# Variation in Growth Rates Across Plant Families and Environments

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*So neither he who plants nor he who waters is anything, but only God who gives the growth, 1 Corinthians 3:7*

# Abstract

The seedling stage is an important part of a plant's life cycle. The seedling determines whether or not the plant will establish and reach maturity, will grow tall and deep enough to out-compete enough of the surrounding vegetation to become a strong and healthy plant and survive the many stresses that may easily damage a young seedling that has not yet developed the protection and reserves found in mature plants. One particular focus of seedling Ecology is growth rates. This is currently a fast-moving topic, with the introduction of a new method of calculating growth rates. Therefore, this thesis investigates growth rates on an inter-specific scale, with particular interest in calculating growth, as: a log-linear formula based on biomass and time; the sum of its growth components and the non-linear size-corrected relative growth rate. Growth rates are investigated in relation to various mild and fatal stressors, such as nutrient and herbivory stress to see if the different methods of calculating growth can enhance our understanding of Ecology.

This thesis found that seed mass is not a key factor explaining the differences in growth rates between growth forms (Chapter 2). It found that plant survival of stressful environments is based on a complex interaction of seed mass, growth rate and biomass (Chapter 3) and that biomass is very important in surviving a sub-optimal and then an extreme stressor (Chapter 4). The components of growth are potentially size-biased, creating a possible problem when attempting to compare the relative importance of each component across different environments (Chapter 5). Additionally, experimental standardisation (Chapter 2) and modelling single vs multiple traits (Chapter 3) are also questioned.

Plant traits are important and useful determinants of plant growth. Understanding variance in plant growth can help us to understand functioning on a population and community level more effectively.

# Chapter 1

# Introduction

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Many species have been labelled with a relative growth rate (RGR; Grime *et al.*, 1988), which is currently the most frequently used method of calculating seedling growth. However, growth can vary considerably within a species and between different environments; therefore it is important to consider growth in relation to the environment currently inhabited. The introduction firstly briefly describes some methods of calculating growth, a parameter that is more difficult to accurately measure than may be initially suspected, and then variance in growth is introduced. This is followed by effects of the environment, particularly in relation to stress, before focusing more specifically on the research aims of the thesis, and providing a brief summary of the research chapters (Chapter 2-5).

### *Current Situation*

The relative growth rate (RGR) is the measure of the increase in biomass of an organism, per unit of biomass and per time, and is sometimes referred to as the 'classic' approach of calculating growth. It is commonly used across ecology, either as a parameter to quantify the effect of a certain variable, or as a trait that can explain other variables. RGR is used as a parameter, for example, to quantify the effect of a treatment on pre-flowering plants, such as fertilisers and pathogens, and is used in the CSR theory, to group plants functionally. RGR is used as a trait, for example in the fast-slow continuum (Franco & Silvertown, 1996), which states that slow-growing species are more likely to survive stress events than fast-growing species and is also used in studies of invasive species, where RGR is a predictor of whether an introduced species will become invasive. Despite the widespread use of growth rates, researchers have been attempting to find an accurate way to calculate growth for a long time.

***The History of Growth Calculations***

Plant growth analysis has been developing since the work of Blackman (1919). One method of calculating growth is the absolute growth rate (AGR), calculated as:

$$AGR = \frac{dW}{dt} \qquad \text{eqn. 1}$$

where W = biomass, t = time and *d* is the change between the different measurements. AGR, however, assumes that growth is linear over time, which is rarely observed, and therefore introduces inaccuracies to the results. This is partially addressed by RGR, which assumes that growth is log-linear over time:

$$RGR = \frac{d\log(W)}{dt} \qquad \text{eqn. 2}$$

A functional approach to measuring RGR calculates the product of the growth components. In this case, growth is typically separated into the following components:

- Net assimilation rate (NAR), which is the increase in biomass per unit of leaf area over time, and is a proxy for photosynthesis;
- Specific leaf area (SLA), which is the leaf area per unit of leaf biomass and
- Leaf mass ratio (LMR), which is the ratio of leaf biomass to total plant biomass.

Leaf area ratio (LAR) can also be used as a component, and is the product of SLA and LMR. RGR is usually calculated as the product of its components when the components are of interest in other aspects of the data analysis. See Chapter 5 for a further description and demonstration of this functional method.

Whilst eqn 2 is currently the most commonly used method to calculate the growth rate, growth is not usually exponential, but decreases over time (Hunt & Lloyd, 1987).

There seems to be a lack of awareness of this limitation, and allowances are rarely made in the interpretation of the results. Typically, two biomass measurements are taken: the initial biomass, which is usually the seed mass, and the final biomass, which are then incorporated into eqn. 2, producing a log-linear association between the variables. However, when multiple measurements are taken across the growth period, the resulting association is often non-linear (Turnbull *et al.*, 2008). This is problematic because the RGR formula cannot always distinguish between two plants that are on different growth curves, and two plants that are at different points on the same growth curve (Figure 1.1). Therefore, RGR cannot differentiate between plant growth and initial size, and thus cannot determine whether or not two plants have the same growth strategy. Attempts to account for changes in RGR with time and size include choosing seedlings that are similar in size (Norgren, 1996).

One response at the modelling stage of calculating growth has been to use a polynomial approach, which can fit a curve to the data (Poorter & Lewis, 1986). However, this solution produces other problems, most noticeably the question of which degree of polynomial to use, which produces differing and subjective responses from researchers (see Poorter, 1989). A further response was to create a ‘combined’ approach, which connected the ‘classic’ and the polynomial approaches of calculating growth (Poorter & Lewis, 1986). However, in a comparison of the polynomial, combined approach and a third method, the Richards function, it was concluded that the latter fitted growth data better and was recommended for use in future studies of plant growth (Poorter & Garnier, 1996). At the authors’ own admission, the Richards function, which produces a generalised logistic curve, is difficult to model, and the method has not been widely accepted. Today, RGR remains the ‘classic’ and popular way to calculate seedling growth.

