monthly rainfall of 16 tropical forest species. Variables were log10-transformed. The main structuring variables (PC scores ≥ |0.30|) determined after varimax raw rotation for each principal component are highlighted in bold; (%) represents the percentage of the total variance accounted for by each component 144

Acknowledgements

First of all I would like to thank my family that has been created during my candidature. My wife Alice has been extremely supportive throughout and I thank her for her level headed approach in prioritising my obligations to both work and family. To my children Bonita and Mischa a big thank you for accepting that Dad could not always be around due to field and work commitments. I would also like to thank my parents who have always believed that I could achieve my dreams and without that initial support I would not have begun my career as a rainforest ecologist.

A huge thank you to Will Edwards; who above all has had the patience to deal with me. I regard Will as a friend, mentor and supervisor. He has made me a better person than who I was when I initially began my undergraduate degree in 1998. I now often see myself making decisions and in retrospect realise that there is an element of Will’s thought process within each decision. This acknowledgement section is too small to list all the key areas where Will has assisted and mentored me through the PhD process so I simply say; thank you. Peter Franks was a breath of fresh air throughout my PhD. Someone who was always encouraging whilst maintaining the highest degree of professionalism. His analytical thought process and attention to detail are two main areas which greatly assisted with the publication of some of the chapters presented here. Within the Cairns campus everyone was supportive and offered their perspective on how I should go about completing my PhD. Had I listened to everyone maybe this PhD would not have taken so long. Thanks to other PhD students especially Matt Pye, Mark Harrington and Romina Rader who were always willing to rebound ideas and listen to my constant moaning with regards to my children never sleeping the night

before. In general, a big thank-you to JCU Cairns. The structure of my undergraduate degree taught me that to understand ecological processes one should have a deep grounding in the physiology of an organism. Although most of these academics taught zoology at the time, they had a big influence on how I developed my research questions; especially Professor Andrew Krockenberger and Associate Professor Jamie Seymour. Professor Paul Gadek was the first person to introduce me to the fact that desiccation sensitive seeds existed, and for that third year essay question I'm most appreciative. Outside the JCU fraternity a special thank you to The Royal Botanic Gardens, Kew, England, who assisted with the gathering of information on desiccation sensitive seeds and the methodology to identify these species. Joe Wright from the Smithsonian Institute (Panama) for taking the time to edit and add suggestions to Chapter 3. Thank you also to Prof Angela Moles (UNSW) and Assoc Prof Susan Laurance (JCU) who added suggestions to improve Chapter 5.

Statement of the Contribution of Others

Nature of Assistance	Contribution	Names
Intellectual support	Project design Statistical analyses Manuscript assistance	Associate Professor Will Edwards
		Associate Professor Peter Franks
Financial support	Scholarship	Australian Research Council

Contribution of others to manuscript production

Chapter No.	**Publication**	**Names**
2	Hill, J & Edwards, W. Decreased community-wide seed production associated with extreme drought in the tropical rainforests of northern Australia (*in prep*)	Associate Professor Will Edwards Centre for Tropical Environmental and Sustainability Studies College of Science & Engineering James cook University
3	Hill, J & Edwards, W. (2010) Dispersal of desiccation-sensitive seeds is not coincident with high rainfall in a seasonal tropical forest in Australia. *Biotropica* 42, 271–275	
4	Hill, J., Edwards, W & Franks, PJ. (2010) How long does it take for different seeds to dry? *Functional Plant Biology* 37, 575-583	Associate Professor Peter Franks Future Fellow Centre for Carbon, Water and Food Faculty of Agriculture and Environment University of Sydney
5	Hill, J., Edwards, W & Franks, PJ. (2012) Size is not everything for desiccation-sensitive seeds. *Journal of Ecology* 100, 1131–1140	

ABSTRACT

Climate change is now regarded as one of the most significant mechanisms contributing to the sixth mass extinction currently occurring on Earth. Increases in both minimum and maximum temperature, increases in the severity of extreme events such as severe tropical cyclones and drought episodes, changes in precipitation patterns and an increase in the duration of the dry season in seasonal tropical regions, are key threatening processes. Tropical forest communities in Australia are extremely vulnerable to these effects as phenological patterns in seed production, seed release and the transition from seed to seedling life stages are intimately timed to current rainfall patterns. Thus, the changes to existing rainfall patterns predicted under climate change scenarios threaten the future composition of these diverse forests. In this thesis I examine the phenophase of seed fall and seed production in a seasonal tropical forest in Cairns, Australia, and explore the germination requirements of a group of species that produce desiccation sensitive seeds. This group, in particular, are underrepresented in current studies, but are potentially most at risk from changes in moisture availability because increasingly dry conditions could substantially reduce numbers of seeds successfully germinating.

To examine phenological patterns in response to seasonal and inter-annual differences in weather patterns, seed production was monitored weekly over four years via 72 seed traps within a 2ha permanent plot at the Smithfield Conservation Park (SCP). Concentration of species' mean dispersal periods within the wet season suggested

intra-annual patterns in seed release corresponded to periods of high moisture availability. Inter-annual variability in seed rain also responded to moisture. My results demonstrate a decrease in seed production during a severe drought associated with the with very low moisture availabilities, with 24/42 species experiencing their lowest levels of seed production in association with 2002 El Niño event. Previous evidence has shown an increase in seed production in association with El Niño Southern Oscillation (ENSO) events in aseasonal and seasonally moist tropical forests. The results I present are counter to results previously shown from other parts of the world, being the first to report a negative impact of ENSO on seed output and suggest the ability to predict the effects of changing global climates on plant reproductive output may be location- and event-specific.

Almost 50% of all seed plants produce desiccation sensitive seeds. In Australia, only three species had been identified prior to the beginning of this thesis within a seasonal tropical rainforests such as SCP; previous hypotheses suggest that these species should time seed release to periods within the year where seed survival prior to germination is high; i.e. that seed dispersal should coincide with wet season. Using the 100-seed Test I identified 24 species that produce desiccation sensitive seeds and tested the hypothesis that dispersal periods of desiccation sensitive seeded species were coincident with the wet season. Unlike similar forests, there was no support for this pattern. Only four species had mean dispersal dates in the wet season. A further four species had dispersed seed across the dry/wet season and were considered wet season dispersers. The remaining 16 species all had dispersal dates centred in the dry season. There was no evidence that release of desiccation sensitive seeds was concentrated within the wet season. Intensification of seasonal aridity may increase mortality in desiccation sensitive seeds.

Reduction in rainfall and the intensification of dry season moisture deficit threaten to expose desiccation sensitive seeds to greater potential negative effects of desiccation. Coupled with the time of release, just how quickly potential to successfully germinate decreases when seeds are exposed to dry conditions will influence future recruitment success. To address this I investigated the rate of seed moisture loss in the same 24 species (see above) and tested the common hypothesis that seeds conform to a simple negative exponential model of moisture loss with time. A negative exponential model described moisture loss in 14 species, but was not the best model for the remaining 10 species. Moisture loss in eight species was best described by a double-negative exponential model, and by a double-linear model in the remaining two species. One way that desiccation sensitive seeds may reduce moisture loss and maintain viability is by reducing surface area to volume (SA:V) ratio. Indeed, desiccation sensitive seeds are generally larger than orthodox seeds, and this is proposed to reflect pressure to reduce SA:V ratio. One prediction from this is that seed mass should predict the rate of desiccation between and within species. I tested this hypothesis by calculating the time constant (t 0.368) for all seeds and used these values to test if seed mass could predict the rate of desiccation. Within species the time to a given state of desiccation could be predicted by seed mass for eight species. Between species there was no relationship between desiccation rate and seed mass. Combined with evidence above that no single simple model of moisture loss can explain pattern observed in all species, this evidence suggests that seed structural features may be more important than seed mass in prolonging desiccation.

To investigate correlations among seed traits considered influential in the retention of viability prior to germination, I examined critical water content (Wr50), the mean seed water content when a cohort of seeds reaches 50% mortality, desiccation rate (t0.368), the time taken for each seed to reach a relative water content of 36.8%, mean time to germination (TG), the time taken for 50% of seeds within a cohort to germinate; and specific relative water content (WM), the proportion of available water to dry seed mass; two morphological traits: seed size (MD), dry seed mass and seed coat ratio (SCR), the proportional mass between the seed coat and dry seed mass; and one phenological variable: mean monthly rainfall at mean time of seed dispersal (RM) in sixteen plant species that produce desiccation sensitive seeds from a seasonal tropical forest in Cairns, Queensland. Regression analysis revealed a significant negative relationship between RM and SCR, indicating that species that had dispersal periods at times of high moisture availability invested proportionally less into seed coats. No other pairwise trait combinations were significantly related. I also examined trait combinations in a multivariate context via principal component analysis (PCA). PCA revealed two axes of trait space. The first axis was associated with the traits SCR, TG, t0.368, RM and WM, and explained 44.1% of the variation between species. There were strong positive loadings for SCR, TG and t0.368, and strong negative loadings for RM and WM. Traits with strong loadings on the second axis (total variance 21%) were MD and Wr50 and

WM. There were strong positive loadings for Wr50 and strong negative loadings for MD and WM. Most importantly; my findings reveal a first principal component axis of trait variability among desiccation sensitive seeds that is orthogonal to size. The implication of this finding is that seed size alone may not account for pre-germination viability in desiccation sensitive seeds. Seed size alone has been considered one of the principal dimensions defining plant life-history strategy space, but this work has almost exclusively been conducted outside of moist tropical areas and used few species that produce desiccation sensitive seeds. My results suggest that the traits I have measured might be important in surviving pre-germination environmental conditions, independent of the advantages and/or disadvantages that size has been shown to confer on seed survival and seedling establishment, and should be considered in attempts to predict long-term persistence and management of these species under expected changes in moisture availabilities.

Throughout this thesis I describe how species exhibit different responses to a decrease in moisture availability. It is now certain that these conditions will be experienced in coming years and decades. My results show that we may anticipate a decrease in seed production associated with years of extreme dry conditions, and differential levels of susceptibility to pre-germination mortality of seeds due to desiccation will influence species differently. For those species most susceptible, the predicted impacts of climate change have the potential to eliminate some species from current vegetation communities and it is expected that many species will experience either a reduction in range size, or at the most extreme extinction. If we wish to conserve many of these species, management plans will need to be developed. I performed the work detailed in this thesis in the hope that these findings will be instructive prioritising conservation management decisions. I encourage further research to investigate the recruitment of desiccation sensitive seeds under a range of drought conditions.

Chapter 1: General Introduction

Introduction

Human-induced climate change is a major threat to global biodiversity (Butchart *et al*. 2010, Cardinale *et al.* 2012). Indeed, climate change is regarded as one of the most significant mechanisms contributing to species extinction events (Barnosky *et al.* 2011). The magnitude and rate of species loss predicted to occur are now so great that it is considered to possibly represent the sixth great episode of species loss ever experienced in the history of life on earth (Barnoski *et al.* 2011).

Survival for many species will be reliant on either an ability to adapt to changes in climate (Hoffman *et al*. 2011, Bellard *et al*. 2012) or to migrate in order to continually locate themselves within regions that will present conditions representing currently existing climatic envelopes (e.g. Phillips *et al.* 2004, Moss *et al.* 2010, Liu *et al*. 2011, Renwick *et al*. 2012). Unfortunately, migration is unlikely for many species that currently exist in isolated refugia (Skov and Svenning 2004, Wang *et al*. 2009, Normand *et al*. 2011). For species existing under these conditions, the ability of physiological and/or morphological traits to allow persistence will determine whether a species will survive changes in physiological stresses that are predicted under current climate change scenarios (Jump and Peňuelas 2005).

There is strong and consistent evidence that Australia will experience changes in weather associated with climate change (IPCC 2002, IPCC 2007, The Garnaut Review 2011, 2011, IPCC 2012, IPCC AR5, 2014). These effects will include; (a) increases within the range of 2-5˚C for both minimum and maximum temperatures before the end of the century, (b) an increase in the severity of extreme events such as severe tropical cyclones and drought episodes, (c) changes in precipitation patterns reducing current average rainfall by the end of the century, and (d) an increase in the duration of the dry season combined with a shorter, albeit more intense, wet season in seasonal tropical regions (Lewis *et al.* 2006, Fauset *et al.* 2012; IPCC AR5, 2014).

Alarmingly, evidence supports the predictions that the impacts of climate change have already begun to be experienced on the Australian continent (Steffen *et al*. 2013, IPCC AR5, 2014, CSIRO and BOM State of the Climate 2014). For example, in Australia, seven of the ten warmest years on record have occurred since 1998 with 2013 being the warmest (BoM and CSIRO 2014). While average conditions show clear indication of change, the incidence of extreme events associated with them have also risen (Trenberth 2011, Villafuerte *et al*. 2015). For example, the frequency of extreme flood, drought and storm events have increased within the past decade and across all continents (Trenberth 2011, Villafuerte *et al*. 2015), and are expected to further increase in frequency and intensity (BOM 2008, Steffen *et al*. 2013; BoM and CSIRO 2014). These changes pose major threats for both plant and animal species because they present conditions outside of their thermal and moisture tolerance ranges (Hoegh-Guldberg *et al*. 2008, Hughes 2011, Dunlop *et al*. 2012). While these threats are pervasive and likely to influence biodiversity in all regions of the globe, in this thesis I specifically address these possible effects on plant species in lowland tropical rainforest in Australia.

Climate Change and tropical rainforest in Australia

Climate change is the biggest threat to the current distribution of tropical rainforest within North Queensland, Australia (Hilbert *et al*. 2001, Hughes 2011). The total area of the Wet Tropics bioregion is less than 100 000 km^2 and is bordered by dry sclerophyllous vegetation to its north, south and west and by the Pacific Ocean to the east. Studies investigating biological responses to climate change within the Wet Tropics bioregion have overwhelmingly concentrated on individual animal species and/or faunal assemblages (Williams *et al.* 2003, Thomas *et al*. 2004, Shoo *et al.* 2006, VanDerWal *et al.* 2009, Laurance *et al.* 2011, Krockenberger *et al.* 2012). For example, greater frequency of the recurrence of temperature extremes has been proposed to place many animal species at risk by exposing than to conditions outside their place thermal niche (Williams *et al*. 2003, Thomas *et al*. 2004, Laurance *et al*. 2011, Krockenberger *et al*. 2012), resulting in either extinction or dramatic reduction in distribution (Hilbert *et al.* 2001, Williams *et al*. 2003). Primarily, this is because increasing temperature is hypothesised to result in species distributional shifts to higher elevations (Williams *et al.* 2003, Thomas *et al.* 2004, Parmesan 2006, Shoo *et al.* 2006, Colwell *et al*. 2008, Isacc *et al*. 2009, VanDerWal *et al*. 2009, Cahill *et al*. 2012).

How climate change will affect plant communities is less certain, because there have been far fewer studies and much less information relating individual plant species or plant communities to elevated warming, changing patterns of climatic seasonality and increased CO^2 levels (Stork *et al*. 2007). Evidence of population responses to changing climates have focused on expansion and contraction of range size, earlier initiation of phenological processes (i.e. bud burst, flowering etc.) and changes in community composition (Parmasen 2003). Like animal species, plants too will be exposed to temperature increases and potential increases in evapotranspiration as climate change intensifies, and the persistence of plant populations will be dependent on their ability to either migrate or adapt to these changes in climate (Walther 2003, Jump and Peňuelas 2005, Berg *et al*. 2010).

While few studies explicitly dealing with Australian Wet Tropics exist, increases in temperatures coupled with higher evapotranspiration rates during the dry season and changes in seasonal rainfall are also predicted to alter species composition in this area (Fauset *et al*. 2012, Lewis *et al*. 2006). This is because these effects encourage the expansion of sclerophyllous, fire- and drought-tolerant species at the expense of mesic drought-intolerant ones (Hilbert *et al*. 2001, Colwell *et al*. 2008). Ultimately, this has been proposed as a mechanism that will reduce overall species diversity within areas now covered by lowland tropical rainforest. Nevertheless, unlike the animal species for which detailed information and predictions exist, lack of information on individual plant species, and plant communities means that at present it is unclear exactly how plant species will respond, and even more difficult to predict which species will be most negatively affected by anticipated changes. If we wish to conserve many of the plant species currently existing in Australian lowland tropical rainforests, it is imperative that assessment be made on the likelihood of survival and attempts be made to identify priority areas for possible conservation management. This has been the main driver of the work described in this thesis.

Two important processes directly affecting plant species persistence are particularly poorly understood in the Australian rainforest context. These are: (1) intra- and inter-annual patterns of flowering and seed release, and (2) the transition from seed to seedling life stages. As both of these attributes of plant reproductive success are intimately associated with environmental conditions, changes in climatic regimes can be expected to exert significant effects on them. In the following two sections I outline the available evidence of these processes from studies across the world to highlight the potential link between them and environmental conditions.

Flowering and seed production in response to climate

Phenological patterns include the timing of vegetative processes such as leaf development and leaf senescence, and the timing of reproductive functions such as flowering and seed production (Newstrom *et al.* 1994, Corlett and LaFrankie, 1998). The timing of reproductive events is often correlated with changes in the environment, such as photoperiod (Bortchert 1989), temperature (Parmasan and Choe 2003), water availability (Borchert 1994) or irradiance (van Schaik *et al.* 1993). The benefit of timing reproduction to environmental cues is that seed dispersal and seedling establishment occur during periods that are most conducive to supporting the survival of offspring (Garwood 1983). Often environmental cues occur on an annual timescale, such as initiation of spring which gives rise to bud burst and new leaf development in deciduous forests (Borchert 1994). However, this is not the case for evergreen and semi-deciduous vegetation communities. In these situations, more subtle cues that signify onset of conditions favourable for reproduction often exist, and these events may be separated by many years. This has led to phenological patterns in reproduction occurring over annual and inter-annual time frames.

Annual and inter-annual patterns in reproduction within tropical forests have been reported to be strongly influenced by seasonality in two abiotic factors important in setting rates of photosynthesis; moisture availability (Borchert 1994, 1996, Sakai *et al.* 2006, Brearley *et al.* 2007), and levels of irradiance (van Schaik *et al.* 1993, Wright and van Schaik, 1994, Saleska *et al.* 2003, Zimmerman *et al.* 2007). For example, the occurrence of El Nino episodes has been shown to trigger dramatic changes in seed production. Over fourteen years Curran *et al*. (1999) investigated the synchronous flowering, seed rain and recruitment of 54 species of Dipterocarp across 90000 ha in West Kalimantan. On four occasions all 54 species synchronously flowered and recorded high fruit set a process known as mast-fruiting. Each mast fruiting event coincided with an El Nino episode. The proposed advantage of mast fruiting over such a large spatial and temporal scale is predator satiation. By producing an abundance of seeds every few years, populations of seed predators are not abundant enough destroy all seeds, with seed predators being satiated overlooking many seeds (Curran and Leighton 2000). Consequently high numbers of seedlings are recruited. In a 9.5 year study examining seed rain at Barro Colorado Island, Wright *et al*. (1999) recorded significant increases in fruit production during El Niño years. For example, during the 1992 El Nino episode in Panama >65 % of 138 plant species recorded their greatest amount of fruit set over the 9.5 year study period. High levels of fruit production was attributed to the increased number of cloudless days experienced during El Nino episode, increasing (PAR) levels and photosynthetic energy gain, hence increasing energy available for seed and fruit production (Wright *et al*. 1999).

Seed – seedling transition in response to climate

Germination is a critical phase in the survival of plant species and is the first of the physiological responses of plant life-history under the influence of the environment (Baskin and Baskin 1998, Bell 1999, Fenner 2005). To avoid periods of adverse environmental conditions many species produce seeds that are released in a state of dormancy (Donohue, 2005). Across species there is a spectrum of dormancy mechanisms. Five broad classes of dormancy exist and reflect varying morphological and physiological properties of seeds (Baskin and Baskin 2004,Nikolaev 2004). Each class has been further broken down into a number of sub – classes according to how different treatments break dormancy (Finch-Savage and Leubner-Metzger 2006). These types of seeds are traditionally known as “orthodox”. Other species produce seeds that are regarded as non-dormant (Farnsworth 2000). In these species, germination occurs within days after dispersal (Baskin and Baskin 1998).

The main difference between desiccation sensitive and orthodox seeds is that the embryo of a desiccation sensitive seed remains metabolically active throughout development (Finch-Savage and Leubner-Metzger 2006). Orthodox seeds internally desiccate during development and enter a phase of metabolic dormancy (Farnsworth 2000, Finch-Savage and Leubner-Metzger 2006). This enables orthodox seeds to persist in soil seed banks.

Baskin and Baskin (2004) have identified 5 broad classes of dormancy each defined by either/or a combination of morphological and physiological barriers that delay germination. These barriers are removed by external stimuli often linked to seasonal changes in environmental conditions (Kozlowski and Pallardy 2002, Cochard and Jackes 2005, Finch-Savage and Leubner-Metzger 2006). For example, in tropical environments subject to periods of seasonal aridity an increase in soil moisture is sufficient to break dormancy. By delaying germination until periods of high soil moisture, seedling establishment and survivorship are enhanced (Kozlowski and Pallardy 2002, Cochard and Jackes 2005). Unlike orthodox seeds where dormancy breaking mechanisms link germination with appropriate environmental conditions, survival in desiccation sensitive seeds is very much reliant on seeds being released from the parent plant at times most suitable for germination and establishment, since the non-dormant state requires rapid germination shortly after dispersal if the seed is to survive and successfully produce seedling (Baskin and Baskin 2004). Seed desiccation sensitivity is thus a potential high-risk regeneration strategy (Pritchard *et al*. 2004), since a prolonged dry spell co-incident with time of seed release has the potential to result in the death of entire cohort of seeds.

Seed desiccation sensitivity is particularly relevant for tropical forest conservation. The highest diversity of species that produce desiccation sensitive seeds is found in tropical rainforests. On a global scale, estimates suggest that species that produce desiccation sensitive seeds comprise approximately 50% of the world’s flora within evergreen rain forest (Daws pers comm.; Tweddle *et al.* 2003). For example, Vanquez-Yanes *et al*. (1999) recorded seed viability in a mature Mexican rainforest community and found that of 150 trees nearly all produced desiccation sensitive seeds (although exact numbers were not given). In Australia, the proportion of species with desiccation sensitive seeds in the tropical rainforests of North Queensland is unknown. The distinct seasonal climate of these rainforests may suggest that there is fewer of these species compared with equatorial rainforest where seasonal fluctuations are not as well defined.

Climate Change, seed production and seed desiccation sensitivity

Climate change poses a major challenge for tropical rainforest communities which comprise species with desiccation sensitive seeds. This is because desiccation sensitive seeds are reliant on favourable environmental conditions for successful germination and seedling establishment and it is these conditions (i.e. temperature and moisture availability) that are expected to change as CO_2 concentrations increase within the atmosphere. For example, seedlings exposed to a longer period of moisture deficit potentially increasing rates of mortality, whilst the dispersal of seeds may occur during times that are not favourable for germination (Walck *et al*. 2011). There is a real risk that the representation of these species within some tropical rainforest communities will decrease. At a regional scale, species within the tropical rainforests of North Queensland are particularly susceptible as these forests are regarded as refugia from Gondwanaland and there is limited suitable habitat available for the colonisation of these species. To understand the risk posed to this group of species it is crucial to understand how climate change is predicted to affect these forest communities; and how plant populations are responding globally to climate change.

Overriding the expectation of increases within the range of 2-5˚C for both minimum and maximum temperatures before the end of the century and changes in precipitation patterns that suggest an increase in the duration of the dry season combined with a shorter, albeit more intense, wet season, one of the largest unknown consequences of climate change will be the impact of extreme events. Extreme climatic event can have measurable impacts on many aspects of plant communities (Delissio and Primack 2003). Of most importance is the ability of droughts to influence community composition through mortality at both the seedling and sapling stage, and change flowering and fruiting phenology (Curren *et al*. 1999, Wright *et al.* 1999, Delissio and Primack 2003, Engelbrecht and Kursar 2003, Condit *et al*. 2004). For example, in 2002/2003 north-east Queensland experienced a severe drought. The drought caused extremely high levels of mortality of rainforest seedlings of approximately 80% y-1, much higher than that recorded in other forests (Edwards and Krockenberger 2006).

As stated above, increased average seed rain has been recorded in the Neotropics and in South East Asia during periods of drought associated with El Nino events. This may suggest drought conditions expected under change will be beneficial. Unfortunately, this is unlikely.

Justification of Research

Plant species that produce desiccation sensitive seeds within the rainforest communities of the Wet Tropics are particularly vulnerable to the impacts of climate change for several reasons;

1) They lack a dormancy mechanism that would enable them to avoid unfavourable environmental conditions;

2) The duration and severity of the dry season is expected to increase potentially exposing desiccation sensitive seeds to conditions that will not support successful recruitment;

3) Migration to areas of suitable habitat outside of the Wet Tropics is unlikely given the distance between rainforest communities along the east coast of Australia.

4) Many species that produce desiccation sensitive seeds are long-lived and take comparatively longer periods of time to reach reproductive maturity. This decreases the ability of this group to evolve traits that would enable them to adapt to changing climates.

5) The increased severities of extreme drought events associated with El Niño episodes will potentially further restrict the germination of species with desiccation sensitive seeds.

There have been numerous attempts to model how vegetation communities will respond to changing climates (Lavorel *et al.* 1998, Westoby *et al.* 1998, Guisan and Zimmerman 2000, Walker *et al.* 2006). Many of these use plant attributes to predict groups of species that are either more resilient or susceptible. Plant attributes include; tree height (Poorter *et al*. 2006, Falster and Westoby 2003*)*, specific leaf area (SLA) (Reich *et al*.

1998, Wright *et al*. 2004), and seed size (Moles and Westoby 2004). Of these seed size is used as proxy for life-history trade-offs associated with regeneration stage. There is good reason to use seed size in this context since it is known to be correlated with survival through many of the hazards plants face during establishment, including shade, competition with established plants or other seedlings, burial under soil or litter, nutrient deprivation, drought, and herbivory (Westoby *et al*. 2002). These attributes have been interpreted to represent the regeneration niche of individual species (Silvertown 2004). Studies describing the trade-offs associated with seed size have concentrated on temperate species, most of which produce long-lived orthodox seeds. Thus, these studies neglect the many tropical species that produce desiccation sensitive seeds. More study is needed on this group of plant species.

Perhaps most knowledge of desiccation sensitivity has been generated as part of the Millennium Seed Bank Project; an ambitious plan by Royal Botanical Gardens, Kew is to collect and preserve 10% of the world’s seed bearing flora in an ex-situ conservation strategy (Millennium Seed bank Project). As a function of this, investigations into desiccation sensitivity have primarily concentrated on physiological aspects associated with their preservation or lack thereof (Daws *et al*. 2004).

Within the past 10 years investigations examining ecological attributes within natural vegetation communities have been initiated (Pritchard *et al*. 2004) in attempts to describe the relationship between desiccation sensitivity, the conditions associated with the timing of seed release and functional attributes of seeds. For example, Pritchard *et al*. (2004) examined 10 plant species found at eight dryland forests in Africa (Kenya, Burkina Faso and Tanzania). Three species were found to be desiccation sensitive, all of which shed fruit/seed during the wettest months of the year, coupled with large seed masses and rapid germination. In contrast, desiccation tolerant species were found to produce seeds with lower comparative masses, had slower germination rates and were shed year round irrespective of rainfall patterns (Prichard *et al*. 2004).

Nevertheless, information about desiccation sensitive seeds and the species that produce them is still very limited. In Australia there are very few species have been identified as producing desiccation sensitive seeds. For example, at the commencement of this PhD, the Royal Botanical Gardens at Kew, England identified three species as producing desiccation sensitive seeds; *Idiospermun australensis* (S.T. Blake) *Castanosperum austral*e (A. Cunn. and C. Fraser *ex* Hook.) and *Syzigium australe* (B. Hyland.) This low number of species identified as producing desiccation sensitive seeds is unlikely to reflect biological reality given the expectation that nearly 50% of rainforest species produce these seeds as most species are restricted to rainforest ecosystems (Tweddle *et al.* 2003).

In this thesis I have attempted to increase understanding of desiccation sensitivity in Australian wet tropics. Since there is no information on the phenology of desiccation sensitive seeds within the Wet tropics bioregion we have little basis on which to predict the potential effects that climate change may have on this group of species. It is now certain that the duration and severity of the dry season will increase within some regions that contain tropical forest communities (IPCC2007, Nichols 2008). Changing precipitation patterns may disadvantage the recruitment of some species that produce desiccation sensitive seeds, especially if increasing dry season conditions increase the risk that desiccation sensitive seeds are dispersed into desiccating and potentially lethal environments.

Components of this thesis

The thesis is divided into four data chapters and a synthesis chapter. The four data chapters (Chapters 2 – 5) are presented as manuscripts which have been published, or submitted for publication. Chapter 2 identifies phenological trends in seed dispersal using seed rain data collected from 72 seed traps monitored weekly over a four year period. In this chapter I identify a community-wide peak in seed dispersal that coincides with the wet season. I also examine seed production recording the number of species that have low seed output over a given year to assess inter-annual pattern in seed production as function of environmental conditions. During an extreme ENSO related drought episode in 2002, 28/40 species recorded their lowest seed output. The findings from this chapter highlight that phenology and seed output are correlated with moisture availability and that a decrease in seed production may be expected under an increased frequency of extreme climatic events (i.e. drought) Chapter 3 identifies species that occur within the study site that produce desiccation sensitive seeds. In doing so I broaden the knowledge of species that produce desiccation sensitive seeds within

Australian tropical forests. I also record the phenological patterns of these species and demonstrate that many of these desiccation sensitive species disperse seeds during the driest period of the year. I highlight recent climate models that predict that the duration of the wet season will decrease in the future, exposing many desiccation sensitive species to an increased period of moisture deficit that will potentially reduce regeneration opportunities. Chapter 4 explores water loss in desiccation sensitive seeds as a mechanism to prolong seed viability under dry conditions. In this chapter I identify three different models to describe seed water loss among species; a negative exponential model, a double negative exponential model and a double linear model. I also demonstrate that water loss is not related to seed mass between species or within species. I conclude by suggesting that other structural components of the seed, primarily the seed coat, may be a better predictor of water loss in desiccation sensitive seeds. Chapter 5 quantifies a number of traits of desiccation sensitive seeds and is a direct test of the conclusion from Chapter 5. I measured four physiological traits: critical water content (Wr50), desiccation rate (t0.368), mean time to germination (TG), and specific relative water content (WM), two morphological traits: seed size (MD), dry seed mass and seed coat ratio (SCR), and one phenological variable: mean monthly rainfall at mean time of seed dispersal (RM). Principle component analysis revealed that SCR, TG, *t*0.368, RM and WM, were more important for surviving pre-germination environmental conditions, independent of the advantages and/or disadvantages that size has been shown to confer on seed survival and seedling establishment. Finally, Chapter 6 provides a synthesis of the results from this study, outline the limitations of this study and possible research questions that could be addressed in the future.

Chapter 2. Decreased seed production associated with severe drought in a seasonal tropical forest.

