Assessing temperature thresholds for germination

Project 04SC1-13h



Banksia caleyi inflorescence

Final Report

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Project Summary



Seed germination is a plant trait that can determine colonisation ability for species that rely on seeds for post-disturbance regeneration. It is a high risk phase in a plant's life cycle and vulnerable to shifts in environmental parameters. Abrupt changes in temperature associated with a changing climate may limit recruitment of obligate seeding species and render them vulnerable to decline and extinction.

Using a Grant GRD1 Temperature Gradient Plate purchased for the Project by the South Coast Natural Resource Management Inc. through Australian Government funding we assessed temperature constraints on germination for 22 endemic and mainly obligate seeding *Banksia* species (Proteaceae) predominantly restricted to the South Coast Region. Forty-nine temperature combinations between 5 and 40 °C allowed us to accurately profile temperature requirements for successful germination. Contour plots were created that highlight species response to optimal and sub-optimal germination temperatures for germination. Mean time to germination was calculated for all temperature combinations where germination was recorded.

Our results indicate that many *Banksia* species confined to the southern coastal areas of Western Australia have a wide physiological tolerance for high fluctuating temperatures during germination, although average temperatures for optimum germination (mean of high and low temperature fluctuations) for all species fall between 10 and 19 °C. A number of common, but geographically restricted species, such as *B. praemorsa, B. oreophila, B. dryandroides* and *B. quercifolia*, have more narrow temperature windows for germination than at least one of the rarer species profiled (eg. *B. verticillata*). The conservation-listed *B. solandri* also demonstrated a narrow range of temperatures over which germination occurred. Mean time to maximum germination (100% for all species except *B. quercifolia* and *B. brownii*) ranged from 8 days for *B. leptophylla* to 23 days for *B. quercifolia*.

This seed-based approach to determining critical physiological thresholds can be used as a climate-change vulnerability predictor that will help detect potentially at risk species and improve management efficiency for prioritising conservation actions. Ideally climate data sampled from each collection site would enhance the overall outcomes of this project and field investigations would confirm timing and success of germination events.

Background

Human-induced climate warming is a major immediate threat to biodiversity. Climate affects the life cycle of plants and drives the distribution of plant communities. This occurs in conjunction with edaphic, topographic and biotic factors that are often correlated with climatic variables. Plant traits, such as the timing of biological activities and processes, are also affected by climate. Germination is a plant trait that can determine colonization ability for species that rely on seeds for post-disturbance regeneration (obligate seeders). After moisture, temperature is arguably one of the most important climatic variables for seeds since it synchronises germination to environmental conditions most suitable for seedling establishment, and for many species temperature-mediated germination cues are important to ensure species persistence in the wild. Climate also interacts with other stresses such as fire, changed hydrology and disease that currently impact on the survival of endemic and geographically restricted plant species in the South West of Western Australia.

Predicting how species will respond to a warming climate from plant functional traits such as germination can help identify species vulnerable to extinction. Plants with limited dispersal ability and slow reproductive rate are likely to be threatened by these changes. Some restricted species may even disappear under even small global temperature increases, and species already restricted to the physical limits of their range, like higher latitudes and altitudes are presumed with some certainty to be at risk of extinction.

In order to predict which obligate seeding species will be negatively affected, and which will benefit, requires the identification of temperatures under which germination might become limited. Mean temperature shifts may have marginal impact but the extreme shifts may magnify effects. If species cannot tolerate temperatures warmer than they currently experience in their temperature envelope, then they may be at risk.

The Grant GRD1 Temperature Gradient Plate is a bi-directional temperature gradient system used to investigate the responses of small organisms, like seeds, to temperature. This equipment was purchased by the South Coast NRM Inc. through Australian Government funding specifically for the Department of Environment and Conservation to investigate the responses of native seeds to minimum, maximum and mean constant and fluctuating temperatures.

This one-year project aimed to investigate the vulnerability of seeds of a range of predominantly obligate-seeding species to changes in the simple environmental parameter of temperature for germination, providing a medium to long term biodiversity outcome for the immediate investment of the GRD1. For management purposes, understanding whether plant species can tolerate elevated temperature conditions during recruitment will assist in planning for recovery and restoration of critical plant species and their habitats.

Species selection

Sensitivity to environmental change depends on species genetic and phenotypic plasticity, life history characteristics and ecological niche and geographic distribution. Obligate seeding species with specific niche requirements, limited dispersal ability and already at the limits of their geographic range may be highly sensitive to changes in environments. *Banksia* (family Proteaceae) is one of the most characteristic genera in South West Western Australia. It is represented by 157 species, almost all endemic to the region. *Banksia* are considered to be confined to the South West by climate, and are naturally restricted in WA to areas with >250mm rainfall isohyet. Many species are listed as conservation-dependant.



