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Investigating the seed ecology, functional traits and ecological strategies of Australian annual plants to create diverse, flowering green roofs

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Abstract

A diverse vegetation layer on green roofs is important to achieving and maximising green roof ecosystem services. Previous green roof research has mostly examined succulents and perennial plants with less attention to annuals and few green roofs incorporate annuals in the planting designs. This is despite annual species having an important role in some natural habitats with conditions that are analogous to green roofs. Their ability to escape seasonally harsh conditions as seeds, fast and cheap establishment via sowing, rapid growth, and the biodiversity and aesthetic value of their colourful flowers are also potential advantages of using annual plants on green roofs. Australia is a continent with a diverse annual flora with colourful and attractive flowering species and high potential to be used in horticultural applications such as green roofs. Therefore, the objective of this project is to develop an attractive flowering, resilient plant palette of Australian annual species suitable for green roofs in Mediterranean-like climates. However, to achieve this, we need more knowledge regarding annual species seed germination, functional traits and ecological strategies.

Seed germination capacity is one of main constraints in seed-based projects. A high proportion of wild plant species in Australia produce seeds that are dormant upon maturity and will not germinate without a suitable pre-treatment. Increasing knowledge about factors such as seed mass and climate variables that may influence or predict germination percentage is helpful if species are to be used in horticultural applications, like green roofs. To do so, we commercially sourced seeds of 58 Australian annual species from a wide range of habitats and performed germination tests using different germination stimulants and temperatures in growth chambers. Only 20 out of 58 species germinated regardless of the applied treatments and no optimal treatment was found for all species. No relationship was found between seed mass, and germination percentage and speed. Lower germination was observed in species from hotter and drier environments as well as environments with more variable rainfall. Moreover, germination speed was greater in species from drier environments and environments with lower climate variability. Finding significant relationships between climate variables and germination attributes, and no relationship between seed mass and germination, shows that climate is a better indicator of germination and could be a more useful way to select annual species for horticultural applications than seed mass.

The plant functional traits of plant species growing on green roofs are major factors determining the ecosystem services green roofs provide. Moreover, flowering plants which can

quickly achieve high cover, growth and biomass are desirable for aesthetic reasons and to meet green roof construction guidelines. Annual species are generally drought escapers with acquisitive strategy and ‘fast’ traits, rapid growth and flowering. However, since annuals grow successfully in different habitats and climates, it is expected that they have different strategies to live and complete their life-cycle. To quantify plant strategies of annuals and evaluate the ecosystem services they may offer on green roofs and to develop a trait-based plant palette of annuals for green roofs, I undertook a common garden experiment with 18 annual plant species (successfully germinated in the previous experiment). It explored the relationships among traits related to drought resistance and resource acquisition, competitive and reproductive ability and also aimed to determine whether differences in plant strategies would influence their growth rate, shoot biomass and flowering time. Species with higher acquisitive strategy were more competitive, fast growing and produced higher final biomass. Two opposing strategies were observed in the studied annual species in regard to their flowering time and relative growth rate. Plant species also showed a trade-off between their flowering time and specific leaf area (SLA). Fast flowering species with higher SLA were considered less drought resistance (drought escapers), as earlier flowering and higher SLA are both evolutionary responses of annual species to escape stress. Fast growing species with resource acquisitive strategy could be more desirable on green roofs as they have higher water use and therefore, stormwater mitigation ability. It is also more preferable to select species with a range of time to flowering onset and SLA to achieve a long-term flowering community with different levels of drought resistance.

Finally, I created a seed mixture using 16 species which we found had higher than 50% germination in the earlier study. I then sowed them in extensive green roof modules, to assess the effects of water availability on annual plant growth and diversity. Species abundance and richness were not significantly different between irrigation levels but showed a significant reduction through the experiment. Plant cover was significantly higher when the mixes were irrigated at a higher frequency; however, > 80% plant cover was achieved in all irrigation treatments. At the community level, functional diversity was unaffected by irrigation frequency. This study showed that annual species seed mixes can have good germination and establishment on green roof substrate and attain high species and functional diversity and cover with little irrigation.

Declaration

This is to certify that

- (i) the thesis comprises only my original work,
- (ii) due acknowledgement has been made in the text to all other material used; and
- (iii) the thesis is fewer than 80,000 words, exclusive of tables, illustrations, maps, bibliographies and appendices

Zahra Saraeian

Date: 22/02/2023

Preface

This Ph.D. thesis consists of three data chapters, one of which is published, and others are being prepared for submission. These papers were all primarily work of me, Zahra Saraeian, who conceived, designed and implemented the research, collected and analysed the data in the laboratory and glasshouse, prepared the manuscripts and wrote this thesis. All co-authors supervised the various stages of the Ph.D. research, contributed data or knowledge, or supported writing and revisiting the manuscripts. The data chapters are written with “we” whereas the introduction and synthesis are written with “I”.

Chapter 2 – Under preparation

Saraeian, Z., Farrell, C., Hirst, M., & Williams, N. S., (under prep). Relationships between seed mass, germination attributes and climate of natural distribution in Australian annual species

Chapter 3 – Under preparation

Saraeian, Z., Farrell, C., & Williams, N. S., (under prep). Quantifying plant functional traits and life strategies of Australian annual species to inform green roof plant selection

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I would like to express my deepest appreciation to my supervisors Nick and Claire for the ongoing support throughout this journey. I am really thankful for the patience, guidance and encouragements they offered me during my Ph.D. for my personal upliftment and my future life and career.

A big thank to my peers, friends and colleagues for sharing this Ph.D. journey with me over years. I am glad I was part of the amazing community of people at Burnley campus as I made friends for life and will always cherish the company of these people throughout life. Special thanks go to my friends Anu, Julia and Peiwen, whom without their endless support, this stage of my life would be way more challenging. Great appreciation also goes to nursery staff Sascha Andrusiak, Nick Osborne, Rowan Berry and Brett Hough. Also, many thanks to Megan Hirst, and John Delpratt for their technical support and Lisa Wittick, who is always there to help.

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Table of contents

Abstract	ii
Declaration	iv
Preface	v
Acknowledgements	vi
List of Figures	x
List of Tables.....	xiii
1 Introduction and literature review	1
1.1 Green roofs	1
1.2 Plants on green roofs	1
1.2.1 Succulents	1
1.2.2 Expanding the plant palette beyond succulents	3
1.2.3 Annual plant species	4
1.2.4 Annual plants on green roofs	5
1.2.5 Australian annual plants for green roof applications	8
1.3 Annual species germination attributes: Relationships among seed germination, seed mass and climate of natural distribution	10
1.4 Annual species life strategies: Relationships among plant functional traits and performance metrics (relative growth rate, shoot biomass and flowering time).....	13
1.5 Annual species on green roofs: Irrigation frequency for a diverse plant community	14
1.6 Research objectives and thesis outline	16
2 Relationships between seed mass, germination attributes and climate of natural distribution in Australian annual species	19
Abstract:	19
2.1 Introduction	20
2.2 Materials and methods	23
2.2.1 Seed mass and viability.....	23
2.2.2 Germination tests	23
2.2.3 Climate variables	24
2.2.4 Statistical analysis.....	25
2.3 Results	26
2.3.1 Seed mass and climate of natural distribution	26
2.3.2 Germination attributes	29
2.3.3 Seed mass and germination attributes.....	29

2.3.4	Germination attributes and climate of natural distribution	30
2.3.5	Germination attributes and climate groups	34
2.4	Discussion	36
2.4.1	Seed mass and climate of natural distribution	36
2.4.2	Seed mass and germination attributes	36
2.4.3	Germination attributes and climate of natural distribution	37
2.5	Conclusions	40
3	Quantifying plant functional traits and life strategies of Australian annual species to inform green roof plant selection	41
	Abstract:	41
3.1	Introduction	42
3.2	Materials and methods	44
3.2.1	Plant functional traits	47
3.2.2	Plant performance metrics (growth rate, shoot biomass and flowering time) ...	48
3.2.3	Data analysis	48
3.3	Results	49
3.3.1	Plant functional traits	49
3.3.2	Plant performance metrics (relative growth rate, shoot biomass and flowering time)	50
3.3.3	Relationships between functional traits and plant performance metrics	53
3.3.4	Relationships among plant performance metrics	53
3.4	Discussion	57
3.4.1	Drought resistance strategy: Relationship between specific leaf area and flowering time.....	57
3.4.2	Acquisitive-conservative strategy	57
3.4.3	Gemination speed.....	58
3.4.4	Relationship between specific leaf area and relative growth rate.....	59
3.4.5	Relationship between relative growth rate and flowering time	59
3.4.6	Green roof application	60
4	Green roofs sown with an annual plant mix attain high cover and functional diversity regardless of irrigation frequency	62
	Abstract:	62
4.1	Introduction	63
4.2	Materials and Methods	66
4.2.1	Study location and climate	66
4.2.2	Experimental design.....	67

4.2.3	Data collection and analysis.....	71
4.3	Results	72
4.3.1	Seedling emergence, diversity, abundance and richness	72
4.3.2	Plant cover	75
4.3.3	Plant traits, community weighted mean trait values, and functional diversity ..	76
4.4	Discussion	79
4.4.1	Plant cover	79
4.4.2	Plant traits	79
4.4.3	Seedling emergence, diversity, abundance and richness	80
4.4.4	Functional diversity	81
4.5	Conclusions and recommendations for green roofs	82
5	Synthesis.....	83
5.1	Summary results and conclusions	84
5.2	Study limitations	86
5.3	Implications of my research for green roofs	87
5.4	Opportunities for future research	88
6	References	90
7	Appendix A – Chapter 2.....	112
8	Appendix B – Chapter 3.....	120
9	Appendix C – Chapter 4.....	127

List of Figures

Figure 1.1. Shallow substrate extensive green roofs covered with succulents, in Milwaukee, Wisconsin, United States (source: liveroof.com).	2
Figure 1.2. Examples of annual plant species growing in Victoria, Australia: top left) <i>Rhodanthe corymbiflora</i> , Mount Korong National Conservation Reserve, top right) <i>Ptilotus exaltatus</i> , Terrick Terrick National Park, down) <i>Xerochrysum</i> sp. Mount Hope. Photos are taken by Zahra Saraeian during fieldwork investigating green roof habitat templates in northern Victoria	9
Figure 1.3. Thesis framework comprised of three data chapters illustrating research objectives addressed in each chapter.....	18
Figure 2.1. Relationships for 58 Australian annual species between their mean seed mass (log-transformed; y-axis) and 16 bioclimatic variables of their natural distribution across Australia (obtained from the Atlas of Living Australia, 2018). Regression line and coefficient of determination (R^2) are shown for significant relationships (P -value < 0.05).	28
Figure 2.2. The results of principal component analysis (PCA) of climate variables (refer to Table 2.1 for the bioclimatic variables codes) for 20 Australian annual species which germinated in at least one of the germination treatments. Each of the plant species in the six climate groups are labelled with the first letters of their scientific names (refer to appendix A1 for species names and codes). Shaded ellipses show the six groups (with group centroids) based on the climate of species' natural distribution (16 climate variables represented in Tables 2.1 and 2.2) obtained from hierarchical clustering using Euclidean distance and complete linkage method. See appendix A3 for the variation of climate variables in each climate group..	31
Figure 2.3. Relationships for 20 Australian annual species (which germinated in at least one of the germination treatments) between germination percentage, germination lag (LAG time) and germination rate (T_{50}) and multivariate climate variables (PC1, PC2 and PC3 generated from PCA of 16 bioclimatic variables) of their natural distribution across Australia (obtained from the Atlas of Living Australia, 2018). Regression lines are obtained from generalized linear models using the mean values of all germination treatments. Refer to Table 2.3 for the P -values of the relationships. Figures are shown for significant relationships (P -value < 0.05).	33
Figure 2.4. Differences in mean germination attributes (a) maximum germination percentage, (b) minimum germination lag (LAG time) and germination rate (T_{50}) among climate groups (created by cluster analysis; refer to Figure 2.2). Letters indicate significant differences among means for each germination attribute, where non-significant differences share at least one letter (one-way ANOVA and Tukey's post-hoc tests). Relationships for annual mean temperature and annual precipitation (c) and annual precipitation and precipitation seasonality (d) are shown for the 20 species (indicated by markers) in the six climate groups based on their natural distribution. Polynomial regression lines and associated R^2 values between climate variables are provided in figures. Climate data was obtained from the Atlas of Living Australia (ALA, 2018).	35
Figure 3.1. Principal component analysis (PCA) of seven functional traits for 18 Australian annual species grown under non-limiting common garden conditions. Panel a shows trait variability along first (PC1) and second principal component (PC2) and Panel b along second (PC2) and third principal component (PC3), with the proportion of explained trait variances in each axis shown in parentheses. Species abbreviations show their position along the PCs (species scores). Refer to Table 3.1 for the abbreviations of the species names. Species have been shown with different colours based on k-means clustering results: Group A (red), B (blue)	

and C (green). The end of the arrows indicates components' loadings for each trait: SLA: specific leaf area, LDMC: leaf dry matter content, LT: leaf thickness, Height: plant height, SM: seed mass, T50: germination rate, LAG: germination lag, and log indicates log transformed data.	52
Figure 3.2. Standardized major axis regression of the relationships between first principal component (PC1) and a) relative growth rate and b) shoot dry weight of 18 Australian annual plant species grown under non-limiting common garden conditions. Coefficient of determination (R^2) and P-values are shown.	53
Figure 3.3. Standardized major axis (SMA) regressions among relative growth rate, flowering time and shoot biomass of 18 Australian annual plant species (black) and their groupings defined by cluster analysis: Group A: red (n = 9), group B = blue (n = 5), and group C = green (n = 4). Coefficient of determination (R^2) and P-values are shown for all relationships. SMA regression lines are plotted when significant (P-value < 0.05). n = sample size.	54
Figure 3.4. Standardized major axis (SMA) regressions between a) relative growth rate and specific leaf area (SLA; mm ² mg ⁻¹), b) shoot dry weight and SLA, and c) flowering time and SLA of 18 Australian annual plant species (black) and their groupings defined by cluster analysis: Group A: red (n = 9), group B = blue (n = 5), and group C = green (n = 4). Coefficient of determination (R^2) and P-values are shown for all relationships. SMA regression lines are plotted when significant (P-value < 0.05). n = sample size.	56
Figure 4.1. Daily maximum (red) and minimum (blue) temperatures during the green roof module experiment from January (summer) to July (winter) 2020, measured at Olympic Park weather station, Melbourne. Data for maximum temperature on 2 April 2020 is missing.	66
Figure 4.2. Green roof modules at the experimental site of University of Melbourne, Burnley Campus, three (A), six (B) and nine (C) months after sowing (April, July, Sep 2020, respectively).	70
Figure 4.3. Mean number of seedlings of each species in each module (0.25 m ²) two months after sowing and before the start of the irrigation treatments. Error bars indicate the standard error of the mean and the dashed line shows the total expected number of seedlings, based on germination testing and sowing rate.	73
Figure 4.4. Mean number of individuals (abundance) of each species in each module (0.25 m ²) five months after sowing (end of the experiment) in response to the three irrigation frequencies: 2 (pink), 4 (green), and 6 (yellow) days between irrigation. Error bars indicate the standard error of the mean. There were no significant differences between irrigation frequencies for each species (Tukey's post-hoc test; P-value < 0.05). The dashed line shows the total expected number of seedlings, based on germination testing and sowing rate.	74
Figure 4.5. Species abundance, richness, and Shannon diversity index of the annual plant mix among the three irrigation frequencies: 2, 4, and 6 days between irrigation, measured at two (striped bar) and five (solid bar) months after sowing. Error bars indicate the standard error of the mean. Significant differences are indicated by dissimilar letters above each bar (Tukey's post-hoc test; P-value < 0.05); upper-case letters denote significant differences among irrigation frequencies at two-months and lower-case letters denotes significant differences among irrigation frequencies at five-months. Asterisks indicate significant differences between two- and five-month measurements within each irrigation frequency (Tukey's post-hoc test; *** P-value ≤ 0.001; ** P-value ≤ 0.01; * P-value ≤ 0.05).	75
Figure 4.6. Plant cover (%) in green roof modules sown with a mixture of 16 annual plant species at the end of the experiment (five months after sowing) with the three irrigation	

frequencies (2, 4, and 6 days between irrigation). Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies are indicated by dissimilar letters above each box (Tukey's post-hoc test; $P\text{-value} < 0.05$). 76

Figure 4.7. Community weighted mean (CWM) of plant traits in green roof modules sown with a mixture of 16 annual plant species with the three irrigation frequencies (2, 4, and 6 days between irrigation). Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies are indicated by dissimilar letters above each box (Tukey's post-hoc test; $P\text{-value} \leq 0.05$)...77

Figure 4.8. Functional diversity indices of plant traits in green roof modules sown with a mixture of 16 annual plant species with the three irrigation frequencies (2, 4, and 6 days between irrigation). Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. There were no significant differences between irrigation frequencies (Tukey's post-hoc test; $P\text{-value} < 0.05$).....78

List of Tables

Table 1.1. Summary of green roof publications using annual plants on green roof across different climate zones showing plant life-forms, the type of plant (plant origin), climate classification of where the study was conducted and the main research goals of the study.....	7
Table 1.2. A summary of seed size effects on plants in different life stages.....	11
Table 2.1. Bioclimatic variables, attributing codes, and definitions (from ANUCLIM version 6.1) expressing the climate of natural distribution of 58 Australian annual species across Australia (obtained from the Atlas of Living Australia, 2018).....	27
Table 2.2. Principal component analysis (PC1, PC2 and PC3) of 16 bioclimatic variables related to the natural distribution of the 20 Australian annual species in this study across Australia (obtained from the Atlas of Living Australia, 2018).....	30
Table 2.3. Generalized linear models of germination percentage (GP), germination lag (LAG time) and germination rate (T_{50}) in relation to seed mass and multivariate climate variables (PC1, PC2 and PC3 generated from PCA) of the species' natural distribution across Australia (obtained from the Atlas of Living Australia, 2018).	32
Table 3.1 Characteristics of the 18 Australian annual species including family and mean values ($\pm$ standard deviation) and ranges (min - max) of annual mean temperature (AMT) and annual precipitation (AP) for species occurrence records held at the Atlas of Living Australia (ALA, 2018).	46
Table 3.2. Principal component analysis (PC1, PC2 and PC3) of seven functional traits of 18 Australian annual species grown under non-limiting common garden conditions. Eigenvalues of the PCs, the proportion of explained variance of all three meaningful PCs (eigenvalues >1) and trait loadings on the PCs are shown. SLA: specific leaf area, LDMC: leaf dry matter content, LT: leaf thickness, Height: plant height; SM: seed mass, T_{50} : germination rate, LAG: germination lag. Log indicates log transformation.	51
Table 4.1. Characteristics of the 16 Australian annual species used in the seed mix including family, germination percentage, number of seeds used in the seed mix for each module, and their climate of origin: mean values ($\pm$ standard deviation) and ranges (min - max) of annual mean temperature (AMT) and annual precipitation (AP) for species occurrence records in the Atlas of Living Australia (ALA, 2018). Germination pre-treatments were applied to six species to maximize germination.	69

1 Introduction and literature review

1.1 Green roofs

High-density urban areas restrict the availability of ground level green space, necessitating a search for new alternatives (Shafique et al., 2018). Rooftops comprise 40 – 50% of the impermeable surfaces in urban areas and provide an opportunity to augment ground level vegetation lost to urbanisation (Shafique et al., 2018). As human-engineered ecosystems, green roofs are designed to support plants on rooftops and are typically composed of light-weight growing substrate, water retention fabric, filter fabric, drainage layer, root barrier and waterproof membranes (Besir & Cuce, 2018). Incorporating green roofs into the urban landscape is a way of introducing vegetation to make built environments more sustainable and liveable (Williams et al., 2014). Green roofs have many potential environmental and social benefits, including mitigating the urban heat island effect (Wong et al., 2021), storm water management (Rey et al., 2020; Zhang et al., 2018), improving urban air quality (Shafique et al., 2018), increasing longevity of roof membranes (Rowe, 2011), thermal benefits for buildings and surrounding environments (Fleck et al., 2022; Zinzi & Agnoli, 2012), increasing urban biodiversity by providing habitat for wildlife (Filazzola et al., 2019; Williams et al., 2014; Wooster et al., 2022), bringing visual appeal to cities, and well-being benefits such as the psychological restoration of urban residents (Lee et al., 2015; Mesimäki et al., 2019; White & Gatersleben, 2011; Williams et al., 2019). However, many of these benefits require healthy plants and good coverage on green roofs (Lundholm et al., 2015).

1.2 Plants on green roofs

1.2.1 Succulents

In temperate climate regions of Europe and North America, shallow substrate green roofs (usually < 20 cm deep) are widespread, due to their light weight and developed green roof industry (Shafique et al., 2018; Williams, Rayner, et al., 2010). These shallow green roofs (also known as extensive green roofs) are therefore easier to retrofit on existing roofs, which may not have the load-bearing capacity required to support green roofs with deeper substrates (intensive green roofs). Extensive green roofs are generally inaccessible and have lower maintenance requirements, including reduced irrigation, and can be designed for wildlife conservation and biodiversity purposes (Catalano et al., 2018; Oberndorfer et al., 2007). However, shallow substrate depths of extensive green roofs mean that water is often limiting

and coupled with high exposure to wind and solar radiation, create a hostile environment for plant growth and survival (Dunnett & Kingsbury, 2008; Kazemi & Mohorko, 2017). This stressful environment limits the types of plant species which can survive, particularly in hot and arid areas and Mediterranean-type climate regions, where vegetation can experience drought stress (Caneva et al., 2015; Rayner et al., 2016; Vahdati et al., 2017; Van Mechelen et al., 2014). A comprehensive study of both planted and spontaneous vegetation on green roofs in North Western Europe with Mediterranean climate revealed that succulent plants, *Sedum* species in particular, are found most frequently (Van Mechelen et al., 2014). Succulents have good survival on green roofs due to their conservative water use strategies, CAM photosynthesis, shallow root systems and thick leaves with waxy leaf cuticles that enable them to survive the harsh conditions of extensive green roofs (Snodgrass & Snodgrass, 2006; Vandegrift et al., 2019). *Sedum* species are easily propagated via cuttings or seed, commercially available and able to achieve rapid surface coverage on green roofs when irrigated during the first growing season (Durhman et al., 2007; Getter & Rowe, 2006; Monterusso et al., 2005; Snodgrass & Snodgrass, 2006) (Figure 1.1).



Figure 1.1. Shallow substrate extensive green roofs covered with succulents, in Milwaukee, Wisconsin, United States (source: liveroof.com).

Sedum species on green roofs have been widely investigated for their hydrological performance (rainfall retention, runoff and quality) (Gong et al., 2021; Kemp et al., 2019; Liu et al., 2020),

thermal performance (substrate cooling and energy saving) (Butler & Orians, 2011; Coutts et al., 2013; Jim, 2014), and biodiversity impact (Hoch et al., 2019; Madre et al., 2013; Partridge et al., 2020; Pearce & Walters, 2012). However, green roofs with only succulent plants (*Sedum* species in particular), offer comparatively low biodiversity and ecosystem services benefits such as substrate and air cooling and runoff reduction (Blanusa et al., 2013; Cook-Patton & Bauerle, 2012; Li et al., 2018). Succulents also have lower aesthetic values than herbaceous plants. For example, in the United Kingdom, White and Gatersleben (2011) compared people's preferences for different vegetation types and showed that a 'Meadow Roof' with tall flowering plants was more preferred and attractive than a 'Sedum roof'. This has also been shown in warmer climates, for example Fernandez-Cañero et al. (2013) showed that Sedum-only green roofs were the least preferred vegetation in a study where participants in Spain compared digital images of green roofs designed with different types of plants (succulents, ornamental grasses, shrubs, turf lawn and small trees). Additionally, Lee et al. (2014) showed green roofs with taller, green grassy and flowering vegetation were highly preferred by Australian office workers compared to succulents on green roofs. This is consistent with diverse, prairie style or grassland green roofs promoting greater fascination, sense of calmness and well-being, and creative, meditative thinking than sedum-style green roofs (Loder, 2014).

1.2.2 Expanding the plant palette beyond succulents

To expand the palette of plant species used on extensive green roofs, researchers generally focus on evaluating plant species originating from natural habitats with conditions analogous to extensive green roofs (Caneva et al., 2015; MacDonagh & Shanstrom, 2015; Van Mechelen et al., 2014). This 'Habitat template approach' suggests that suitable green roof plant species can be found in microclimates or habitats with similar biotic and abiotic conditions to those on extensive green roofs (Lundholm, 2006), including plants from grasslands, rock outcrops and coastal cliffs or barrens (Farrell et al., 2012; Mandel et al., 2016; Nagase & Tashiro-Ishii, 2018). For example, Sutton et al. (2012) surveyed North American prairies and grassland habitats, which had similar stressful conditions to extensive green roofs, to come up with lists of potential species including grasses or grass-like plants and forbs, which could be used on green roofs. Many of these studies have also experimentally evaluated perennial plants for green roofs, including those from rocky outcrops in southern Australia (Farrell, Szota, et al., 2013), coastal plant communities in Japan (Nagase & Tashiro-Ishii, 2018), local plants from the semiarid Northwest region of Cordoba, Argentina (Cáceres et al., 2018), and plants of dry prairies of Chicago, Illinois USA (Ksiazek-Mikenas et al., 2021). While annual plants have not

been widely investigated using this approach, Van Mechelen et al. (2014) identified a wide number of annual plant species which could be suitable for green roofs, in a survey of Mediterranean open habitat plant communities in the south of France. However, while they reported annual plants constitute a major part of Mediterranean vegetation and could be good candidates for roof greening, they have not been evaluated widely for their performance on green roofs.

1.2.3 Annual plant species

Annual plant species complete their life-cycle (germination, flowering, setting seeds and death) within one growing season (Dunnett, 2015; Mulroy & Rundel, 1977; Snodgrass & Snodgrass, 2006). Annual plants are often ruderal species, typical of high disturbance habitats, and are characterised by rapid growth, short life-span and high investment of resources in reproduction (seed production) (Grime, 1974; Pierce et al., 2017). Annuals, such as desert ephemerals, generally escape drought stress by completing their life-cycle before the onset of dry conditions (Hossain et al., 2016; Kooyers, 2015; Nemeskéri et al., 2012). Persistence of annual plant communities which escape drought stress is through dormant seeds which germinate when conditions are again favourable (Philippi, 1993).

Annuals can be divided up into two groups based on their germination and growth periods: winter annuals and summer annuals. Winter annuals germinate in autumn after a triggering rainfall event, overwinter as seedlings or immature plants, and flower in spring or early summer before dying in summer (Donohue, 2009; Newman, 1963). In contrast, summer annuals typically germinate after the first heavy rainfall in spring and seedling growth, flowering, seed maturation, dispersal and death occurs between spring and autumn (Donohue, 2009; Mulroy & Rundel, 1977). The germination of annuals is restricted to when soil moisture and temperatures are suitable (Bell, 1999; Gutterman, 1993; Mulroy & Rundel, 1977). Studies in Californian deserts, which receive both summer and winter precipitation, have shown winter annual seedlings germinate in moist soil under low temperatures, while summer annual seedlings emerge under high temperatures (Gutterman, 1993). Spring flowering species of northern Western Australia (winter annuals) germinate at lower temperatures in the glasshouse, while autumn flowering species (summer annuals) germinate at higher temperatures (Mott, 1972). In Australia, northern regions generally receive summer rainfall, while southern and western Australia receive winter rainfall, the rain that triggers the germination of annual species (Bell et al., 1993). In arid central Australia, however, rainfall is sporadic, and winter rainfall leads to

the germination of annual forbs, while summer rains favour the germination of grasses (Jurado & Westoby, 1992). The bimodal pattern of annual rainfall in arid regions (lower than 200 mm annual rainfall) of Western Australia has led to the evolution of both summer and winter annual floras in these regions (Mott, 1974).

1.2.4 Annual plants on green roofs

Using annual plants on green roofs has several potential advantages. Firstly, establishing an annual plant palette by sowing seeds allows a large area to be covered with little labour and potentially lower costs than planting (McDavid, 2012; Sutton, 2013). For example, Sutton (2013) showed that in the USA the labour cost of seeding native grasses on green roofs is 40% lower than planting *Sedum* plants as plugs and the cost of seeds and their transportation was also estimated to be lower. Establishing wildflowers from seeds (1 g m⁻²) on ground-level landscapes in urban environments in UK costs about £0.18 m⁻², while using nursery grown plants costs approximately £7.00 m⁻² (Scott, 2004).

Annuals also grow and flower rapidly, providing ground cover and nectar and pollen in the spring and early summer for pollinators, owing to their prolific and colourful flowers (Dunnett, 2015). Many annual species have visually attractive flowers and people are attracted to flowers on green roofs (Haviland-Jones et al., 2005; Lee et al., 2014). Annual plants can also reseed from year to year to enable their population to persist in the longer-term (Dunnett, 2015). Re-seeding is also a desirable characteristic as plants can colonise bare areas of substrate and prevent weed invasion (Zhang et al., 2014). Annuals can persist on green roofs over generations due to their ability to avoid harsh conditions by staying dormant as seeds (Dunnett, 2015; Thuring & Dunnett, 2019). This has been supported by Vanstockem et al. (2018) who found a high proportion of annuals' seeds in extensive green roofs seed banks. Annuals (with ruderal strategy) can also colonize spaces on green roofs which become available periodically and therefore remain present (Ksiazek-Mikenas & Köhler, 2018; Zhang et al., 2021). However, the success of annual plants on green roofs depends on the existing plant diversity. For example, a study on 129 extensive green roofs in Northern Belgium showed that annual species can only thrive on exposed roofs with a low planted functional diversity, because high functional diversity of plant communities on green roofs may decrease the coverage of spontaneously colonizing annuals by occupying a large number of niches (Vanstockem, Somers, et al., 2019).

Annual plants have been investigated on green roofs as communities with no other life-forms (Nagase & Dunnett, 2013; Vasl et al., 2019) or in combination with *Sedum* species (Kircher,

2004; Schindler et al., 2018; Schindler et al., 2019; Vasl et al., 2017) or herbaceous perennials (Dvorak, 2021; Ondoño et al., 2015) (Table 1.1). For example, Nagase and Dunnett (2013) evaluated a diverse annual flowering meadow on an unirrigated 7 cm-deep extensive green roof in Sheffield, UK. While this roof established and flowered for 3 – 4 months, they suggested that this plant palette may not be suitable for other climates and needed to be evaluated. Germination capacity and growth of a mixture of six annual and perennial semi-arid Mediterranean species was investigated in a pot experiment using three artificial green roof substrates. The results showed that some of the studied species (both annual and perennial) can provide permanent cover throughout the year (Ondoño et al., 2015). Also, as both annual and *Sedum* species offer some advantages on green roofs, Vasl et al. (2017) investigated the interaction between these two plant life-forms in a Mediterranean climate over three growing seasons. Strong competition between *Sedum sediforme* and annuals reduced annual species biomass and diversity, and reduced *S. sediforme* cover and flowering. However, the authors found that the combination of both *S. sediforme* and annual species results in longer plant coverage and flowering, and improved green roof services such as habitat provision, cooling, and stormwater runoff mitigation through greater water retention.

In hotter climates, solar panels are thought to increase green roof microhabitat diversity and soil moisture. Therefore, Schindler et al. (2018) evaluated annual plants growing with *Sedum* species in a non-irrigated green roof to explore the impact of solar panels on vegetation abundance and diversity. Solar panels decreased air temperature at ground level through shading (but not substrate temperature) and increased soil moisture heterogeneity (but not average soil moisture), resulting in greater *Sedum* growth and flowering duration of annual species (Schindler et al., 2018). Few studies have also evaluated annuals as a part of seed mixes sown on green roofs (Benvenuti, 2014; MacIvor et al., 2013; Olly et al., 2011) (Table 1.1).

Table 1.1. Summary of green roof publications using annual plants on green roof across different climate zones showing plant life-forms, the type of plant (plant origin), climate classification of where the study was conducted and the main research goals of the study.

Reference	Plants life-form	Type of plant	Köppen climate classification	Research focus
Nagase and Dunnett (2013)	Annual species	Both native and non-native species	Temperate Maritime (Cfb)	Exploring the possibility of establishing annual plant species on an extensive green roof through direct sowing, and finding an appropriate sowing rate and watering regime to use in the first growing season
Vasl et al. (2019)	Annual species	Local species	Mediterranean (Csa)	Investigating heterogeneity-diversity relationship by exploring the effect of substrate heterogeneity on plant mix diversity growing on green roof
Kircher (2004)	Annual and <i>Sedum</i> species	Not mentioned	Humid Continental (Dfb)	Achieving high plant coverage on green roof via direct sowing of annuals or planting <i>Sedum</i> cuttings and exploring the effect of fertilization on plant coverage, abundance and height
Vasl et al. (2017)	Annual and one <i>Sedum</i> species	Local species	Mediterranean (Csa)	Studying the interactions (competition and facilitation) of annual plants with a <i>Sedum</i> species growing on green roof
Schindler et al. (2018)	Annual and one <i>Sedum</i> species	Local species	Mediterranean (Csa)	Investigating the advantages of integrating photovoltaic panels into a green roof (mutual effects), including changes in vegetation and arthropod abundance and diversity as a result of the presence of solar panels, in addition to changes in electricity production due to the presence of vegetation on the roof
Schindler et al. (2019)	Annual and one <i>Sedum</i> species	Local species	Mediterranean (Csa)	Comparing the cooling effects of green roofs composed of <i>Sedum</i> only or <i>Sedum</i> + annual species
MacIvor et al. (2013)	Annual and perennial species	Both native and non-native species	Humid Continental (Dfb)	Evaluating the effects of irrigation (none, timed, and moisture sensor) and growing media (organic and FLL) on vegetative cover, above ground biomass and plant diversity of grass/forb plant mix and <i>Sedum</i> mix, growing on green roof
Benvenuti (2014)	Annual and perennial species	Native species	Mediterranean (Csa)	Evaluating a wildflower covered roof to explore the fitness of country landscape in urban environment, their agronomic performances, vegetation dynamics and biodiversity
Ondoño et al. (2015)	Annual and perennial species	Native species	Mediterranean Semiarid (BSH and BSk)	Assessing the germination capacity and subsequent development of plant species on different substrate types with different physio and biochemical properties
Olly et al. (2011)	Annual and perennial species + one <i>Sedum</i> species	Dominated by indigenous species	Temperate Maritime (Cfb)	Studying the potential of green roofs with low substrate depth to mitigate urban habitat loss, and the effects of substrate depth on plant assemblages in the first growing season

1.2.5 Australian annual plants for green roof applications

In recent years, there has been a great deal of interest in using native plants on green roofs (see Azeñas et al., 2019; Brandão et al., 2017; Kokkinou et al., 2016; Kolk et al., 2020; MacIvor & Lundholm, 2011; Mandel et al., 2016; Monterusso et al., 2005; Nagase & Tashiro-Ishii, 2018; Rayner et al., 2016; Razzaghmanesh et al., 2014; Rey et al., 2020; Williams, Hughes, et al., 2010). This is mainly because native species are adapted to the local environment, are less likely to become invasive, provide resources for native fauna, and aesthetically match the natural landscape (Butler et al., 2012). Australia is a continent with a high level of plant diversity and 85 – 92% of all plant species are endemic (Chapman, 2009; Hopper & Gioia, 2004). Annual plants contribute greatly to the plant diversity in many Australian ecosystems across different climates including arid regions of Western Australia and South Australia, chenopod shrublands of southern Australia (semi-arid Mediterranean), temperate eucalypt woodlands of southern Australia, and lowland temperate grasslands of south-eastern Australia (Beard, 2015; Keith, 2017) (Figure 1.2). As a result, their seed bank characteristics (Scott & Morgan, 2012b), germination (Dwyer & Erickson, 2016; Merritt et al., 2006; Morgan et al., 2020; Scott & Morgan, 2012a; Towers et al., 2022), functional traits and community assembly (Dwyer et al., 2014; Dwyer et al., 2015; Dwyer & Laughlin, 2017), competition/facilitation and coexistence (Bimler et al., 2018; Staples et al., 2016; Stouffer et al., 2018; Towers et al., 2020; Wainwright et al., 2018) have been a focus of research in recent years. For example, Towers et al. (2022) investigated the effect of light on seed germination of 12 annual plant species growing along a gradient of seed bank conditions, in southwest Western Australia. Information on germination characteristics and traits is essential to create annual plant mixes for direct seeding in horticultural applications such as green roofs. However, despite the diversity of annual plant species in Australia and their vast distribution, which likely reflects their adaptability to different environmental conditions, they have not yet been evaluated on green roofs.



Figure 1.2. Examples of annual plant species growing in Victoria, Australia: top left) *Rhodanthe corymbiflora*, Mount Korong National Conservation Reserve, top right) *Ptilotus exaltatus*, Terrick Terrick National Park, down) *Xerochrysum* sp. Mount Hope. Photos are taken by Zahra Saraeian during fieldwork investigating green roof habitat templates in northern Victoria

While annual plants provide opportunities for broadening the diversity of flowering plants on green roofs, there may be challenges with establishing and supporting annual plants on green roofs in Mediterranean-type climates or where summers are hot and dry (Du et al., 2019; Guo et al., 2021). The performance of annual plants on green roof will also vary depending on their germination, growth, drought resistance strategies and their interaction in communities.