This chapter is currently in preparation for submission as "*Hill, J & Edwards, W. Decreased community-wide seed production associated with extreme drought in the tropical rainforests of northern Australia*"

Introduction

Phenological patterns include the timing of vegetative processes such as leaf development and leaf senescence, and the timing of reproductive functions such as flowering and seed production (Newstrom *et al.* 1994, Corlett and LaFrankie 1998). Annual and inter-annual patterns in reproduction within tropical forests have been reported to be strongly influenced by seasonality in two abiotic factors important in setting rates of photosynthesis; moisture availability (Borchert 1994, 1996, Sakai *et al.* 2006, Brearley *et al.* 2007), and levels of irradiance (van Schaik *et al.* 1993, Wright and van Schaik 1994, Saleska *et al.* 2003, Zimmerman *et al.* 2007).

Whether timing of reproductive events arise as a function of light or water limitation may be related to the intensity and duration of seasonal drought experienced (van Shaik *et al.* 1993, Zimmerman *et al.* 2007). For example, 60% (12/20) tropical forest sites described as strongly seasonal have been shown to exhibit peaks in flowering during the onset of the wettest period of the year (van Sheik *et al.* 1993). Similarly, studies conducted in forests that experience seasonal rainfall patterns also report a correlation between fruit/seed dispersal and the onset or occurrence of periods of high rainfall (Lieberman 1982, White 1994, Murali 1997, Bollen and Donati 2005). In the above studies authors invoke moisture limitation during periods of seasonal drought as the principle mechanism setting phenological patterns. Alternatively, Zimmerman *et al.* (2007) reported that a higher than expected number of species dispersed seeds during the dry season in a seasonally moist forest on Barro Colorado Island (BCI), Panama, suggesting light, rather than moisture, was most limiting for seed production. Indeed, light limitation may be very important in tropical forests that experience long periods of constant cloud cover which limits photosynthesis.

In contrast, concentration of phenological patterns during the wet season is less prevalent in locations where rainfall is considered only weakly seasonal; 33% (8/24) of sites with weak rainfall seasonality exhibited flowering peaks coincident with the wet season (van Sheik *et al.* 1993). This is true also for fruiting and seed release periods. For example, community-wide fruit production was reported to be uniformly distributed throughout the year in four sites in the aseasonal Atlantic tropical forests of Brazil (Morelleto *et al.* 2000) as well as in tropical forests of Mt Kinabalu, Borneo (Kimura *et al.* 2001) and El Verde, Puerto Rico (Zimmerman *et al.* 2007).

Unlike other locations throughout the world, Australian tropical, seasonally dry forests are dominated by evergreen tree species (Bowman and Prior 2005, Heise-Pavlov *et al.* 2008), rather than deciduous ones. One explanation is that locations within Australia where these forests exist is more extreme in terms of rainfall seasonality and variability, which favours the evergreen habit because maintaining leaves maximises CO_2 assimilation over longer periods (Bowman and Prior 2005). Given this fundamental difference in physiology it is unknown whether results from phenological studies conducted in other parts of the world can be used to predict responses in Australian seasonally dry forests. Identifying factors that initiate or limit seed and fruit production within tropical forests will be crucial for conservation because annual patterns of moisture and light availability are expected to change with changing global climates (Hulme and Viner 1998, Malhi and Wright 2004, BOM 2007, IPCC 2014).

Phenological patterns can also vary at inter-annual scales. The greatest source of inter-annual climate variation affecting most bioregions that sustain tropical forests is the El Niño Southern Oscillation (ENSO) (Kiladis and Diaz 1989, Malhi and Wright 2004). ENSO events are associated with high temperatures, increased levels of irradiance, reduced annual precipitation and extended dry seasons (Curran *et al.* 1999, Wright *et al.* 1999, Asner and Townsend 2000, Laurance and Williamson 2001, Sakai 2002, Sakai *et al.* 2006, Wright and Calderón 2006, Brearley *et al.* 2007, Malhi and Wright 2004, Condit *et al.* 2004). Drought conditions associated with ENSO have been shown to increase tree and seedling mortality both directly (Leighton and Wirawen 1986, Condit *et al.* 1995, Laurance *et al.* 2001, Edwards and Krockenberger 2006) and indirectly through increased likelihood of the occurrence of wildfires (Leighton and Wirawen 1986, Slik and Eichhorn 2003, Kobayashi *et al.* 2005, Edwards and Krockenberger 2006). However, little is known about how phenological patterns respond to inter-annual variability in conditions, especially those associated with ENSO.

Predictions about how phenological processes in tropical forests respond to ENSO events are uncertain. Mild drought conditions and reduced cloud cover associated with ENSO events has been shown to increase community wide reproduction and/or coincide with seed production in species that reproduce at supra-annual intervals (Curran *et al.* 1999, Wright *et al.* 1999, Wright and Calderón 2006, Sakai 2006, Bearley *et al.* 2007). These reports are specific to aseasonal and mildly seasonal tropical forests of Borneo and BCI, Panama. Nevertheless, under extreme drought conditions plant reproduction might be expected to decrease especially in all tropical forests that are strongly seasonal (Wright and Calderón 2006) due to the negative effects of prolonged moisture deficit on photosynthesis.

In January 2002 I began a monitoring program to study patterns in seed and fruit production in a seasonal tropical forest (see methodology for definition) in Queensland Australia. At the site, seed rain was monitored over four years. In this study I examine three components that combine to generate potential intra-annual patterns in the timing of seed dispersal and compare these results against findings from other tropical forest sites. The three components are; (a) the duration and timing of seed dispersal periods in individual species, (b) temporal concordance between species in the timing of seed dispersal, and (c) the community level concentration of seed dispersal during the year. Moreover, during 2002/03 (the first years of our study) most of the Australian continent experienced a severe drought in association with an ENSO event (Nicholls 2004). Thus, I was in a unique position to compare inter-annual phenological patterns of fruit and seed production between ENSO-related drought and non drought years.

Specifically, I ask three main questions: (i) Are patterns in the timing of seed release similar for all species? (ie. is seed dispersal seasonal), and if so, (ii) Within what season is seed dispersal most concentrated?, and (iii) What was the influence of drought associated with the 2002/03 ENSO event on seed production?

Methodology

The study was conducted within the Smithfield Conservation Park (SCP) (16º48´S, 145º41´E) north Queensland, Australia (Figure 1). The SCP borders the Wet Tropics World Heritage Area and covers approximately 270 ha of lowland tropical rain forest. Major vegetation types represented include; Mesophyll Vine Forest, Complex Notophyll Vine Forest and Simple Notophyll Vine Forest (Stanton and Stanton 2003). The site is seasonal, based on Leigh’s (2004) broad definition of mean monthly precipitation less than 100mm in one, or more months. Under this scheme, forests are considered aseasonal if total monthly precipitation >100mm in all months of the year and seasonal when rainfall totals <100mm in any month, in recognition that 100mm represents the

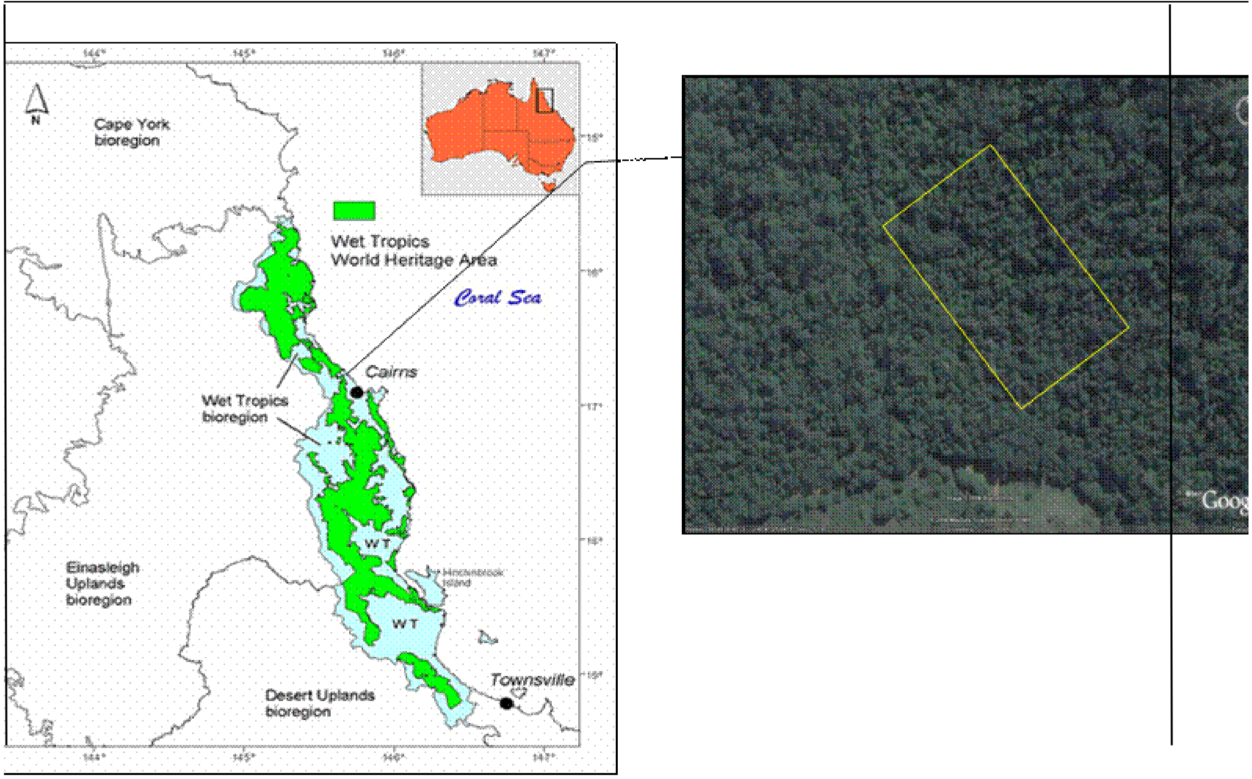


Figure 1 Location of Smithfield Conservation Park (SCP) within the Wet Tropics World Heritage Area in North Queensland (-16.8106, 145.6813) (*Map courtesy of the Wet Tropics Management Authority*).

level below which water lost due to evapo-transpiration exceeds precipitation, resulting in moisture deficit (Leigh 2004). The forest is comprised predominately of evergreen tree and vine species with a heterogeneous canopy approximately 25-35m in height. Species (and higher taxa) richness is high, and we have recorded 130 tree and vine species from 49 plant families. Taxonomic composition is typical for these forest types, with high species richness in the families Lauraceae, Myrtaceae, Meliaceae, Rutaceae and Sapindaceae. The study site was a two-hectare area of Mesophyll Vine Forest

(Stanton and Stanton, 2003). All species were divided into broad groups according to dispersal mechanism; those species requiring biotic assistance to disperse fruit (assisted), or those that require wind to aid in fruit/seed dispersal (non-assisted). Species with non-assisted dispersal were those that produced fruits or seeds containing features promoting dispersal via wind (i.e. samaras, plumes attached to seed etc.).

We recorded seed rain by collecting seeds and fruits that dispersed into 72 seed traps over four years (1 January 2002 – 1 January 2006). Seed traps were constructed of a circular wire frame with a conical, polyethylene funnel suspended approximately 0.2 m above the ground. The diameter of each trap was 0.80 m, resulting in a total catch area of approximately 0.5 m^2. Traps were positioned above permanently secured collecting pots (0.1 m high). Fine mesh bags were inserted within each pot, into which all material falling into seed traps was collected. The 72 traps were positioned in a grid-like arrangement. Six traps were regularly located along each of 12 north-south transects and the mean distance between any two adjacent traps was 15.57m (± 0.3 m SE). Seed production was assessed weekly. All material falling into traps was returned to the laboratory where seeds and fruits were sorted and identified to species using local botanical keys (Hyland *et al.* 2002, Cooper and Cooper 2004). Seed dispersal was measured by the number of seeds that were deposited into traps. In cases where fruits, rather than single seeds, were collected all fruits were dissected to determine total seed numbers. Very small seeds that could not be identified under 10×magnification (i.e. figs and orchids) and seeds from the Poaceae were excluded. I acknowledge that exclusion of small seeded species and grasses (as well as our trapping method) will mean that our sample does not necessarily reflect the entire plant community. While I am confident that the analyses describes phenological pattern in the species included in the study (see below), I make no claim that these will be similar in all other species.

To position the site with respect to other tropical rain forest sites throughout the world we began by characterising the strength of seasonality. I calculated Walsh's pre humidity index (W.I.) as an estimate of seasonality (Walsh 1996) using 71 years (1943-2014) of monthly rainfall records taken at Cairns airport (approximately 10 km from the study site) (Aust BOM). Walsh's index is calculated by scoring rainfall totals for each month (2 for months with > 200 mm precipitation; 1 for months between 199 mm – 100 mm; -1 for months ranging from 51- 100 mm and -2 for months < 50 mm) and summing monthly scores over each year. For months averaging less than 100 mm but preceded by a month exceeding 100 mm 0.5 is added. High positive scores are characteristic of aseasonal forests where monthly rainfall totals rarely, if ever, fall below 100 mm. Low (even negative) scores are characteristic of seasonal forests and reflect the number of months that experience average rainfall below 100 mm. Although simple, W.I. has been used to divide the 18 forests plots within the Centre for Tropical Forest Science (CTFS) network into nine aseasonal and nine seasonal sites (Leigh 2004) and we tested whether W.I. value generated for the SCP site could be assumed to come from either of the two groups of CTFS sites via two independent *t*-tests.

Intra-annual patterns in seed dispersal.

To identify phenological patterns (i.e. the concentration of seed production) we first tested if species had an aggregated period of seed release and if so, within which particular months. We used this information to then test whether seed dispersal periods were randomly distributed throughout the year, or were concentrated within either the wet or dry seasons. As expected, the number of seeds recorded for each species was not uniform and many species were represented by very few records. Under these circumstances, using species with very few records in attempts to determine phenological patterns will clearly lack precision. The approach of excluding species due to insufficient data was based on that of Zimmerman *et al.* (2007) who used seed rain records collected from seed traps in El Verde (Puerto Rico) and BCI (Panama). In El Verde and BCI traps are positioned along linear walking trails in a random stratified manner. To ensure that the species for which analyses were conducted were likely represented by greater than one individual, Zimmerman *et al.* (2007) excluded species if they were recorded in ≤ 5% of all traps (i.e. El Verde $n_{traps} = 6$; BCI $n_{traps} = 10$) and represented by less than 16 records. Mean distance between traps in this study (15.57m), and the minimum distance between traps on BCI and El Verde (15.7m) are similar, however, these study traps were arranged on a grid. The grid arrangement of traps could result in seeds from a single individual reaching a greater number of traps in our study than at El Verde or BCI. To account for this I selected species for inclusion in analysis using a more restrictive set of criteria. Species were excluded if they were represented by less than 20 records and/or occurred in less than 20% ($n_{traps} = 14$) of traps.

For all species that fulfilled the above criteria a circular statistical approach was used to examine annual patterns in seed production. A similar statistical approach was adopted as Wright and Calderon (1995), Zimmerman *et al.* (2007) and Ting *et al.* (2008). Circular statistical techniques are required when data are arranged on a circular scale (such as weeks within years) and the designation of high and low values are arbitrary. For example, a series of weekly records that encompass December-January will include weeks with both high and low values when considered on a Julian time scale, and a simple linear mean will not reflect true indication of central tendency (Zimmerman *et al.* 2007). Weekly census records were converted to the corresponding angle (week 1 = 6.92º) and weighted by the number of seeds recorded after which mean angles (*a*) and confidence limits were calculated. These estimates were back transformed to an estimate of the mean time (week). Vector lengths (*r*) were also calculated as a measure of concentration around the mean angle. To examine whether seed rain in each species was significantly concentrated within a particular time of the year, I tested for uniformity in the distribution of seed rain records for each species via individual Rayleigh's tests. Species that showed significant deviation from uniformity and returned mean weekly angles (and standard error) that corresponded to periods falling between May and November (weeks 18-47) were classified as dry season dispersers, while those species with mean weekly angles between December and April (weeks 48-52; 1-17) were classified as wet season dispersers. Species that did not demonstrate a significant concentration, could not be ascribed to a "season" and were not included. In total, this was one species. To compare trends in the concentration of seed release between aseasonal and mildly seasonal tropical forests the vector lengths calculated by Zimmerman *et al.* (2007) for El Verde and BCI were used to compare with those obtained from SCP. A t-test was used to examine differences between sites. To examine whether patterns of seed rain was concentrated within either the wet or dry seasons we examined deviations from expected values of the number of species in wet or dry season based on the assumption of no temporal concentration within any season via a log–likelihood (*G*) test.

Inter-annual patterns in seed dispersal.

To examine inter-annual responses we asked whether there were consistent patterns in variation in seed production between species across years. Species were classified into two groups according to the number of years that seeds were recorded; 1) recorded in all, or three of four years, and 2) recorded in one or two years only. Because the data collection began in January 2002, the 2002 wet season records (December – April) did not cover the same period as wet seasons used to characterise seed rain in later years. Thus, total seed rain for species dispersing primarily in December-January may have been low during 2002 because our sampling period did not include December 2001. To account for this I removed all species in which the standard error of the mean angle included dates spanning December-January. Of the original 50 species used to assess temporal concentration, four were omitted on this basis. Of the remaining 46 species, 28 were recorded for all four years and 13 species were recorded in three of the four years. The remaining five species, recorded in one or two years only, were not considered further. All years were scored (2002 – 2005) for the number of species in which either seed rain records were lowest or absent in one of four years and examined divergence from expected frequencies via a log-likelihood (*G*) test. Deviations were then examined for significance by calculating the Freeman-Tukey (FT) deviates and corresponding *P* values associated with each cell (Sokal and Rohlf 1995). Finally, I considered the possibility that drought might affect species with dry season versus wet season seed fall differently. To test this possibility I performed a log-linear analysis similar to above but included “period of dispersal” as well as “year” as a main effect (Sokal and Rohlf 1995).

To link above results (see below) to inter-annual variability in rainfall I then assessed the level of rainfall experienced within each of the study years against the mean rainfall expected for the area. I compared total rainfall recorded in each of our study years (2002 – 2005) against the frequency distribution of total annual rainfall generated from the 70 year dataset via simple *Z* test after *ln* transformation. Because the wet season included December (see Results), yearly totals were generated from the continuous period December – November.

Results

Annual mean (± SE) precipitation was 1991 mm (± 305.2 mm) in Cairns. Five months (December–April) have mean monthly rainfall >100 ml and were classified as representing the wet season, and seven months (May–November) have mean monthly rainfall totals >100 ml and represent the dry season (Figure 2). In comparison to other study sites within tropical rain forests across the world, the Cairns area is highly seasonal. Mean Walsh's Index calculated for SCP was -2.7, significantly lower than W.I.'s recorded from aseasonal (mean W.I. = 20.87; $t_7 = 36.68$, $P < 0.0001$) or seasonal (mean W.I. = 6.62; $t_7 = 4.59$, $P < 0.01$) forests in the CTFS (Figure 3).

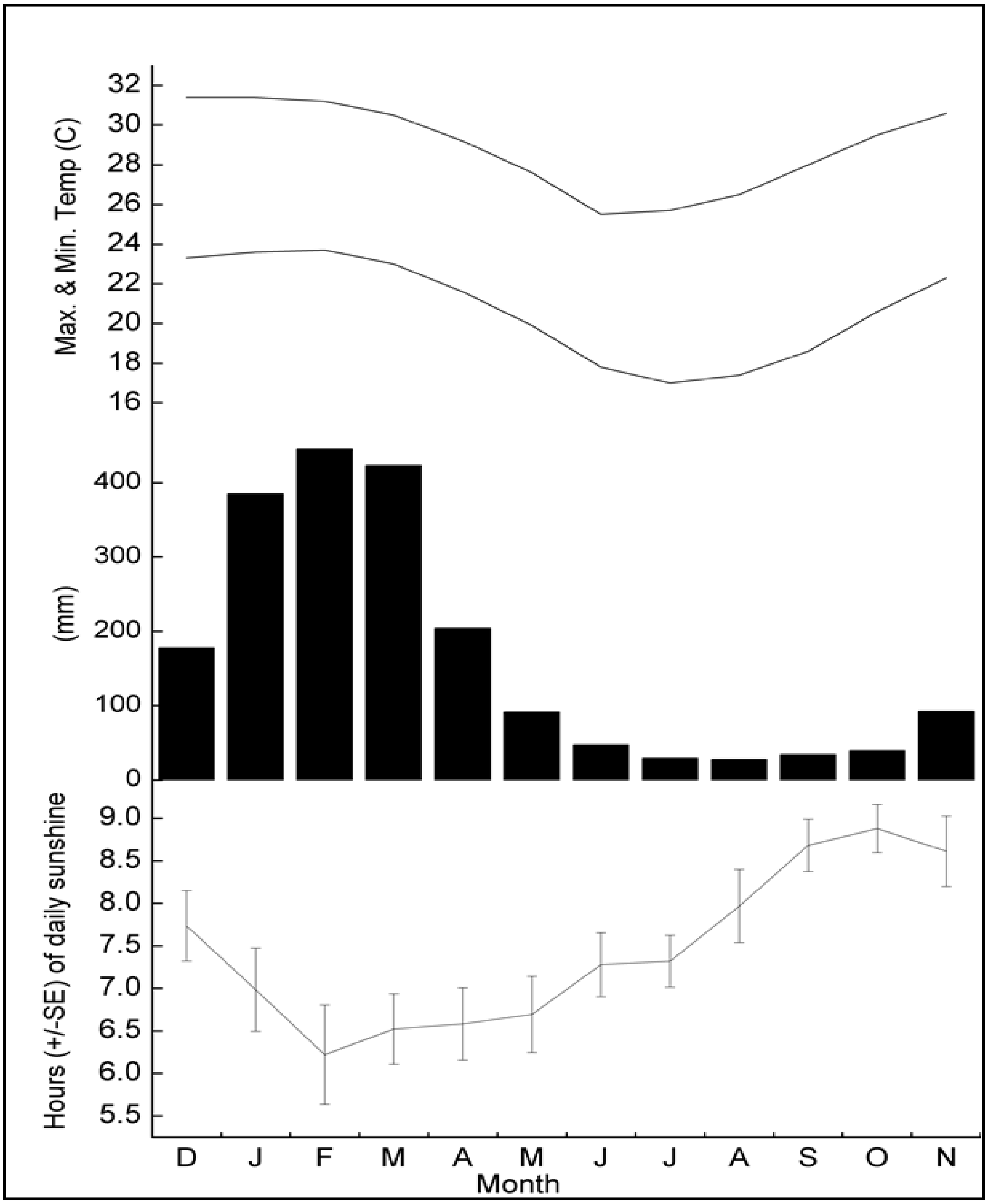


Figure 2 Meteorological data from the Cairns Airport situated 14km south of the study site. Figure represents the mean monthly rainfall, the mean minimum and maximum monthly temperature and the mean hours of sunlight per month. (data supplied by the *Australian Bureau of Meteorology*).

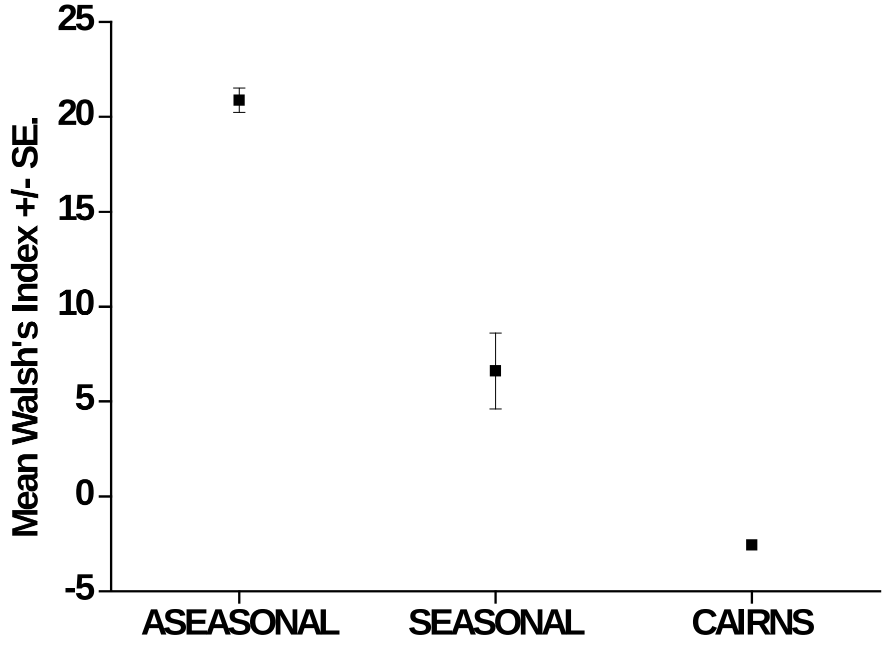


Figure 3 A comparison of the mean Walsh's seasonality index (± SE) between aseasonal tropical forest sites (n=9), seasonal tropical forest sites (n=9) and Cairns, Queensland. Data for tropical forest sites was obtained from the CTFS network (Leigh 2004).

Intra-annual seed dispersal.

We collected a total of 75332 seeds representing 205 species and morphospecies. Most species were represented by very few seeds (i.e. 58 species from a single seed only and 99 species were represented by five seeds or less). Fifty species met the criteria of being recorded on 20 or more occasions and within at least 20% of the traps

(Table 1). Of these, seven were recorded as requiring an abiotic agent for dispersal whilst the majority (43) required biotic agents. All but one species, the vine *Cayratia trifoliata* (L.) Domin. displayed a significant deviation from uniformity in the period of seed release (Figure 4a),and the degree of concentration was similar to that reported in other seasonal forests. The mean vector length (± SE) was 0.75 (± 0.03) was not significantly different to the mean vector length determined in seasonal forests of BCI, Panama (0.74 ± 0.02; $t_{233} = -0.156$, $P = 0.98$), although it was significantly higher than mean vector length calculated for the aseasonal forest of El Verde (0.57 ± 0.02; $t_{110} = -4.32$, $P < 0.001$).

Twenty-eight of the 49 species (57%) had mean (± SE) seed release periods within the wet season, even though no species were recorded in April (last month of wet season). As a result, we found no support for the hypothesis that temporal concentration in seed release was randomly distributed throughout the year. A significantly higher than expected number of species had mean dispersal periods concentrated within the five month wet season (n = 28) as opposed to the seven month dry season (n = 21) ($G_1 = 4.74$, $P < 0.01$, Figure 4 a and b).

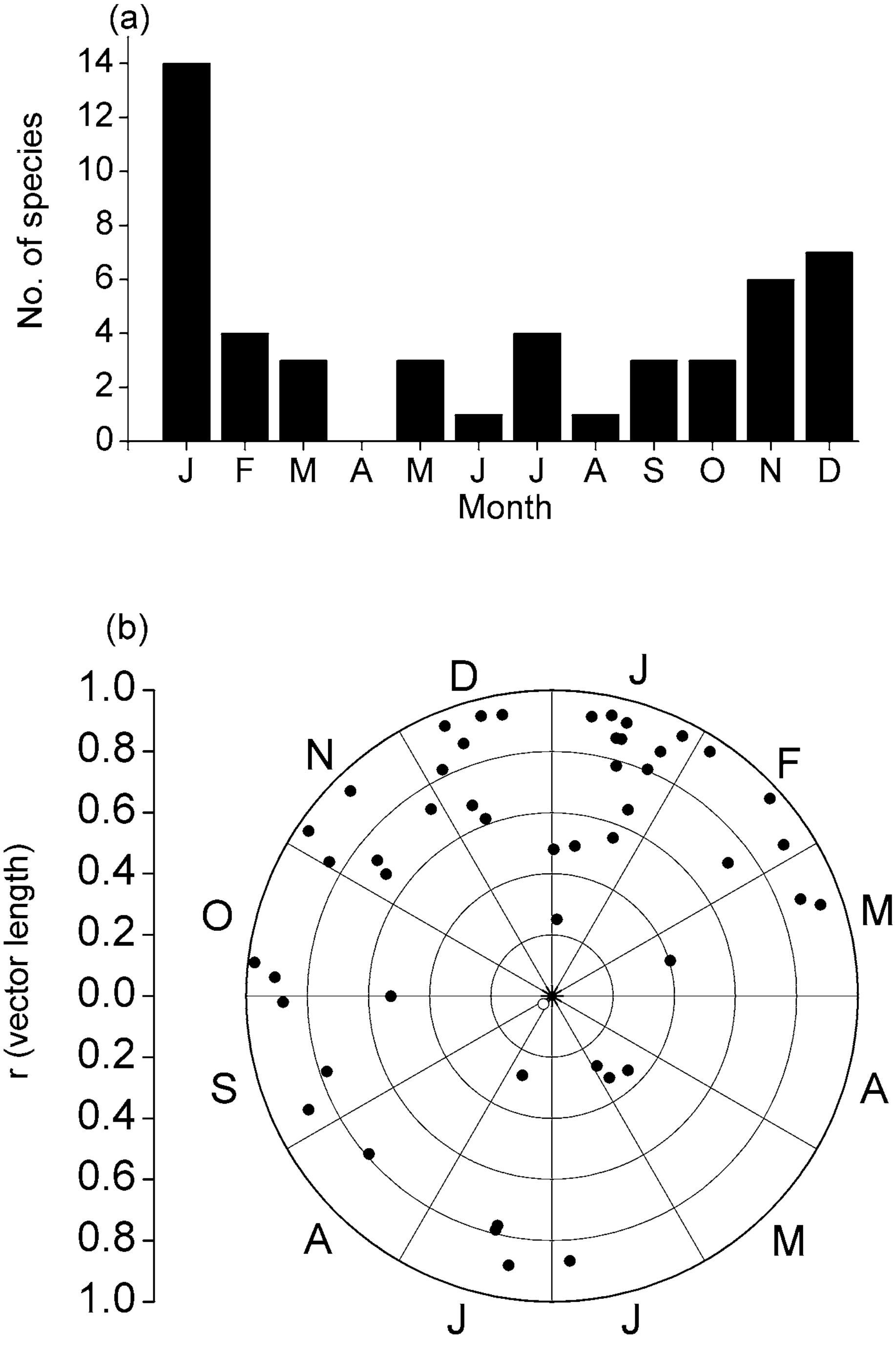


Figure 4 Pattern of temporal concentration in seed release. **4*a***). Frequency distribution of dispersal periods within months. **4*b***). A polar plot with mean angles and vector. Vector angles represent mean weeks and vector lengths represent the temporal

concentration. Vector lengths range from 0 to 1, 0 indicating a perfectly uniform distribution, 1 representing temporal concentration within in a single week (increments are 0.2). Closed circles represent species displaying a significant seasonal distribution in seed rain and open circles represent species displaying a uniform distribution as determined via Rayleigh's test.

Inter-annual seed dispersal

There was a consistent pattern of variability in inter-annual seed production among species. Twenty-four of the 41 species recorded either their poorest year of seed production or were not recorded at all in 2002, while nine, six and two species recorded their poorest years during 2003, 2004 and 2005 respectively. Overall, there was a significant deviation between observed and expected frequencies between years (G_3 =25.53, $P < 0.0001$). Examination of the FT deviates identified significant positive deviation between the observed and expected cell frequencies for 2002 and a significant negative deviation in 2005 (Table 2.) (Figure5). There was no significant deviation between the observed and expected cell frequencies during 2003 or 2004 (Table 2.).

Table 1 The timing of seed release (weeks), for 50 plant species within a seasonal tropical forest in the Smithfield Conservation Park, Queensland. Also present is the number of seeds collected for each species and the number of years where seeds were collected.