Banksia are long-lived woody perennials with slow reproductive rates and low dispersal ability, and they are often sensitive to abiotic factors in the landscape (e.g. temperature and fire). Species in the genus considered to be most vulnerable to stresses (e.g. climate change) are the non-lignotuberous species that rely on seeds for recruitment. In the fire-prone vegetation communities of the South West 60% of *Banksia* species (*sensu stricto*) are obligate seeders. In many of these communities *Banksia* are keystone species. They provide structural support and habitat for numerous other organisms and are an important food source for both vertebrate and invertebrate fauna. Any alterations to the health and viability of *Banksia* populations may directly impact on ecosystem function. For these reasons, members of the genus *Banksia* were chosen for this Project.



Project Officer Susanne Schreck collecting fruiting cones of Banksia solandri, Stirling Range National Park

Key Investment areas addressed by this Project

Employment and investigation

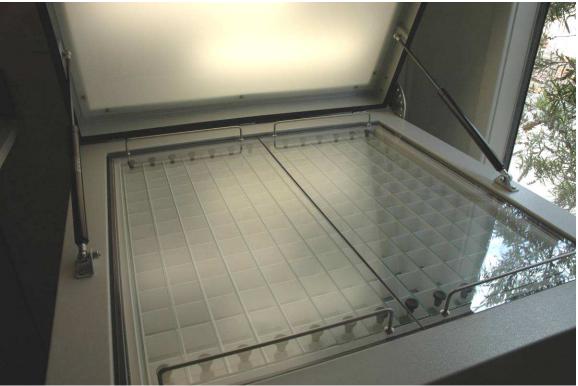
The Project employed a 0.2 FTE to conduct seed investigations using the GRD1 in addition to in-kind support from the DEC Threatened Flora Seed Centre. Fruiting cones of 22 endemic *Banksia* species were collected from the wild between July 2008 and June 2009 (Appendix 1), predominantly from species endemic to the South Coast Region (Appendix 2). With the exception of *B. grandis*, all species are fire-intolerant, do not contain a lignotuber and hence regenerate only from seeds. Cones were sampled from a minimum of 20 individuals within each *Banksia* population. Seeds were extracted by subjecting cones to a gas flame from a blow torch until the follicles cracked open. Cones were then soaked in water for 6 hours before being placed to dry in a warm location. The time taken for seeds to extract from fruiting cones varied between species, ranging from immediately after burning to the requirement for a week of drying. Seed samples from each collection were weighed to the nearest 1 µg after reaching equilibrium with ambient laboratory conditions (ca. 20°C).



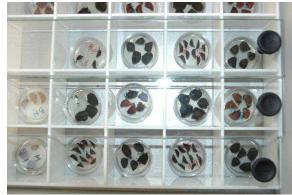
Project Officer Susanne Schreck conducting seed investigations on the GRD1 temperature gradient plate

Seeds were sown on the surface of 1 % w/v water agar on 35 mm Petri dishes and placed in individual temperature cells on the GRD1. In total the GRD1 can provide 169 different temperature combinations. In each experiment four species were tested therefore providing each species with 49 diurnally fluctuating temperatures ranging from approximately 5 to 40°C. For each temperature either 5 or 10 seeds were used, depending

on seed size. Actual temperatures experienced by seeds were determined by five thermistors on the temperature gradient plate connected to a data logger (Grant Instruments Squirrel 2020 Series). All environments received irradiance for 12 hd⁻¹ (fluorescent white light ~50 μ mol m ⁻² s ⁻¹). Germination was scored every two days and defined as visible radicle emergence. Germination tests ran for up to 45 days at which time remaining seeds were subjected to a cut-test to determine viability. Seeds with hard, white endosperm and embryo were scored as potentially viable.



Temperature cells on the GRD1 temperature gradient plate



Petri dishes within individual GRD1 temperature cells

Each individual seed was considered a statistically independent unit (ie each seed could either live or die). Sigma Plot for Windows Version 10 (2006 Systat Software Inc©) was used to create contour plots from germination data. The mean and magnitude of the temperature fluctuations under which seeds germinated was generated to enable comparison between temperature cells. Mean time to germinate (MTG) was calculated for all temperature combinations where germination occurred using the equation: $MTG = \sum (n \times d) / N$ where: n = number of seeds germinated between scoring intervals; d = the incubation period in days at that time point and N = total number of seeds germinated.