1.3 Annual species germination attributes: Relationships among seed germination, seed mass and climate of natural distribution

Direct seeding is an effective way of establishing annual plants on green roofs and has been used in previous green roof research involving annuals. One of the main constraints for effective and efficient direct seeding relates to seed germination capacity (and the level of seed dormancy) (Merritt et al., 2007). This can be an issue when developing annual plant seed mixtures for green roofs in new regions using native plants. Many Australian plant species produce seed that is dormant upon maturity and will not germinate without proper pre-treatment (Dwyer & Erickson, 2016; Hoyle et al., 2008; Kildisheva et al., 2019). Therefore, increasing knowledge about the factors influencing germination (such as seed mass and climate variables) is helpful if species are to be used in horticultural applications like green roofs. Germination tests and the reported germination percentages also allow for the accurate calculation of sowing rates and a more realistic assessment of the performance of the seed lot after sowing.

Seed germination is a critical transition from the relatively safe seed stage into the more vulnerable seedling stage when plants are susceptible to environmental hazards (Larson et al., 2015; Yi et al., 2019). Germination and seedling establishment are key phases explaining plant species distribution patterns and community composition (Felton et al., 2021; Huang et al., 2016; Saatkamp et al., 2011). To cope with environmental unpredictability during establishment, many plants have developed some degree of seed dormancy which prevents germination (Cross et al., 2018; Willis et al., 2014).

Germination is controlled by both intrinsic (i.e., seed mass) and extrinsic (i.e., climate variables) factors (Dwyer & Erickson, 2016; Saatkamp et al., 2019). Seed mass is a fundamental characteristic of a plant's life history that influences seed germination (and dormancy) (Huang et al., 2016; Kahmen & Poschlod, 2008; Leishman et al., 2000), as seed size indicates nutrient reserves of the seed (Leishman et al., 2000) and has impacts on various stages of plant life-cycle, summarized in Table 1.2. Generally, larger seeds germinate faster and at higher rates than smaller seeds (Andersson, 1996; Cordazzo, 2002; Huang et al., 2016; Pake & Venable, 1996; Simons & Johnston, 2000), although there are exceptions (Bu et al., 2007; Harel et al., 2011; Wang et al., 2008). Research has also correlated larger seed size with higher germination under water limitation (Daws et al., 2008).

Several studies have shown relationships between seed mass and environmental factors including temperature (Dainese & Sitzia, 2013; Moles et al., 2014; Murray et al., 2004; Tautenhahn et al., 2008), precipitation (Dubuis et al., 2013; Stromberg & Boudell, 2013), light intensity and soil nutrients (Dainese & Sitzia, 2013; Leishman et al., 2000; Sonkoly et al., 2017). Harsher environments are usually associated with greater seed mass, as bigger seeds have a greater chance to successfully grow under adverse conditions. This is in accordance with Muller-Landau (2010) tolerance/fecundity trade-off, which is based on stress tolerance ability of large seeds. According to the tolerance/fecundity trade-off, benign environments, where most plants are able to grow and regenerate, are mostly occupied by small-seeded species due to their greater fecundity. Whereas large-seeded plants will capture the more stressful regeneration sites where small seeds are unable to recruit due to harsh and stressful conditions (Muller-Landau, 2010).

Table 1.2. A summary of seed size effects on plants in different life stages

Plant life stage	Seed size Effects	References
Seed production	Seed size/number trade-off	Leishman (2001)
Dormancy	Smaller seeds are more likely to be dormant	Huang et al. (2016); Leishman et al. (2000)
Seed dispersal type	Dispersal strategies (wind, animal, water, unassisted) are different for seeds with different sizes	Moles et al. (2007)
Seed persistence (or survival) in soil	Smaller seeds constitute higher proportion of soil seed bank, although this rule is not universal	Leishman and Westoby (1998); Thompson et al. (1993); Volis and Bohrer (2013)
Seedling emergence	Larger seeds have higher emergence rate due to their greater energy reserves that enables them to germinate from deeper burial depth	Benard and Toft (2008); Pearson et al. (2002)
Seedling survival	Large seededness is positively correlated with seedling establishment and survival	Coomes and Grubb (2003); Metz et al. (2010)
Subsequent growth to adulthood	The advantages of large seededness are long-lasting and continue to later stages i.e., reproduction	Martínez-López et al. (2020); Metz et al. (2010)

Seed germination and dormancy are also regulated by climatic factors such as temperature (Bell, 1999; Cross et al., 2018; Hicks et al., 2019; Ooi et al., 2009; Xu et al., 2014), precipitation

(Barga et al., 2017; Clauss & Venable, 2000; Torres-Martínez et al., 2017) and their variability (Donohue et al., 2010; Escobar et al., 2018; Rubio de Casas et al., 2017; Torres-Martínez et al., 2017; Yi et al., 2019). Seed germination rate is higher in warmer environments compared to cooler environments, since high temperatures accelerate dormancy release for many physically or physiologically dormant seeds (Ooi et al., 2009). For example, Cross et al. (2018) found that seeds of annual *Byblis* species collected from warmer regions of North Kimberley, Australia, had higher germination percentage than seeds of species from colder parts. Dwyer and Erickson (2016) also investigated six winter annual plant species growing in two climatically distinct woodland fragments of southwest Australian floristic region (wheatbelt subregion). They found higher germination probabilities for populations of species originating from northern sites with warmer temperatures in winter and summer and higher aridity level, compared to populations originating from cooler southern sites. For 134 common annual and perennial species of the eastern Tibetan Plateau, higher temperatures also significantly increased germination percentage (Xu et al., 2014). Conversely, perennial Crofton weed populations grown under lower temperatures in higher elevations of southwest China showed higher germination percentage compared to populations of lower elevations with higher temperatures (Li & Feng, 2009).

Studies investigating precipitation have found higher germination percentage with slower germination speed in species from mesic habitats compared to species from drier habitats. For example, Jurado and Westoby (1992) demonstrated that annual and perennial species of Australian arid zones germinated faster than species from mesic areas in Australia. They also found that fast germination was positively associated with low germinability. In addition, the results of a 4-year field study on desert annuals indicated that species from xeric environments have lower germination percentage (Clauss & Venable, 2000). In contrast, along an aridity gradient in the Mediterranean region, Harel et al. (2011) found higher germination fractions for annual species at drier sites. Variability in temperature and precipitation also affect germination through changing the onset of drought, such that species from unpredictable (highly seasonal) environments generally have lower germination percentage compared to species from more predictable environments (Martins et al., 2019; Rubio de Casas et al., 2017).

Seeds of Australian native species have different types of dormancy and therefore there is no standard method for improving their germination (Erickson, 2015). Germination is a critical stage in annual species life-cycle and seed mass and climate variables have undisputable

impacts on it, as discussed before. Although there has been research on Australian annual species germination, there are still many other species which have not been studied and there is no information on their germination and the relationship between their germination, seed mass and climate of their natural distribution. This information would increase our knowledge regarding plant selection for seed-based projects (such as green roofs) in different climatic regions by predicting seed germinability from easily accessible climate data and seed size.

1.4 Annual species life strategies: Relationships among plant functional traits and performance metrics (relative growth rate, shoot biomass and flowering time)

After successful germination, plant species need to survive the harsh conditions on green roofs to provide ecosystem services, which relies on healthy vegetative growth and high coverage (Azeñas et al., 2018; Lundholm et al., 2015). Drought is one of the main challenges that plants need to overcome to survive and grow successfully on green roofs (Du et al., 2019; Rayner et al., 2016). Plant species have adapted to drought by evolving different strategies. Species with drought avoidance and tolerance strategies develop leaves with dense hairs and thick waxy cuticles, lower stomatal conductance or tiny leaves with smaller and less stomata, lower specific leaf area (SLA) and higher leaf dry matter content (LDMC), and deeper root systems to resist drought (Chu & Farrell, 2022; Hossain et al., 2016; Ilyas et al., 2021). While these traits (conservative or ‘slow’ traits) enable the species to cope with water limitations, they come at the price of reduced growth (Chu & Farrell, 2022; Hossain et al., 2016; Liu et al., 2012). Drought avoidance and tolerance strategies are mainly employed by perennial species, whereas annual species escape drought stress by completing their life-cycle before drought and survive as seeds in the soil seed bank (Berger et al., 2016; Volaire, 2018). Thus, they use their resources for rapid growth and flowering and employ an acquisitive resource-use strategy with ‘fast’ traits such as thinner leaves with high SLA and low LDMC (Kooyers, 2015; Matesanz et al., 2020; Shavrukov et al., 2017).

Along with coping with drought conditions on green roofs, it is preferable for plant species to provide rapid growth and coverage and have flowers, as these features improve cooling (Franzaring et al., 2016), rainfall retention (Nagase & Dunnett, 2012), aesthetic value and habitat provision (Benvenuti, 2014) on green roofs. Previous studies on annual species have demonstrated that annual species can achieve high coverage and biomass on green roofs, even when water is limited (Nagase & Dunnett, 2013).

While annual species generally employ ‘fast’ traits and accelerated growth and reproduction when exposed to stress, their vast distribution over climatically distinct environments (Blumenthal et al., 2020; Kooyers et al., 2015; Matesanz et al., 2020) indicates that annuals likely have diverse functional traits and strategies to establish, grow, and reproduce. For example, 30 annual species distributed along a rainfall gradient (mean annual rainfall between 120-580 mm yr⁻¹) exhibited two- to five-fold variation in their interspecific leaf traits and 26-fold variation in their growth rate (Kurze et al., 2021). Populations of California wild radish, growing along an aridity gradient (~ 290 mm annual precipitation and 11.5 °C average temperature to 1200 mm and 17 °C), also exhibit significantly different intraspecific trait values associated with drought escape and drought tolerance strategies (Welles & Funk, 2020). These studies indicate the possibility of interspecific and intraspecific trait variability and diverse plant strategies in annual species.

Functional traits determine the position of plant species along a trade-off between resource acquisition and conservation (or ‘fast-slow’ spectrum), drought resistance level and competitive and reproductive ability (Reich, 2014; Westoby, 1998; Wright et al., 2004). These are important strategies of plant species to survive and grow successfully on green roofs as well. As functional traits and plant growth and survival relate to the ecosystem services plant species provide on green roofs (Lundholm et al., 2015; Xie et al., 2018), understanding the trait variation in Australian annual plant species is important to evaluate their suitability for green roofs.

1.5 Annual species on green roofs: Irrigation frequency for a diverse plant community

High plant diversity is desired on green roofs due to the greater ecosystem services and visual appeal compared to monocultures or low-diversity plant communities (Cardinale et al., 2011; Lundholm, 2015). Diverse plantings on green roofs are also likely to support higher faunal diversity, including insects and animals such as bees, soil invertebrates and birds (Knapp et al., 2019). Higher plant diversity could also increase long term performance of green roofs by reducing plant community susceptibility to climatic fluctuations, diseases, and weed invasion (Heim & Lundholm, 2014; MacIvor et al., 2016). In order to create a diverse green roof community, Cook-Patton and Bauerle (2012) suggested that considering species functional traits is more useful than increasing species richness *per se*, as functional diversity, the extent of trait dispersion within a community, is a central element of biodiversity, and strongly determines ecosystem functioning (Cadotte et al., 2011; Díaz & Cabido, 2001; Petchey &

Gaston, 2002). Plant communities with higher functional trait diversity would likely provide greater ecosystem services on green roofs (Cook-Patton & Bauerle, 2012; Van Mechelen, Van Meerbeek, et al., 2015; Xie et al., 2018). For example, in a modular experiment on rooftops in Toronto, MacIvor et al. (2018) showed that increasing phylogenetic diversity of plant communities (as a predictor of functional diversity) reduced mean roof surface temperature (higher roof cooling) and increased rainwater capture (better stormwater management).

In natural ecosystems, plant functional traits and their composition (calculated by community weighted mean trait values and functional diversity) generally change along environmental gradients due to filtering on the regional species pool. Plant communities evolving under contrasting habitat conditions will have different plant trait values (Cunningham et al., 1999; Dwyer & Laughlin, 2017; Lhotsky et al., 2016; Reich et al., 2003; Westoby et al., 2002; Wright et al., 2002; Xu et al., 2018). Water availability is one of the main drivers of contrasting habitat conditions and niche segregation, causing differences in species composition and plant trait distribution (Cornwell & Ackerly, 2009). For example, plants in drier habitats usually have shorter stature and smaller leaves (Gross et al., 2013; Mc Donald et al., 2003; Nunes et al., 2017). Water availability also affects species diversity, abundance, and cover (Miranda et al., 2009; Vahdati et al., 2017). Consequently, water availability influences the community weighted means of functional traits and functional diversity in both natural and green roof ecosystems (Azeñas et al., 2018; Nunes et al., 2017). This effect is mainly due to the differences in plants' drought resistance strategies and their functional traits (Griffin-Nolan et al., 2019; Nunes et al., 2017).

Leaf traits and plant height are frequently used to evaluate functional diversity in natural (Midolo et al., 2021; Spasojevic & Suding, 2012) and green roof ecosystems (Vanstockem, Bastiaens, et al., 2019; Xie et al., 2018). These traits are important due to their contribution to ecosystem services such as stormwater capture, cooling, and warming effects (substrate temperature), nutrient uptake and aboveground biomass and canopy density (productivity indices) (Lundholm et al., 2015; Xie et al., 2018). For example, a positive relationship was found between the ability to capture water runoff and plant height for twelve species of sedums, forbs and grasses (Nagase & Dunnett, 2012). Lundholm et al. (2015) also reported positive relationships between plant height and specific leaf area (SLA) with canopy density, with higher canopy density resulting in warmer substrate temperature in winter. Therefore, plant height and SLA were positively related to winter substrate temperature.

Water scarcity is one of the main factors that make extensive green roofs hostile for plants, especially during hot and dry summer months (Williams et al., 2021). Irrigation is needed on green roofs for the survival and growth of non-succulent plant species, especially in regions with Mediterranean-type climates (Du et al., 2019; Guo et al., 2021; Rayner et al., 2016; Zhang et al., 2014), and during the establishment phase (Nagase & Dunnett, 2013; Van Mechelen, Dutoit, et al., 2015a; Vandegrift et al., 2019). For example, in Melbourne, Australia, Du et al. (2019) evaluated 15 shrub species (from a broad range of climates) growing in unirrigated green roof modules and found that the hot and dry conditions in summer resulted in the mortality of many shrubs and suggested summer irrigation was essential for green roofs in hot and dry climates. In humid continental East Lansing, Michigan, USA, Vandegrift et al. (2019) evaluated 22 plant species including native, drought tolerant herbaceous perennials and grasses, and sedums, for eight years on ~ 5, 10 and 20 cm deep green roofs irrigated only for establishment. While all five *Sedum* species were alive after 8 years, only seven of the 17 herbaceous perennials and grasses were alive. Zhang et al. (2021) reported that only one of the 18 forb species originally planted on an extensive green roof in Beijing, China, with temperate monsoon climate could survive the unirrigated conditions on green roof, due to the seasonal climate and spring drought. This indicates that irrigation is likely to be needed to establish annuals on extensive green roofs in hot and dry climates like southern Australia.

However, given the scarcity of water for irrigation (Williams, Rayner, et al., 2010), there is a gap in research regarding how much irrigation is needed to maintain diverse annual plant mixtures with good coverage and high functional diversity, and consequently provide high levels of ecosystem services and visual appeal.

1.6 Research objectives and thesis outline

The overall objective of this thesis is to develop an annual-based green roof planting palette for Australian conditions. This requires understanding: 1) species differences in germination, dormancy, and seed traits 2); species differences in plant functional traits and life strategies (e.g., drought resistance, resource acquisition and reproduction); and 3) how plant establishment, coverage, species abundance and richness (community composition) and functional diversity of a direct-seeded mixture of annual plants is affected by water availability (irrigation).

This thesis consists of this introduction, three experimental chapters and a concluding synthesis chapter. The objectives of the three experimental chapters (Figure 1.3) are to:

- 1- Determine if seed mass and germination percentage and germination speed of Australian annuals changes according to climate variables (temperature, rainfall and seasonality) of where they are naturally distributed (*Chapter 2*). For this purpose, I evaluated the germination (germination percentage, lag and rate) of 58 Australian annual plant species under different treatments and related it to their seed mass and the climate of their natural distribution.
- 2- Describe the strategies of Australian annual species by investigating their functional traits and relationships with their performance metrics (relative growth rate, shoot biomass and flowering time) (*Chapter 3*). I evaluated 18 Australian annual species in a common garden experiment and measured their functional traits (related to resource acquisition, drought resistance and reproduction) and plant performance metrics to inform their applicability on green roofs.
- 3- Evaluate the effect of irrigation frequency on plant cover, species abundance and richness, and functional diversity of a mixture of sown annual species under green roof conditions (*Chapter 4*). To achieve this, I sowed a mixture of 16 Australian annual species onto 30 green roof modules and irrigated them with three different irrigation frequencies: 2 days, 4 days and 6 days between irrigation. I evaluated their coverage and diversity in response to watering.

The synthesis chapter integrates the key results of my thesis, discusses implications for green roof design and outlines the limitations and future research opportunities.

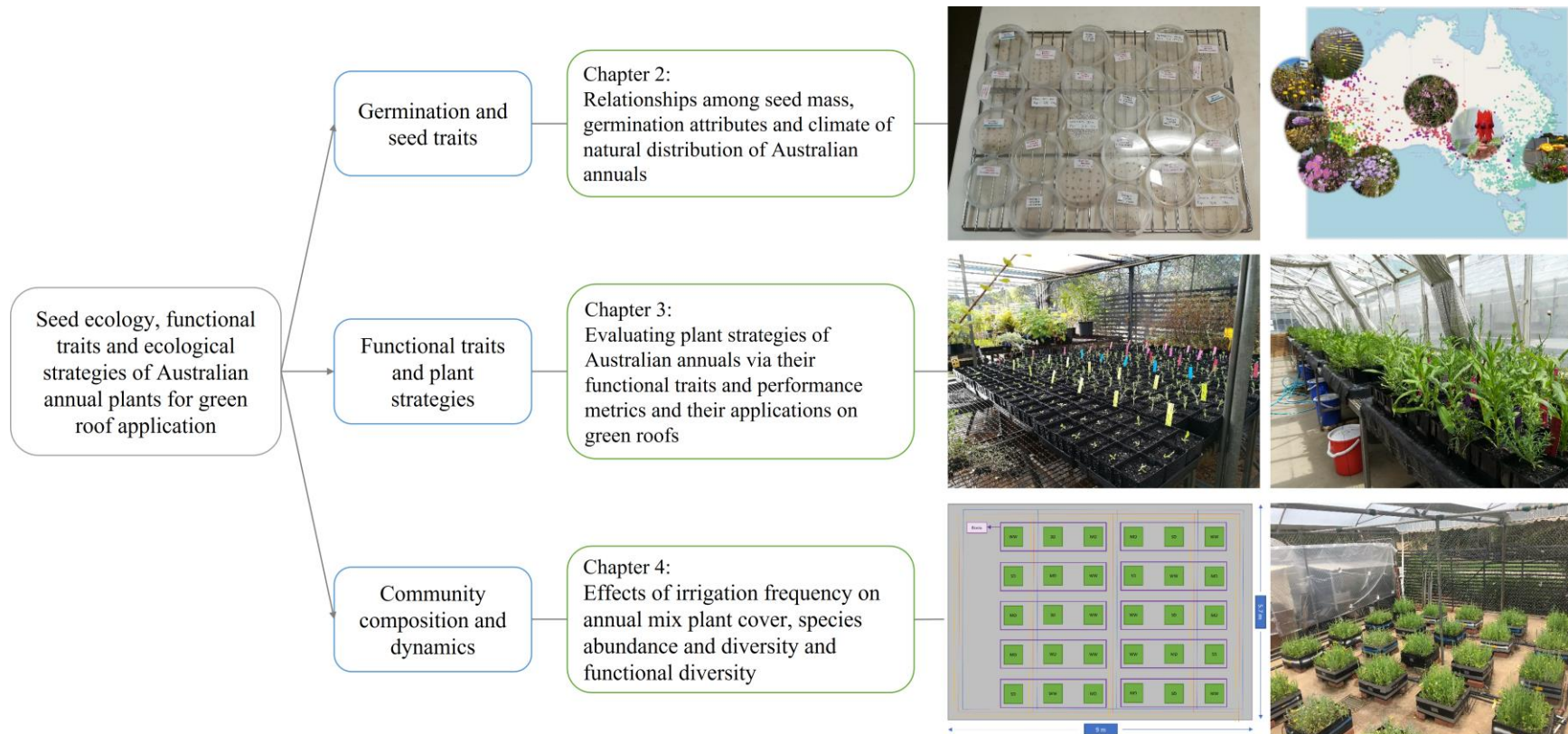


Figure 1.3. Thesis framework comprised of three data chapters illustrating research objectives addressed in each chapter.

2 Relationships between seed mass, germination attributes and climate of natural distribution in Australian annual species

Abstract:

Germination, a critical stage in plant's life-cycle, is under the strong influence of climate and seed reserves. Considering the importance of this stage, we explored the relationships between seed mass, germination attributes (germination percentage, lag, and rate) and the climate of species' natural distribution of Australian annual species. For this purpose, seeds of 58 Australian annuals from over a wide range of habitats were sourced from seed companies and seed mass was measured following protocols. Climate data for the species' natural distribution were obtained from Atlas of Living Australia. Germination tests were performed under different treatments in growth chambers to break the potential dormancy of species. The following relationships were explored using statistical analyses: (1) seed mass and climate of natural distribution, (2) seed mass and germination attributes and (3) germination attributes and climate of natural distribution. We found greater seed mass in species from hotter, drier, and/or environments with higher annual temperature variation. We found no relationship between seed mass, and germination percentage and speed. Lower germination was observed in species from hotter and drier environments as well as environments with more variable rainfall. Moreover, germination speed was greater in species from drier environments and environments with lower climate variability. In conclusion, climate has stronger relationships with germination attributes than seed mass. In dry environments, the trade-off between germination percentage and speed (lower germination percentage with faster germination speed) ensures a future seed bank; while in climates with high rainfall seasonality, bet-hedging has evolved to avoid complete germination prior to unfavourable conditions therefore avoiding seedling mortality and providing a seed bank when conditions improve.

2.1 Introduction

Germination and seedling establishment are two critical phases in a plant's life-cycle and can determine species distribution patterns and community composition (Huang et al., 2016; Saatkamp et al., 2011). Germination is strongly influenced by the surrounding environment (Donohue et al., 2010) as seeds require specific environmental cues to trigger germination. These differences in germination patterns of viable seeds indicate adaptation to the environment a given species may experience over time (Martins et al., 2019). For example, in environments with unfavourable or unpredictable climates (e.g., deserts) a fraction of available seeds do not germinate, even under optimal conditions, and remain in a state of dormancy. This phenomenon has been coined *bet-hedging* (Finch-Savage & Leubner-Metzger, 2006; Gremer et al., 2016), a strategy which helps buffer plant populations in the face of adverse conditions post germination (Long et al., 2015). Seed dormancy must be alleviated so that seeds can respond to environmental or germination cues (Downes et al., 2014; Merritt et al., 2007). Temperature is an important regulator of seed germination (Baskin & Baskin, 1998; Bell, 1999; Xu et al., 2014), and generally, in environments with warmer mean temperatures, species have seeds with lower dormancy level compared to species in cooler environments (Fernández-Pascual et al., 2013; Huang et al., 2014; but see Li & Feng, 2009). Other environmental factors such as precipitation and light also influence seed germinability and dormancy (Barga et al., 2017; El-Keblawy, 2017; Torres-Martínez et al., 2017). Generally, species from mesic environments have lower level of dormancy in their viable seeds, but slower germination speed compared to species from drier environments (Clauss & Venable, 2000; Fernández-Pascual et al., 2013; Xu et al., 2014). Climate seasonality is another factor affecting seed germination through its direct effect on soil moisture and onset of favourable conditions (Donohue et al., 2010; Rubio de Casas et al., 2017; Yi et al., 2019). Generally, species from environments with higher climatic seasonality have lower immediate germination percentage after dispersal, due to a higher level of seed dormancy as a bet-hedging strategy in response to climatic unpredictability, compared to species from more predictable environments (Martins et al., 2019; Rubio de Casas et al., 2017).

Along with climatic factors, seed mass has long been regarded as a key trait influencing germination (Kahmen & Poschlod, 2008; Rubio de Casas et al., 2017). Seed mass differs among species due to differences in embryo nutrient reserves provisioned by the maternal plants (Wang et al., 2016) in which larger seeds generally germinate faster and in higher proportions than smaller seeds (Huang et al., 2016; Kahmen & Poschlod, 2008). Variation in

seed mass across taxa can be related to differences in environmental conditions (Dainese & Sitzia, 2013; Moles et al., 2007). Associations between seed mass and environmental factors have been reported for temperature (Dainese & Sitzia, 2013; Moles et al., 2014), precipitation (or moisture availability) (Dubuis et al., 2013; Stromberg & Boudell, 2013), light / shade and soil nutrition (Leishman et al., 2000). In this context, greater seed mass is often associated with less favourable or harsher environmental conditions (reviewed in Guo et al., 2010). For example, greater seed mass is generally associated with higher annual mean temperature and/or lower annual rainfall. However, this pattern does not always hold as some studies have found negative or no significance relationship between seed mass and aridity (Gallagher & Leishman, 2012; Moles et al., 2014; Swenson et al., 2012). The probability of surviving adverse conditions is higher for seedlings originating from larger seeds, through seedling size effects (bigger seeds develop larger seedlings), reserve effects (larger seeds deploy their reserve more slowly) and/or metabolic effects (lower potential relative growth rate of larger seeds is preferable under unfavourable conditions) (Leishman et al., 2000). In addition, larger sized seeds have greater reserves to allocate to root development providing access to soil moisture at greater depths (Leishman & Westoby, 1994). Consequently, species with bigger seeds are more likely to successfully grow and reproduce in harsh environments. Following on, species with smaller seeds may be excluded or filtered from habitats with harsh climates.

Prior research has highlighted a connection between seed mass, germination, and climatic conditions. However, it is important to test all three factors together rather than individually to reveal interactive effects (Dainese & Sitzia, 2013; Fernández-Pascual et al., 2013; Martins et al., 2019). To better understand these relationships, and to explore the inconsistencies found in previous research, in this study we use species' natural distribution rather than one specific habitat or ecosystem (e.g., deserts) by using broad climatic gradients that include multiple climatic factors.

Seed mass and seed germination are important traits in annual plants, as the seed unit is the connecting link from one year to the next (Gremer et al., 2016). Seed mass, dormancy and germination strategies, phenotypic plasticity and community composition of annual species, are the subject of numerous studies in different climates from arid (El-Keblawy, 2017; Gremer et al., 2016) to semi-arid (Scott & Morgan, 2012a), semi-arid Mediterranean (Jimenez et al., 2011), Mediterranean (Azcarate et al., 2010; Dwyer et al., 2015), continental (Rees et al., 1996), temperate continental (Yi et al., 2019), and cold-humid Alpine areas (Bu et al., 2018). Globally, annuals are widely distributed and occur in many different habitats such as deserts

(Angert et al., 2010; Clauss & Venable, 2000), grasslands (Arellano & Peco, 2012), coastal dunes (Coomes et al., 2002; Rees, 1995), wetlands and vernal pools (Carta et al., 2013; Kraft et al., 2014), and mountains (Chen et al., 2018). Annuals are an often-overlooked life-form despite being an important component of many plant communities. Few studies have investigated how seed mass and germination attributes of annual plants vary with climate.

Considering the potential interacting effects of climate and seed mass on germination, we investigated how seed mass and germination attributes relate to the climate of natural distribution for Australian annual species from different environments (with disparate climates). Australia has a wide-ranging climate, from arid, to temperate and tropical (Peel et al., 2007). Average daily annual mean temperature generally decreases along the latitudinal gradient from north to south and aridity increases from coastal to interior regions (Bureau of Meteorology, 2016). Such broad climatic gradients provide a useful framework to relate differences in seed mass and germination attributes of annual plants to the climate of their natural distribution.

In this study, we explored relationships among seed mass, germination attributes (germination percentage, lag, and rate), and the climate of natural distribution for 58 Australian annuals. We hypothesised that seed mass would be greater in annual plants distributed in hotter and drier environments and that germination percentage would be lower in species from cooler and/or drier environments and less climatically predictable environments. And further, we were interested if faster germination occurred for species from drier compared to wetter environments.

2.2 Materials and methods

2.2.1 Seed mass and viability

Seeds of 58 Australian annual species from eight families (Appendix A1) that occur across Australia in a wide range of habitats and climates (Appendix A2) were sourced commercially from “Nindethana” (<http://www.nindethana.net.au>) and “Australian Seed” (<http://www.australianseed.com>) seed companies in 2017 – 2018. The vast range of distribution across different habitats of these species and their short, unpredictable lifecycles made it impractical to collect the seeds directly for our study. Seeds were stored in a refrigerator at 6 °C prior to viability and germination testing.

Seed mass was defined as the oven-dry weight of seeds after removing all its accessories (e.g., wings, pappus, comas). For seed mass, four replicates each with 50 seeds, or one replicate of 100 seeds (depending on seed size or availability) for each species was oven-dried at 80 °C for 72 hrs and weighed to obtain one-seed weight (mg) (Pérez-Harguindeguy et al., 2013). Seed viability (the proportion of germinable seeds) was determined using a cut test (Commander et al., 2009) on three replicates of 20 seeds per species (Appendix A1). The presence or absence of endosperm was evaluated by sectioning seeds under a dissecting microscope. Filled seeds (seeds containing fully developed embryos/endosperm) were considered viable, while empty seeds or seeds with shrivelled endosperm were considered non-viable.

2.2.2 Germination tests

To quantify germination of each species, four replicates of 25 randomly selected seeds (unsterilized) were placed on the surface of 1% agar in a 9 cm petri dish and incubated in a growth chamber (providing cool white, fluorescent light). Petri dishes were sealed with parafilm to prevent evaporation.

Germination was scored every day for 31 days and defined as the emergence of the radicle from the seed coat. Germination attributes determined in this experiment were:

1. Germination percentage (GP), which was calculated as follows:

$$GP = \frac{\text{number of germinated seeds}}{(\text{number of seeds per treatment} \times \text{Percent viability})} \times 100$$

2. Germination lag (LAG time), days to first germination from the beginning of the experiment.

3. Germination rate (T_{50}), days taken from the commencement of the experiment to germination of 50% of all germinated seeds (Coolbear et al., 1984).

To alleviate any potential dormancy and trigger germination, and achieve the highest germination possible for each species, germination tests were conducted using five different combinations of incubation temperature and germination stimulants. For all tests, the light conditions in the chambers were maintained at a 12/12 h light/dark photoperiod. Temperature regimes for the germination treatments were informed by prior research on Australian species (Aleman et al., 2015; Bell, 1999; Facelli et al., 2005; Jurado & Westoby, 1992; Morgan, 1998; Plummer & Bell, 1995). Two germination stimulants, gibberellic acid (GA_3) and karrikinolide (KAR1; 3-methyl-2H-furo [2,3-c] pyran-2-one; a smoke derived compound) were used to break the dormancy and/or promote germination as these have been shown to be commonly used for these purposes in Australian species (Aleman et al., 2015; Commander et al., 2009; Hoyle et al., 2008; Probert et al., 2009). The five germination test treatments were:

1. Control 20/10 °C: Untreated seeds with alternating temperature regime of 20/10 °C.
2. Control 25/15 °C: Untreated seeds with alternating temperature regime of 25/15 °C.
3. Gibberellic acid 20/10 °C: Seeds pre-soaked overnight in a 250 mg/L GA_3 solution before moving to agar plates, then incubated at 20/10 °C.
4. Gibberellic acid 25/15 °C: Seeds pre-soaked in 250 mg/L GA_3 solution incubated at 25/15 °C.
5. Karrikinolide 20/10 °C: Seeds pre-soaked for 24 h in a 0.67 μ M concentration of KAR1 before moving to agar plates, then incubating at 20/10 °C.

2.2.3 Climate variables

For each species, the climate of their natural distribution was determined using 16 bioclimatic variables (Xu & Hutchinson, 2011) obtained from each known location (occurrence point) in Australia using the Atlas of Living Australia (ALA) (<http://www.ala.org.au>) (Table 2.1). These variables express temperature (degree Celsius), precipitation (mm), radiation ($Mj/m^2/day$) and moisture index (0: dry – 1: saturated) data from 1960 – 1990. The mean value for each climate variable was calculated from all distribution records of each species. These measures were used to express the potential range of climate experienced by each species across their natural distribution to explore how mean values of climate variables relate with seed mass.

2.2.4 Statistical analysis

One-way ANOVA and Tukey's post-hoc tests (P -value < 0.05) were used to analyse differences in germination attributes of each species among treatments. For this analysis, the proportion corresponding to the germination percentage (GP) was logit transformed, and seed mass and T_{50} were log-transformed before analysis to meet assumptions of statistical tests. Before transformation, 0.5% were decreased from 100% germination percentage values and 0.5% were added to 0% germination percentage values to make the transformation possible. As LAG time data did not satisfy assumptions of normality even after transformation, non-parametric Kruskal-Wallis and Mood's Median tests were performed to analyse differences among treatments.

The germination attributes (GP, LAG time and T_{50}) were used as response variables in separate linear mixed effect models (MMs), with plant species as random factors and treatments as fixed factors, to determine significant differences among treatments. Fisher's LSD post-hoc tests were performed if the P -value of the treatments were significant (P -value < 0.05). Log-transformed LAG time was used for the purpose of this analysis.

Relationships between seed mass and climate variables were investigated using simple linear regressions.

Principal components analysis (PCA) was performed on climate variables to reduce the relatively large number of correlated variables into a smaller set, for examining the association between climate and seed mass with germination attributes. Generalized linear models were used to explore relationships among multivariate climate variables (PC1, PC2 and PC3 generated from the PCA) and seed mass with germination attributes (GP, LAG time and T_{50}), with *Gaussian* error distribution and *identity* link function for GP (logit transformed) and *Gamma* distribution with *inverse* link function for LAG time and T_{50} . Mean values of all germination treatments were used in these models. Including seed mass in our model was a way of partly controlling a potential confounding factor in our study i.e., different maternal provisioning among our plant species. Species with zero germination in all the treatments were excluded from these analyses.

Following the PCA, to compare the germination attributes of plant species from different natural distributions, a hierarchical cluster analysis with Euclidean distance and complete linkage was performed on mean values of 16 climate variables for the 20 germinated species using *Factoextra* package in R programming language (Kassambara & Mundt, 2017), to cluster

the species into climate groups, with species from more similar climate assigned to the same group (Figure 2.2, Appendices A2 and A3). One-way ANOVA and Tukey's post-hoc tests (P -value < 0.05) were used to compare the differences in germination attributes among different climate groups.

Statistical analysis was carried out in MINITAB v.19 and R statistical software version 4.1.2 (R Development Core Team, 2018). Figures were created using Microsoft Excel and *ggplot2* package (Wickham, 2016) within R.

2.3 Results

2.3.1 Seed mass and climate of natural distribution

For the 58 species used in this study, seed mass ranged from 0.0002 mg (*Isotoma scapigera*) to 6.1173 mg (*Swainsona formosa*; Appendix A1). The 58 species were distributed over a wide range of climates (Figure 2.1). Annual mean temperature ranged from 13.45 – 26.09 °C (*Craspedia variabilis* and *Heliotropium paniculatum* respectively) and annual precipitation had a range of 236.68 – 816.67 mm (*Calotis cymbacantha* and *Asteridea pulverulenta* respectively). The species also came from areas with different rainfall predictability, as precipitation seasonality varied from 18.61 – 105.22% (*Xerochrysum viscosum* and *Heliotropium paniculatum* respectively).

Seed mass was positively correlated with temperature and radiation variables (Figure 2.1), including temperature annual range ($R^2 = 0.26$, P -value < 0.001), mean temperature of the wettest quarter ($R^2 = 0.11$, P -value = 0.010), mean temperature of the warmest quarter ($R^2 = 0.13$, P -value = 0.005), and annual mean radiation ($R^2 = 0.12$, P -value = 0.009). Seed mass was negatively correlated with variables related to precipitation and soil moisture (Figure 2.1), including annual precipitation ($R^2 = 0.16$, P -value = 0.002), precipitation of the wettest quarter ($R^2 = 0.16$, P -value = 0.002), precipitation of the coldest quarter ($R^2 = 0.18$, P -value = 0.001), and annual mean moisture index ($R^2 = 0.18$, P -value = 0.001). Seed mass was also negatively related to isothermality ($R^2 = 0.22$, P -value < 0.001) and positively related to temperature seasonality ($R^2 = 0.27$, P -value < 0.001). However, the range of values for these two variables were very narrow in our distribution sites (0.443 – 0.532 and 1.01 – 2.11% respectively), therefore, temperature variability in our study was mainly defined by annual temperature range (Table 2.1).

Table 2.1. Bioclimatic variables, attributing codes, and definitions (from ANUCLIM version 6.1) expressing the climate of natural distribution of 58 Australian annual species across Australia (obtained from the Atlas of Living Australia, 2018).