Species	**No. of seeds trapped**	**Dispersal mechanism**	**Mean angle (*a*)**	**Standard Error of Mean**	**Mean Vector (*r*)**	**Rayleigh's Test (P)**	**Week**	**Season**	**Years**
Acacia polystachya	178	Assisted	350.0°	1.586°	0.934	<.0001	51	wet	4
Agyrodendron peralatum	237	Non-assisted	22.2°	3.451°	0.658	<.0001	4	wet	4
Alphitonia whitei	576	Assisted	337.4°	2.133°	0.674	<.0001	49	wet	4
Alstonia muelleriana	41	Non-assisted	334.2°	5.577°	0.821	<.0001	49	wet	4
Blepharocarya involucrigera	902	Non-assisted	8.1°	0.77°	0.922	<.0001	2	wet	4
Brombya platynema	145	Assisted	147.3°	12.118°	0.272	<.0001	22	dry	4
Calamus australis	47	Assisted	24.0°	4.34°	0.873	<.0001	4	wet	3
Calamus radicalis	89	Assisted	15.2°	3.191°	0.87	<.0001	3	wet	3
Carallia brachiata	73	Assisted	315.4°	2.378°	0.939	<.0001	46	dry	3
Cayratia trifoliata	364	Assisted	225.6°	56.093°	0.038	0.594	33		4
Chionanthus ramiflora	311	Assisted	273.9°	1.422°	0.908	<.0001	40	dry	4
Cissus hastata	135	Assisted	3.7°	13.69°	0.251	<.001	1	wet	4
Connarus conchocarpus	72	Assisted	176.1°	3.581°	0.868	<.0001	26	dry	3

Cordia dichotoma	291	Assisted	345.8°	1.151°	0.943	<.0001	50	wet	4
Cryptocarya mackinnoniana	83	Assisted	200.5°	15.668°	0.278	< 0.01	29	dry	4
Decaspermum humile	3457	Assisted	193.5°	0.666°	0.787	<.0001	28	dry	3
Diplocyclos palmatus	905	Assisted	229.1°	1.294°	0.79	<.0001	34	dry	3
Diploglottis diphyllostegia	1304	Assisted	276.4°	0.341°	0.977	<.0001	40	dry	3
Dysoxylum pettigrewianum	54	Assisted	22.9°	5.098°	0.804	<.0001	4	wet	4
Elaeocarpus bancrofttii	51	Assisted	8.7°	10.68°	0.496	<.0001	2	wet	3
Endiandra hypotephra	62	Assisted	268.7°	3.686°	0.879	<.0001	39	dry	3
Endiandra longipedicilliata	91	Assisted	306.4°	5.389°	0.672	<.0001	45	dry	3
Euroshinus falcata	86	Assisted	338.4°	2.02°	0.948	<.0001	49	wet	3
Flagellaria indica	529	Assisted	14.0°	1.32°	0.868	<.0001	3	wet	4
Ganophyllum falcatum	362	Assisted	12.1°	1.076°	0.938	<.0001	2	wet	4
Glossocarya hemiderma	8679	Non-assisted	73.4°	1.03°	0.404	<.0001	11	wet	4
Gossia myrsinocarpa	831	Assisted	21.1°	2.315°	0.553	<.0001	4	wet	4
Hippocratea barbata	56	Non-assisted	251.4°	5.389°	0.776	<.0001	37	dry	1
Hypserpa laurina	95	Assisted	339.5°	5.937°	0.618	<.0001	50	wet	4
Jasminum didymum	37	Assisted	15.6°	6.549°	0.781	<.0001	3	wet	2
Macaranga subdentata	46	Assisted	145.0°	17.746°	0.327	<.001	21	dry	3
Macaranga tanarius.	96	Assisted	301.1°	3.331°	0.849	<.0001	44	dry	4
Mallotus polyadenos	596	Assisted	47.9°	0.66°	0.961	<.0001	7	wet	4
Melodorum uhrii	477	Assisted	327.1°	2.081°	0.727	<.0001	48	dry	4

Muehlenbeckia rhyticarya.	206	Assisted	193.3°	2.849°	0.771	<.0001	28	dry	2
Myristica globosa	310	Assisted	307.9°	2.613°	0.722	<.0001	45	dry	4
Neosipicaea jucunda	142	Non-assisted	15.4°	1.903°	0.925	<.0001	3	wet	4
Passiflora suberosa	1469	Assisted	134.4°	2.938°	0.348	<.0001	20	dry	4
Polyscias australiana	980	Assisted	56.9°	0.819°	0.905	<.0001	9	wet	4
Polyscias elegans	42406	Assisted	244.9°	0.142°	0.877	<.0001	36	dry	4
Pouteria chartacea	42	Assisted	269.9°	10.986°	0.526	<.0001	39	dry	3
Ptychosperma elegans	38	Assisted	0.7°	12.86°	0.479	<.001	1	wet	3
Rhamnella vitiensis	333	Assisted	32.9°	0.997°	0.951	<.0001	5	wet	4
Scolopia braunni	105	Assisted	53.0°	4.497°	0.721	<.0001	8	wet	4
Solanum torvum.	74	Assisted	340.7°	3.457°	0.873	<.0001	50	wet	4
Terminallia seriocarpa	166	Assisted	26.6°	1.402°	0.951	<.0001	4	wet	4
Tetracera nordtiana	3904	Assisted	68.8°	0.478°	0.872	<.0001	10	wet	4
Trichosperma pleitostigma	1407	Non-assisted	304.1°	0.438°	0.96	<.0001	44	dry	4
Viticipremna queenslandica	56	Assisted	189.1°	3.658°	0.892	<.0001	28	dry	2
Xanthophyllum octandrum	32	Assisted	71.2°	4.658°	0.927	<.0001	11	wet	2

The interaction term in the log-linear analysis between the main effects ‘period of seed release’ and ‘year’ was not significant ($\chi^2_3 = 4.25$, $P = 0.236$). Low seed output occurred during 2002, and was experienced equally between species that dispersed seeds during the wet and dry seasons. The main effect “period of seed release” was not significant ($\chi^2_1 = 0.22$, $P = 0.64$). This is in contrast to our demonstration that a greater than expected number of species were identified as wet season dispersers when considered across the entire 49 species (above), and indicates that the majority of species removed from this analyses (those that produced seeds in one or two years only) were wet season dispersers. The results for the main effect “year” were, of course, identical to the result of the *G* test described above.

There was strong concordance between the number of species producing lowest seed output in 2002 and rainfall conditions during that time. Total annual rainfall for 2002 was 721mm, the lowest on record and a significant negative deviation from the distribution derived from all other 71 years ($Z = -3.2$, $P < 0.01$) (Table 3). This arose because precipitation in both the wet and dry seasons were significantly lower than the seasonal averages (wet season = 602.8mm; $Z = -3.3$, $P < 0.01$; dry season = 118.2mm; $Z = -2.46$, $P < 0.01$). The *Z* scores for total annual rainfall and for wet and dry season precipitation were not outside of the 95% CI for years 2003 – 2005 (Table 3).

Table 2 Observed and expected frequencies of the combined number of species recording lowest seed rain in each of the four study years and the number of species that failed to produce seeds in one of the four study years. Also shown is the log-likelihood G – test for significant deviation between observed and expected frequencies and the post-hoc Freeman-Tukey deviates identifying years where significant deviations occurred. Positive values for the Freeman-Tukey deviates indicate greater than expected numbers of species, negative values fewer than expected (** significant at P < 0.01).

Group	**Year**			
	2002	**2003**	**2004**	**2005**
Species recorded in all years.				
Observed	24	9	6	2
Expected	10.25	10.25	10.25	10.25
G =25.53, df = 3, *P* = 0.000012				
Freeman-Tukey deviates	+3.4**	-0.31	-1.38	-3.33**

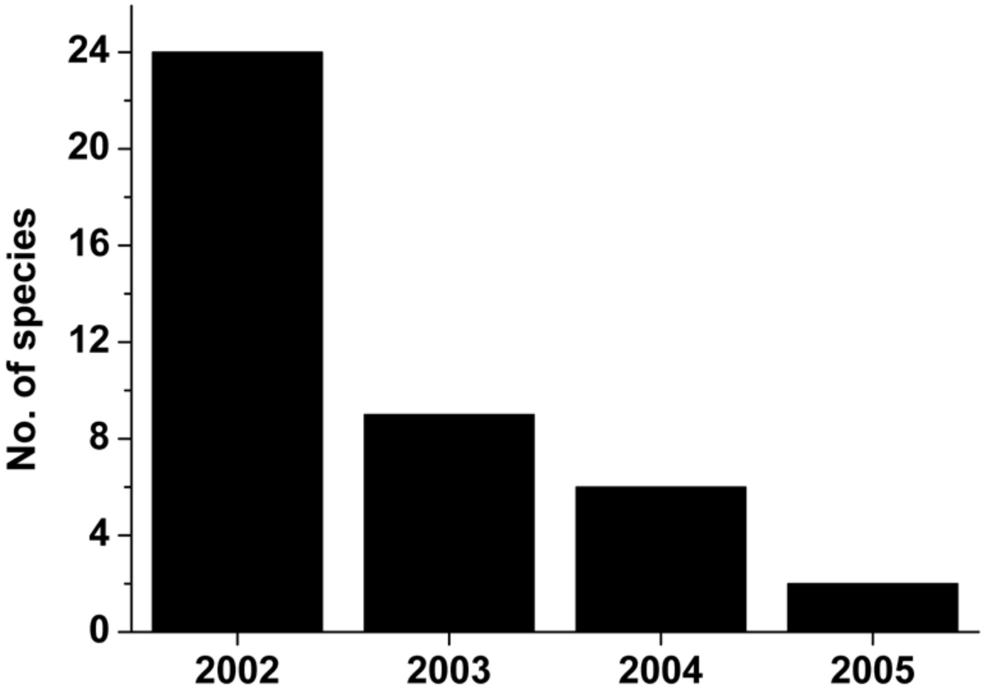


Figure 5 The distribution of species with poor seed production across years, showing highest frequencies of poor performance during the year 2002. Frequency distribution of the numbers of species with poor seed production within a given year.

Table 3 Total annual rainfall and rainfall during "wet" and "dry" seasons for Cairns Airport, 10 km SE of Smithfield Conservation Park (SCP) Cairns over four years of study (2002-2005). Also shown are the 70 year mean values. Z-scores are the results of the comparisons of rainfall recorded in each season in each year against the frequency distribution of rainfall in wet or dry seasons generated from the 70 year dataset, and Walsh's seasonality Index (W.I.) for each study year.

			Year		70 yr
	2002	2003	2004	2005	mean
Total rainfall (mm)	721	1279	2777	1471	2014
Total rainfall (mm) Wet Season	602.8	1076.6	2556	1252	1655
Z	-3.3	-1.18	+1.5	-0.72	
P	> 0.01	N.S	N.S	N.S	
Total rainfall (mm) Dry Season	118.2	202.6	221.6	218.8	362
Z	-2.49	-1.21	+1	-1.03	
P	> 0.01	N.S	N.S	N.S	
Walsh's Seasonality Index	-9	-5.5	-5.5	-5.5	-2.5

Discussion

These results demonstrate that patterns of seed release within the tropical forest of SCP were associated with intra- and inter-annual moisture availability (rainfall). First, seed release periods for 49 species were not randomly distributed with respect to the wet and dry seasons. There was a significant concentration of species with mean seed release dates during the five month wet season. Second, and perhaps more importantly, the between-year analyses revealed a significant negatively association between seed production and drought conditions associated with 2002 ENSO event. During the 2002 ENSO total precipitation was extremely low and a higher number of species than expected either produced their lowest seed output compared with the following three years; or were not recorded in 2002, even though seeds were caught in all three subsequent years. The interpretation of these findings are that; (i) intra-annual variability in patterns of rainfall result in seed dispersal phenologies that are concentrated within the wet season for the majority of species, and (ii) influence of ENSO-related drought may result in decreased seed production.

In this study 49/50 species displayed aggregated periods of seed release. Other tropical forests have also reported significant aggregation in seed rain for the majority of species. For example, on BCI, another seasonal forest type, 183/186 species displayed concentrated seed release during some period within the year (Zimmerman *et al.* 2007) and this is proportionally equivalent to my findings. In aseasonal forests a similar pattern exists, but its strength is generally weaker. For example, at El Verde 62/65 species have concentrated seed release periods. However, the degree of temporal concentration differs based on strength of seasonal effects. For example, the mean vector length (r) determined for species occurring at SCP was not significantly different to that calculated for BCI, but both were significantly higher than that recorded from El Verde where seasonal effects are less strong.

In this study more species than expected displayed mean seed release dates within the five month wet season (December - April), while fewer species than expected occurred during the seven month dry season (May - November). Similar patterns of fruit production aggregated within a particular season have been observed in other seasonal tropical forests, although concentration within seasons differs between studies (Frankie *et al.* 1974, Opler *et al.* 1980, Lieberman 1982, White 1994, Murali 1997, Bollen and Donati 2005). For example, high numbers of species have been reported to disperse fruits and seeds during the wet season in seasonal tropical forests in Africa (Lieberman 1982, White 1994, Bollen and Donati 2005) and Asia (Murali 1997), while production coincident with the dry season has been reported in Costa Rica (Frankie *et al.* 1974) and BCI (Zimmerman *et al.* 2007). Whether or not a consistent pattern across sites might be expected is uncertain since van Sheik *et al.* (1993) examined 20 sites displaying rainfall seasonality and reported community peaks in seed production during wet periods in eight of these locations (van Sheik *et al.* 1993). This suggests that differences between sites may depend on local conditions experienced and be context and site specific.

Numerous possible mechanisms have been proposed to explain phenological patterns. For example, synchronous flower and fruit development result in higher rates of pollination success (Augspurger 1981, Sakai 2002) as well as escape from pre- and post-dispersal seed predation (Augspurger 1981, Curran and Leighton 2000). The proposed mechanism linking reproduction to the dry season on BCI is light limitation, due to high level of cloud cover during the wet season that reduces the potential for photosynthetic activity (Zimmerman *et al.* 2007). Light limitation may be particularly important at BCI, since the wet season spans seven months. A similar result has also been reported in the tropical dry forests of Hacienda La Pacifica (HLP), Costa Rica (Frankie *et al.* 1974). The HLP site also experiences a seven month wet season and is also presumably associated with a high degree of cloudiness. One obvious difference that may explain why the results differ from BCI and HLP is the relative lengths of the wet and dry seasons. At both BCI and HLP there is a seven month wet season and five month dry season. For the SCP site, the reverse is true.

Annual precipitation is highly concentrated in the Cairns region and periods of seasonal drought may be much longer than that experienced at other seasonal tropical sites. For example, mean monthly rainfall totals within the December-April wet season account for, on average, 82% of the annual precipitation. Further, the combined average rainfall for the three driest months is just 65mm (± 9 mm) and it is not uncommon for monthly rainfall totals during this period to fall below 10 mm. The longer duration and intensity of the annual dry season at SCP compared to other tropical locations is reflected in the significantly lower W.I. values compared to all rainforest plots within the CTFS. The dry season is also associated with a high proportion of cloud-free days, during which light limitation is unlikely to operate. My finding that seed dispersal was concentrated during the wettest period of the year suggests that water availability may be most important in setting phenological processes in this forest.

There are two hypotheses for why seed production and dispersal may be timed to avoid moisture deficit. First, many tropical plants produce seeds that are desiccation sensitive (Vanquez-Yanes and Orozco-Segovia 1993; Farnsworth 2000, Tweddle *et al.* 2003). In seasonally dry tropical forests, pressures to avoid highly desiccative conditions may select for seed release during the wettest period of the year (Pritchard *et al.* 2004). Second, selection may have operated to link seed release with the greatest opportunity for seedlings to become established prior to moisture deficits associated with the next dry season (Garwood 1983). Germination and establishment during periods of high moisture availability allow seedlings to develop deeper root systems that can mitigate top soil moisture deficits throughout periods of seasonal drought (Marod *et al.* 2002). This is especially true for species that grow in seasonal tropical forests where the development of deep root systems in early seedling growth enhances survival during drought conditions (Engelbrecht *et al.* 2002). My results do not allow distinction between these alternatives at present. Nevertheless, this does not diminish the association between seed dispersal in the majority of species and the occurrence of the wet season. This suggests a general phenomenon of seed dispersal phenology associated with patterns of moisture availability in this highly seasonal forest that may not be common to other sites.

Seed output also varied as a function of inter-annual differences in moisture availability. We found significantly more species than expected by chance produced their poorest seed output during the 2002 El Niño drought event. A negative association between seed output and ENSO was also not predicted based on previous work. For example, at BCI the past six ENSO events over the period 1986 -2004 have also been those years in which the highest number of species have been recorded producing fruit (Wright and Calderón 2006). Moreover, ENSO events have also been shown to coincide with mass flowering in species that reproduce at super-annual timescales in Southeast Asia (Ashton *et al.* 1988; Curran *et al.* 1999; Sakai 2002; Sakai *et al.* 2006; Bearley *et al.* 2007), and reduced levels of seed production have been linked to the cooler La Niña phase on BCI (Wright and Calderón 2006) and in Sabah, Borneo (Wong *et al.* 2005). Our analysis is in conflict with these findings, and, as far as I am are aware, represent the first documentation of a decrease in seed production associated with an ENSO event.

One possible explanation for the discrepancy between these results and previous studies is the magnitude of the drought conditions experienced. Previous studies have suggested that when ENSO events result in extreme water deficits, moisture availability has the potential to become the most important factor limiting net primary productivity

(Wright and Calderón 2006) and should have negative effects on reproductive potential. In Australia, the 2002 ENSO event coincided with perhaps the worst drought since European settlement (Nicholls 2004). Although the 2002 ENSO event itself was not considered strong based on Southern Oscillation Index (SOI) values (Hoerling and Kumar 1997; Huang *et al.* 2000) deviations in rainfall totals from seasonal averages were extreme. The significant negative deviation for total precipitation during both the wet and dry seasons during 2002 suggest that potential effects of moisture limitation were likely to be very severe with respect to the long-term average conditions and that moisture deficit was overriding in setting reproductive effort.

There are two potential influences operating in the data that must be considered. First, (as discussed in Methods) the sampling protocol was biased against species that produced small seeds. I was not able to report on the seed production in species that produce smaller seeds (i.e. Ficus species), or grasses. Furthermore species that produce large seeds generally produce fewer of them (Westoby *et al.* 1992). Hence it is likely that large seeded species were also under-represented in the analyses due to low numbers of seeds captured over the four year sampling period. I caution readers against interpreting these results to imply all species in the community respond in the same way, but do not consider that this diminishes the overall findings.

Second, in December 2002 a low intensity wild fire burnt through the accumulated leaf litter across approximately 55% of the 2ha plot (Edwards and Krockenberger 2006).

Although seedling mortality was high, mortality to trees was not, and most trees and small saplings in areas in which the fire passed were able to produce new leaves (Marrinan *et al.* 2005). Even so, there is a possibility that seed rain could have been affected by the fire event. We do not consider that this influenced the overall result, however. If tree mortality due to fire had operated to reduce seed production and dispersal we would have expected seed rain to decrease after December 2002. The fact that most species recorded their lowest seed outputs prior to December 2002 negates this concern.

Climate change is predicted to alter patterns of intra-annual rainfall (Malhi and Wright 2004). In particular, the duration of the dry season in tropical north Queensland is predicted to increase (BOM 2007, Dunlop and Brown 2008), intensifying patterns of intra-annual moisture availability and increasing the frequency of ENSO events. The results suggest that the majority of plant species we studied release seeds during times of high moisture availability and experience reduced seed output under ENSO-related drought conditions. More importantly, none of the results reported were predicted from studies in other locations and across other ENSO events. This discrepancy highlights the potential idiosyncratic nature of responses to extreme climatic conditions and the need to co-ordinate studies across multiple sites before generalities of how tropical rain forests will respond to future climates can be proposed.

In the following chapter I investigate a group of plant species that rely on moisture availability. Desiccation sensitive seeds lose viability once seed moisture contents drop below a specific threshold (Farnsworth 2000). Changing climates may be extremely detrimental to their persistence within seasonal rainforests.

Chapter 3. Dispersal of desiccation sensitive seeds is not coincident with high rainfall in a seasonal tropical forest in Australia.

This chapter has been published *as "Hill, J & Edwards, W. (2010) Dispersal of desiccation-sensitive seeds is not coincident with high rainfall in a seasonal tropical forest in Australia. Biotropica 42, 271–275*."

Introduction.

Seed release periods for plant species that produce desiccation sensitive seeds have been shown to coincide with periods of high precipitation (i.e. during wet seasons) in seasonal tropical forests (Pritchard *et al.* 2004, Daws *et al.* 2005, Yu *et al.* 2008). For example, on Barro Colarado Island (BCI), Panama, Daws *et al.* (2005) showed that all but five of thirty-two species that produce desiccation sensitive seeds released seeds during months in which the average rainfall exceeded 100mm. The adaptive significance of wet season dispersal is that conditions conducive to seed dehydration and subsequent seed mortality are avoided (Farnsworth 2000, Pritchard *et al.* 2004, Daws *et al.* 2006, Berjak and Pammenter 2008).

Predicted increases in temperatures and intensification of seasonality (the majority of total annual precipitation occurring within narrower temporal bands) in association with global climate change (Malhi and Wright 2004, BOM 2007, Dunlop and Brown 2008, IPCC 2014) suggest that plant species that produce desiccation sensitive seeds will be potentially at risk of reduced recruitment probabilities. This is because increased average temperatures should increase the rate of seed desiccation (decreasing the time seeds remain viable) and more concentrated rainfall patterns will expose seeds of some species to periods of increased aridity outside of that which currently exists.

Predictions associated with climate models for tropical north east Australia suggest seasonal aridity will increase (BOM 2007, Dunlop and Brown 2008). Whether or not this might represent a significant threat to species with desiccation-sensitive seeds currently occurring in these locations is completely unknown at present. Across the entire tropical forests of north Australia, seed desiccation sensitivity has been documented in just three species; *Idiospermun australensis* (S.T. Blake) (Franks and Drake 2003) *Castanosperum austral*e (A. Cunn. and C. Fraser *ex* Hook.) and *Syzigium australe* (B. Hyland) (pers.com. M. Daws, The Royal Botanic Gardens, Kew). The lack of information is almost certainly due to the limited number of investigations rather than reflecting biological reality, since global estimates suggest that recalcitrant seeded species may represent approximately half (48%) of all seed-producing plants within tropical forests (Tweddle *et al.* 2003).

In this study I test desiccation sensitivity in seeds of 32 species in an Australian tropical forest community. I use this information, together with estimates of seed release periods to assess the widely reported hypothesis that dispersal periods of desiccation sensitive seeded species are coincident with the wet season. In doing so, I increase the information available on the number of tropical tree species that produce desiccation sensitive seeds. I also use this information to examine and highlight the potential impact of a predicted increase in seasonality and a reduction in total rainfall for this important, but neglected, group of species. For example, if most desiccation sensitive seeded species display seed dispersal phenologies timed to coincide with the wet season, as has been shown the general case in other tropical forest communities (Daws *et al.* 2005, Pritchard *et al.* 2004, Yu *et al.* 2008), then future intensification of seasonality may have little adverse effects on their abilities to maintain positions within these communities. If, however, this is not the case, increased seasonality and mean temperatures could negatively influence recruitment and establishment opportunities. Under this scenario, conservation efforts aimed at maintaining species composition may need to include direct management.

Methodology.

Seed desiccation sensitivity was assessed for 32 plant species. Species were chosen on the criterion that seeds could be collected from a minimum of five adult individuals and that sufficient information to estimate seed release periods was available. Species chosen represented a diverse range of fruit types (i.e. nuts, drupes, capsules, follicles, berries, samaras), seed dispersal morphologies (i.e. fleshy or dry fruits, aril present, winged seeds) (Cooper and Cooper 2004) and thus dispersal modes (i.e. vertebrate vectors and wind) (Table 4). All seeds were obtained from freshly dispersed fruit or individual seeds, collected on daily visits to known adult trees. The number of seeds collected per species varied between 40 and 60. Seeds were stored in polyethylene bags containing an equal mass of vermiculite and water and stored in a refrigerator set at 10˚C. Seed storage occurred for three days at which time seeds were regarded as fully hydrated. Seeds from each species were randomly and equally divided into two groups: (i) fully hydrated control; and (ii) desiccation treatment.

Seeds within the control group were cleaned and immediately placed into individual compartments partially filled with 1% agar (germination medium) within two or three (depending on seed size) larger polyethelene containers, and housed within a temperature controlled room set at 24º ± 1ºC. Seeds within the desiccation treatment were placed in individual compartments filled with 15 g of silica gel (desiccant) and dried to a constant mass. The relative humidity (RH) within compartments was monitored on consecutive days (Vaisala HMP44 temperature and humidity probe, Vaisala Inc.) and desiccant was replaced whenever RH within a single compartment rose above 10%. The mass of each seed in each desiccation treatment was recorded every second day until there was no detectable change in mass, at which time we considered each seed as fully desiccated. These were then transferred to germination media as described above. Germination was scored every two days over a four month period and was determined at the time the radicle had emerged approximately 2 mm (Pritchard *et al.* 2004a,b). Desiccation-sensitivity (recalcitrance) was ascribed for all species in which seeds germinated within the control, but not the desiccation treatment (Pritchard *et al.* 2004b).

Of the 32 species, seeds from six species germinated within the desiccation and control treatments and were not classified as desiccation-sensitive. Seeds from two species *Endiandra cowleyana* (F.M. Bailey) and *Agyrodendron peralatum* (Edin *ex* Boas) did not germinate in either treatment and could not be confidently assigned to either group. These species were not considered further. Twenty-four species failed to produce a single germinant after desiccation and were thus classified as producing desiccation-sensitive seeds (Table 5).

Information on the temporal pattern of seed release for species came from two sources. For one group (n=15), timing was determined from records of fruit/seed dispersal recorded between January 2002 - December 2005 from 72 x .5 m^2 seed traps within a 2ha study site in SCP. The total number of seeds dispersed by each species was used to calculate the mean monthly angle of dispersal and associated vector length (measure of dispersion) using circular statistics (Kovach 2006) (Table 4). The second group of species (n=9) were not recorded in the above study. For these species the temporal pattern of seed release was determined by recording the presence or absence of recently dispersed fruits and seeds between December 2005 and February 2007 (Table 5). Daily visits to known trees were made during the entire fruiting period until there was no evidence of continued fruit/seed fall. To ensure that the timing of all possible fruiting events were considered, all known trees of all species were visited weekly thereafter. Species were defined as wet season dispersers if the mean dispersal period and collection occurred during the months of Dec-Apr, and dry-season dispersers if mean dispersal periods fell between May - Nov. A simple G-test was used to test for deviation from expected frequencies of species identified as either wet- or dry-season dispersers under the hypothesis of no temporal concentration.

Results.

Only four desiccation-sensitive species exhibited dispersal completely restricted to the wet season. A further four species had mean dispersal periods within the dry season but the dispersal period included part of the wet season (Figure 6; Table 6). These eight species were all considered wet-season dispersers. The remaining 16 species had mean dispersal periods centred within the dry season (Table 6). There was no evidence that release of desiccation-sensitive seeds was concentrated within the wet season. The number of species that dispersed seeds within the wet season was not significantly different from expectation of random dispersal periods within the year (G_1 = 0.7; P = 0.409). While a greater number of species dispersed seeds during the dry season, the dry season spans seven months, resulting in no significant difference between the observed and expected frequencies of species between seasons.

Table 4 The mean monthly angle of dispersal and associated vector length for 15 species recorded between January 2002 - December 2005 from 72 x .5 m2 seed traps within a 2ha study site in SCP.

Family	Species	Mean Month of dispersal	Mean angle	Vector length
Connaraceae	*Connarus conchocarpus*	July	6.60	0.868
Lauraceae	*Cryptocarya mackinnoniana*	August	6.83	0.255
Lauraceae	*Cryptocarya murrayi*	November	10.56	0.866
Lauraceae	*Endiandra bessaphila*	August	7.94	0.930
Lauraceae	*Endiandra hypotephra*	October	9.64	0.820
Lauraceae	*Endiandra leptodendron*	November	10.28	0.914
Lauraceae	*Endiandra longipedicellata*	November	10.90	0.747
Meliaceae	*Dysoxylum pettigreweanum*	January	0.70	0.873
Myristicaceae	*Myristica globosa*	November	10.70	0.678
Myrtaceae	*Syzigium kuranda*	August	7.50	0.944
Oleaeceae	*Chionanthus ramiflorus*	October	9.46	0.922
Proteaceae	*Helicia australasica*	November	10.94	0.952
Sapindaceae	*Diploglottis diphyllostegia*	October	9.65	0.969
Sapindaceae	*Mishocarpus exangulatus*	August	7.10	0.988
Sapindaceae	*Toechima erythrocarpum*	October	10.00	0.967

Table 5 The temporal pattern of seed release was determined by recording the presence or absence of recently dispersed fruits and seeds between December 2005 and February 2007

	Months of the Year											
Species	J	F	M	A	M	J	J	A	S	O	N	D
Garcinia warreni	■	■										■
Diospyros hebecarpa									■	■		
Dysoxylum allecium							■	■				
Pittosporum rubiginosum	■	■	■	■	■							
Castanospora. alphandii										■	■	
Cupaniopsis flagellaformis									■	■		
Cupaniopsis foveolata										■	■	
Palaquium.galactoxylon										■	■	
Agyrodendron. polyandrum	■											■

Discussion.

Based on previous studies I expected a significant concentration associated with the wet season. The null result in the test suggests that the widely held view that dispersal periods for desiccation-sensitive seeds is coincident with the wet season does not hold in this forest. The lack of evidence for aggregated seed release periods is at present unique to this forest community. Only eight species in this study were considered wet season dispersers. Although unusual, dry season dispersal has been recorded for a few species within the seasonal tropical forests. For example, Daws *et al*. (2005) identified five species dispersing seeds during the dry season at BCI and Yu *et al.* (2008) identified one from eight species with dry season dispersal in Yunnan Province. How common dry season dispersal is in other seasonal locations is not known at present.

