Developing a Tool to Identify Plant Species at Risk of Climate Change

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ABSTRACT

Climate change forecasts predict that there will be increased temperatures and altered rainfall patterns within the next decades. These conditions will increase pressure on plant populations that are already under threat. We have developed a screening tool using osmotic solutions containing polyethylene glycol (PEG) to simulate water stress over a range of different temperatures. This method was used to assess the impact of climate change on seed germination of seven of South Australia's vulnerable and endangered species. The germination profiles varied for each species and pinpointed those with greater sensitivity to increases in temperature and water stress. *Derwentia decorosa* was found to be the species most susceptible to higher temperatures and water stress whereas *Eucalyptus bicostata* retained high levels of germination at the highest temperature (35°C) and was least affected by decreasing water potential. We propose that the screening method could be used as a two-step process, firstly using temperature to identify species that have a narrow range for germination and secondly to apply water stress treatments to those species. This approach would be more efficient and would reduce the amount of seed needed to test each combination of temperature and water stress treatments.

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 as plant populations. This project focuses on screening the germination of seeds from vulnerable and threatened species for their response to changes in temperature and water stress. By using germination as an indicator of regeneration capability under simulated conditions of climate change we aim to identify species at risk of decline under predicted climate change scenarios.

Atmospheric CO_2 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 CO_2 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 CO_2 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. Solar radiation levels are also expected to increase in winter and spring in southern Australia resulting in decreased soil moisture through evaporation (Suppiah et al 2006).

We have used a two-phased approach to develop screening methods that simulate altered climate conditions in the future to determine the levels of temperature and water stress that will affect germination. The first is a method to estimate the germination rates of several species in response to a range of static and diurnal temperatures using a thermogradient plate. The Australian Flora Foundation and the Native Vegetation Council have funded this component of the project and the results will be presented in separate reports. The second approach addresses germination rates in response to water stress in combination with a range of temperatures and is the focus of this report.

Polyethylene glycol (PEG) is a polymer produced in a range of molecular weights. Lagerwerff et al., (1961) were the first to demonstrate that PEG could be used to modify the osmotic potential of solutions and induce plant water deficit in a uniform manner, which could be used in experimental protocols. Large molecular weight PEG does not penetrate the plant cells and induces water stress because water is withdrawn from the cells via osmosis (Hohl and Peter 1991). PEG (4000 to 8000) has been commonly used to

induce controlled drought stress in physiological experiments measuring plant growth and seed germination.

Although PEG can be used to simulate sustained water stress in solution, it is difficult to relate the osmotic potential of PEG solutions to the amount of rainfall experienced in a given area. Water potential in the soil varies significantly between soil types and is affected by rainfall and other factors such as solar radiation, amount of cloud cover, relative humidity and seasonal and diurnal temperature fluctuations. The type of soil will also affect the rate of water retention between rainfall events and hence the water potential profiles over time. However, decreases in rainfall will lead to corresponding decreases in soil water potential. Field capacity (-0.01 to -0.03 MPa) is the state of the soil after water drains from the soil via gravity and what remains is held in the soil pores by capillary action. Water is available to plants from this state up to approximately -1.5 MPa when plants can no longer extract water from the soil and is known as the permanent wilting point. For this study we have selected a range of water potentials between field capacity and the permanent wilting point to simulate different water stress conditions for germination.

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. 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 and moisture. 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. In this study we have investigated the effects of water stress and temperature on a range of endangered species from different habitats and with different plant forms. The aim of the study is to determine whether screening procedures for germination in altered climate change conditions can be used to identify the species at most risk of decline.

MATERIALS AND METHODS

Seed collection and evaluation

Seeds were collected from the following species between 2008 and 2010: *Acacia spooneri*, *Brachyscome diversifolia*, *Derwentia decorosa*, *Eucalyptus bicostata*, *Oreomyrrhis eriopoda*, *Pultenaea graveolens* and *Veronica parnkalliana*. Collection sites are shown in Table 1. After cleaning and quantifying, seeds were stored in a controlled environment room at 15°C at 40% RH. Seed viability was estimated after cut-testing 50 seeds. Preliminary experiments were conducted to identify whether the seeds were easy to germinate or had a dormancy mechanism that was easily overcome. These were carried out on 1% (w/v) agar plates in an incubator set for spring/autumn conditions (12 h 10ºC dark/ 12 h 20ºC light). Hard seeded species were treated by nicking the seed coat with a scalpel, or submerging in boiling water for 1-2 minutes. Treatments to overcome dormancy included supplementing the agar plates with potassium nitrate (100 mg/L) or gibberellic acid (250 mg/L). Species descriptions were adapted from the Electronic Flora of South Australia provided by the State Herbarium of South Australia.

Effect of temperature and water potential on germination

Seeds were placed onto three pieces of Advantec 2, 70 mm filter paper in a 90 mm glass petri dish and irrigated with 6 mL of water (control) or PEG-8000 solution. Plates were sealed using cling film to reduce evaporation. To overcome physiological dormancy, control and PEG solutions were supplemented with KNO_3 (100 mg/L) for *Brachyscome diversifolia* seeds, and GA_3 (250 mg/L pH 6.5) for *Veronica parnkalliana* and *Derwentia decorosa* seeds. *Acacia spooneri* and *Pultenaea graveolens* were nicked to overcome physical dormancy. Plates were placed in incubators set at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. A diurnal light source set for12 h intervals was present in all the incubators except for the one set at 35°C. Germination was scored when the emerging radicle or cotyledon ≥ half the seed length and these were removed after scoring. Plates were scored weekly for five weeks. To avoid concentration of the PEG solutions over the course of the experiment, seeds were transferred with the top piece of filter paper into clean plates containing 2 new pieces of filter paper and 5mL of fresh PEG solution with or without supplements every fortnight.