Bioclimatic variable code	Variable	Definition
Bio01	Annual mean temperature	---
Bio03	Isothermality	The mean diurnal range (Bio02) divided by the annual temperature range (Bio07)
Bio04	Temperature seasonality (CV)	The temperature Coefficient of Variation (C of V) is the standard deviation of the weekly mean temperatures expressed as a percentage of the mean of those temperatures
Bio07	Temperature annual range	Max-Min or Bio05-Bio06
Bio08	Mean temperature of wettest quarter	---
Bio09	Mean temperature of driest quarter	---
Bio10	Mean temperature of warmest quarter	---
Bio11	Mean temperature of coldest quarter	---
Bio12	Annual precipitation	---
Bio15	Precipitation Seasonality (CV)	The Coefficient of Variation (C of V) is the standard deviation of the weekly precipitation estimates expressed as a percentage of the mean of those estimates
Bio16	Precipitation of wettest quarter	---
Bio17	Precipitation of driest quarter	---
Bio18	Precipitation of warmest quarter	---
Bio19	Precipitation of coldest quarter	---
Bio20	Annual mean radiation	---
Bio28	Annual mean moisture index	Calculated from precipitation and evapotranspiration values obtained from the climate surfaces in conjunction with the soil type and maximum soil water availability values. It is a measure of relative soil moisture, ranges from 0 (dry) to 1 (saturated)

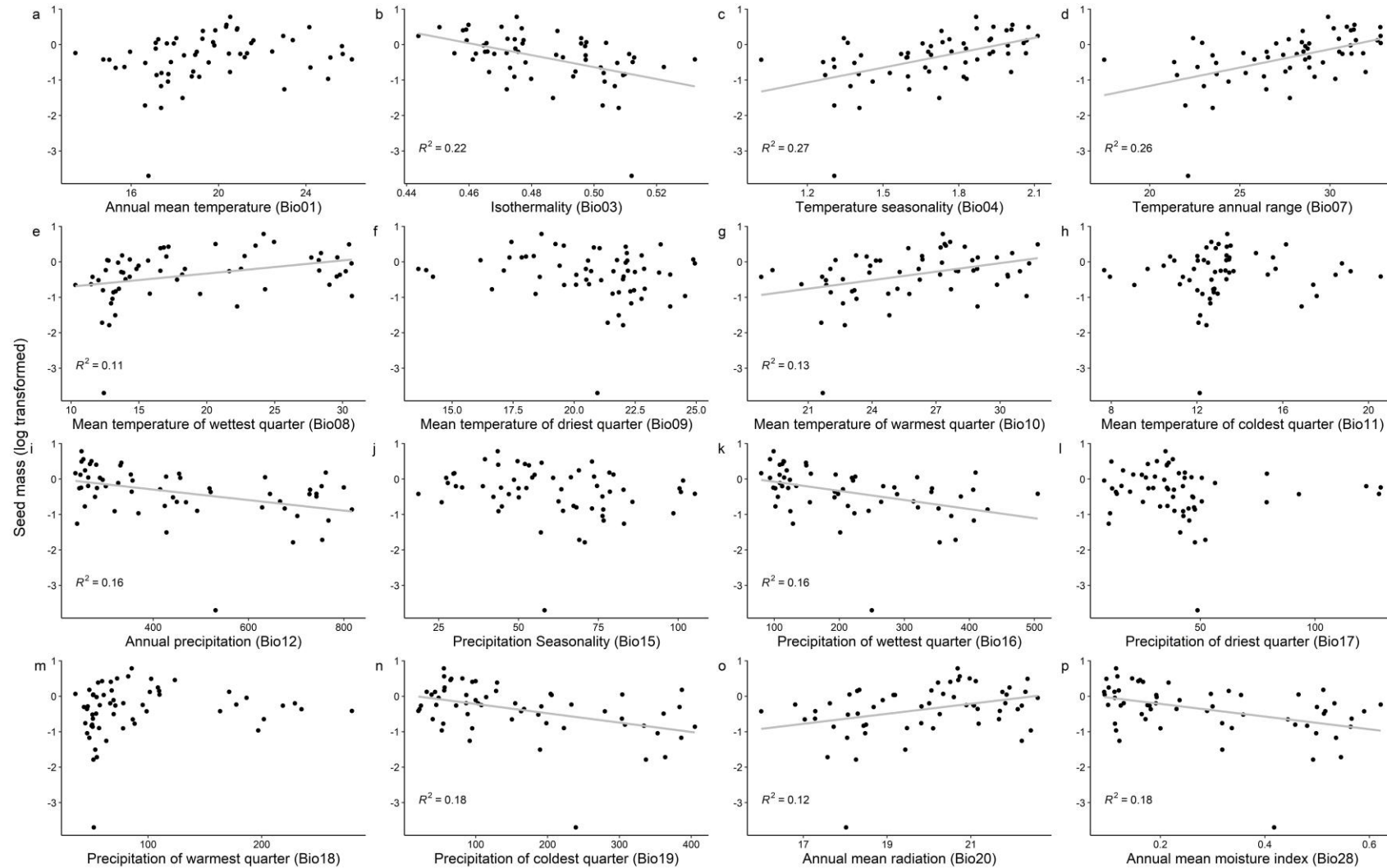


Figure 2.1. Relationships for 58 Australian annual species between their mean seed mass (log-transformed; y-axis) and 16 bioclimatic variables of their natural distribution across Australia (obtained from the Atlas of Living Australia, 2018). Regression line and coefficient of determination (R^2) are shown for significant relationships (P -value < 0.05).

2.3.2 Germination attributes

Viability testing across all species showed 60 – 100% seed viability. However, only 20 of 58 species germinated in the germination tests (Appendix A1) regardless of treatments applied (Appendix A4). Three of the 20 species, *Goodenia microptera*, *G. vilmorinae* and *Heliotropium tenifolium*, showed dormancy and only germinated in treatments with germination stimulants (GA₃ or KAR1; Appendix A4). Of the species that germinated, germination percentage (mean values) in all tests ranged from 6 to 98%, LAG time ranged from 1 to 19 days and T₅₀ from 1.1 to 15.5 days (Appendices A4 - A6).

Mixed model analysis of germination for the 20 species which germinated in our study showed no significant difference (P -value = 0.293) in GP between incubation temperatures (20/10 °C and 25/15 °C), whereas there were significant differences (P -value < 0.001) among non-treated, gibberellin-treated, and karrikinolide-treated seeds, with karrikinolide-treated seeds having the highest germination percentage (Appendix A7). However, when the three dormant species (*G. microptera*, *G. vilmorinae* and *H. tenifolium*) which required germination stimulants were removed from the analysis, there was no difference among non-treated, gibberellin-treated and karrikinolide-treated seeds.

LAG time and T₅₀ were highly correlated ($R^2 = 0.73$, P -value < 0.001), therefore, we refer to them collectively as germination speed. In general, species incubated at higher temperatures (25/15 °C) had significantly (P -value < 0.001, appendix A7) higher germination speed (lower LAG time and T₅₀) compared to those incubated at lower temperatures (20/10 °C) (Appendix A7). Among germination stimulants, KAR1 and GA₃ significantly (P -value < 0.001) increased germination speed (decreased LAG time and T₅₀) compared to untreated seeds (Appendix A7).

2.3.3 Seed mass and germination attributes

While relationships between seed mass and climate variables were performed on all 58 species in our study, we were not able to use all species to look at relationships among seed mass and germination attributes, due to lack of germination in germination tests. Seed mass varied 90-fold among the 20 species which germinated (Appendix A1). However, mixed effect models showed that seed mass was not related to any of the germination attributes in our study (Table 2.3).

2.3.4 Germination attributes and climate of natural distribution

Principal component analysis was used to reduce the number of climate variables to examine how multiple climate variables together relate with germination attributes. The PCA created three meaningful principal components (eigenvalues > 1) which together explained 95.4% of the variation in climate variables (Table 2.2).

Table 2.2. Principal component analysis (PC1, PC2 and PC3) of 16 bioclimatic variables related to the natural distribution of the 20 Australian annual species in this study across Australia (obtained from the Atlas of Living Australia, 2018).

	PC1	PC2	PC3
Eigenvalue	9.50	3.86	1.89
Percentage variation explained	59.4	24.2	11.8
Eigenvectors			
Annual mean temperature (Bio01)	0.300	0.178	0.101
Isothermality (Bio03)	-0.123	0.397	0.037
Temperature seasonality (Bio04)	0.248	-0.308	-0.048
Temperature annual range (Bio07)	0.286	-0.212	0.011
Mean temperature of wettest quarter (Bio08)	0.286	0.012	0.307
Mean temperature of driest quarter (Bio09)	0.107	0.375	-0.357
Mean temperature of warmest quarter (Bio10)	0.317	0.097	0.045
Mean temperature of coldest quarter (Bio11)	0.258	0.298	0.085
Annual precipitation (Bio12)	-0.281	0.134	0.287
Precipitation Seasonality (Bio15)	0.141	0.445	0.095
Precipitation of wettest quarter (Bio16)	-0.209	0.337	0.247
Precipitation of driest quarter (Bio17)	-0.249	-0.254	0.264
Precipitation of warmest quarter (Bio18)	0.081	-0.018	0.695
Precipitation of coldest quarter (Bio19)	-0.281	0.173	-0.197
Annual mean radiation (Bio20)	0.319	0.081	0.012
Annual mean moisture index (Bio28)	-0.316	0.042	0.107

The first principal component (PC1) explained more than 59% of the total variance and precipitation and moisture index variables were negatively loaded along this axis, while temperature and radiation variables were positively loaded along PC1. Annual mean radiation, annual mean temperature and mean temperature of warmest quarter had the highest positive loadings along PC1, and annual mean moisture index, annual precipitation and precipitation of coldest quarter had the highest negative loadings (Table 2.2, Figure 2.2). The second principal

component (PC2) accounted for 24% of the total variation, and the highest positive loadings were related to precipitation seasonality, isothermality, mean temperature of driest quarter and precipitation of wettest quarter (Table 2.2, Figure 2.2). Precipitation of warmest quarter had the highest loading in PC3 which explained 12% of climate variation (Table 2.2).

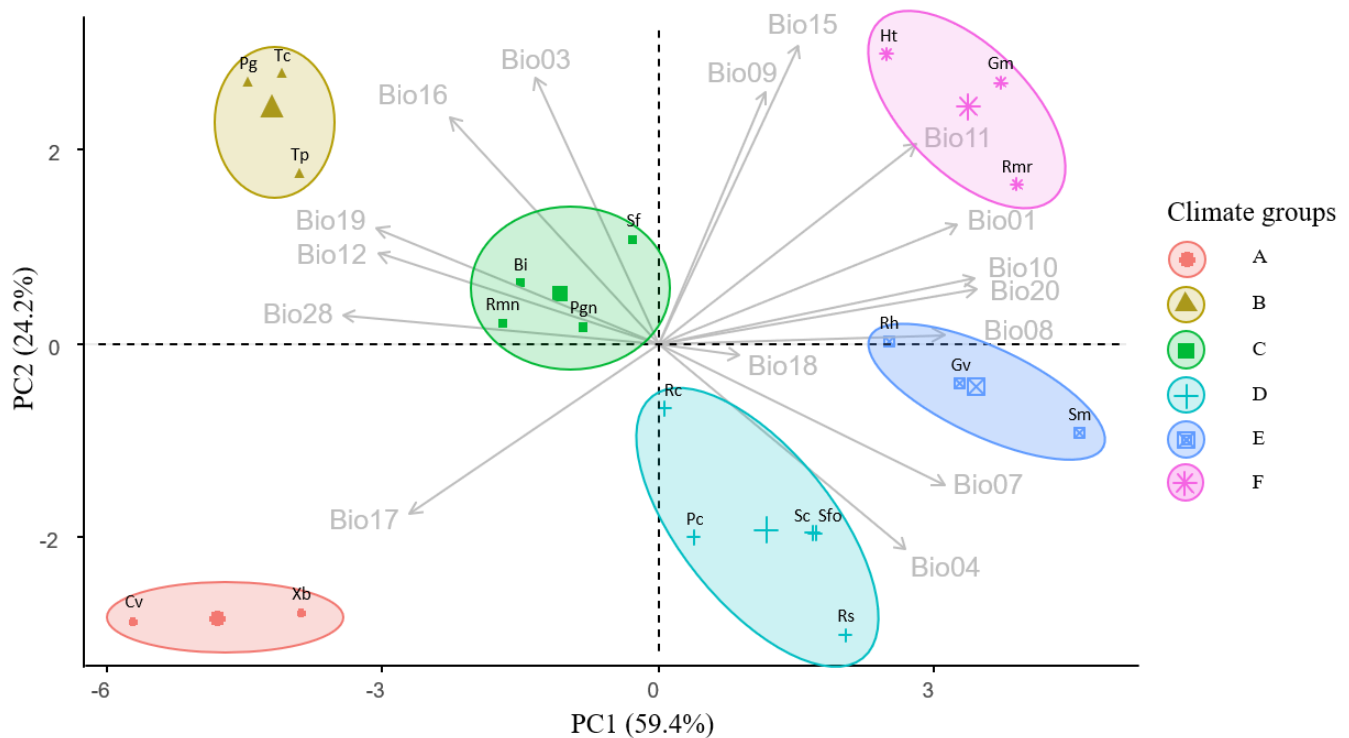


Figure 2.2. The results of principal component analysis (PCA) of climate variables (refer to Table 2.1 for the bioclimatic variables codes) for 20 Australian annual species which germinated in at least one of the germination treatments. Each of the plant species in the six climate groups are labelled with the first letters of their scientific names (refer to appendix A1 for species names and codes). Shaded ellipses show the six groups (with group centroids) based on the climate of species' natural distribution (16 climate variables represented in Tables 2.1 and 2.2) obtained from hierarchical clustering using Euclidean distance and complete linkage method. See appendix A3 for the variation of climate variables in each climate group.

Generalized linear models showed significant negative relationships between GP and PC1 and PC2 (Table 2.3, Figure 2.3). This suggests that germination percentage increases with precipitation and moisture index and decreases with temperature and solar radiation. Moreover, the data showed that environments with higher seasonality in their rainfall, contained seeds with lower germination percentage (Figure 2.3). LAG time and T_{50} were negatively related to PC1, and positively related with PC2 (Table 2.3, Figure 2.3). Therefore, germination speed (both LAG time and T_{50}) is mainly related to rainfall and its variation (Table 2.3). Relationships between germination percentage and germination rate and PC3 were significant, but weak (Table 2.3, Figure 2.3).

Table 2.3. Generalized linear models of germination percentage (GP), germination lag (LAG time) and germination rate (T_{50}) in relation to seed mass and multivariate climate variables (PC1, PC2 and PC3 generated from PCA) of the species' natural distribution across Australia (obtained from the Atlas of Living Australia, 2018).

	GP (logit transformed)			LAG time			T_{50}		
	Estimate	SE	P-value	Estimate [†]	SE	P-value	Estimate [†]	SE	P-value
PC1	-0.314	0.072	3.22e-05***	0.015	0.004	0.001**	0.008	0.003	0.003*
PC2	-0.362	0.123	0.004*	-0.045	0.007	2.33e-08***	-0.025	0.004	1.12e-07***
PC3	0.423	0.160	0.010*	0.026	0.010	0.014`	0.016	0.006	0.006*
Seed mass	-0.051	0.171	0.765	-0.014	0.012	0.279	0.008	0.008	0.311
Model R ²		0.32			0.42			0.46	

Asterisks denote the following significant levels: *** P -value < 0.000, ** P -value < 0.001, * P -value < 0.01, ` P -value < 0.05.

[†] Estimate values are inversed due to *inverse* link used in GLMs for LAG time and t_{50} .

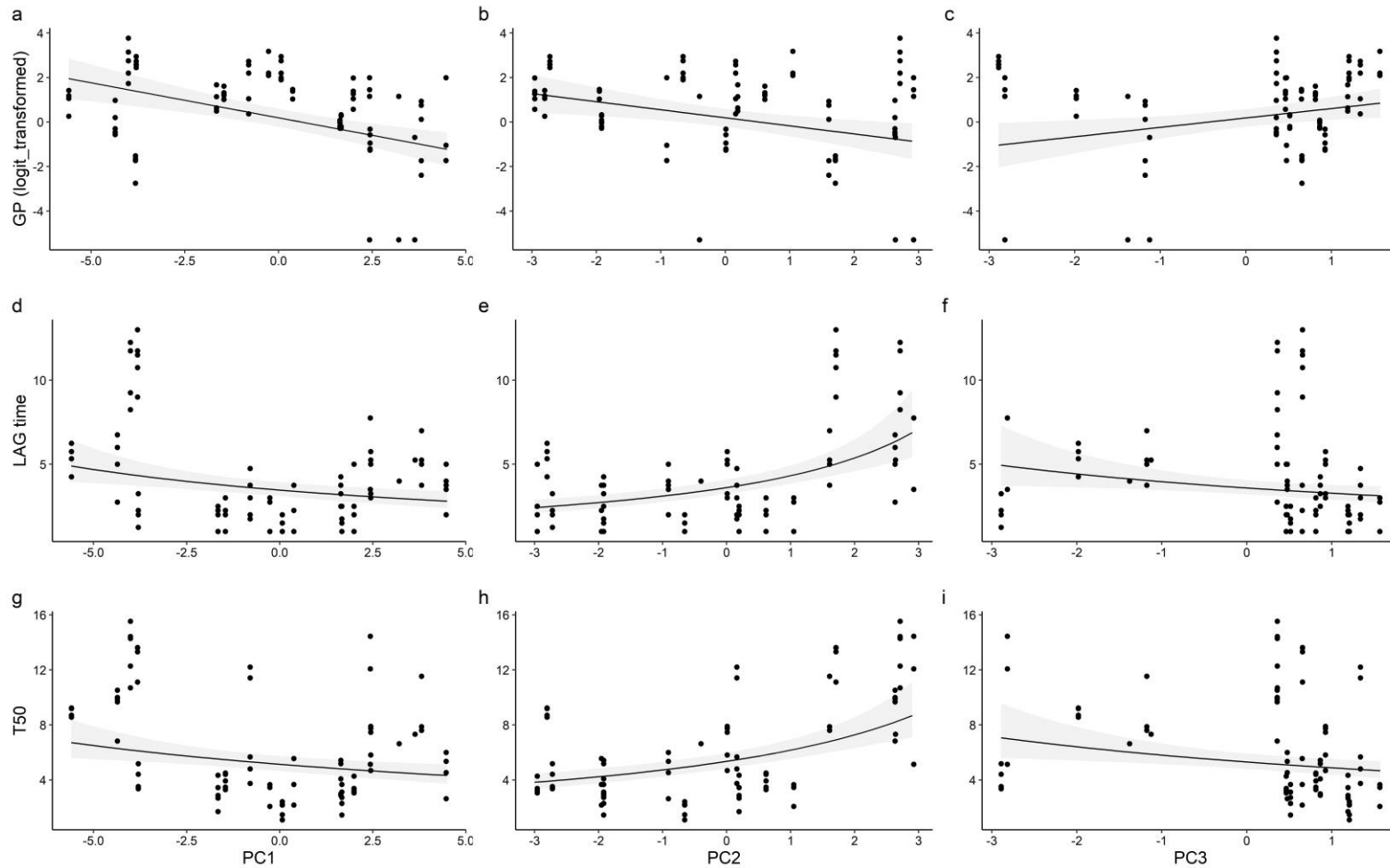


Figure 2.3. Relationships for 20 Australian annual species (which germinated in at least one of the germination treatments) between germination percentage, germination lag (LAG time) and germination rate (T_{50}) and multivariate climate variables (PC1, PC2 and PC3 generated from PCA of 16 bioclimatic variables) of their natural distribution across Australia (obtained from the Atlas of Living Australia, 2018). Regression lines are obtained from generalized linear models using the mean values of all germination treatments. Refer to Table 2.3 for the P-values of the relationships. Figures are shown for significant relationships (P -value < 0.05).

2.3.5 Germination attributes and climate groups

Cluster analysis separated the 20 plant species into six climate groups, labelled A-F (Appendix A3, Figure 2.2). To compare the germination attributes among the six climate groups, we used the maximum germination percentage ($G_{\max}$) for each species in the germination test, plus the shortest time for LAG time ($LAG_{\min}$) and T_{50} ($T_{\min}$) to capture the fastest germination speed of each species regardless of germination treatments. Maximum germination percentage was only significantly different between groups C and F (P -value = 0.021). There were no other differences among climate groups (Figure 2.4a). There were significant differences in climate group means for germination speed (both LAG time and T_{50} ; Figure 2.4b). Group B had the lowest germination speed (highest LAG time and T_{50}), while groups C and D had the highest. As mentioned above, the relationship between germination attributes and climate of natural distribution showed that precipitation seasonality was the main climate variable in determining germination speed (Table 2.3). Therefore, to better understand the changes in germination speed, climate group pairs were chosen with similar annual precipitation, but different precipitation seasonality to compare the germination speed of groups that receive the same amount of rainfall per year while have different predictability in their rainfall. Looking at groups A and B (high precipitation), C and F (medium precipitation), and D and E (low precipitation), we found that germination speed was significantly higher ($LAG_{\min}$ and $T_{\min}$ were lower) when predictability of the climate was higher (precipitation seasonality was lower) (Figures 2.4b and 2.4d).

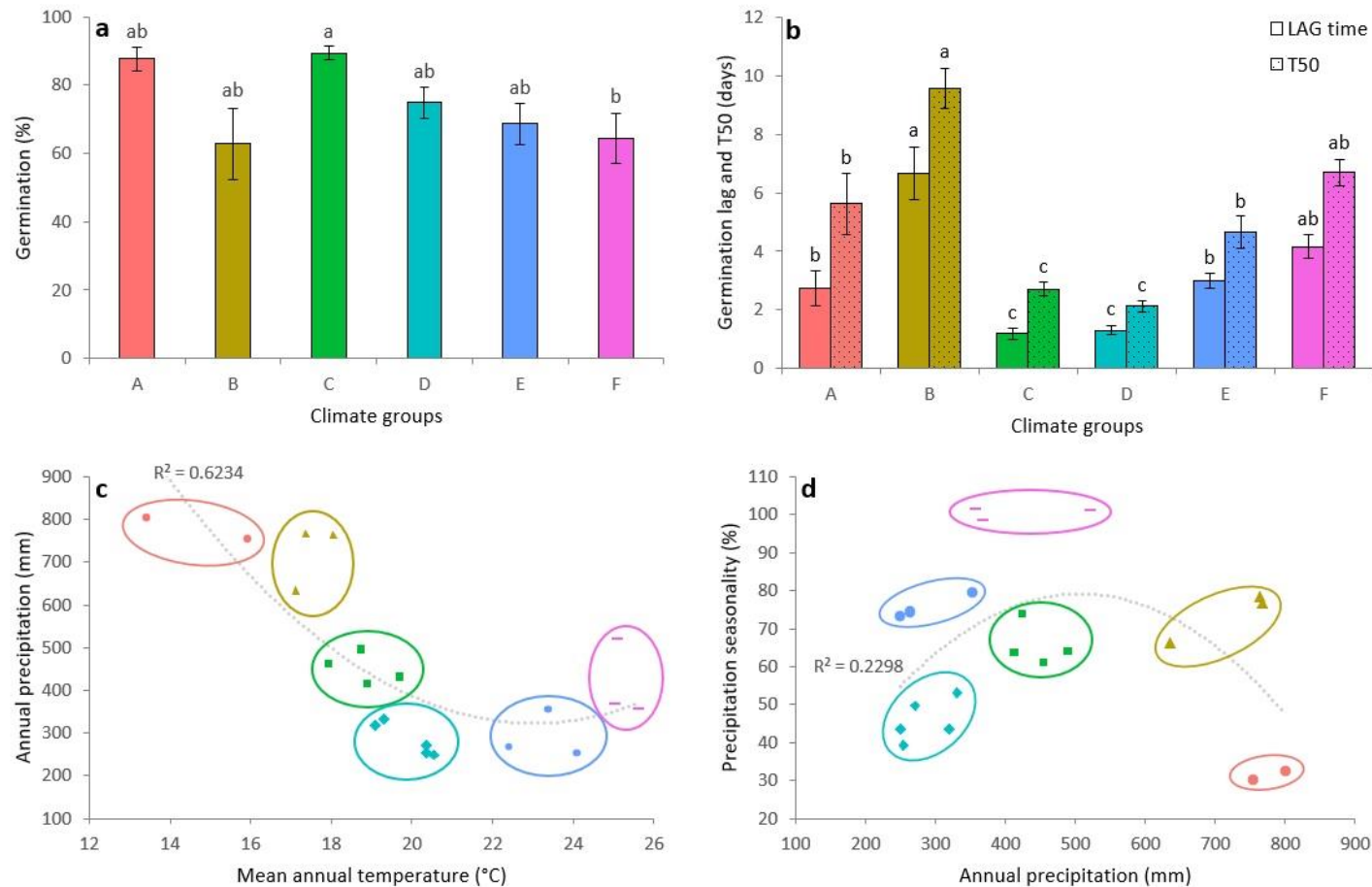


Figure 2.4. Differences in mean germination attributes (a) maximum germination percentage, (b) minimum germination lag (LAG time) and germination rate (T_{50}) among climate groups (created by cluster analysis; refer to Figure 2.2). Letters indicate significant differences among means for each germination attribute, where non-significant differences share at least one letter (one-way ANOVA and Tukey's post-hoc tests). Relationships for annual mean temperature and annual precipitation (c) and annual precipitation and precipitation seasonality (d) are shown for the 20 species (indicated by markers) in the six climate groups based on their natural distribution. Polynomial regression lines and associated R^2 values between climate variables are provided in figures. Climate data was obtained from the Atlas of Living Australia (ALA, 2018).

2.4 Discussion

Using Australian annual plant species distributed across different habitats in Australia, we explored relationships among seed mass, germination attributes and climate. In particular, we examined relationships between: (1) seed mass and climate of natural distribution, (2) seed mass and germination attributes and (3) germination attributes and climate of natural distribution.

2.4.1 Seed mass and climate of natural distribution

Our investigation of 58 species of Australian annual plants indicates that seed mass was related to the climate of natural distribution. Seed mass was greater for species from hotter and/or drier environments (i.e., harsher climatic conditions) (Dainese & Sitzia, 2013; Leishman et al., 2000; Tautenhahn et al., 2008). Lower seed mass was associated with cooler environments and/or environments with greater water availability (through higher rainfall or higher soil moisture). This concurs with previous studies that have generally found seed mass varies across climate gradients with species from warmer (Dainese & Sitzia, 2013; Dubuis et al., 2013; Gallagher & Leishman, 2012; Moles et al., 2014; Murray et al., 2004; Swenson et al., 2012; Wang et al., 2008), and more arid environments (areas with lower rainfall or lower soil moisture) (Dubuis et al., 2013; Murray et al., 2004; Stromberg & Boudell, 2013; Tautenhahn et al., 2008; Wang et al., 2008; Wright & Westoby, 1999) having larger seeds.

Seed mass was greater for species from environments with greater annual temperature variation but was not related to the precipitation seasonality of the environment. The limited number of studies exploring the relationship between climate variability (seasonality) and seed mass show inconsistent results (Malhado et al., 2015; Swenson et al., 2012). However, this may be due to the species investigated in our study as they are a subset of Australian annuals due to the lack of seed availability.

We acknowledge that these relationships are drawn from non-randomly chosen species from across Australia, and caution must be taken in the generalisation of our results. More research is needed on larger number of Australian annual species from various climates to gain a broader understanding of the relationship between seed mass and climate.

2.4.2 Seed mass and germination attributes

In our study, germination percentage and speed were not related to seed mass. Previous studies have shown mixed results (positive, negative and no correlation) for the relationships between

seed mass and germination attributes (germination percentage and speed) (Huang et al., 2016; Wang et al., 2016; Yi et al., 2019). For example, Walder and Erschbamer (2015) found no relationship between seed mass and germination percentage for 28 species of Alpine plants. Likewise, seed mass of 20 common species (mainly annuals) of the Inner Mongolia Autonomous Region, China, was not related to their germination percentage (Yi et al., 2019). However, Wang et al. (2016) showed that seed mass of 109 (annual and perennial) plant species from north-eastern China had a significant negative relationship with germination percentage. Regarding germination speed, Huang et al.'s (2016) finding showed faster germination for large-seeded desert annuals in Arizona, USA, while Chen et al. (2018) found a positive relationship between seed mass (of 1119 species from mountains of Southwest China) and time to germination. These contradictions in relationships between seed mass and germination attributes may occur for different reasons. Firstly, the range of seed mass and life history strategies vary among studies. The range of seed mass in our study, which focused only on annuals, was skewed toward lighter seeds (0.0002 – 6.1173 mg), whereas for Wang et al.'s (2008) study (showing a negative relationship between seed mass and germination percentage), the seed mass (including annual, biennial and perennial species) ranged from 0.06 – 63.50 mg. Secondly, climatic conditions for the species ranges can have a greater impact on seed germination (dormancy) than the effects of seed traits such as seed mass (Jurado & Flores, 2005; McIvor & Howden, 2000). For example, it has been hypothesized that germination of species from temperate climates is more dependent on environmental conditions than seed mass (Yi et al., 2019). While no relationship was found between germination attributes of 20 Australian annuals and seed mass in our study, we found significant relationships between germination attributes and climate variables of their natural distribution. Therefore, for our species, climate had a stronger relationship with germination attributes than seed mass.

2.4.3 Germination attributes and climate of natural distribution

Germination attributes were related to the climate of natural distribution for our annual species. Seed germination is partly under the regulation of climate, and therefore, differences in climatic conditions among species environments are one determinant of variation in plant species germination attributes (Fernández-Pascual et al., 2013). The timing of germination might vary both within a season (germination speed) and among seasons (germination percentage or dormancy) (Simons & Johnston, 2000, 2006). Generally, species from colder and/or drier sites have lower germination than species from hotter and/or wetter sites (Clauss & Venable, 2000; Fernández-Pascual et al., 2013; Walder & Erschbamer, 2015). In our study, the relationships

between germination percentage and climate variables, based on long-term climate data from the locations of a plant's natural distribution, showed lower germination for species from warmer and drier environments. While lower germination in species with more arid climate of natural distribution is consistent with most studies (i.e., Clauss & Venable, 2000), lower germination in species from warmer environments contradicts many other studies (i.e., Walder & Erschbamer, 2015). However, Li and Feng (2009) showed perennial Crofton weed populations from higher elevations (lower temperature) of southwest China had greater germination than populations from lower elevations (higher temperature).

Inconsistent relationships between temperature of origin or natural distribution and germination may be due to differences in study design. Firstly, some studies have focused on relationships between germination and temperatures during the period of seed maturation and not annual temperatures (e.g. Donohue et al., 2008; Fenner, 1991; Schmutz et al., 2006). Secondly, the effect of long-term climatic conditions at the seed source on germination is species-specific (Meineri et al., 2013); therefore, relationship between temperature and germination is strongly influenced by the investigated species (Dwyer & Erickson, 2016). Thirdly, the temperature range of different habitats, or type of habitats (e.g., tropical, desert, Mediterranean, etc.) where the seeds are sourced or distributed, may change the direction of the relationship between germination and temperature. For example, in our study, annual mean temperature ranged from 13 – 25 °C (for germinated species), whereas in a study by Meineri et al. (2013) who showed a positive relationship between seed source temperature and germination for *Veronica officinalis*, temperature ranged from 8 – 11 °C.

We found lower germination percentage in Australian annual species from environments with higher rainfall variability. This is supported by the findings of Rubio de Casas et al. (2017) for legumes on a global scale and Torres-Martínez et al. (2017) on an annual herb endemic to vernal pools of California. This result reflects the bet-hedging strategy for germination in fluctuating environments (Adondakis & Venable, 2004; Gremer et al., 2016) where plants have evolved to avoid germination during unfavourable conditions or transient favourable conditions which may lead to seedling mortality (Long et al., 2015). The bet-hedging strategy also increases long-term fitness of plant species by forming a soil seed bank of un-germinated seeds (Clauss & Venable, 2000; Saatkamp et al., 2014). This is an important strategy for annual plants, as complete failure in reproduction would lead to extinction if there is no seed bank due to lack of other persistence mechanisms (Adondakis & Venable, 2004). Therefore, for

Australian annuals in climates with higher rainfall variability, bet-hedging is an important strategy to maintain persistence.

Despite contrasting climatic conditions among the climate groups we identified, we only found significant differences in maximum germination percentage ($G_{\max}$) between group C and F. Low $G_{\max}$ in group F was mainly because of low germination percentage of *Goodenia microptera* (33%), a species that germinated only after being treated with an application of karrikinolide. The reason behind lower differences between germination attributes in different climate groups compared to differences in individual climate variables could be the presence of climate variables with positive and negative relationships with germination attributes together, in one climate group. Maximum germination percentage did not reach 100% in any of the climate groups. Some seeds would always stay dormant and be incorporated into the soil seed bank as a bet-hedging strategy. This strategy, as previously mentioned, spreads the risk of extinction over time as only a proportion of seeds would germinate as a result of any particular rainfall event (Gremer & Venable, 2014).

We observed faster germination in species from drier climates compared to wetter climates and also in species from climates with lower variability in their rainfall, regardless of aridity. This is supported by previous studies looking at germination speed across climatic gradients. For example, Jurado and Westoby (1992) showed that for 105 annual and perennial plant species from a range of ecosystems across Australia, species from arid zones had faster germination than from other areas. Rapid germination was also observed in *Caragana* species (small tree or woody perennial shrub) from drier environments compared to humid ones (Fang et al., 2017). Xu et al. (2014) found seeds of 134 annual and perennial plant species collected from a south slope of eastern Tibetan Plateau with harsher conditions showed faster germination in comparison with a north slope. While rapid germination provides a longer growing season for seedlings and opportunities to take advantage of limited rainfall events, it can also be risky as conditions may deteriorate and lead to seedling mortality. On the other hand, slow germination as a form of bet-hedging is favoured when rapid germinators may die as a result of a catastrophe (Baskin & Baskin, 2014; Donohue et al., 2010). In our study, annual species from dry environments showed lower germination percentage. The trade-off between the percentage and speed of germination (i.e., lower percentage but faster germination) in these environments, allows for the development of a soil seed bank which can maintain a population over time. While species from more variable environments (environments with high rainfall unpredictability) showed stronger bet-hedging, by possessing lower and slower germination.

2.5 Conclusions

Our study has increased the understanding of how Australian annual plants may germinate under different conditions given their natural distribution and seed attributes. Climatic gradients provide a natural opportunity for researchers to examine the potential impacts of different climatic conditions on plant traits. Our findings show that significant variations in seed mass and germination attributes (germination percentage, lag, and rate) exist along climatic gradients. We did not find a relationship between seed mass and the germination attributes we tested. Knowledge of how germination and seed mass change with climatic conditions, regardless of the species, can help predict the distribution of plant species under future climate change scenarios. Future research on how climate of natural distribution may relate to, or influence seed mass and germination attributes could be extended to other plant life-forms to determine whether the relationship we have found for annual species applies to longer-lived species that may be less affected by environmental variability.

3 Quantifying plant functional traits and life strategies of Australian annual species to inform green roof plant selection

Abstract:

The plant functional traits of species growing on green roofs are major factors determining the ecosystem services green roofs provide. In addition, flowering plants which can quickly achieve high cover through rapid growth and high shoot biomass are desirable for aesthetic reasons and to meet green roof construction guidelines. Annual species generally escape drought by completing their life-cycle before the onset of dry conditions and typically have an acquisitive strategy with ‘fast’ traits, rapid growth and flowering. However, annuals grow successfully in different habitats and climates and therefore, it is expected that they have different strategies to live and complete their life-cycle. To quantify plant strategies of annuals and evaluate the ecosystem services they may offer on green roofs and to develop a trait-based plant palette of annuals for green roofs, I undertook a common garden experiment with 18 annual plant species. It explored the relationships among traits related to drought resistance and resource acquisition, competitive and reproductive ability and also aimed to determine whether differences in plant strategies would influence their growth rate, shoot biomass and flowering time. Species with higher acquisitive strategy were more competitive, fast growing and produced higher final biomass. Two opposing strategies were observed in the studied annual species in regard to their flowering time and relative growth rate; one group of annuals showed faster flowering with faster growth and later flowering with slower growth (species at the higher end of relative growth rate spectrum), while the other group had faster flowering with slower growth and later flowering with faster growth. Plant species also showed a trade-off between their flowering time and specific leaf area (SLA). Fast flowering species with higher SLA were considered less drought resistant (drought escapers), as earlier flowering and higher SLA are both evolutionary responses of annual species to escape stress. Fast growing and resource acquisitive plants could be more desirable on green roofs as they have higher water use and therefore higher stormwater mitigation ability. In addition, it is preferable to select species with a range of time to flowering onset and SLA, in order to achieve a long-term flowering community with different levels of drought resistance.

3.1 Introduction

Extensive green roofs are constructed ecosystems which can provide a range of ecosystem services such as urban cooling (Wonga et al., 2003), stormwater mitigation (Zhang et al., 2018), urban biodiversity habitat (Wooster et al., 2022) and aesthetic value (Williams et al., 2019). However, their shallow substrate depth (< 20 cm deep), wide temperature fluctuations, high exposure to wind and solar radiation, and water scarcity all create challenging environments for plant growth and survival (Kazemi & Mohorko, 2017; Rayner et al., 2016). Poor plant survival on green roof leads to lower vegetation growth, coverage and aesthetics, which in turn reduces the provision of ecosystem services (Azeñas et al., 2018; Lundholm et al., 2015).

Drought is one of the main constraints on plant growth and survival on green roofs (Du et al., 2019; Rayner et al., 2016). Plant species have evolved different strategies to cope with drought conditions which are expressed through traits such as sclerophylly (low specific leaf area), thick leaves, waxy leaf cuticles, sunken stomata, Crassulacean acid metabolism (CAM) photosynthetic pathway and succulence (Durhman et al., 2006; Hossain et al., 2016; Liu et al., 2012). Many of these leaf traits enable species to survive limited water availability and are generally associated with slow growth (Chu & Farrell, 2022; Hossain et al., 2016; Liu et al., 2012), as they require greater investment in leaf structural tissues (Berger et al., 2016; Gremer et al., 2013; Kooyers, 2015).