Plant species response

Individual seed weights (average of 20 seeds) for the 22 species ranged from a mean of 10.2mg for the smallest seeds (*B. pulchella*) to 146.5mg for *B. caleyi* (Appendix 1). Seed weights for *B. baueri* varied with collection location, with seeds from the Stirling Range weighing almost twice that of seeds from the wheatbelt (142mg vs 78mg respectively).

Contour plots highlight the response of each species to optimal and sub-optimal germination temperatures (Appendix 3). Points of equal percentage germination are connected by germination isopleths. Constant temperatures are presented on the diagonal gradient from the bottom left hand corner of the diagram (lowest temperature \pm 5°C) to the maximum constant temperature of \pm 40°C at the top right hand corner of the diagram. All point above and below the diagonal line represent a range of fluctuating temperatures with amplitudes from 2°C to 35°C.



The majority of seedlings produced during this project were used in research into species susceptibility to the pathogen *Phytophthora cinnamomi*. Seedlings of the rare *Banksia brownii* will be reintroduced into the wild as part of the DEC's ongoing recovery process for threatened species in Western Australia.

When the mean of each temperature fluctuation was calculated, maximum per cent germination occurred for all species between 10.4 and 19.1°C (Appendix 4), with an average of 15°C over all species. B. dryandroides displayed the lowest optimum mean temperature for germination at 10.4°C and *B. media* the highest at 19.1°C. Germination was high for a number of species when the magnitude of the temperature fluctuation was large, provided the minimum temperature was less than 15°C. In many cases of high germination with large fluctuations, mean time to germination was also high, suggesting sub-optimal conditions. Of the 22 species investigated B. leptophylla appeared to have the largest tolerance for high temperature fluctuations, with *B. speciosa* and *B. media* also displaying good tolerance. Each of these three species had their optimum mean temperature above 16°C. Banksia *leptophylla* is one of the two species investigated that occurs north of Perth, therefore experiencing higher temperatures in its natural habitat than species from the South Coast Region. The large difference in seed weights between the B. baueri collections did not translate into any significant differences in germination response from the collections made from across the species' geographic range. The inland collections from Tarin Rock and the Stirling Range displayed a marginally higher, but not significant, optimum mean temperature for germination than seeds from East Mt Barren on the coast. Banksia solandri, B. praemorsa, B. oreophila, B. dryandroides and B. quercifolia had narrower temperature windows for germination than at least one of the rarer species profiled (eg. B. verticillata), with B. solandri perhaps the most temperature limited. Mean temperatures for germination derived from all temperature combinations are presented graphically for each species (Appendix 5).

Mean time to germination when per cent germination was maximum (100% for all species with the exception of *B. quercifolia* and *B. brownii*) ranged from 8 days for *B. leptophylla* to 23 days for *B. quercifolia* (Appendix 4). Averaged over all species, mean time to maximum germination was 16 days. Germination in the quickest possible time is often considered a good strategy to reduce competition after fire. Slow germination may indicate sub-optimal conditions and may result in seedlings being out-competed for moisture, nutrients and light.

Project outcomes

In summary, a number of *Banksia* species confined to the South Coast Region demonstrated a high degree of plasticity in germination response to temperatures, suggesting that portions of the seed population for these species may already be 'pre-adapted' to higher germination temperatures, providing sufficient resilience to tolerate a warming climate. It also possibly suggests that the temperature tolerances of these species are wider than the climatic envelopes they currently occupy. Alternatively, germination of these species might not necessarily be restricted to the cooler months of the year when rainfall is guaranteed. It may be that sufficient moisture from later rainfall events and cloud cover, particularly in the South Coast Region, may assist survival of later germinating seed, providing sufficient moisture for evading summer drought. Unfortunately, a warming, drying climate may alter existing weather patterns and effect a change in germination timing. From this investigation, a number of *Banksia* species have been identified as potentially suitable candidates for monitoring responses to future climate change due to their narrow temperature thresholds for germination. Banksia solandri, already restricted in its geographic distribution to the Stirling Range, appears to be quite temperature limited for germination and will require closer examination. This research has also recognized a number of common species that may warrant further investigation including Banksia praemorsa, B. dryandroides, B. quercifolia and B. oreophila. None of these species are currently listed as threatened, though their geographic distribution is limited (in particular B. praemorsa), and like most Banksia species, they are susceptible to Phytophthora cinnamomi and aerial cankers and the effects of too frequent fire. Long-term on ground ecological monitoring of populations of these species may be warranted in the future. If temperatures rise substantially then some species with narrow temperature windows for germination like these may suffer declines. These species may persist under sub-optimal climatic conditions, although high mortality of seedlings post-fire, in conjunction with pressure from disease, may gradually erode population size. Climate data from each collecting sites would assist with interpretation of the data, as would field investigations into the timing of natural seedling recruitment for each species.