PEG solutions were prepared to simulate a range of water stress scenarios; a very low water potential near to field capacity (-0.1 MPa), a low level (-0.25 MPa), a medium level (-0.5 MPa) and a moderately high level (-1.0 MPa). The solutions were prepared separately for each incubator using the formula [PEG] = $(4-(5.16\psi T) - 560\psi+16)^{0.5})/(2.58T-280)$ where T = temperature (C); [PEG] = PEG 8000 g/g H2O and ψ = water potential (bars) from Michel (1983).

Seeds were placed in 3 replicate plates containing 25 seeds each for all treatments. However, with endangered species it is not always possible to obtain enough seeds for large experiments and there were some treatments not tested due to insufficent seed. *D. decorosa* was not tested at the highest PEG treatment (-1.0 MPa) as well as the PEG levels -0.5 MPa and -1.0 MPa at 30°C and 35°C. *A. spooneri* was limited to 20 seeds per replicate for the PEG treatments in the 30°C and 35°C incubators. The 35ºC treatment was not used for *B. diversifolia* because previous experiments showed that this species has a light requirement for germination.

RESULTS

Species descriptions and seed properties

The species used in this study underwent preliminary testing to determine conditions that were conducive to germination. Two species *E. bicostata* and *O. eriopoda* had no dormancy and germinated readily on water agar plates. The hard seeded species *P. graveolens* and *A. spooneri* had physical dormancy that was overcome by nicking the seed coat or by submerging in boiling water. The other species had physiological dormancy, which was over come by the addition of gibberellic acid. *B. diversifolia* also had a positive germination response to the addition of potassium nitrate. The results from these tests were used to determine the treatments used for the water stress experiments. Descriptions of the plants used and their seed characteristics of their seeds are summarised in Table 1.

Acacia spooneri

Family: Leguminosae

Collection site: Southern Flinders Ranges

Status: Vulnerable

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, with seeds ripening in early summer.
Seed properties: Seeds were 96% viable, approximately 3.5 mm long with an investing embryo. They have physical dormancy that was overcome by nicking the seed-coat or submerging in boiling water for 1-2 minutes.

Brachyscome diversifolia

Family: Compositae

Collection site: Southern 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.
Seed properties: Seeds were 96% viable, approximately 2.2 mm long with a linear embryo. They have physiological dormancy but germinated in the presence of potassium nitrate or gibberellic acid.

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.
Seed properties: Seeds were 78% viable, approximately 2.5 mm long with linear under-developed embryos. They are physiologically dormant but germinated in the presence of gibberellic acid.

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.
Seed properties: Seeds were 94% viable, approximately 1.8 mm long with a folded embryo. They were non-dormant.

Oreomyrrhis eriopoda

Family: Umbelliferae

Collection site: Southern 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.
Seed properties: Seeds were 100% viable, approximately 4.1 mm long with linear underdeveloped embryos. They were non-dormant.

Pultenaea graveolens

Family: Leguminosae

Collection site: Southern Mount Lofty Ranges

Rating: None

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.
Seed properties: Seeds were 98% viable, approximately 3.4 mm long with a bent embryo. They have physical dormancy that was overcome by nicking the seed coat or submerging in boiling water for 60 seconds.

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.
Seed properties: Seeds were 96% viable, approximately 1.7 mm long with an underdeveloped linear embryo. They have physiological dormancy but germinated in the presence of gibberellic acid.

Table 1. Species descriptions and seed properties determined by preliminary evaluations.

Predicted temperature and rainfall data under different climate change scenarios

Changes in temperature and rainfall data predicted for the Adelaide Mount Lofty Region and the Northern and Yorke Regions are shown in Tables 2 and 3. Data were sourced from Suppiah et al (2006) using a scenario that CO_2 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 both regions the largest temperature increase was likely to be experienced in summer, with rainfall reduction more prevalent in winter and spring.

Region	**Annual**	**Summer**	**Autumn**	**Winter**	**Spring**
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
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

Table 2. Range of temperature changes in °C predicted for 2070 on a path that stabilises CO_2 at 550 ppm by the year 2150.

Region	Annual	Summer	Autumn	Winter	Spring
Adelaide and Mount Lofty	-19 to -1	-8 to +4	-6 to +1	-20 to -4	-40 to -4
Northern and Yorke	-17 to -1	-17 to +14	-12 to +4	-21 to -3	-40 to -4

Table 3. Range of rainfall changes in percentage predicted for 2070 on a path that stabilises CO_2 at 550 ppm by the year 2150.

Effect of temperature and water stress on seed germination

The seed germination profiles under increasing temperature and water stress conditions for the species used in this project are shown in Figure 1. The temperatures that were conducive to the highest germination levels for all of the species were 10°C and 15°C. Higher temperatures inhibited germination in *D. decorosa*, *V. parnkalliana*, *B. diversifolia* and *O. eriopoda*. However, for the hard seeded shrubs inhibition was observed at 25°C and above. Germination for *E. bicostata* seeds was still high at 35°C in the control -0.1 Mpa, -0.25 Mpa and -0.5 Mpa treatments. In contrast, no germination was scored in the 5°C treatments except for the control, very low and low water stress treatments in *B. diversifolia* and the very low and low water stress treatments in *A. spooneri* during the five week period.

Germination was completely inhibited at the highest water stress level (-1.0 MPa) in all of the species tested. The second highest level of water stress (-0.5 MPa) was more strongly inhibitory in the forb species *D. decorosa*, *V. Parnkalliana*, *B. diversifolia* and *O. eriopoda* than the hard seeded shrubs or the eucalypt. The low level water stress level (-0.25 MPa) did not appear to reduce germination in *A. spooneri* and *E. bicostata* but had a negative effect on the germination of the other species. Lower levels of germination in *B. diversifolia* were observed in the very low level of water stress (-0.10 MPa) compared to the control (0 MPa) at all of the temperatures tested but this was not so clearly observed in the other species.