Drought strategies also differ among life-forms. Perennial species adopt a conservative resource-use strategy (“slow” traits) and lower relative growth rate (RGR) when water is limited. Whereas annual species typically escape drought by completing their life-cycle before the start of the drought conditions and guarantee population persistence by remaining dormant as seeds in the soil seed bank (Berger et al., 2016; Volaire, 2018). Annual species typically have fast growth and acquisitive strategy with ‘fast’ traits such as high specific leaf area, leaves with thinner lamina and low tissue density, low water-use efficiency and leaf dry matter content. They also adjust their vegetative and reproductive growth according to water availability and show developmental plasticity through shorter plant life-cycle and earlier reproductive (flowering) phenology (Kooyers, 2015; Matesanz et al., 2020; Shavrukov et al., 2017).

In addition to coping with drought on green roofs, flowering plants which can quickly achieve high cover through rapid growth and high shoot biomass are desirable when choosing plants for green roofs. This is because these factors strongly influence the ecosystem services

provided by green roofs (Lee et al., 2014; Lundholm & Williams, 2015; Van Mechelen, Van Meerbeek, et al., 2015). For example, plant biomass and coverage positively impact cooling (Franzaring et al., 2016; Jim, 2012) and water runoff reduction (Nagase & Dunnett, 2012). Lundholm et al. (2010) also found that greater plant biomass was associated with higher reflection of solar radiation and consequently, lower surface temperature on a green roof. Flower traits are also important in bringing visual appeal to urban areas, while attracting pollinators by providing nectar and pollen (Benvenuti, 2014; Van Mechelen, Van Meerbeek, et al., 2015). Annual species can achieve high growth and biomass (shoot weight), and also colourful, long-term flowering on green roofs, even under low water availability (Nagase & Dunnett, 2013).

While annuals generally complete their life-cycle before drought occurs, there is likely to be a wide range of variation in growth, biomass and reproduction, due to their wide distribution. Annual species grow successfully in different types of environments and inhabit a wide range of habitats, along gradients of water and resource availability (Blumenthal et al., 2020; Brouillette et al., 2014; Dwyer & Erickson, 2016; Kurze et al., 2021; López et al., 2020; Sotomayor & Gutierrez, 2015; Welles & Funk, 2020), temperature (Matesanz et al., 2020), land use intensity (Dirks et al., 2017) and latitudinal or altitudinal gradients (Mitchell-Olds & Schmitt, 2006; Tabas-Madrid et al., 2018). Therefore, it is expected that annual plants will have different functional traits and strategies to live and complete their life-cycle.

Plant strategies can be quantified by measuring functional traits. Westoby (1998) highlighted three key-traits under the title of “leaf-height-seed (LHS) plant ecology strategy scheme”. Leaf traits are key to the leaf economic spectrum (LES) (Wright et al., 2004), which itself is a part of the fast-slow plant economic spectrum (PES) (Reich, 2014), and determines plant resource uptake dynamics and resistance to drought stress (Blumenthal et al., 2020; Kooyers, 2015). While annuals generally fall on the acquisitive side of the PES, previous research on annual plants growing along a gradient of resource availability (e.g., precipitation) has shown significant population differences in leaf traits (Kurze et al., 2021; Mason & Donovan, 2015). For example, populations of the annual species, *Helianthus anomalus*, sourced from southerly (with hotter drier climate and lower soil fertility) and northerly (colder wetter climate and higher soil fertility) sites of Utah, USA, had a range of resource-use leaf traits from acquisitive to conservative, respectively (Brouillette et al., 2014). Plant height is related to the size of the plant, light absorption, competitive ability and seed dispersibility (Westoby et al., 2002). In annual crop species, greater plant height is known to play a role in competitiveness mainly

through increased light interception and the shading of shorter plants (weeds) suppressing their growth (Blackshaw, 1994; Lemerle et al., 2001; Zerner et al., 2008). Seed mass and germination attributes are associated with reproductive strategy, dispersal capability and seedling survivorship (Chen et al., 2020; Coomes & Grubb, 2003; Reich, 2014; Westoby, 1998). Plant species growing in warmer and drier environments usually have larger seeds, which shows the higher tolerance of larger seeds under harsh conditions (Dainese & Sitzia, 2013; Wang et al., 2008). Germination speed in annual species also varies according to the environmental conditions they experience (Jurado & Westoby, 1992; Xu et al., 2014).

To survive in different environments, annual plant species are likely to employ different fast-slow traits, drought resistance, and reproductive strategies, that will be reflected in their functional traits. Gaining a better understanding of these will assist the development of trait-based plant palettes for green roofs in different regions and microclimates. Therefore, we undertook a common garden experiment with 18 annual plant species to explore relationships among traits related to drought resistance and resource acquisition (mainly specific leaf area, leaf dry matter content and leaf thickness), competitive (plant height) and reproductive ability (seed mass and germination attributes including germination lag and rate). We also determined whether differences in strategies influenced their growth rate, shoot biomass and flowering time, as these are key performance criteria for plant selection on green roofs.

3.2 Materials and methods

Seeds of 18 Australian annual plant species were commercially sourced from “Nindethana” (<http://www.nindethana.net.au>) and “Australian Seed” (<http://www.australianseed.com>) seed companies in 2017 – 2018 (Table 3.1). It was impractical to collect the seeds directly for our study because these 18 species are distributed across geographically distant habitats and have short, unpredictable life-cycles. Communication with the suppliers indicated the seeds of each species were collected from different plants. The 18 species belong to 10 genera and 4 families (mainly Asteraceae; Table 3.1). These species were chosen from a pool of 58 species which could germinate under one of the following conditions: Control 20/10 °C, Control 25/15 °C, Gibberellic acid 20/10 °C, Gibberellic acid 25/15 °C, or Karrikinolide 20/10 °C (refer to Chapter 2).

We used a common garden approach to grow these plants and evaluate their traits, growth, biomass and flowering time. We grew the plants from climatically different seed origins under controlled, non-limiting glasshouse conditions to minimize environmentally induced variation

in traits (Gorne et al., 2020). The experiment was conducted at the University of Melbourne, Burnley Campus, Melbourne, VIC, Australia. Seeds (pre-treated if needed, as determined in Chapter 2) were sown individually in propagation punnets (0.216 L, C18 – 8 Cell Imperial – Propagation punnets, Garden City Plastics®) filled with seed raising mix (containing 10% washed coarse sand, 10% sieved coir peat, and 80% medium pine bark by volume), with additives per m³ equal to 1.5 kg wetting agent (SaturAid®, Debco Pty Ltd., Australia) and 1 kg dolomite lime. Sowing of all species took place from November 2018 to January 2019 and the punnets were placed in the nursery under shade and watered with an automatic irrigation system for germination. After the appearance of the first pair of true leaves, seedlings were transplanted into larger pots (0.9 L Square pots: 11.5 cm length × 9.8 cm width × 10.8 cm height, Garden City Plastics®) filled with 10 cm of potting mix (containing 12.5% washed coarse sand and 87.5% medium pine bark by volume), with additives per m³ equal to 4 kg controlled release fertiliser (Macracote® Colonizer Plus (Red), N:P:K 15:3:9, plus trace elements, 8 – 9 M, Sunpalm Australia Pty Ltd., Australia), 1.5 kg wetting agent (SaturAid®, Debco Pty Ltd., Australia) and 1 kg dolomite lime. After another two weeks of growth under the shade, plants were then moved into a climate-controlled glasshouse and arranged in a randomized complete block design with 20 replicates for each species (which was later reduced in the case of some species due to pest damage) and watered manually every day to pot capacity. Plants were harvested upon flowering to measure their functional traits. Plant harvest took place from January to July 2019 (except for *Calandrinia eremaea* which was harvested in August 2019). Daily mean temperature and humidity of the glasshouse were measured using a ThermoChron iButton. Daily mean temperature ranged from 14.2 – 27.1 °C, and daily mean humidity ranged from 43.9 – 90.2 % during the experiment.

Table 3.1 Characteristics of the 18 Australian annual species including family and mean values ($\pm$ standard deviation) and ranges (min - max) of annual mean temperature (AMT) and annual precipitation (AP) for species occurrence records held at the Atlas of Living Australia (ALA, 2018).

Scientific name	Species names abbreviations	Family	AMT ($^{\circ}$ C)		AP (mm)	
			Mean ($\pm$ SD)	Range	Mean ($\pm$ SD)	Range
<i>Brachyscome iberidifolia</i>	Br_iber	Asteraceae	18.8 ($\pm$ 2.3)	11.9-27.1	492 ($\pm$ 261.6)	155-1216
<i>Calandrinia eremaea</i>	Cal_erem	Montiaceae	17.6 ($\pm$ 1.9)	9.7-26.3	368 ($\pm$ 145.6)	154-1218
<i>Calandrinia flava</i>	Cal_flav	Montiaceae	20.8 ($\pm$ 0.9)	19.6-23.4	265.5 ($\pm$ 31.3)	202-307
<i>Craspedia variabilis</i> *	Cra_var	Asteraceae	13.5 ($\pm$ 2.5)	4.1-22.6	799.7 ($\pm$ 256.8)	197-2190
<i>Podolepis canescens</i>	Pod_can	Asteraceae	19.1 ($\pm$ 2.2)	12.9-24.2	319 ($\pm$ 121.4)	164-870
<i>Podolepis gracilis</i>	Pod_gra	Asteraceae	17.4 ($\pm$ 1.3)	13.7-20.4	767.5 ($\pm$ 176.9)	244-1205
<i>Podotheca gnaphalioides</i>	Pod_gna	Asteraceae	18.9 ($\pm$ 1.4)	15.3-23.6	414.4 ($\pm$ 169.5)	198-1111
<i>Rhodanthe chlorocephala</i> subsp. <i>rosea</i>	Rho_chl	Asteraceae	19.3 ($\pm$ 1.4)	13.7-25.2	331.1 ($\pm$ 131.1)	172-1030
<i>Rhodanthe humboldtiana</i>	Rho_hum	Asteraceae	22.5 ($\pm$ 1.8)	16.8-27.1	263.4 ($\pm$ 66.8)	182-801
<i>Rhodanthe manglesii</i>	Rho_man	Asteraceae	18 ($\pm$ 1.6)	11.9-21.4	456.7 ($\pm$ 186.6)	230-1280
<i>Rhodanthe stricta</i>	Rho_str	Asteraceae	20.4 ($\pm$ 1.8)	14.1-27.1	253.3 ($\pm$ 89.5)	139-1245.7
<i>Schoenia cassiniana</i>	Sch_cass	Asteraceae	20.3 ($\pm$ 1.6)	15.4-27	270.4 ($\pm$ 71.6)	154-1030
<i>Schoenia filifolia</i> subsp. <i>subulifolia</i>	Sch_fil	Asteraceae	19.7 ($\pm$ 1.1)	16.3-20.4	426.9 ($\pm$ 190.5)	329-1038.7
<i>Schoenia macivorii</i>	Sch_mac	Asteraceae	24.2 ($\pm$ 0.8)	21.3-24.8	249.7 ($\pm$ 29.1)	215-327
<i>Swainsona formosa</i> *	Swa_form	Fabaceae	20.5 ($\pm$ 2.9)	13.2-27.9	249.5 ($\pm$ 83.4)	148-1030
<i>Trachymene coerulea</i> *	Tra_coer	Araliaceae	18.1 ($\pm$ 1.5)	14.1-22.8	762.1 ($\pm$ 196)	195-1151
<i>Trachymene pilosa</i>	Tra_pil	Araliaceae	17.1 ($\pm$ 1.4)	10.9-22.2	634.6 ($\pm$ 225.1)	206.8-1252
<i>Xerochrysum bracteatum</i> *	Xer_brac	Asteraceae	16 ($\pm$ 3.2)	3.4-27.7	753.2 ($\pm$ 374.6)	157-3197

*Occurs in both annual and short-lived perennial forms

3.2.1 Plant functional traits

Plant functional traits related to drought resistance and resource acquisition (specific leaf area, leaf dry matter content and leaf thickness), competitive ability (plant height) and reproduction (seed mass, germination lag and germination rate) were quantified after harvesting the plants. Plants were harvested at the onset of flowering (appearance of the first flower bud), thereby standardizing measurements at the same plant developmental stage. Functional traits (except seed mass and germination attributes) and plant performance metrics were measured on 10 – 20 mature plants (per species) following protocols (Pérez-Harguindeguy et al., 2013), under non-limiting conditions. Sample size was different between species due to losing plants to pests. Individuals that appeared heavily attacked by insects, pathogens or other organisms were not used for trait measurements. For plants which did not flower, traits were measured after six months of growing under controlled conditions (end of the experiment). In those cases, a number of individuals were kept longer to measure flowering time.

Leaf traits were measured on five young fully expanded leaves selected randomly from each plant (10 plants per species). Leaves were individually weighed and photographed to measure single-sided leaf area using ImageJ software (Rasband, 1997 – 2018). Leaf samples were oven-dried at 80 °C for 48 hrs to determine leaf dry weight. Leaf areas were used to calculate specific leaf area (SLA; $\text{mm}^2 \text{mg}^{-1}$). Leaf dry matter content (LDMC) was calculated as the ratio of leaf oven-dry mass to leaf fresh mass (mg g^{-1}). Leaf thickness (LT) was approximated by dividing the leaf fresh weight by leaf area. Plant height (cm) was measured from soil level to the tip of the longest vertical shoot (Pérez-Harguindeguy et al., 2013). Seed mass (SM), germination lag (LAG time) and germination rate (T_{50}) data were obtained from our previous experiment. LAG time and T_{50} have been obtained from species growing in Control 20/10 °C treatment (no germination stimulants), except for *Podotheca gnaphalioides* and *Schoenia macivorii* which germinated using karrikinolide and gibberellic acid, respectively (refer to Chapter 2). Seed mass was measured on four replicates of 50 seeds, or one replicate of 100 seeds (depending on seed size or seed availability) for each species which were put in 80 °C oven for 72 hrs and weighed to obtain the weight of a single seed (Pérez-Harguindeguy et al., 2013). LAG time was calculated as the number of days to first germination from the beginning of the germination test. T_{50} was recorded as the number of days from the beginning of the germination test to the time that 50% of the seed in each replicate had germinated (Coolbear et al., 1984).

3.2.2 Plant performance metrics (growth rate, shoot biomass and flowering time)

Flowering time (days) was recorded as the time between seed emergence to the appearance of the first flower bud. Relative growth rate was calculated from shoot dry weight (shoot biomass) at the end of the experiment. Above-ground (shoot) living portions of the plant (i.e., stem, leaves, flowers excluding dead materials or leaves) were harvested and oven dried at 80 °C for 48 h to determine shoot dry weight. Relative growth rate (RGR in g g⁻¹ day⁻¹) was calculated by the following equation:

$$RGR = \frac{\ln(SDW_2) - \ln(SDW_1)}{t_2 - t_1}$$

where SDW_2 was final plant shoot dry weight at time t_2 (final harvest time), and SDW_1 was considered to be zero as it was the initial shoot dry weight at time t_1 , when seeds were emerged (Hoffmann & Poorter, 2002).

3.2.3 Data analysis

Statistical analyses were performed using MINITAB v.19 and R statistical software version 4.1.2 (R Development Core Team, 2018). Transformations (Log10) were applied to some trait data before analysis to ensure normality. Descriptive statistics including minimum, maximum, mean, median, outliers and the spread of data were calculated for each trait and each species. Averaged trait values per species were used for the rest of the analyses. Principal component analysis (PCA) (using *FactoMineR* (Lê et al., 2008) and *factoextra* (Kassambara & Mundt, 2017) packages) and k-means clustering with Euclidean distance (using *NbClust* (Charrad et al., 2014) and *ClusterR* (Mouselimis, 2022) packages) were used to identify major axes of trait covariation and to group species based on their functional trait similarities. The optimal number of clusters was determined using the “silhouette” method (Rousseeuw, 1987). Clustering was performed to further investigate the relationships among plant performance metrics and trait-performance relationships in functionally dissimilar plant groups. One-way ANOVA and Fisher’s LSD post-hoc tests (P -value < 0.05) were used to analyse performance metrics’ differences among groups. Pearson’s correlation was used to explore relationships between species scores along significant principal components (eigenvalue > 1) obtained from PCA and the plant performance metrics of growth rate, shoot biomass and flowering time. Standardized major axis (SMA) regression (using *smatr* package (Warton et al., 2012)) was then used to plot the best fitted line for significant relationships. SMA regression was also used to determine

bivariate relationships between plant performance metrics and functional traits, both when all species were included and for each group (determined by the cluster analysis) separately (see appendix B1 for the contribution of phylogeny in these relationships). SMA is recommended to summarize the relationship between two variables when there is no clear response and dependant variable to use one variable to predict the other, and it estimates the best fitting line between variables by minimizing the error associated to variation in both variables (Warton et al., 2006). Figures were created using *ggplot2* package (Wickham, 2016) within R.

3.3 Results

3.3.1 Plant functional traits

The 18 annual species had great variation across their leaf, height, and seed traits (Appendix B2). Specific leaf area (SLA) ranged from 11.2 (*Schoenia filifolia*) to 40.9 mm² mg⁻¹ (*Rhodanthe stricta*). Leaf dry matter content (LDMC) ranged from 27 mg g⁻¹ (*Calandrinia flava*) to 196 mg g⁻¹ (*Rhodanthe humboldtiana*). Leaf thickness (LT) ranged from 0.1 mm (*R. stricta*) to 1.4 mm (*C. flava*). Plant height varied over nearly nine orders of magnitude across the 18 species, from 91 mm (*Calandrinia eremaea*) to 817 mm (*R. humboldtiana*). Seed mass ranged from 0.04 mg (*C. eremaea*) to 6.12 mg (*Swainsona formosa*). Germination lag (LAG) ranged from 1.8 days (*Podotheca gnaphalioides*) to 12.2 days (*Trachymene coerulea*) and germination rate (T₅₀) varied from 2.4 days (*R. chlorocephala*) to 14.3 days (*T. coerulea*; Appendix B2).

The seven plant functional traits were correlated along three principal components (eigenvalue > 1), which together explained 85% of the variation in plant traits (Table 3.2). The first principal component (PC1) accounted for 42.4% of the variation and leaf dry matter content, plant height and seed mass were positively associated with this axis, while leaf thickness was negatively associated (Table 3.2, Figure 3.1). Therefore, larger scores along PC1 correspond with taller plants that have large seed mass and greater LDMC. Whereas smaller scores along PC1 are associated with species with thicker leaves. The second axis (PC2) explained 26.8% of the total variation in plant traits and was positively associated with lag time and T₅₀, both germination-related traits (Table 3.2, Figure 3.1a). The third axis (PC3) accounted for 15.9% of the variation and was associated with SLA (Table 3.2, Figure 3.1b).

3.3.2 Plant performance metrics (relative growth rate, shoot biomass and flowering time)

The lowest relative growth rate was observed in *Schoenia macivorii* ($-0.03 \text{ g g}^{-1} \text{ day}^{-1}$), and the highest RGR was observed in *Rhodanthe chlorocephala* ($0.02 \text{ g g}^{-1} \text{ day}^{-1}$; Appendix B2). The fastest flowering time was observed in *S. macivorii* which flowered in 61 days, while it took 207 days for *Podothea gnaphalioides* to flower. The lowest shoot biomass (0.1 g) was produced by *S. macivorii*, while *R. humboldtiana* was the largest (13.2 g).

Table 3.2. Principal component analysis (PC1, PC2 and PC3) of seven functional traits of 18 Australian annual species grown under non-limiting common garden conditions. Eigenvalues of the PCs, the proportion of explained variance of all three meaningful PCs (eigenvalues >1) and trait loadings on the PCs are shown. SLA: specific leaf area, LDMC: leaf dry matter content, LT: leaf thickness, Height: plant height; SM: seed mass, T_{50} : germination rate, LAG: germination lag. Log indicates log transformation.

	PC1	PC2	PC3
Eigenvalue	2.97	1.87	1.11
Percentage variation explained	42.4	26.8	15.9
Cumulative variation explained	42.4	69.2	85.1
Eigenvectors			
SLA (log)	0.225	0.066	-0.854
LDMC (log)	0.529*	0.062	0.320
LT (log)	-0.552	-0.089	0.220
Height	0.448	-0.012	0.339
SM (log)	0.398	-0.057	-0.008
T_{50} (log)	-0.067	0.701	0.049
LAG (log)	-0.031	0.699	0.036

*Trait loadings > |0.38| are marked in bold. This number has been calculated according to the expected average contribution of the traits on the principal components.

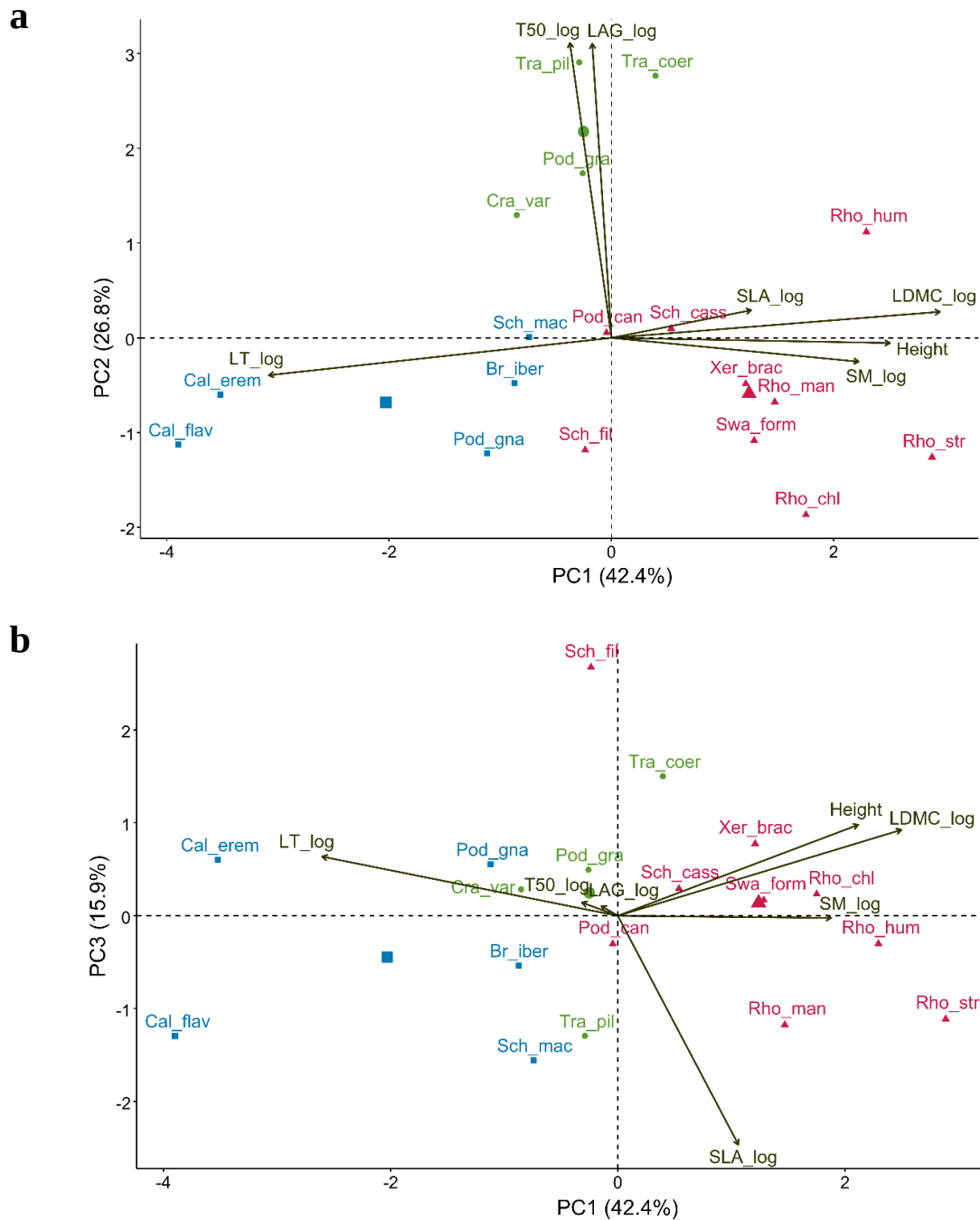


Figure 3.1. Principal component analysis (PCA) of seven functional traits for 18 Australian annual species grown under non-limiting common garden conditions. Panel a shows trait variability along first (PC1) and second principal component (PC2) and Panel b along second (PC2) and third principal component (PC3), with the proportion of explained trait variances in each axis shown in parentheses. Species abbreviations show their position along the PCs (species scores). Refer to Table 3.1 for the abbreviations of the species names. Species have been shown with different colours based on k-means clustering results: Group A (red), B (blue) and C (green). The end of the arrows indicates components' loadings for each trait: SLA: specific leaf area, LDMC: leaf dry matter content, LT: leaf thickness, Height: plant height, SM: seed mass, T₅₀: germination rate, LAG: germination lag, and log indicates log transformed data.

3.3.3 Relationships between functional traits and plant performance metrics

Both RGR and shoot dry weight were significantly and positively related to the score of species along PC1 axis (leaf thickness, LDMC, plant height and seed mass; Figure 3.2a and b). However, they were not significantly related to PC2 (T_{50} and LAG) and PC3 (SLA; Appendix B3). Flowering time was not significantly related to any of the PCs (Appendix B3).

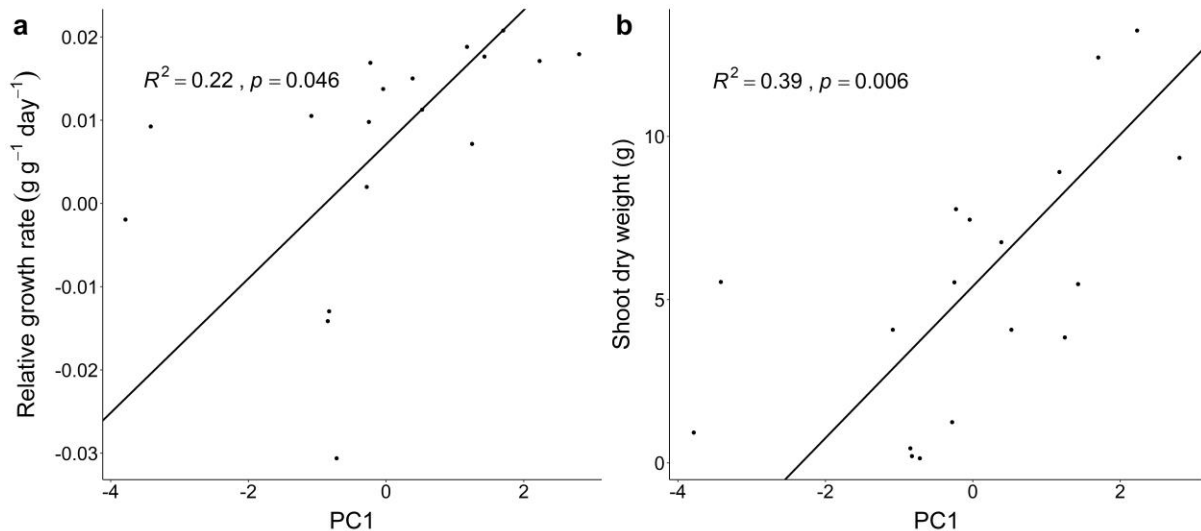


Figure 3.2. Standardized major axis regression of the relationships between first principal component (PC1) and a) relative growth rate and b) shoot dry weight of 18 Australian annual plant species grown under non-limiting common garden conditions. Coefficient of determination (R^2) and P-values are shown.

Our annual species exhibited large ranges of plant functional traits and performance metrics, and consequently created a fast-slow spectrum. Clustering divided the annual plants into three distinct groups: groups A, B and C (Figure 3.1). Plants in group A (all from Asteraceae family except *Swainsona formosa*) had greater plant height, LDMC, relative growth rates and shoot biomass (Appendix B4, Figure 3.1). Group B (including the two succulent species: *Calandrinia eremaea* and *Cal. flava*) had the thickest leaves and group C (with the two slow germinating *Trachymene* species) were the slowest germinators (Appendix B4, Figure 3.1). There were no significant differences among groups for flowering time (Appendix B4).

3.3.4 Relationships among plant performance metrics

Investigating the relationships among plant performance metrics showed that RGR was significantly and positively related to shoot biomass (Figure 3.3b; black line). However, flowering time was not related to RGR (Figure 3.3a, $p = 0.12$) or shoot biomass (Figure 3.3c, P -value = 0.47).

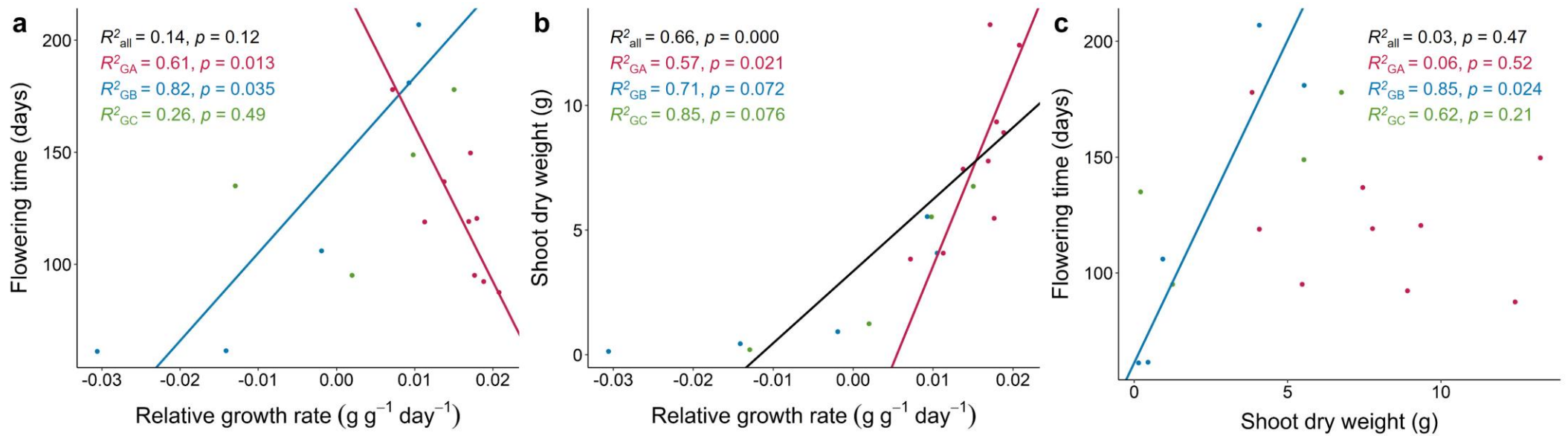


Figure 3.3. Standardized major axis (SMA) regressions among relative growth rate, flowering time and shoot biomass of 18 Australian annual plant species (black) and their groupings defined by cluster analysis: Group A: red ($n = 9$), group B = blue ($n = 5$), and group C = green ($n = 4$). Coefficient of determination (R^2) and P -values are shown for all relationships. SMA regression lines are plotted when significant (P -value < 0.05). n = sample size.

Relationships among plant performance metrics were different when considering functional groups, compared to the relationships when all species were included. While there was no relationship between RGR and flowering time across all species (Figure 3.3a), two opposing relationships were observed between these metrics in groups A and B. In group A, flowering time decreased with RGR. In other words, species with higher growth rates (group A), flowered earlier (Figure 3.3a; red line). In contrast, flowering time increased with RGR for group B (Figure 3.3a; blue line). There was no significant relationship between flowering time and RGR for group C (Figure 3.3a, $p = 0.49$). In regard to the relationships between RGR and shoot biomass, higher growth rates were associated with greater shoot biomass in group A (Figure 3.3b; red line) but was not significant for groups B (Figure 3.3b, $p = 0.072$) and C (Figure 3.3b, $p = 0.076$). Shoot biomass and flowering time were positively correlated for group B, with smaller species flowering earlier (Figure 3.3c; blue line). However, there were no significant relationships between shoot biomass and flowering time for groups A (Figure 3.3c, $p = 0.52$) and C (Figure 3.3c, $p = 0.22$).

When all species were included, RGR and shoot dry weight were not significantly related to PC3 (mainly represented by SLA; Appendix B3). However, using functional groups revealed negative relationships between RGR and shoot dry weight with SLA in group B (Figure 3.4a and b; blue lines). Analysing the relationships between plant performance metrics and functional traits in groups also showed that the negative relationship between flowering time and SLA in the all-species analysis (Figure 3.4c; black line), was only significant in groups B and C (Figure 3.4c; blue and green lines, respectively).

RGR and flowering time were not related to seed mass, germination attributes, plant height, LDMC and leaf thickness, in the group analysis, except for the positive relationship between RGR and plant height in group A (Appendices B5 and B6).

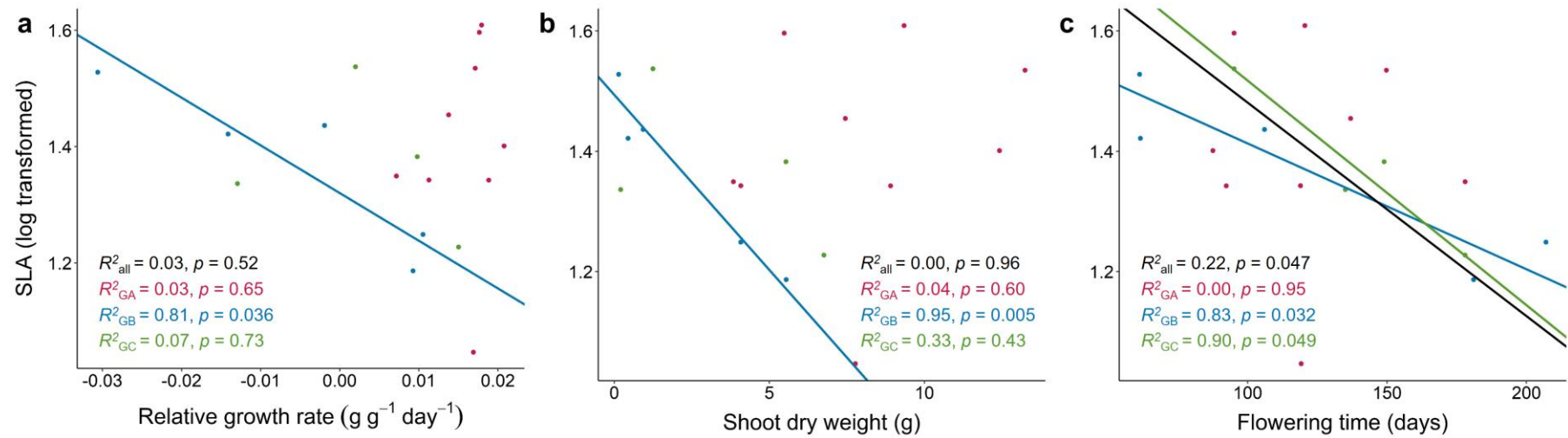


Figure 3.4. Standardized major axis (SMA) regressions between a) relative growth rate and specific leaf area (SLA; $\text{mm}^2 \text{ mg}^{-1}$), b) shoot dry weight and SLA, and c) flowering time and SLA of 18 Australian annual plant species (black) and their groupings defined by cluster analysis: Group A: red ($n = 9$), group B = blue ($n = 5$), and group C = green ($n = 4$). Coefficient of determination (R^2) and P-values are shown for all relationships. SMA regression lines are plotted when significant ($P\text{-value} < 0.05$). n = sample size.

3.4 Discussion

High trait dimensionality suggests greater variety of ecological strategies which can be employed by plant species to confront challenges (Laughlin, 2014; Wright et al., 2007). In this common garden study, covariation patterns in functional traits and their correlation with performance metrics revealed different drought resistance, resource-use and reproductive strategies adopted by Australian annual plant species.

3.4.1 Drought resistance strategy: Relationship between specific leaf area and flowering time

Our results indicated a trade-off between specific leaf area (SLA) and flowering time. A negative relationship between flowering time and SLA has also been observed in populations of an annual wild radish growing on the West Coast of North America (Welles & Funk, 2020). Earlier flowering and higher SLA are both evolutionary responses of annual species to escape stress (Brouillette et al., 2014; Sherrard & Maherali, 2006; Welles & Funk, 2020). Empirical research on *Arabidopsis thaliana* and other C₃ annual species (Lovell et al., 2013; Mckay et al., 2003) have often shown a genetic correlation between earlier flowering and reduced water-use efficiency, indicating a trade-off between drought escape and avoidance strategies (Mckay et al., 2003). Later flowering and more efficient water use means that plants are able to survive longer into the dry period (Kooyers et al., 2021). We did not directly measure water-use efficiency in our plants; however, it is likely that in our annual plants, faster flowering species with higher SLA would have lower drought resistance, and escape drought earlier by completing their lifecycle faster, while later flowering species with lower SLA likely have higher drought resistance. Species with higher drought resistance are more favoured under drier conditions, as they can take advantage of a longer growing season to gain resources.

3.4.2 Acquisitive-conservative strategy

Our study partly supported the resource acquisition-conservation trade-off (the fast-slow economics spectrum) (Reich, 2014). The more acquisitive strategy in our species was represented by taller species with bigger and thinner leaves and greater seed mass, having higher relative growth rate (RGR) and biomass. While shorter plants with thicker and smaller leaves and seeds, had lower RGR and biomass and had a more conservative strategy. Taller plants have greater ability to capture light and consequently grow faster (de la Riva et al., 2016). Fast growing species with more acquisitive traits acquire resources more rapidly and therefore have greater biomass (Reich, 2014; Wright et al., 2004). However, SLA was not contributing

to this resource-use axis and was independent from other leaf traits. This decoupling of SLA and leaf dry matter content (LDMC) has been observed in other studies of annuals. For example, Kurze et al. (2021) explored trade-offs in 12 leaf traits related to resource acquisition-conservation in 30 winter annual species distributed along a rainfall gradient in a Mediterranean climate and found a decoupling between “structural” (including LDMC) and “carbon gain” traits (including SLA). Another study of plant communities (including both annuals and perennials) growing along a gradient of soil metal toxicity in a subtropical climate (Delhaye et al., 2020) also showed that SLA and LDMC were also not correlated along the same axis. This indicates that SLA and LDMC might be related to different functions in annual species, such that SLA is related to leaf photosynthesis, while LDMC is related to leaf structure (leaf hydraulic function) (Delhaye et al., 2020; Griffith et al., 2016; Kurze et al., 2021). Taller plants with bigger seeds and bigger leaves with greater dry matter content also represents greater competitive ability according to the CSR strategies proposed by Grime (1974, 1977) (Pierce et al., 2013; Pierce et al., 2017). Along with greater height, seed mass and LDMC, rapid and higher production of biomass may also confer a competitive advantage (Liancourt et al., 2009).