Although laboratory experiments may be imperfect in their ability to simulate the many forces that act on seeds during germination they are able to provide a controlled, mechanistic approach to forecasting species response to a single driver of climate change such as temperature. Such data can inform management decisions when planning for conservation to ensure that climate-driven extinction of vulnerable species does not occur. Where basic understanding of factors limiting species persistence is not known then knowledge of tipping points or thresholds can offer assistance. Germination is just one plant trait that may provide a key to vulnerability.



Acknowledgements

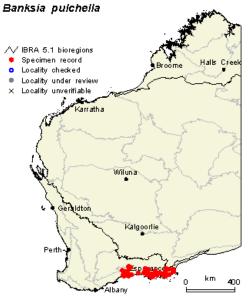
This research was supported by Australian Government Natural Heritage Trust funding secured from the Australian Government through the South Coast Natural Resource Management Inc (Project 04SC1-13h).

The Department of Environment and Conservation manages lands and waters in Western Australia for the conservation of biodiversity at ecosystem, species and genetic levels, including management for the renewable resources they provide, and for the recreation and visitor services they can sustainably support.

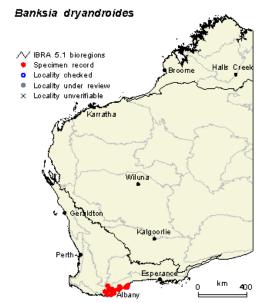
Appendix 1. List of endemic *Banksia* species investigated including collection location, life history and seed weight (average of 20 seeds).

Species	Collection location	Method of persistence	Life form	Flowering period	Seed weight (mg)
Banksia aculeata	Stirling Range	Non-sprouter	shrub	summer	111.1 ± 3.0
Banksia baxteri	Stirling Range	Non-sprouter	shrub	summer	69.3 ± 3.2
Banksia baueri	Stirling Range	Non-sprouter	shrub	winter	142.1 ± 4.0
Banksia baueri	East Mt Barren	Non-sprouter	shrub	winter	111.6 ± 5.0
Banksia baueri	Tarin Rock	Non-sprouter	shrub	winter	78.4 ± 3.0
Banksia benthamiana	Dalwallinu	Non-sprouter	shrub	summer	17.5 ± 0.7
Banksia blechnifolia	NW Ravensthorpe	Non-sprouter	prostrate	spring	62.9 ± 1.7
Banksia brownii	Albany	Non-sprouter	tree	winter	29.6 ± 0.9
Banksia caleyi	Stirling Range	Non-sprouter	shrub	spring	146.5 ± 7.0
Banksia dryandroides	Wellstead	Non-sprouter	shrub	spring/summer	14.7 ± 0.5
Banksia grandis	Albany	Sprouter	tree	spring/summer	61.4 ± 2.3
Banksia laevigata	Ravensthorpe	Non-sprouter	shrub	spring	28.3 ± 9.0
Banksia lemanniana	Ravensthorpe	Non-sprouter	shrub	spring/summer	74.0 ± 3.5
Banksia leptophylla	Greenhead	Non-sprouter	shrub	summer/autumn/winter	44.8 ± 0.4
Banksia media	Esperance	Non-sprouter	shrub	autumn/winter	38.5 ± 1.9
Banksia nutans	Esperance	Non-sprouter	shrub	summer	33.8 ± 1.1
Banksia oreophila	Stirling Range	Non-sprouter	shrub	winter	19.1 ± 0.8
Banksia petiolaris	Jerdacuttup	Non-sprouter	prostrate	spring	69.1 ± 2.6
Banksia praemorsa	Albany	Non-sprouter	shrub	spring	36.9 ± 1.4
Banksia pulchella	Esperance	Non-sprouter	shrub	all year	10.2 ± 0.4
Banksia quercifolia	Denmark	Non-sprouter	shrub	autumn/winter	15.3 ± 0.5
Banksia solandri	Stirling Range	Non-sprouter	shrub	spring	48.4 ± 1.9
Banksia speciosa	Esperance	Non-sprouter	shrub/tree	all year	110.1 ± 4.3
Banksia verticillata	Albany	Non-sprouter	shrub	summer/autumn	17.5 ± 0.6

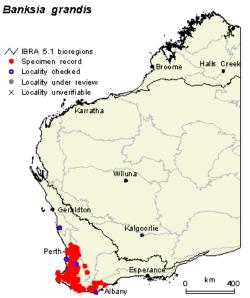
Appendix 2. Geographic distribution of *Banksia* species investigated. Mapping by Paul Gioia. Images used with the permission of the Western Australian Herbarium, Department of Environment and Conservation (<u>http://florabase.dec.wa.gov.au/help/copyright</u>). Accessed on Tuesday, 23 June 2009.