Figures 2 to 8 show the rate of germination for each of the treatments at the different temperatures over time for each of the species. Interestingly in *A. spooneri* germination at 5°C started at 5 weeks and was only observed in PEG solutions -0.1 and -0.25 Mpa. We will continue to monitor germination on these plates to see the extent of germination in all the treatments. In general the rate of germination was slower at the higher levels of PEG, however, different levels of osmoticum were inhibitory for some species and not others. The temperature had an effect on germination rates at the different water stress levels. When germination occurred, the rate appeared to be highest for all treatments at the optimum temperatures for germination, 10°C and 15°C. This has also been reported for other species where osmotic potential had more effect at temperatures above and below the optimum for germination (Hughes *et al*, 1984).

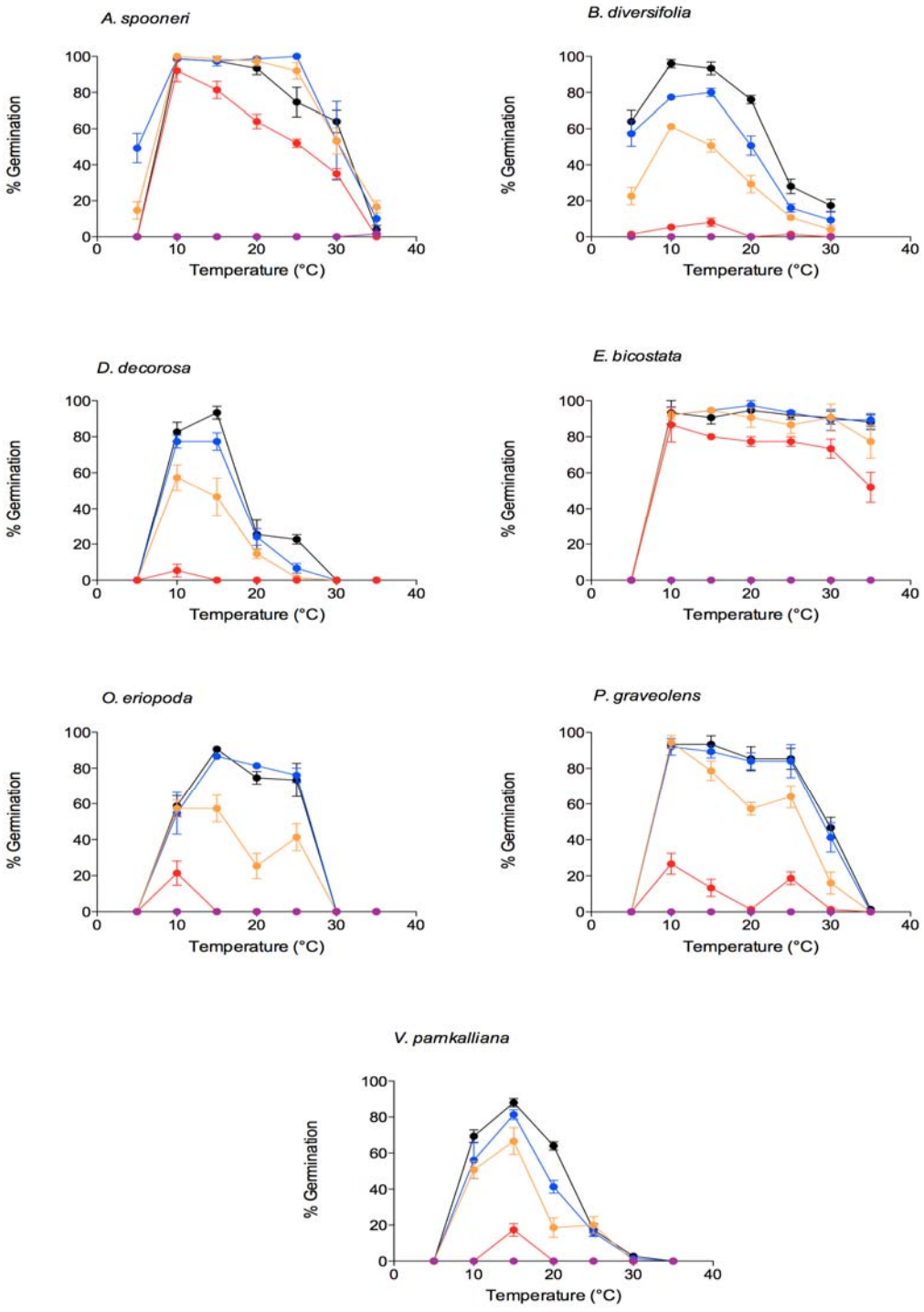

Figure 1. Maximum germination of seeds at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red), and -1.00 MPa (purple).