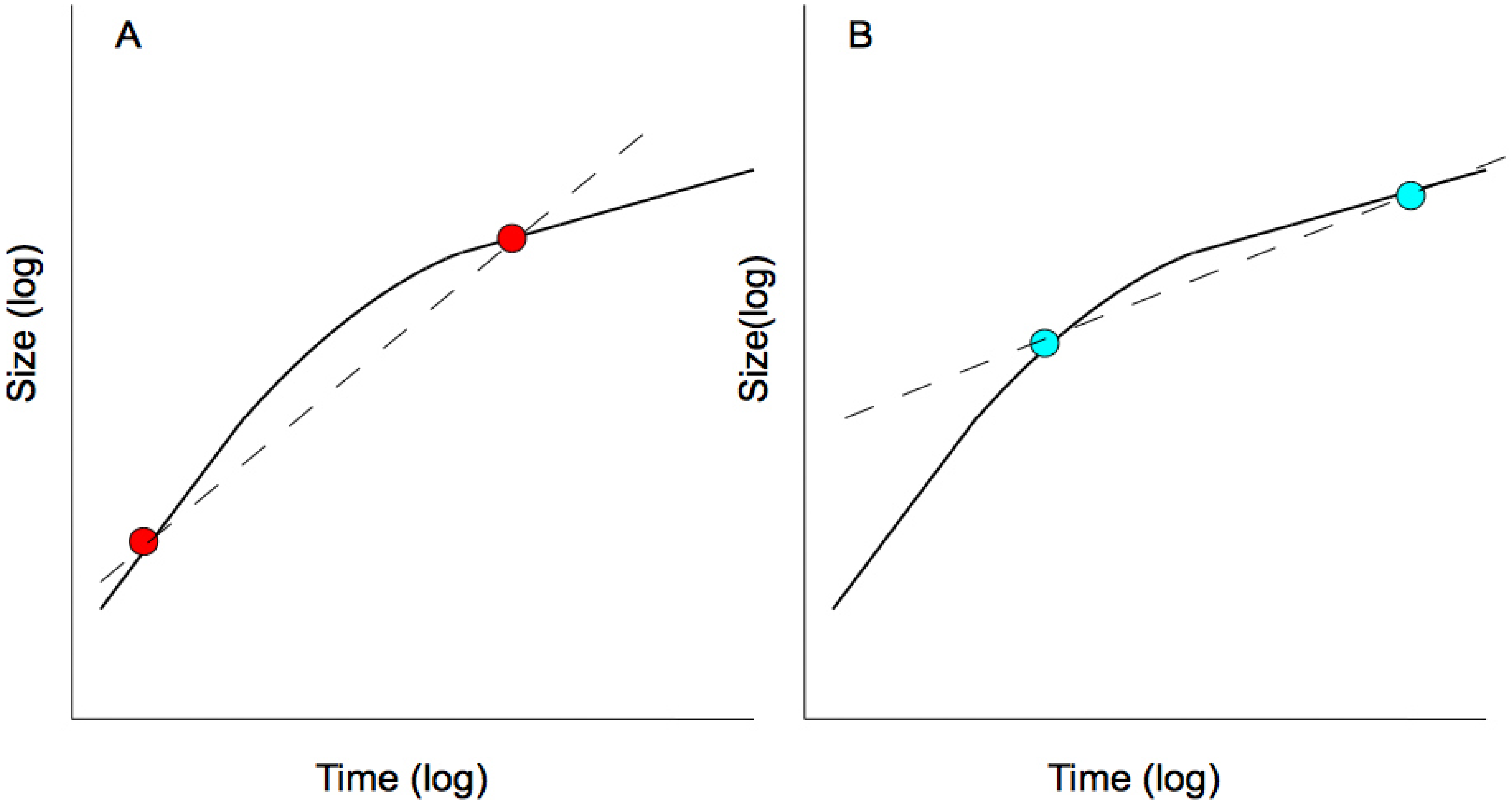

Figure 1.1: Diagram of plant growth over time. The solid black line represents the actual growth curve of a plant. The coloured dots show the points where plant size was calculated for a classic RGR analysis. Plants A (red) and B (blue) are on the same growth curve, but plant A has a smaller initial size. Because the classic RGR analysis (modelled using the black dotted line) does not acknowledge the curve, it is wrongly concluded that plant A is an inherently faster-growing plant.

The newest proposed method for calculating growth rates is the size-corrected relative growth rate (SGR), which can account for the non-independence of size found in the classic RGR formula (Metcalf *et al.*, 2006; Turnbull *et al.*, 2008; Paine *et al.*, 2012). Instead of the classic log-linear model, SGR is calculated using a non-linear mixed-effects model, which can account for the shape of the growth curve. Importantly, this new method has produced different results to those seen when using the classic method.

Turnbull *et al.* (2008) applied the new size-corrected formula, as well as the classic formula to the correlation between seed mass and RGR. In agreement with the majority of the literature (Shipley & Peters, 1990; Maranon & Grubb, 1993), when using the classic formula, they found that as seed mass increased, RGR decreased. However, when they applied the SGR method, the correlation was positive (i.e. seedlings with larger seed masses grew quicker). They concluded that size-correcting growth rates had a large influence over the experimental results.

Since the publication of Turnbull *et al.*'s (2008) paper, other studies have also shown that size-correcting RGR significantly affects the outcome of research in this area of ecology. For example, the biological significance of size-correcting RGR was demonstrated in a study of the costs of fast plant growth (Rose *et al.*, 2009). When using the conventional method of calculating RGR, no significant costs or benefits of fast-growth were observed. With SGR, however, a greater reproduction rate was observed in fast-growing plants. Once an environmental stressor was introduced (defoliation), the fast-growing plants suffered a greater loss of fitness than the slow-growing plants, due to decreased survival and reproductive rate. The data provided by this size-corrected dataset also provides supporting evidence for the fast-slow continuum theory (Franco & Silvertown, 1996), although the RGR method found no evidence for the theory. Other examples where the results from using RGR and SGR differ include the effects of plant defence on growth (Paul-Victor *et al.*, 2010) and a study on the growth components (Rees *et al.*, 2010). It is currently unknown how many areas of ecology that currently utilise the classic RGR formula would be significantly affected by using a size-corrected RGR formula.

However, while using general mixed-effect modelling allows for flexibility to choose the growth curve that best fits the data, it shares a problem with the Richard's function, in that growth curves are more difficult to calculate than the classic RGR. As research outcomes are impacted by the use of non-linear growth models, the cost of the extra effort to calculate them relative to RGR may be outweighed by the benefits of having accurate results.

### ***Variance in Growth***

Some species intrinsically grow faster than others, however, within a species, there is variance in growth between individuals when grown in near-optimal conditions. Ricklefs and Peters (1981) stated,

*"Among individuals in natural populations, variation is the rule".*

Differences between species are even more extensive (Grime & Hunt, 1975), and can be caused by a variety of physiological and environmental factors. Entire books have been written about why different species have different growth rates (e.g. Lambers *et al.*, 1998). Physiologically, variance in growth can be caused by a wide range of variables (Figure 1.2). Environmentally, variance in growth can be caused by responses to: light, nutrients, water, temperature, pH, rooting substrate, competition, herbivory, disturbance, microbes and disease. Understanding why and how growth varies can increase our understanding of seedling establishment; seedling survival; invasion; competition; conservation and responses to climate change. It therefore has wider significance in Biology.

Whilst fast-growth, and therefore also high resource-capture and competitive ability, can improve a seedling's likelihood of surviving to reproductive maturity, many species are intrinsically slow-growing. This could be for an assortment of reasons. As slow-growing species are often found in unproductive environments, one idea is that slow-growth reduces the likelihood of over-exploiting the limited resources, while another suggests that slow-growth enables the storage of resources, in preparation for a later deficiency. The shortfalls of these ideas are discussed in Lambers and Poorter (1992).

There is also some plasticity in growth rates (Zou *et al.*, 2009), although the extent of the plasticity and the associated costs are not yet clear.

### ***Environment***

Seed mass is affected by factors ranging from the maternal plant (Castro, 1999) to latitude (Moles *et al.*, 2007). Seed persistence and germination are strongly governed by the environment (Fenner & Thompson, 2005). Productivity is also one of many factors in the environment that can affect seeds and seedlings. Stress in the environment, such as low nutrients, low light, high competition, herbivory, fungal attack and human impact can affect seedling size, growth rate and survival. Stressors are particularly damaging to young seedlings, which may not yet have developed chemical resistance to herbivory or deep roots to protect against nutrient depletion, drought or high wind. It is becoming more important to understand these stressors as the environment changes, due to climate change and increased urbanisation.

