

**Developing a screening procedure to determine the
impact of climate change on seed germination in
threatened native plant species**



Derwentia decorosa

FINAL REPORT TO THE AUSTRALIAN FLORA FOUNDATION

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ABSTRACT

Climate change forecasts predict that there will be increased temperatures and altered rainfall patterns within the next decades, which is likely to exert pressure on plant populations that are already under threat. We have developed a screening tool using a thermogradient plate to produce a range of different temperatures to assess the impact of climate change on seed germination of thirteen South Australian threatened species. The germination profiles varied for each species and pinpointed those with greater sensitivity to increases in temperature. This screening method could be used to identify plant populations at risk of decline and contribute to management decisions regarding in situ and ex situ conservation strategies.

INTRODUCTION

The long term changes in temperature and rainfall that are predicted by current climate change modelling are likely to have a negative impact on native plant populations, many of which are already under threat due to pressures such as reduced habitat and competition with introduced species. Seed germination is a critical step for the regeneration of many plant species and their continuing survival in their natural environment. By using germination as an indicator of regeneration capability over a range of different temperatures we aim to identify species at risk of decline under predicted climate change scenarios.

Atmospheric CO₂ is increasing, it was recorded at 280 ppm in pre-industrial times and >385 ppm in 2008 with a 70% increase since 1970 (Steffen et al, 2009). This continuing trend will result in a range of environmental changes including increasing temperatures, altered rainfall patterns and rising sea levels. One of the difficulties in providing long-term climate predictions is due to the unknown levels of continuing emissions and resulting levels of atmospheric CO₂ in the future. Forecasts in Australia are increased minimum and maximum temperatures in all seasons with an expected average 1 °C rise between 1990 and 2030 in coastal areas and 2 °C inland. Predicted changes beyond that depend largely on the levels of CO₂ emissions. The two major outcomes predicted for South Australia are a general increase in temperature and a decrease in rainfall within the

next few decades. It is expected that temperature will increase in all seasons and rainfall will decrease in annual averages as well as in winter and spring. The forecast also includes increases in the frequency of extreme events such as storms and wild fires, which could adversely affect populations of endangered species (Suppiah et al 2006).

The effects of climate change on Australian flora are likely to be multifaceted. Patterns of lifecycle events such as flowering, fertilisation, seed set, dispersal, dormancy periods and germination are at risk of being altered (Hedhly et al 2008; Ooi et al 2009). In this project we have focused on seed germination, as it is a key step in plant regeneration and is known to be sensitive to changes in temperature (Burmeier & Jensen 2008; Milbau et al 2009). Seeds are dispersed at maturity and may germinate readily or persist in the soil seed bank for months, or years, before germination is triggered by specific environmental cues. The requirements for seedling emergence vary between plant species and the range of temperatures conducive to germination may be wide or quite narrow (Beardsell and Richards, 1987).

Little is known about the germination requirements of many Australian native species (Baskin and Baskin, 2003), including rare species with restricted habitats in South Australian regions. In addition, the effect of changes in climactic conditions on seed germination in these species is currently unknown. In this study we developed a method to screen the germination of threatened species from different habitats with different plant forms, over a range of temperatures. Germination rates in response to a range of static and diurnal temperatures were recorded and indicated which species were inhibited by, or tolerant of, high temperatures. The aim of the study was to determine whether screening procedures for germination under different temperature regimes could be used to identify species at risk of decline under forecast climate conditions.

MATERIALS AND METHODS

Seed collection and evaluation

A list of threatened plant species identified as being at potential risk from the impact of climate change was developed. A range of plant habits: trees, mid-storey and understorey species, were included to cover a diverse representation of plant types and vegetation profiles. Two key criteria were used to identify plant species for the research project:

- 1) Being listed as threatened (endangered, vulnerable and/or rare) under the South Australian National Parks and Wildlife Act, 1972.
- 2) Having a limited distribution within a submontane environment.

During 2008/2009, 14 plant species (Table 1) were located in their native environment and had seed of a suitable quantity and quality (deemed as viability > 75%) collected and made available for the project.

After cleaning and quantifying, seeds were stored in a controlled environment room at 15 °C at 15% RH. Seed viability was estimated after cut-testing 50 seeds. Embryo types were determined after dissection according to Baskin and Baskin 2003. Preliminary experiments were conducted to identify whether seeds were easy to germinate or had a dormancy mechanism that could be overcome. These were carried out using 25 seeds sown on 1% (w/v) water agar plates with and without the addition of potassium nitrate (100 mg/L) or gibberellic acid (250 mg/L) in an incubator set for spring/autumn conditions (12 h 10 °C dark/ 12 h 20 °C light). Germination was recorded weekly and scored when the radicle had elongated to at least half the seed length. The treatments for seed germination in larger scale experiments were determined on the results from these experiments. Water agar plates were used for the non-dormant species that germinated readily within six weeks without the addition of supplements. For some species with physiological dormancy, potassium nitrate treatment was used. Gibberellic acid was used where the potassium nitrate treatment did not produce a satisfactory germination response (Table 2).

Table 1. List of plant species with their collection region, rating and plant descriptions.

Acacia gunnii



Family: Leguminosae

Collection site: Mount Lofty Ranges

Status: Rare

Plant and seed photos by SASCC

Description: Small rigid profuse prickly sprawling shrub 20-80 cm high with triangular phyllodes. Simple axillary globular whitish yellow flower heads occurring in May-September. Seeds are mottled without arils and ripen in early summer.

Acacia spooneri



Family: Leguminosae

Collection site: Southern Flinders Ranges

Status: Endemic (SA)

Plant and seed photos by SASCC

Description: Open shrub growing up to 2m with obovate phyllodes. Inflorescences are axillary glabrous racemes with globular, yellow flower-heads. Flowering occurs in October to December. Seeds are dark brown with white arils, ripening in early summer.

Billardiera uniflora



Family: Pittosporaceae

Collection site: Mount Lofty Ranges

Status: Endemic (SA)

Seed photos by SASCC

Description: Twiner or creeper up to 1m with alternate linear to narrow-lanceolate leaves. Flowers occur in September to November. Petals are 12-16 mm long and are cream or white, occasionally infused with purple. Flowers are solitary or in groups of two or three. Fruit is a berry with brown seeds 2-3 mm long, ripening in autumn.

Brachyscome diversifolia



Family: Compositae

Collection site: Mount Lofty Ranges

Rating: Endangered

Plant and seed photos by SASCC

Description: Perennial herb up to 45 cm high with several stems. Leaves are sessile, glandular, pubescent and oblanceolate. Daisy flower heads are approximately 20 to 25 mm in diameter with white rays. Flowering occurs in late spring with seed ripening and dispersal during summer.

Derwentia decorosa



Family: Scrophulariaceae

Collection site: Southern Flinders Ranges

Status: Rare

Plant and seed photos by SASCC

Description: Shrub growing up to 1 m high. Leaves are sessile, linear to ovate-linear, with a serrate margin. Inflorescences are long axillary racemes, flowers 10-16 mm long with a white corolla, sometimes with purple striations in the centre. Flowers are seen from July to November with seeds ripening in late spring /early summer.

Eucalyptus dalrympleana ssp dalrympleana



Family: Myrtaceae

Collection site: Mount Lofty Ranges

Status: Rare

Plant photo sourced from ornamental-trees.co.uk

Seed photo by SASCC

Description: Single stemmed trees to 33 m high with smooth, orange to reddish bark, shedding in strips to reveal a white waxy layer. Flowers occur in summer in umbels of 3 with white stamens. Seeds are contained within the fruit, a hardened capsule, until the valves open allowing dispersal.