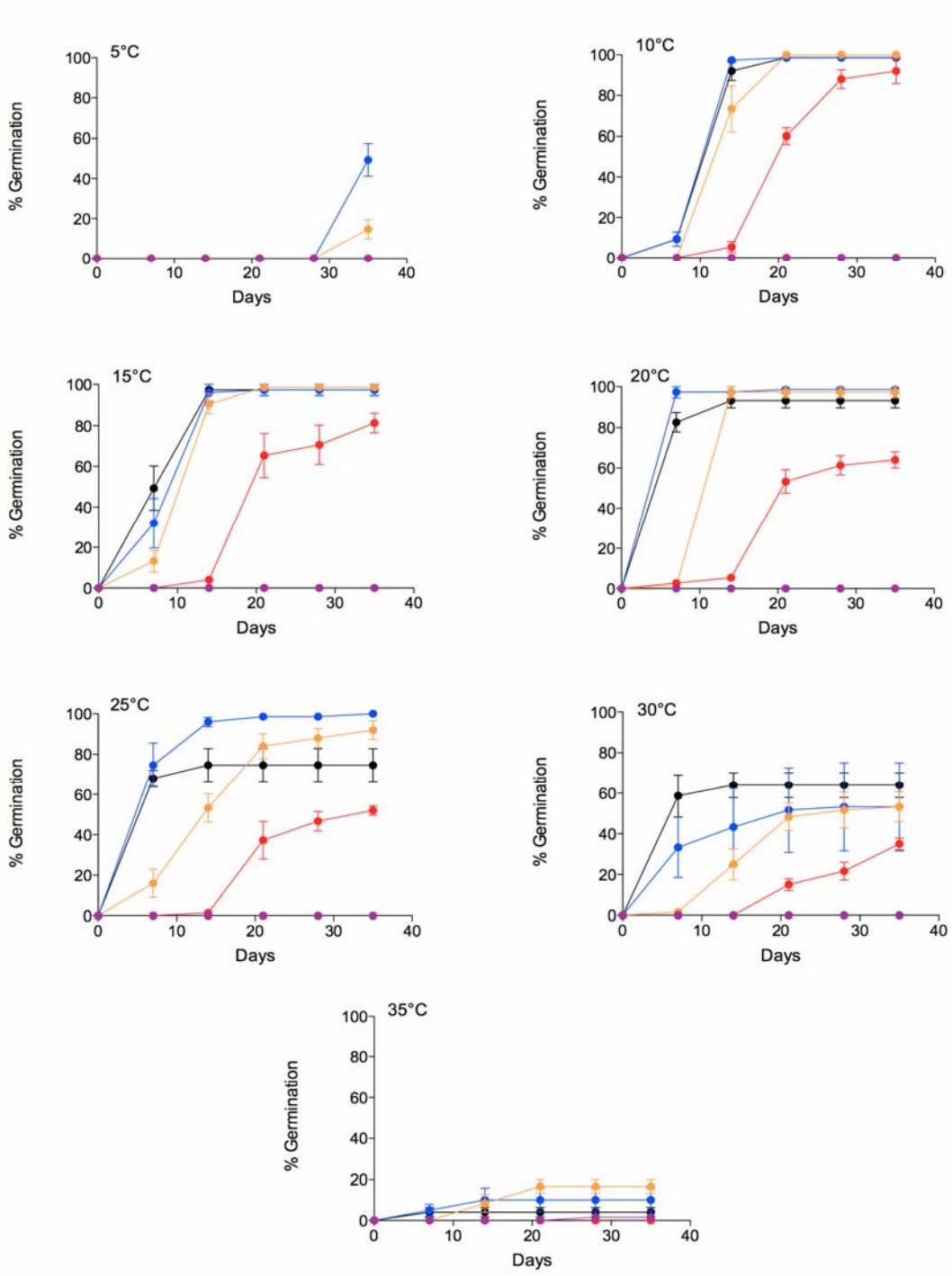

Figure 2. Germination of *A. spooneri* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black) -0.10 MPa (blue) -0.25 MPa (yellow) -0.50 MPa (red) and -1.00 MPa (purple).