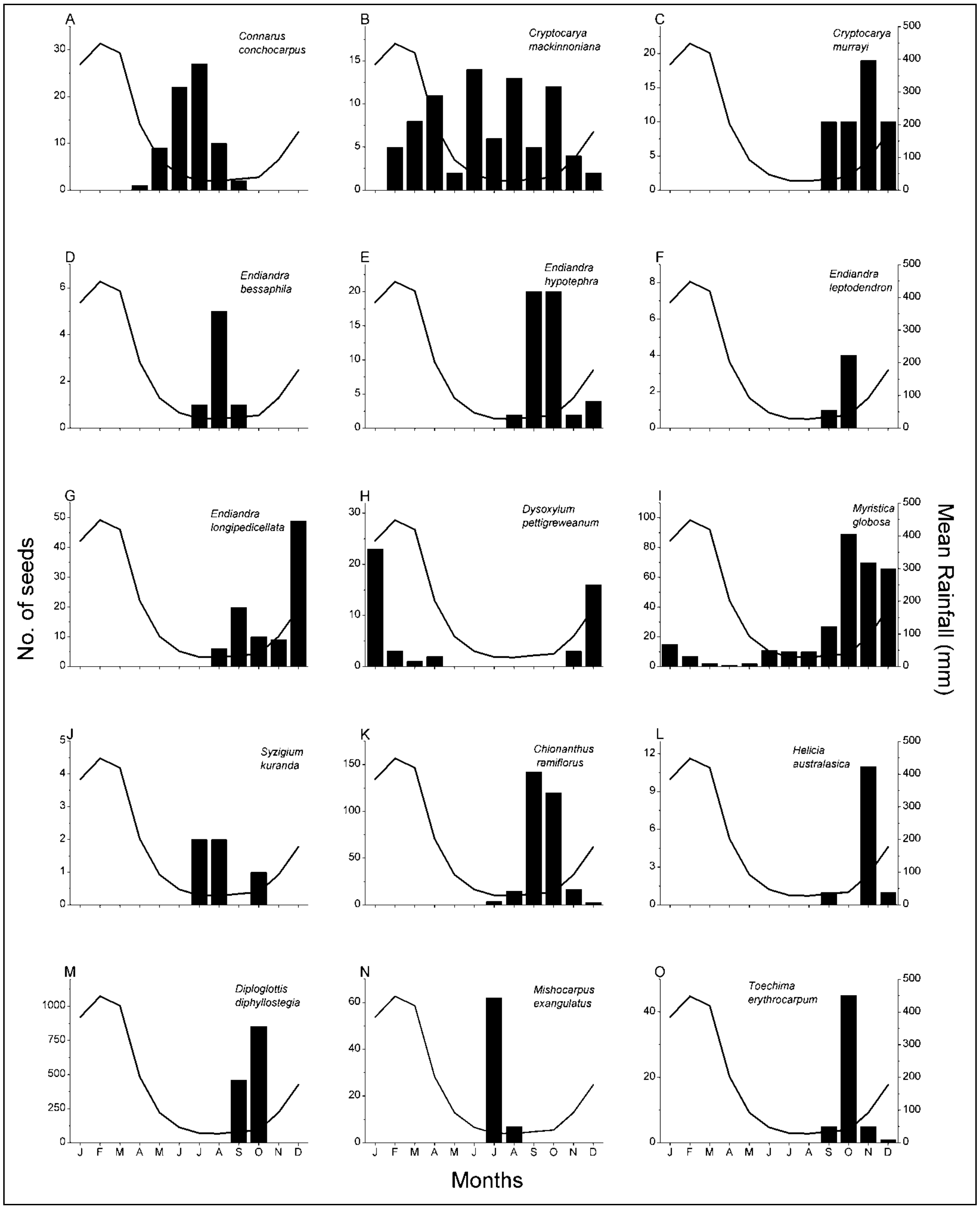


Figure 6 A - O. The monthly distribution of seeds dispersed from 15 rainforest plant species that produce recalcitrant seeds. Columns represent the cumulative monthly number of seeds over a four year period (2002-2005) using seed traps (see methods). The solid line represents the average rainfall (70 yrs).

In this study almost half of all species came from just two families; Sapindaceae (n=7) and Lauraceae (n=7). Furthermore, five Lauraceae species came from a single genus; *Endiandra*. Relatedness amongst species could influence the result of the test if closely related species display similar seed physiology with respect to desiccation sensitivity and similar seed release periods due to shared ancestry. I do not consider that this possibility was important in determining the outcome we demonstrate, however. For example, of the five *Endiandra* species, three were identified as dry season dispersers, one as wet season disperser and one intermediate (wet/dry). Furthermore, one species (*E. cowleyana*) produced no germinants in either of the desiccation or control treatments and was not included in the analyses. Similarly, the seven Sapindaceae species were not restricted to a single seed physiology class or season of dispersal. Although six species were identified as dry season dispersers and were classified as producing desiccation sensitive seeds differences between co-familiar and congeneric species suggest that desiccation-sensitivity and dispersal may be labile, while full phylogenetic analyses covering a much larger sample set would be required to explore this in detail.

Defining dry season months as those with mean rainfall below 100mm may also have influenced the findings. For example, Pritchard *et al*. (2004) identified a potential relationship between desiccation sensitivity, dispersal during periods of high moisture availability and large seed mass (above 0.5g) in central Africa. Unlike this study (and others), Pritchard *et al*. (2004) used an average monthly rainfall of 60mm to define seasonality, reporting that no species dispersed desiccation-sensitive seeds when monthly rainfall averages were below 60mm. I assigned seasonality based on monthly averages above/below 100mm for two reasons. First, general monthly evaporation in tropical forests exceeds 100mm and precipitation rates below this should correspond to months experiencing net moisture lost (Leigh 2004) and second, to allow a direct comparison with results reported in Daws *et al*. (2005) for BCI, Panama. In Daws *et al*. (2005) a significantly greater number of desiccation sensitive seeds were released during the wet season similar to Pritchard *et al*. (2004). If I considered dry season months as those below 60mm as in Pritchard *et al*. (2004) five months (June - October) are still identified as the dry season and 12 (50%) species had dispersal periods centred within these months.

That some species release desiccation-sensitive seeds during the dry season must imply an ability to germinate and persist under conditions experienced during this time. Little is known about the direct mechanism(s) by which this is achieved, and (more importantly) whether these mechanisms will continually enable these seeds to survive, germinate and establish as seedlings under changing climates. There are a number of possible mechanisms by which desiccation sensitive seeds may survive dry seasons. At the individual seed (proximate) level, directed dispersal to microsites of high moisture availability, the retention of fleshy a endocarp/aril or inclusion within animal faeces may all buffer seeds against dehydration. These last two factors are relevant to fleshy fruited species only, and unlikely to be applicable to wind dispersed, dry fruited species we identified. Future field comparisons should be made among more species that have structures than can aid in moisture retention. One further possibility also exists.

Potentially other selective pressures such as the predator avoidance/ satiation (Augspurger 1981, Curran and Leighton 2000) may have a stronger influence on the timing of seed release in these species. The ultimate mechanism setting dispersal periods should be a response to the life-time reproductive success of parent plants. If germination and establishment success during relatively mild dry seasons counteracts possible establishment failure during extremely dry years, inter-annual variability in monthly precipitation may be more important than the mean. More studies on inter-annual variability in seed fate and its relationship to moisture availability and seed traits will be required to answer these questions.

Species that produce desiccation-sensitive seeds represent a large component of tropical floras. In the context of predictions associated with changing global climates, our finding that a wide range of species release desiccation-sensitive seeds during the dry season is potentially alarming. Higher temperatures will increase the rate of desiccation, the effects of which may be compounded by lower dry season precipitation and a reduction in the duration of the wet season (BOM 2007, Dunlop and Brown 2008). Seed survival and seedling establishment in species with desiccation-sensitive seeds may become more vulnerable to failure because the proximate conditions experienced by individual seeds within any given year are likely to become drier and the return time (years) between potentially favourable, mild dry seasons is likely to increase. Objective 5b of The Global Strategy for Plant Conservation is to, “Improve long-term conservation, management and restoration of plant diversity, plant communities, and the associated habitats and ecosystems, in situ..., and, where necessary to complement in situ measures, ex situ...” (The Global Strategy for Plant Conservation, 2010). At present, desiccation-sensitive seeds are unable to be stored at sub-zero temperatures without cellular damage (Prichard 2004), limiting the use of ex-situ seed banks in their long-term conservation. Techniques to store the embryonic axes of desiccation sensitive seeds at sub-zero temperatures have been achieved (Pence 1995, Berjak and Pammenter 2007). Similarly cyropreservation of meristems has also enabled the long-term preservation of some species with desiccation sensitive seeds (Englemann 2009), although currently they appear too costly and time consuming to be applied to a broad range of species, and are generally reserved for species that have direct economic importance to human societies (Prichard 2004). Storage of embryonic axes and cryopreservation of meristems is therefore unlikely to be used as a tool for conserving the majority of native species that produce desiccation-sensitive seeds at present (although future advances may enable a broader application of these methods). Given the current situation, conserving this important component of tropical rainforest flora may predominately be reliant on *in-situ* measures. The dilemma for conservation initiatives is that changing climates will expose desiccation sensitive seeds to unfavourable conditions. With limited resources, the future challenge may be to prioritise which species will receive conservation focus. One possible way forward may be to combine information about dispersal periods, desiccation sensitivity and desiccation rates in order to rank species in terms of likely threat predictions of future climates present.

In the next chapter I begin to investigate seed traits that may enable desiccation sensitive seeds to survive during dry conditions. The most obvious way forward is to ask how quickly desiccation sensitive seeds lose water. Seed desiccation may conform to a single model of water loss whereby there is no distinct difference between species. Alternatively selection may have influenced some species to desiccate at slower rates so that they may persist in unfavourable environments.

Table 6. The fruit type, dispersal syndrome ((+) *assisted dispersal*; (-) *non-assisted dispersal*), desiccation sensitivity ((+) *sensitive to desiccation*; (-) *not sensitive to desiccation*), trapping method, season of dispersal, and germination success of control and dehydrated treatments of seed-lots from 32 tropical forest plant species from Cairns, Australia.

Family	Species	Fruit Type	Dispersal syndrome	Desiccation sensitive.	Phenology Method	Season	Germination Success (control)	Germination success (desiccation treatment)
Araliaceae	*Polyscias elegans*	Drupe	+	-	Seed trap	Dry	2/30	5/30
Celestraceae	*Hippocratea barbata*	Capsule[a]	-	-	Seed trap	Dry	27/30	26/30
Clusiaceae	*Garcinia warreni*	Drupe	+	+	Focal	Wet	20/20	0
Connaraceae	*Connarus conchocarpus*	Follicle[b]	+	+	Focal / Seed trap	Dry	27/30	0
Dillineaceae	*Tetracera nordtiana*	Capsule[b]	+	-	Seed trap	Wet	1/30	4/30
Ebenaceae	*Diospyros hebecarpa*	Berry	+	+	Focal	Dry	15/20	0
Lauraceae	*Cryptocarya mackinnoniana*	Nut	+	+	Focal / Seed trap	Dry/Wet	7/30	0
Lauraceae	*Cryptocarya murrayi*	Berry	+	+	Focal / Seed trap	Dry/Wet	20/20	0
Lauraceae	*Endiandra bessaphila*	Drupe	+	+	Focal / Seed trap	Dry	20/20	0
Lauraceae	*Endiandra cowleyana*	Drupe	+	?	Focal / Seed trap	Wet	0	0
Lauraceae	*Endiandra hypotephra*	Drupe	+	+	Focal / Seed trap	Dry	20/20	0

Lauraceae	*Endiandra leptodendron*	Drupe	+	+	Focal / Seed trap	Dry	20/20	0
Lauraceae	*Endiandra longipedicellata*	Drupe	+	+	Focal / Seed trap	Dry/Wet	20/20	0
Meliaceae	*Dysoxylum allecium*	Capsule[b]	+	+	Focal	Dry	5/30	0
Meliaceae	*Dysoxylum pettigreweanum*	Capsule[b]	+	+	Focal / Seed trap	Wet	20/20	0
Myristicaceae	*Myristica globosa*	Capsule[b]	+	+	Focal / Seed trap	Dry/Wet	7/20	0
Myrtaceae	*Syzigium kuranda*	Berry	+	+	Focal / Seed trap	Dry	30/30	0
Oleaeceae	*Chionanthus ramiflorus*	Drupe	+	+	Focal / Seed trap	Dry	26/30	0
Pittosporaceae	*Pittosporum rubiginosum*	Capsule	+	+	Focal	Wet	30/30	0
Rutaceae	*Helicia australasica*	Drupe	+	+	Focal / Seed trap	Dry	20/20	0
Proteaceae	*Flindersia acuminata*	Capsule[a]	-	-	Seed trap	Dry	15/30	19/30
Sapindaceae	*Castanospora alphandii*	Berry	+	+	Focal	Dry	18/20	0
Sapindaceae	*Cupaniopsis flagelliformis*	Capsule[b]	+	+	Focal	Dry	21/30	0
Sapindaceae	*Cupaniopsis foveolata*	Capsule[b]	+	+	Focal	Dry	11/20	0
Sapindaceae	*Diploglottis diphyllostegia*	Capsule[b]	+	+	Focal / Seed trap	Dry	26/30	0
Sapindaceae	*Ganophyllum falcatum*	Berry	+	-	Focal / Seed trap	Wet	20/20	9/20

Sapindaceae	*Mishocarpus exangulatus*	Capsule[b]	+	+	Focal / Seed trap	Dry	30/30	0
Sapindaceae	*Toechima erythrocarpum*	Capsule[b]	+	+	Focal / Seed trap	Dry	20/20	0
Sapotaceae	*Palaquium galactoxylon*	Drupe	+	+	Focal	Dry	14/20	0
Sterculiaceae	*Argyrodendron polyandrum*	Samara[a]	-	+	Focal	Wet	19/20	0
Sterculiaceae	*Argyrodendron peralatum*	Samara[a]	-	?	Focal / Seed trap	Wet	0	0
Verbenaceae	*Clerodendron tracyanum*	Drupe	+	-	Seed trap	Dry	2/30	6/30

[a] *winged seeds*; [b] *aril presen*

Chapter 4. How long does it take for different seeds to dry?

This chapter has been published as "*Hill, J., Edwards, W & Franks, PJ. (2010) How long does it take for different seeds to dry? Functional Plant Biology 37, 575-583."*

Introduction.

Many tropical plant species produce seeds that lose viability with dehydration and they are termed desiccation-sensitive. Desiccation-sensitive seeds lose viability once the relative seed water content decreases below a specific threshold. Prolonged exposure to dry conditions is unsuitable for germination and results in a loss of viability (Farnsworth 2000, Tweedle *et al*. 2003). Most investigations into desiccation intolerance of desiccation sensitive seeds have been driven by the need to extend seed storage life in agricultural species (*see* Chin and Roberts 1980, Leprince *et al*. 1999, Dussert *et al.* 1999, Liang and Sun 2000). However, there is increasing interest in the broader significance of desiccation sensitive seeds in natural vegetation systems, particularly in relation to conservation of rare species (Horne and Kahn 2000, Franks and Drake 2003) and community processes (Sun 1999, Dussert *et al*. 1999, Pritchard *et al.* 2004$_{a}$, Daws *et al.* 2005, 2006$_{a}$, Yu *et al*. 2008). For example, phenological patterns of the time of seed release for desiccation sensitive species generally occur during the wettest period of the year with few species dispersing seeds within drier months (Dussert *et al*. 1999, Pritchard *et al.* 2004$_{a}$, Daws *et al.* 2005, Yu *et al*. 2008).

How quickly desiccation sensitive seeds lose water is an important determinant in their ability to tolerate periods of seasonal drought and subsequently contribute to regeneration processes once appropriate conditions are encountered. Thus, recruitment in species that produce desiccation sensitive seeds may alter under novel environmental stresses. For example, scenarios predicting changing global climate patterns suggest greater, more intense periods of seasonal drought within the seasonal tropical forests of north east Queensland, Australia (Dunlop and Brown 2008). This represents a greater risk for species that produce desiccation sensitive seeds, since changing climates may reduce the likelihood of seedling production and result in changes in relative abundances of species based on abilities of seeds to withstand periods of moisture stress.

Amongst species the degree of desiccation sensitivity varies and is best described as a continuum from high to low tolerance (Sun 1999, Berjak and Pammenter 2008). Desiccation sensitive seeds have varying thresholds of seed viability. These are often described as critical water contents and relate to the amount of water loss that leads to a loss of viability. Of equal importance is the rate in which water is lost from the seed. This trait determines the persistence time a seed can withstand desiccation prior to death. Despite its potential importance for determining species persistence under increasingly drier climates, very little is known about the most basic property of desiccation in desiccation sensitive seeds: how whole seeds lose water, and whether patterns of water loss are consistent in all species.

The simplest way to describe the time course of desiccation of an initially hydrated seed is;

$$W_{r(t)} = W_{r0} e^{-\left(\frac{t}{T}\right)} \qquad (1)$$

where $W_{r(t)}$ is the relative seed water content at any time t (in days) as a fraction of the total available water at full hydration (i.e. ranging between 0 and 1), W_{r0} is W_r at $t = 0$, and T (in days) is the time constant (a product of the hydraulic capacitance of the seed and the resistance to moisture loss). In the case of fully hydrated seeds at the commencement of desiccation, $W_{r0} = 1$, and hence equation (1) may be reduced to

$$W_{r(t)} = e^{-\left(\frac{t}{T}\right)}. \qquad (2)$$

Equations (1) and (2) are variants of a simple exponential decay function. There is some evidence that desiccating seeds do indeed follow this pattern. For example, Dussert *et al.* (1999) reported a negative exponential curve described the pattern of desiccation in the seeds of *Coffea canephora* and a similar pattern has been shown in the seeds of *Citrus suhuiensis* (Makeen *et al.* 2006). However, given that seeds of desiccation sensitive species are structurally diverse, and that T is not necessarily constant during seed desiccation (due perhaps to changing resistance or capacitance), it is unlikely that the simple, single-compartment exponential model (equation (2)) is representative of all desiccation sensitive seeds.

Predicting the potential consequences for seed survival arising from increased aridity and seasonality will require understanding the temporal pattern of seed moisture loss across species. To date there have been no detailed investigations of $W_{r(t)}$ across a large and diverse group of species. In this study I investigate patterns of seed moisture loss in 24 species of rainforest plants that produce desiccation sensitive seeds (Chapter 3), and examine the generality of a simple negative exponential model relating seed desiccation and time. Specifically, I examine three predictions about seed desiccation patterns that arise from the model above. I first test the generality of equation (2), i.e. that all seeds conform to an exponential mass (or relative mass) versus time relationship during desiccation under the same environmental conditions (Hypothesis 1). It might also be expected that, because hydraulic capacitance increases with seed size, larger seeds have larger T and therefore take longer to desiccate i.e. seed dry mass (M_D) correlates with T (Hypothesis 2). The proportionality between hydraulic capacitance and seed size would depend on corresponding proportionalities in seed structure, so we apply Hypothesis 2 only to seeds of the same species. Across species, structural diversity of seeds is likely to disrupt such a relationship, i.e. across species M_D does not predict T (Hypothesis 3).

Methods.

Seed collection and preparation

A total of 20–30 freshly dispersed fruit or seeds, depending on availability, were collected for each species. For species that produce drupes and nuts, seeds were extracted from fallen fruit and seeds from capsules were obtained from the forest floor. To ensure that only freshly fallen specimens were collected, daily visits were made to known trees of each species during their respective seed release periods. All seeds were classified as exalbuminous as storage cotyledons were present. Immediately upon collection, seeds/fruits were transported to a laboratory where any remaining endocarp or aril was removed.

Seeds were then stored in moist vermiculite within a zip-lock polyethylene bag at 10˚C within a refrigerator for a maximum period of 2 weeks at which point seeds were regarded as fully hydrated (Pritchard *et al*. 2004b). Seeds were removed from storage and cleaned by rinsing under H^2O to remove any remaining vermiculite. All surface moisture was then removed from the seeds using laboratory tissue and seeds were placed on a laboratory bench for 30min to equilibrate with room temperature (23.5±1˚C).

Time to desiccation.

After equilibration the mass of each fully hydrated individual seed was determined (i.e. M_0). Seeds were then desiccated by placing them in individual compartments (50mm x 50mm x 55mm) each filled with 15 g of silica gel (desiccant) housed within larger polyethylene boxes. The mass of each seed (M_s) was recorded every second day until there was no further detectable change in seed mass across three consecutive measures, at which point I considered seeds to have reached absolute dry weight (M_D). Time (in days) to reach M_D (i.e. time to full desiccation), was identified as t_D. I then calculated that point representing 1% remaining seed moisture.

All desiccation studies were performed in a closed, dark cupboard at a constant temperature (24ºC). Relative humidity in desiccating chambers was assessed at each mass determination using a Vaisala HMP44 temperature and humidity probe and was maintained between 5.5% and 10% by replacing the desiccant when RH reached 10%. During mass determinations a limited number of seeds (six in total) exhibited indications of predation (i.e. larval emergence). These seeds were immediately discarded, resulting in a sample size of 28 seeds for *Chionanthus ramiflora* (Roxb.) and *Diploglottis diphyllostegia* (F.M. Bailey), 29 seeds for *Cupaniopsis flagelliformis* (Radlk.) and 19 seeds for *Garcinia warrenii* (F. Muell.).

Determining relative seed water content.

For each sampling interval t, W_r was calculated for all seeds in all species according to

$$W_r = \frac{M_s - M_D}{M_0 - M_D} \quad . \qquad (3)$$

Testing for generality of the single exponential decay model

To investigate the generality of the single negative exponential curve to describe the pattern of seed desiccation across species (hypothesis 1) I fitted equation (2) to individual plots of mean W_r versus t using data from all 24 species. The adequacy of equation (2) to describe the data was examined using the following standard diagnostics: constant variance and predicted residual error sums of squares (PRESS). The PRESS statistic is generated by summing the squares of the prediction errors (the difference between predicted and observed values) for each observation, with that point deleted from the computation of the regression equation. Thus, the smaller the PRESS statistic, the better the fit of the model.

Evaluation of the statistical information from fitting equation (2), as well as observations of the fitted line (see Results) revealed that the single exponential model was potentially inadequate for ten of the 24 species. The strong inflection point evident in the desiccation plots for these species suggested a two-phase process, as would occur if the seeds comprised two compartments with different moisture loss characteristics. We therefore tested the fit of two alternative two-phase models for those four species: (i) a double negative exponential model, and (ii) a double linear model. The double negative exponential model is of the form

$$W_{r(t)} = ae^{-\left(\frac{t}{T_1}\right)} + be^{-\left(\frac{t}{T_2}\right)} \qquad (4)$$

where $a+b=1$ and both a and b are ≥ 0. The double linear model is of the form

$$W_{r(t)} = (c_1 + c_2) + (d_1 + d_2)t \qquad (5)$$

for $0 \leq t < T1$, otherwise

$$W_{r(t)} = c_2 + td_2 \quad , \qquad (6)$$

where c_1 and d_1 are the offset and slope, respectively, of a line describing moisture loss from the first compartment, and c_2 and d_2 are the offset and slope, respectively, of a line describing moisture loss from the second compartment. T1 is the inflection point, i.e. the point of abrupt change in rate of moisture loss.

All models were fitted using the nonlinear curve fit procedure in Sigmaplot version 10.0 (Systat software 2006). The procedure uses the Marquardt-Levenberg algorithm to estimate the parameters that give the best fit between the equation and the data via iteration (Press *et al.* 1986). I constrained each of the model fits to pass through the point (0,1) under the simple assumption that the moisture content was equal to 100% at time t=0. For the double linear model represented by equations (5) and (6), Sigmaplot was used to fit a two-part linear model (yielding Sigmaplot output variables Y1, Y2, Y3 and T1), from which c_1, c_2, d_1, and d_2 were obtained.

Where the single exponential model was a poor fit, the appropriateness of the two-phase models were examined in two ways. First, by examining dependency values for fitted parameters in the more complex model. Dependency values are calculated as one minus the variance of the estimated parameter when other parameters are held constant, expressed as a proportion of the variance in that parameter when other parameters are allowed to vary. When dependencies approach or equal unity, then estimated parameters are strongly dependent on one another, indicating that the higher order equation is over-parameterised and the less complex model should be accepted. Second, by testing for an improvement of the fit of each of the alternative models (double exponential or double linear) to the fit of the simple negative exponential model. The change in explanatory power between models was examined via a simple *F*-test comparing the sum of squares of the residuals from the two model fits.

Based on these results, I calculated T for the fourteen species that conformed to the negative exponential model and equivalent estimates of T ($t_{0.368}$) from the double negative exponential and double linear models for the remaining ten species.

Testing for dependence on seed mass

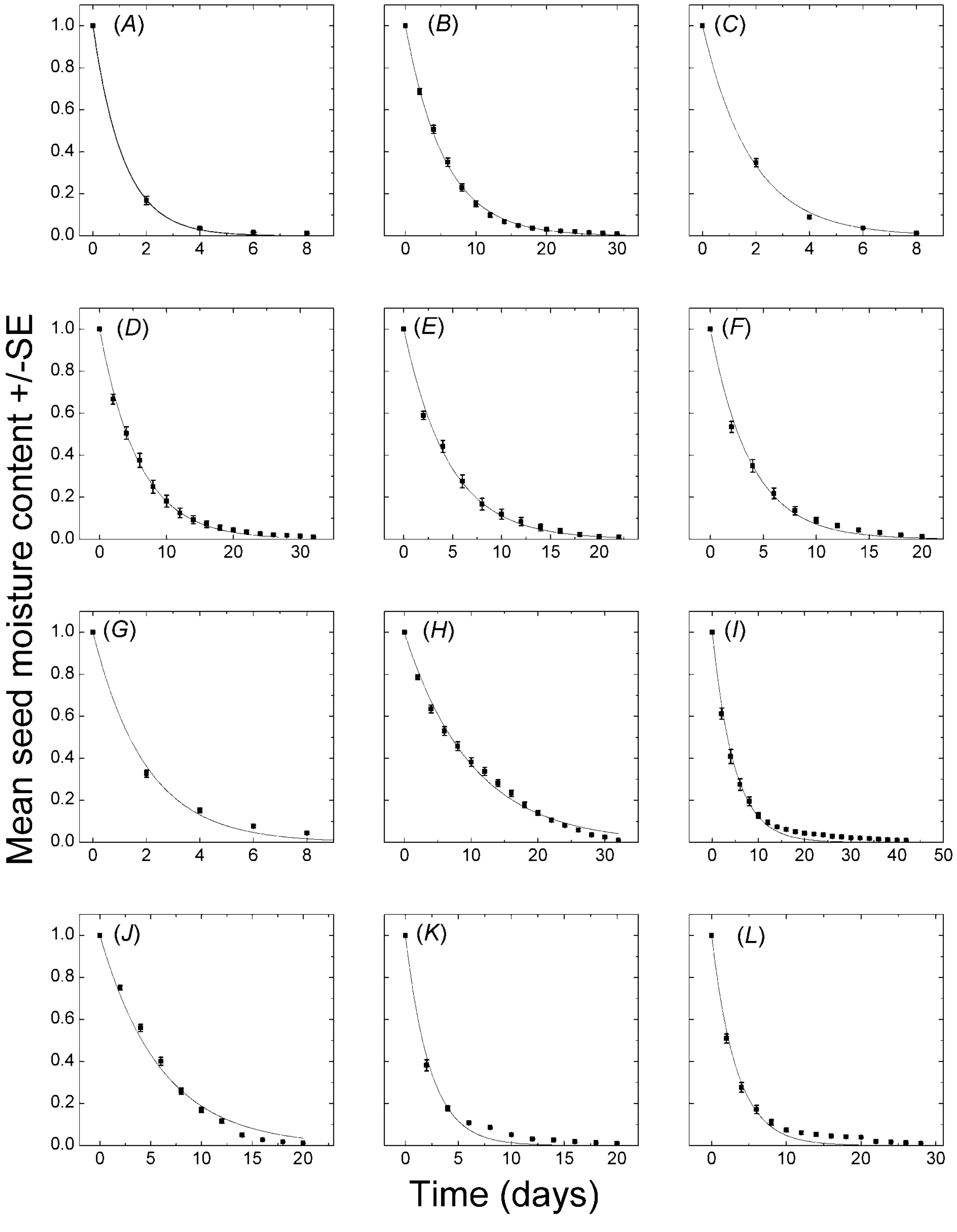
The time constant T in equation (2) (for species that conformed to the single exponential decay model), or the modelled time $t_{0.368}$ to reach W_r=0.368 (based on the equivalent fractional water loss corresponding to $t=T$ in the single exponential decay model), were used to examine association between desiccation rate and seed mass. To examine the relationship between seed mass variation and desiccation within species (Hypothesis 2), I assessed the relationship between T (or $t_{0.368}$) and

M_D using each individual seed within each species, with Bonferroni corrected P to account for the number of tests performed. Dependence of T (or $t_{0.368}$) on seed mass differences between species (Hypothesis 3) was tested by regressing ln mean T (or $t_{0.368}$) against ln mean M_D.

Results.

Superficially, the single exponential model seemed to describe the data well, based on non-linear regression output. In all species, the R^2 values associated with the fits were consistently high (range 0.99 *Helicia australasica* (F. Muell.), to 0.88 *Agyrodendron polyandrum* (L.S. Sm)).

Nevertheless, the quality of the fit generally declined as time to 1% desiccation increased. For example, Table 7 lists species ordered (top to bottom) with respect to increasing days required to reach $W_r = 0.01$ ($t_{0.01}$) (as indicated by the denominator degrees of freedom, Table 7). The PRESS statistic also increased with increasing $t_{0.01}$, demonstrating a decreasing trend in the model's ability to explain the data associated with increases in the time required for seeds to desiccate. Moreover, visual inspection of the fitted models (see Figure 7) and residual plots (not shown) indicated that the single negative exponential model was potentially not the best possible description of the relationship between W_r and t in ten species: *Cryptocarya murrayi* (F. Muell.), *Endiandra hypotephra* (F. Muell.), *Castanospora alphandii* (F. Muell.), *Agyrodendron peralatum* (Edin ex Boas), *A. polyandrum*, *Mishocarpus exangulatus* (Radlk.)*, Myristica globosa* (W.J. de Wilde)*, Cupaniopsis foveolata* (Radlk.), *C. flagelliformis* and *C. ramiflora* (Figure 7*C-F*). Of these species, the double exponential model well described W_r versus t for eight (dependencies of fitted parameters <1; Table 8, Figure 7), but was inadequate in describing W_r versus t for *C. flagelliformis* and *C. ramiflora* (dependencies of fitted parameters equal to one). However, the W_r versus t relationship for *C. flagelliformis* and *C. ramiflora* was well described by the double linear model (dependencies of fitted parameters <1; Table 8, Figure 7*E-F*). The three types of W_r versus t relationship identified (single exponential, double exponential, double linear) appeared to be related to the time taken to reach $W_r = 0.01$ (1% relative water content), in that the single exponential model best fitted species with quickest desiccation rate, whereas the double exponential and the double linear model generally took the longest to desiccate to $W_r = 0.01$.