Eucalyptus bicostata



Family: Myrtaceae

Collection site: Mid Northern SA

Rating: Vulnerable

Plant and seed photos by SASCC

Description: Trees growing up to 45 m tall with multi-stems. Juvenile leaves are thin, grey and glaucous but are dark green, long and glossy in adult form. Sessile buds and fruits occur in umbels of three and are warty and glaucous. Inflorescences have white stamens. Seeds are contained within the fruit, a hardened capsule, until the valves open allowing dispersal.

Festuca benthamiana



Family: Gramineae

Collection site: Southern Flinders Ranges

Rating: Rare

Seed photos by SASCC

Description: Grass species, slender with erect stiff stems. Leaves up to 20 cm long with a blue-green tinge. Seeds ripening in early summer.

Oreomyrrhis eriopoda



Family: Umbelliferae

Collection site: Mount Lofty Ranges

Rating: Endangered

Plant and seed photos by SASCC

Description: Erect pubescent perennial herb, approximately 30 cm high. Umbels are composed of 12-35 flowers with white petals and purplish anthers. Flowering occurs in late spring with seed ripening and dispersal in early summer.

Pultenaea graveolens



Family: Leguminosae

Collection site: Mount Lofty Ranges

Rating: Endemic (SA)

Plant photo by R de Kok ANBG Photo No.: a.13055

Seed photo by SASCC

Description: Small erect branched shrub, to 1 m high. Flowers (5-8 mm long) are papilionaceous (characteristic of pea flowers) with yellow standard, yellow or tinted purple wings and a red to crimson keel. Flowering occurs in spring with seed ripening and dispersal during summer.

Veronica derwentiana ssp homalodonta



Family: Scrophulariaceae

Collection site: Mount Lofty Ranges

Rating: Endangered

Plant and seed photo by SASCC

Description: Perennial with long erect ascending branches to 2 m high. Leaves up to 14 cm long and 4 cm wide, long-acuminate narrowly recurved. Inflorescences occur in October to January and are long dense axillary racemes. Corollas are pale blue to pale mauve with 4 lobes. Capsules are ovoid 3.5-2.6 mm, ripening in autumn.

Veronica gracilis



Family: Scrophulariaceae

Collection site: South East

Status: Vulnerable

Plant photo sourced from home.vicnet.net.au

Seed photos by SASCC

Description: Slender perennial herb with erect branches 4-35 cm long. Leaves are linear up to 6 mm long and recurved. Racemes are 2-5 flowered, corollas up to 6 mm long with rounded lobes. Flowers occur October to December with seed ripening in summer.

Veronica parnkalliana



Family: Scrophulariaceae

Collection site: Southern Flinders Ranges

Status: Endangered

Plant and seed photos by SASCC

Description: Perennial herb about 30-40 cm high. Leaves are opposite, sessile and obovate-elliptic with pairs of scattered long narrow teeth. Flowering occurs in September-October. Approximately 5 to 20 flowers are borne on racemes and have predominantly white petals with purple striations. Seed ripens and disperses during summer.

Wurmbea uniflora



Family: Liliaceae

Collection site: Mount Lofty Ranges

Status: Endangered

Plant photo sourced from University of Tasmania
Seed photo by SASCC

Description: Plants 4 -17 cm to the top of the inflorescence. The two leaves are narrow and linear at the base of the stem. Flower is solitary, occasionally two, white to pinkish when older, occurring in September to January. Seed ripens in late spring to summer.

Effect of temperature on germination; thermogradient plate in the one-way mode.

Agar plates containing seeds were placed in a Temperature Gradient Plate (Model GRD1, Grant Instruments (Cambridge) Ltd, UK) with a photoperiod of 12 h light/dark supplied by a fitted light hood as shown in Figure 1. Three replicate plates containing 25 seeds were used per row when the plate was used in the one-way mode with the temperature gradient programmed from 4 °C at the front to 40 °C at the back. Seeds were placed in 50 mm diameter Petri dishes containing 10 mls of water agar (1% w/v) with, or without supplements of either KNO₃ (100 mg/L) or GA₃ (250 mg/L) (as indicated in Table 2). Seeds were scored for germination on a weekly basis for 6 weeks. However for *W. uniflora* 75 seeds were added per dish to minimise the space this species occupied on the plate as it was anticipated that it would require a long incubation time to achieve germination. Germination was scored for 11 weeks for this species. The thermogradient

plate accommodated 13 rows of plates and generated average temperatures in °C for each row as follows: 5.6, 8.4, 11.3, 14.2, 17.1, 20.0, 22.9, 25.8, 28.6, 31.5, 34.3, 37.2 and 40.1. The standard error for each row was between 0.1 °C and 0.2 °C.



Figure 1. Agar plates containing 25 seeds each in the Temperature Gradient Plate.

Effect of temperature on germination; thermogradient plate in the two-way mode.

The thermogradient plate in the two-way mode was set up for a 12 h photoperiod with the light hood on and the gradient set from 5 ° to 40 °C from front to back. The direction was then changed for the 12 h night regime with no light and the gradient set from 5 °C to 40 °C from left to right (Figures 4-10). Petri dishes (100 cm diameter) were placed in an 8 x 8 grid making 64 individual temperature combinations. Four species were placed in each plate using 4 way divided X plate Petri dishes (Falcon) with 25 seeds in each section as shown in Figure 2. A 10 mL volume of water agar (1% w/v) with, or without supplements KNO₃ (100 mg/L) or GA₃ (250 mg/L) was used per section as a matrix. Seven species were used for these experiments: *E. bicostata*, *E. dalrympleana* ssp *dalrympleana*, *B. diversifolia*, *P. graveolens*, *F. benthamiana*, *D. decorosa* and *O. eriopoda*. Treatments used for each species are shown in Table 2. Based on results in the one-way mode (Figure 3), species sensitive to higher temperatures were not placed in the

positions over 30 °C. These were *D. decorosa*, *F. benthamiana* and *O. eriopoda*. Germination was scored weekly for 6 weeks.

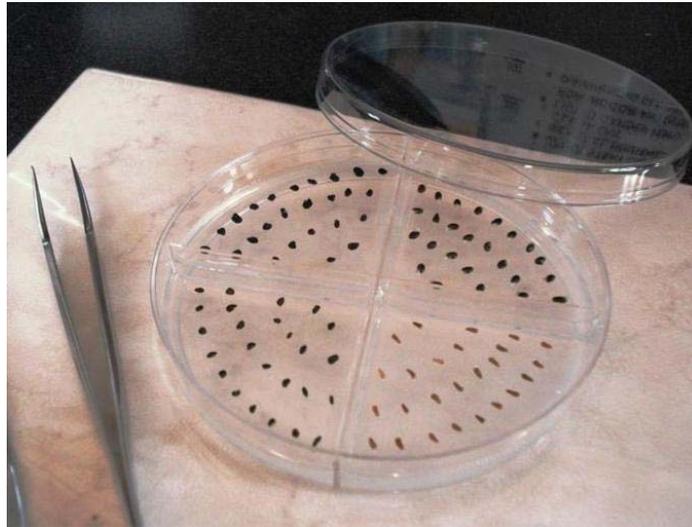


Figure 2. Seeds sown onto agar in a 4-way divided X plate. Clockwise from top right: *P. graveolens*, *B. diversifolia*, *E. dalrympleana* ssp *dalrympleana* and *E. bicostata*.