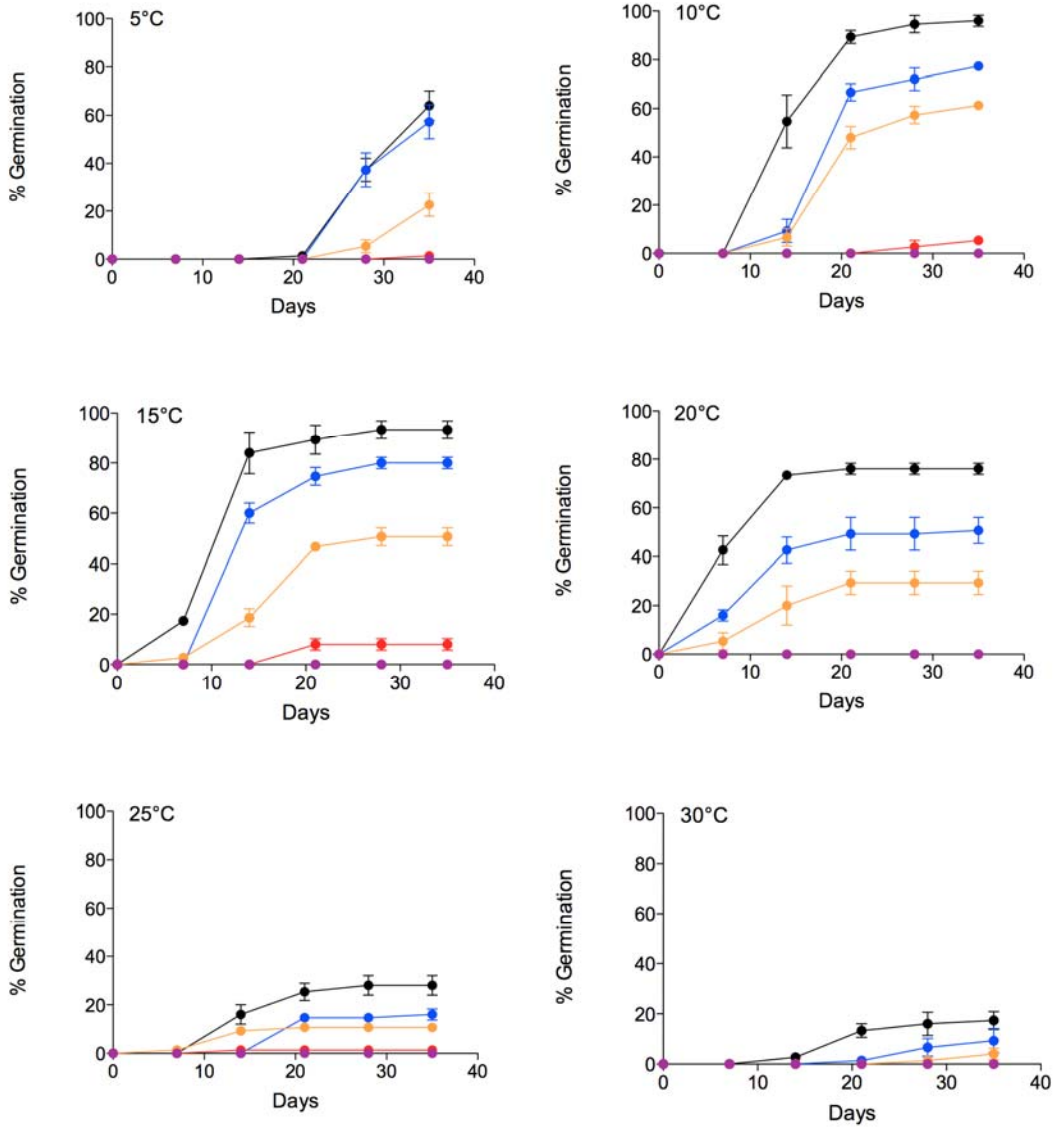

Figure 3. Germination of *B. diversifolia* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red) and -1.00 MPa (purple).

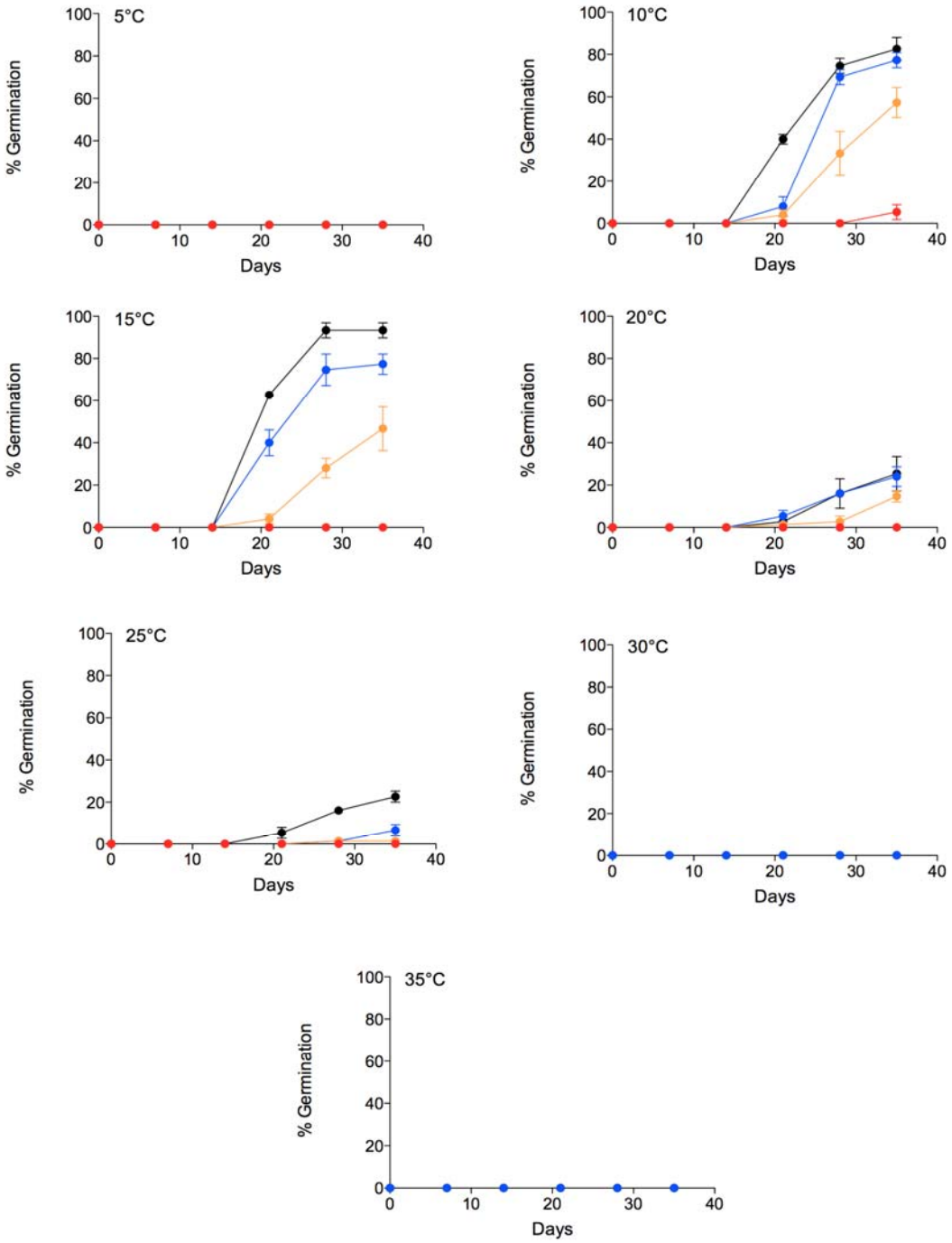

Figure 4. Germination of *D. decorosa* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red) and -1.00 MPa (purple).