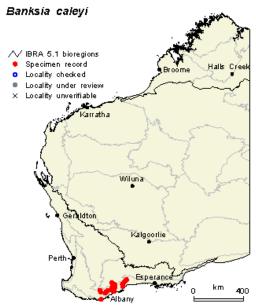




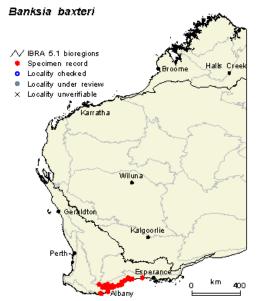
Map by Paul Gioia, WA Herbarium. Current at February 26, 2009



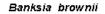
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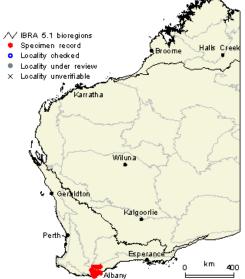


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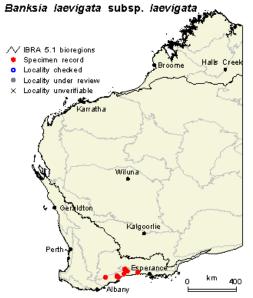


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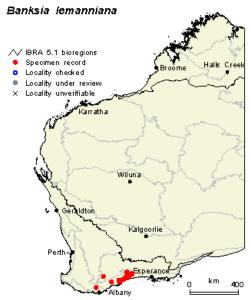




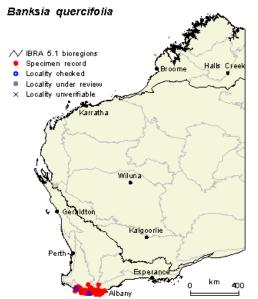
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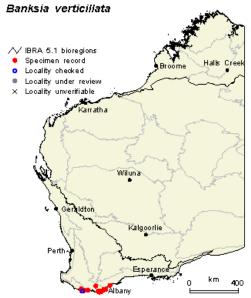
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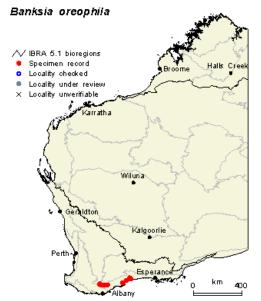
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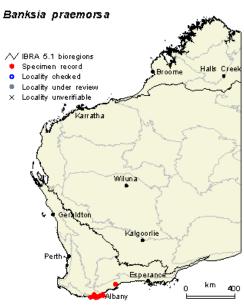
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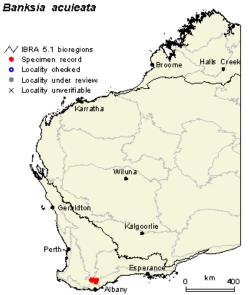
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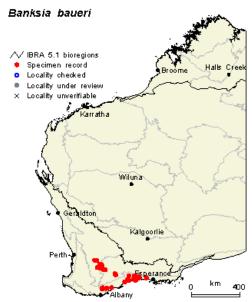
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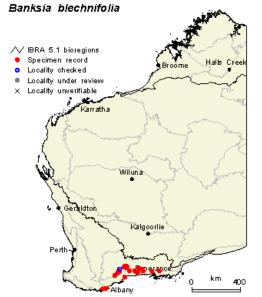
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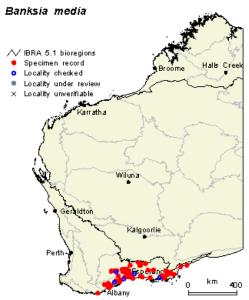
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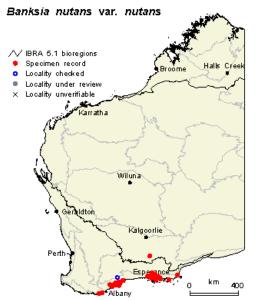
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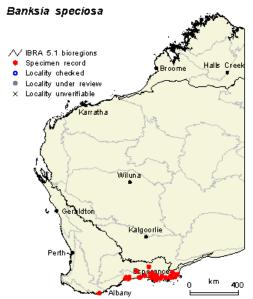
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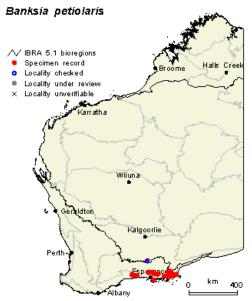
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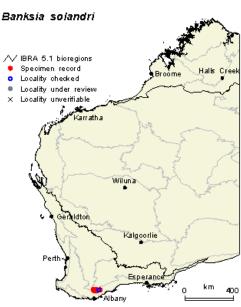
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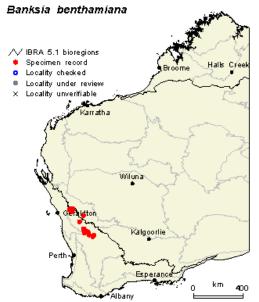
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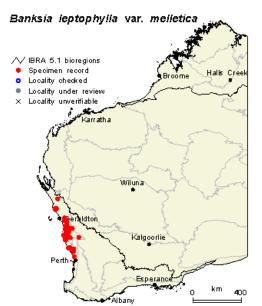
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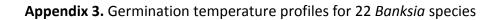