### ***Thesis Summary***

The aim of this thesis is to study variance in growth. This is demonstrated in Figure 1.2, which shows the areas of biology and plant traits that may explain variance in growth. More than one of these variables are incorporated into each chapter. An additional aim through this work is to gain a better understanding of how beneficial the SGR method is. The thesis studies RGR in two directions – breaking it down into its components and building it up to look at the effects of size-correcting the RGR on a larger scale, to see if the new methodology can tell us more about different areas of ecology.

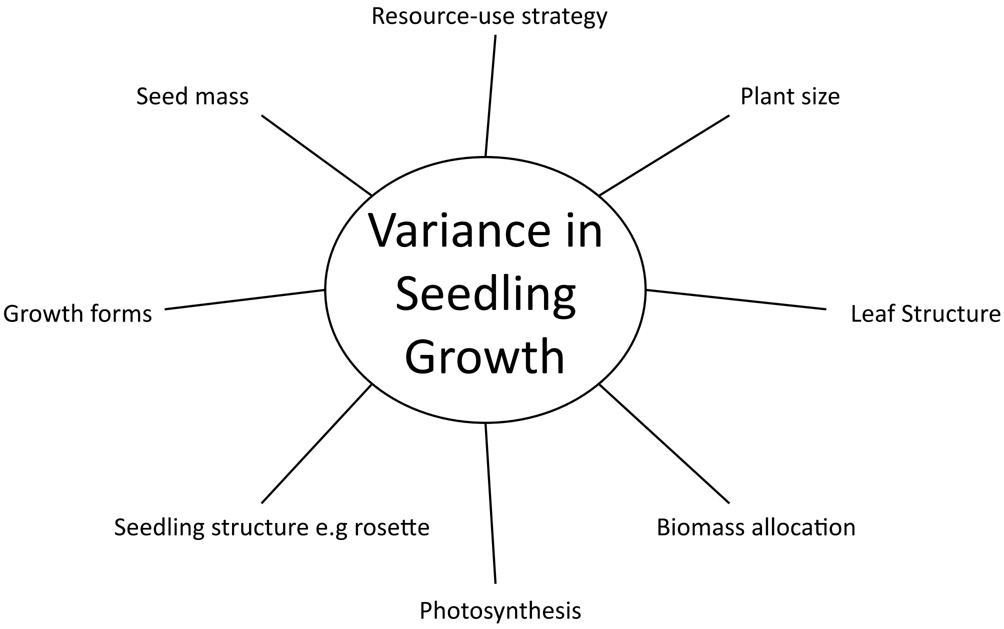

Figure 1.2: Spider diagram of physiological variables that can impact seedling growth. The thesis focuses around the central idea of understanding the causes of variance in seedling growth

Below are the main objectives and key questions asked throughout the thesis, along with the chapter where each question is tested:

**Objective 1:** To understand more about the physiology of variance in seedling growth

**Questions:**

Is variance in growth between growth forms caused by differences in seed mass? (Chapter 2)

Does growth or biomass explain more variance in survival rates? (Chapter 3)

Do species responses to one environment prepare them for a change in environment? (Chapter 4)

Does NAR account for most of the variance in growth once the size-bias in NAR has been accounted for? (Chapter 5)

**Objective 2:** Does growth affect survival of stressful conditions?

**Questions:**

Do fast- and slow-growing species respond differently to stressful environments? (Chapter 3 & 4)

Do large and small seeded species respond differently to stressful environments? (Chapter 4)

**Objective 3:** To study the components of RGR (net assimilation rate, NAR and leaf area ratio, LAR), using size-independent methods, to reveal which components are the most important parameters in determining growth

**Questions:**

Is NAR the most important factor in determining the RGR in near-optimal conditions?

Is NAR is more important when the variance in size increases (because the formulae for RGR and NAR are similar)? (Chapter 5)

Growth can vary between different environments, so the first step in the thesis was to assess whether decisions that are made when designing greenhouse experiments significantly affect growth rates (Chapter 2). This is useful to know when comparing results across different experiments, and is therefore of use to the rest of this thesis. This chapter primarily asks if the differing growth rate between growth forms is caused by seed mass. The question is important in relation to theories behind growth strategy (e.g. Grime, 1977; Tilman, 1988), which have previously ignored the effect of seed mass on growth differences between growth forms and growth strategies, even though seed mass varies with both growth form (Westoby *et al.*, 1996; Moles *et al.*, 2005) and growth rates (Shipley & Peters, 1990; Maranon & Grubb, 1993).

The fast-slow continuum suggests that a low-RGR is an adaptation to high-stress environments (Franco & Silvertown, 1996). The independent effects of SGR and biomass on a species' probability of surviving an environmental stress event were investigated (Chapter 3). This was made possible by using SGR, as it enabled biomass and growth to be investigated as separate variable, which is not possible with the more size-dependent RGR.

The results of that experiment lead to the question of what would happen with 2 levels of environmental treatment i.e. a less simple situation that is more representative of seedlings in the field. (Chapter 4)

The final data chapter (Chapter 5) investigates whether or not variance in growth is largely due to variance in NAR. The explanatory power of NAR in describing growth may be a side-effect of the size-dependency of NAR and the size-bias of RGR. This was a good opportunity to utilise the SGR, but growth was still log-linear. It instead highlights the continued usefulness of RGR in some situations and marks the importance of understanding the growth pattern currently being studied, as they can vary greatly.

The above objectives summarise the key questions and sub-questions contained within this thesis. Other important issues are also addressed throughout, such as standardising experimental designs (Chapter 2) and the use of additional explanatory variables to those being directly studied to improve model reliability (Chapter 3). This thesis includes 451 different species, grown in near-optimal conditions and/ or under the stressors of: light, water, nutrients and simulated herbivory. The large range represents the need to understand general patterns that hold across multiple conditions and to understand when and why differences between the groups occur.

Over the last decade, the accuracy of the conventional method of calculating the RGR has been questioned. However, it was only in 2008 that this miscalculation has been shown to lead to a misinterpretation of experimental results (Turnbull *et al.*, 2008). The extent and implications of this new method of calculating RGR is unknown. Therefore, more research needs to be conducted in this area, ideally approaching the problem from a different perspective, which is what this thesis starts to address. RGR is used in many areas of ecology, conservation and agronomy. As a result, size-correction of RGR may also have implications for the application of experimental results in some aspects of these specialities.

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# Chapter 2

# Does Seed Mass Drive the Differences in RGR Between Growth Forms?

## Introduction

Relative growth rate (RGR) varies widely between species [Poorter & Remkes, 1990; Hunt & Cornelissen, 1997). In a large screening experiment, Grime and Hunt (1975) grew 130 species in standardised, 'optimal' conditions (no competition, plentiful light and nutrients), and found that even in this environment there was a 6-fold variation in maximum RGR between species. As a result of this variation, RGR is considered to be a useful metric for separating species into functional groups. For example, in Grime's CSR theory, RGR is a central parameter determining a species' strategy, with fast-growing species being classified as ruderals or competitors, and slow-growing species as stress tolerators (Grime, 1977; Grime, 2002). Similarly, RGR is also a key trait in Tilman's theories (Tilman, 1988), and he argued that differences in allocation determine RGR. Thus both theories predict a link between RGR and growth form, with woody species having lower RGRs than herbaceous species (see also Hunt & Cornelissen, 1997; Wright & Westoby, 2001). The link between RGR and growth form is therefore central to many of the most influential ideas in plant ecology, and has been found repeatedly in experimental studies (Hunt & Cornelissen, 1997, Galmes *et al*., 2005).