(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
Mean seed moisture content +/-SE
Time (days)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
0
10
20
30
0
5
10
15
20
0
10
20
30
40
50

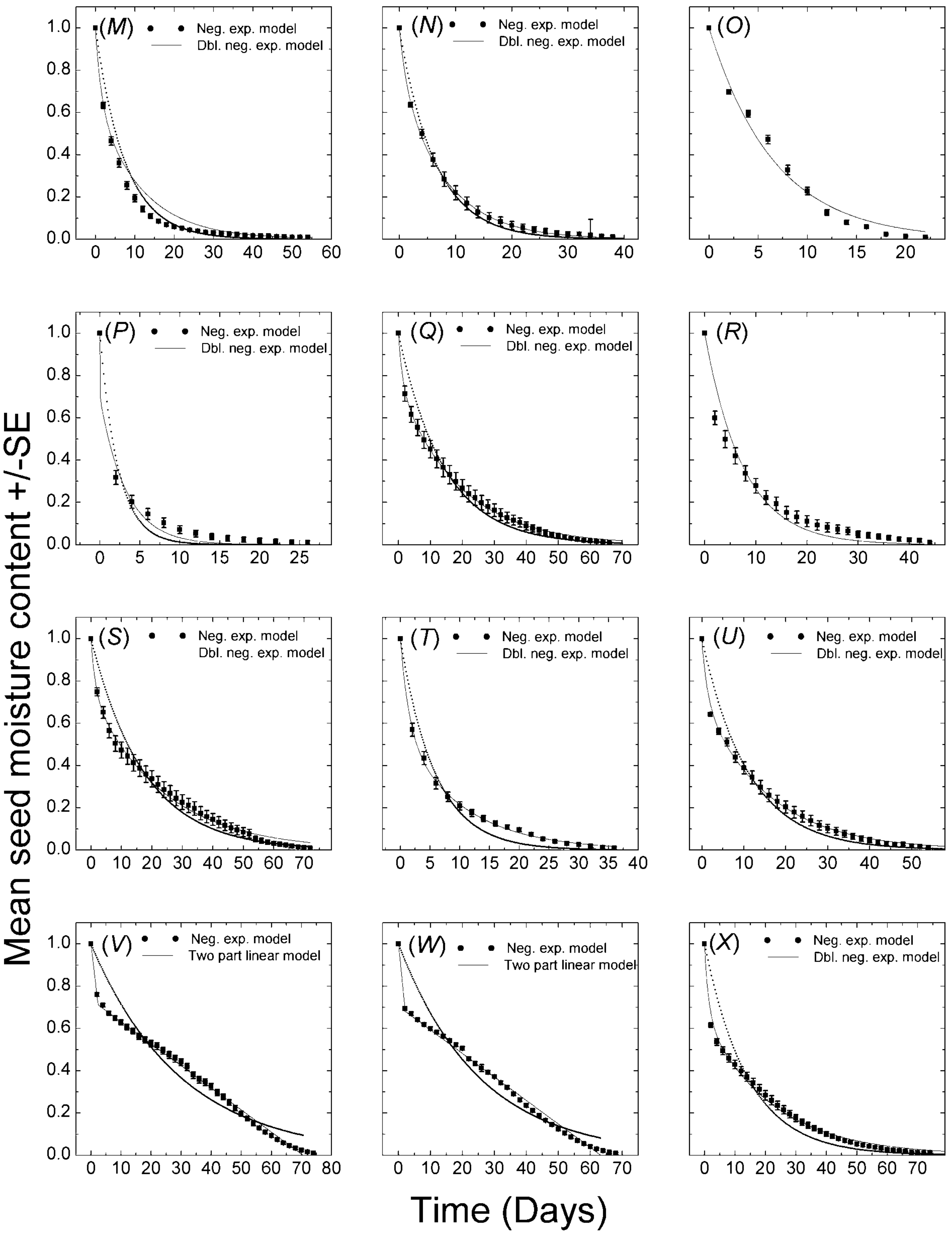


Figure 7 Time course of seed desiccation to 1% mean (± SE) relative seed moisture content (W_r) for 24 rainforest plant species that produce desiccation sensitive seeds in north Queensland, Australia, and the fit of a negative exponential model (equation 3). The model fit is represented as a solid line for species where the fit was deemed acceptable, otherwise the fit appears as a dashed line. Species codes are as follows (*A*) *Cryptocarya mackinnoniana*, (*B*) *Helicia australasica*, (*C*) *Endiandra*

leptodendron, (*D*) *Toechima erythrocarpum*, (*E*) *Dysoxylum alliaceum*, (*F*) *Dysoxylum pettigrweanum*, (*G*) *Diploglottis diphyllostegia*, (*H*) *Syzygium kuranda*, (*I*) *Agyrodendron peralatum*, (*J*) *Endiandra bessaphila*, (*K*) *Garcinia warrenii*, (*L*) *Connarus conchocarpus*, (*M*) *Palaquium galactoxylon*, (*N*) *Myristica globosa*, (*O*) *Cryptocarya murrayi*, (*P*) *Endiandra longipedicellata*, (*Q*) *Endiandra cowleyana*, (*R*) *Castanospora alphandi*, (*S*) *Endiandra hypotephra*, (*T*) *Cupaniopsis foveolata*, (*U*) *Chionanthus ramiflora*, (*V*) *Mischocarpus exangulatus*, (*W*) *Agyrodendron polyandrum*, (*X*) *Cupaniopsis flagelliformis*

Table 7. Results of fitting a simple negative exponential decay model to the seed desiccation plots (Wr versus t) and the time to 63.21% relative seed moisture loss (T or *t*0.368) for 24 species of rain forest trees from north Queensland, Australia. The constant variance and predicted residual error sums of squares (PRESS) were used to evaluate the strength of the relationship (see Methods).

Species	R^2_{adj}	*df*	F	P	Con. Var.	PRESS
Cryptocarya mackinnoniana	0.99	1, 4	3059.88	<0.0001	Pass	0.002
Helicia australasica	0.99	1, 4	20876.71	<0.0001	Pass	0.001
Endiandra leptodendron	0.98	1, 8	1374.41	<0.0001	Pass	0.012
Toechima erythrocarpum	0.99	1, 10	1603.93	<0.0001	Pass	0.008
Dysoxylum alliaceum	0.99	1, 10	941.90	<0.0001	Pass	0.014
Dysoxylum pettigrweanum	0.99	1, 10	876.06	<0.0001	Pass	0.014
Syzygium kuranda	0.98	1, 10	723.28	<0.0001	Pass	0.021
Diploglottis diphyllostegia	0.99	1, 11	2088.13	<0.0001	Pass	0.007
Agyrodendron peralatum	0.96	1, 13	313.35	<0.0001	Pass	0.064
Endiandra bessaphila	0.99	1, 14	1229.84	<0.0001	Pass	0.013
Garcinia warrenii	0.99	1, 15	11858.11	<0.0001	Pass	0.002
Connarus conchocarpus	0.99	1, 15	439.93	<0.0001	Pass	0.056
Palaquium galactoxylon	0.99	1, 16	6121.73	<0.0001	Pass	0.005
Myristica globosa	0.94	1, 18	351.54	<0.0001	Pass	0.077
Cryptocarya murrayi	0.99	1, 20	1391.71	<0.0001	Pass	0.026
Endiandra longipedicellata	0.99	1, 21	2457.09	<0.0001	Pass	0.012
Endiandra cowleyana	0.95	1, 22	474.20	<0.0001	Pass	0.081
Castanospora alphandi	0.98	1, 29	2226.16	<0.0001	Pass	0.024

Endiandra hypotephra	0.95	1, 27	472.36	<0.0001	Fail	0.119
Cupaniopsis foveolata	0.96	1, 32	729.21	<0.0001	Fail	0.109
Chionanthus ramiflora	0.90	1, 33	317.88	<0.0001	Pass	0.255
Mischocarpus exangulatus	0.95	1, 34	680.58	<0.0001	Fail	0.129
Agyrodendron polyandrum	0.89	1, 36	291.31	<0.0001	Fail	0.270
Cupaniopsis flagelliformis	0.91	1, 36	393.23	<0.0001	Pass	0.236

For eight species there was a significant linear relationship between seed dry mass and $t_{0.368}$ (Table 9). For the remaining 16 species no pattern between seed dry mass and $t_{0.368}$ was evident. Thus, within a species, the rate of decline in W_r as seeds desiccate may not always depend on mass alone. I considered that one possible explanation for the lack of a significant relationship between seed dry mass and $t_{0.368}$ within some species and significant relationship in others may have been differences in the range of seed sizes collected. If, for example, the range of seed masses sampled differed between species, the ability to detect a significant relationship might differ between species based on this effect. In order to test this hypothesis we first calculated the co-efficient of variation (CV) of seed mass for each species and then tested for differences in the distribution of coefficients of variation between two groups of species; those showing a significant relationship and those not, via Mann-Whitney U test. There was no evidence that the inability to detect significant relationship between seed mass and time was related to differences in the variance associated with a range of seed masses sampled in each species (Mann-Whitney $U = 62$, $Z = -0.09$, $P = 0.93$).

Across the 24 species, $t_{0.368}$ ranged from 1.15 days in *H. australasica* to 38 days in *C. flagelliformis* (Table 7). However, there was no evidence that differences in $t_{0.368}$ across species was related to mean seed dry mass. Regression of ln $t_{0.368}$ against ln mean M_D; ($F_{1,22} = 0.002$, $P = 0.96$) (Figure 8a), explained almost none of the variation in $t_{0.368}$ ($R^2 < 0.0001$). This result was consistent when only species for which a significant relationship between seed dry mass and $t_{0.368}$ were used in analysis ($F_{1,6} = 0.23$, $P = 0.64$, $R^2 = 0.037$) (Figure 8b).

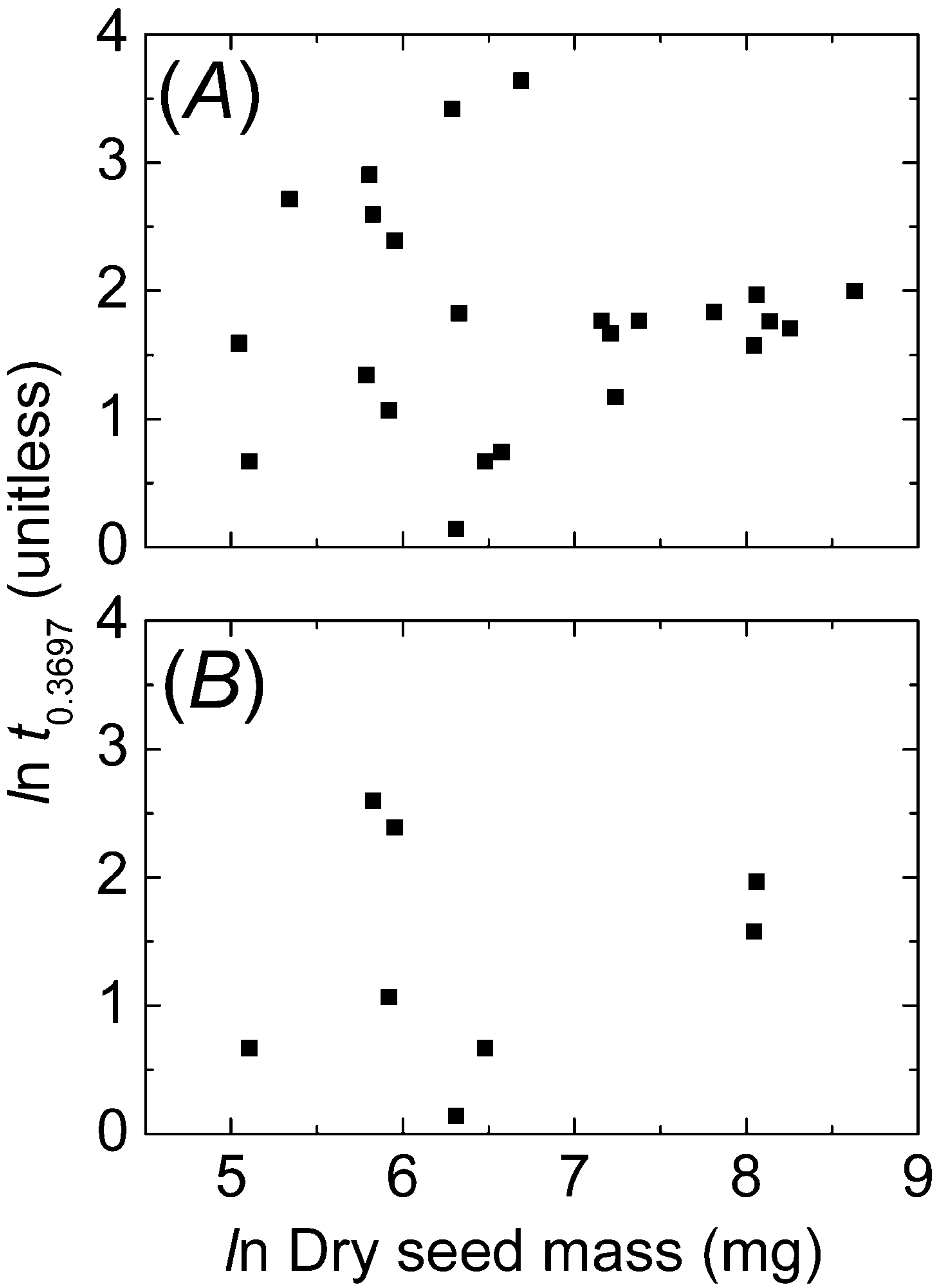


Figure 8 The relationship between the estimated time constant (T or t0.3679) (ln) associated with seed desiccation and the mean dry seed mass (ln) of, A) twenty-four rainforest plant species; and, B) the eight species that recorded a significant positive correlation within species between T or t0.3679 and dry seed mass, from a seasonal tropical forest, Queensland, Australia.

Table 8. Results of fitting either a double negative exponential, or two-piece linear models to the ten species that did not conform to the single negative exponential model. Also shown are the results of the F-tests to determine significance of increase in explanatory power of more complex model.

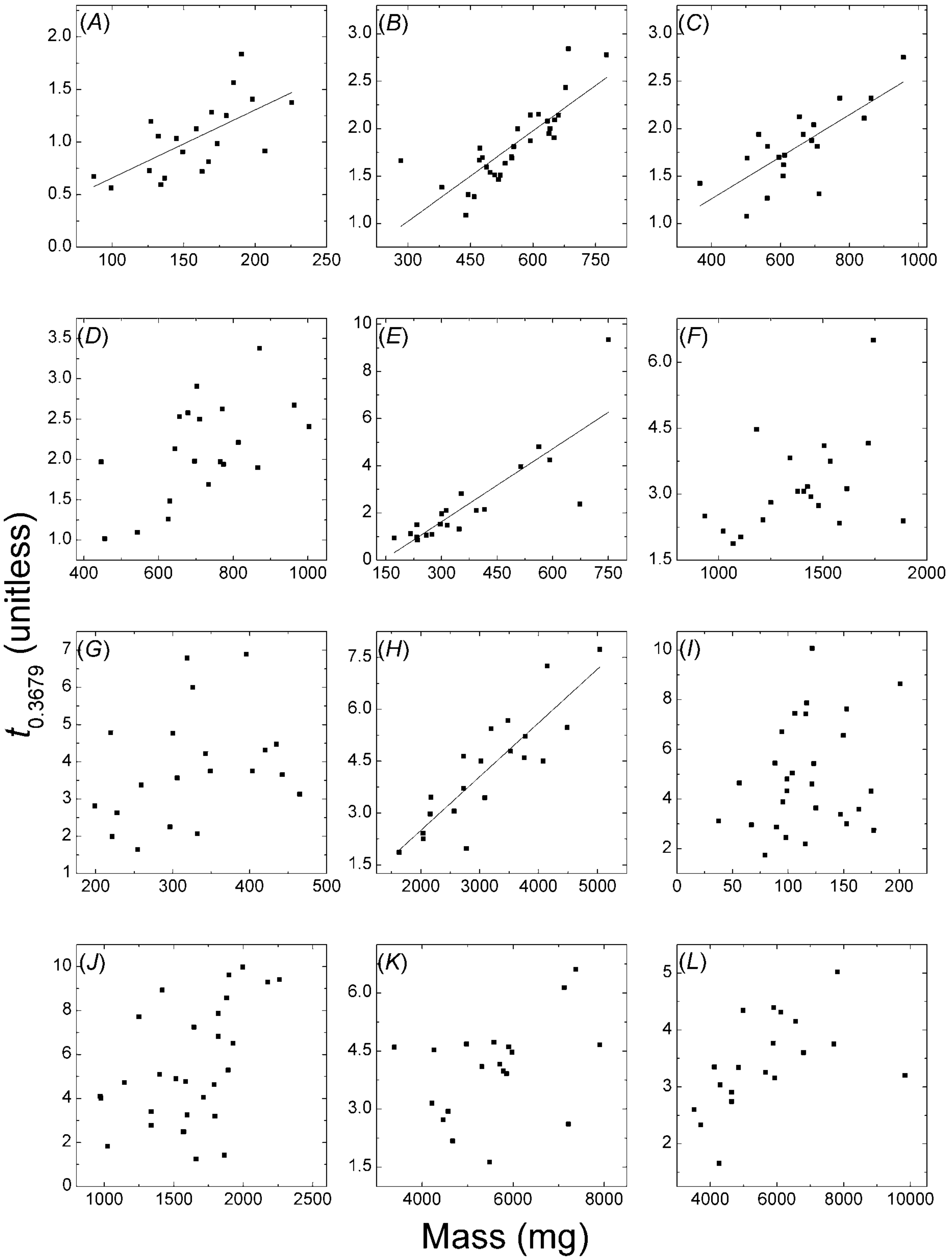
Species	Alternative model. Negative exponential versus…	Parameter	Parameter dependencies	df	F	P	Conclusion
Agyrodendron peralatum	Double negative exponential	$a = 0.469$ $T_1 = 6.070$ $b = 0.531$ $T_2 = 0.662$	$a = 0.971$ $T_1 = 0.944$ $b = 0.951$ $T_2 = 0.817$	3, 15	2614.6	<0.0001	Double negative exponential a sig. better fit.
Myristica globosa	Double negative exponential	$a = 0.441$ $T_1 = 0.651$ $b = 0.559$ $T_2 = 0.092$	$a = 0.883$ $T_1 = 0.756$ $b = 0.955$ $T_2 = 0.850$	3, 16	5.7	0.0135	Double negative exponential a sig. better fit.
Castanospora alphandi	Double negative exponential	$a = 0.154$ $T_1 = 0.058$ $b = 0.846$ $T_2 = 6.488$	$a = 0.901$ $T_1 = 0.729$ $b = 0.946$ $T_2 = 0.874$	3, 23	63.4	<0.0001	Double negative exponential a sig. better fit.

Cryptocarya murrayi	Double negative exponential	a = 0.163 T_1 = 0.054 b = 0.836 T_2 = 7.277	a = 0.890 T_1 = 0.716 b = 0.938 T_2 = 0.912	3, 15	145.1	<0.0001	Double negative exponential a sig. better fit.
Endiandra hypotephra	Double negative exponential	a = 0.740 T_1 = 16.280 b = 0.260 T_2 = 0.035	a = 0.799 T_1 = 0.933 b = 0.630 T_2 = 0.479	3, 23	2170.6	<0.0001	Double negative exponential a sig. better fit.
Cupaniopsis foveolata	Double negative exponential	a = 0.260 T_1 = 1.134 b = 0.740 T_2 = 21.162	a = 0.630 T_1 = 0.534 b = 0.824 T_2 = 0.948	3, 33	3315.2	<0.0001	Double negative exponential a sig. better fit.
Mischocarpus exangulatus	Double negative exponential	a = 0.221 T_1 = 0.700 b = 0.644	a = 0.480 T_1 = 0.390 b = 0.752	3, 33	155.3	<0.0001	Double negative exponential a sig. better fit.

		$T_2 = 26.06$	$T_2 = 0.946$				
Agyrodendron polyandrum	Double negative exponential	$a = 0.221$	$a = 0.492$	3, 33	1421.6	<0.0001	Double negative exponential a sig. better fit.
		$T_1 = 0.832$	$T_1 = 0.467$				
		$b = 0.778$	$b = 0.829$				
		$T_2 = 0.043$	$T_2 = 0.707$				
Chionanthus ramiflora	Two-piece linear	$c_1 = 0.299$	$Y_1 = 0.002$	3, 31	255.7	<0.0001	Two-piece linear a sig. better fit.
		$c_2 = 0.701$	$Y_2 = 0.432$				
		$d_1 = -0.144$	$Y_3 = 0.249$				
		$d_2 = -0.111$	$T_1 = 0.275$				
Cupaniopsis flagelliformis	Two-piece linear	$c_1 = 0.267$	$Y_1 = 0.028$	3, 34	491.1	<0.0001	Two-piece linear a sig. better fit.
		$c_2 = 0.733$	$Y_2 = 0.460$				
		$d_1 = -0.108$	$Y_3 = 0.246$				
		$d_2 = -0.010$	$T_1 = 0.333$				

Discussion.

In this study I showed that the single negative exponential model of seed moisture loss is not the best fit model describing the relationship between W_r versus t in all species. In this regard, the negative exponential model cannot be considered general. Hence, I reject hypothesis 1. In addition to the single negative exponential model, our results identified two alternative models that were better descriptions of seed moisture loss within ten species. These were: (1) a double negative exponential function; and (2) a double negative linear function. To the best of our knowledge, this is the first demonstration of a two-phase process in seed desiccation studies. Seed dry mass did not predict $t_{0.368}$ between species discounting a relationship between seed mass and desiccation. Within species seed dry mass was a strong predictor of $t_{0.368}$ for 8/24 species. We therefore partially accept hypothesis 2, and accept hypothesis 3.


(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
$t_{0.3679}$ (unitless)
Mass (mg)

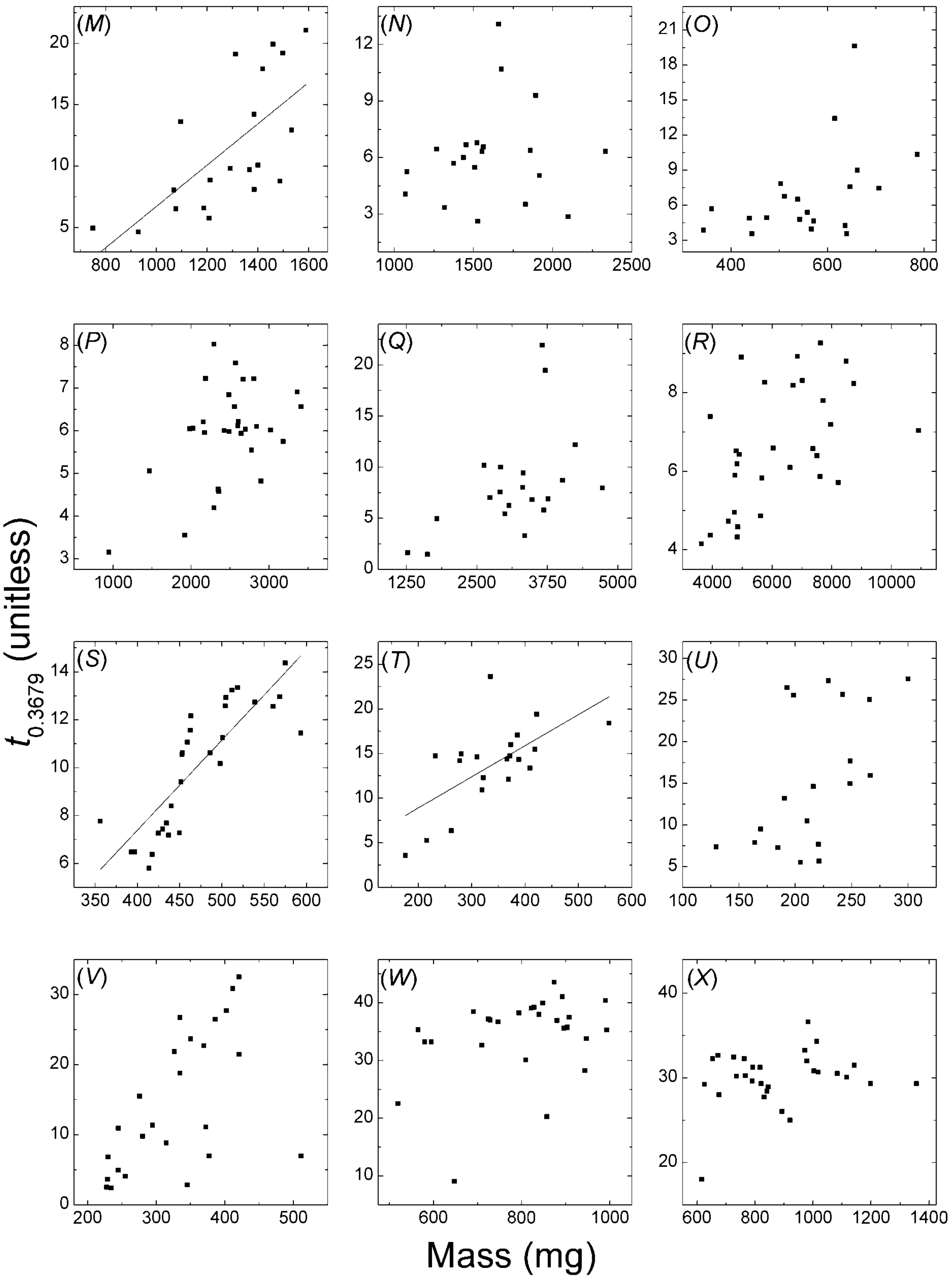


Figure 9(A-X). The relationship between seed dry mass and the time constant (T or t0.368) within 24 desiccation sensitive seeded rainforest plant species. The plotted regression equations represent those species where the relationship was significant. Species codes as in Figure7.

Table 9 The relationship between the time constant ($t_{0.368}$) and mean dry seed mass for 24 plant species located within the seasonal tropical forests of Cairns, Australia. The Boferroni correction for multiple tests was used to identify Significant relationships were identified using the Bonferroni correction for multiple tests (i.e. sig. $P > 0.0026$) and are highlighted in bold**.**

Species	t0.368 (days)	Mean dry seed mass (mg)	R^2_{adj}	df	F	P
Cryptocarya mackinnoniana	**1.95**	**165**	**0.65**	**1, 28**	**54.10**	**<0.0001**
Helicia australasica	**1.15**	**551**	**0.40**	**1, 18**	**13.71**	**<0.001**
Endiandra leptodendron	**1.95**	**651**	**0.55**	**1, 18**	**24.67**	**<0.0001**
Toechima erythrocarpum	3.82	326	0.05	1, 18	2.01	0.173
Dysoxylum alliaceum	6.25	2473	0.20	1, 28	8.10	0.008
Dysoxylum pettigreweanum	2.10	717	0.29	1, 18	8.84	0.008
Diploglottis diphyllostegia	4.90	156	0.03	1, 26	1.83	0.189
Syzygium kuranda	7.35	5608	0.23	1, 28	9.48	0.005
Agyrodendron peralatum	**2.91**	**373**	**0.69**	**1, 18**	**42.73**	**<0.0001**
Endiandra bessaphila	3.21	1393	0.16	1, 18	4.75	0.042
Garcinia warrenii	5.50	3848	0.11	1, 17	3.14	0.094
Connarus conchocarpus	**10.90**	**385**	**0.68**	**1, 28**	**63.34**	**<0.0001**
Palaquium galactoxylon	5.84	1598	0.04	1, 18	0.25	0.619
Myristica globosa	5.30	1355	0.20	1, 28	8.14	0.008
Cryptocarya murrayi	6.21	560	0.17	1, 18	4.92	0.039
Endiandra longipedicellata	**4.83**	**3122**	**0.74**	**1, 18**	**55.18**	**<0.0001**
Endiandra cowleyana	7.13	3169	0.20	1, 18	5.90	0.025

Castanospora alphandii	5.81	3416	0.23	1, 16	6.42	0.021
Endiandra hypotephra	**5.84**	**1283**	**0.42**	**1, 18**	**14.96**	**<0.001**
Cupaniopsis foveolata	15.07	209	0.25	1, 17	7.14	0.016
Chionanthus ramiflora	38.00	805	0.01	1, 27	1.36	0.253
Mischocarpus exangulatus	18.25	332	0.30	1, 28	11.23	0.002
Agyrodendron polyandrum	**13.39**	**339**	**0.38**	**1, 18**	**12.60**	**<0.001**
Cupaniopsis flagelliformis	30.50	539	0.09	1, 27	3.91	0.058

The expectation that a single negative exponential model underlies patterns of seed water loss is based on the assumption that seeds desiccate according to the physical laws governing water vapour loss from a moist material in association with differences between water vapour pressure in the moist seed interior and the surrounding air (see Introduction). This model considers the moist seed to have a homogeneous interior with uniform diffusion properties. The finding of both double negative exponential and double linear functions questions the generality of this assumption. There are several reasons why it cannot be assumed that all desiccation sensitive seeds should follow the classical pattern of moisture loss represented by equations 1-2. Physical changes to the seed’s internal and surface properties may cause changes in resistance to moisture loss, and hence T, as the seed dries. This might occur as seeds undergo shrinkage and shrivelling during desiccation. Alternatively, non-uniformity of the moisture environment within the seed, perhaps due to compartmentalisation of moisture within different physical structures, may also contribute to non-conformity to equation (2). Further studies are required to investigate these alternatives.

As time to 1% desiccation increased the appropriateness of the single negative exponential model decreased. There was a trend between the length of time required to dry to 1% seed moisture content and the ability of the single negative exponential model to describe the pattern of seed moisture loss. For example, of the ten species with an alternative model seven species (Figure 7R-X) took the longest period of time to reach to 1% seed moisture content. The time taken to reach 1% moisture ranged between 34-74 days (*A. peralatum* and *A. polyandrum*) for species where the double negative exponential models were the best fit while double negative linear models best described *C. flagelliformis* and *C. ramiflora* (74 and 68 days respectively). In contrast, the range of times to reach 1% moisture for species described by single negative exponential function was between 8 (*H. australasica*) and 44 days (*Endiandra cowleyana* (F.M. Bailey)). This may not be unexpected. The nature of the two-phase model is that it is best considered as two (potentially) independent processes, representing different rates of moisture loss. In these species, an initial rapid rate of moisture loss from one compartment is offset by a much slower rate in the second compartment. Thus, the operation of the second compartment prolongs the total amount of time required to fully desiccate, resulting in greater times required to reach 1% moisture.

Since all seeds were desiccated in a uniform environment in the study, the lack of relationship between desiccation rate and seed mass between species suggests that seed size alone is not a good measure on which to predict rates of decline in W_r in comparisons between species. Nevertheless, within species we found a positive relationship between desiccation rate and seed mass (although the relationship was significant in 8/24 species tested) in line with our prediction based on; (i) the dependence of seed hydraulic capacitance on seed mass when all other structural properties of the seed scale with mass, and (ii) constant resistance to moisture loss, i.e. resistance per unit seed area remains unchanged in larger seeds (Figure 9). The results suggest that, at least within some species, larger seeds are potentially able to tolerate drought conditions better than smaller seeds.

It is unlikely that seeds used in this experiment would be exposed to such drying conditions within natural environments. The approach was not to assess the ability of seeds to withstand desiccation within a natural setting, but instead to asses if differences existed between species that co-exist within the same forest community. The method of drying used in this study has been described as a slow drying method (Farrant *et al*. 1985; Farrant *et al*. 1989; Pammenter *et al*. 1998). Slower drying rates tend to yield higher mortality values opposed to intermediate or rapid rates at a given seed moisture content. In natural systems the rate of desiccation is potentially much less than what we have used here (R.H. 10%), even so one would expect that under drought field conditions the slower rate of desiccation would result in higher critical water thresholds.

In the face of potentially drier conditions within tropical forests associated with changing global climates, there is an urgency to understand the role of seed desiccation sensitivity in processes determining individual species’ distributions. Within tropical forests the distribution of plant species has been shown to be strongly associated with moisture availability (Engelbrecht *et al.* 2007). Predictions that drought episodes within tropical forests may both intensify and become more common (IPCC 2014) may thus represent a threat to the distribution and persistence of desiccation sensitive seeded species. For example, vulnerability to desiccation means that desiccation sensitive seeds may have a relatively brief residence in the seed bank. This may limit their potential for dispersal (Pammenter and Berjak 2000), a mechanism that has been suggested to contribute to the restricted distributions that desiccation sensitive species often exhibit (Vazquez-Yanes and Orozco-Segovia 1993; Pritchard *et al.* 2004$_a$; Daws *et al.* 2005). Similarly, some species with desiccation sensitive seeds produce dormant seeds that require long periods of moist incubation for dormancy to break (Daws *et al.* 2006$_b$). The predicted restriction in seasonal precipitation to fewer months would also place species with dormant seeds at risk of not having sufficient time in moist incubation for dormancy to break.