RESULTS

Species descriptions and seed properties

The species used in this study underwent preliminary testing to determine viability, embryo type and conditions that were conducive to germination (Table 2). Four species: *E. bicostata*, *E. dalrympleana* ssp *dalrympleana*, *F. benthamiana* and *O. eriopoda* germinated readily on water agar plates without pretreatment or chemical supplementation. The hard seeded species *P. graveolens*, *A. gunnii* and *A. spooneri* had physical dormancy that was overcome by nicking the seed coat. The other species had physiological dormancy, which was overcome by the addition of either potassium nitrate or gibberellic acid. The species *W. uniflora* and *B. uniflora* were very slow to germinate with or without the addition of supplements. Both of these species had linear embryos that were very underdeveloped (embryo length: seed length ≤ 0.5). When incubated on water agar plates in a spring/autumn regime (12 h 10°C dark/ 12 h 20°C light) for 7 weeks followed by a winter temperature regime (5 °C for 4 h /15 °C for 20 h with 14 h

dark/ 10 h light) *W. uniflora* started to germinate after 14 weeks. Comparatively, *B. uniflora* only yielded 4% germination after 20 weeks in spring/autumn conditions and was therefore not included in further temperature gradient experiments due to its low germination rate.

Table 2. Preliminary evaluation of seed collections included cut testing to estimate viability and dissection to identify embryo type. Treatments that were conducive to germination are listed for each species.

Species	Viability (%)	Embryo type	Dormancy type	Treatment
<i>A. gunnii</i>	88	Investing	Physical	Nicked seed coat
<i>A. spooneri</i>	96	Investing	Physical	Nicked seed coat
<i>B. diversifolia</i>	94	Linear	Physiological	KNO ₃ (100 mg/L)
<i>B. uniflora</i>	100	Linear underdeveloped	Physiological	Water agar
<i>D. decorosa</i>	78	Linear underdeveloped	Physiological	GA ₃ (250 mg/L)
<i>E. bicostata</i>	94	Folded	Non-dormant	Water agar
<i>E. dalrympleana</i> <i>ssp dalrympleana</i>	96	Folded	Non-dormant	Water agar
<i>F. benthamiana</i>	84	Lateral	Non-dormant	Water agar
<i>O. eriopoda</i>	100	Linear underdeveloped	Non-dormant	Water agar
<i>P. graveolens</i>	98	Bent	Physical	Nicked seed coat
<i>V. derwentiana</i> <i>ssp homalodonta</i>	92	Linear	Physiological	KNO ₃ (100 mg/L)
<i>V. gracilis</i>	96	Linear underdeveloped	Physiological	GA ₃ (250 mg/L)
<i>V. parnkalliana</i>	96	Linear underdeveloped	Physiological	GA ₃ (250 mg/L)
<i>W. uniflora</i>	94	Linear underdeveloped	Physiological	Water agar

Germination of seeds over a range of static temperatures.

The seed germination profiles over the range of temperatures generated by the thermogradient plate to produce a one-dimensional gradient varied between the 13 species tested and are shown in Figure 3. *W. uniflora* had the most limited temperature range where germination occurred with a sharp peak at 11.3 °C. In contrast, *E. bicostata* had good germination levels over the whole temperature range from 5 °C – 40 °C. The other eucalypt, *E. dalrympleana* ssp *dalrympleana* also germinated over a wide range of temperatures but the levels decreased above ~32 °C and almost no germination was observed at 40 °C.

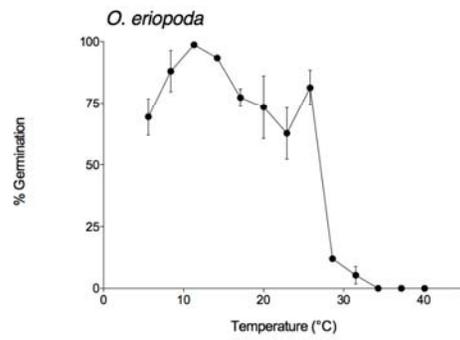
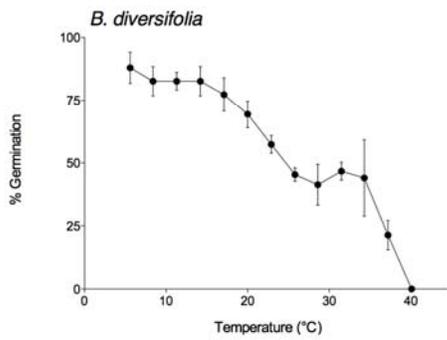
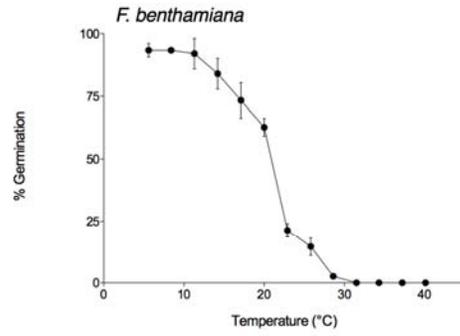
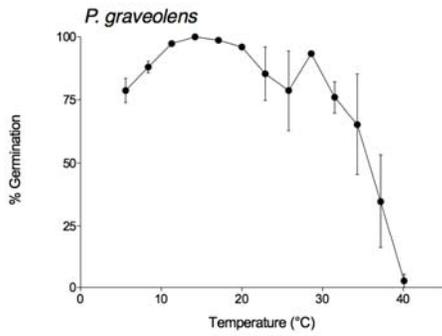
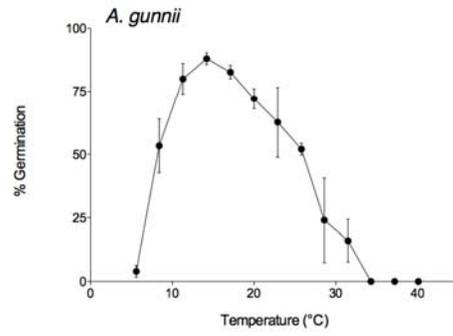
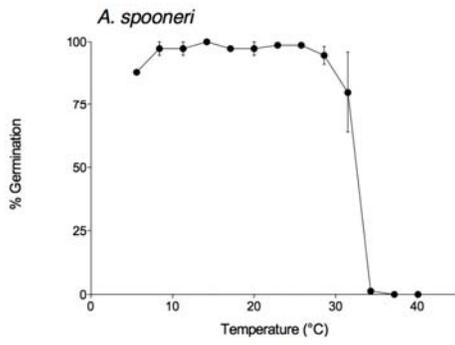
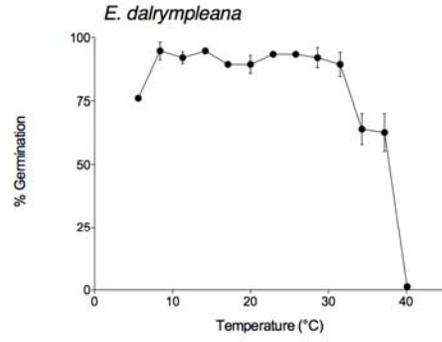
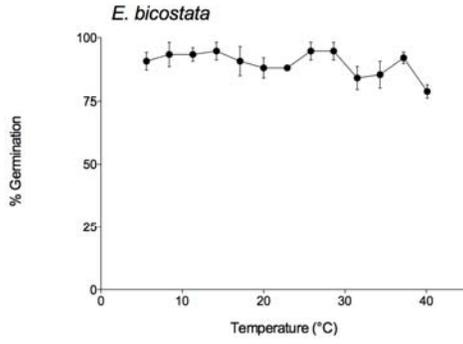
Some species (*W. uniflora*, *F. benthamiana*, *D. decorosa* and *V. derwentiana* ssp *homalodonta*) showed increased sensitivity to higher temperatures and yielded less than 25% germination at temperatures above 25 °C. The highest levels of germination were at the cooler end of the thermogradient plate with maximum germination achieved between 8 °C and 11 °C. Other species varied in their responses to the cooler end of the plate but in most cases germination was inhibited at the warmer end at 35 °C and above. The exceptions were *B. diversifolia*, *E. dalrympleana* ssp *dalrympleana*, *E. bicostata* and *P. graveolens* as germination was still recorded at temperatures over 35 °C in these species. For all the species tested, high germination was achieved at the third position on the plate which had an average temperature of 11.3 °C.