3.4.3 Germination speed

Germination speed was independent of other functional traits or performance metrics in our study. Annual species show higher seed dormancy (a form of escape strategy), but faster germination in drought conditions (Clauss & Venable, 2000; Jurado & Westoby, 1992; Xu et al., 2014), compared to favourable conditions. Fast germination after rainfall or irrigation provides a longer growing season and opportunities to take advantage of rare rainfall events, although it can also lead to seedling death if conditions deteriorate (Donohue et al., 2010; Gremer et al., 2016). However, in environments with unpredictable climatic conditions, plants that exhibit a bet-hedging strategy (slow or inconsistent germination) may be advantaged, as rapid germinators may die due to catastrophic conditions (Donohue et al., 2010). Seed dormancy and slow germination lead to the creation of a seed bank which can maintain populations over time and restore vegetation cover after periods of extreme conditions (Thompson, 2000).

3.4.4 Relationship between specific leaf area and relative growth rate

Contrary to the world-wide leaf economics spectrum (LES), we found negative or insignificant relationships between RGR and SLA, when all species were included or in the groups defined by cluster analysis. Consistent with our results, Kimball et al. (2013), who investigated populations of four winter annual species growing at two climatically different sites, found that relationships between SLA and RGR were absent in most cases. Welles and Funk (2020) also found that under well-watered conditions, wild radish growth rate was negatively associated with SLA. These studies and our results indicate a deviation of annual species from the LES concept (Dirks et al., 2017; Kurze et al., 2021), as according to the LES, SLA and RGR are positively related (Donovan et al., 2011; Onoda et al., 2017; Wright et al., 2004). This is likely the result of the drought escape strategy used by annual species which does not require plants to invest resources in costly organs to resist drought (Berger et al., 2016).

3.4.5 Relationship between relative growth rate and flowering time

While the relationship between RGR (and shoot biomass) and flowering time was absent when all 18 annual species were included in the analysis, separating groups based on their functional traits (via cluster analysis) revealed distinct relationships. In Group A, which had the highest RGR and was on the more acquisitive side of the PC1 axis, faster growing species (higher RGR) flowered earlier while attaining higher biomass compared to slower growing species. This is consistent with drought escape and the acquisitive strategy (Volaire, 2018). Species in group A appeared to allocate their resources to rapid growth and successful reproduction before the onset of drought. A model describing flowering time in annual species developed by Guilbaud et al. (2015) predicted that it is associated with vegetative growth rate, and that plants with intrinsically higher RGR will flower earlier.

Conversely, species in Group B, which were short, had the thickest leaves and were located on the more conservative side of PC1 axis, faster growing species flowered later, and later flowering species produced greater biomass compared to earlier flowering species. Therefore, earlier flowering species were smaller and later flowering species produced larger biomass. The former is an adaptation to drier environments (Aronson et al., 1992) consistent with their growth strategy and the latter could be considered to have a more drought avoidance-like strategy in which accumulating more vegetative biomass prior to flowering either enables the plant to stay alive for long enough to complete reproduction during a terminal drought and/or confers greater water-use efficiency (Kooyers et al., 2021). Species in group C did not show

any correlations between their growth rate, flowering time and final biomass. These species were positioned in the middle of our resource acquisition conservation axis.

We acknowledge that these relationships are drawn from a small number of species within each of our plant groups, and caution must be taken in the generalisation of our results. More research is needed under both well-watered and water-deficit conditions on larger number of Australian annual species from various climates and families to gain a broader understanding of their life strategies.

3.4.6 Green roof application

Trait values of plant species growing on green roofs are the major factors determining the ecosystem services they offer (Lundholm et al., 2015; Xie et al., 2018). Regarding water runoff reduction, fast growing and resource acquisitive plants could be more desirable on green roofs as they use more water per unit time and therefore replenish the rainfall storage capacity of the green roof between rain events (Schrieke & Farrell, 2021; Szota et al., 2017). Water use of green roof plants also generally increases with plant biomass (Du et al., 2018; Farrell, Szota, et al., 2013; Nagase & Dunnett, 2012). Consistent with these statements, Schrieke and Farrell (2021) found that among nine common spontaneous species growing on green roofs, species with fast traits (e.g., higher growth rate, bigger leaves and greater biomass) have higher water usage than slow species. Plant height has also been shown to be positively related to stormwater capture on green roof (Lundholm et al., 2015; Nagase & Dunnett, 2012). Plant height, through its positive relationship with growth rate, is also indirectly related to phosphate and nitrate removal (Lundholm et al., 2015). Therefore, if reducing water runoff is the main purpose of green roof installation, our plant species with higher scores along PC1 axis should be considered more suitable, because they provide higher runoff water quality and retention due to being taller, having higher RGR and greater biomass. However, green roofs having plant communities with higher functional diversity (functionally dissimilar plant groups) would have less inter-specific competition and higher resilience to environmental stress (Cook-Patton & Bauerle, 2012; Heim & Lundholm, 2014).

Green roofs are harsh environments for seed germination due to high temperatures and free draining substrates and therefore can resemble arid environments (Dunnett & Kingsbury, 2008; Harp et al., 2015). If irrigation is available during germination and establishment, annuals could germinate fast and achieve rapid coverage (Saraeian et al., 2022). Otherwise, especially in unpredictable environments, seeds do not germinate and remain ungerminated in the soil

(Dalling et al., 2011). Exploring the above ground vegetation and the seed bank of 109 extensive green roofs in Belgium showed that seed banks, consisting mainly of species with transient seed banks, can develop on green roofs, but with significantly lower seed density and longevity compared to seed banks in urban gardens (Vanstockem et al., 2018). The study found that annual plant species make up a large part of both the above ground vegetation and the seed bank (21% and 36%, respectively) (Vanstockem et al., 2018). This is mainly because annual species escape stress (e.g., drought) by staying dormant as seeds in the soil (Sotomayor & Gutierrez, 2015). therefore, both fast and slow germinators can be selected for green roofs depending on the water availability and weather conditions.

Finally, selecting plant species with different flowering times (and SLA ranges, due to its negative relationship with flowering time) would help to create a longer floral display on green roofs. Flowering time and SLA may also indicate varying levels of drought resistance (water-use efficiency) in our Australian annual species. Annual plants with higher SLA (drought escapers) would flower earlier in the growing season, whereas species with lower SLA (higher drought resistance) would likely resist moderate levels of drought stress in late spring and early summer and therefore, continue growing and flower later into the growing season. A long flowering plant palette on green roofs is likely to be more favourable given that flowers impact on people's preferences for vegetation on green roofs (Haviland-jones et al., 2005; Lee et al., 2014). In the absence of greater knowledge of Australian annuals flowering time and growth periods, SLA values could provide a useful trait for designing annual plant communities for green roofs.

The functional traits and plant performance metrics and their mixed relationships in our studied Australian annual species shows that different life strategies (drought resistance, resource-use and reproductive strategies) can exist in annuals. In the 18 species we evaluated, there were annual species which were more acquisitive and competitive (taller species with bigger and thinner leaves and greater seed mass, higher relative growth rate and biomass), species which escape drought earlier through faster flowering and others which would likely better resist drought and flower later. The diversity in annual species strategies likely reflects the diverse natural environments where these annuals grow and is likely to improve their performance and resilience on green roofs.

4 Green roofs sown with an annual plant mix attain high cover and functional diversity regardless of irrigation frequency

Abstract:

Annual plant species have great potential on green roofs as many are highly attractive, fast and cheap to establish via sowing and can provide rapid cover and growth, which is important for ecosystem service provision. While irrigation is essential for survival and growth of annual plants in seasonally hot or dry climates, it is also important to minimize water use as availability is often limited. Therefore, we evaluated how irrigation frequency affects plant cover, species abundance, richness and diversity, plant traits and functional diversity of a 16 species mixture of Australian annual species ($4 \text{ g m}^{-2} \sim 2100 \text{ seeds m}^{-2}$) sown onto thirty 0.25 m^2 green roof modules. The experiment was carried out in Melbourne, Australia, from January (summer) to July (winter) 2020. After a 2-month irrigated establishment phase (to ensure germination and seedling establishment), three irrigation treatments (2, 4 and 6 days between irrigation) were applied to the modules for three months. Plant cover was reduced at lower irrigation frequency (6 days), but $\geq 80\%$ plant cover was achieved in all irrigation treatments. There was no effect of irrigation frequency on species abundance and richness; however, abundance, richness and diversity reduced over time, likely due to competition effects. Plant height and leaf area were also reduced by lower irrigation frequency. At the community level, functional diversity was unaffected by irrigation frequency. Our results indicate that green roofs sown with a mixture of annual plants can achieve good plant coverage, as recommended by green roof guidelines, and maintain high diversity when minimally irrigated in their first growing season.

4.1 Introduction

The environmental and social benefits green roofs provide (Mesimäki et al., 2019; Shafique et al., 2018) depend on a healthy vegetation layer with good coverage (Cook & Larsen, 2021; Lee et al., 2014; Williams et al., 2019). However, the harsh environments of green roofs limit the types of plants which can survive hot and dry summer conditions (Rayner et al., 2016; Vahdati et al., 2017). Due to their adaptation to shallow substrates, low water usage and high stress tolerance (Getter & Rowe, 2006), succulent plants, especially *Sedum* species, are commonly grown on extensive green roofs (Van Mechelen et al., 2014). Unfortunately, green roofs composed of only succulents have relatively low biodiversity and limited ecosystem services such as cooling, stormwater management, habitat provision for local flora and fauna, and aesthetic value (Blanusa et al., 2013; Cook-Patton & Bauerle, 2012; Lee et al., 2014; Li et al., 2018). In terms of aesthetic value, White and Gatersleben (2011) compared people's preferences for different vegetation types and found that a "Sedum roof" was not attractive, while a "Meadow roof" was more preferred. Similarly, Lee et al. (2014) showed that Australian office workers preferred taller, green grassy and especially flowering vegetation compared to succulent vegetation. More diverse, prairie style or grassland green roofs have also been shown to promote greater fascination, sense of calmness and well-being, and creative, meditative thinking than sedum-style green roofs (Loder, 2014).

To expand the number of plant species used on extensive green roofs beyond the regularly used succulents, researchers have attempted to find plant species originating from natural habitats with analogous conditions to those on extensive green roofs (Caneva et al., 2015; MacDonagh & Shanstrom, 2015; Van Mechelen et al., 2014). This process called the habitat template approach (Lundholm, 2006), suggests that plant species appropriate for green roofs can be found in microclimates or habitats with similar biotic and abiotic conditions to those on extensive green roofs (Lundholm, 2006). This approach has been used by researchers in various regions with different climates (Ksiazek-Mikenas et al., 2021; Nagase & Tashiro-Ishii, 2018; Sutton et al., 2012) including Mediterranean-type climates (Cáceres et al., 2018; Caneva et al., 2015; Farrell et al., 2012) and has resulted in suggested plants, particularly native species, suitable for green roofs. However, these species are mostly perennials and few studies have evaluated annual plants despite their potential to perform well on green roofs (Van Mechelen et al., 2014).

One way to increase the diversity and aesthetic appeal of green roofs is to use more flowering species, including annual wildflower species. The flowers of many annual species provide nectar and pollen for pollinators and have been shown to add visual attraction to extensive green roofs (Dunnett, 2015). As annual plants complete their life-cycle within one growing season; they germinate, grow, bloom, and set seeds under favourable conditions and are dormant as seeds during adverse conditions (Mulroy & Rundel, 1977), they are likely to persist on green roofs. Few studies have investigated the establishment of annual plants (including native species) on green roofs (or in green roof module experiments) as mixtures solely of annual species (Nagase & Dunnett, 2013; Vasl et al., 2019) or along with *Sedum* species (Kircher, 2004; Schindler et al., 2019; Vasl et al., 2017) or herbaceous perennials (Bates et al., 2013; Benvenuti, 2014; MacIvor et al., 2013; Ondoño et al., 2015). For example, Nagase and Dunnett (2013) found that it is possible to establish a 3 – 4 month flowering annual plant mix (non-native) on unirrigated extensive green roofs in the maritime climate of Sheffield, UK. Vasl et al. (2017) also demonstrated the possibility of improving green roof performance and services (cooling effect and storm water runoff mitigation) in Mediterranean climates, by combining local annuals and *Sedum* species. Using annual plants on green roofs can also have economic advantages as they can be quickly sown across large roofs. For example, Sutton (2013) found 40% lower labor costs for seeding native grasses compared to direct planting of small *Sedum* plants (plugs) on green roofs, in addition to the lower cost of seeds. Annual plants can also provide rapid coverage of substrate and prevent weed invasion during their vegetative phase. Long-term studies have shown that in the absence of regular maintenance, vegetation communities on green roofs shift towards annual species (or ruderals) (Catalano et al., 2016; Köhler, 2006; Thuring & Dunnett, 2019), indicating that annual species are likely to persist on green roofs over multiple generations (Vanstockem et al., 2018).

In natural environments, annual plants germinate following sufficient rainfall, with further precipitation after germination promoting survival and growth (Brown, 2002; Gao et al., 2014). In artificial environments, irrigation can trigger seed germination in annual plants under favourable growing temperatures (Funk et al., 2015; Wolf & Young, 2016). In green roofs, irrigation is critical for the survival and growth of non-succulent plant species in regions with Mediterranean-type climates (Du et al., 2019; Guo et al., 2021; Vandegrift et al., 2019; Zhang et al., 2014). For example, Rayner et al. (2016) showed that in Melbourne, Australia, all 17 forb and grass species died during summer on an unirrigated 12 cm deep green roof due to hot and dry conditions, whereas in Sheffield, UK, annual meadows can be established on extensive

green roofs without irrigation due to greater and more regular precipitation of their maritime climate (Nagase & Dunnett, 2013). Therefore, in climates with seasonally hot and or dry seasons, irrigation will be essential to grow annuals on green roofs. However, as water resources available for irrigating urban green areas can be scarce, especially in drier environments, it is important to minimise irrigation, but at the same time ensure green roofs have good coverage and diversity (Van Mechelen, Dutoit, et al., 2015b; Williams, Rayner, et al., 2010).

Differences in species' responses to water availability due to differences in drought resistance and traits (Azeñas et al., 2019; Vandegrift et al., 2019) will affect species abundance, diversity and cover (Miranda et al., 2009; Vahdati et al., 2017), and consequently community structure and function (Azeñas et al., 2018; Nunes et al., 2017). Green roofs with higher species and functional trait diversity are more resilient to environmental stress (Heim & Lundholm, 2014; MacIvor et al., 2016; Nagase & Dunnett, 2010) and provide greater aesthetic value (Lee et al., 2014; MacIvor et al., 2016) and ecosystem services (Lundholm, 2015; Van Mechelen, Van Meerbeek, et al., 2015; Xie et al., 2018). Plant height and leaf traits have been used to explore variation in functional diversity in natural (Midolo et al., 2021; Spasojevic & Suding, 2012) and green roof ecosystems (Vanstockem, Bastiaens, et al., 2019; Xie et al., 2018). Plant height has been found to increase competitive and dispersal ability and negatively respond to environmental stress (Garnier & Navas, 2012; Nunes et al., 2017), whereas leaf traits are associated with light capture and photosynthesis, water and nutrient acquisition and resistance to water stress (Onoda et al., 2017). These traits also contribute to green roof functioning (Lundholm et al., 2015). For example, lower plant height was found to be associated with cooler substrate temperatures (Xie et al., 2018), while greater plant height was positively related to higher stormwater capture (Lundholm et al., 2015).

The suitability of using annual species on extensive green roofs in seasonally hot and/or dry climates, and the lowest frequency of irrigation needed to result in an acceptable coverage and diversity is largely unexplored. While a mixture of perennials and annuals would be required to provide cover all year round, this study examined mixtures of annuals only to examine their performance in green roof applications. Therefore, we conducted an experiment using green roof modules sown with a mixture of 16 annual plant taxa in Melbourne, Australia. While Melbourne has a maritime temperate climate with warm summers and cool, wet, winters, it often can experience prolonged summer heat waves and drought periods. These hot and dry conditions are exacerbated on green roofs, where substrate temperatures can exceed 65 °C and

evaporative demand regularly exceeds rainfall for long periods of time (Rayner et al., 2016). We applied three different irrigation frequencies (days between irrigation) to evaluate how irrigation frequency affects: 1) plant cover, species abundance, richness and diversity; 2) plant traits of individual species and 3) plant traits and functional diversity at the community level. Another aim was to determine the lowest irrigation frequency which results in an acceptable coverage and diversity of annuals on green roofs.

4.2 Materials and Methods

4.2.1 Study location and climate

A green roof module experiment with a complete randomized block design and three irrigation frequencies (2, 4, and 6 days between irrigation) was established at the Burnley Campus, University of Melbourne, Australia (37°82' S, 145°02' E). The experiment was conducted from January (summer) to July (winter) 2020, where daily mean minimum temperature ranged from 1.7 – 11.7 °C and maximum temperature from 17.4 – 42.9 °C (Figure 4.1). Previous germination trials with the annual plant species used in this experiment (refer to Chapter 2) showed that daily temperature in January would be appropriate for their germination (Saraeian *et al.*, unpublished; Figure 4.1). Therefore, we commenced the experiment in January at a time when temperatures were optimal, approximately 20 °C monthly mean temperature (Bureau of Meteorology, 2020).

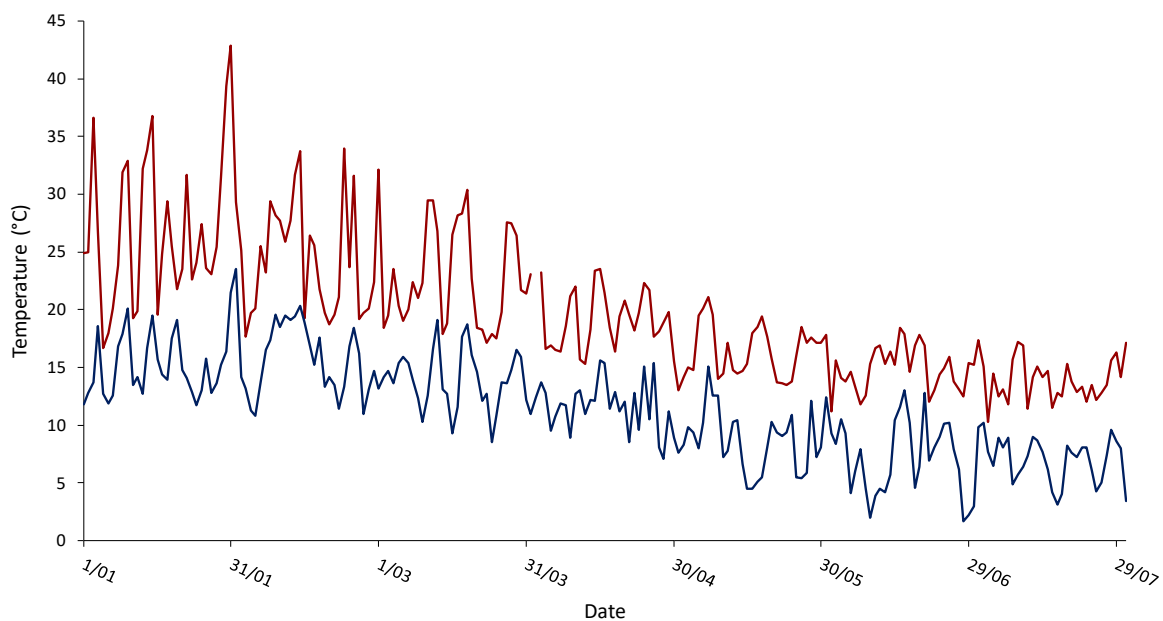


Figure 4.1. Daily maximum (red) and minimum (blue) temperatures during the green roof module experiment from January (summer) to July (winter) 2020, measured at Olympic Park weather station, Melbourne. Data for maximum temperature on 2 April 2020 is missing.

4.2.2 Experimental design

Thirty experimental green roof modules (HDPE boxes, 50 cm × 50 cm × 20 cm depth) were installed under a rainout shelter. Each module consisted of a 30 mm deep drainage layer (VersiCell® 3050, Elmi Pty Ltd), a layer of geotextile and 100 mm deep scoria-based green roof substrate (60% scoria ≤ 8 mm, 20% 7 mm scoria, and 20% coir) (Farrell, Ang, et al., 2013), to mimic the construction of a typical extensive green roof.

Seeds of 16 Australian annual species, from a range of different climates (Table 4.1, Appendix C1) were sourced from two commercial seed suppliers “Nindethana” (<http://www.nindethana.net.au>) and “Australian Seed” (<http://www.australianseed.com>). The germination rate of all species was quantified by germinating the seeds on agar at 20 °C for four weeks. All seeds had germination rates greater than 50% (Table 4.1).

Germination pre-treatments were applied to the seeds of six species to maximize germination, based on the results of our previous study (Saraeian *et al.*, unpublished) (Table 4.1). Seeds of *Goodenia vilmorinae*, *Podotheca gnaphalioides*, *Rhodanthe margarethae* and *Swainsona formosa* were soaked in a 0.67 µM concentration of karrikinolide (3-methyl-2H-furo [2,3-c] pyran-2-one, Toronto Research chemicals), for 24 hrs, and seeds of *Heloiotropium tenuifolium* and *Schoenia macivorii* were soaked overnight in a 250 mg L⁻¹ Gibberellic acid (GA3) solution and then redried at room temperature for one day prior to sowing.

Thirty seed mixes (one for each module) were made, each containing 25 seeds each of the 11 species that had germination rates > 80% and 50 seeds each of the five species with germination rates 50 – 65% (Table 4.1). The final sowing rate was 525 seeds per module (~1 g, ≈ 400 germinable seeds), equivalent to ~ 4 g m⁻² or ~ 2100 seeds m⁻². This sowing rate was based on previous studies using seeds of annual or perennial plant species for green roofs (Benvenuti, 2014; Nagase & Dunnett, 2013; Van Mechelen, Dutoit, et al., 2015b; Vasl et al., 2017). Modules were sown on 8th January (summer) 2020, with seeds combined with 100 ml fine sand to assist spreading across each module surface. Seeds were then covered with an additional 2 mm layer of sand to protect them from drying out. Fifty g m⁻² of controlled slow release fertiliser (Osmocote® Pro, Everris Australia Pty Ltd.: 15 N:1.3 P:10 K) was added to the substrate surface of each module, six weeks post-sowing.

Irrigation was applied with an automatic irrigation system with four shrubblers® 360° adjustable flow (Antelco Pty Ltd.) placed at four corners of each module to ensure even watering. Modules were irrigated for one minute three times per day (9 L d⁻¹ module⁻¹) for the

first two months after sowing to ensure germination, seedling emergence and establishment. After this phase, ten replicate modules were assigned to one of three irrigation treatments: 2 days, 4 days, and 6 days between irrigation events (irrigation frequencies) and received 1 min of irrigation (approximately 3 L water) per event (Figure 4.2). The amount of water applied at each irrigation event was sufficient to restore substrate moisture to pot capacity, as evidenced by drainage from modules after watering.

Table 4.1. Characteristics of the 16 Australian annual species used in the seed mix including family, germination percentage, number of seeds used in the seed mix for each module, and their climate of origin: mean values ($\pm$ standard deviation) and ranges (min - max) of annual mean temperature (AMT) and annual precipitation (AP) for species occurrence records in the Atlas of Living Australia (ALA, 2018). Germination pre-treatments were applied to six species to maximize germination.

Scientific name	Family	AMT ($^{\circ}$ C)		AP (mm)		Germination (%)	Number of seeds	Germination pre-treatment
		Mean ($\pm$ SD)	Range	Mean ($\pm$ SD)	Range			
<i>Brachyscome iberidifolia</i>	Asteraceae	18.8 ($\pm$ 2.3)	11.9-27.1	492 ($\pm$ 261.6)	155-1216	84%	25	----
<i>Craspedia variabilis</i> *	Asteraceae	13.5 ($\pm$ 2.5)	4.1-22.6	799.7 ($\pm$ 256.8)	197-2190	91%	25	----
<i>Goodenia vilmoriniae</i>	Goodeniaceae	23.4 ($\pm$ 1.9)	19.1-27.2	353.1 ($\pm$ 103.7)	198-1488	63%	50	Karrikinolide
<i>Heliotropium tenuifolium</i> *	Asteraceae	25.1 ($\pm$ 2)	17.8-28.5	520.5 ($\pm$ 229.1)	156-1788	59%	50	Gibberellic acid
<i>Podotheca gnaphalioides</i>	Asteraceae	18.9 ($\pm$ 1.4)	15.3-23.6	414.4 ($\pm$ 169.5)	198-1111	90%	25	Karrikinolide
<i>Rhodanthe chlorocephala</i> subsp. <i>rosea</i>	Asteraceae	19.3 ($\pm$ 1.4)	13.7-25.2	331.1 ($\pm$ 131.1)	172-1030	90%	25	----
<i>Rhodanthe humboldtiana</i>	Asteraceae	22.5 ($\pm$ 1.8)	16.8-27.1	263.4 ($\pm$ 66.8)	182-801	83%	25	----
<i>Rhodanthe manglesii</i>	Asteraceae	18 ($\pm$ 1.6)	11.9-21.4	456.7 ($\pm$ 186.6)	230-1280	80%	25	----
<i>Rhodanthe margarethae</i>	Asteraceae	25 ($\pm$ 1.5)	21.7-27.6	369 ($\pm$ 53.2)	203-443	65%	50	Karrikinolide
<i>Rhodanthe stricta</i>	Asteraceae	20.4 ($\pm$ 1.8)	14.1-27.1	253.3 ($\pm$ 89.5)	139-1245.7	85%	25	----
<i>Schoenia cassiniana</i>	Asteraceae	20.3 ($\pm$ 1.6)	15.4-27	270.4 ($\pm$ 71.6)	154-1030	49%	50	----
<i>Schoenia filifolia</i> subsp. <i>subulifolia</i>	Asteraceae	19.7 ($\pm$ 1.1)	16.3-20.4	426.9 ($\pm$ 190.5)	329-1038.7	90%	25	----
<i>Schoenia macivorii</i>	Asteraceae	24.2 ($\pm$ 0.8)	21.3-24.8	249.7 ($\pm$ 29.1)	215-327	92%	25	Gibberellic acid
<i>Swainsona formosa</i> *	Fabaceae	20.5 ($\pm$ 2.9)	13.2-27.9	249.5 ($\pm$ 83.4)	148-1030	52%	50	Karrikinolide
<i>Trachymene coerulea</i> *	Araliaceae	18.1 ($\pm$ 1.5)	14.1-22.8	762.1 ($\pm$ 196)	195-1151	93%	25	----
<i>Xerochrysum bracteatum</i> *	Asteraceae	16 ($\pm$ 3.2)	3.4-27.7	753.2 ($\pm$ 374.6)	157-3197	95%	25	----
Total number of seeds							525	

---- no germination pre-treatment has been used for these species

* Occurs in both annual and short-lived perennial forms



Figure 4.2. Green roof modules at the experimental site of University of Melbourne, Burnley Campus, three (A), six (B) and nine (C) months after sowing (April, July, Sep 2020, respectively).

4.2.3 Data collection and analysis

Species richness (number of species present) and abundance (number of individuals) were determined during plant surveys using a square sampling frame (50 × 50 cm) which was the same size as the module and divided into 25 quadrats (10 × 10 cm). In each module, plants were identified and counted in six randomly selected quadrats (24% of the module area, excluding the module corners). Surveys were performed twice: two months after sowing prior to starting irrigation treatments (seedling emergence; March 2020), and again five months after sowing at the end of the experiment (June 2020). Species richness and abundance were used to calculate Shannon-Wiener diversity index (H') as a measure of community structure, using the formula:

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

where S is the total number of species (species richness), and p_i is the proportion of individuals of species i (relative abundance). The Shannon index measures the diversity of the community and higher values indicate greater diversity. This index is sensitive to both species richness and abundance and has higher values with greater richness, and/or more even abundance (Pla et al., 2012).

At the end of the experiment, plant cover (percentage of substrate surface covered by plants) was quantified from photos taken 1.5 m above modules surface. Photos were processed using ImageJ software (Rasband, 1997 – 2018) and percentage plant cover was calculated by dividing green pixels in the photo divided by the module area.

Plant traits including plant height, leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC) and leaf thickness were measured for each species at the end of the experiment following standard protocols (Pérez-Harguindeguy et al., 2013). Individuals with pest damage were excluded from trait measurements. Four species were excluded from analyses as they were completely damaged or lost to pests, drought, or competition, or because they did not grow to an adult plant, and we could not measure their traits.

To evaluate how irrigation frequency affects community trait composition, we calculated community weighted mean trait values (CWMs) and functional diversity (FD) of our plant mixes. CWMs express the mean trait values in communities by the absolute or relative abundance of the species (Lavorel et al., 2008; Ricotta & Moretti, 2011). CWM values were calculated for each trait in each irrigation frequency, using the “weighted.mean” function of

dplyr package (Wickham et al., 2021) within the R programming language (R Development Core Team, 2018). Functional diversity indices of plant mixes in each irrigation frequency were calculated using “dbFD” function of *FD* package (Laliberté et al., 2014). As functional diversity indices including functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), and functional dispersion (FDis) are complementary and describe different features of functional diversity, a combination of these indices was used (Laliberté & Legendre, 2010; Villéger et al., 2008). High levels of functional diversity indicate greater species complementarity in resource use within communities (Mouchet et al., 2010).

All statistical analyses were performed using MINITAB v.19. One-way ANOVA and Tukey-HSD post-hoc tests (P -value < 0.05) were used to compare the abundance of each plant species among irrigation frequencies. Linear mixed effect models (LMMs) and Tukey-HSD post-hoc tests (P -value < 0.05) were used to determine significant differences in all measures among irrigation frequencies, with block as a random factor and irrigation treatments (three irrigation frequencies) as fixed factors. Comparison of abundance, richness, and Shannon index between the two measurement times (two and five months after sowing) for each irrigation frequency was obtained by performing LMMs for each frequency of irrigation, with time as a fixed factor and block as a random factor. Figures were created using Microsoft Excel and “ggplot” function of the *ggplot2* package (Wickham, 2016) in R statistical software version 3.5.1 (R Development Core Team, 2018).

4.3 Results

4.3.1 Seedling emergence, diversity, abundance and richness

Two months after sowing, 51 % of seeds had germinated, resulting in 269.8 ± 9.4 seedlings per module (1079 ± 37.5 seedlings m^{-2}). Seedling emergence varied greatly among the 16 species (Figure 4.3). *Podothea gnaphalioides* had the lowest mean seedling abundance (4.0 ± 0.8), followed by *Goodenia vilmorinae* (10.6 ± 2). While *Xerochrysum bracteatum* and *Schoenia cassiniana* had the highest seedling abundance (22.8 ± 4.3 and 22.0 ± 4.2 , respectively) (Figure 4.3). These two species were also the most abundant at the end of the experiment (five months after sowing), whereas *Heliotropium tenuifolium* had the lowest abundance at the end of the experiment (Figure 4.4). There was no significant effect of irrigation frequency on species abundance at the end of the experiment (Figure 4.4). However, regardless of the irrigation frequency there was a significant ($P < 0.05$) loss of individuals (plant death) in five (*G.*

vilmorinae, *H. tenuifolium*, *Rhodanthe margarethae*, *Schoenia macivorii* and *Swainsona formosa*) of the 16 species between the two- and five-month measurements.

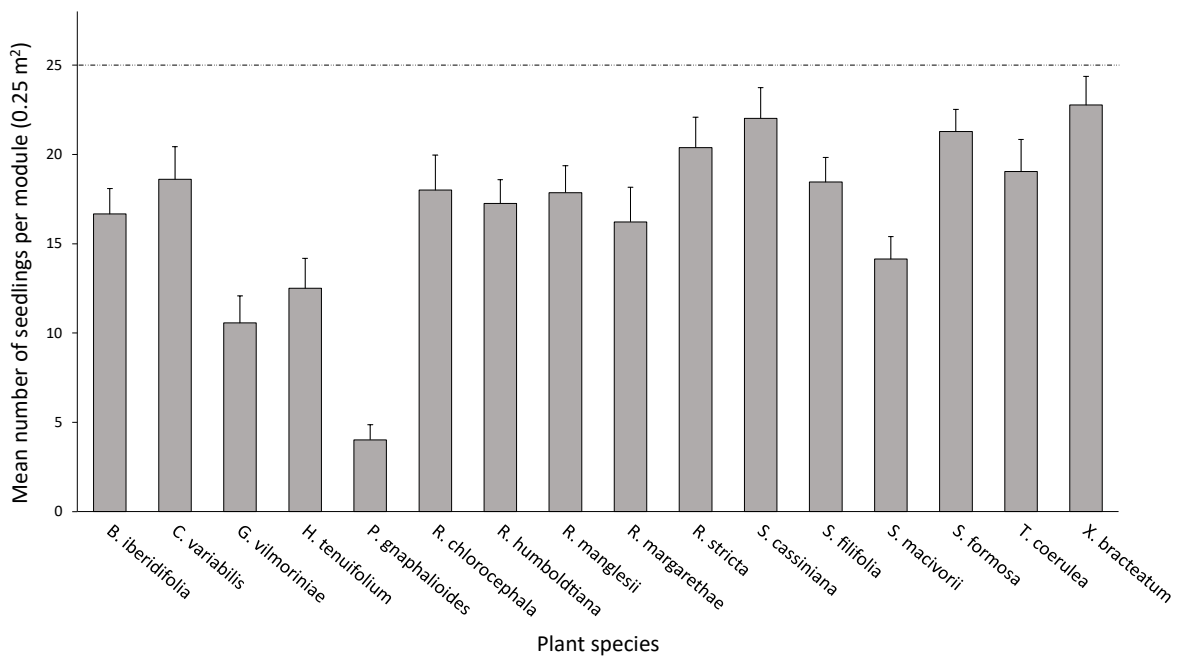


Figure 4.3. Mean number of seedlings of each species in each module (0.25 m²) two months after sowing and before the start of the irrigation treatments. Error bars indicate the standard error of the mean and the dashed line shows the total expected number of seedlings, based on germination testing and sowing rate.

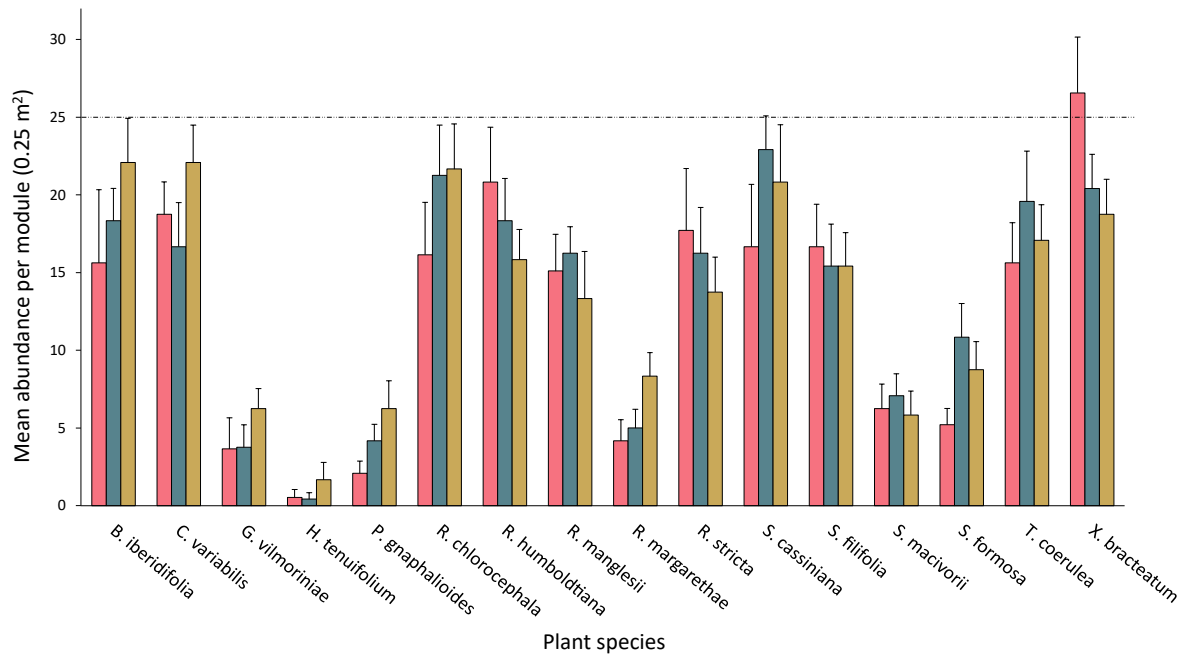


Figure 4.4. Mean number of individuals (abundance) of each species in each module (0.25 m²) five months after sowing (end of the experiment) in response to the three irrigation frequencies: 2 (pink), 4 (green), and 6 (yellow) days between irrigation. Error bars indicate the standard error of the mean. There were no significant differences between irrigation frequencies for each species (Tukey's post-hoc test; P -value < 0.05). The dashed line shows the total expected number of seedlings, based on germination testing and sowing rate.