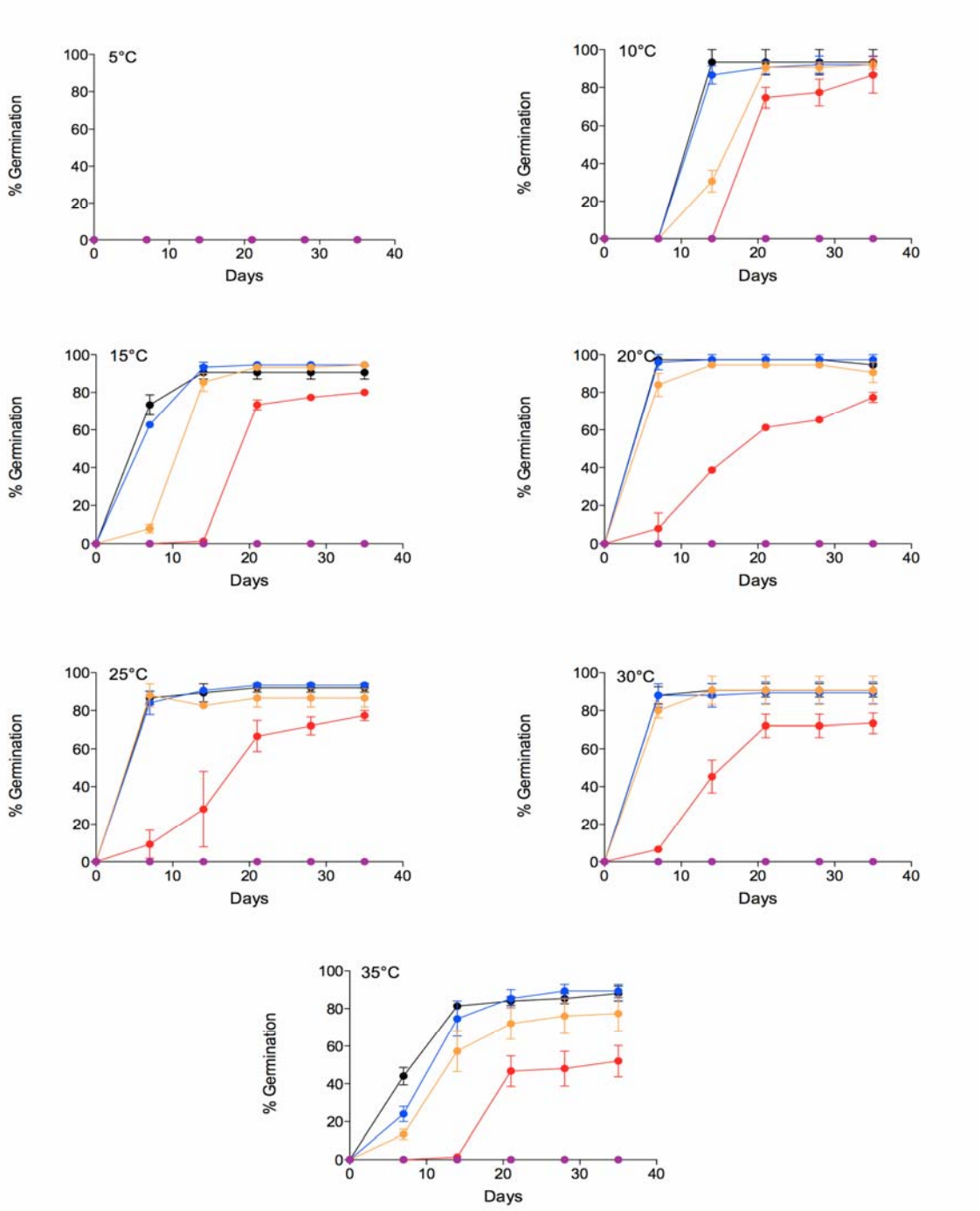

Figure 5. Germination of *E. bicostata* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials;
0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red) and -1.00 MPa (purple).