Germination of seeds over a range of diurnal temperature combinations.

The results obtained from running the thermogradient plate in the two-way mode are shown in Figures 4 to 10. In general seed germination for each species had a similar pattern in the diurnal system as in the one-way mode (Figure 3). *E. bicostata* had good levels of germination at all temperature combinations and this was also observed in *E. dalrympleana* ssp *dalrympleana* except at the constant 40 °C position, which resulted in low germination in this species. *B. diversifolia* seeds had negligible germination in any of the positions that had 40 °C as a night or day temperature and in *P. graveolens*,

germination was also reduced at the positions with 40 °C in combination with temperatures 25 °C and above. *D. decorosa*, *F. benthamiana* and *O. eriopoda* seeds were subjected to temperature ranges between 5 and 30 °C. *D. decorosa* was the most sensitive to higher temperatures and germination was reduced at positions exposed to 25 °C and 30 °C. *O. eriopoda* had low germination at static temperatures over 25 °C but in the diurnal experiment good germination was observed at temperatures of 25 °C and 30 °C as one of the diurnal temperatures. However, germination was inhibited at the constant 30 °C position as well as the 30 °C day/25 °C night. *F. benthamiana* also was able to germinate at higher temperatures than on the one-way experiment, but was reduced in nearly all of the treatments with a 30 °C component.

Climate Change Impact On Germination



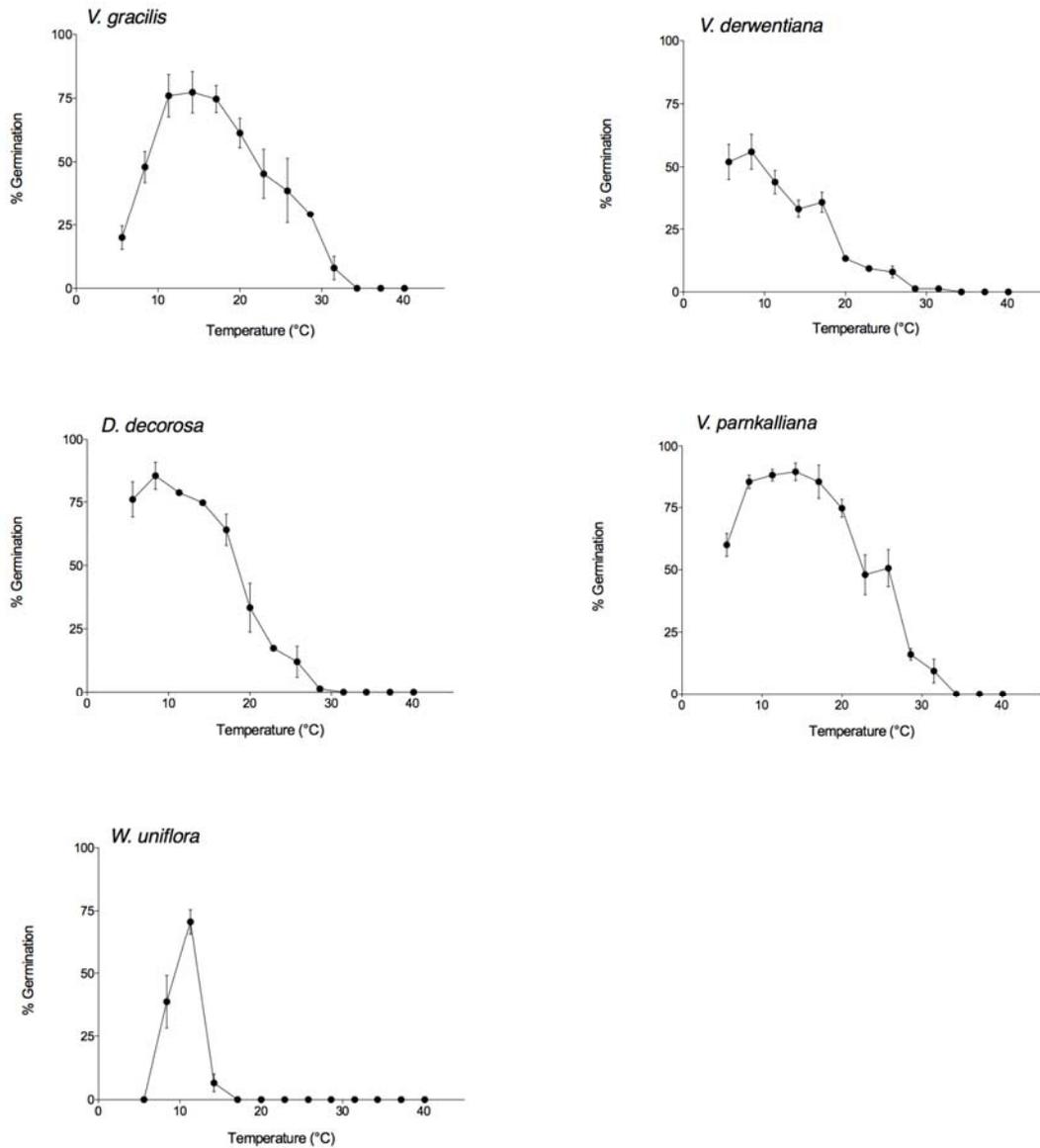


Figure 3. Seed germination of 13 different species over a range of static temperatures generated on a thermogradient plate operating in one-way directional mode. Germination (%) was recorded after 6 weeks (11 weeks for *W. uniflora*). Data plotted are means for each temperature with SE bars (n=75).