Shannon diversity index was highest in the 6 days irrigation frequency and decreased significantly with 2 days irrigation frequency. However, neither abundance nor richness was affected by irrigation frequency. Shannon diversity, abundance and richness all decreased over the experimental period (between two- and five-month measurements) in all irrigation frequencies (Figure 4.5).

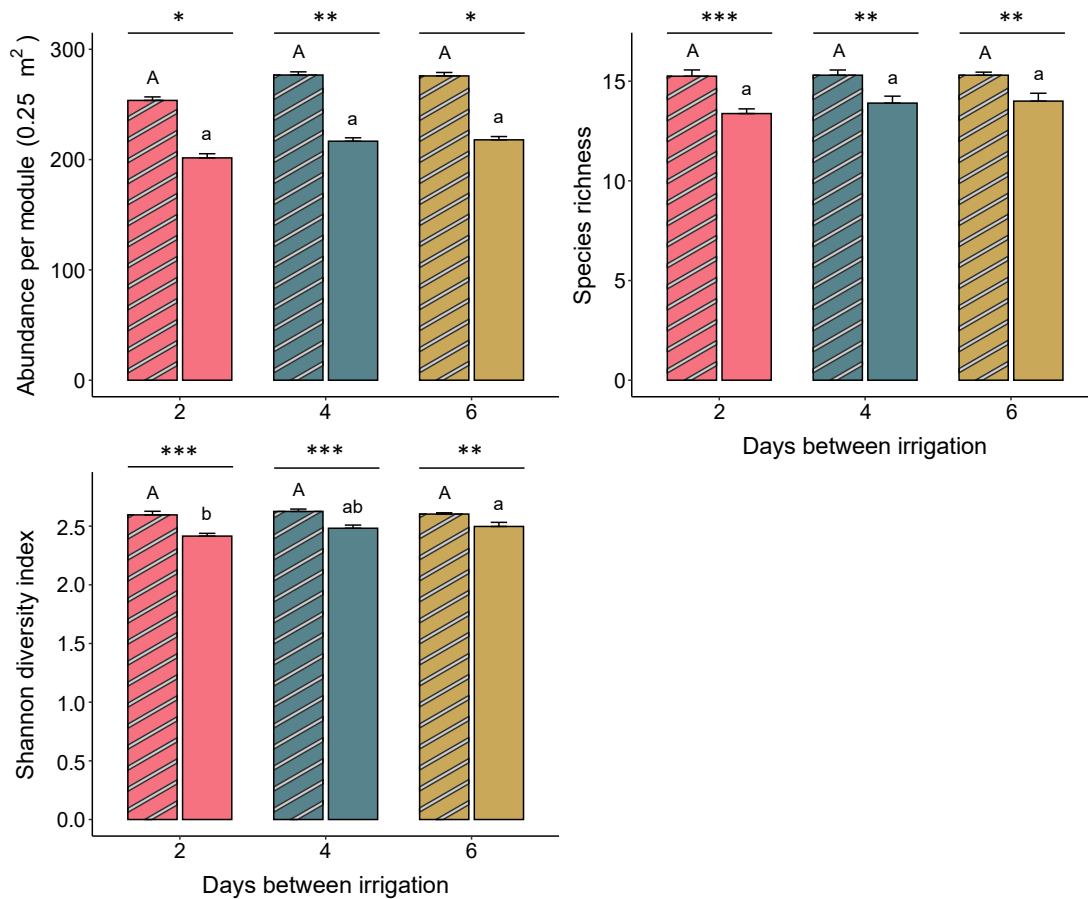


Figure 4.5. Species abundance, richness, and Shannon diversity index of the annual plant mix among the three irrigation frequencies: 2, 4, and 6 days between irrigation, measured at two (striped bar) and five (solid bar) months after sowing. Error bars indicate the standard error of the mean. Significant differences are indicated by dissimilar letters above each bar (Tukey's post-hoc test; P -value < 0.05); upper-case letters denote significant differences among irrigation frequencies at two-months and lower-case letters denotes significant differences among irrigation frequencies at five-months. Asterisks indicate significant differences between two- and five-month measurements within each irrigation frequency (Tukey's post-hoc test; *** P -value ≤ 0.001; ** P -value ≤ 0.01; * P -value ≤ 0.05).

4.3.2 Plant cover

Plant cover in all irrigation frequencies was greater than 81%. However, pairwise comparisons found a significant effect of irrigation treatment on plant cover. There was significantly greater plant cover in 2 days (89.3%) than 4 days (83.8%) and 6 days between irrigation (81.7%), however, these differences were minimal (5.4% and 7.6% respectively; Figure 4.6).

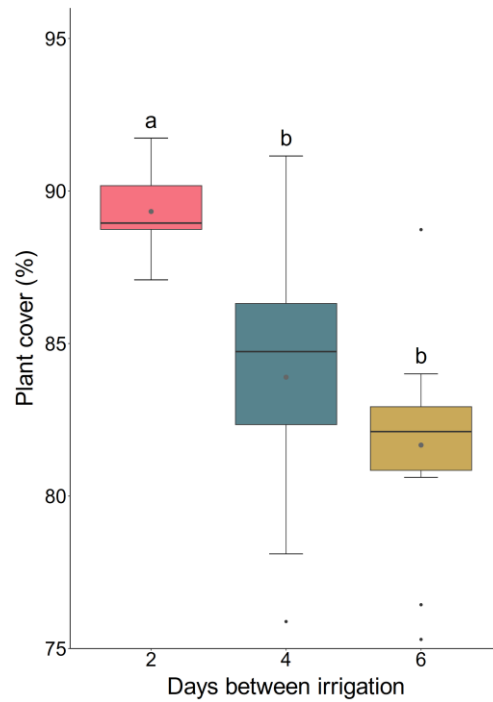


Figure 4.6. Plant cover (%) in green roof modules sown with a mixture of 16 annual plant species at the end of the experiment (five months after sowing) with the three irrigation frequencies (2, 4, and 6 days between irrigation). Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value < 0.05).

4.3.3 Plant traits, community weighted mean trait values, and functional diversity

Plant height decreased significantly in response to decreasing irrigation frequency (more days between irrigation events) in all species except *X. bracteatum*. For five of the species, height decreased significantly with each reduction in irrigation frequency. In five species, height did not differ significantly between 2 days and 4 days irrigation frequencies but became significantly shorter when the irrigation frequency decreased to 6 days, whereas the height of *S. cassiniana* decreased from 2 to 4 days irrigation frequency but did not decrease further when irrigation frequency was reduced to 6 days. Appendix C2).

Leaf area was not significantly different between irrigation frequencies in all species except for *S. cassiniana* and *Trachymene coerulea*, where LA decreased with increasing days between irrigation, and *Rhodanthe margarethae* where LA was significantly higher in 4 days compared to 6 days irrigation frequency (Appendix C3). For a small number of species, there were significant differences in specific leaf area, leaf dry matter content and leaf thickness among irrigation frequencies but their changes were mixed (Appendices C4 - C6).

Community weighted mean (CWM) plant height significantly decreased (F -value $_{2,17.21} = 27.63$, P -value < 0.001) with 6 days irrigation frequency (Figure 4.7). CWM leaf area also decreased (F -value $_{2,25} = 3.3$, P -value $= 0.053$) with reduced irrigation frequency, with the lowest value in 6 days irrigation. CWM SLA, LDMC and leaf thickness did not show significant differences among irrigation frequencies (Figure 4.7).

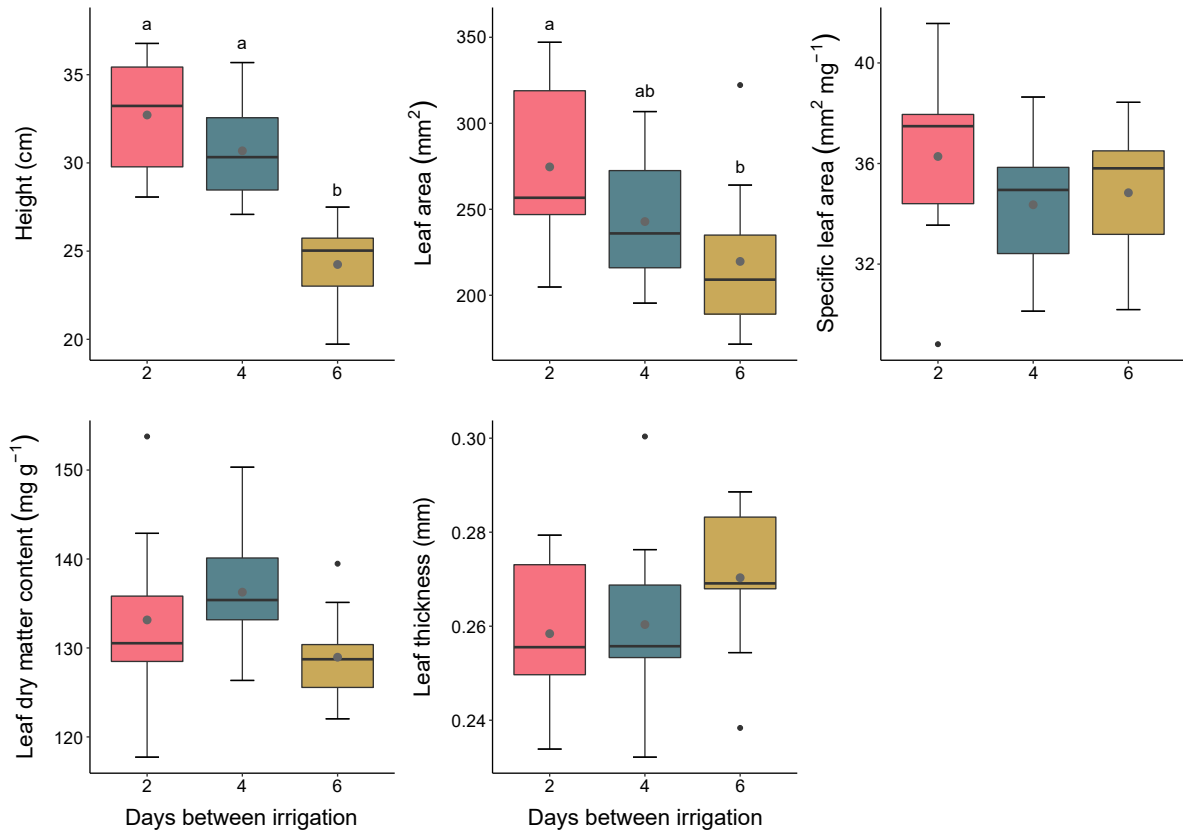


Figure 4.7. Community weighted mean (CWM) of plant traits in green roof modules sown with a mixture of 16 annual plant species with the three irrigation frequencies (2, 4, and 6 days between irrigation). Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value ≤ 0.05).

While there were some significant differences in the responses of individual traits to irrigation frequency, there were no significant differences in functional diversity indices among irrigation frequencies (Figure 4.8). Functional evenness was around 0.8, which implies a regular distribution of mean species traits within the trait space.

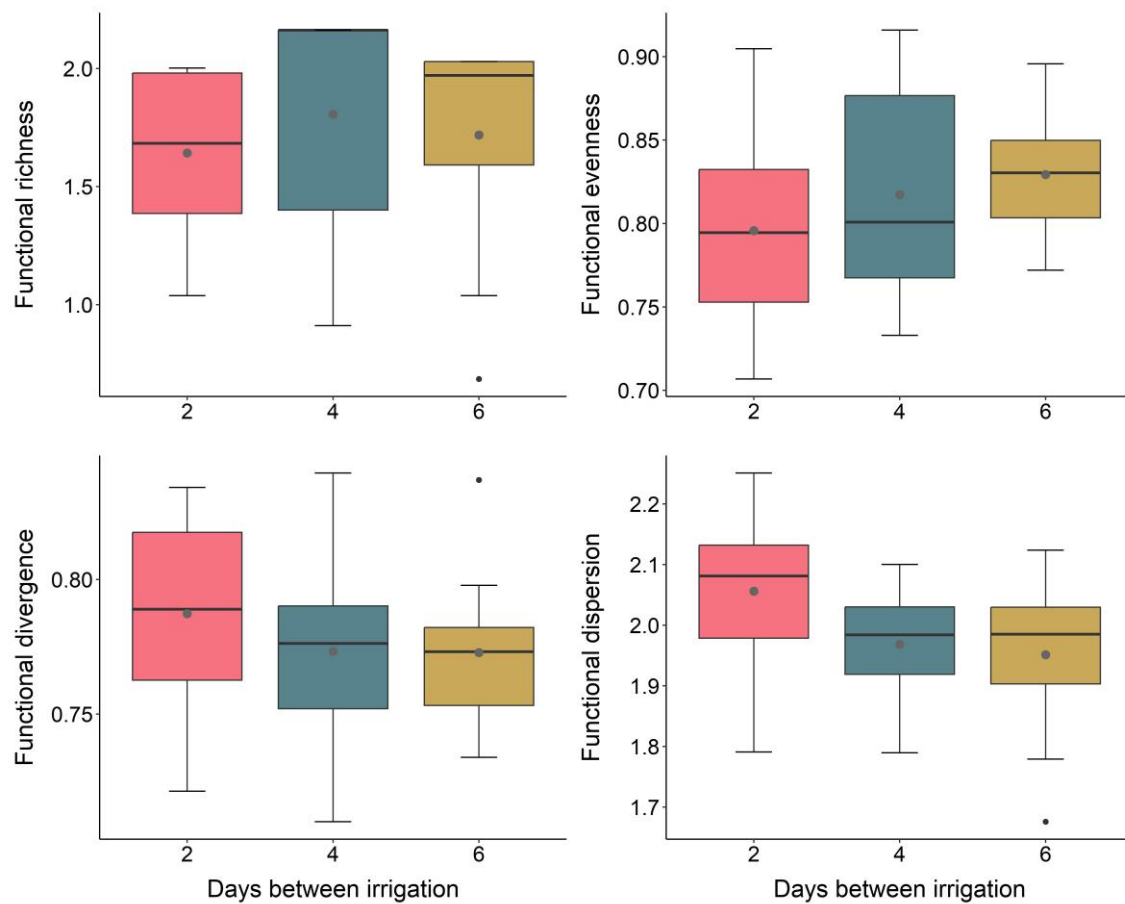


Figure 4.8. Functional diversity indices of plant traits in green roof modules sown with a mixture of 16 annual plant species with the three irrigation frequencies (2, 4, and 6 days between irrigation). Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. There were no significant differences between irrigation frequencies (Tukey's post-hoc test; P -value < 0.05).

4.4 Discussion

4.4.1 Plant cover

High plant cover on green roofs is important to minimize substrate loss, prevent weed invasion and to maximise ecosystem services and aesthetic values (Lundholm & Williams, 2015; Nagase et al., 2013). In our green roof modules sown with a mixture of 16 annual plant species, reduced irrigation frequency resulted in lower plant cover of annuals. Reduced plant cover with reduced water availability was shown in a 2-year green roof modular experiment with perennial species in a Mediterranean climate (Azeñas et al., 2018). In contrast, MacIvor et al. (2013) found no effect of water availability on plant cover of an annual and perennial grass/forb seed mix in humid continental Toronto, Canada. However, in their study, drought tolerant species became dominant when water was limiting and filled the gaps left by species which had died, and therefore plant cover was maintained. In our study, it is important to note that the reduction in plant cover with reduced irrigation frequency, while significant, was minor ($< 8\%$) and all modules achieved greater than 80% plant cover. The $\geq 80\%$ cover in all of our modules at the end of the experiment satisfies the German FLL and North American guidelines for the recommended vegetation cover on green roofs (Dvorak, 2011) and shows that our plant mix of Australian annual species can provide sufficient cover in their first growing season.

4.4.2 Plant traits

Plants were shorter and had smaller leaf areas when irrigation frequency was reduced, for both individual species and at the community level. However, the other leaf traits we measured, including SLA, LDMC and leaf thickness, were unaffected by irrigation frequency. Our findings of reduced plant height and leaf area with lower water availability are consistent with other green roof studies. For example, annual plants which had natural rainfall only were shorter than annual plants which were irrigated in the maritime climate of Sheffield, UK (Nagase & Dunnett, 2013). A green roof experiment in Mediterranean Spain also found that lower water availability (50% of field capacity) resulted in smaller leaf area in three out of five perennial species (Azeñas et al., 2019). Changes in plant height and leaf area in our study are also consistent with previous research on annual plants along a natural precipitation gradient in Morgan Hill, California (Lambrecht et al., 2017) or where water availability has been experimentally manipulated (Matesanz et al., 2020). As shorter plants with smaller leaves have reduced water consumption and water loss from leaves (Matesanz et al., 2020), it is likely that

reduced height and leaf area enabled the annual species in our mix to maintain high cover under the lowest irrigation frequency.

4.4.3 Seedling emergence, diversity, abundance and richness

Greater irrigation frequency (2 days between irrigation) resulted in slightly but significantly lower plant diversity in our experiment. In mixtures of grasses, legumes and forbs in experimentally field-grown grassland communities, reduced diversity has been attributed to greater plant cover which creates more shade (Hautier et al., 2009) and intensifies competition for light (Rajaniemi, 2003). In plant communities consisting of both annual and perennial species, faster growing or taller species have also been shown to competitively exclude shorter species (Lamb et al., 2009). This indicates that in our study, greater vegetative cover and canopy height with greater irrigation frequency (2 days irrigation frequencies) likely reduced light availability to shorter species, resulting in lower species diversity than 4 days and 6 days irrigation frequencies.

Overall, we observed greater than 50% seedling emergence, resulting in ≥ 1000 seedlings m^{-2} after two months in all of our modules (sown with $4 \text{ g seeds m}^{-2} \approx 2100 \text{ seeds m}^{-2}$). This number of seedlings is greater than reported in other green roof studies ($70 - 1000 \text{ plants m}^{-2}$) with greater sowing rates ($5 - 10 \text{ g m}^{-2}$) (e.g., Benvenuti, 2014; Molineux et al., 2015; Walker & Lundholm, 2018), and is likely due to the daily irrigation of our modules during the first two months of our experiment. However, between establishment (two months) and the end of our experiment (five months), there was an approximate 20% reduction in abundance in all modules (from ~ 1080 to $\sim 800 - 870 \text{ plants m}^{-2}$), likely due to self-thinning. Greater plant density limits access to space, light, soil nutrients and moisture, and increases mortality through competition (Falster et al., 2008). Self-thinning in seed-sown plant communities dominated by forbs has been reported previously. For example, in urban parks in Sheffield, UK, meadows sown with 15 forbs and two grasses showed 72% plant loss over three years in plots sown at high density (sowing rate of $1380 \text{ seeds m}^{-2}$; 2.5 g m^{-2}) compared to 55% loss in plots sown at low density (690 seeds m^{-2} ; 1.25 g m^{-2}) due to self-thinning (Hitchmough & de la Fleur, 2006). Therefore, it is likely that lower sowing rates of annuals on green roofs will result in similar species abundance to our study, without loss of species over time due to self-thinning.

Irrigation frequency had no effect on species abundance or richness at the end of our experiment, despite having significantly lower plant cover in the lowest irrigation frequency. However, while significant, the difference in plant cover between the highest and lowest

irrigation frequencies was only 7%. Another reason for the lack of difference in species abundance and richness among irrigation treatments is that water availability was not a limiting factor during seedling emergence and establishment, when plants are more susceptible to low water availability, and changes in their abundance have high impact on future community composition (Lloret et al., 2004). It is also likely that the lowest irrigation frequency in our experiment was not severe enough to result in loss of individuals or species. For example, Miranda et al. (2009) showed that 25% reduction in rainfall did not change the abundance and richness of annual species growing along in a wide range of habitats in southeast Spain; whereas 50% reduction resulted in significantly fewer individuals and species. In a green roof experiment with forb and grass mixtures in Sheffield, UK, Nagase and Dunnett (2010) observed decreasing irrigation frequency from watering once every week to once every two weeks after a 6-month irrigated establishment phase did not affect survival. However, significant reductions in survival were observed when irrigation was reduced to once every three weeks, because plants wilted and died (Nagase & Dunnett, 2010). However, in our experiment, weather conditions were relatively benign and it is likely that our lowest irrigation frequency (6 days) would negatively affect plant survival during summer months.

4.4.4 Functional diversity

Functional diversity of the annual plant mix was unaffected by irrigation frequency, likely because of the lack of any effect on abundance and richness (the two components of the functional diversity indices calculation) (Laliberté & Legendre, 2010; Mouchet et al., 2010; Schleuter et al., 2010). Several green roof studies have suggested that higher functional diversity (or phylogenetic diversity, as a proxy of functional diversity) would likely improve ecosystem services delivery of green roofs (Cook-Patton & Bauerle, 2012; MacIvor et al., 2016; MacIvor et al., 2018; Van Mechelen, Van Meerbeek, et al., 2015). For example, Xie et al. (2018) found that plant functional and phylogenetic diversity was positively related to green roof cooling. Combinations of different plant life-form groups have been shown to optimize summer roof cooling and water capture on green roof compared to monocultures (Lundholm et al., 2010). While green roof studies have not looked at changes in functional diversity in response to different water availabilities, ecological studies have shown variable effects of drought on functional diversity. For example, species' spatial distribution models have shown that in Australia, plant species grown under drought stress (extreme or seasonal dryness) had the lowest functional diversity compared to species of wetter and warmer regions (Andrew et al., 2021). On the contrary, in North America, drought resulted in greater functional diversity

in six native grasslands (Griffin-Nolan et al., 2019) and no change in Californian herbaceous plant communities (Copeland et al., 2016). These inconsistencies have been attributed to differences in trait selection and functional diversity indices as well as drought severity (Griffin-Nolan et al., 2019). In our study, as functional diversity of the annual plant mixes did not change with irrigation frequency, it is likely that the plant mix will provide the same function on green roofs when irrigated less.

4.5 Conclusions and recommendations for green roofs

Regardless of irrigation frequency, all modules sown with the mixture of 16 annual plant taxa achieved > 80% plant coverage, high species abundance and diversity. While self-thinning occurred over time, likely due to competition for light and space, irrigation frequency had no effect on species abundance and richness. However, plants had reduced height and leaf area in the lowest irrigation frequency. This indicates that lower irrigation frequency can sustain diverse annual plant mixtures on green roofs during benign weather conditions. However, higher irrigation frequencies are likely to be needed during hot summer months in climates where rainfall is sporadic and/or limited. Irrigation frequency did not change the functional diversity of the annual plant mix in the first growing season. However, plant diversity and cover may change in subsequent growing seasons as the longevity of annual plant communities on green roofs will depend on seed production and their subsequent germination. While some of the annual species failed to establish in green roof modules, most species established well and showed good growth and coverage. Due to the high level of self-thinning we observed, we suggest that a lower sowing rate could be used to establish annual plant mixes on green roofs, which would reduce the cost of establishment. However, plants should be irrigated frequently during germination and seedling emergence. Most species flowered six months after sowing and likely produced viable seeds to ensure persistence on the green roof. However, longer-term studies are needed on green roofs to assess seed germination in the next growing season to determine if re-sowing is needed, or whether annual plant communities can be self-regenerating or some species would produce lots of viable seeds, while others will not, which would result in a dramatic shift in community composition in the future. Although annuals can provide high coverage and diversity on green roofs during their first growing season, mixing them with perennial plant life-forms such as succulents, would provide sustained vegetation cover and flowering when annuals are dormant.

5 Synthesis

This chapter summarises the main findings of my thesis and discusses implications for green roof design and management. I then outline future research opportunities that are worth considering to continue improving our knowledge regarding how annual species can be used more widely in green roof projects.

The overall aim of this thesis was to better understand the potential of using Australian annual plants on green roofs and develop an annual-based green roof planting palette. This is because annual plants offer some advantages on green roofs, but as yet have not been used for this purpose in Australia and are the subject of very little research. Annual species are important plant life forms globally and Australia has a rich annual flora. Many annual species are distributed over arid and semi-arid parts of the country (Beard, 2015; Keith, 2017), which is a useful feature when selecting plant species for green roofs using the habitat template approach (Lundholm, 2006).

To achieve this aim, I started with the first and most critical stage in plant growth which is germination. Because low seed germination can hamper efforts to grow a diverse plant community via sowing, I explored seed germination attributes of Australian annual plant species using different treatments, and the relationship between germination attributes with seed mass and climate variables, two factors which influence seed germination and dormancy. Knowledge of how germination attributes change with seed mass and the climatic conditions of where they are distributed can help select plant species for seed-based horticulture projects such as green roofs. In addition, I investigated functional traits and plant strategies of the annual species, as these influence the ecosystem services green roofs deliver and can be used to help develop a trait-based plant palette for green roofs (Lundholm et al., 2015; Xie et al., 2018). Lastly, because green roofs with higher species and functional trait diversity are more resilient to environmental stress and provide greater ecosystem services and aesthetic value (Heim & Lundholm, 2014; MacIvor et al., 2016; Nagase & Dunnett, 2010; Van Mechelen, Van Meerbeek, et al., 2015), I evaluated how community composition and functional diversity of green roof modules sown with an annual plant mix could change with different irrigation frequencies.

5.1 Summary results and conclusions

I conducted a germination trial using the seeds of 58 Australian annual species with three pre-treatments (control, gibberellic acid and karrikinolide) and two different temperature regimes (20/10 °C and 25/15 °C). I then investigated if the climate of the species' natural distribution (obtained from the Atlas of Living Australia) relates to the mass of the seeds or their germination attributes (germination percentage, lag and rate) and which climate variables have the greatest influence. In addition, I examined if seed mass affects germination percentage or speed.

I found that species from hotter and drier environments and environments with greater annual temperature variation (harsher climatic conditions) had greater seed mass. However, greater seed mass does not lead to higher or faster germination and a more successful seed-based project. I also found that annual species from drier environments and species from environments with higher rainfall variability, had lower germination (after taking viability into account). However, contrary to our expectations, species from hotter environments had lower germination percentages. Faster germination speed was observed in species from drier environments and environments with lower rainfall variability. Therefore, species from drier environments showed a trade-off between germination percentage and speed (i.e., lower percentage but faster germination). This trade-off likely allows the development of a persistent soil seed bank, where not all seeds of a species germinate when conditions are good, which can maintain a population over time. Species of environments with high rainfall variability had a stronger bet-hedging strategy demonstrated by a lower germination percentage and slower germination. The overall results also showed that germination attributes in annuals are more influenced by climate than seed mass. This chapter provided some insights about the treatments which can improve germination in Australian annual plant species and indicated that climate variables might be more useful than seed mass to select species for seed-based projects.

I used gibberellic acid and karrikinolide, two germination stimulants which have been found previously to be very effective in breaking seed dormancy and promoting seed germination for Australian native species (Aleman et al., 2015; Commander et al., 2009; Hoyle et al., 2008; Probert et al., 2009). However, only 20 of my 58 species germinated and these additives or temperatures could not break the dormancy of the rest of the species. This shows the necessity of more research on Australian species' seed germination triggers. Knowledge of optimum treatments to improve germination capacity along with reliable germination reports will

facilitate the calculation of more accurate sowing rates for seed-based horticultural and restoration projects and better assessment of the performance of seed lots after sowing (Kildisheva et al., 2020).

Next, I performed a common garden experiment with 18 annual species that germinated in the previous chapter and measured their plant functional traits related to drought resistance and resource acquisition (specific leaf area - SLA, leaf dry matter content and leaf thickness), competitiveness (plant height) and reproductive ability (seed mass and germination attributes including germination lag and rate). I also explored the relationship between functional traits and plant performance metrics of annual species (relative growth rate, shoot biomass and flowering time).

Relationships among plant traits and performance metrics revealed that different life strategies (drought resistance, resource-use and reproductive strategies) exist in Australian annuals. In the studied annuals, more acquisitive species had fast traits, higher growth and greater biomass, while more conservative strategy was represented by slow traits, lower relative growth rate and shoot biomass. Higher resource acquisition and faster growth is desirable on green roofs as this can increase transpiration and therefore increase their stormwater mitigation ability. Species that had higher SLA and flowered earlier could be categorised as having a drought escape strategy, while later flowering species with lower SLA would likely better resist drought and survive longer in dry conditions. Using annual plants with a range of time to flowering and different SLAs (as indicators of drought resistance) would extend the flowering period and drought resistance of the plant palette which is desirable aesthetically and for biodiversity.

Lastly, I explored how annual plants perform as a community in green roof modules when receiving different irrigation frequencies. This part of my thesis has been recently published in *Urban Forestry and Urban Greening* (Saraeian et al., 2022). For this experiment, a mixture of 16 Australian annual species (with higher than 50% germination and sufficient number) was made and sown onto thirty green roof modules and irrigated with three different irrigation regimes: 2, 4, and 6 days between irrigation. While lower irrigation frequency (6 days between irrigation) reduced plant cover significantly, all modules had higher than 80% cover at the end of the experiment. This meets the criteria for recommended vegetation cover on green roofs in widely used green roof guidelines. This is a promising result, considering the importance of high plant cover to green roof ecosystem function and aesthetics. It also suggests that good plant cover can be achieved with Australian annuals in water limited situations such as green

roofs. All modules experienced a decline in the abundance, richness and diversity of annual species over time, likely due to competition effects. This implies that a lower sowing rate could be used, which would still result in a high cover, abundance and richness of plant species. High functional diversity is another key factor in determining the functionality and aesthetic value of green roofs and also their resilience to environmental stresses (Lee et al., 2014; Nagase & Dunnett, 2010; Xie et al., 2018). In this experiment, functional diversity at the community level was unaffected by irrigation frequency. These results suggest that green roofs sown with annual mixtures can achieve high plant cover and maintain diversity when minimally irrigated. I have to acknowledge that the climatic conditions were relatively benign during this experiment. Consequently, if green roof using Australian annuals is constructed in environments with unpredictable and long dry seasons, higher irrigation frequency may be needed to keep the annual community alive longer.

5.2 Study limitations

The following limitations were experienced during the course of this study and need to be acknowledged:

Due to their wide distribution across different, often remote, habitats and short, unpredictable lifecycles, it was impractical to collect the seeds of my studied annual species directly and therefore, they were sourced commercially. However, the suppliers could not provide information of the exact collection location of all species and therefore, I used the climate of natural distribution instead of the climate of origin to investigate the relationship between seed mass and germination attributes of annual species with climate variables. While climate of natural distribution represents the variation of climate that species experience, using climate of origin would provide more accurate and less variable information about the climate where the specific populations grow. Collaboration among seed collectors or producers, seed supply companies, botanical gardens and local community seedbanks might be useful to collect more information about the seeds. These collaborations would provide researchers with easier access to the seeds of higher number of plant species and their collection location climate data for further investigation.

The inability to break the dormancy of seeds for the majority of the species purchased, restricted the number of species I could work with in the following experiments and the small sample size in the third chapter (common garden experiment), limited the strengths of the conclusions.

Finally, strict Covid-19 lockdowns in Melbourne, Australia, significantly disrupted my data collection for the last chapter. The initial plan for the green roof module experiment was collecting the abundance and richness data of the species more frequently and soil water content regularly during the course of the experiment. However, during Covid-19 pandemic, students had almost no access to the Burnley campus, where the experiment was conducted.

5.3 Implications of my research for green roofs

My results indicate great potential of Australian annual species to be used on extensive green roofs, as they can germinate and establish successfully in green roof substrates (if water is present during germination and establishment phase through precipitation or irrigation) and grow successfully on shallow substrates (10 cm) with low irrigation while keeping their cover and diversity high. However, higher irrigation frequencies are likely to be needed during hot summer months in climates where rainfall is sporadic and/or limited. Therefore, this study has broadened the number of potential plant species which can be used by designers in green roof plant palettes. Annual species can be seeded directly which is a cheaper and faster method compared to planting tubestock by hand (Sutton, 2013). Annuals also have the potential to be combined with hardy ground covers to provide year-round vegetation cover and flowering while providing greater ecosystem services in comparison with succulent-only roofs.

Australian annual species with colourful flowers would make green roofs more aesthetically pleasing with mental well-being benefits (Lee et al., 2014) and nectar and pollen provision for pollinators (Dunnett, 2015). This could also demonstrate the attraction of the Australian flora and encourage greater green roof implementation.

In Australia, the majority of green roofs are intensive and built on newly constructed apartment and office buildings, and there are few extensive green roofs (Williams et al., 2021; Williams, Rayner, et al., 2010). There are also a low number of green roof retrofits in Australia (Wilkinson & Dixon, 2016; Williams, Rayner, et al., 2010). Australia is following the lead of cities around the world having mandatory policies for green roof construction by developing comprehensive policies and programs such as City of Melbourne's 'Green Our City Strategic Action Plan' (City of Melbourne, 2017), City of Sydney 'Green roofs and walls policy' (City of Sydney, 2014a), and 'Green roofs and walls policy implementation plan' (City of Sydney, 2014b). The City of Melbourne has developed and introduced the 'green factor tool', a method to assess the greenery score of green infrastructure integration into new developments (Bush et al., 2021). This would likely encourage designers and developers to look for cheaper, lighter,

and low-maintenance type green roofs to integrate into new buildings, such as pre-planted sedum mats, a widespread extensive green roof style in North America and Western Europe (Durhman et al., 2007; Getter & Rowe, 2009; Van Mechelen et al., 2014). As well as direct seeding, annual species have the potential to be used in designing roll-out mats which can be easily implemented on green roofs, with light weight, fast establishment and low maintenance, while offering colourful and attractive flowers and higher ecosystem services compared to sedum only green roofs.

Australian annual species can also be used in other horticultural projects on ground level such as wildflower urban meadows due to their attractive colourful flowers (Hoyle, 2015). Research has shown the effect of urban green infrastructure on human well-being (Nielsen & Hansen, 2006; Thwaites et al., 2005). Planting species with colourful flowers in urban parks receive people preferences and appreciation (Hitchmough & de la Fleur, 2006; Hoyle et al., 2017). Australian annual species have impressive flowers with different colours and are perfect options to bring visual attraction to urban landscape. Combining winter and summer annuals may create succession of flowering and a dramatic vegetation for longer time.

5.4 Opportunities for future research

While this study demonstrated the suitability of Australian annual species for green roofs, having a reliable supply of good quality seed, from a large and diverse range of herbaceous species, is the initial and essential requirement for such projects relying on seed (Merritt et al., 2007). The native seed supply chain is not very reliable in Australia and providing large quantities of seeds for seed-based projects is a challenge (Hancock et al., 2020). Therefore, more research is needed on the conditions required to break seed dormancy and facilitate the production of seeds in large quantities. For example, both warm and cold stratification has been shown to alleviate seed dormancy in Australian (Merritt et al., 2007) or North American species (Kildisheva et al., 2019). Access to seed for a higher number of species from Australia's rich annual flora would broaden the options for designing artificial mixtures that suit the environmental conditions where a horticultural project is undertaken.

If readily germinable species are available, optimal sowing time is a major factor in determining the success of the seed-based project, because adverse post-germination environmental conditions may result in establishment failure (Turner et al., 2006). Moreover, seed enhancement technologies such as priming can be used to improve and synchronize germination and allow faster establishment and reduce weed density (Brown et al., 2021).

My study showed that annual species can successfully grow and provide high coverage and diversity on green roofs in their first growing season. However, more research is needed to investigate the performance of annual species in subsequent generations, the germination potential of the seeds produced and their persistence (seed longevity) in green roof substrates. This is important if the population is to be sustained. In addition, due to the drought escape strategy of annuals, mixing annuals with perennial ground cover species or succulents with high stress tolerance should be investigated to ensure sustained vegetation cover and flowering when annuals are dormant. The interaction between annuals and succulents might differ under well-watered and water-deficit conditions (Butler & Orians, 2011). Succulents might facilitate the growth of annual species under drought, by cooling the substrate or increasing substrate water content by suppressing evapotranspiration (Butler & Orians, 2009). However, competition may occur under favourable conditions due to competing over resources, such as water and light (Vasl et al., 2017). Therefore, long-term studies are needed to evaluate the best combination of annual species with other plant life forms in green roof conditions to attain the best results.