In addition to growth form, RGR is associated with several other traits. In particular, seed mass is often negatively correlated with RGR, so large seeded species tend to have lower RGRs (Maranon & Grubb, 1993; Agboola, 1996; Milberg *et al*., 1998). A meta-analysis of six studies (Shipley & Peters, 1990) demonstrated the generality of the negative relationship between RGR and seed mass, suggesting that it is robust to differences in experimental protocol and the pool of species used. Like RGR, seed mass is linked with growth form, with herbaceous species usually having smaller seed masses than woody species (Leishman *et al*., 2000). As a result of this, it is possible that the relationship between RGR and growth form is a consequence of variation in seed mass, rather than the differences in growth strategy. Recently Turnbull *et al.* (2012) have re-evaluated the seed mass: RGR relationship by calculating RGR at a common seedling mass so allowing comparison between species of different masses. We were unable to use this approach with published estimates of RGR, and so compare growth forms at a common seed mass.

To do this, we built a database of published studies that contained measures of RGR, and augmented this with additional information on life history, Köppen-Geiger climate classification and seed mass. Comparing the results of different studies is problematic, due to differences in experimental protocols (e.g. duration of the study and the pot volume) and other unmeasured factors. In order to account for this, we used a mixed modelling approach incorporating study-specific covariates and random effects.

## Methods

### ***Data collection***

The 'Web of Knowledge' (Thomson Reuters, 2012) and ‘Scopus’ (Elsevier, 2012) databases were searched for papers containing the words 'seed mass' or 'seed size' and 'relative growth rate' or 'growth rate' on 20th January, 2012. The following variables were recorded per species per study:

1. The relative growth rate. This was either calculated using linear regression or as:

RGR = $(\log W_t - \log W_0) / t$

where $W_0$ is the initial plant mass, $W_t$ is the final plant mass and t is the number of days between the two measurements (Hunt, 1990). All measurements were expressed as g $g^{-1}$ $day^{-1}$.

2. Seed mass, in mg. Where the seed mass was not presented in the original paper, the average seed mass for the species was obtained from either the Ecoflora database (Fitter & Peat, 1994), the KEW Seed Information Database (KEW, 2008) or Grime *et al.* (1988).
3. The duration of the study was measured as the number of days between germination and the final harvest.
4. Pot volume was recorded in $cm^3$.
5. The growth forms of each species (forb, graminoid, shrub or tree) were recorded, using data from: the original paper, Grime *et al.* (1988) or the PLANTS Database (USDA & NRCS, 2012).
6. Studies were assigned to the Köppen-Geiger climate classification (continental, dry, moderate, or tropical) based on their location (Peel *et al.*, 2007).

Where studies had multiple treatment groups (e.g. multiple light levels), only the results from the control treatments (i.e. plentiful light, nutrients and water) were recorded. The final dataset consisted of 761 species (431 unique speciies) from 45 studies (see Appendix 1 for details).

### ***Statistics***

A linear mixed-effects model for variation in RGR was developed in R (R Development Core Team, 2011) using the lme4 package (Bates *et al.*, 2011). These models allow the dependence on covariates and unmeasured study-specific factors to be explored. Specifically, in this case, different studies were performed using different protocols and under different environmental conditions, and so study-specific random effects were included in the models. In addition to these, we also included study-specific covariates (e.g. duration and pot volume). Information on pot volume was not available for ≈25% of the species, and so we initially developed models ignoring pot volume. The conclusions from models with and without pot volume were, however, similar, and so we only present analyses ignoring pot volume; see Appendix 2 for models including pot volume. All continuous variables were log transformed (base 10).

The statistical significance of the relationships between RGR and the study specific covariates, and between the covariates was assessed using a mixed model with a study-specific random intercept. We then generated a sample from the posterior distribution of the parameters from the fitted model using Markov Chain Monte Carlo methods (based on the mcmcsamp function in lme4 and pvals.fnc in languageR).

## Results

### *Relationships between Experimental Variables*

In agreement with previous research, woody plants do appear to have lower RGRs than herbaceous species (Figure 2.1a). There were also associations with the study-specific covariates: study duration and pot volume. RGR was negatively correlated with both the duration of the study and pot volume (Figure 2.1b & c). Similarly, seed mass was also correlated with these covariates, with studies of longer duration, in larger pots, typically using larger seeded species (Figure2. 1d & e). As expected, studies using larger pots were usually of longer duration (Figure 2.1f). In tropical studies RGR is lower, and these studies typically use larger seeded species (Figure 2.2a and b). Tropical studies are also typically performed in large pots and are of long duration (Figure 2.2c and d). Consistent with expectations from the literature (Westoby *et al*., 1996), woody species (trees and shrubs) had higher seed masses than the herbaceous species (forbs and graminoids, Figure 2.3), which could potentially bias comparisons between growth forms.

### *Model Selection*

A linear mixed-effects model was developed to explain the effects of growth form and seed mass on RGR, using: seed mass, growth form, duration of the study, Köppen-Geiger climate classification, and study (which groups the data by their original paper). The initial model assumed that variation in RGR was explained by seed mass, growth form, Köppen-Geiger climate classification and duration, with study-specific correlated intercepts and seed mass slopes (Table 2.1: Model 1). Removing the duration of the study from the model improved the fit (Table 2.1: Model 1 *vs* 2), possibly because the effect of duration was confounded with the study random effect. Likewise removal of the Köppen-Geiger climate classification improved the fit of the models (Table 2.1: Model 2 vs 3); therefore both variables were removed from the model. Inspection of the fitted parameters suggested there was little difference between the forbs and graminoids, and between the shrubs and trees, and so they were combined into two groups, the herbaceous and woody species. This grouping improved both the AIC and BIC (Table 2.1: Model 3 *vs* 4). Including an interaction between herbaceous and woody, and seed mass did not improve the fit of the model (Table 2.1: Model 4 *vs* 5), however, making the study-specific intercepts and seed mass slopes independent did (Table 2.1: Model 4 *vs* 6).

In the final mixed-effects model, log(RGR) declined linearly with log(seed mass), but the herbaceous and woody species had different intercepts, so woody species had a consistently lower average RGR (Figure 2.4a). There was no evidence for an interaction between seed mass and growth form (Table 2.1: Model 4 *vs* 5), suggesting that RGR declines with seed mass at the same rate in both groups. Importantly, this means that at any common seed mass, woody species do indeed grow more slowly than herbaceous species. All study-specific slopes were negative (Figure 2.4b).