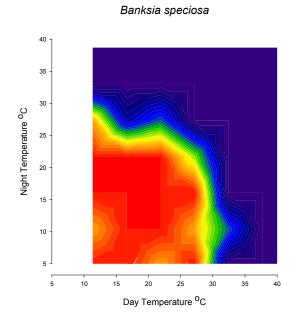


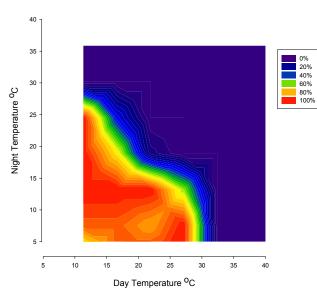
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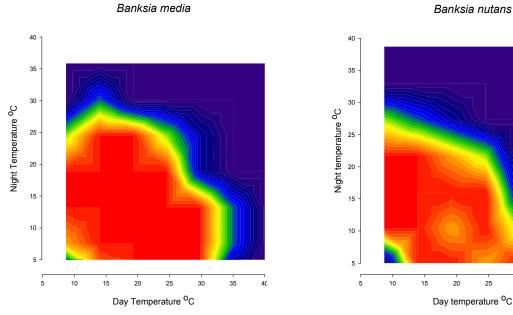
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Banksia nutans

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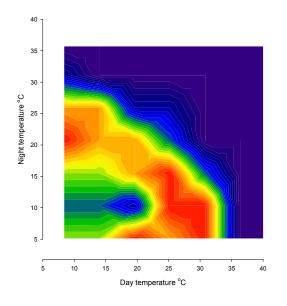
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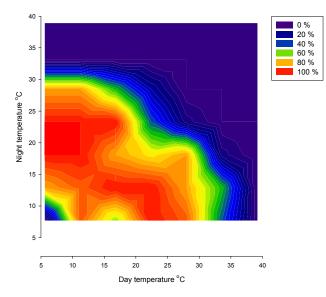
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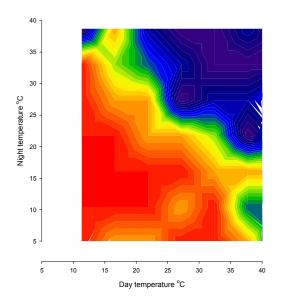
Banksia aculeata

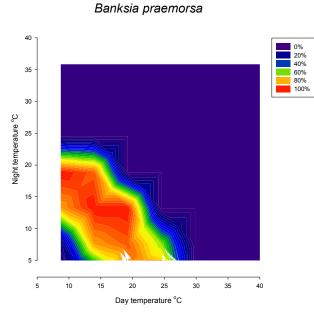
Banksia benthamianna





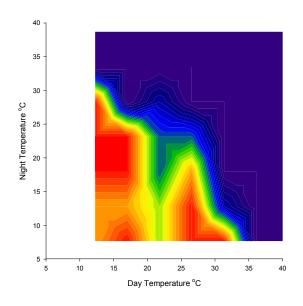
Banksia leptophylla

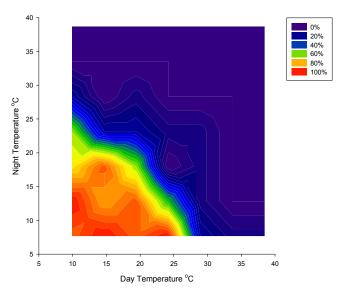




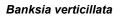
Banksia lemanniana

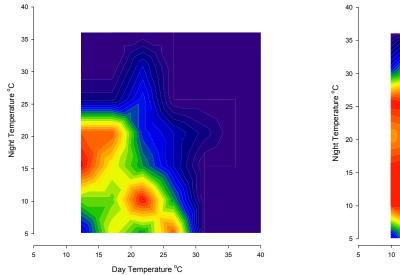
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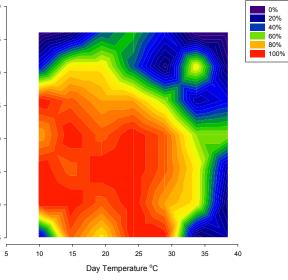