6 References

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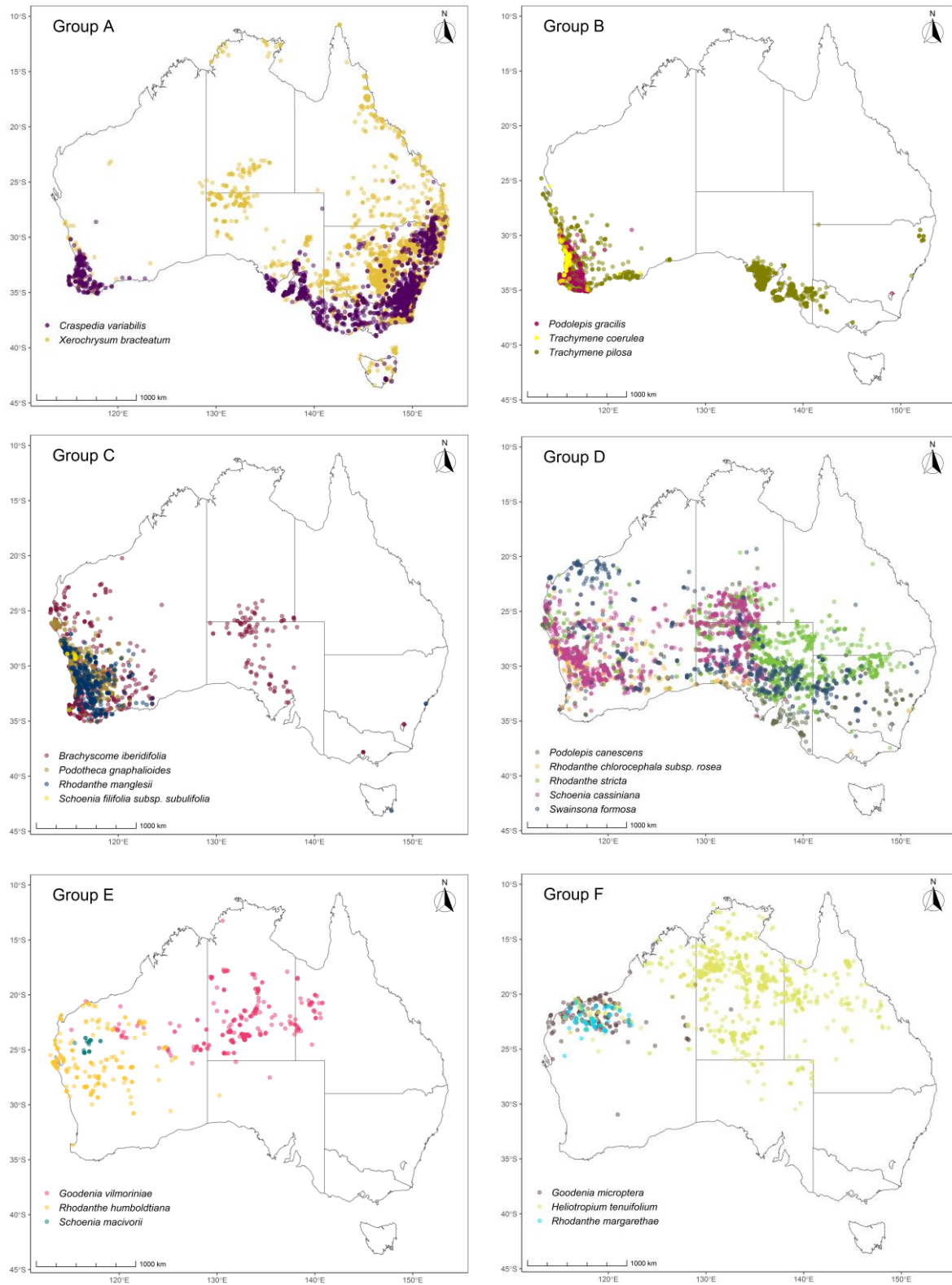
7 Appendix A – Chapter 2

Appendix A1. Characteristics of the 58 Australian annual species including family, seed mass, viability percentage (determined by cut test prior to germination test), germination status (if species have germinated in at least one of the germination treatments), and mean values ($\pm$ standard deviation) and ranges (min - max) of annual mean temperature (AMT) and annual precipitation (AP) for species occurrence records held at the Atlas of Living Australia (ALA, 2018).

Scientific name	Family	AMT (°C)		AP (mm)		Seed mass (mg)	Viability percentage	Germination status
		Mean ($\pm$ SD)	Range	Mean ($\pm$ SD)	Range			
<i>Angianthus milnei</i>	Asteraceae	23 ($\pm$ 1.4)	21.3-26.6	240.3 ($\pm$ 39.6)	186-560	0.055	100	
<i>Asteridea pulverulenta</i>	Asteraceae	17.2 ($\pm$ 1.3)	14.9-20.3	816.7 ($\pm$ 173)	314-1252	0.139	100	
<i>Bellida graminea</i>	Asteraceae	19.8 ($\pm$ 0.7)	16-21.4	294.1 ($\pm$ 22.9)	242-423	0.956	100	
<i>Brachyscome iberidifolia</i>	Asteraceae	18.8 ($\pm$ 2.3)	11.9-27.1	492 ($\pm$ 261.6)	155-1216	0.127	90	✓
<i>Bulbine semibarbata</i>	Liliaceae	17.2 ($\pm$ 1.9)	7.9-24.6	454.3 ($\pm$ 190.4)	154-1740	1.420	100	
<i>Calotis cymbacantha</i>	Asteraceae	19.3 ($\pm$ 1.5)	14.3-27.2	236.7 ($\pm$ 67.5)	148-1233	1.467	100	
<i>Calotis multicaulis</i>	Asteraceae	21 ($\pm$ 2)	15.6-27.6	244.8 ($\pm$ 58.5)	150-726	0.550	100	
<i>Calotis plumulifera</i>	Asteraceae	21.3 ($\pm$ 1.8)	13.6-27.8	248.8 ($\pm$ 74.6)	140-838	0.573	100	
<i>Craspedia variabilis</i>	Asteraceae	13.5 ($\pm$ 2.5)	4.1-22.6	799.7 ($\pm$ 256.8)	197-2190	0.584	85	✓
<i>Erymophyllum ramosum</i> subsp. <i>involucratum</i>	Asteraceae	21.2 ($\pm$ 1.7)	18.5-25.4	354.8 ($\pm$ 160.1)	196-734	0.441	100	
<i>Erymophyllum ramosum</i> subsp. <i>ramosum</i>	Asteraceae	19.5 ($\pm$ 2.1)	15.4-27	277.8 ($\pm$ 53.7)	193-765	0.315	80	
<i>Flaveria australasica</i>	Asteraceae	24.2 ($\pm$ 2.9)	11.6-28.9	449.4 ($\pm$ 213.3)	138-2018	0.227	100	
<i>Goodenia microptera</i>	Goodeniaceae	25.6 ($\pm$ 1.2)	18.3-27.7	356.5 ($\pm$ 128.4)	195-1270	0.910	75	✓
<i>Goodenia vilmorinae</i>	Goodeniaceae	23.4 ($\pm$ 1.9)	19.1-27.2	353.1 ($\pm$ 103.7)	198-1488	1.343	100	✓
<i>Heliotropium paniculatum</i>	Asteraceae	26.1 ($\pm$ 2.4)	20.5-29	742.5 ($\pm$ 290.5)	203-1465	0.386	100	
<i>Heliotropium tenuifolium</i>	Asteraceae	25.1 ($\pm$ 2)	17.8-28.5	520.5 ($\pm$ 229.1)	156-1788	0.433	100	✓
<i>Hyalosperma cotula</i>	Asteraceae	17.7 ($\pm$ 1.5)	14.2-25.5	702.2 ($\pm$ 191)	201-1180	0.091	100	
<i>Hyalosperma glutinosum</i>	Asteraceae	20.5 ($\pm$ 1)	17.9-23.6	282 ($\pm$ 38.5)	201-386	0.562	90	
<i>Hyalosperma praecox</i>	Asteraceae	15.3 ($\pm$ 1.4)	11.4-20.1	468.6 ($\pm$ 112.9)	259-916	0.223	100	
<i>Hyalosperma simplex</i>	Asteraceae	15.7 ($\pm$ 0.7)	15.2-17.2	666.1 ($\pm$ 139.1)	367-829	0.235	95	
<i>Isotoma hypocrateriformis</i>	Campanulaceae	17.4 ($\pm$ 1.5)	14.1-24.2	693.3 ($\pm$ 239.5)	193-1252	0.016	85.71	
<i>Isotoma scapigera</i>	Campanulaceae	16.8 ($\pm$ 1.2)	14.6-20.2	530.8 ($\pm$ 215.6)	283-1097	0.000	100	
<i>Leucochrysum fitzgibbonii</i>	Asteraceae	20.5 ($\pm$ 1.5)	17.2-27.2	256.6 ($\pm$ 74.2)	154-1014	0.170	95	
<i>Leucochrysum stipitatum</i>	Asteraceae	21.5 ($\pm$ 1.2)	17.9-26.2	263.7 ($\pm$ 60.9)	154-1263	1.117	60	
<i>Myriocephalus guerinae</i>	Asteraceae	20.8 ($\pm$ 1.1)	18.4-24.3	267.2 ($\pm$ 44.4)	199-718	2.681	100	
<i>Podolepis canescens</i>	Asteraceae	19.1 ($\pm$ 2.2)	12.9-24.2	319 ($\pm$ 121.4)	164-870	0.125	100	✓
<i>Podolepis gracilis</i>	Asteraceae	17.4 ($\pm$ 1.3)	13.7-20.4	767.5 ($\pm$ 176.9)	244-1205	0.068	80	✓
<i>Podolepis lessonii</i>	Asteraceae	18.3 ($\pm$ 1.7)	13.6-24.5	427.7 ($\pm$ 189.9)	195-1151	0.031	ND	

<i>Podotheca angustifolia</i>	Asteraceae	16.6 (±1.4)	12.6-23.2	441.5 (±179.8)	197-1219	0.305	ND	
<i>Podotheca chrysantha</i>	Asteraceae	18.4 (±0.5)	15.4-20.3	729.1 (±93.9)	269-963	0.500	70	
<i>Podotheca gnaphalioides</i>	Asteraceae	18.9 (±1.4)	15.3-23.6	414.4 (±169.5)	198-1111	0.518	100	✓
<i>Pterochaeta paniculata</i>	Asteraceae	17.4 (±1.3)	11.9-22.2	628.4 (±217)	263-1117	0.159	100	
<i>Rhodanthe charsleyae</i>	Asteraceae	21.6 (±1.2)	15.6-26	247.2 (±56.2)	142-434	1.329	86.66	
<i>Rhodanthe chlorocephala</i> subsp. <i>rosea</i>	Asteraceae	19.3 (±1.4)	13.7-25.2	331.1 (±131.1)	172-1030	2.446	100	✓
<i>Rhodanthe humboldtiana</i>	Asteraceae	22.5 (±1.8)	16.8-27.1	263.4 (±66.8)	182-801	0.640	100	✓
<i>Rhodanthe manglesii</i>	Asteraceae	18 (±1.6)	11.9-21.4	456.7 (±186.6)	230-1280	1.087	95	✓
<i>Rhodanthe margarethae</i>	Asteraceae	25 (±1.5)	21.7-27.6	369 (±53.2)	203-443	0.109	92	✓
<i>Rhodanthe polygalifolia</i>	Asteraceae	17.6 (±1.2)	14.1-22.3	288.2 (±67.4)	149-505	1.089	ND	
<i>Rhodanthe pygmaea</i>	Asteraceae	17.1 (±1.3)	13.1-23.5	326.5 (±96.1)	149-1020	0.778	100	
<i>Rhodanthe sterilecens</i>	Asteraceae	23 (±1.1)	19.1-25.7	256.9 (±39)	194-404	1.772	90	
<i>Rhodanthe stricta</i>	Asteraceae	20.4 (±1.8)	14.1-27.1	253.3 (±89.5)	139-1245.7	3.647	100	✓
<i>Schoenia cassiniana</i>	Asteraceae	20.3 (±1.6)	15.4-27	270.4 (±71.6)	154-1030	3.202	100	✓
<i>Schoenia filifolia</i> subsp. <i>subulifolia</i>	Asteraceae	19.7 (±1.1)	16.3-20.4	426.9 (±190.5)	329-1038.7	1.174	100	✓
<i>Schoenia macivorii</i>	Asteraceae	24.2 (±0.8)	21.3-24.8	249.7 (±29.1)	215-327	3.127	100	✓
<i>Senecio pinnatifolius</i> var. <i>latilobus</i>	Asteraceae	17.8 (±1.7)	14.5-23.2	742.4 (±227.5)	212-1219	0.325	100	
<i>Senecio pinnatifolius</i> var. <i>maritimus</i>	Asteraceae	15 (±1.5)	8.5-20.2	727.8 (±326.3)	242-2135	0.377	100	
<i>Stylidium calcaratum</i>	Stylidiaceae	16.6 (±1.5)	12.2-21	754.5 (±187.1)	296-1216	0.019	100	
<i>Swainsona formosa</i>	Fabaceae	20.5 (±2.9)	13.2-27.9	249.5 (±83.4)	148-1030	6.117	100	✓
<i>Trachymene coerulea</i>	Araliaceae	18.1 (±1.5)	14.1-22.8	762.1 (±196)	195-1151	1.527	100	✓
<i>Trachymene pilosa</i>	Araliaceae	17.1 (±1.4)	10.9-22.2	634.6 (±225.1)	206.8-1252	1.129	100	✓
<i>Trianthema pilosa</i>	Aizoaceae	25.7 (±1.7)	16.3-28.8	518 (±325.2)	152-1481	0.546	100	
<i>Velleia connata</i>	Goodeniaceae	20.9 (±3.3)	14.1-28	333.1 (±101.9)	177-1343	2.892	100	
<i>Velleia rosea</i>	Goodeniaceae	19.9 (±1.1)	16.1-23.7	280.4 (±42.2)	189-816	2.549	100	
<i>Vittadinia humerata</i>	Asteraceae	19 (±1)	16.8-21.5	300.6 (±30.2)	215-382	0.633	100	
<i>Waitzia nitida</i>	Asteraceae	18.8 (±1.7)	13.7-24.3	424.1 (±164)	198-1034	0.175	ND	
<i>Waitzia suaveolens</i> var. <i>suaveolens</i>	Asteraceae	17.7 (±1.4)	14.4-24	675.8 (±202)	229-1252	0.148	66.66	
<i>Xerochrysum bracteatum</i>	Asteraceae	16 (±3.2)	3.4-27.7	753.2 (±374.6)	157-3197	0.633	100	✓
<i>Xerochrysum viscosum</i>	Asteraceae	14.7 (±2.1)	5.5-20.9	644.8 (±176)	313-1784	0.381	65	

ND, not determined



Appendix A2. Natural distribution locations (occurrence points) for 20 Australian annual species (which germinated in at least one of the germination treatments in our study) across Australia. Distribution data obtained from the Atlas of Living Australia (ALA, 2018). Species are classified into six climate groups labelled A-F, based on cluster analysis (refer to Figure 2.2).

Appendix A3. Means of long-term climate variables of natural distribution for 20 Australian annual species across Australia, obtained from the Atlas of Living Australia (ALA, 2018). Species are classified into six climate groups based on cluster analysis (refer to Figure 2.2). Refer to Table 2.1 for the bioclimatic variable codes.

Plant species code	Bioclimatic variables	Bio01	Bio03	Bio04	Bio07	Bio08	Bio09	Bio10	Bio11	Bio12	Bio15	Bio16	Bio17	Bio18	Bio19	Bio20	Bio28
Plant species																	
Group A																	
Cv	<i>Craspedia variabilis</i>	13.45	0.47	1.60	25.19	12.56	13.94	19.32	7.67	799.73	32.35	284.58	128.60	177.54	233.08	16.80	0.62
Xb	<i>Xerochrysum bracteatum</i>	15.95	0.48	1.68	26.48	18.39	13.61	22.03	9.69	753.20	30.38	267.43	122.27	229.08	154.94	17.63	0.53
Group B																	
Pg	<i>Podolepis gracilis</i>	17.37	0.51	1.37	23.00	12.95	22.33	22.63	12.62	767.47	76.67	406.70	45.18	48.37	385.93	18.04	0.54
Tc	<i>Trachymene coerulea</i>	18.07	0.50	1.34	22.40	13.76	22.76	23.24	13.38	762.05	78.45	409.52	43.28	48.34	386.72	18.34	0.51
Tp	<i>Trachymene pilosa</i>	17.12	0.50	1.36	22.91	12.67	21.75	22.28	12.32	634.57	66.42	319.71	46.12	51.78	303.91	18.10	0.46
Group C																	
Bi	<i>Brachyscome iberidifolia</i>	18.77	0.49	1.60	26.33	15.80	21.57	24.75	12.96	492.05	63.95	244.92	38.73	56.93	222.67	19.48	0.34
Pgn	<i>Podotheca gnaphalioides</i>	18.93	0.49	1.69	27.40	13.70	22.49	25.29	12.84	414.36	63.42	205.64	35.93	47.02	191.17	19.82	0.30
Rmn	<i>Rhodanthe manglesii</i>	17.95	0.50	1.66	27.01	12.76	22.17	24.18	12.03	456.70	60.84	219.41	43.17	51.57	205.62	19.19	0.35
Sf	<i>Schoenia filifolia</i>	19.75	0.50	1.74	28.43	14.32	24.87	26.36	13.49	426.93	73.67	224.46	30.19	36.07	204.15	20.34	0.30
Group D																	
Pc	<i>Podolepis canescens</i>	19.11	0.47	1.82	29.14	19.50	18.42	25.74	12.25	319.02	43.72	124.71	42.52	78.05	95.07	20.10	0.20
Rc	<i>Rhodanthe chlorocephala</i>	19.28	0.49	1.73	28.48	16.59	21.04	25.70	12.87	331.06	53.26	149.13	35.42	56.04	129.60	20.22	0.22
Rs	<i>Rhodanthe stricta</i>	20.36	0.46	2.01	31.35	24.95	17.43	27.66	12.67	253.28	39.49	93.36	38.04	82.60	55.91	20.73	0.12
Sc	<i>Schoenia cassiniana</i>	20.34	0.47	1.94	30.93	20.65	19.23	27.40	13.00	270.38	49.70	114.06	31.08	72.76	81.33	20.97	0.15
Sfo	<i>Swainsona formosa</i>	20.53	0.48	1.87	29.89	24.18	18.66	27.31	13.41	249.48	43.49	98.95	34.82	85.61	55.58	20.68	0.12
Group E																	
Gv	<i>Goodenia vilmorinae</i>	23.40	0.48	1.92	31.43	29.61	17.85	29.85	15.55	353.06	79.74	194.22	20.76	171.36	31.68	22.31	0.11
Rh	<i>Rhodanthe humboldtiana</i>	22.45	0.47	1.77	28.52	22.52	22.86	28.92	15.66	263.37	74.58	133.74	15.65	78.21	86.71	21.83	0.13
Sm	<i>Schoenia macivorii</i>	24.15	0.45	2.08	32.79	30.48	23.54	31.77	16.15	249.65	73.00	114.80	11.25	102.70	65.95	22.36	0.10
Group F																	
Gm	<i>Goodenia microptera</i>	25.64	0.50	1.69	28.62	30.65	24.94	31.36	18.76	356.53	101.55	220.98	8.11	186.62	48.28	22.60	0.11
Ht	<i>Heliotropium tenuifolium</i>	25.12	0.51	1.59	28.84	29.80	20.08	30.36	18.46	520.45	100.85	341.56	13.21	234.94	21.57	22.12	0.18
Rmr	<i>Rhodanthe margarethae</i>	25.01	0.48	1.83	30.29	30.68	24.54	31.25	17.58	368.99	98.51	223.19	10.66	196.85	52.74	22.43	0.12

Appendix A4. Germination percentage (mean $\pm$ SE; $n = 4$) of 20 annual species which germinated in at least one of the germination treatments.

Species	Untreated		GA ₃		KAR1
	20/10 °C	25/15 °C	20/10 °C	25/15 °C	20/10 °C
<i>Brachyscome iberidifolia</i>	83.3 $\pm$ 4.6 ^{a†}	77.8 $\pm$ 5.9 ^a	76.7 $\pm$ 2.1 ^a	78.9 $\pm$ 4.9 ^a	73.3 $\pm$ 4.6 ^a
<i>Craspedia variabilis</i>	76.5 $\pm$ 4 ^a	74.3 $\pm$ 5.1 ^a	56.5 $\pm$ 8.2 ^a	----- [‡]	80.5 $\pm$ 4.4 ^a
<i>Goodenia microptera</i>	0 ^b	0 ^b	0 ^b	0 ^b	33.3 $\pm$ 7.7 ^a
<i>Goodenia vilmorinae</i>	0 ^b	0 ^b	0 ^b	0 ^b	76 $\pm$ 2.3 ^a
<i>Heliotropium tenuifolium</i>	0 ^b	0 ^b	76 $\pm$ 3.7 ^a	88 $\pm$ 2.8 ^a	81 $\pm$ 5 ^a
<i>Podolepis canescens</i>	80 $\pm$ 4.6 ^a	-----	81.3 $\pm$ 7.5 ^a	-----	73.8 $\pm$ 5.2 ^a
<i>Podolepis gracilis</i>	72.5 $\pm$ 8.3 ^a	55 $\pm$ 5.8 ^{ab}	42.5 $\pm$ 6 ^b	36.3 $\pm$ 1.3 ^b	38.8 $\pm$ 3.1 ^b
<i>Podothea gnaphalioides</i>	59 $\pm$ 1.9 ^b	94 $\pm$ 2.6 ^a	74 $\pm$ 5.3 ^{ab}	93 $\pm$ 3 ^a	90 $\pm$ 2.6 ^{ab}
<i>Rhodanthe chlorocephala</i> subsp. <i>rosea</i>	95 $\pm$ 1 ^a	88 $\pm$ 2.8 ^b	94 $\pm$ 1.2 ^{ab}	87 $\pm$ 1.9 ^b	90 $\pm$ 2 ^{ab}
<i>Rhodanthe humboldtiana</i>	42 $\pm$ 1.2 ^a	28 $\pm$ 2.8 ^{ab}	23 $\pm$ 4.7 ^b	22 $\pm$ 3.5 ^b	36 $\pm$ 5.9 ^{ab}
<i>Rhodanthe manglesii</i>	84.2 $\pm$ 4.2 ^a	65.3 $\pm$ 3.6 ^b	62.1 $\pm$ 4.7 ^b	65.3 $\pm$ 7 ^b	75.8 $\pm$ 3.4 ^{ab}
<i>Rhodanthe margarethae</i>	8.2 $\pm$ 4.7 ^c	67.9 $\pm$ 8.4 ^a	14.9 $\pm$ 1.4 ^{bc}	53 $\pm$ 9 ^{ab}	71.7 $\pm$ 4.2 ^a
<i>Rhodanthe stricta</i>	88 $\pm$ 5.2 ^a	80 $\pm$ 3.3 ^{ab}	74 $\pm$ 2.6 ^{ab}	64 $\pm$ 3.3 ^b	78 $\pm$ 2.6 ^{ab}
<i>Schoenia cassiniana</i>	50 $\pm$ 2.6 ^a	49 $\pm$ 3.4 ^a	43 $\pm$ 10.2 ^a	52 $\pm$ 7.1 ^a	45 $\pm$ 7 ^a
<i>Schoenia filifolia</i>	96 $\pm$ 1.6 ^a	-----	90 $\pm$ 2 ^a	-----	89 $\pm$ 5 ^a
<i>Schoenia macivorii</i>	26 $\pm$ 3.8 ^b	15 $\pm$ 2.5 ^b	88 $\pm$ 1.6 ^a	88 $\pm$ 3.3 ^a	26 $\pm$ 2.6 ^b
<i>Swainsona formosa</i>	43 $\pm$ 3.4 ^a	57 $\pm$ 3.4 ^a	54 $\pm$ 3.8 ^a	45 $\pm$ 5 ^a	58 $\pm$ 6.8 ^a
<i>Trachymene coerulea</i>	90 $\pm$ 2.6 ^{ab}	98 $\pm$ 1.2 ^a	85 $\pm$ 4.4 ^b	94 $\pm$ 1.2 ^{ab}	96 $\pm$ 1.6 ^{ab}
<i>Trachymene pilosa</i>	18 $\pm$ 2.6 ^a	16 $\pm$ 2.8 ^{ab}	15 $\pm$ 3.8 ^{ab}	6 $\pm$ 2 ^b	18 $\pm$ 2.6 ^a
<i>Xerochrysum bracteatum</i>	93 $\pm$ 1.9 ^a	93 $\pm$ 3.4 ^a	94 $\pm$ 2.6 ^a	95 $\pm$ 1 ^a	92 $\pm$ 1.6 ^a

† Letters indicate significant differences among means for each species, where non-significant differences share at least one letter (one-way ANOVA and Tukey's post-hoc tests).

‡ No germination tests were conducted in these cases due to lack of seeds

Appendix A5. Germination lag (mean $\pm$ SE; $n = 4$) of 20 annual species which germinated in at least one of the germination treatments.

Species	Untreated		GA ₃		KAR1
	20/10 °C	25/15 °C	20/10 °C	25/15 °C	20/10 °C
<i>Brachyscome iberidifolia</i>	3 ^{a†}	2 ^b	2.3 $\pm$ 0.5 ^{ab}	2 ^b	1 ^c
<i>Craspedia variabilis</i>	6.3 $\pm$ 0.3 ^a	5.8 $\pm$ 0.3 ^a	5.3 $\pm$ 0.3 ^a	----- [‡]	4.3 $\pm$ 0.3 ^b
<i>Goodenia microptera</i>	No G.	No G.	No G.	No G.	5.3 $\pm$ 0.8
<i>Goodenia vilmorinae</i>	No G.	No G.	No G.	No G.	4
<i>Heliotropium tenuifolium</i>	No G.	No G.	7.8 $\pm$ 1 ^a	3.5 $\pm$ 0.3 ^b	7.8 $\pm$ 0.3 ^a
<i>Podolepis canescens</i>	3.8 $\pm$ 0.3 ^a	-----	2.3 $\pm$ 0.3 ^b	-----	1 ^c
<i>Podolepis gracilis</i>	6.8 $\pm$ 0.3 ^a	5 $\pm$ 0.4 ^{cd}	6 ^b	5 ^c	2.8 $\pm$ 1 ^d
<i>Podotheca gnaphalioides</i>	4.8 $\pm$ 0.5 ^a	2 ^c	3.8 $\pm$ 0.5 ^{abd}	3 ^b	1.8 $\pm$ 0.8 ^d
<i>Rhodanthe chlorocephala</i> subsp. <i>rosea</i>	2 ^a	2 ^a	1.5 $\pm$ 0.3 ^{ab}	1 ^b	1 ^b
<i>Rhodanthe humboldtiana</i>	5.3 $\pm$ 0.3 ^a	3.3 $\pm$ 0.3 ^{bc}	5.8 $\pm$ 0.6 ^a	5 $\pm$ 0.9 ^{ab}	3 ^c
<i>Rhodanthe manglesii</i>	2.5 $\pm$ 0.3 ^a	2 ^a	2.3 $\pm$ 0.3 ^a	2 ^a	1 ^b
<i>Rhodanthe margarethae</i>	19 $\pm$ 6.9 ^{a^}	5.3 $\pm$ 0.5 ^b	7 $\pm$ 1.2 ^{ab}	5 $\pm$ 0.4 ^b	3.8 $\pm$ 0.8 ^b
<i>Rhodanthe stricta</i>	2 ^b	2.5 $\pm$ 0.3 ^b	5 ^a	2 ^b	1 ^c
<i>Schoenia cassiniana</i>	4.3 $\pm$ 0.3 ^a	2.5 $\pm$ 0.3 ^b	3.8 $\pm$ 0.3 ^a	2.5 $\pm$ 0.3 ^b	3.3 $\pm$ 0.3 ^b
<i>Schoenia filifolia</i>	3 ^a	-----	2.8 $\pm$ 0.3 ^a	-----	1 ^b
<i>Schoenia macivorii</i>	3.8 $\pm$ 0.5 ^b	4 ^b	5 ^a	2 ^c	3.5 $\pm$ 0.3 ^b
<i>Swainsona formosa</i>	2.5 $\pm$ 0.3 ^a	1.5 $\pm$ 0.3 ^a	1.8 $\pm$ 0.5 ^a	1.8 $\pm$ 0.3 ^a	1 ^a
<i>Trachymene coerulea</i>	12.3 $\pm$ 0.3 ^a	9.3 $\pm$ 0.5 ^b	12.3 $\pm$ 0.6 ^a	8.3 $\pm$ 0.3 ^b	11.8 $\pm$ 0.3 ^a
<i>Trachymene pilosa</i>	13 ^a	9 ^c	11.8 $\pm$ 0.3 ^b	10.8 $\pm$ 0.8 ^b	11.5 $\pm$ 0.5 ^b
<i>Xerochrysum bracteatum</i>	3.3 $\pm$ 0.3 ^a	2 ^b	3.3 $\pm$ 0.3 ^a	2.3 $\pm$ 0.3 ^b	1.3 $\pm$ 0.3 ^c

† Letters indicate significant differences among means for each species, where non-significant differences share at least one letter (one-way ANOVA and Tukey's post-hoc tests).

‡ No germination tests were conducted in these cases due to lack of seeds

No G.: No germination occurred under these treatments and therefore no LAG time was recorded

^ In this case, only two out of four replicates germinated. Therefore, 31 days has been recorded for those two replicates to enable calculating a mean of four replicates

Appendix A6. Germination rate (mean $\pm$ SE; $n = 4$) of 20 annual species which germinated in at least one of the germination treatments.

Species	Untreated		GA ₃		KAR1
	20/10 °C	25/15 °C	20/10 °C	25/15 °C	20/10 °C
<i>Brachyscome iberidifolia</i>	4.5 $\pm$ 0.3 ^{a†}	3.3 $\pm$ 0.1 ^b	4.4 $\pm$ 0.3 ^{ab}	3.5 $\pm$ 0.2 ^{ab}	3.9 $\pm$ 0.4 ^{ab}
<i>Craspedia variabilis</i>	9.2 $\pm$ 0.3 ^a	9.2 $\pm$ 0.4 ^a	8.6 $\pm$ 0.3 ^a	----- [‡]	8.7 $\pm$ 0.6 ^a
<i>Goodenia microptera</i>	No G.	No G.	No G.	No G.	7.3 $\pm$ 0.8
<i>Goodenia vilmorinae</i>	No G.	No G.	No G.	No G.	6.6 $\pm$ 0.6
<i>Heliotropium tenuifolium</i>	No G.	No G.	14.4 $\pm$ 1.2 ^a	5.1 $\pm$ 0.3 ^b	12.1 $\pm$ 0.8 ^a
<i>Podolepis canescens</i>	5.6 $\pm$ 0.3 ^a	-----	3.7 $\pm$ 0.3 ^b	-----	2.2 $\pm$ 0.1 ^c
<i>Podolepis gracilis</i>	10.5 $\pm$ 0.7 ^a	9.9 $\pm$ 0.1 ^{ab}	10 $\pm$ 0.6 ^{ab}	9.7 $\pm$ 1.7 ^{ab}	6.8 $\pm$ 0.3 ^b
<i>Podotheca gnaphalioides</i>	11.4 $\pm$ 1.4 ^a	3.7 $\pm$ 0.3 ^b	12.2 $\pm$ 2.3 ^a	4.8 $\pm$ 0.2 ^b	5.7 $\pm$ 0.4 ^b
<i>Rhodanthe chlorocephala</i> subsp. <i>rosea</i>	2.4 $\pm$ 0.1 ^a	2.2 $\pm$ 0.0 ^a	2.2 $\pm$ 0.1 ^a	1.5 $\pm$ 0.2 ^b	1.1 $\pm$ 0.0 ^b
<i>Rhodanthe humboldtiana</i>	7.5 $\pm$ 0.7 ^{ab}	4.7 $\pm$ 0.2 ^b	7.8 $\pm$ 0.3 ^a	7.9 $\pm$ 1.2 ^{ab}	5.8 $\pm$ 0.8 ^{ab}
<i>Rhodanthe manglesii</i>	4.3 $\pm$ 0.3 ^a	2.7 $\pm$ 0.1 ^b	3.4 $\pm$ 0.3 ^{ab}	2.9 $\pm$ 0.4 ^b	1.7 $\pm$ 0.1 ^c
<i>Rhodanthe margarethae</i>	No G./little G.	7.6 $\pm$ 0.6 ^b	little G.	7.9 $\pm$ 0.9 ^b	11.5 $\pm$ 0.4 ^a
<i>Rhodanthe stricta</i>	3.2 $\pm$ 0.1 ^{ab}	3.2 $\pm$ 0.1 ^{ab}	4.3 $\pm$ 0.1 ^a	3.1 $\pm$ 0.4 ^b	3.4 $\pm$ 0.4 ^{ab}
<i>Schoenia cassiniana</i>	5.4 $\pm$ 0.4 ^a	3 $\pm$ 0.2 ^b	5.2 $\pm$ 0.2 ^a	2.9 $\pm$ 0.2 ^b	4.1 $\pm$ 0.3 ^a
<i>Schoenia filifolia</i>	3.5 $\pm$ 0.0 ^a	-----	3.7 $\pm$ 0.2 ^a	-----	2.1 $\pm$ 0.2 ^b
<i>Schoenia macivorii</i>	5.4 $\pm$ 0.8 ^a	4.5 $\pm$ 0.4 ^a	4.5 $\pm$ 0.1 ^a	2.6 $\pm$ 0.3 ^b	6 $\pm$ 0.9 ^a
<i>Swainsona formosa</i>	3.7 $\pm$ 0.5 ^a	3.1 $\pm$ 0.5 ^a	2.7 $\pm$ 0.3 ^a	2.3 $\pm$ 0.1 ^{ab}	1.5 $\pm$ 0.2 ^b
<i>Trachymene coerulea</i>	14.3 $\pm$ 0.6 ^a	12.3 $\pm$ 0.4 ^b	14.4 $\pm$ 0.6 ^a	10.7 $\pm$ 0.2 ^b	15.5 $\pm$ 0.3 ^a
<i>Trachymene pilosa</i>	13.6 $\pm$ 0.5 ^a	11.1 $\pm$ 1.2 ^a	13.6 $\pm$ 0.9 ^a	Little G.	13.3 $\pm$ 0.8 ^a
<i>Xerochrysum bracteatum</i>	4.4 $\pm$ 0.1 ^a	3.4 $\pm$ 0.1 ^b	5.2 $\pm$ 0.2 ^a	3.5 $\pm$ 0.2 ^b	3.4 $\pm$ 0.2 ^b

† Letters indicate significant differences among means for each species, where non-significant differences share at least one letter (one-way ANOVA and Tukey's post-hoc tests).

‡ No germination tests were conducted in these cases due to lack of seeds

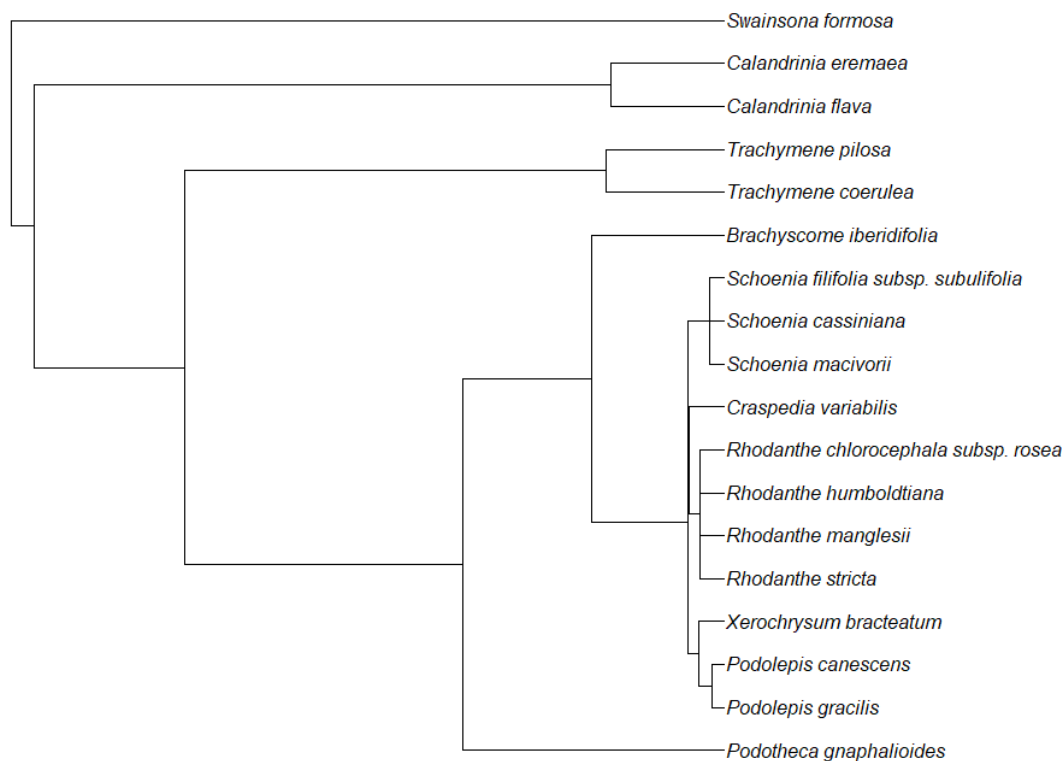
No G. or little G.: No germination occurred under these treatments or germination lower than 10% was recorded and therefore no T₅₀ was calculated.

Appendix A7. Means of germination attributes of 20 annual species under different germination treatments.