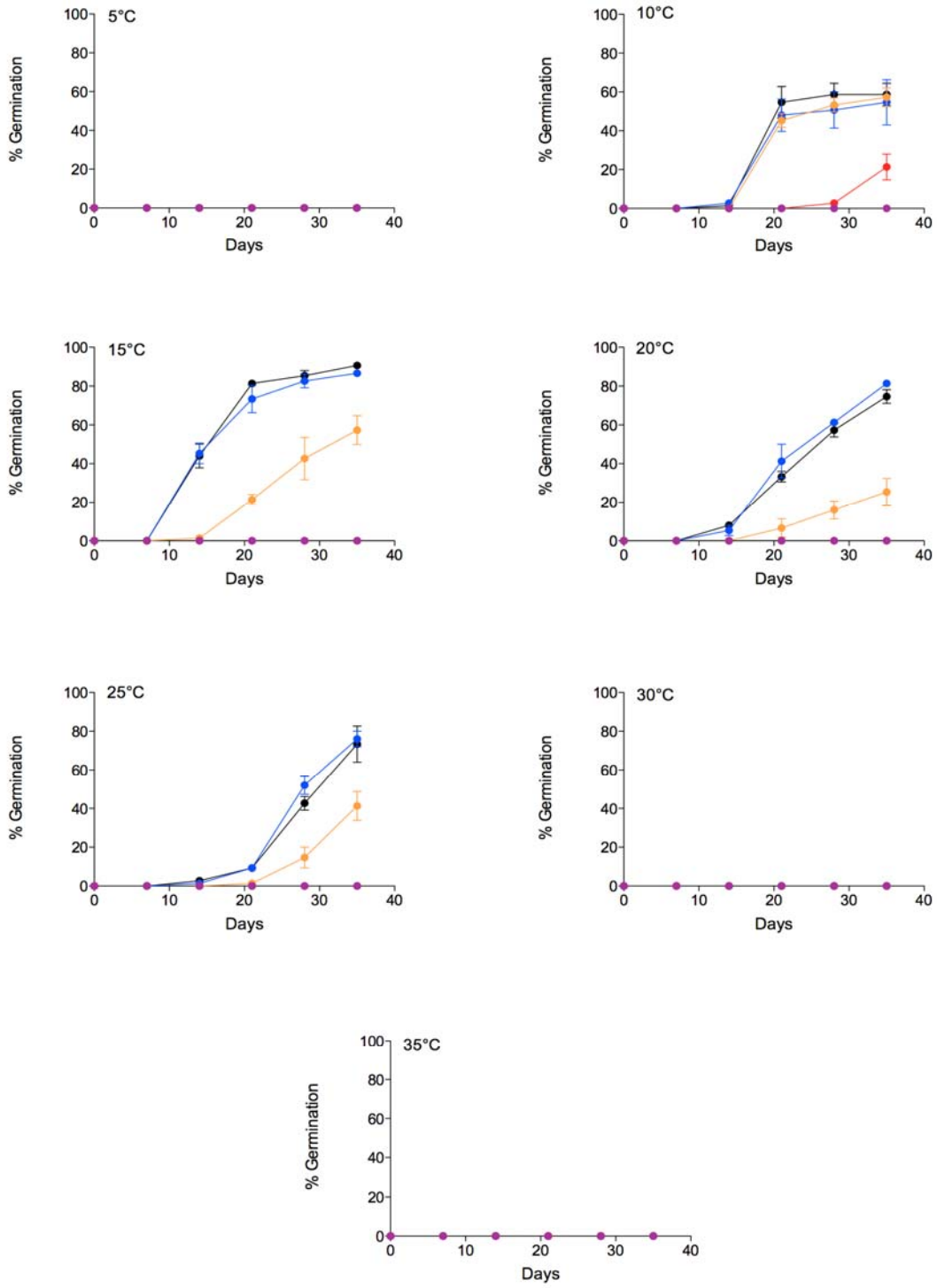

Figure 6. Germination of *O. eriopoda* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red) and -1.00 MPa (purple).

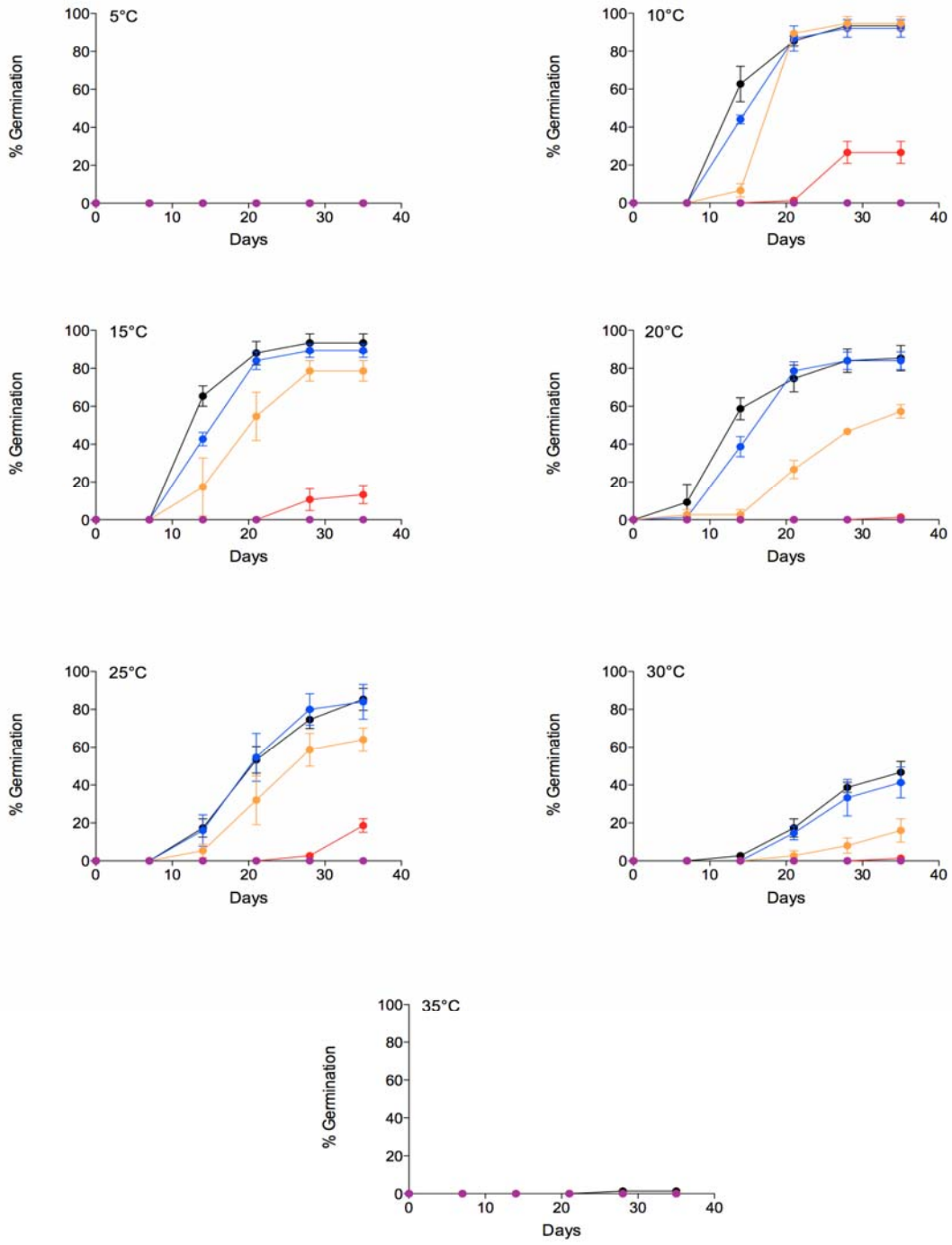

Figure 7. Germination of *P. graveolens* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red) and -1.00 MPa (purple).