Banksia quercifolia

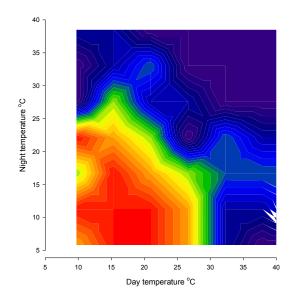


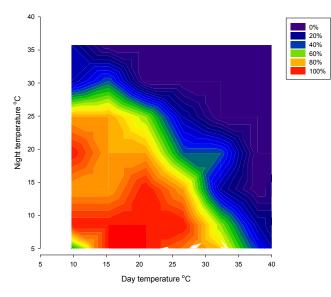




Banksia grandis

Banksia caleyi





Banksia baxteri

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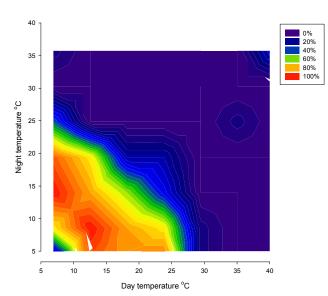
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Day temperature °C

Night temperature °C



Banksia dryandroides

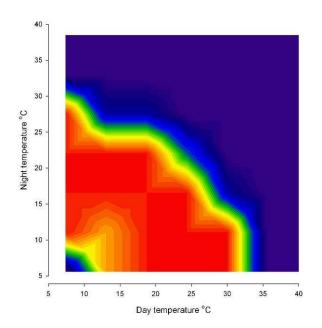
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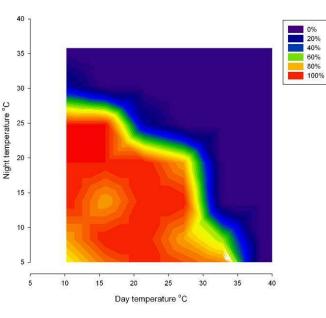
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Banksia baueri EMB