Table 2.1: Model selection, for the linear mixed effects model of RGR. RGR, seed mass and duration were all log transformed (base 10), KG = Köppen-Geiger climate classification.

| No | Model | AIC | BIC |
|---|---|---|---|
| 1 | RGR ~ seed mass + duration + growth form +KG + (seed mass \| Study) | -275.3 | -215.6 |
| 2 | RGR ~ seed mass + growth form + KG + (seed mass \| Study) | -277.2 | -222.1 |
| 3 | RGR ~ seed mass + growth form + (seed mass \| Study) | -279.4 | -238.1 |
| 4 | RGR ~ seed mass + Herb or Woody + (seed mass \| Study) | -293.4 | -261.2 |
| 5 | RGR ~ seed mass * Herb or Woody + (seed mass \| Study) | -288.0 | -251.2 |
| **6** | **RGR ~ seed mass + Herb or Woody + (1\|Study) + (0 + seed mass \| Study)** | **-295.2** | **-267.7** |

The terms in brackets indicate study-specific random effects. The (1 | Study) term specifies study-specific intercepts, the (seed mass | Study) term specifies study-specific correlated slopes and intercepts, while (0 + seed mass | Study) specifies study-specific uncorrelated slopes and intercepts. AIC and BIC are both measures of model fit, with different penalties on model complexity (the number of parameters estimated).

Table 2.2: Parameters of the final mixed-effects model for variation in RGR (Table 2.1; Model 6).

| Fixed effects: | Estimate | Standard Error | t value |
|---|---|---|---|
| Woody | -1.211 | 0.053 | -23.052 |
| Herb | -1.012 | 0.054 | -18.732 |
| Seed mass | -0.107 | 0.015 | -7.084 |
| | | | |
| Random effects: | Variance | Standard Deviation | |
| Study | 0.100 | 0.317 | |
| Seed mass | 0.004 | 0.060 | |
| Residual | 0.030 | 0.172 | |

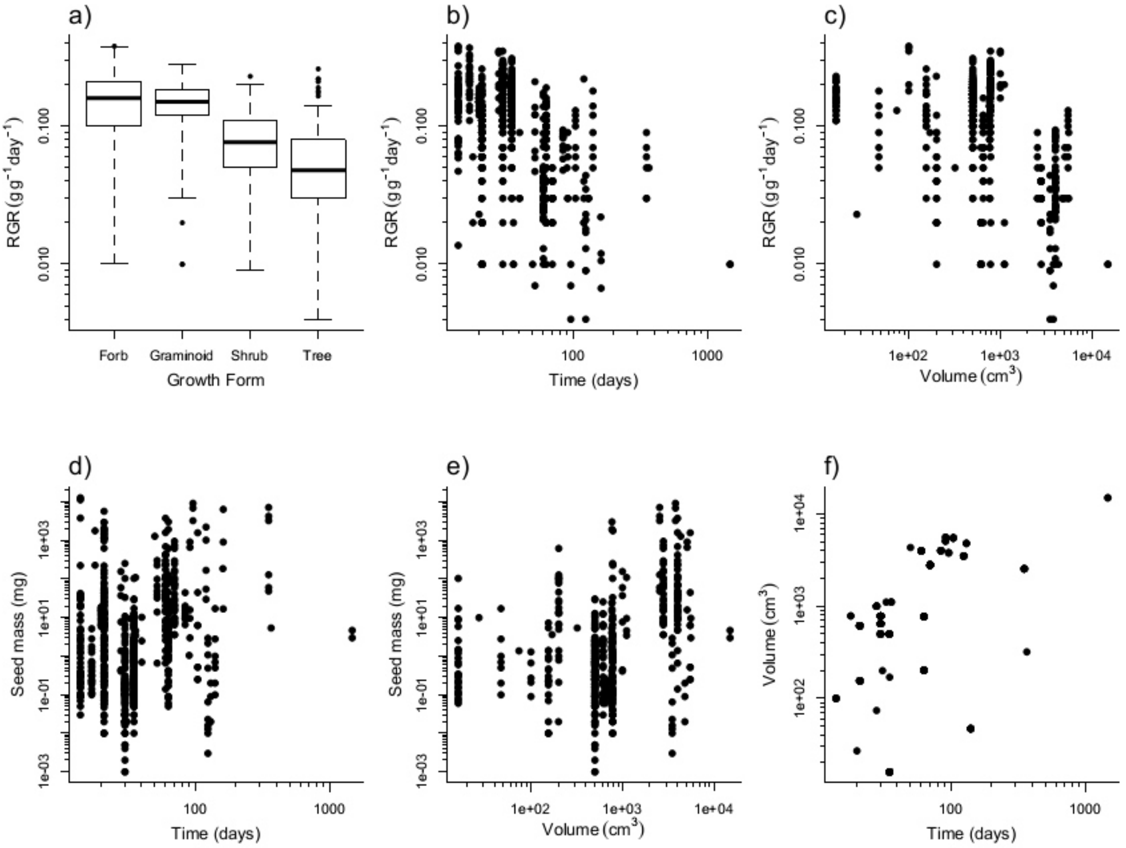

Figure 2.1: The relationships between the experimental variables. a) RGR and growth form (p<0.0001), b) RGR and duration (p<0.0001), c) RGR and pot volume (p<0.0001), d) Seed mass and duration (p<0.001), e) seed mass and volume (p<0.0002) and f) Volume and duration (p<0.0001). All variables except growth form are displayed on the log scale. p-values generating a sample from the posterior distribution of the parameters of the fitted model using Markov Chain Monte Carlo methods; see text for details.