In previous studies seed desiccation has been assessed using different methodologies and different seed components and are often focused on single species. For example, studies of the physiological aspects of recalcitrance rarely use entire seeds, working instead with embryonic axes (Leprince *et al.* 1999; Liang and Sun 2000; Liang and Sun 2001) cotyledons (Leprince *et al.* 1999; Sun and Liang 2001), whole embryos (Dussert *et al.* 1999) and dissected seeds (Yu *et al.* 2008). Given the range of techniques and structures used to examine desiccation sensitivity, comparisons of the rate and pattern of drying in whole seeds have been difficult to undertake. In this study I imposed a common desiccation environment across whole seeds in 24 species, allowing comparison between them. Overall, two lines of evidence from our results suggest that desiccation in desiccation sensitive seeds may not be simple, nor easily generalised across species. These are: (1) the demonstration that the pattern of seed moisture loss cannot always be assumed to be a simple, single negative exponential function, (2) the lack of any relationship between the rate of desiccation and mean seed mass between species, (i.e. different species have fundamentally different desiccation curves irrespective of mass differences between them). Alternatively, once the pattern of moisture loss is known for a given species, individual seed mass may provide some measure of desiccation time. This is most likely due to similarities of structural properties of seeds within a species. The three models we present describing different patterns of moisture loss from the desiccation sensitive seeds of 24 rainforest tree species, and potentially other models for different species, will assist in assessing the differential impact of changing forest moisture regimes on different species.

In the following Chapter I calculate a range of seed traits that are potentially expressed in species with mean dispersal dates within the dry season. I also examine whether seed mass alone is a good trait to predict resilience in desiccation sensitive seeds.

Chapter 5. Size isn't everything for desiccation sensitive seeds.

This chapter has been published as "*Hill, J., Edwards, W & Franks, PJ. (2012) Size is not everything for desiccation-sensitive seeds. Journal of Ecology 100, 1131–1140*."

Introduction

Seed size has often been investigated with respect to the advantages and disadvantages size confers on post-germination success; that is, in the establishment and subsequent growth of seedlings (Foster 1986, Coomes and Grubb 2003, Moles *et al.* 2004, Moles and Westoby 2006). For example, seedlings produced from large seeds have greater tolerance to defoliation (Armstrong and Westoby 1993), shade (Osunkoya *et al.* 1994, Leishman and Westoby 1994a, Saverimuttu and Westoby 1996, Niinemets 2006), drought (Leishman and Westoby 1994b, Grubb and Metcalfe 1996, Lloret *et al.,* 1999) and grow taller as seedlings, enabling them to out-compete seedlings produced from smaller seeds (Coombs and Grubb 2003, Kisdi and Geritz 2003). As such, mass or size has been the main, and often only, measure used to characterize seeds in studies that attempt cross-species comparisons (Osunkoya *et al.,* 1994, Hammond and Brown 1995, Ter Steege and Hammond 2001, Moles *et al.,* 2004, 2005, Willis and Hulme 2004, Pluess *et al.* 2005).

In contrast, comparatively few studies examine the potential role of size (or other seed traits) in pre-germination survival. Of those that do, relationships between seed size and pre-germination success focus largely on spatial dispersal (e.g. Soons *et al.* 2008, Schupp *et al.* 2010), temporal persistence in the face of seed predation (e.g. Paine and Beck 2007, Hautier *et al.* 2010) or, for seeds that accumulate in soils, the retention of viability within soil seed banks (e.g. Dalling *et al.* 1998).

Estimates suggest that almost 50% of all tropical plant species produce desiccation sensitive seeds (Vazquez-Yanes and Orozco-Segovia 1993, Tweedle *et al.* 2003). Desiccation sensitive seeds are shed with high seed moisture contents, maintain metabolic function throughout the period prior to germination and do not establish seed banks (Franks and Drake 2003). These seeds are at high risk of mortality should they disperse to unfavourable (desiccating) locations and selection for traits that enable them to avoid critically low water contents prior to germination may be at least as important as size is for post-germination survival. To date, however, we know little about the traits that may confer tolerance to desiccation prior to germination in species with desiccation sensitive seeds, nor how traits co-vary among species.

Seed size has been suggested as a proxy for desiccation tolerance (Dickie and Pritchard 2002, Daws *et al.* 2005, 2006a). This is based on the assumption that seed water loss can be described by a simple negative exponential relationship, and, because hydraulic capacitance and the ratio of volume to surface area increase with size, larger seeds may take longer to dry (Foster 1986, Metcalfe and Grubb 1997;

Dussert *et al.* 1999, Daws *et al.* 2005, Makeen *et al.* 2006). The principle of larger seed mass conferring greater desiccation tolerance appears to play out across co-occurring species, to the extent that, as a group, desiccation intolerant species have larger mean seed mass than desiccation tolerant species. For example, Dickie and Pritchard (2002) reported that the mean seed mass of desiccation sensitive species was 3958 mg (n = 205), over an order of magnitude greater than desiccation tolerant species (329 mg) (n = 839), a pattern that was later supported in a similar analysis using phylogenetic comparisons from semi-deciduous forest in Panama (Daws *et al.,* 2005). Nevertheless, comparison of means between desiccation sensitive and desiccation tolerant groups of species provides little information on the attributes of desiccation sensitive seeds, including how they may differ between species or whether size *per se* should be considered the principal response to desiccation pressure. Indeed size may not be the principal attribute determining survival of desiccation-sensitive seeds. In Chapter 4 I showed that seed size could not always predict the rate of desiccation among 24 species that produce desiccation sensitive seeds in a seasonal tropical forest community in Cairns, Queensland. Within these species size was only significantly positively related to desiccation in 8 out of the 24.

In species with desiccation sensitive seeds, selection for reduced rate of desiccation has likely acted upon multiple correlated seed traits, rather than size alone (Reich *et al.* 2003, Laughlin *et al.* 2010). At present, I know of only five studies that have attempted to assess combinations of traits in desiccation sensitive seeds (Dussert 1999, Pritchard *et al.* 2004, Daws *et al.* 2005, 2006, Yu *et al.* 2008). Besides size, we identify six other attributes of seeds (and their production) that are potentially important for the survival of desiccation sensitive seeds. These include four physiological traits: critical water content (W_{r50}), relative water content (W_M), desiccation rate ($t_{0.368}$) and mean time to germination (T_G); one morphological trait: seed coat ratio (SCR); and one phenological variable: the water availability at the time of seed release from parent (hereafter mean monthly rainfall at time of seed release (R_M)).

The W_{r50} is an estimate of the relative water content at which desiccation sensitive seeds can retain 50% viability. Previous evidence has shown that the time taken to reach W_{r50} varies between species and populations and is strongly influenced by local climatic conditions (Dussert *et al.* 2000, Sun and Liang 2001, Berjek and Pammenter 2008). Seed relative water content is sometimes measured as the amount of water a fresh seed has at time of dispersal, expressed relative to seed dry mass. Although this measure does not readily indicate how hydrated the seed is, it has been shown to be positively correlated with W_{r50} (Yu *et al.* 2008) such that seeds with high water contents at maturity also lose viability at higher W_{r50}.

The potential rate of desiccation is indicated by the time constant, $t_{0.368}$, for the exponential rate of water loss from full hydration (Chapter 4). For seeds with more complex time-courses of desiccation, $t_{0.368}$ is the time taken to lose 63.2% of the water available at full seed hydration. Higher $t_{0.368}$ values should enable desiccation-sensitive seeds to tolerate longer periods under drought conditions, taking longer to reach W_{r50}.

Desiccation sensitive seeds germinate quickly (low T_G) (Pammenter and Berjak 2000, Pritchard *et al.* 2004a, 2004b). Short T_G decreases the length of time a desiccation sensitive seed remains exposed to potentially lethal conditions (Pammenter and Berjak 2000). SCR is simply the seed coat proportional to dry seed mass. Desiccation sensitive species exhibit relatively low SCR in comparison to desiccation tolerant species (Pritchard *et al.* 2004a; Xiu *et al*., 2014). It has been suggested that low SCR must be associated with rapid germination to avoid dry conditions (Pritchard *et al.* 2004a). While advantages and disadvantages of both T_G and SCR have been proposed in comparisons between desiccation-tolerant and desiccation-sensitive seeds, little is known about the variability in these traits among species with desiccation sensitive seeds.

Finally, whole-plant phenological patterns may be shaped by the requirement to reduce pre-germination mortality. Mean seed release times (R_M) for desiccation sensitive seeds generally coincide with months of high precipitation, a mechanism thought to offset the risk of seed dehydration (Pritchard *et al.* 2004a; Daws *et al.* 2005; Yu *et al.* 2008; Xiu *et al*. 2014). This was not the case in the Australian tropics, however (Chapter 3). Even so, all studies report some species that release seeds during dry periods. That any species releases desiccation sensitive seeds outside of periods of highest moisture availability argues against strong selection for phenological timing in desiccation sensitive species.

Correlations between seed size and other seed attributes have been used to suggest that size might be sufficient to describe differences in desiccation tolerance prior to germination between species. Here I ask the simple question: does seed size capture a plant strategy axis associated with all traits that may contribute to pre-germination drought tolerance, as it has been shown to describe post-germination differences in tolerance to hazards among seedlings (Muller-Landau 2010)?

Methods

A total of 140 freshly dispersed fruit or seeds were collected for each of 16 species typical of these vegetation type (Table 10). For species that produce drupes and berries, seeds were extracted from fallen fruit. For all other fruit categories seeds were obtained from freshly fallen specimens collected during daily visits to known trees. Species selection was determined by the number of fruiting individuals available. Fruits were collected from a minimum of seven individuals from each species. Eleven species produced fleshy fruits and the remaining five species produced capsules where seeds were presented with arils.

Seeds/fruits were transferred to the laboratory where they were stored at 10 ºC in moist vermiculite within a zip-lock polyethylene bag (following Daws *et al.* 2005) until required numbers were sampled to begin the experiments. All seeds/fruits were collected within a ten-day period, reducing the amount of time seed was stored. At this time, seeds were removed from storage and rinsed in H_2O to remove any vermiculite from the seed coat. All surface moisture was then removed and seeds were left on a laboratory bench for 30 minutes to equilibrate with room temperature

(23.5 ± 1 ºC). Seeds were randomly allocated into seven treatment groups each with 20 seeds. The randomisation ensured that all treatments had similar mean seed masses and similar variances.

Mean seed dry mass, specific water content at full hydration and SCR

Fully hydrated seeds in one seed lot were weighed (M_0) and then fully desiccated by oven-drying (80 ºC) for five days. They were then re-weighed to determine seed dry mass, M_D. Specific seed relative water content at full hydration, W_M, was then calculated as

$$W_{\mathrm{M}} = \frac{M_0 - M_{\mathrm{D}}}{M_{\mathrm{D}}} \qquad \text{eqn 1}$$

Mean M_D was later used for determining W_r, $t_{0.368}$ and W_{r50} (see below). The seed coat and/or endocarp was removed and the SCR was determined as a proportion of total dry seed mass. These seeds were not used in germination treatments.

Seed relative water content and $t_{0.368}$

In a second seed lot, the time-course of desiccation was tracked by placing seeds in a desiccating environment and periodically measuring W_r, as it declined from 1.0

(fully hydrated) to 0 (fully desiccated). W_r was measured according to (Franks and Drake 2003; Hill *et al.* 2010):

$$Wr = \frac{M_S - M_D}{M_0 - M_D} \qquad \text{eqn 2}$$

where M_S represents the seed mass at given time, M_D equals final seed dry mass (determined as per full desiccation treatment described above) and M_0 is mass of the fully hydrated seed, i.e. at the commencement of desiccation. W_r was recorded every second day until there was no change in M_S. I fitted negative exponential, double negative exponential and two-piece linear fits to the time-series of W_r to determine which model best described the pattern of desiccation in each species. In all cases, the negative exponential model was the best fit and parameters estimated from that model were used to determine $t_{0.368}$ (Chapter 4).

Critical water content and mean germination time (W_{r50}, T_G)

One seed lot was immediately sown on 1% agar gel to assess germination success in fully hydrated seeds, i.e. at $W_r = 1.0$ (0% desiccation). The remaining four seed lots were placed into desiccating environments constructed from polyethylene boxes each filled with 15 g of silica gel (desiccant). Each seed was placed in a separate compartment (one seed per compartment) within the polyethylene boxes. Relative humidity within compartments was regularly assessed using a Vaisala (Helsinki, Finland) HMP44 temperature and humidity probe and was maintained between 5.5

and 10% by replacing the desiccant when relative humidity reached 10%. The boxes were kept in a temperature-controlled laboratory maintained at 24 °C. Our goal was to generate a range of target seed relative water contents representing desiccation treatments of W_r = 0.8, 0.6, 0.4 and 0.2, corresponding to 20%, 40%, 60% and 80% desiccation, respectively. We calculated the time to reach each desiccation treatment from the negative exponential model determined above for each species. When the prescribed W_r was reached, all seeds were transferred from the desiccation treatments to the 1% agar germination medium (as above). All germination treatments were carried out in a temperature controlled room set at 24 °C, exposed to a 12 h photoperiod using standard florescent lights. Treatments were examined every second day over a three-month period and germination was recorded when the radicle had emerged 2 mm (Pritchard *et al.* 2004b). Seeds that did not germinate after a three-month period were deemed non-viable and not considered further. I controlled fungal infestation within treatments by administering a broad spectrum aerosol fungicide (Baycor 300, Bayer$^{©}$) where necessary.

Mean time to germination, T_G, for each species and treatment was calculated as

$$T_G = \frac{\Sigma(Dn)}{\Sigma(n)} \qquad \text{eqn 3}$$

where *D* is the number of days since seeds were sown and *n* is the number of seeds that germinate on day *D* (Pritchard et al. 2004b).

Critical seed water content, W_{r50}, was determined as the lowest W_r at which more than 50% of seeds can still germinate. The viability of recalcitrant seeds subjected to desiccation usually declines in a sigmoidal fashion with increasing desiccation (Dussert *et al*. 1999). To determine W_{r50}, mortality curves were generated by fitting the following Boltzmann sigmoid function to plots of seed germination success (as the proportion of seeds that germinate at any given W_r) versus W_r treatment:

$$y = A_1 + \frac{A_2 - A_1}{1 + 10^{\left(\frac{\log x_0 - x}{p}\right)}} \qquad \text{eqn 4}$$

where y is the proportion of seeds that germinate, x is the W_r treatment, A_1 is the lower asymptote, A_2 is the upper asymptote, p is the slope generated between the estimated proportion of germinated seeds at 10 and 90% water contents, and x_0 is W_{r50}. The upper asymptote, A_2, was fixed as the proportion of seeds that germinated within the control (fully hydrated) treatment and the lower asymptote A_1 was fixed at 0. In some cases there was a higher number of germination events after treatments had been exposed to desiccation (three species; *Syzygium kuranda, Cupaniopsis foveolata* and *Chionanthus ramiflora*), and a sigmoidal curve would not fit the full data set (this is not unusual and other reports have shown increased germination success in some species after slight drying, see for example, Pritchard *et al.* (2004a), in which 3 out of 10 species displayed greater germination after a period of storing in vermiculite). In these cases, the desiccation treatment with the highest number of germinants was used to set the upper value of 1 (A_1) and all other treatments expressed as proportions of this group.

Moisture availability at the time of peak seed release was based on information on the temporal pattern of seed release from records of fruit/seed dispersal recorded between January 2002 and December 2005 from 72 x 0.5 m^2 seed traps within a 2-ha area of the study site. The total number of seeds dispersed by each species was used to calculate the mean monthly angle of dispersal and associated vector length (measure of dispersion) using circular statistics (Kovach 2006). We used the mean vector angle to determine the month corresponding to peak seed release and matched this with mean monthly rainfall estimated from 70 y of monthly data taken at Cairns airport (10 km from the study site).

Analysis

Linear regressions were used to test for significant bivariate relationships between all seed traits. Because 7 individual traits leads to 21 unique regression analyses, Bonferroni correction was used to set alpha for each test (i.e. $P < 0.002$). To examine the relationship between species across traits, we used principal component analysis (PCA) to ordinate species in trait space with a varimax function to rotate the axis and improve the factor loadings within each principal component. The number of significant principal components was determined by eigenvalues above 1. Following Laughlin *et al*. (2010), all traits with eigenvalues > |0.30| were considered significant structuring variables on PC axes. Variables that did not conform to a normal distribution were $\log_{10}$ transformed where necessary prior to analysis.

Results

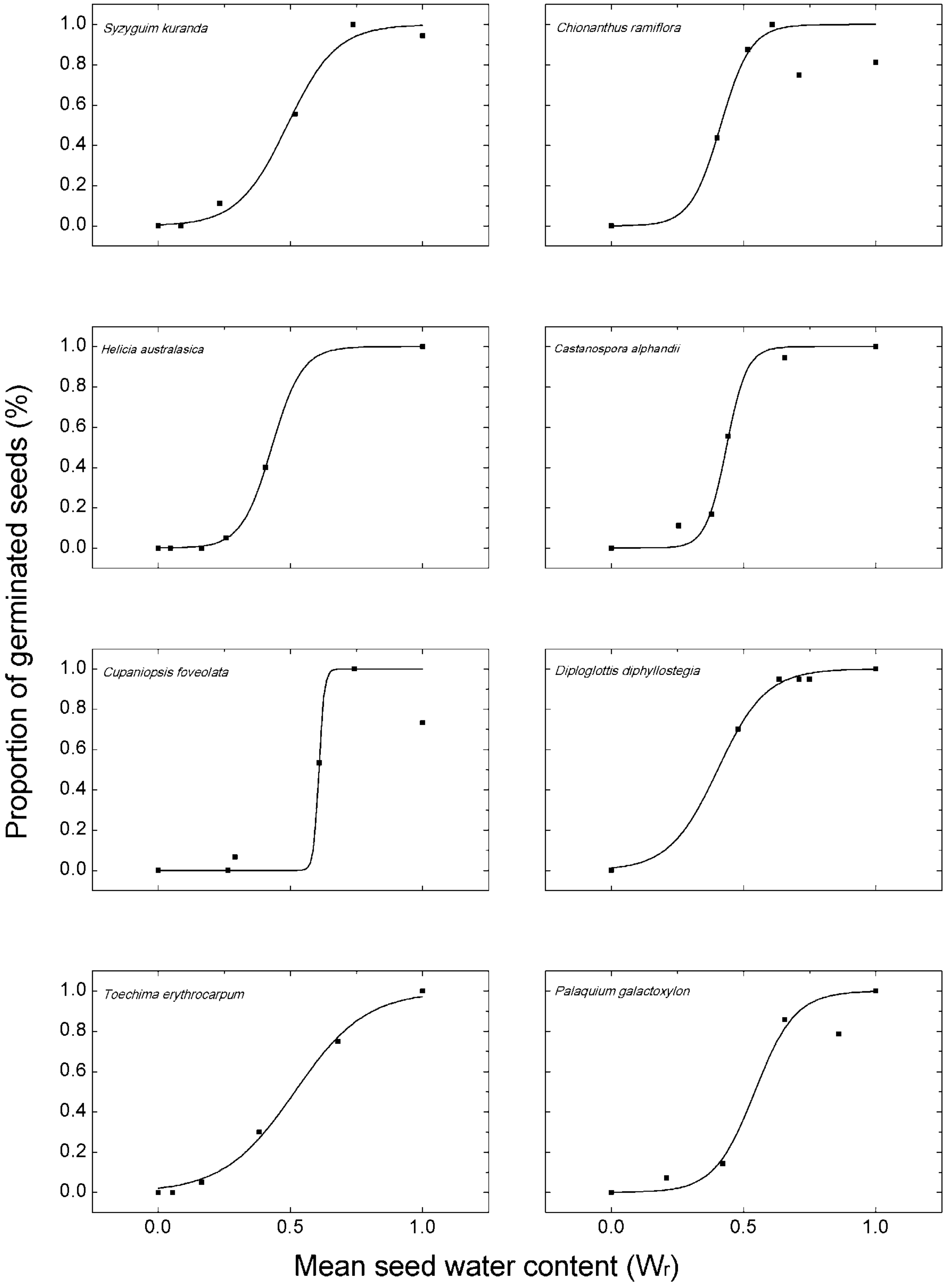
Considerable variation existed between the seed traits of each species (Table 10). Mean seed mass varied from the smallest seeded *Diploglottis diphyllostegia* (156 mg) to largest, *Syzygium kuranda* (5608 mg). Similarly, there was a wide spread of values across all other seed traits. For example, the range of critical water contents (W_{r50}) varied between 0.715 for *Dysoxylum pettigrewianum* to 0.331 for *Myristica globosa* (Table 11, Figure 10). Even closely related species (i.e. from the same genera) exhibited different W_{r50} values. For example, the four *Endiandra* species had estimated W_{r50} values that varied across a range representing 20% difference in seed water content; from 0.551 for *Endiandra leptodendron* to 0.348 for *Endiandra hypotephra*. Desiccation rates also varied between species (Table 10). The time constant $t_{0.368}$ ranged from 1.15 days for *Helicia australasica* to 30.3 days for *Chionanthus ramiflora.* Nevertheless, desiccation rates were generally fast and the majority of species (14) had $t_{0.368}$ values of less than 10 days.

The mean time to germination in fully hydrated seeds also spanned almost an order of magnitude, from 6.79 days for *Toechima erythrocarpum* to 49.88 days for *Syzygium kuranda* (Table 10). The majority of species germinated within 15 days, although 4 species, *Myristica globosa*, *Diploglottis diphyllostegia*, *Chionanthus* *ramiflora* and *Syzygium kuranda* all displayed mean time to germination greater than 20 days. Similar variability was recorded for the other three traits: W_M, SCR and R_M (Table 10).

Table 10 Traits of sixteen rain forest plant species that produce desiccation sensitive seeds. All are from a lowland tropical rain forest community in north Queensland. Traits are: Wr50 (seed relative water content at which 50% of a cohort of seeds become unviable); TG (time to germination in fully hydrated seeds); MD (seed dry mass); *t*0.368 (time constant for exponential decline of Wr under the desiccation treatment); SCR (ratio of seed coat and/or endocarp to embryo); and WM (the specific relative water content as defined in eqn 1). Mean values, n = 20 (see text for further details). (Species authorities are in accordance with Bostock and Holland 2010).

Species	**Family**	**W_{r50}**	**T_G (days)**	**M_D (mg)**	**$t_{0.368}$ (days)**	**SCR**	**W_M ($g.g^{-1}$)**
Castanospora alphandii	Sapindaceae	0.435	9.6	3416	5.8	0.186	0.989
Chionanthus ramiflora	Oleaceae	0.413	39.4	805	38.0	0.29	0.648
Cryptocarya murrayi	Lauraceae	0.448	7.4	560	6.2	0.225	0.896
Cupaniopsis foveolata	Sapindaceae	0.607	9.2	209	15.1	0.198	0.742
Diploglottis diphyllostegia	Sapindaceae	0.402	32.8	156	4.9	0.144	0.973
Dysoxylum pettigrewianum	Meliaceae	0.715	11.0	717	2.1	0.057	0.953
Endiandra bessaphila	Lauraceae	0.468	13.0	1393	3.2	0.174	0.861
Endiandra hypotephra	Lauraceae	0.348	10.4	1283	5.8	0.21	0.861
Endiandra leptodendron	Lauraceae	0.551	8.9	651	1.9	0.105	0.880
Endiandra longipedicellata	Lauraceae	0.364	11.3	3122	4.8	0.169	0.998
Garcinia warrenii	Clusiaceae	0.419	9.1	3848	5.5	0.05	1.322
Helicia australasica	Proteaceae	0.429	7.2	551	1.1	0.174	1.089
Myristica globosa	Myristicaceae	0.336	22.3	1355	5.3	0.339	0.523

Palaquium galactoxylon	Sapotaceae	0.542	8.4	1598	5.8	20.4	0.921
Syzygium kuranda	Myrtaceae	0.482	49.4	5608	7.3	30.3	0.862
Toechima erythrocarpum	Sapindaceae	0.516	6.8	326	3.8	13.6	0.897


Syzyguim kuranda
Chionanthus ramiflora
Helicia australasica
Castanospora alphandii
Cupaniopsis foveolata
Diploglottis diphyllostegia
Toechima erythrocarpum
Palaquium galactoxylon
Proportion of germinated seeds (%)
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.5
1.0
Mean seed water content (Wr)

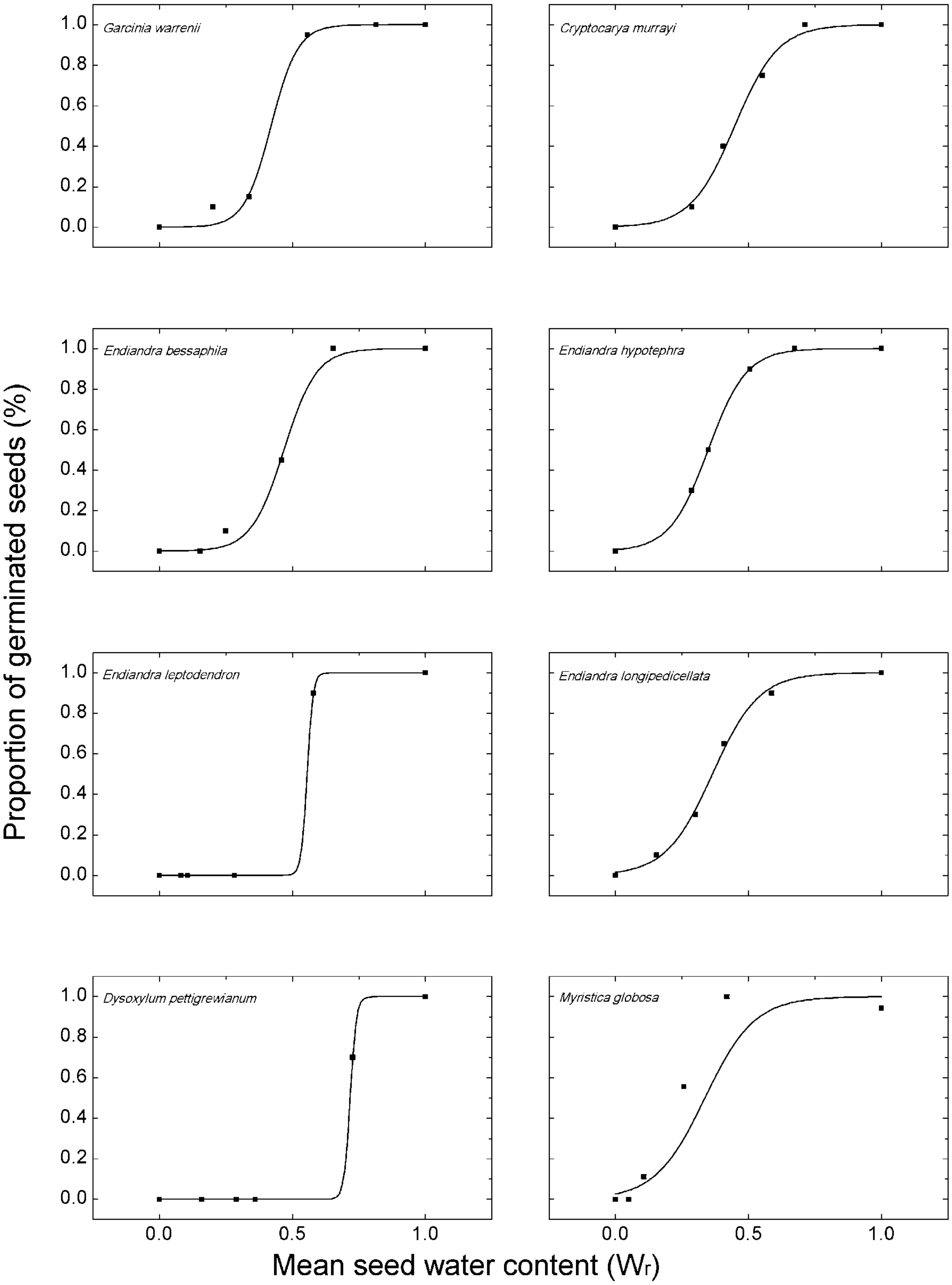


Figure 10 Seed mortality curves for 16 rain forest species that produce desiccation sensitive seeds in north Queensland, Australia. The y-axis represents the proportion of seeds germinating and the x-axis is the mean seed relative water content, Wr. Each point is based on germination in a seedlot of 20 seeds subjected to a range of desiccation treatments (Wr = 1.0, 0.8, 0.6, 0.4 and 0.2; see text for details).

Relationships between seed traits

There was only one significant relationship between seed traits (Table 11). Monthly rainfall was negatively related to SCR (i.e. species with greater relative investment into seed coats had dispersal periods during months of low precipitation ($R^2 = 0.58$, $F_{1,\ 15} = 19.0$, $P < 0.001$)) (Figure 11). There were no other significant bivariate relationships between traits and no traits were significantly related to M_D.

PCA identified two principal components with eigenvalues > 1. Combined, these two axes explained approximately 65% of the total variance in the species-trait correlation matrix (Table 12). The first principal component accounted for 44.1% of the total variance and identified five variables with factor loadings > |0.30|. There were strong negative loadings on R_M and W_M and strong positive loadings for SCR, T_G and $t_{0.368}$ (Table 12). That is, species at one end of this spectrum have mean dispersal periods during months of low rainfall, produce seeds that have comparatively lower moisture contents, greater relative investment in seed coat, take longer to germinate and desiccate at slower rates than do seeds from species at the opposite end of this axis (Figure 12). In part, the axis loadings also reflect the single significant bivariate relationship (above). That is, R_M and SCR were negatively related, and have opposite signs on PC1.

The second principal component explained approximately 21% of the variation between traits (Table 12). There was a strong positive weighting on W_{r50} and strong negative loadings for M_D and W_M. That is, larger seeded species tend to release their seeds at maturity with greater water contents, and these species are also able to withstand desiccation to lower W_r (i.e. have lower W_{r50}).

Discussion

The analysis revealed two principal axes separating species in trait space. The first PC was associated with strong negative loadings on average monthly rainfall (RM) and specific water content (WM) and strong positive loadings for seed coat ratio (SCR), mean time to germination (TG) and desiccation rate ($t_{0.368}$). This axis represents a spectrum in which species that disperse seeds during periods of low moisture availability (low mean RM values) have comparatively lower moisture contents, greater relative investment in seed coat, take longer to germinate and desiccate at slower rates than seeds from species at the opposite end of this axis. This axis is in agreement with expectations from previous reports based on comparison between desiccation sensitive and desiccation tolerant species (Daws *et al*. 2005). In general, I consider that this axis might represent a primary response to moisture availability in terms of the relative investment in seed coat. Recall that the single significant bivariate relationship, between RM and SCR, was negative. Assessed in this way, species with greater relative investment in seed coats had dispersal periods during months of low precipitation. Investment in SCR is a measure of relative protection of the embryo, either from predation and pathogens, or from drying (Pammenter and Berjak 2000, Pritchard *et al*. 2004a) and, indeed, length of time taken to desiccate to a given water content was positively associated with relative investment in seed coat. Other traits associated with PC1 are also in agreement with expectation based on SCR although they are not significant. For example, TG was positively associated (similar sign on PC1) with SCR, most likely due to the longer time required for radicle to penetrate seed coat or because any requirement to imbibe moisture might take longer with higher SCR.