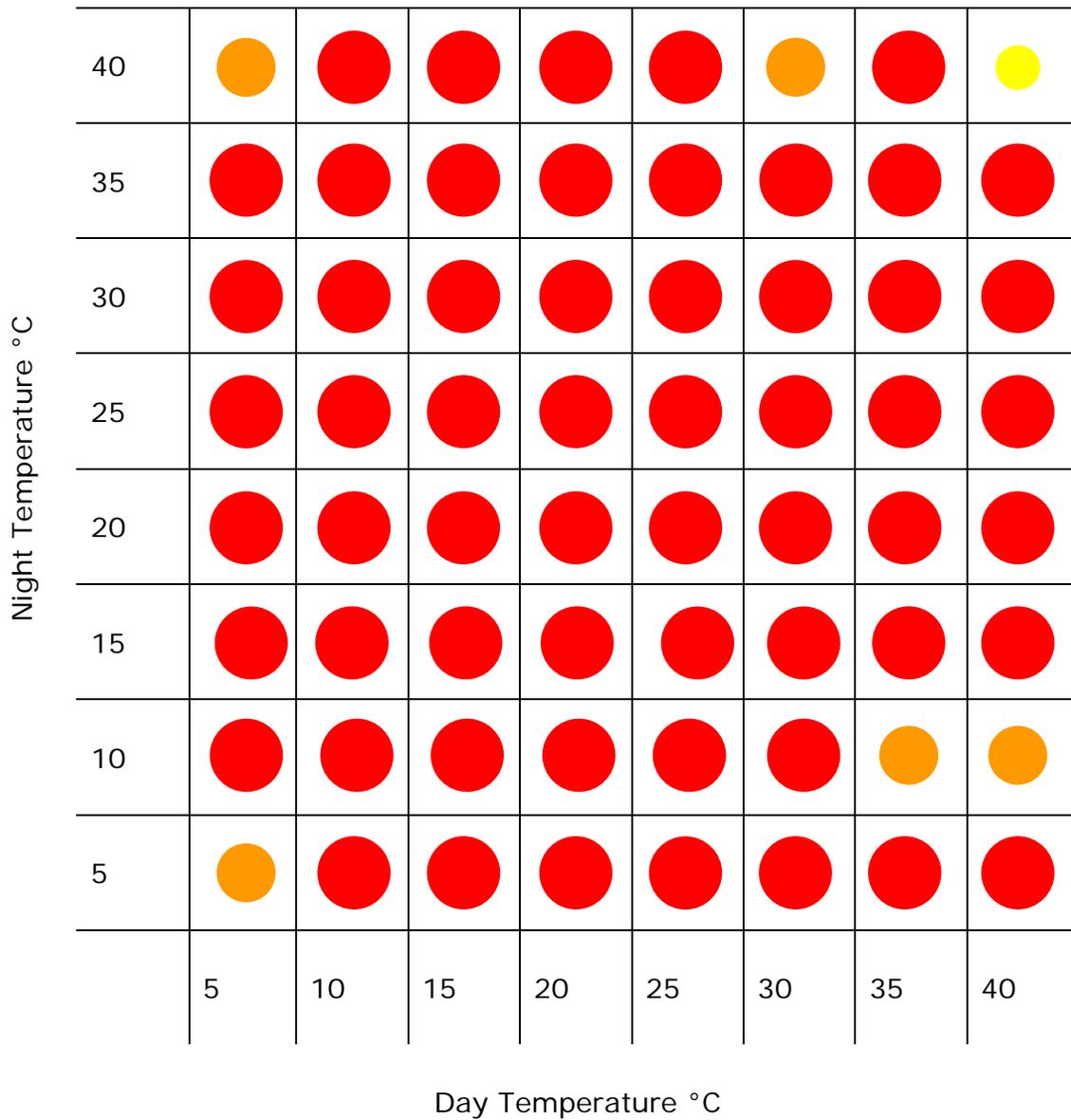


Figure 4. Germination of *E. bicostata* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate (n=25).

81-100% , 61-80% , 41-60% , 21-40% , 1-20% .

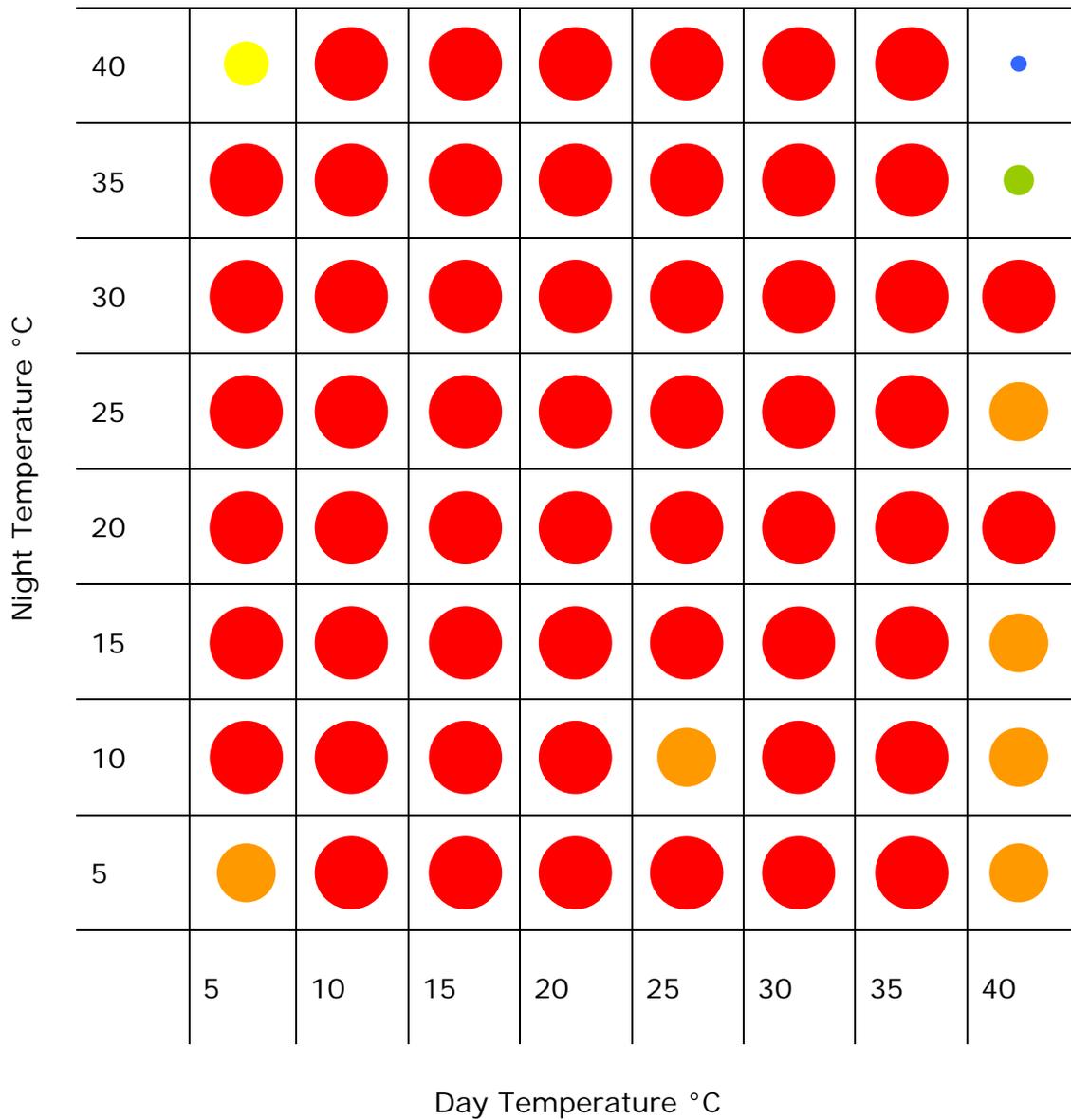


Figure 5. Germination of *E. dalrympleana* ssp *dalrympleana* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate (n=25).

81-100% , 61-80% , 41-60% , 21-40% , 1-20% .