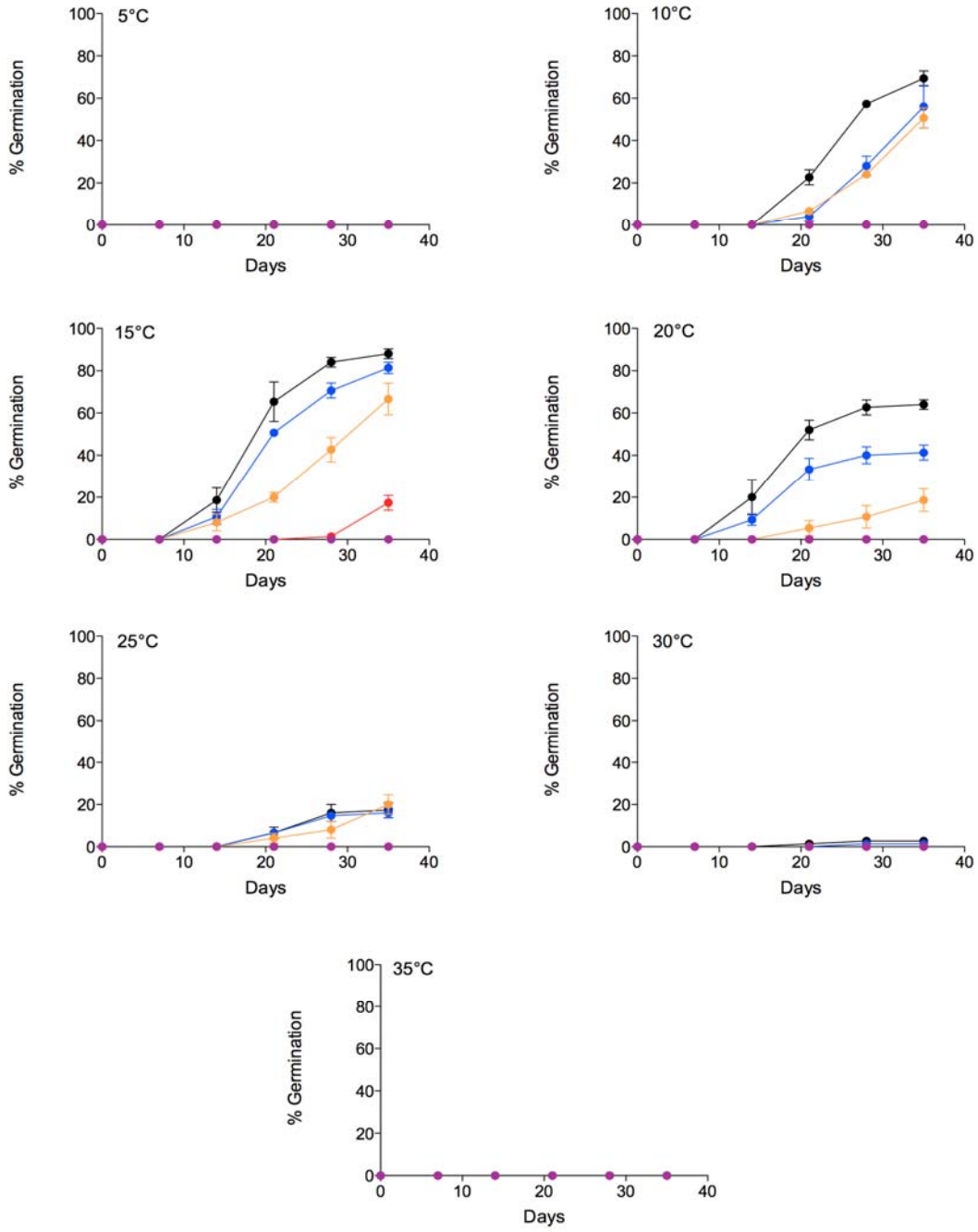

Figure 8. Germination of *V. parnkalliana* over 5 weeks at the temperatures indicated with irrigation using PEG solutions with decreasing osmotic potentials; 0 MPa (black), -0.10 MPa (blue), -0.25 MPa (yellow), -0.50 MPa (red) and -1.00 MPa (purple).

DISCUSSION

The screening procedure generated a variety of germination profiles in response to the range of temperature and water stress conditions for each species. From this group of species, *D. decorosa* had the narrowest temperature range (between 10 ºC and 20ºC) where high levels of germination occurred and was inhibited at the medium level (-0.5 MPa) of water stress. In contrast, *E. bicostata* seeds had the capacity to germinate under a wide set of temperatures (10 ºC to 35 ºC) and decreasing water potentials (0 Mpa to -0.5 MPa). The results show that this method can determine species that are more likely to be at risk in a changing climate.

The soil moisture content in the natural environment is under constant flux, increasing with rain and then reducing again as water leaves the soil through draining, evaporation and transpiration with soil type influencing these processes. In the laboratory we have used a set of static temperatures and water potentials within the environmental range to predict which species are at risk of not regenerating. From this data set we have observed several important aspects of germination; the rate of germination under the different treatments which is an important indicator of the capacity for a rapid response to rainfall events, the optimum temperatures for germination indicating the seasons where germination is most likely, the range of temperatures and water stress conditions conducive to germination which mark the levels that cannot be exceeded for healthy germination to occur. The most useful outcome of this screening method is to identify species most sensitive to temperature and water stress so these populations can be monitored in situ and effectively conserved ex situ through seed banking.

From these results plant structure may indicate temperature sensitivity as the forb species had no, or very reduced germination at 30°C with or without water stress treatments. The shrubs with physically dormant seeds had reduced germination at 35°C and seeds from the eucalyptus tree had high germination at 35°C. 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.

The forb species also had greater response to water stress than the other plants, shown by their responses to the medium water stress level (-0.5 MPa) which were very low and only observed at 10°C or 15°C. It appears from this that sensitivity to temperature and water stress may be linked, although further research is needed on an extended range of plants to define this relationship. However, for the purposes of a screening method it may be advantageous to use a two-step approach. Firstly to screen with temperature to identify species that have a narrow range for germination and secondly to apply the more time-consuming water stress treatments. This would be a more streamlined process capable of dealing with larger numbers of species and would also reduce the amount of seed needed for the screen.

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 endangered 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 a screening procedure can identify species that are more vulnerable to changes in temperature and water stress. With further development this has the potential to be a useful tool to assist in management decisions involving in situ and ex situ conservation strategies in the face of a changing environment.

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