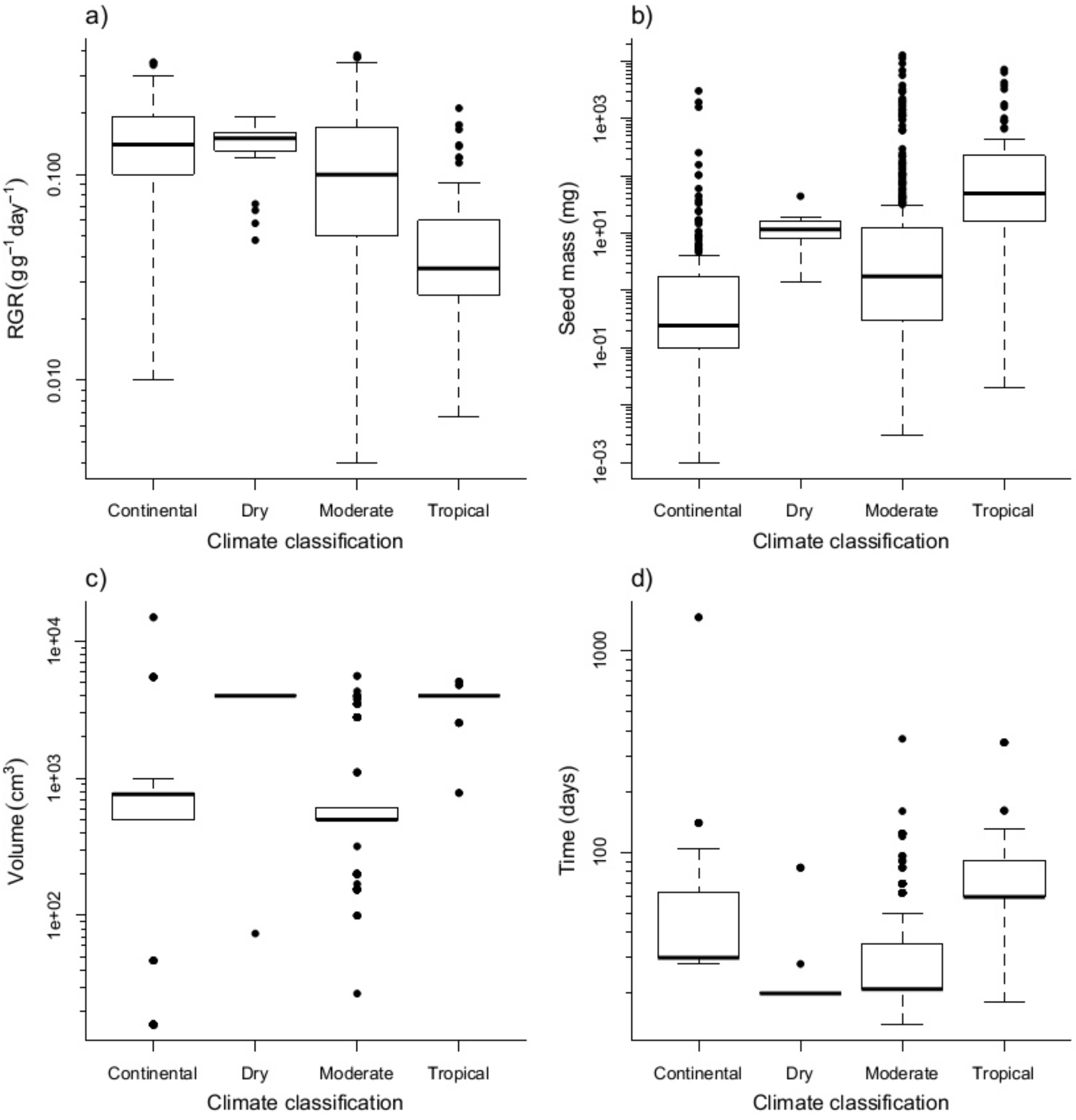

Figure 2.2: The relationships with climate classification for a) RGR, b) seed mass, c) pot volume, and d) duration. In all cases p<0.0001, using p-values generated using Markov Chain Monte Carlo methods; see text for details.

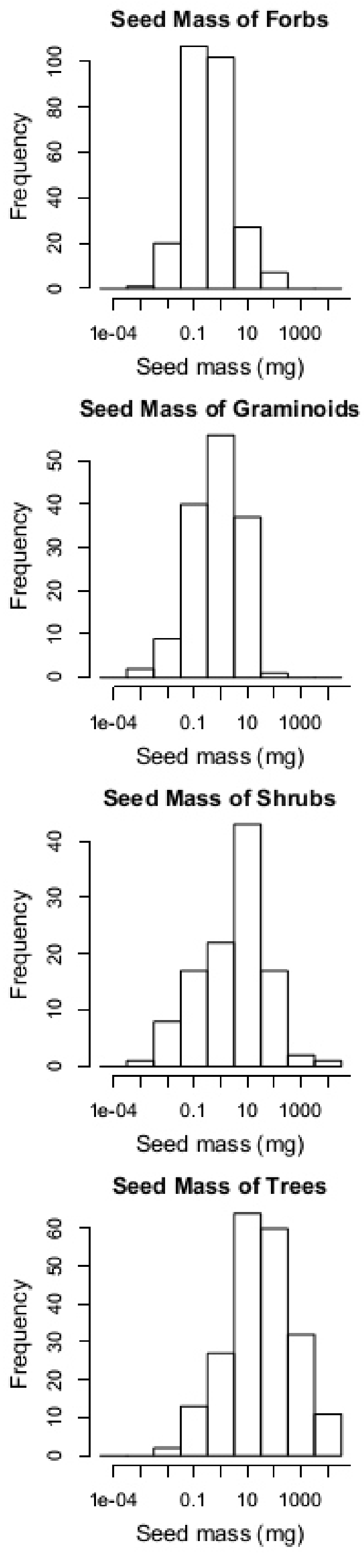

Figure 2.3: Histogram of seed mass, subdivided into forbs, graminoids, shrubs and trees ($p<0.0001$); p-value generated using Markov Chain Monte Carlo methods, see text for details.

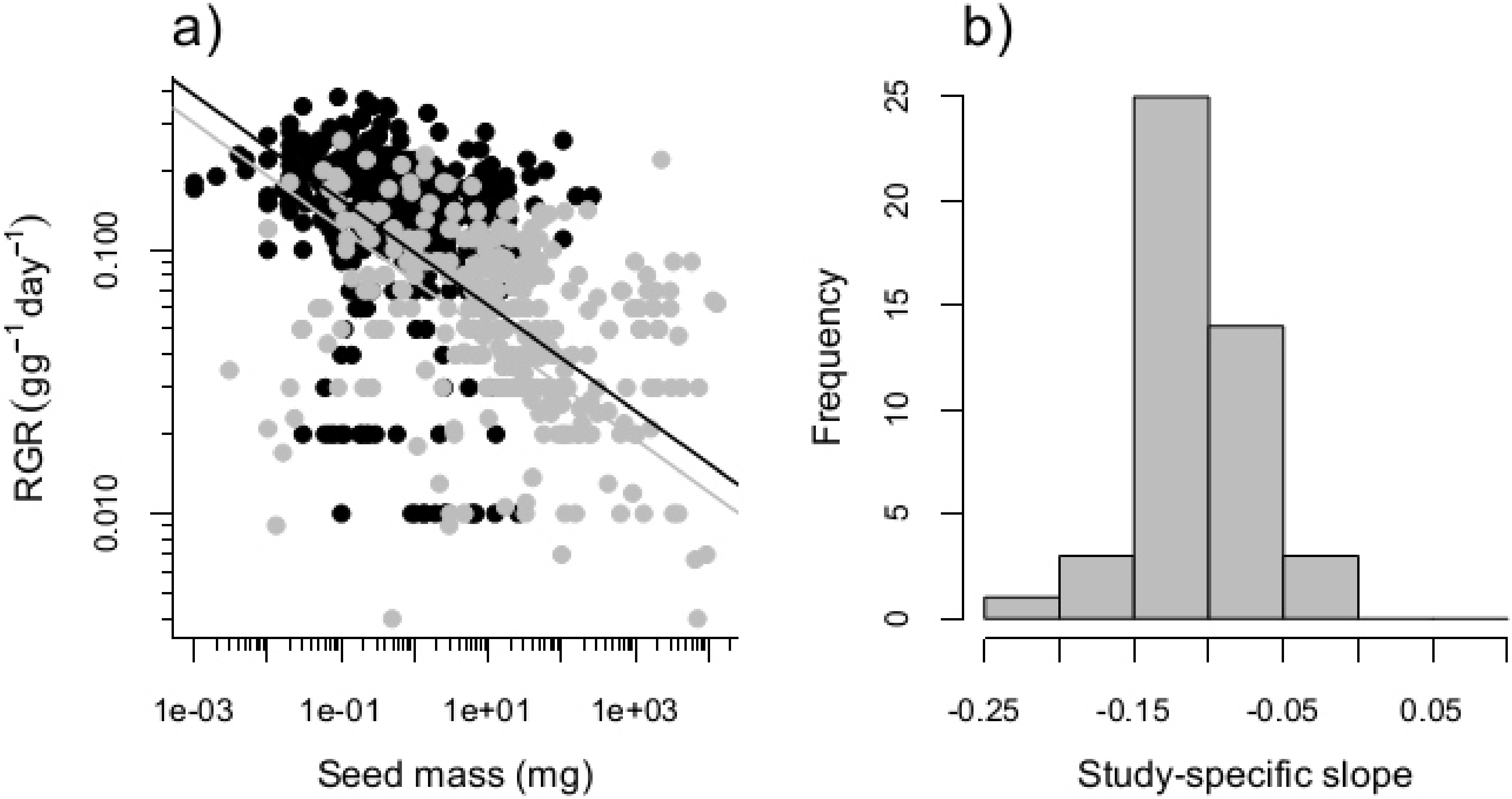

Figure 2.4: a) Relationship between RGR and seed mass. Herb = black, woody = grey. The solid lines use the fixed effects from the final mixed-effects model (Model 6, Table 2.1). b) Study-specific slopes for the log(RGR) *vs* log(Seed mass) regressions.