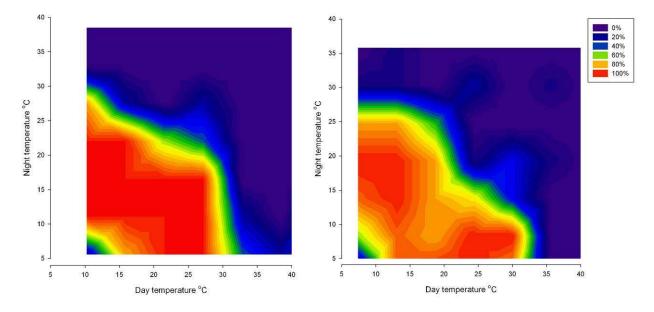
Banksia baueri SRNP





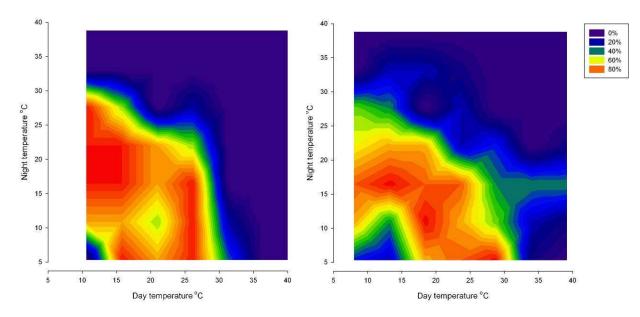
Banksia baueri Tarin Rock

Banksia laevigata



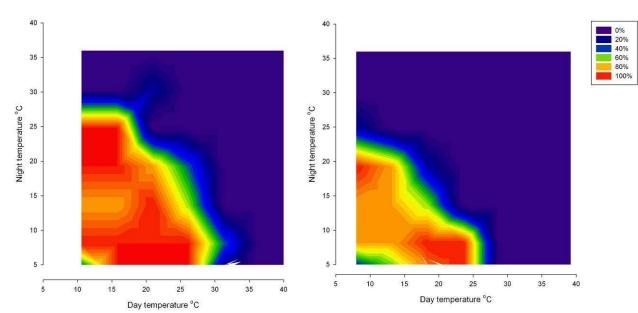
Banksia blechnifolia

Banksia brownii



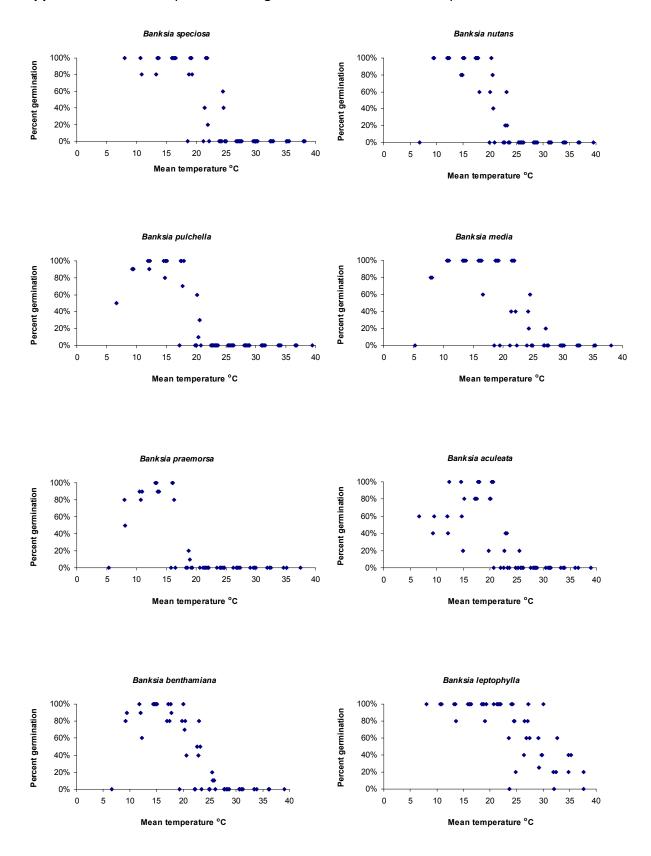
Banksia petiolaris





Appendix 4. Mean time to germination and temperatures (mean and magnitude of fluctuation) at maximum germination for 22 *Banksia* species. Note *B. baeuri* collections – SRNP Stirling Range, TR Tarin Rock and EMB East Mt Barren

	Temperature °C				Mean Time to	
Species	Day	Night	Magnitude	Mean	Germination (MTG)	Germination
Banksia aculeata	18.6	5.1	14.5	12.4	17.6	100%
Banksia baxteri	6.9	16.7	9.8	11.8	14.0	100%
Banksia baueri (SR)	15.9	19.3	3.4	17.6	14.3	100%
Banksia baueri (TR)	15.9	16.6	0.7	16.2	12.9	100%
Banksia baueri (EMB)	13.1	16.6	3.5	14.8	15.4	100%
Banksia benthamiana	22.4	7.7	14.7	15.1	16.6	100%
Banksia blechnifolia	15.8	16.5	0.6	16.1	18.4	100%
Banksia brownii	13.2	16.5	3.3	14.8	14.8	90%
Banksia caleyi	9.7	19.4	9.7	14.6	16.2	100%
	15.4	8.5	6.9	12.0	16.2	100%
Banksia dryandroides	6.8	14	7.1	10.4	21.3	100%
Banksia grandis	21.0	5.8	15.2	13.4	11.4	100%
Banksia laevigata	13.1	13.8	0.7	13.4	20.1	100%
Banksia lemanniana	12.3	18.0	5.7	15.1	17.4	100%
Banksia leptophylla	25.2	7.7	17.5	16.5	8.0	100%
Banksia media	13.9	18.9	5.0	16.4	12.6	100%
	19.3	18.9	0.4	19.1	12.6	100%
	24.5	7.6	17.0	16.1	12.6	100%
	24.5	13.2	11.3	18.9	12.6	100%
Banksia nutans	19.3	16.1	3.2	17.7	13.0	100%
Banksia oreophila	9.9	12.8	2.9	11.4	22.1	100%
Banksia petiolaris	21.0	8.1	12.9	14.5	17.4	100%
Banksia praemorsa	11.2	15.5	4.3	13.4	19.9	100%
	16.8	15.5	1.3	16.2	19.9	100%
Banksia pulchella	11.3	18.9	7.5	15.1	11.6	100%
Banksia quercifolia	21.8	10.3	11.5	16.0	22.7	90%
Banksia solandri	18.4	8.1	10.3	13.2	22.6	100%
Banksia speciosa	16.6	16.1	0.6	16.3	13.8	100%
Banksia verticillata	19.4	15.4	4.0	17.4	15.5	100%



Appendix 5. Mean temperatures for germination for 22 Banksia species