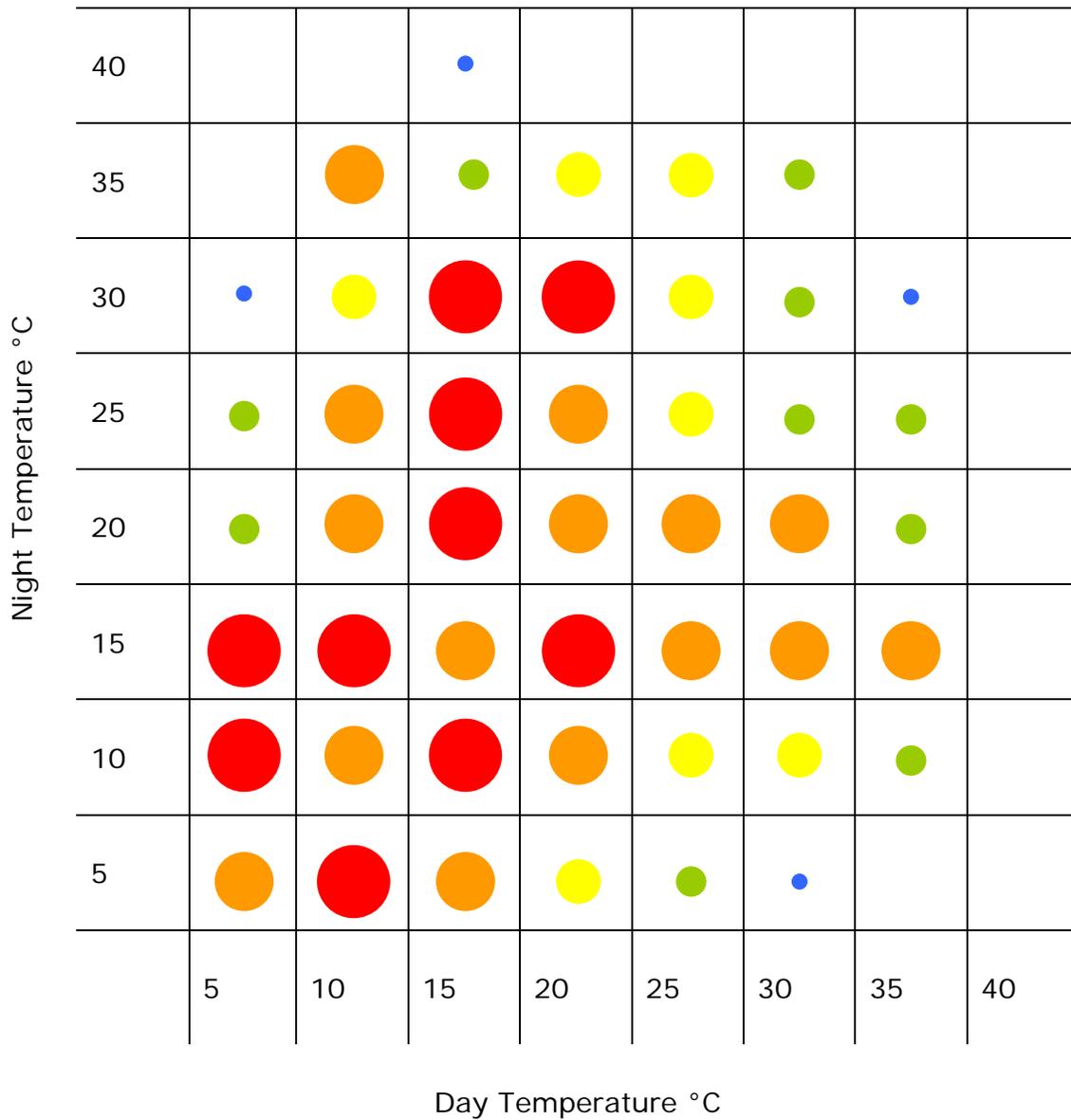


Figure 6. Germination of *B. diversifolia* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate (n=25). No circle = 0% germination.

81-100% ●, 61-80% ●, 41-60% ●, 21-40% ●, 1-20% •.

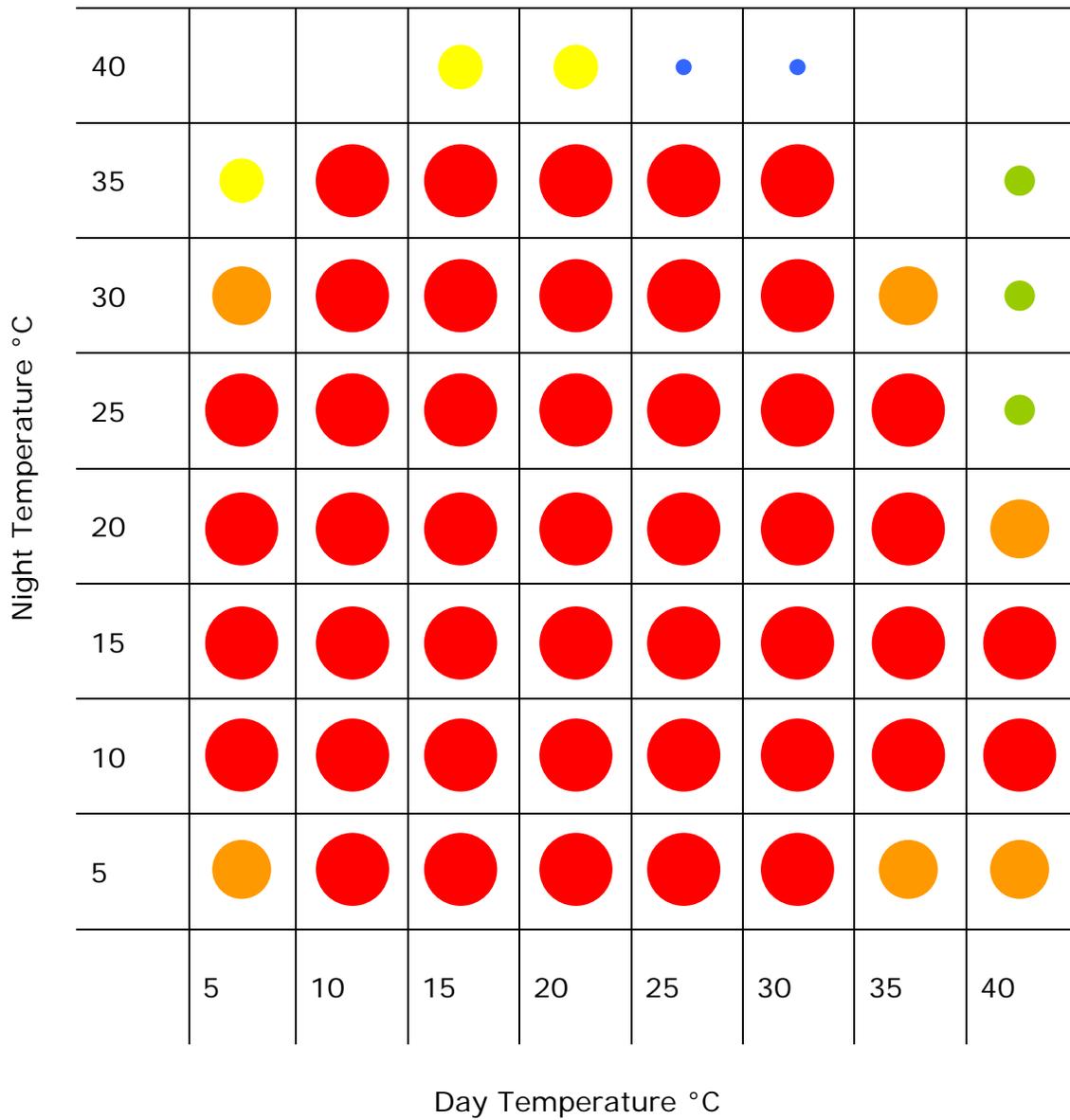


Figure 7. Germination of *P. graveolens* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate (n=25). No circle = 0% germination.

81-100% , 61-80% , 41-60% , 21-40% , 1-20% .