TG, SCR and t0.368 all had high negative loadings on PC1, while RM was positively loaded on PC1. Thus, mean time to germination was longer for species with dispersal dates during periods of low water availability. At present, the adaptive significance of this finding is unclear, although longer TG may be advantageous if the temporal shift between dispersal and germination results in seedling establishment closer to defined wet season where water availability is higher (Farnsworth 2000, Pritchard *et al*. 2004a, b, Daws *et al*. 2006, Berjak and Pammenter 2008).

The findings based on SCR may provide an explanation of how desiccation sensitive seeds tolerate dispersal during dry seasons. Recent investigations by Xia *et al*. (2012) has shown that the pericarp delays seed desiccation in *Quercus schottkyana* and *Q. franchetii* during unseasonal dry spells enabling pre-germination survival (Xia *et al*, 2012). Further investigations examining potential differences in the unique structure of the seed coat may reveal mechanism(s) that delay the loss of water from desiccation-sensitive seeds. It may also help explain the occurrence of desiccation sensitive species in tropical drylands (Danthu *et al*. 2000, Tweedle *et al*. 2003).

The second principal component explained approximately 21% of the variation between species. There was a strong negative weighting on MD and WM. That is, PC2 represents between-species variability in seed size, and species producing larger seeds tend to be released with greater seed water contents. The analysis also showed that larger seeds are able to withstand desiccation to lower percentage water levels (i.e. a significant positive loading for Wr50), an opposite result to that shown previously (Yu *et al*. 2008). Specific seed water content at full hydration (WM) was also positively associated with RM on PC1, but MD and RM were not related. Furthermore, we found no evidence that MD was related to t0.368 across species in contrast to other studies linking higher MD with reduced rates of decline in Wr in desiccating environments (Dussert *et al*. 1999, Makeen *et al*. 2004).

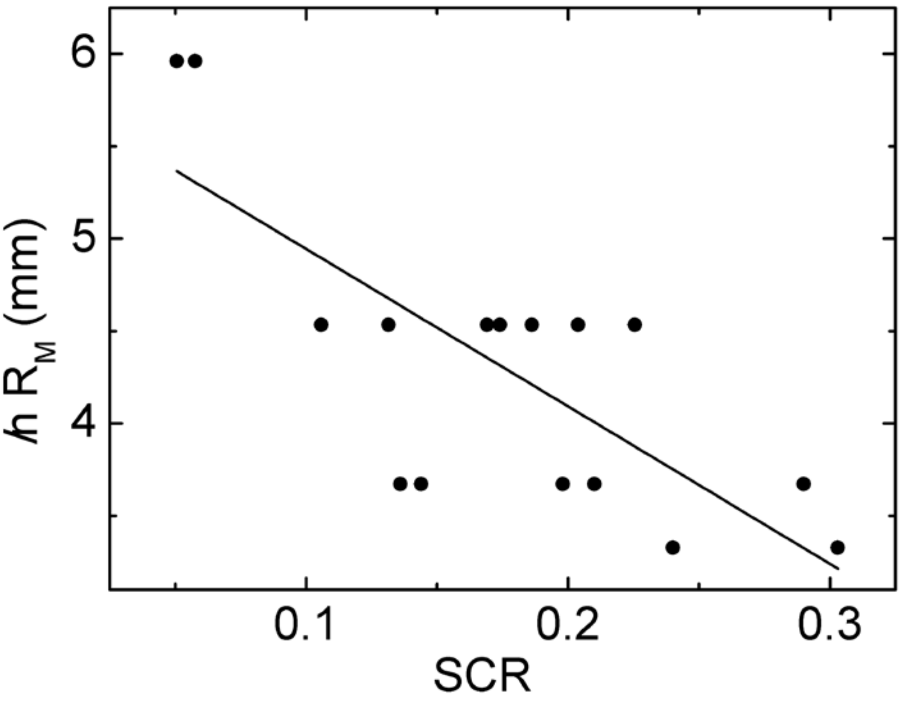


Figure 11 A significant negative relationship between log mean monthly rainfall at seed shedding, *l*n(R_M), and SCR. This was the only significant relationship found in bivariate

regressions between seed traits among 16 species producing desiccation sensitive seeds in north Queensland, Australia.

The study represents the first investigation to simultaneously examine variability in important attributes of desiccation sensitive seeds and show that a single solution for avoiding pre-germination mortality is unlikely. Previous studies have examined seed traits of desiccation sensitive seeds (Parmenter and Berjak 2000, Dickie and Pritchard 2002, Franks and Drake 2003, Pritchard *et al*. 2004a, Daws *et al.* 2005, 2006a, 2006b, Yu *et al*. 2008; Xiu *et al*. 2012). Other studies have assumed either: (i) size as a predictor of desiccation rate (Dussert *et al*. 1999, Makeen *et al*. 2004), or, in cases where multiple traits have been examined, (ii) size is the main predictor variable against which other traits have been assessed (Yu *et al*. 2008).

Table 11 Bivariate relationships between seed traits of sixteen tropical tree species that produce desiccation sensitive seeds within a tropical forest community, Cairns, Australia. The only significant relationship (SCR × R_M) is indicated in bold. R_M is mean monthly rainfall in the month of seed dispersal; W_{r50} (seed relative water content at which 50% of a cohort of seeds become unviable); T_G (time to germination in fully hydrated seeds); M_D (seed dry mass); $t_{0.368}$ (time constant for exponential decline of W_r under the desiccation treatment); SCR (ratio of seed coat and/or endocarp to embryo); and W_M (the specific relative water content as defined in eqn 1). Bonferroni corrected $P = 0.002$

Variables	R^2	$F_{1,15}$	P
$M_D \times W_{r50}$	0.06	0.03	0.84
$M_D \times T_G$	0.001	0.16	0.70
$M_D \times$ SCR	0.02	0.31	0.67
$M_D \times t_{0.368}$	0.01	0.71	0.68
$M_D \times W_M$	0.009	0.13	0.72
$M_D \times R_M$	0.03	0.52	0.48
$W_{r50} \times T_G$	0.07	1.18	0.29
$W_{r50} \times$ SCR	0.06	0.92	0.35
$W_{r50} \times t_{0.368}$	0.06	1.04	0.32
$W_{r50} \times W_M$	0.0001	0.0002	0.98
$W_{r50} \times R_M$	0.06	0.98	0.33
$T_G \times$ SCR	0.22	3.99	0.06
$T_G \times t_{0.368}$	0.22	3.98	0.06
$T_G \times W_M$	0.103	1.61	0.22
$T_G \times R_M$	0.21	3.71	0.07
SCR × $t_{0.368}$	0.15	2.45	0.14
SCR × W_M	0.15	2.51	0.13
SCR × R_M	**0.58 (neg)**	**19.0**	**0.0006**
$t_{0.368} \times W_M$	0.17	2.95	0.11
$t_{0.368} \times R_M$	0.19	3.40	0.08
$W_M \times R_M$	0.28	5.39	0.03

The dispersal of desiccation sensitive seeds within the drier months of the year is unusual. Annual patterns in seed dispersal within seasonal tropical forest tend to peak at the onset of the wet season (Garwood 1983). This enables a seed adequate time to germinate and become established prior to the onset of the dry season. One explanation for dry season dispersal may be to take advantage of years when rainfall is higher than average. Events such as La Niña are often associated with an increase in rainfall and an earlier onset of the ‘wet season’ (Wright and Calderon 2006). In these years, dry season dispersal would enable seeds to become well established before the onset of the next dry season perhaps conferring an advantage over individuals that are dispersed and germinate at a later date (i.e. wet season). Similarly, early establishment may be advantageous during La Niña events when the probability of a tree fall gap is potentially greater associated with either increased cyclone activity or heavy precipitation events.

Table 12. Principal component scores of seed traits and mean monthly rainfall of 16 tropical forest species. Variables were log10-transformed. The main structuring variables (PC scores ≥ |0.30|) determined after varimax raw rotation for each principal component are highlighted in bold; (%) represents the percentage of the total variance accounted for by each component

	PC1	PC2
Eigenvalue (%)	3.08 (44.1)	1.47 (21)
Eigenvector		
W_{r50}	-0.24	**0.42**
T_G	**0.42**	-0.27
R_M	**-0.42**	-0.10
SCR	**0.51**	-0.07
M_D	0.03	**-0.74**
$t_{0.368}$	**0.37**	0.14
W_M	**-0.44**	**-0.39**

In short, the findings demonstrate a principal axis of trait variability among desiccation sensitive seeds that is orthogonal to seed size. Seed size is recognised as one leading dimension defining plant strategies (Westoby 1998, Jakobson and Eriksson 2000, Wright *et al*. 2007, Laughlin *et al*. 2010), although under these schemes, post-germination success (seedling tolerance to environmental hazards) has been the primary measure against which seed size has been assessed (Muller-Landau 2010). Selection for pre-germination tolerance of mild desiccation may be equally as important as post-germination success in the adaptation of desiccation sensitive seeds to conditions of reduced or unpredictable rainfall. An ability to demonstrate “that two trait dimensions are orthogonal (not correlated) is at least as important … as demonstrating that they are correlated” (Wright *et al.* 2007, p. 1004) because orthogonal axes provide insight into potentially independent plant strategy dimensions (Wright *et al*. 2007). Size may confer advantages for desiccation sensitive seeds when compared against desiccation tolerant ones (Dickie and Pritchard 2002; Daws *et al.* 2005). However, our results suggest that within desiccation sensitive seeds, other traits (i.e. SCR, t0.368, TG and RM) might be most important for surviving pre-germination environmental conditions, independent of the advantages and/or disadvantages that size confers on seed survival and seedling establishment.

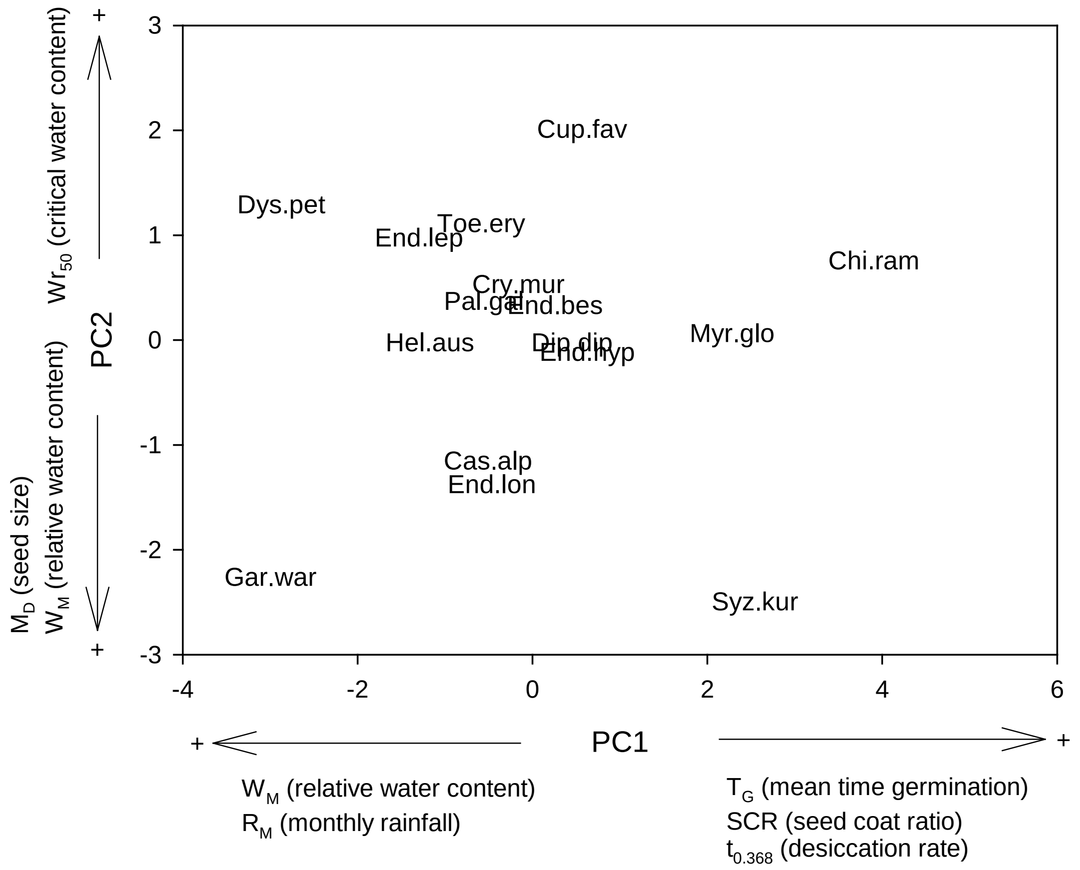


Figure 12. Plot of species in seed trait space defined by two principal components. Labels and arrows show the trait variables that exhibited eigenvector scores > |0.30|. The two significant (eigenvalues >1) principal components explain 65% of the overall variance in the species-trait correlation matrix. Species symbols are: Cas.alp, *Castanospora alphandii;* Chi.ram, *Chionanthus ramiflora;* Cry.mur *Cryptocarya murrayi;* Cup.fov, *Cupaniopsis foveolata;* Dip.dip, *Diploglottis diphyllostegia;* Dys.pet, *Dysoxylum pettigreweanum;* End.bes, *Endiandra bessaphila;* End.hyp, *Endiandra hypotephra*; End.lep, *Endiandra leptodendron*; End.lon, *Endiandra longipedicellata*; Gar.war, *Garcinia warrenii*; Hel.aus, *Helicia australasica*; Myr.glo, *Myristica globosa*; Pal.gal, *Palaquium galactoxylon*; Syz.kur, *Syzygium kuranda*; and Toe.ery, *Toechima erythrocarpum.*

Understanding trait variability in desiccation sensitive seeds and their possible role in the maintenance of pre-germination viability will be required if we wish to conserve many of the current populations of tropical plant species. In addition to the changes associated with climate, tropical rain forest are being altered by direct habitat loss and fragmentation. The replacement of once large areas of primary forest with secondary growth (Laurance 2004) and introducing edge-related effects that can cause changes in physical variables such as wind penetration and temperature may also cause reduction in rainfall at the local scale (Laurance 2004). Long-term plant survival appears more likely for species that can disperse widely and recruit in regrowth forests on abandoned lands (Chazdon *et al*. 2009). Recruitment in regrowth forests will strongly favour drought-resistant species that can withstand the hotter and drier conditions of this habitat (Chazdon 2008). If climate changes occur on the scale predicted, rainfall in tropical forests in Australia, and many other parts of the world, will become more seasonal and temperatures will increase (IPCC 2007, Dunlop and Brown 2008, Solomon *et al*. 2009). All of these factors increase the probability of desiccation and will pose higher pre-germination mortality risks for species that produce desiccation sensitive seeds. The analysis identifies a suite of physiological and morphological traits that, when viewed together, could be used to better assess the vulnerability of these species.

Chapter 6. Synthesis and implications of climate change on desiccation sensitive seeds

Synthesis

Changing climates will have a profound impact on the recruitment of plant species in rainforest plant communities (Colwell *et al*. 2008). Within the Wet Tropics in Australia, the impacts of rising temperatures are predicted to result in a reduction in the overall extent of the area covered by rainforest vegetation (Dunlop *et al*. 2012). Most of the interest and research on implications of changing climates for Australian tropical rainforests have focused on fire, and shifting range extents. For example, the anticipated decrease in rainforest area is most often proposed to occur in response to increased fire intensities and frequencies along rainforest edges enabling savanna woodland encroachment (Olivares *et al*. 2015). Plant species are predicted to migrate along elevational gradients in response to higher temperatures (Walther 2003, Jump and Peňuelas 2005, Berg *et al*. 2010). Little attention has focused on the other consequences of climate change, in particular, the anticipated increase in the duration and severity of the dry season and an increase in the severity of ENSO events (Steffen *et al*. 2013, IPCC AR5, 2014). The aim of this thesis was to focus on how changes of moisture availability associated with changing climates might affect an important plant demographic measure; recruitment. To do so, I investigated the possible effects of drying conditions over an annual and inter-annual timeframe. I did this by examining seed production and phenological patterns of seed release, as well as attributes allowing seeds to persist within desiccating environments in the Smithfield Conservation Park (SCP) Cairns, Queensland, Australia.

It is vitally important that we develop deeper understanding of how plant reproduction and recruitment are likely to respond to increased aridity. There are strong links between reproductive phenology and moisture availability in seasonal rainforests (Borchert 1994, 1996, Sakai *et al.* 2006, Brearley *et al.* 2007). On a global scale numerous reports document the timing of seed dispersal with the onset of the wet season (Lieberman 1982, White 1994, Murali 1997, Bollen and Donati 2005). I showed that this pattern in seed dispersal timing holds true for the rainforest community at SCP; where 28/49 species had mean dispersal dates centred in the five month wet season (Chapter 2). The most accepted explanation for wet season dispersal is that dispersal during periods of high available moisture ensures germination and seedling establishment prior to the next period of seasonal aridity (Garwood 1983). If, as predicted, the length of the dry season increases, some plant species with current dispersal times in the wet season may not have adequate time to establish sufficient root systems allowing them to avoid drought-induced mortality.

Plant composition in seasonal rainforests is strongly associated with a species ability to survive periods of seasonal drought (Engelbrecht *et al.* 2007, Curran *et al*, 2013, Paz *et al*, 2015). One trait often cited as an adaptation to seasonal drought is deep root systems (Poorter and Markesteijn 2008, Paz *et al*, 2015). Species that are able to develop deeper root systems have a distinct advantage over species that produce shallower roots post germination. Although seedling morphology and physiology were not investigated in this thesis it would be an ideal topic for further research. Species with wet season dispersal may have a range of seedling traits that enable them to persist through the dry season and it may be predicted that taxa that display rapid root growth (or higher proportional allocation to roots over shoots) will have a greater chance of survivorship when exposed to a lengthening dry season.

Inter-annual variability in seed rain also responds to moisture availability (Curran *et al.* 1999; Wright *et al.* 1999; Wright and Calderón 2006; Sakai 2006; Bearley *et al.* 2007). Whilst previous evidence has shown an increase in seed production in association with El Niño drought events (Ashton *et al.* 1988; Curran *et al.* 1999; Sakai 2002; Sakai *et al.* 2006; Bearley *et al.* 2007), my results demonstrate a decrease in seed production during an ENSO event. One plausible explanation why my findings are in contrast to other studies is that the 2002 drought event experienced in tropical Australia was an event of greater severity (Nicholls 2004) than had been included in any previous analysis. There is very little information on the impacts of severe drought in rainforest prior to the 1997/98 El Niño event. Drought events are not common in rainforests and it is difficult to gauge the severity of one event without evidence from past events. In this thesis I used 70 years of rainfall records to demonstrate the severity of drought. Indeed, 2002 was the driest year (both wet and dry season) ever recorded. My results are instructive, because under current climate change models the severity of El Niño events are expected to increase in all locations across the globe. Thus, the decrease in seed production I showed at SCP may be a reflection of how other rainforest communities will respond to more severe El Niño drought events in the future.

Dispersal during the wet season also favours the survival of desiccation sensitive seeds (Daws *et al.* 2005; Pritchard *et al.* 2004; Yu *et al.* 2008). Global estimates suggest that approximately 50% of all seed-bearing flora produce seeds that are desiccation sensitive (Vanquez-Yanes and Orozco-Segovia 1993, Tweddle *et al.* 2003, Hamilton *et al*. 2013). In Australia, however, only three species had been identified prior to beginning this thesis (pers. comm M Daws). I tested 32 plant species from SCP for seed desiccation sensitivity. Of these, 24 species from eight plant families were characterised as producing desiccation sensitive seeds (Chapter 3). The high proportion of species that produce desiccation sensitive seeds reflects that this regenerative strategy is more widespread within Australian rainforests than previously recorded. Indeed, these results were used by Hamilton *et al*. (2013) to estimate that 50% of all Australian rainforest species produce desiccation sensitive seeds.

The timing of seed dispersal of species producing desiccation sensitive seeds has been correlated with periods of high moisture availability (Pritchard *et al*. 2004; Daws 2005, Yu *et al*, 2008). Dispersal under these conditions sustain high seed moisture contents which are vital to retain viability (Pritchard *et al*. 2004). Surprisingly, when I investigated dispersal periods for desiccation sensitive species alone (Chapter 3), I found that only 8/24 species had mean dispersal dates centred within, or overlapping, the wet season. The remaining 16 species all had mean dispersal dates centred within the dry season. In the context of predictions associated with changing global climates, this result is potentially alarming in context of retention of viability in desiccation sensitive seeds. For example, predictions for tropical Australia suggest a contraction (and possible intensification) of the wet season, and, conversely, an increase in the length, the degree of aridity and the temperatures experienced during the dry season. Higher temperatures will undoubtedly increase the rate of desiccation, the effects of which may be compounded by lower dry season precipitation and a reduction in the duration of the wet season (BOM 2007, Dunlop and Brown 2008).

One explanation why seeds are dispersed during the dry season could be that during the La Nina cycle of the ENSO the dry season is shorter and less severe. Dispersal of seeds during these wetter months would give the seedling the greatest length of time to become established prior to the next dry season. Investigations examining if phenological patterns are correlated to La Nina events, or examining the relative rates of seedling establishment success would be of great benefit. With seasonal aridity predicted to become more severe, future La Nina events may be the only opportunity for successful germination and seedling establishment in some species. This type of evidence will require similar long-term data as I have collected and report on here, but over substantially longer periods of time. I strongly encourage this type of research project.

An additional and complimentary approaches to identifying and conserving potentially at-risk species will be based on the attributes they possess; ie. plant functional traits These functional traits include plant height, leaf longevity, seed size, photosynthesis per leaf dry mass, specific leaf area etc. (*see* Kattge *et al*. 2011 for a comprehensive list of functional traits). In my study I concentrated on seed attributes. Across studies, perhaps the most commonly recorded seed trait is seed size (e.g.

Osunkoya *et al*. 1994, Hammond and Brown 1995, Ter Steege and Hammond 2001, Moles *et al*. 2004, 2005, Willis and Hulme 2004, Pluess *et al*. 2005). Indeed, size itself is one of the main axes of the Leaf-Height-Size (LHS) plant strategy scheme proposed to capture a significant component of the variation on performance across species (Westoby 1998; Jakobson and Eriksson 2000; Wright *et al*, 2007; Laughlin *et al*, 2010).

With respect to seed size, plants that produce larger seeds are predicted to be more resilient to change and recover from perturbations compared with species that produce smaller seeds. Whilst the benefits of large seed size have been documented post germination, previously there has been no evidence that large seed size confers any advantage prior to germination in desiccation sensitive seeds (Foster 1986, Coomes and Grubb 2003, Moles *et al.* 2004, Moles and Westoby 2006). The results from Chapter 4 and 5 clearly show seed traits other than size are far more important for the survivorship of desiccation sensitive seeds. In fact, these results question the general applicability of current plant strategy schemes that do not consider attributes effecting pre-germination survival. This is especially true in tropical plant communities which are less sampled, and contain many more species that produce desiccation sensitive seeds, than temperate floras.

The PCA conducted in chapter 5 highlighted four seed traits other than seed size that were advantageous prior to germination in the dry season. The first PC was associated with strong negative loadings on average monthly rainfall and specific water content and strong positive loadings for seed coat ratio, mean time to germination and desiccation rate. This axis represents a spectrum in which species that disperse seeds during periods of low moisture availability (low mean RM values) have comparatively lower moisture contents, greater relative investment in seed coat, take longer to germinate and desiccate at slower rates than seeds from species at the opposite end of this axis.

One trait that was not investigated was the time taken for seeds to recover from desiccation. This, I consider, a very interesting possible avenue for future study. Partially dried seeds require moisture (imbibition) and time to repair cellular damage prior to germination (Farnsworth 2000, Berjak and Pammenter 2008). The rate of imbibition and cellular repair could provide insights into how species respond to unseasonal rain events. Furthermore, such investigations may show that larger seeds require a longer timeframe to imbibe hence delaying germination. Unfortunately, none of my experiments were designed to explicitly test this phenomenon, and thus this remains an area that requires further understanding and quantification.

Implications for the future

Plant species composition in the tropical forests will change as a direct result of changing climates (Colwell *et al*. 2008, Thuiller *et al*. 2008). The relative composition of forest communities with species that produce desiccation sensitive seeds can be expected to change over the long term. It is most likely that these will be replaced by a higher proportion of species present producing seeds that are orthodox (i.e. can withstand desiccating post-dispersal environments). This could have cascading effects through trophic food webs as most of the plant species identified in this thesis produce seeds that provide a nutritional reward for animal dispersers (i.e. either fleshy fruited drupes or produce capsules that contain arils attached to the seeds). Changes in number of seeds produced and or timing could: threaten frugivore populations that experience food scarcity in the dry season (Fredriksson *et al*, 2007, Catterall *et al*, 2012, Sanchez *et al*. 2012); alter effectiveness of the dispersal services of frugivores (Mokany et al., 2015); and disrupt life-cycles of many insect species that spend their entire larval stage within these fruits/seeds (Stewart *et al*. 2015), thus disrupting pollination services they provide (Tylianakis *et al*. 2008) These are just four of the many possible changes that alteration of forest composition may bring, and that require future investigation.

FINAL CONCLUSION

It is clear that climate change will have a profound influence on all aspects of the ecology of seasonal rainforests in Australia (and other parts of the world). Of concern is climate change research has primarily focused on seeds that are orthodox (Fenner and Thompson 2005, Duke *et al*. 2011) and how increased temperatures may affect dormancy breaking mechanisms (Daws *et al*. 2012). There are no current discussions addressing how species that produce desiccation sensitive will cope with changes in temperature or alterations in the distribution of annual and inter-annual rainfall patterns. Tropical forests are renowned for the diversity of plants that are unique to this habitat type. If species with desiccation sensitive seeds do account for 50% of total plant diversity (Tweddle *et al*. 2003) then there is an urgent need to firstly understand their ecology and physiology, and secondly understand how climate change will affect this important group of species. To date, only five studies (other than those published within this thesis) Yu *et al*. 2008, Daws *et al*. 2006, Daws *et al*. 2005, Pritchard *et al*. 2004, Dussert *et al*. 1999) have focused on community interactions of species (mainly phenology) that produce desiccation sensitive seeds. It is becoming evident that many species that produce desiccation sensitive seeds will require conservation efforts if they are to survive through changes predicted under climate change scenarios.

Assigning conservation priorities for species with desiccation-sensitive seeds is unlikely given the sheer number of species (Pritchard 2004). At present, desiccation sensitive seeds are unable to be stored at sub-zero temperatures without cellular damage (Prichard 2004, Umarani *et al*, 2015) limiting the use of ex-situ seed banks in their long-term conservation. Techniques to store the embryonic axes and meristems of desiccation-sensitive seeds at sub-zero temperatures have been achieved (Pence 1995, Berjak and Pammenter 2007, Englemann 2009, Berjak and Pammenter 2014), although currently they appear too costly and time consuming to be applied to a broad range of species. Hence, ex-situ conservation is unlikely to be the primary conservation method.

In situ conservation could be used to enable some species to occupy areas where moisture availability is high. Such areas include riparian zones where many species of conservation significance now reside. Yet, for the majority of areas containing seasonal forest, species that produce desiccation sensitive seeds and especially those that dispersal them at times coinciding with most arid conditions will potentially suffer population declines and a restriction of their current distribution (Thuiller *et al*. 2008).

In conclusion, climate change presents obvious challenges for biodiversity. I have shown how community-level seed production decreased during a period of severe drought and how predicted changes in seasonality may affect reproductive output and establishment in species that produce desiccation sensitive seeds. While the scientific aims of the thesis are identified in each chapter, the broader conservation aim of this thesis was to increase awareness of this large number of previously neglected group of plants amongst conservation practitioners. In the future it is hoped that the results will assist in the development of management plans that protect species that produce desiccation sensitive seeds. I hope this study will provide the catalyst for further investigations, especially within threatened ecosystems such as seasonal rainforest (Janzen 1988, Miles *et al*. 2006).

Reference

Achard F, Eva HD, Stibig HJ, Mayaux P, Gallego J, Richards T and Malingreau JP (2002). Determination of deforestation rates of the world's humid tropical forests. *Science* **297**, 999-1002.

Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, Dale M, Diemer M, Gugerli F, Henry GHR, Jones MH, Hollister RD, Jonsdottir IS, Laine K, Levesque KE, Marion GM, Molau U, Molgaard P, Nordenhall U, Raszhivin V, Robinson CH, Starr G, Stenstrom A, Stenstrom M, Totland O, Turner PL, Walker LJ, Webber PJ, Welker JM and Wookey PA (1999) Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecological Monographs* **69**, 491-511.

Armstrong DP and Westoby M (1993) Seedlings from large seeds tolerate defoliation better: A test using Phylogeneticaly Independent Contrasts. *Ecology* **74**, 1092-1100.

Augspurger CK (1981) Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators on *Hybanthus prunifolius* (Violaceae). *Ecology* **62**, 775-788.

Ashton PS, Givnish TJ, and Appanah S (1988) Staggered flowering in the Dipterocarpaceae: new insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *American Naturalist* **132**, 44-66.

Asner GP and Townsend AR (2000) Satellite observation of El Niño effects on Amazon forest phenology and productivity. *Geophysical Research Letters* **7**, 981-984.

Baskin J. M and Baskin C. C. (2004) A classification system for seed dormancy. *Seed Science Research*, **14**, 1-16.

Baskin CC and Baskin JM (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.

Beckage B, Osborne B, Gavin DG, Pucko C, Siccama T and Perkins T (2008) A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 4197-4202.

Bell DT (1999) The process of germination in Australian species. *The Australian Journal of Botany* **47**, 475-517

Berg MP, Kiers ET, Driessenen G, van dar Heijden M, Kooi B.W, Kuenen F, Liefting M, Verhoef HA and Ellers J (2010) Adapt or disperse: understanding species persistence in a changing world. *Global Change biology* **16**, 587-598.

Berjak P and Pammenter NW (2008) From Avicennia to Zizania: Seed Recalcitrance in Perspective. *Annals of Botany* **101**, 213–228.

Berjak P and Pammenter NW (2015) Cryostorage of germplasm of tropical recalcitrant-seeded species: Approaches and problems. *International Journal of Plant Sciences*, **175**, 29-39.

BOM, 2007. Climate change in Australia: technical report. CSIRO, Bureau of Meteorology, Australia.

Bollen A and Donati G (2005) Phenology of a littoral forest of Sainte Luce, southern Madagascar. *Biotropica* **37**, 32-43.

Borchet R (1994) Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology* **75**, 1437-1449.

Borchet R (1996) Phenology and flowering periodicity of neotropical dry forest species: Evidence from herbarium collections. *Journal of Tropical Ecology* **12**, 65-80.

Bostock PD and Holland AE (2010) Census of the Queensland Flora 2010. Queensland Herbarium, Department of Environment and Resource Management, Brisbane.