## Discussion

### *Seed mass and RGR*

There was a highly significant overall negative relationship between RGR and seed mass (Table 2.2, Figure 2.4a) and all the study-specific slopes were negative (Figure 2.4b), which supports the majority of the literature (for example Maranon & Grubb, 1993; Grotkopp et al., 2002) and agrees with the results of the only other published comparative analysis on the relationship between RGR and seed mass (Shipley & Peters, 1990).

Herbaceous species had consistently higher average RGRs than woody species at all seed masses and growth form does not alter the slope of the relationship between RGR and seed mass. As a result, conclusions from previous research that have compared RGR across growth forms, ignoring seed mass, may not be qualitatively affected by not accounting for seed mass. However, because seed mass is correlated with RGR, and seed mass varies between growth forms, failure to account for the effects of seed mass will bias comparisons.

### *Growth forms*

The RGRs of forbs and graminoids were similar, as were the RGRs of shrubs and trees (Figure 2.1a). However, herbs had significantly higher RGRs than woody species (Figure 2.4a), consistent with the widely accepted view that different growth forms have different RGRs (Grime & Hunt, 1975). As this difference holds even when species are compared at a common seed mass, we must consider other causes of variance in seedling RGR between growth forms.

The differing RGR between growth forms has previously been linked to growth components, where RGR is decomposed into: specific leaf area (SLA; leaf area per unit of leaf biomass), leaf mass ratio (LMR; ratio of leaf biomass to total plant biomass) and net assimilation rate (NAR; increase in biomass per unit of leaf area per time). Herbaceous species tend to have higher SLAs than woody species (Wright & Westoby, 2001; Galmes *et al*., 2005), providing greater light absorption per unit of leaf mass, which may contribute to their higher RGR. SLA is often found to account for most of the variance in RGR in studies of herbaceous species (Poorter & Remkes, 1990; Poorter & Van der Werf, 1998). In woody species, however, while a major contribution of SLA is supported by some studies (Huante *et al*., 1995; Cornelissen *et al*., 1998), others find that NAR explains more of the variance in RGR (Veneklaas & Poorter, 1998). The meta-analysis by Shipley (2006) found that NAR was generally the best predictor of RGR, but that as light intensity decreased, the importance of NAR declined and the importance of SLA increased, at least in herbaceous species. The relationship between LMR and RGR is inconsistent, with many non-significant relationships (Reich *et al*., 1998). Therefore explanations for the variance in RGR between growth forms using the components of RGR are currently contradictory and require further research using approaches that account for the effects of plant mass (Rees *et al*., 2010).

### ***Environmental Variables***

Although plants in longer studies had lower RGRs (Figure 2.1b), duration did not improve the model for RGR (Table 2.1), likewise pot volume was also removed from the model (Appendix 2). There was a negative relationship between pot volume and RGR (Figure 2.1c) possibly because plants in large pots tend to be woody species in long-term experiments, which have a lower RGR (Figure 2.1a). The effects of pot volume are unlikely to be a consequence of plants becoming pot bound, as study duration is typically too short for this to occur (Poorter *et al*., 2012). The lack of significant pot volume and duration effects is largely a consequence of these factors varying at the level of the study. Where there is within-study variation in pot volume, large effects are often found (Poorter *et al*., 2012). The regressions between pot volume, duration and other covariates indicate, unsurprisingly, that they are not independent, making it difficult to separate their effects (Figure 2.1).

### ***Study***

RGR varied between studies, for reasons beyond differences in species, seed mass and growth form. This suggests that details of the experimental protocol are important. Previous studies have also shown that other factors that vary between studies, for example light (Poorter, 1999) and nutrients (Burns *et al.*, 1997), also affect RGR, but that even after these variables are accounted for (as far as they can be), substantial variation remains (Shipley, 2006). To fully understand the physiological effects of the variables studied here and the relations between them, the development of a standardised experimental design should be considered. Standardised growth conditions would improve the integrity of comparisons between experiments and produce a clearer outcome across studies and species groups. This would enable more clear and specific conclusions to be drawn from comparative and meta-analyses. However, this may prove difficult on the global scale, as conditions that are optimal for one species may be very sub-optimal for others. Encouraging the ecological community to adopt the same design, which may be difficult or expensive to implement in some environments, may also prove to be an insurmountable challenge.

## Conclusion

RGR varied between growth forms, even at a given seed mass, particularly between the herbaceous and woody species. However, there was little difference between the forbs and graminoids, and between the shrubs and trees. The slopes of the regressions between seed mass and RGR was the same for both herbaceous and woody growth forms, suggesting a consistent difference between the groups. Further work is required to understand why RGR has a common scaling in these different groups, and explain the magnitude of the difference between them.

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**Appendix 1**

**Data Sources**

Table S2.1: A list of the studies used in the analysis

| Authors | HerbWoody | Growth Form | No spp |
|---|---|---|---|
| Agaboola, 1996 | Woody | Tree | 3 |
| Almeida-Cortez et al., 1999 | Herb | Forb & Graminoid | 28 |
| Atkin et al, 1998 | Woody | Tree | 10 |
| Burslem & Miller, 2001 | Woody | Tree | 2 |
| Cambrolle et al., 2011 | Herb | Forb | 1 |
| Campbell & Rochefort, 2003 | Herb & Woody | Forb, Shrub & Tree | 19 |
| Castro et al., 2008 | Woody | Tree | 1 |
| Castro-diez, et al., 2003 | Woody | Shrub & Tree | 22 |
| Choe et al., 1988 | Herb | Forb | 1 |
| Cordazzo, 2002 | Herb | Graminoid | 3 |
| Cornelissen et al., 1996 | Herb & Woody | Graminoid, Shrub & Tree | 83 |
| Grime & Hunt, 1975 | Herb & Woody | Forb, Graminoid, Shrub & Tree | 126 |
| Grotkopp et al., 2002 | Woody | Tree | 29 |
| Gyimah & Nakao, 2007 | Woody | Tree | 3 |
| Huante & Rincon., 1997 | Woody | Tree | 9 |
| Huante et al., 1995 | Woody | Tree | 33 |
| Hunt and Cornelissen, 1997 | Herb & Woody | Forb, Graminoid & Shrub | 59 |
| Jones & Reekie, 2007 | Woody | Tree | 2 |
| Kelly et al., 2009 | Woody | Tree | 3 |
| Khurana & Singh, 2004 | Woody | Tree | 5 |
| Kitajima, 1994 | Woody | Tree | 13 |
| Li et al., 1998 | Herb | Forb | 1 |
| Manning et al., 2009 | Herb | Forb | 5 |
| Maranon & Grubb, 1993 | Herb | Forb | 27 |
| McKenna & Houle, 2002 | Herb | Forb | 1 |
| Metcalfe et al., 2002 | Woody | Tree | 6 |
| Meyer & Carlson, 2001 | Woody | Tree | 1 |
| Milberg et al., 1998 | Woody | Shrub & Tree | 22 |
| Miyazawa & Lechowicz, 2004 | Woody | Tree | 1 |
| Moraes et al., 2010 | Woody | Tree | 1 |
| Norgren, 1996 | Woody | Tree | 2 |
| Padilla et al., 2007 | Woody | Shrub | 3 |
| Poorter, 1999 | Woody | Tree | 14 |
| Poorter & Remkes, 1990 | Herb | Forb | 24 |