Germination attributes	GP	LAG time	T ₅₀
Germination treatments			
Untreated 20/10 °C	55.31 ^{b†}	5.25 ^a	7.38 ^a
Untreated 25/15 °C	56.01 ^b	3.85 ^b	5.53 ^b
GA ₃ 20/10 °C	57.42 ^b	5.09 ^a	7.39 ^a
GA ₃ 25/15 °C	60.65 ^b	3.63 ^b	5.11 ^c
KAR1 20/10 °C	66.10 ^a	3.54 ^c	6.14 ^{bc}

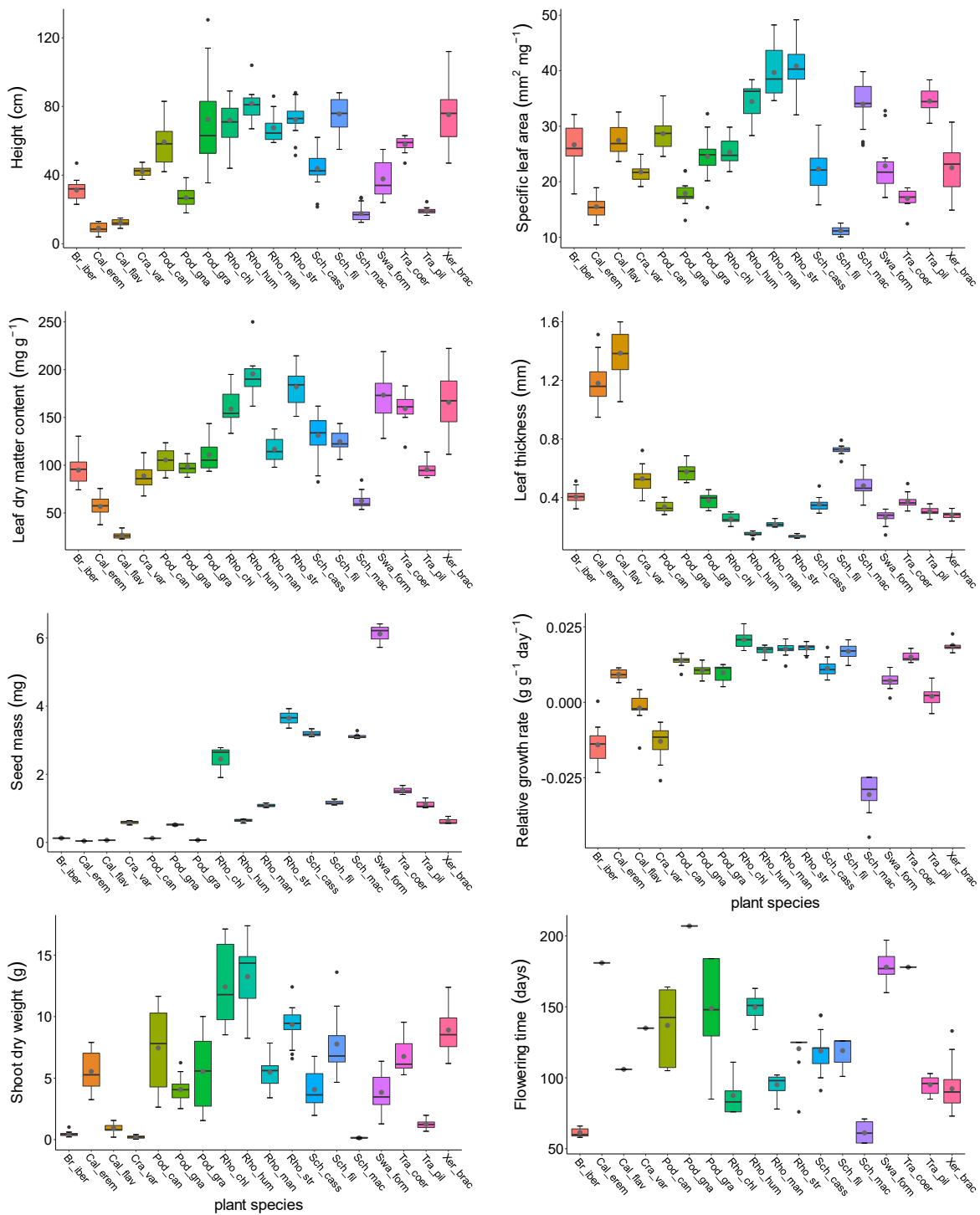
† Letters indicate significant differences among means for each germination attribute, where non-significant differences share at least one letter (mixed effect models and Fisher's LSD post-hoc tests)

8 Appendix B – Chapter 3



Appendix B1. Phylogenetic tree containing the 18 studied plant species.

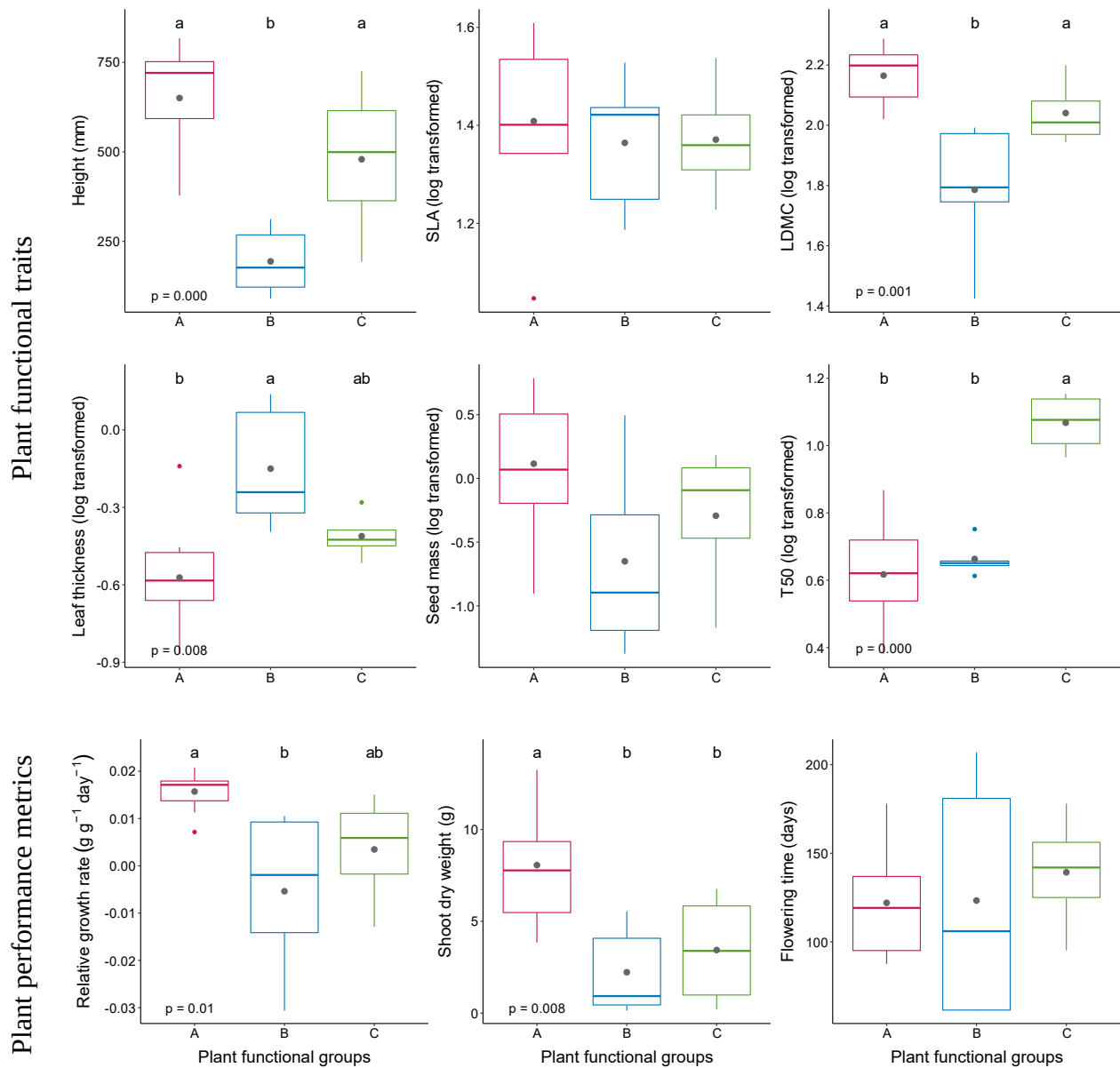
Phylogenetic tree of the 18 studied species was created using *V.PhyloMaker* package (Jin & Qian, 2019). Pagel's Lambda was then calculated using *phytools* package (Revell, 2012) to quantify the strength of phylogenetic signal among the studied species for all traits and performance metrics. However, most of the variables showed weak (and nonsignificant) phylogenetic signals, which means that closely related species exhibit different trait values. Therefore, accounting for phylogeny would not change the results of the regressions.



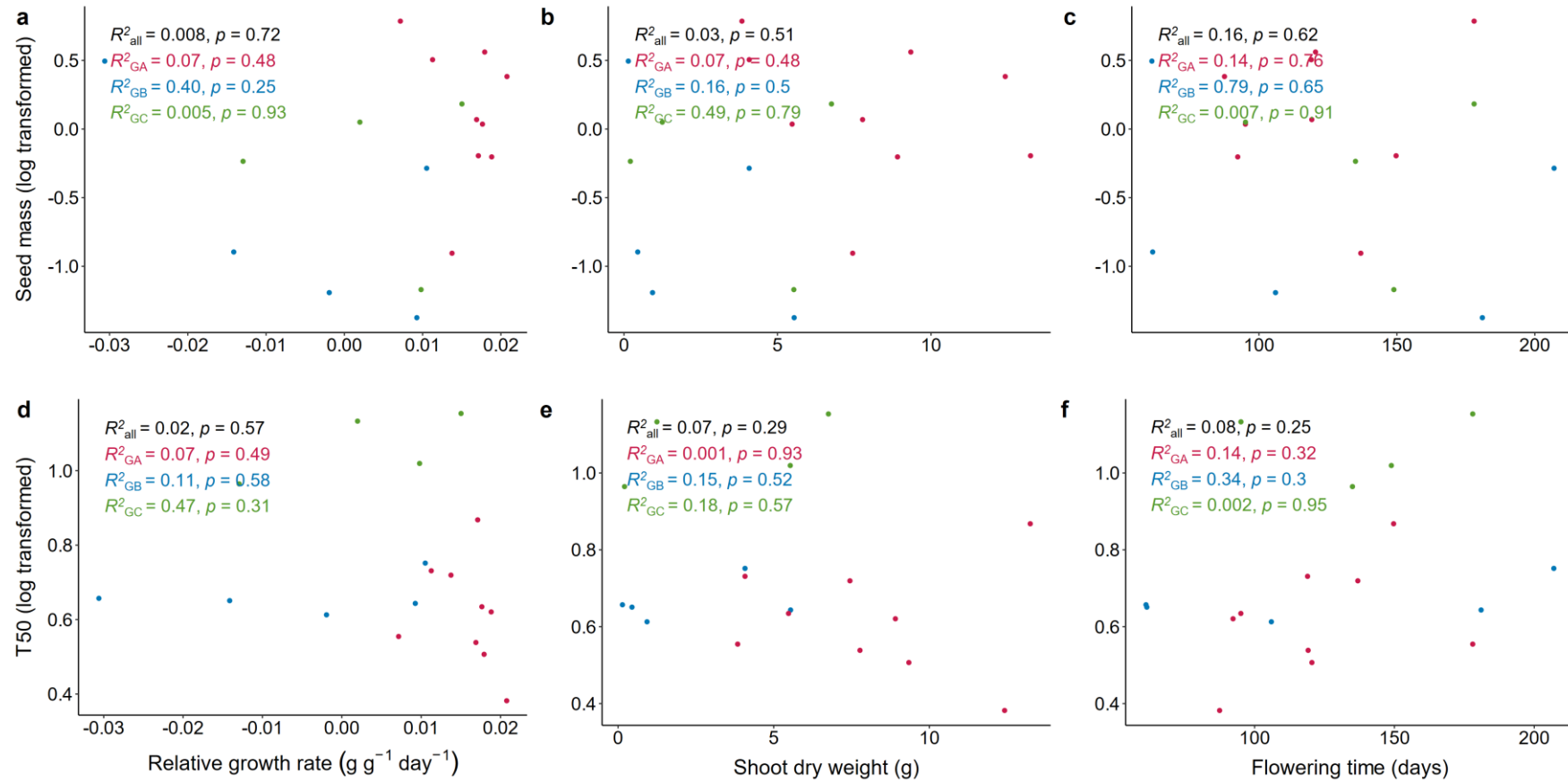
Appendix B2. Boxplots demonstrating functional traits (specific leaf area, leaf dry matter content, leaf thickness, plant height and seed mass) and plant performance metrics (relative growth rate, shoot dry weight and flowering time) values of 18 Australian annual plant species. Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Species are ordered according to their scientific names. Refer to Table 3.1 for the abbreviations of the species names.

Appendix B3. Pearson's correlation coefficient (r) between plant performance metrics (RGR: relative growth rate, SDW: shoot dry weight and Ftime: flowering time) and principal components (PC1, PC2 and PC3) obtained from principal component analysis of seven functional traits (refer to Table 3.2) of the 18 Australian annual species grown under non-limiting common garden conditions. Significant correlations ($P < 0.05$) are marked in bold.

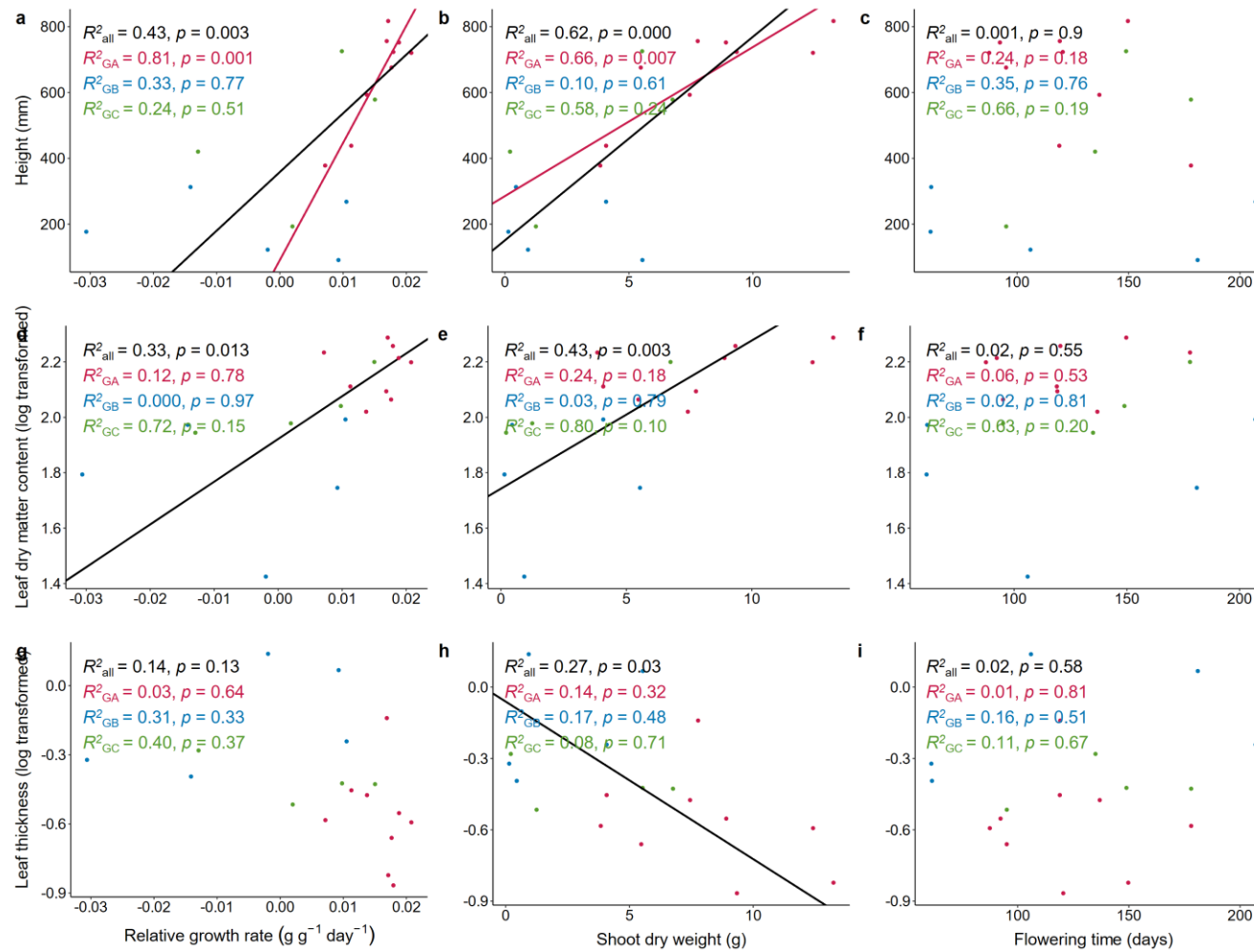
	PC1	PC2	PC3
RGR	0.47	-0.15	0.43
SDW	0.62	-0.19	0.33
Ftime	-0.11	0.12	0.46



Appendix B4. Boxplots illustrating functional traits and plant performance metrics of 18 Australian annual plant species divided into three groups defined by cluster analysis: Group A: red ($n = 9$), group B = blue ($n = 5$), and group C = green ($n = 4$). Significant differences among mean values of each group for each trait are indicated by dissimilar letters above each box (Fisher's post-hoc test; $P\text{-value} \leq 0.05$). SLA: specific leaf area ($\text{mm}^2 \text{mg}^{-1}$), LDMC: leaf dry matter content (mg g^{-1}), leaf thickness (mm), seed mass (mg) and T50: germination rate (days).



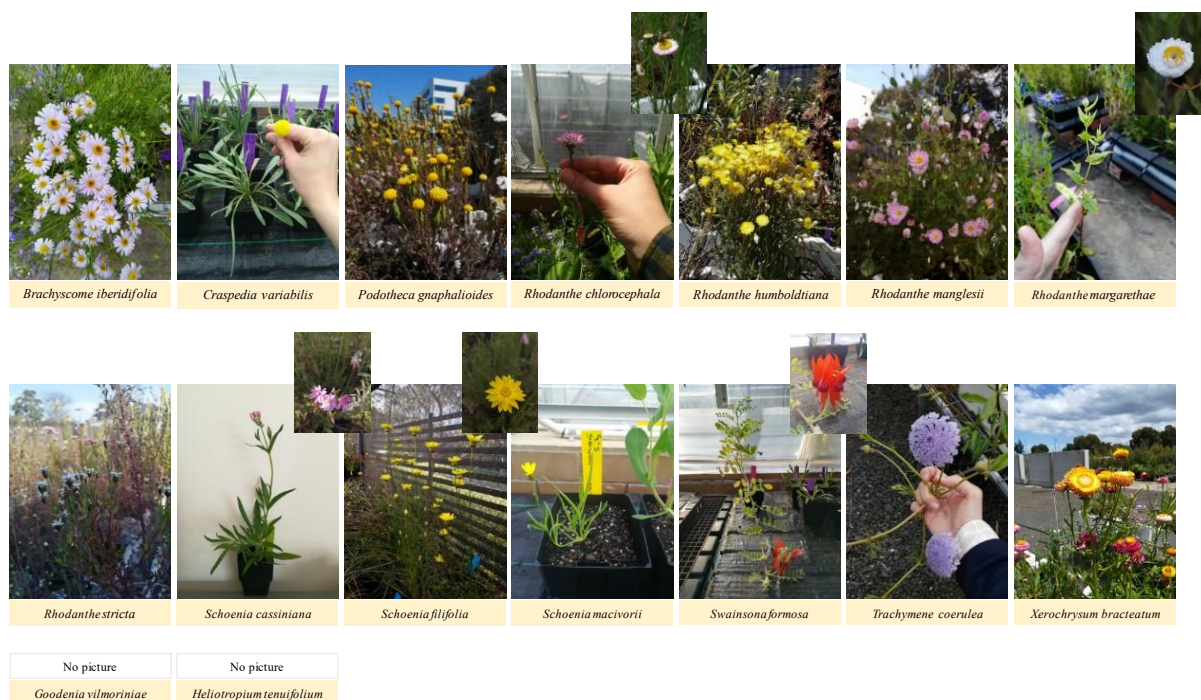
Appendix B5. Standardized major axis (SMA) regressions among a) relative growth rate and seed mass (mg), b) shoot dry weight and seed mass, c) flowering time and seed mass, d) relative growth rate and germination rate (T50; days), e) shoot dry weight and germination rate, and f) flowering time and germination rate of 18 Australian annual plant species (black) and their groupings defined by cluster analysis: Group A: red ($n = 9$), group B = blue ($n = 5$), and group C = green ($n = 4$). Coefficient of determination (R^2) and P-values are shown for all relationships. SMA regression lines are plotted when significant (P -value < 0.05). n = sample size.



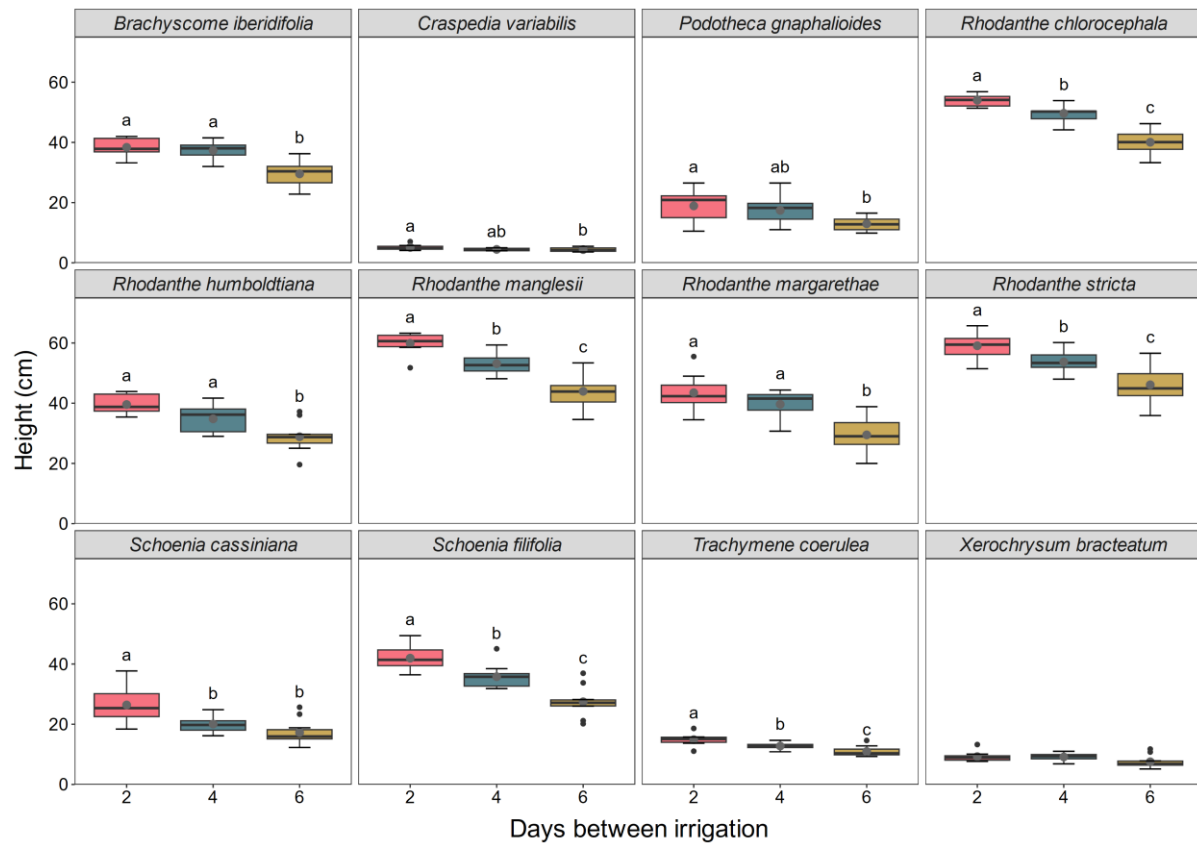
Appendix B6. Standardized major axis (SMA) regressions among a) relative growth rate and height (mm), b) shoot dry weight and height, c) flowering time and height, d) relative growth rate and leaf dry matter content (mg g^{-1}), e) shoot dry weight and leaf dry matter content, f) flowering time and leaf dry matter content, g) relative growth rate and leaf thickness (mm), h) shoot dry weight and leaf thickness and i) flowering time and leaf thickness of 18 Australian annual plant species (black) and their groupings defined by cluster analysis: Group A: red ($n = 9$), group B = blue

(n = 5), and group C = green (n = 4). Coefficient of determination (R^2) and P-values are shown for all relationships. SMA regression lines are plotted when significant (P-value < 0.05). n = sample size.

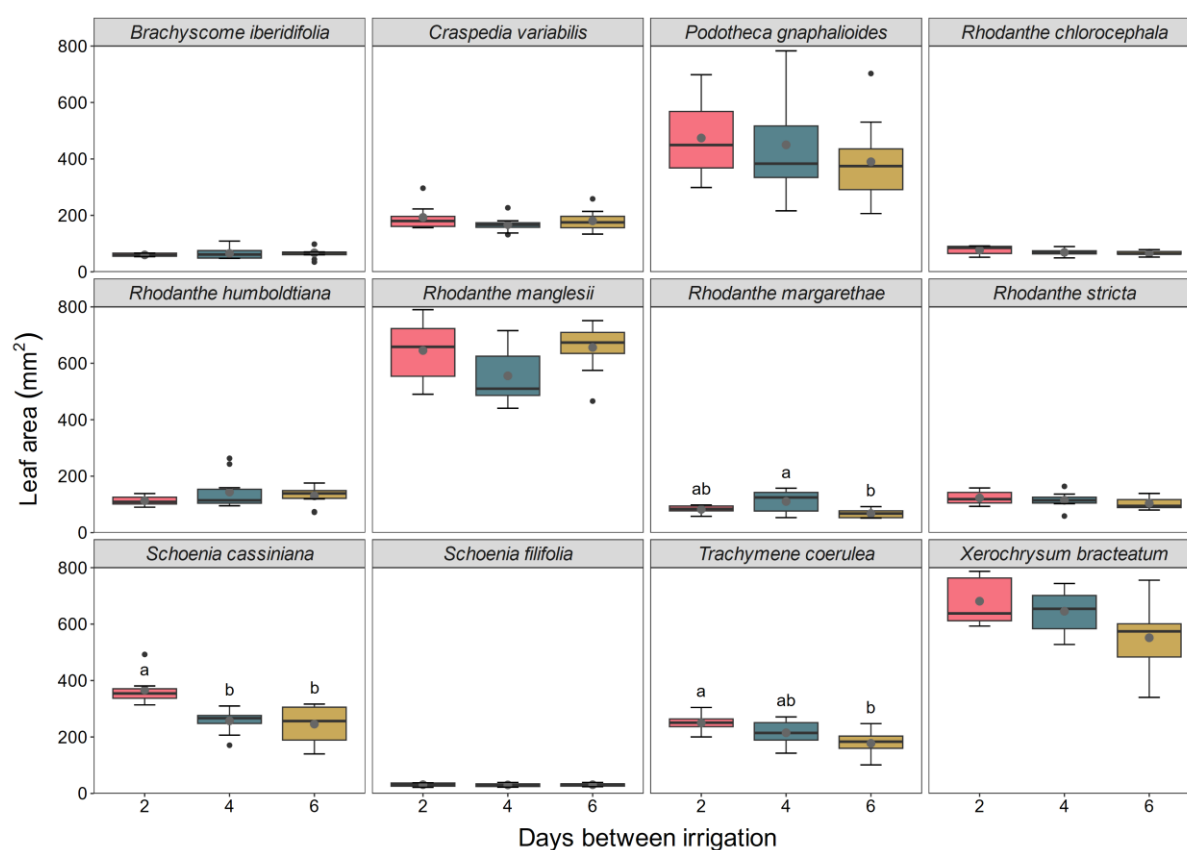
9 Appendix C – Chapter 4



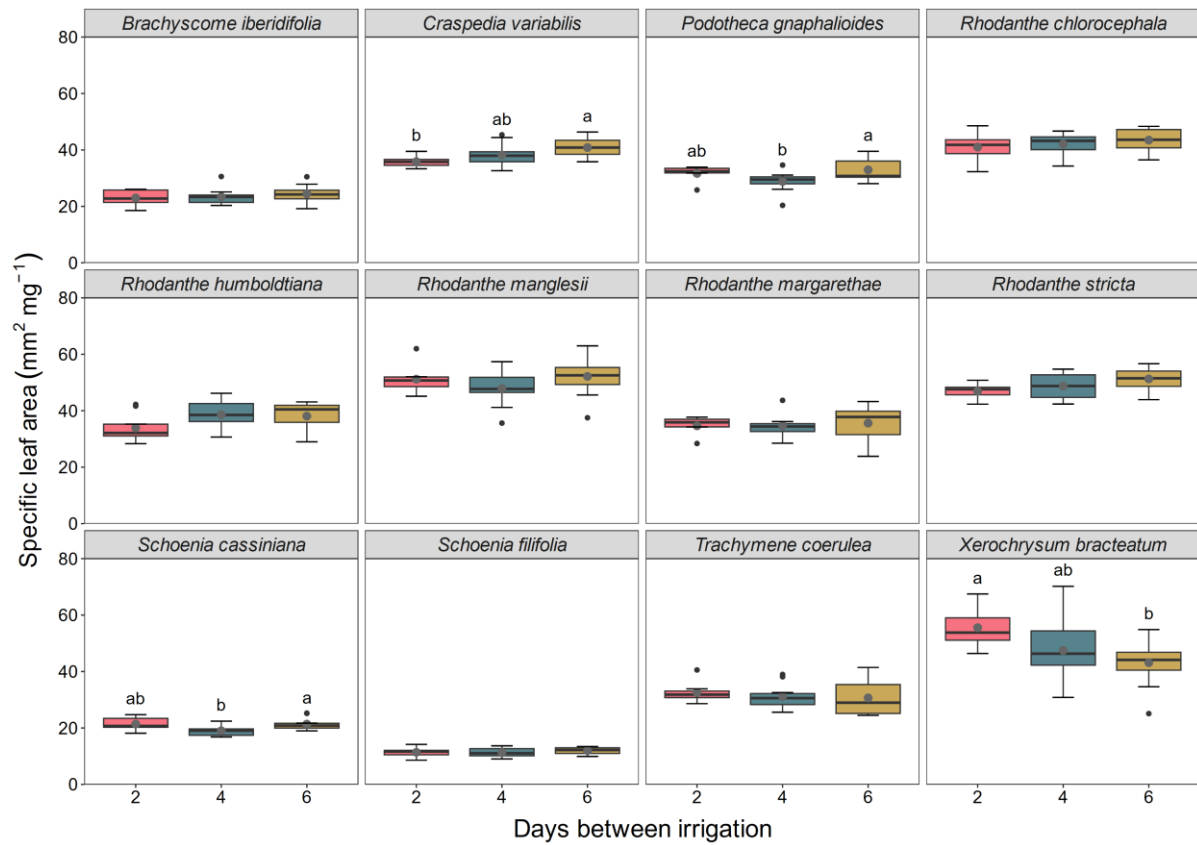
Appendix C1. Sixteen Australian annual species used in the seed mix. Refer to Table 4.1 for more information about plants families, germination percentage, number of seeds used in the seed mix for each module, climate of origin and the germination pre-treatments applied to maximize germination.



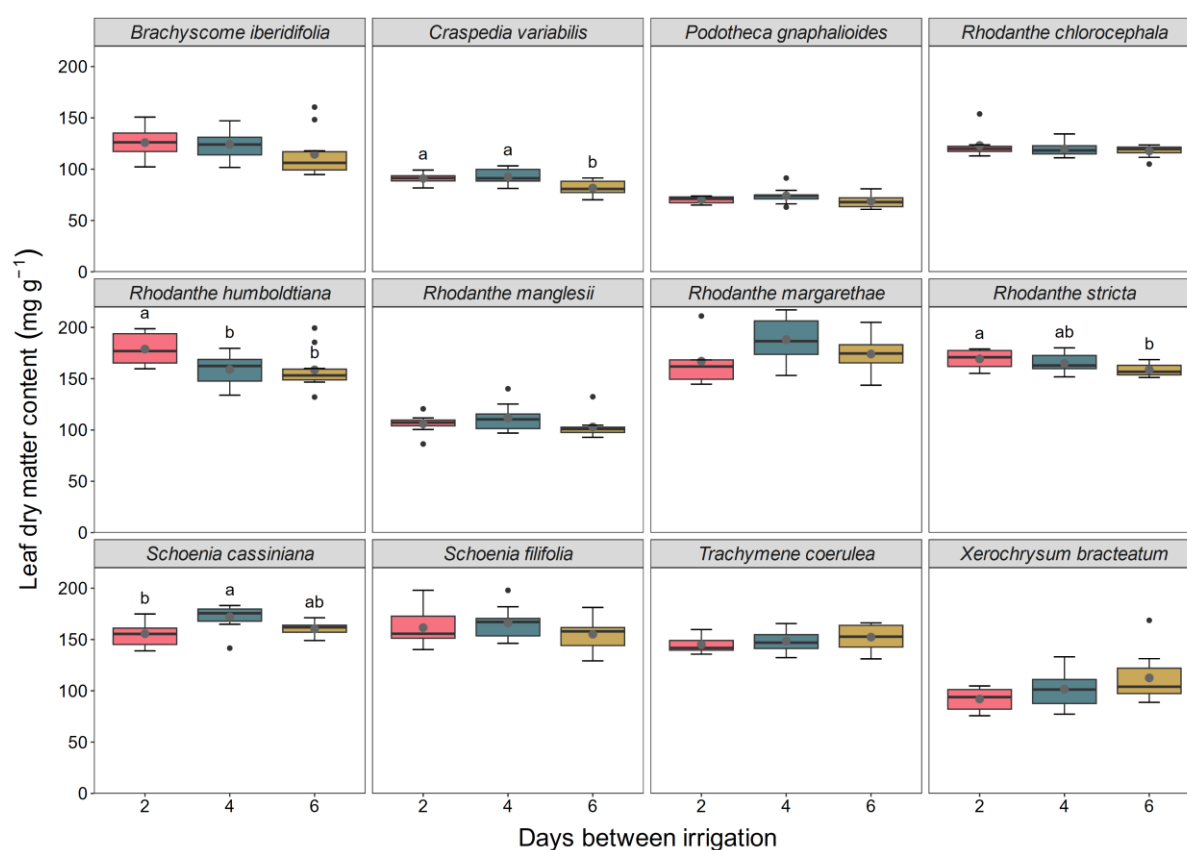
Appendix C2. Mean height of each plant species at the end of the experiment (five month after sowing) in response to the three irrigation frequencies: 2, 4, and 6 days between irrigation. Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies for each species are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value < 0.05).



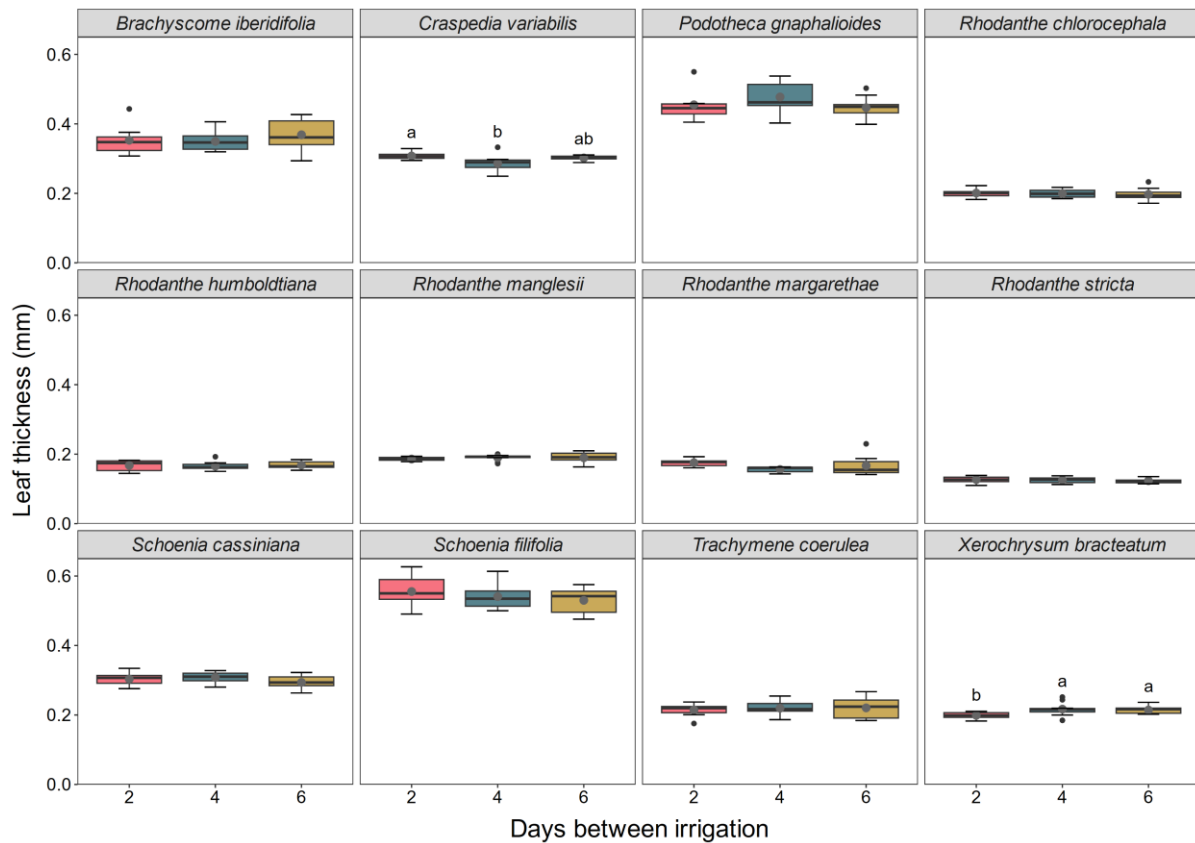
Appendix C3. Mean leaf area of each plant species at the end of the experiment (five month after sowing) in response to the three irrigation frequencies: 2, 4, and 6 days between irrigation. Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies for each species are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value < 0.05).



Appendix C4. Mean specific leaf area of each plant species at the end of the experiment (five month after sowing) in response to the three irrigation frequencies: 2, 4, and 6 days between irrigation. Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies for each species are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value < 0.05).



Appendix C5. Mean leaf dry matter content of each plant species at the end of the experiment (five months after sowing) in response to the three irrigation frequencies: 2, 4, and 6 days between irrigation. Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies for each species are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value < 0.05).



Appendix C6. Mean leaf thickness of each plant species at the end of the experiment (five month after sowing) in response to the three irrigation frequencies: 2, 4, and 6 days between irrigation. Grey circles and black lines inside the boxes show the mean and median (50% quantile) values, respectively. Upper and lower hinges show the 25% and 75% quantiles. Outlying points are shown as black dots. Significant differences between irrigation frequencies for each species are indicated by dissimilar letters above each box (Tukey's post-hoc test; P -value < 0.05).