Brearly FQ, Proctor J, Suriantata , Nagy L, Dalrymple G and Voysey (2007) Reproductive phenology over a 10-year period in a lowland evergreen rainforest of central Borneo. *Journal of Ecology* **95**, 825-839.

Brooks TM, Mittermeier R, Mittermeier CG, da Fonseca GAB, Rylands AB, Konstant WR, Flick P, Pilgrim J, Oldfield S, Magin G and Hilton-Taylor C (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* **16**, 909-923

Cai W, Lengaigne M, Borlace S, Collins M, Cowan T, McPhadden MJ, Timmermann A, Power S, Brown J, Menkes C, Ngari A, Vincent EM and Widlansky MJ (2012) More extreme swings of the south Pacific convergence zone due to greenhouse warming. *Nature* **488**, 365-370.

Catterall CP, Freeman AND, Kanowski J and Freebody K (2012) Can active restoration of tropical rainforest rescue biodiversity? A case with bird community indicators. *Biological Conservation* **146**, 53-61.

Chazdon RL (2008) Beyond deforestation: restoring forests and ecosystem services on degraded lands. *Science* **320**, 1458-1417.

Chazdon R, Peres CA, Dent D, Sheil D, Lugo AE, Lamb D, Stork NE and Miller SE (2009) The potential for species conservation in tropical secondary forests. *Conservation Biology* **23**, 1406-1417.

Chin HF and Roberts EH (1980) *Recalcitrant Crop Seeds*. Kaula Lumpur, Malaysia: Tropical Press Syndicate.

Chuine I and Beaubien EG (2001) Phenology is a major determinant of tree species range. *Ecology Letters* **4**, 500-510.

Cochard R and Jackes BR (2005) Seed ecology of the invasive tropical tree *Parkinsonia aculeate*. *Plant Ecology* 180, 13-31.

Colwell RK, Brehm G, Cardelús CL, Gilman AC and Longino JT (2008) Global Warming, elevational range shifts, and lowland biotic Attrition in the Wet Tropics. *Science* **10**, 258-261.

Condit RS, Hubbell SP and Foster RB (1995) Mortality rates of 205 neotropical tree and shrub species and the impact of a severe drought. *Ecological Monographs* **65**, 419–439.

Condit RS, Aguiler A, Hernandez R, Perez S, Lao G, Angehr, Hubbell SP and Foster RB (2004) Tropical forest dynamics across a rainfall gradient and the impact of an El Niño dry season. *Journal of Tropical Ecology* **20**, 51–72.

Coomes DA and Grubb PJ (2003) Colonization, tolerance, competition and seed-size variation within functional groups. *Trends in Ecology and Evolution* **18**, 283 - 291.

Cooper W and Cooper WT (2004) Fruits of the Australian tropical rainforest. Nokomis Editions, Melbourne.

Corlett RT and La Frankie JV (1998) Potential impacts of climate change on tropical Asian forests through an influence on phenology. *Climatic Change* **39**, 439-453.

Corlett RT (2012) Climate change in the tropics: The end of the world as we know it? *Biological Conservation* **151**, 22-25.

Curran LM, Caniago I, Paoli GD, Astianti D, Kusneti M, Leighton M, Nirarita CE and Haeruman H (1999) Impact of El Niño and logging on canopy tree recruitment in Borneo. *Science* **286**, 2184–2188.

Curran LM and Leighton M (2000) Vertebrate responses to spatiotemporal variation in seed production of mast-fruiting Dipterocarpaceae. *Ecological Monographs* **70**, 101-128.

Curran TJ, Clarke PJ and Warwick NWM (2013) Drought survival of Australian rainforest seedlings is influenced by species evolutionary history and soil type. *Australian Journal of Botany* **61**, 22 -28.

Dalling JW, Swaine MD and Garwood NC (1998) Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forests. *Ecology* **79**, 564-578.

Danthu PA, Gueye A, Boye D, Bauwens D and Sarr A (2000) Seed storage of four Sahelian and Sudanian tree species (*Boscia seneglensis*, *Butryospermum parkii*, *Cordlya pinnata* and *Saba senagalensis*). *Seed Science Research* **10**, 183-187.

Daws MI, Garwood NC, Pritchard HW (2005) Traits of recalcitrant seeds in a semi-deciduous tropical forest in Panamá: some ecological implications. *Functional Ecology* **19**, 874–885

Daws MI, Garwood NC, Pritchard HW (2006$_a$) Prediction of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits and 104 species. *Annals of Botany* **97**, 667-674.

Daws MI, Cleland H, Chmielarz P, Gorian F, Leprince O, Mullins CE, Thanos CA, Vandvik V, Pritchard HW (2006$_b$) Variable desiccation tolerance in *Acer pseudoplatanus* seeds in relation to developmental conditions: a case of phenotypic recalcitrance? *Functional Plant Biology* **33**, 59-66.

Dickie JB and Pritchard HW (2002) Systematic and evolutionary aspects of desiccation tolerance in seeds. Desiccation and Survival in Plants: Drying Without Dying (eds. M Black and HW Pritchard) pp 239-259. CAB International, Wallingford, UK.

Dunlop M and Brown PR (2008) Implications of climate change for Australia's National Reserve System; a preliminary assessment. *Report to the Department of Climate Change, February 2008*. Department of Climate Change, Canberra, Australia.

Dussert S, Chabrillange N, Engelmann F, Hamon S (2000) Quantitative estimation of seed desiccation sensitivity using a quantal response model: application to nine species of the genus *Coffea* L. *Seed Science Research* **10**, 293–300.

Edwards W and Krockenberger AK (2006) Seedling mortality due to drought and fire associated with the 2002 El Niño event in a tropical rain forest in north-east Queensland, Australia. *Biotropica* **38**, 16-26.

Engelbrecht BMJ, Wright SJ and De Steven D (2002) Survival and ecophysiology of tree seedlings during El Niño in a tropical moist forest in Panama. *Journal of Tropical Ecology* **18**, 569-579.

Engelbrecht BMJ, Comita LS, Condit R, Kursar TA, Tyree MT, Turner BL, Hubbell SP (2007) Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* **447**, 80-83**.**

Etterson JR and Shaw RG (2001) Constraint to adaptive evolution in response to global warming. Science **294**, 151–154.

Etterson JR (2004) Evolutionary potential of *Chamaecrista fasciculate* in relation to climate change. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the Great Plains. *Evolution* **58**, 1459–1471.

Falster DS and Westoby M (2003) Plant height and evolutionary games. *Trends in Ecology and Evolution* **18**, 337-343.

Farnsworth E (2000) The ecology and physiology of viviparous and recalcitrant seeds. *Annual Review of Ecology and Systematics* **31**, 107-138.

Farrant JM, Berjak P, Pammenter NW (1985). The effect of drying rate on viability retention of recalcitrant propagules of *Avicennia marina*. *South African Journal of Botany* **51**, 432-438.

Farrant JM, Berjak P, Pammenter NW (1989) Germination-associated events and the desiccation sensitivity of recalcitrant seeds - a study on three unrelated species. *Planta* **178**, 189-198.

Frankie GW, Baker HG and Opler PA (1974) Comparative phenological studies in tropical wet and dry forests in the lowlands of Costa Rica. *Journal of Ecology* **62**, 881-919

Franks PJ and Drake PL (2003) Desiccation-induced loss of seed viability is associated with a 10-fold increase in CO_2 evolution in the seeds of the rare *Idiospermum australiense*. *New Phytologist* **159**, 253-261.

Foster SA (1986) On the adaptive value of large seeds for tropical moist forest trees. A review and synthesis. *The Botanical Review* **52**, 261 - 299.

Garwood NC (1983) Seed-germination in a seasonal tropical forest in Panama – A community study. *Ecological Monographs* **53**, 159-181.

Germaine HL and McPherson GR (1998) Effects of timing of precipitation and acorn harvest date on emergence of *Quercus emoryi*. *Journal of Vegetation Science* **9**, 157-160.

Grubb, P. J. and Metcalfe, D.J. (1996) Adaptation and inertia in the Australian tropical lowland rain-forest flora: Contradictory trends in intergeneric and intrageneric comparisons of seed size in relation to light demand. *Functional Ecology*, **10**, 512-520.

Hammond, D. S. and Brown, V.K. (1995) Seed size of woody-plants in relation to disturbance, dispersal, soil type in wet neotropical forests. *Ecology*, **76**, 2544-2561.

Hamilton KN, Offord CA, Cuneo P and Deseo MA (2013) A comparative study of seed morphology in relation to desiccation tolerance and other physiological responses in 71 Eastern Australian rainforest species. *Plant Species Biology* **28**, 51-62.

Hautier Y, Saner P, Philipson C, Bagchi R, Ong RC and Hector A (2010) Effects of seed predators of different body size on seed mortality in Bornean logged forest. *Plos One*, doi: 10.1371/journal.pone.0011651

Hilbert DW, Ostendorf B and Jenkins MS (2001) Sensitivity of tropical forests to climate change in the humid tropics of north Queensland. *Austral Ecology* **26**, 590–603.

Hoegh-Guldberg O, Hughes l, McIntyre S, Lindenmayer DB, Parmesan C, Possingham HP and Thomas CD (2008) Assisted colonization and rapid climate change. *Science* **321**, 345-346.

Hoerling MP and Kumar A (1997) Origins of the extreme climate states during the 1982-83 ENSO winter. *Journal of Climate*. **10**, 2859-2870.

Horne R and Kahn A (2000) Water loss and viability in (Poaceae) seeds during short-term desiccation. *American Journal of Botany* **87**, 1707-1711.

Huang RH, Zhang RH and Zhang QY (2000) The 1997/98 ENSO cycle and its impact on summer climate anomalies in East Asia. *Advances in Atmospheric Sciences* **17**, 348-362.

Hughes L (2011) Climate change and Australia: key vulnerable regions. *Regional Environmental Change*. **11**, S 189-195.

Hulme M and Viner D (1998) A climate change scenario for the tropics. *Climatic Change* **39**, 145-176.

Hyland BPM, Whiffin T, Christophel DB, Grey B and Elick R (2003) Australian tropical rain forest plants trees, shrubs and vines. CSIRO Publishing, Collingwood.

IPCC (2002) Climate Change and Biodiversity. Technical paper. (Intergovernmental Panel on Climate Change: Geneva, Switzerland.)

IPCC (2007) Climate change 2007: impacts, adaptation and vulnerability (Intergovernmental Panel on Climate Change: Brussels)

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change: Geneva, Switzerland

Jump AS and Penuelas J (2005) Running to stand hill: adaptation and the response of plants to rapid climate change. *Ecology Letters* **8**, 1010-1020.

Keeley JE and Fotheringham CJ (2000) Role of fire in regeneration from seed. In *Seeds: The Ecology of Regeneration in Plant Communities*, ed. M. Fenner, Wallingford:CABI Publishing, pp 311-330.

Keeley JE (1987) Role of fire in seed-germination of woody taxa in California chaparral. *Ecology* **68**, 434-443.

Kiladia GN and Diaz HF (1989) Global climatic anomalies associated with extremes in the southern oscillation. *Journal of Climate* **2**, 1069–1090.

Kisdi E and Geritz SAH (2003) On the coexistence of perennial plants by the competition-colonisation trade-off. *American Naturalist* **161**,350-354.

Körner C and Basler D (2010) Phenology under global warming. *Science* **327**, 1461-1462.

Kobayshi H, Matsunga HT and Hoyano A (2005) Net primary production in Southeast Asia following a large reduction in photosynthetically active radiation owing to smoke. *Geophysical Research Letters* **32**, 1029-1033

Kovach WL (2006) *ORIANA – Circular Statistics for Windows, ver. 2*. Kovach Computing Services, Pentraeth, Wales, UK.

Kozlowski TT and Pallardy C (2002) Acclimation and adaptive responses of woody plants to environmental stresses. *The Botanical review* **68**, 270-334.

Krockenberger AK, Edwards W and Kanowski J (2012) The limit to the distribution of a rainforest marsupial folivore is consistent with the thermal intolerance hypothesis. *Oecologia* **168**, 889-899.

Lan QY, Xia K, Wang XF, Liu JW, Zhao J and Tan YH (2014) Seed storage behaviour of 101 woody species from the tropical rainforest of southern China: a test of the seed-coat ratio-seed mass (SCR-SM) model for determination of desiccation sensitivity. *Australian Journal of Botany* **62**, 305-311.

Laughlin DC, Leppert JJ, Moore MM and Hull Sieg C (2010) A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. *Functional Ecology*, **24**, 493-501.

Laurance WF, Williamson GB, Delamonica P, Oliveira A, Lovejoy TE, Gascon C and Pohl L (2001) Effects of a strong drought on Amazonian forest fragments and edges. *Journal of Tropical Ecology* **17**, 771-785.

Laurance WF and Williamson GB (2001) Positive feedbacks among forest fragmentation, drought, and climate change in the Amazon. *Conservation Biology* **15**, 1529-1535.

Laurance, W.F. (2004) Forest-climate interactions in fragmented tropical landscapes. *Philosophical Transactions of the Royal Society of London B*, **359**, 345-352.

Laurance WF, Cochrane MA, Bergen S, Fearnside PM, Delamonica P, Barber C, D'Angelo S and Fernandes T (2001) Environment–The future of the Brazilian Amazon. *Science* **291**, 438-439.

Laurance WF, Nascimento HEM, Laurance SG, Condit R, D'Angelo S and Andrade A. (2004) Inferred longevity of Amazonian rainforest trees based on a long-term demographic study. *Forest Ecology and Management* **190**, 131-143.

Laurance WF and Wright SJ (2009) Special section: New insights into the tropical biodiversity crisis. *Conservation Biology* ***23***, 1382-1385.

Laurance WF, Dell B, Turton SM, Lawes MJ, Hutley LB, McCallum H, Dale P, Bird M, Hardy G, Prideaux G, Gawne B, McMahon CR, Yu R, Hero JM, Schwarzkopf L, Krokenberger A, Douglas M, Silvester E, Mahony M, Vella K, Saikia U, Wahren CH, Xu Z, Smith B and Cocklin C (2011) The 10 Australian ecosystems most vulnerable to tipping points. *Biological Conservation* **144**, 1472–1480.

Leigh Jr. EG (2004) How wet are the wet tropics? *In* EC Losos and EG Jr. Leigh (eds) Tropical Forest Diversity and Dynamism: Findings from a Large-scale Plot Network. pp. 43-55. The University of Chicago Press, Chicago, USA.

Leighton M and Wirawan N (1986) Catastrophic drought and fire in Borneo tropical rain forest associated with the 1982–83 El Niño Southern Oscillation event. *In* GT Prance (ed.) Tropical Rain Forest and the World Atmosphere, AAAS Selected Symposium 101.). pp. 75-102. American Association for the Advancement of Science, Washington DC.USA.

Leishman MR and Westoby M (1994a) The role of large seed size in shaded conditions: experimental evidence. *Functional Ecology* **8**, 205-214

Leishman MR and Westoby M (1994b) The role of seed size in seedling establishment in dry soil conditions-experimental evidence from semi-arid species. *Journal of Ecology* **82**, 249- 258.

Lenoir J, Gegout JC, Marquet OA, de Ruffray P and Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science* **320**, 1768-1771.

Leprince O, Butinik J, Hoekstra FA (1999) Axes and cotyledons of recalcitrant seeds of *Castanea sativa* Mill. Exhibit contrasting responses of respiration to drying in relation to desiccation sensitivity. *Journal of Experimental Botany* **50**, 1515-1524.

Liang Y and Sun WQ (2000) Desiccation tolerance of recalcitrant *Theobroma cacao* embryonic axes: the optimal drying rate and its physiological basis. *Journal of Experimental Botany* **51**, 1911-1919.

Lieberman D (1982) Seasonality and phenology in a dry tropical forest in Ghana. *Journal of Ecology* **70**, 791-806.

Ller AM and Inouye DW (2010) Effects of climate change on mast-flowering cues in a clonal montane herb, *Veratum tenuipetalum* (Melanthiaceae). *American Journal of Botany* **100**, 519-525.

Lloret F, Casanovas C and Penuelas J (1999). Seedling survival of Mediterranean shrubland species in relation to root: shoot ratio, seed size and water and nitrogen use. *Functional Ecology* **13**, 210-216.

Makeen AH, Normah MN, Dussert S and Clyde MM (2006) Seed moisture characteristics in relation to total lipid content of five *Citrus* taxa using an equilibrium dehydration protocol. *Seed Science and Technology* **34**, 453-464.

Malhi Y and Wright J (2004) Spatial patterns and recent trends in the climate of tropical rainforest regions. *Philosophical Transactions of the Royal Society London Series B* **359**, 311–329.

Marrinan MJ, Edwards W and Landsberg J (2005) Resprouting of saplings following a tropical rainforest fire in north-east Queensland, Australia. *Austral Ecology* **30**, 817-826.

Marod D, Kutintara U, Tanaka H and Nakashizuka T (2002) The effects of drought and fire on seed and seedling dynamics in a tropical seasonal forest in Thailand. *Plant Ecology* **161**, 410-457.

Martinez-Ramos M and Alvarez-Buylla E (1998) How old are tropical rain forest trees? *Trends in Plant Science* **3**, 400-405.

Metcalfe DJ and Grubb PG (1997) The responses to shade of seedlings of very small-seeded tree and shrub species from tropical rain forest in Singapore. *Functional Ecology* **112**, 215-221.

Mokany K, Prasad S and Westcott DA (2014) Loss of frugivore seed dispersal services under climate change. *Nature Communications* **5**, 3971-3971.

Mokany K, Prasad S and Westcott DA (2015) Impacts of climate change and management responses in tropical forests depend on complex frugivore-mediated seed dispersal. *Global Ecology and Biogeography* **24**, 685-694.

Moles AT, Warton DI, Stevens RD and Westoby M (2004) Does a latitudinal gradient in seedling survival favour larger seeds in the tropics? *Ecology Letters* **7**,911-914.

Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB and Westoby M (2005) A brief history of seed size. *Science* **307**, 576-580.

Moles AT and Westoby M (2006) Seed size and plant strategy across the whole life cycle. *Oikos* **113**, 91-105.

Muller-Landau HC (2010) The tolerance-fecundity trade-off and the maintenance of diversity of seed size. *Proceedings of the National Academy of Science* **107**, 4242-4247.

Murali KS (1997) Patterns of seed size, germination and seed viability of tropical tree species in southern India. *Biotropica* **29**, 271-279.

Nathan R and Muller-Landau HC (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* **15**, 278-285.

Newstrom LE, Frankie GW and Baker HG (1994) A new classification for plant phenology based on flowering patterns in lowland tropical rain forest trees at La Selva, Costa Rica. *Biotropica* **26**, 141-159.

Nicholls N (2004) The changing nature of Australian droughts. *Climatic Change* **63**, 323-336.

Niinemets, U. (2006) The controversy over traits conferring shade-tolerance in trees: ontogenetic changes revisited. *Journal of Ecology*, **94**, 464-470.

Nikolaeva MG (2004) On criteria to use in studies of seed evolution. *Seed Science Research* **14**, 315-320.

Normand RE, Ricklefs RE, Flemming SF, Bladt J, Tackenberg O and Svenning JC (2011) Postglacial migration supplements climate in determining plant species ranges in Europe. *Proceedings of the Royal Society London. B* **278**, 3644-3653.

Olivares I, Svenning JC, Van Bodegom BM and Balslev H (2015) Effects of warming and drought on the vegetation and plant diversity in the Amazon Basin. *The Botanical Review* **81**, 42-69.

Opler PA, Frankie GW Baker G (1980) Comparative phenological studies of treelet and shrub species in tropical wet and dry forests in the lowlands of Costa Rica. *Journal of Ecology* **68**, 167-188.

Osunkoya OO, Ash JE, Hopkins MS and Graham AW (1994) Influence of seed size and seedling ecological attributes on shade-tolerance of rain-forest tree species in northern Queensland. *Journal of Ecology* **82**, 149-163.

Paine CET and Beck H (2007) Seed predation by neotropical rain forest mammals increases diversity in seedling recruitment. *Ecology* **88**, 3076–3087.

Pammenter NW, Greggains V, Kioko JI, Wesley-Smith J, Berjak P and Finch-Savage WE (1998) Effects of differential drying rates on viability retention of recalcitrant seeds of *Ekebergia capensis*. *Seed Science Research* **8**, 463-471.

Pammenter NW and Berjak P (1999) A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* **9**, 13-37.

Pammenter NW and Berjak P (2000) Evolutionary and ecological aspects of recalcitrant seed biology. *Seed Science Research* **10**, 301-306.

Pan YD, Birdsey RA, Fang JY, Houghton R R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Candell JG, Ciais P, Jackson RB, Pacala SW, McGuire AD, Piao Sl, Rautianen A, Sitch S and Hayes D (2011) A large and persistent carbon sink in the world’s forests. *Science* **333**, 988-993.

Parmasen C and Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42.

Parmasen C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* **37**, 637–69.

Paz H, Pineda-Garcia F and Pinzon-Perez LF (2015) Root depth and morphology in response to soil drought: comparing ecological groups along the secondary succession in a tropical dry forest. *Oecologia* **179**, 551-561.

Pimm SL and Raven P (2000) Biodiversity – Extinction by numbers. *Nature* **403**, 843-845.

Pluess AR, Schütz W and Stöcklin J (2005) Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species. *Oecologia* **144**, 55–61.

Poorter L and Markesteijn L (2008) Seedling traits determine drought tolerance of tropical tree species. *Biotropica* **40**, 321-331.

Press WH, Flannery BP, Teukolsky SA, Vettering WT (1986) Numerical Recipes Cambridge: *Cambridge University Press*.

Pritchard HW, Daws MI, Fletcher BJ, Game'ne CS, Msanga HP and Omondi W (2004a) Ecological correlates of seed desiccation tolerance in tropical African dryland trees. *American Journal of Botany* **91**, 863-870.

Pritchard HW, Wood CB, Hodges S and Vaumer HJ (2004b) 100-seed test for desiccation tolerance and germination: A case study on 8 tropical palm species. *Seed Science and Technology* **32**, 393-403.

Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleskyn J, Westoby M and Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Science* **164** (Suppl 3), S143-S164.

Rivera G and Borchert R (2001) Induction of flowering in tropical trees by a 30-min reduction in photoperiod: evidence from field observations and herbarium specimens. *Tree Physiology* **21**, 201-212.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C and Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature* **421**, 57–59.

Saatchi SS, Harris NL, Brown S, Lefsky M, Mitchard ETA, Salas W, Zutta BR, Buermann W, Lewis SL, Hagen S, Petrova S, White L, Silman M and Morel A (2011) Benchmark map of forest carbon stocks in tropical regions across three continents. *Proceedings for the National Academy of Sciences of the United States of America* **108**, 9899-9904.

Sakai S (2002) General flowering in lowland mixed dipterocarp forests of South–east Asia. *Biological Journal of the Linnaean Society* **75**, 233–247.

Sakai S, Harrison RD, Mamose K, Kuraji K, Nagamasu H, Yasunari T, Chong L and Nakashizuka T (2006) Irregular droughts trigger mass flowering in aseasonal tropical forests in Asia. *American Journal of Botany* **93**, 1134-1139.

Saleska SR, Miller SD, Matross DM, Goulden ML, Wolfsy SC, Da Rocha HR, De Camargo PB, Crill P, Daube BC, De Freitas HC, Hutyra L, Keller M, Kirchhoff V, Menton M, Munger JW, Pyle EH, Rice AH and Silva H (2003) Carbon in Amazon forests. Unexpected seasonal fluxes and disturbance-induced losses. *Science* **302**, 1554-1557.

Saverimuttu T and Westoby M (1996) Seedling longevity under deep shade in relation to seed size. *Journal of Ecology* **84**,681-689.

Schupp EW, Jordano P and Gomez JM (2010) Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* **188**, 333-353.

Skov F and Svenning JC (2004). Potential impact of climatic change on the distribution of forest herbs in Europe. *Ecography* **27**, 366-380.

Slik JWF and Eichhorn (2003) Fire survival of lowland tropical rain forest trees in relation to stem diameter and topographic position. *Oecologia* **137**, 446-455.

Sodhi NS, Koh LP, Brook BW and Ng PKL (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology and Evolution* **19**, 654-660.

Sodhi NS, Posa MRC, Lee TM, Bickford D, Koh LP and Brook BW (2010) The state and conservation of Southeast Asian biodiversity. *Biodiversity and Conservation* **19**, 317-328.

Solomon S, Plattner GK, Knutti R and Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Science* **106**, 1704-1709.

Sokal RR and Rohlf FJ (1995) 1995 Biometry. W.H. Freeman, New York, USA.

Soons MB and Bullock JM (2008) Non-random seed abscission, long-distance dispersal and plant migration rates. *Journal of Ecology* **94**, 581-590.

Steffen W, Hughes L and Karoly D (2013) The Critical Decade: Extreme (Climate Commission). Commonwealth of Australia (Department of Industry, Innovation, Climate Change, Science,Research and Tertiary Education)

Stanton P and Stanton D (2003) *Various map sheet reports*. Unpublished reports to the Wet Tropics Management Authority, Cairns, Australia.

Stewart AJA, Bantock TM, Beckmann BC, Bjoern C, Botham MS, Hubble D, Roy DB and David B (2015) The role of ecological interactions in determining species ranges and range changes. *Biological Journal of the Linnaean Society* **115**, 647-663.

Stork NE, Balston J, Farquhar GD, Franks PJ, Holtum AM and Liddell MJ (2007) Tropical rainforest canopies and climate change. *Austral Ecology* **32**, 105–112.

Stork NE (2010) Re-assessing current extinction rates. *Biodiversity and Conservation* **19**, 357-371.

Sun WQ (1999) Desiccation sensitivity of sixty-four tropical, subtropical and temperate recalcitrant seeds. *Asian Journal of Tropical Biology* **3**, 9-13.

Sun WQ and Liang Y (2001) Discrete levels of desiccation sensitivity in various seeds as determined by the equilibrium dehydration method. *Seed Science Research* **11**, 317–323.

Systat Software. 2006. *Sigma Plot® 10.0 User’s Guide*. Systat Software Inc. Ca USA.

Thuiller W, Albert C, Araujo MB, Berry PM, Cabeza M, Guisan A, Hickler T, Midgely GF, Paterson J, Schurr FM, Sykes MT and Zimmermann NE 2008. Predicting global change impacts on plant species' distributions: Future challenges. *Perspectives in Plant Ecology, Evolution and Systematics* **9**, 137-152.

Ter Steege H and Hammond DS (2001) Character convergence, diversity, and disturbance in tropical rain forest in Guyana. *Ecology* **82**, 3197-3212.

Trenberth K.E (2011) Changes in precipitation with climate change. *Climate Research* **47**, 123-138.

Tweddle JC, Dickie JB, Baskin CC and Baskin JM (2003) Ecological aspects of seed desiccation sensitivity. *Journal of Ecology* **91**, 294-304.

Tylianakis JM, Didham RK, Bascompte J and Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters* **11**, 1351-1363.

Umarani R, Aadhavan EK and Faisal MM (2015) Understanding poor storage potential of recalcitrant seeds. *Current Science* **108**, 2023-2034.

Van Shaik CP, Terbrough JW and Wright SJ (1993) The phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annual Review of Ecology and Systematics* **24**, 353-377.

Vazquez-Yanes C, Orozco-Segovia A (1993) Patterns of seed longevity and germination in the tropical rainforest. *Annual Review of Ecology and Evolution* **24**, 69-87.

Venable LD and Lawlor L (1980) Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* **46**, 272-282.

Villafuerte MQ and Matsumoto J (2015) Significant Influences of Global Mean Temperature and ENSO on Extreme Rainfall in Southeast Asia. *Journal of Climate* **28**, 1905-1919.

Wang J, Gao P, Kang M, Lowe AJ and Huang H (2009) Refugia within refugia: the case study of a canopy tree (*Eurycorymbus cavaleriei*) in subtropical China. *Journal of Biogeography* **36**, 2156–2164.

Walck JL, Hidayati S, Dixon KW, Thompsons K and Poschold P (2011) Climate change and plant regeneration from seed. *Global Change Biology* **17**, 2145-2161.

Walther G (2003) Plants in a warmer world. *Perspectives in Plant Ecology, Evolution and Systematics* **6**, 169–185.

Walsh RPD (1996) The Environment. *In* P. W. Richards (ed). The Tropical Rainforest: An Ecological Study. pp. 159-285. Press Sydicate. University of Cambridge. Cambridge. UK.

Westoby M, Jurado E and Leishman M (1992) Comparative evolutionary ecology of seed size. *Trends in Ecology and Evolution* **11**, 368-372.

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* **199**, 213-227.

White LJT (1994) Patterns of fruit-fall phenology in the Lopé Reserve, Gabon. *Journal of Tropical Ecology* **10**, 289-312.

Williams RJ, Myers BA, Muller WJ, Duff GA and Eamus D (1997) Leaf phenology of woody species in a north Australian tropical savannah. *Ecology* **78**, 2542-2558.

Williams SE, Bolitho EE and Fox S (2003) Climate change in Australian tropical rainforests: an impending environmental catastrophe. *Proceedings of the Royal Society of London. B* **270**, 1887–1892.

Willis SG and Hulme PE (2004) Environmental severity and variation in the reproductive traits of *Impatiens glandulifera*. *Functional Ecology* **18**, 887-898.

Wong ST, Servheen C, Ambu L and Norhayati A (2005) Impacts of fruit production cycles on Malaysian sun bears and bearded pigs in lowland tropical forest of Sabah, Malaysian Borneo. *Journal of Tropical Ecology* **21**, 627-639.

Wright IJ, Ackerly DD, Bongers F, Harms KE, Ibarra-Manriquez G, Martinez-Ramos M, Mazer SJ, Muller-Landau HC, Paz H, Pitman NCA, Poorter L, Silman MR, Vriesendorp CF, Webb CO, Westoby M and Wright SJ (2007) Relationships among ecologically important dimensions of plant trait variation in seven Neotropical forests. *Annals of Botany* **99**, 1003-1015.

Wright SJ and Van Schaik CP (1994) Light and the phenology of tropical trees. *American Naturalist* **143**, 192–199.

Wright SJ, Carrasco CO, Calderón O Paton S (1999) The El Niño southern oscillation, variable fruit production and famine in a tropical forest. *Ecology* **80**, 1632–1647.

Wright SJ and Calderón O (1995) Phylogenetic patterns among tropical flowering phenologies. *Journal of Ecology* **83**, 937–948.

Wright SJ and Calderón O (2006) Seasonal El Niño and longer term changes in flower and seed production in a moist tropical forest. *Ecology Letters* **9**, 35-44.

Xia K, Daws MI, Stuppy W, Zhou ZK, Pritchard HW (2012) Rates of water loss and uptake in recalcitrant fruits of Quercus species are determined by pericarp anatomy. *PLoS ONE 7*, e47368.

Yu HY, Luedeling E and Xu JC (2010) Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 22151-22156.

Yu Y, Baskin JM, Baskin CC, Tang Y and Cao M (2008) Ecology of seed germination of eight non-pioneer tree species from a tropical seasonal forest in southwest China. *Plant Ecology* **197**, 1-16.

Zimmerman JK, Wright SJ, Calderon O, Pagan MA and Paton S (2007) Flowering and fruiting phenologies of seasonal and aseasonal neotropical forests: the role of annual changes in irradiance. *Journal of Tropical Ecology* **23**, 231-251.