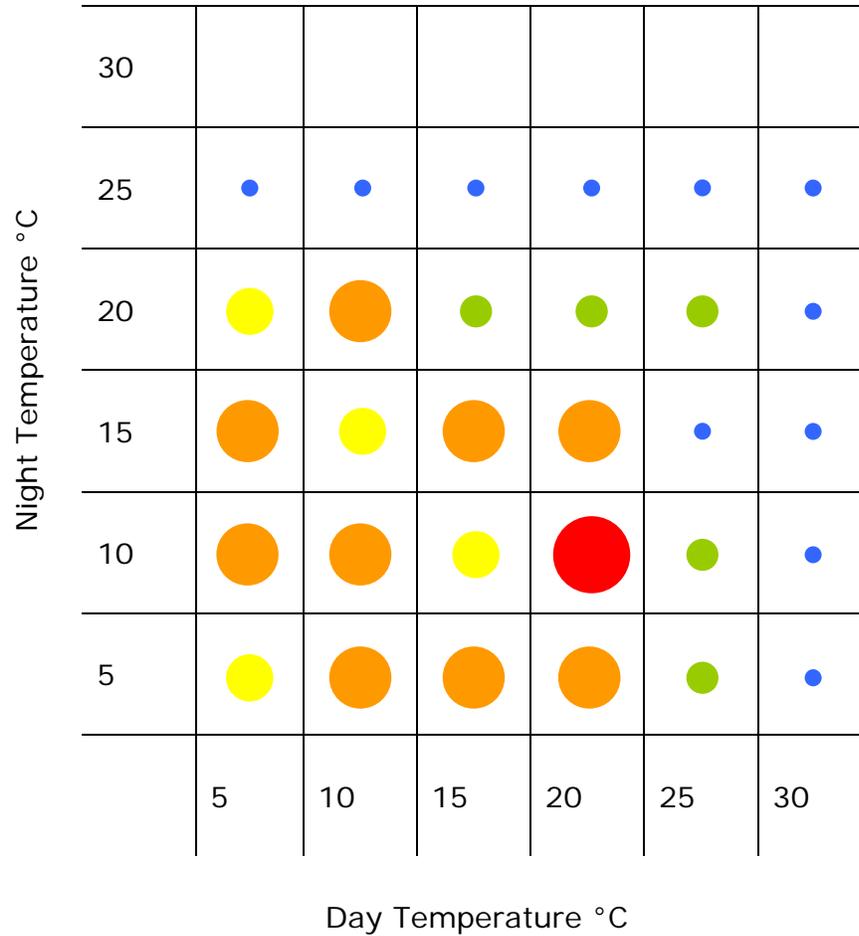


Figure 8. Germination of *D. decorosa* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate (n=25). No circle = 0% germination.

81-100% ●, 61-80% ●, 41-60% ●, 21-40% ●, 1-20% •.

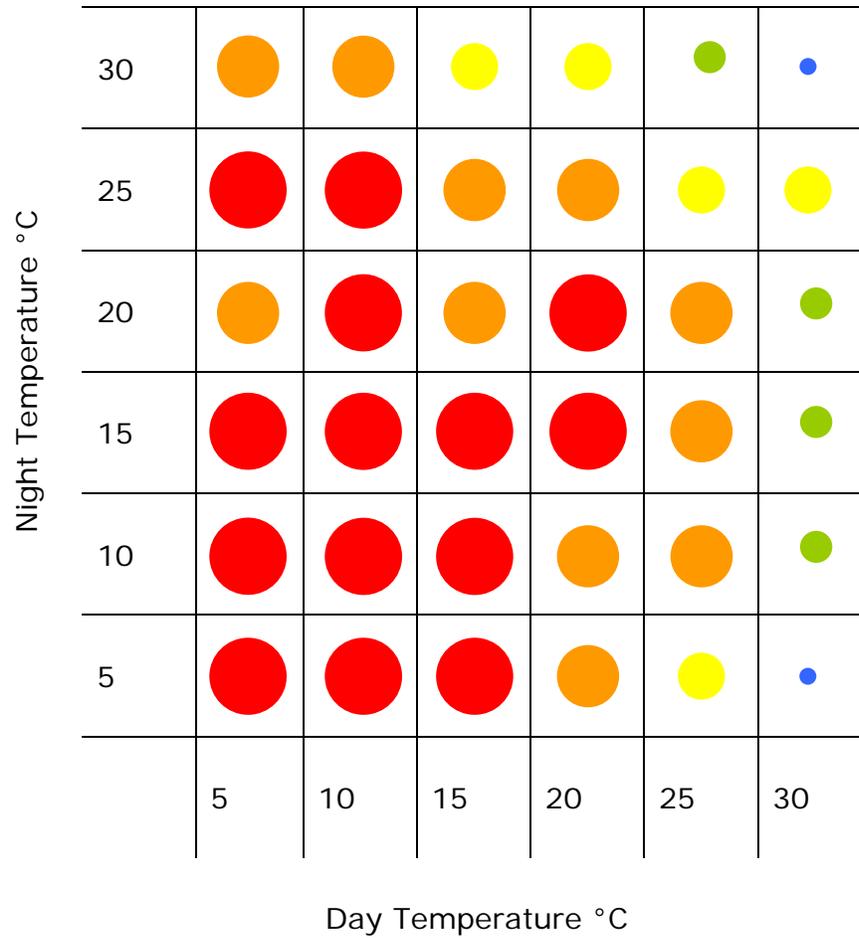


Figure 9. Germination of *F. benthamiana* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate (n=25).

81-100% , 61-80% , 41-60% , 21-40% , 1-20% .

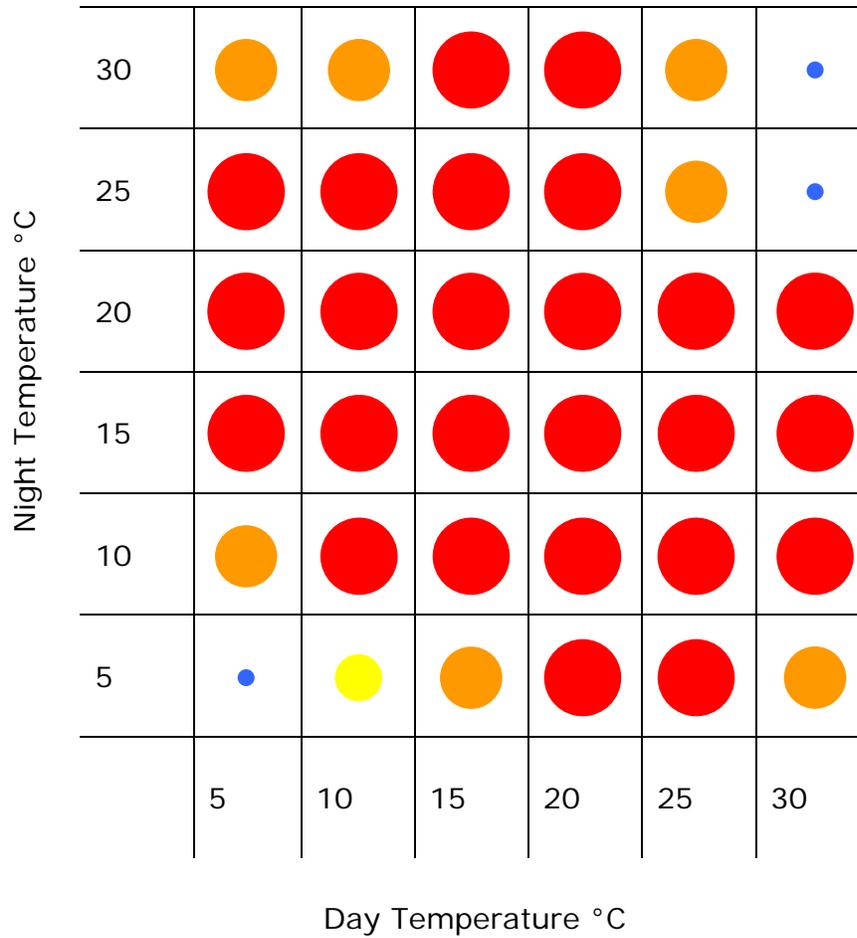


Figure 10. Germination of *O. eriopoda* seeds under different diurnal temperature combinations. Circles indicate the percent germination for each position on the thermogradient plate.

81-100% ●, 61-80% ●, 41-60% ●, 21-40% ●, 1-20% ●.

Predicted temperature data under different climate change scenarios.

Changes in temperature predicted for the Adelaide Mount Lofty Region and the South East, Northern and Yorke Regions are shown in Table 3, along with the species that were collected from these regions. Temperature data were sourced from Suppiah et al (2006) using a scenario that CO₂ concentrations stabilised at 550 ppm by the year 2150. This was the mid range of their predictions between a path that assumed no policies to reduce emissions and a path that stabilised at 450 ppm by the year 2100. For all regions the largest temperature increase is likely to be experienced in summer.

Table 3. Range of temperature changes in °C predicted for 2070 on a path that stabilises CO₂ at 550 ppm by the year 2150.

Species	NRM Region	Annual	Summer	Autumn	Winter	Spring
<i>A. gunnii</i> <i>B. diversifolia</i> <i>E. dalrympleana</i> <i>ssp dalrympleana</i> <i>F. benthamiana</i> <i>O. eriopoda</i> <i>P. graveolens</i> <i>V. derwentiana</i> <i>ssp. homalodonta</i> <i>W. uniflora</i>	Adelaide and Mount Lofty	1.0 to 2.2	0.9 to 2.5	1.0 to 2.3	0.9 to 2.1	1.0 to 2.4
<i>A. spooneri</i> <i>D. decorosa</i> <i>E. bicostata</i> <i>V. parnkalliana</i>	Northern and Yorke	1.1 to 2.4	1.1 to 2.7	1.1 to 2.4	1.0 to 2.4	1.1 to 2.6
<i>V. gracilis</i>	South East	1.0 to 2.2	1.0 to 2.6	1.0 to 2.3	0.9 to 2.0	0.9 to 2.2

DISCUSSION

In this study we have used a series of temperatures within the environmental range to predict which species may have a poor capacity for regeneration under the warmer

conditions that are predicted by climate change models. From this data set we have determined the optimum temperatures for germination for each species. The temperature range conducive to germination is flanked by ceiling temperatures, beyond which germination does not occur, and suboptimal temperatures, where rate of germination is reduced. From this data we can predict the season when seedling emergence is most likely and compare the temperatures that are predicted for that season in the future.

The screening procedure using static temperatures generated a variety of germination profiles. From this group of species, *W. uniflora* had the narrowest temperature range (between 8 °C and 11 °C) where high levels of germination occurred. In contrast, *E. bicostata* seeds had the capacity to germinate under a wide set of temperatures (5 °C to 40 °C). The results indicate that *W. uniflora* seeds are likely to emerge in winter and the prediction of a possible increase of up to 2.1 °C in the Adelaide and Mount Lofty region by 2070 (Table 3) could seriously affect the regeneration of this species in the future. *E. bicostata* seed germination is less likely to be inhibited by the 2070 temperatures predicted for the Northern and Yorke region. Other species that were identified as having low temperature thresholds were two forb species and a grass: *D. decorosa*, *V. derwentiana* ssp *homalodonta* and *F. benthamiana*. The results show that this method can determine which species are more likely to be impacted by increasing temperatures in a changing climate. It is also possible to program a narrower temperature range on the thermogradient plate to gain a more precise determination of the climactic envelope tolerated for seed germination.

In general, the experiments in the two-way mode reflected the findings from the static temperature experiments. The two eucalyptus species germinated over a broader temperature range than the other species tested. *D. decorosa* yielded the lowest level of germination at higher temperatures and was inhibited at the 25 °C range similar to the static temperature experiment. Germination in the *F. benthamiana* and *O. eriopoda* was inhibited in the two-way experiment at constant 30 °C but had higher germination when the day or night temperatures were lower. This shows that, for these species, the effects of higher temperatures were alleviated by a diurnal regime including a cooler period. The

static temperature screen had the advantages that treatments could be replicated and fewer seeds were needed to conduct the experiment.

From these results plant structure may indicate temperature sensitivity as the forb and grass species had no, or very reduced germination at 30 °C, with the exception of *B. diversifolia* that tolerated slightly higher temperatures. The shrubs with physically dormant seeds had reduced germination at 35 °C to 40 °C and seeds from the eucalyptus trees also had high germination at 35 °C (*E. dalrympleana* ssp *dalrympleana*) and 40 °C for (*E. bicostata*). The seeds used in this study were mainly collected from the southern Flinders Ranges and Mount Lofty Ranges where the temperatures are generally cooler than in other parts of the state. It would be interesting to use this method to test a range of other species from different environments to see whether a correlation exists between plant structure and temperature sensitivity in germination.

One of the limitations of working with rare species is the difficulty in obtaining sufficient seed for large experiments. For this project, plant populations had to be located, correctly identified and then revisited at harvest time to obtain enough ripe seed for experimental use. The screen for germination over a wide temperature range requires germination within 4-6 weeks so that a reasonable amount of species can be processed relatively quickly. This involved identifying and overcoming any dormancy mechanisms in the seeds prior to starting the experiments. Germination was achieved within the time frame for most of the species, but further research is needed for on *B. uniflora* to find conditions that increase the rate of seed germination.

Australia has one of the highest rates of species loss in the world and it is likely to keep rising. The predicted patterns of climate change forecast a rapid rate of change punctuated by extreme events with an increase in the frequency and intensity of bushfires and increasingly severe storms (Steffen et al, 2009). Threatened species may not be able to adapt to these scenarios in such a short timeframe. Populations of threatened plant species are often limited to specific habitats and lack the option of migration to more favourable environments. Although the environmental conditions of niche habitats are far more complex than those we create in the laboratory, this study has shown that the

screening procedure can identify species that are more vulnerable to increasing temperatures. This method has the potential to be a useful tool to assist in management decisions involving in situ and ex situ conservation strategies such as site monitoring, relocation programs and seed banking. Another major prediction for climate change in South Australia is a decrease in rainfall. Further studies on the effects of temperature and water stress on seed germination could be developed to consider the implications of rainfall shortages in combination with increasing temperatures on plant regeneration.

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REFERENCES

- Baskin CC and Baskin JM. (2003) Overview and recommendations for future research priorities on native seed dormancy and germination of Australian plants. *Australasian Plant Conservation* 11, 2-9.
- Beardsell D and Richards D (1986) In: *Germination of Australian native plant seed*. Ed PJ Langkamp Inkata Press Melbourne & Sydney.
- Burmeier S and Jensen K. (2008) Is the endangered *Apium repens* (Jaq.) Lag. Rare because of a narrow regeneration niche? *Plant Species Biology* 23, 111-118.
- Hedhly A, Hormaza JI and Herrero M. (2008) Global warming and sexual plant reproduction. *Trends in Plant Science* 14, 30-36.
- Milbau A, Graae BJ, Shevtsova A, Nijss I. (2009) Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany* 104, 287-296.
- Ooi MJ, Auld TD and Denham AJ. (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Global Change Biology* 15-2375-2386.
- Suppiah R, Preston B, Whetton PH, McInnes KL, Jones RN, Macadam I, Bathols J, Kirono D. Climate change under enhanced greenhouse conditions in South Australia. Report by Climate Impacts and Risk Group, CSIRO Marine and Atmospheric Research.

Steffen W, Burbidge AA, Hughes L, Kitching R, Lindenmayer D, Musgrave W, Stafford Smith M and Werner PA. (2009) In: Australia's Biodiversity and Climate Change. (CSIRO Publishing: Collingwood Vic Australia).