|  |  |  |  |
|---|---|---|---|
| Quero et al., 2005 | Woody | Tree | 4 |
| Reich et al., 2003 | Woody | Forb & Graminoid | 34 |
| Roberts et al., 2010 | Herb | Forb | 1 |
| Ruiz-Robleto & Villar, 2005 | Woody | Shrub & Tree | 10 |
| Seibert & Pearce, 1993 | Herb | Forb | 6 |
| Shipley & Keddy, 1988 | Herb & Woody | Forb, Graminoid & Shrub | 28 |
| Shipley & Peters, 1990 | Herb | Forb & Graminoid | 56 |
| Shipley et al., 1989 | Herb | Forb & Graminoid | 23 |
| Villar et al., 1998 | Herb | Graminoid | 21 |
| Walters & Reich, 2000 | Woody | Tree | 7 |
| Walters et al., 1993 | Woody | Tree | 8 |

No species represents the number of species from each paper used in the analysis. This may be fewer than the number of species in the original studies due to unobtainable seed mass or RGR data.

## Appendix 2

### Model selection including pot volume

In this analysis of the data, all entries without pot volume data have been removed. The models including pot volume were not significantly better than the models excluding pot volume, so pot volume was not included in the final model. Model selection was based on the AIC and BIC.

Table S2.2: Model selection, for the linear mixed effects model of RGR including volume. RGR, seed mass, volume and duration were all log transformed (base 10), KG = Köppen-Geiger climate classification.

| No | Model | AIC | BIC |
|---|---|---|---|
| 1 | RGR ~ seed mass + volume + duration + growth form + KG + (seed mass\| Study) | -242.4 | -183.3 |
| 2 | RGR ~ seed mass + volume + growth form + KG + (seed mass \| Study) | -246.7 | -191.9 |
| 3 | RGR ~ seed mass + volume + growth form + (seed mass\| Study) | -256.8 | -214.6 |
| 4 | RGR ~ seed mass + growth form + (seed mass \| Study) | -257.5 | -219.5 |
| 5 | RGR ~ seed mass + Herb or Woody + (seed mass \| Study) | -271 | -241.5 |
| 6 | RGR ~ seed mass * Herb or Woody + (seed mass \| Study) | -267 | -233.3 |
| **7** | **RGR ~ seed mass + Herb or Woody + (1\|Study) + (0 + seed mass \| Study)** | **-272.9** | **-247.6** |

The terms in brackets indicate study-specific random effects. The (1 | Study) term specifies study-specific intercepts, the (seed mass | Study) term specifies study-specific correlated slopes and intercepts, while (0 + seed mass | Study) specifies study-specific uncorrelated slopes and intercepts. The model in bold was chosen as the model which best explained the data.

Table S2.3: Parameters of the final mixed-effects model for variation in RGR (Table S2.2; Model 7).

| Fixed effects: | Estimate | Standard Error | t value |
|---|---|---|---|
| Woody | -1.237 | 0.065 | -18.882 |
| Herb | -1.047 | 0.067 | -15.562 |
| Seed mass | -0.120 | 0.018 | -6.604 |
| | | | |
| Random effects: | Variance | Standard Deviation | |
| Study | 0.115 | 0.339 | |
| Seed mass | 0.004 | 0.064 | |
| Residual | 0.025 | 0.158 | |

# Chapter 3

# Growth and Biomass affect Seedling Survival of Drought and Defoliation

## Introduction

Larger organisms typically have a survival advantage over smaller ones (Cook, 1980), although size effects can become more complicated in relation to rates of predation (Sogard, 1997). Bigger seedlings may experience higher survival rates during stress events, because they are better established (i.e. they have more extensive root systems, as above- and below-ground biomass are usually correlated, Enquist and Niklas, 2002). However, biomass is not the only predictor of survival. Several authors have argued that the probability of survival depends on growth rate, with slow-growing organisms typically exhibiting a survival advantage over fast-growing organisms (Franco and Silvertown, 1996). The fast-slow continuum suggests that slow-growing plants allocate more resources to defence and storage organs, so they can better tolerate various stresses compared with fast-growing plants (e.g. Rose *et al.*, 2009). This means that although being big increases survival, the mechanism by which it is achieved, namely fast growth, has the opposite effect, making it important to separate growth rate and size effects.

It is currently not known whether growth rate or biomass has a greater impact on an organism's likelihood of survival. The primary problem when studying size- and growth-dependent mortality is separating their effects. Biomass and growth are strongly dependent on one another, as it is through growth that a plant increases in biomass, and growth rates are calculated by measuring changes in biomass (Hunt, 1990). Although relative growth rate (RGR) was designed to estimate the rate of growth of an organism independent of its biomass, the classical approach confounds the effects of size and growth rate - a problem that has been realised for some time in multiple fields of ecology, e.g. direct studies of RGR (Norgren, 1996); forestry (South, 1995) and research on competition (Schwinning and Weiner, 1998). RGR assumes that growth is exponential, but when this is not the case, mass can influence RGR (Turnbull *et al.*, 2008).

Metcalf *et al.* (2006) suggested calculating RGR at a common size in order to compare between species, and this approach has been widely used since (Paine *et al.*, 2012, Paul-Victor *et al.*, 2010, Rees *et al.*, 2010, Rose *et al.*, 2009, Taylor *et al.*, 2010). These approaches do not assume that growth is exponential, and therefore allow RGR to be size-independent. RGR at a specific size, termed SGR, is typically calculated using a non-linear model of biomass over time (whereas the conventional RGR method uses a log-linear model). The non-linear approach accounts for the decrease in growth over time, and can therefore characterise growth more precisely, with less size-bias than the log-linear approach. Consequently, SGR enables the effects of size and growth to be separated, allowing us to explore their relative roles in determining survival.

As the effects of seedling growth and biomass can now be separated through the SGR, we can examine which trait contributes more to seedling survival during stress events. Seedlings from three families were exposed to a biotic (defoliation) or abiotic (drought) stressor, with the aim of understanding general seedling responses to stressors. It is hypothesised that big seedlings are more likely to survive a stress event than small seedlings and that slow-growing seedlings are more likely to survive a stress event than fast-growing seedlings. The data set is also used to investigate the effect of seed mass on survival, as it is widely believed that large-seeded species have a survival advantage (Leishman and Westoby, 1994). Studies of variables in isolation are frequently seen in the literature (e.g. Davis *et al.*, 1999), although Walters & Reich (2000) explored the effects of seed mass and RGR on tree seedling survival in a range of light and nutrient treatments, where seedling survival increased with seed mass but not with RGR. Here, SGR, seedling biomass, seed mass and plant family are incorporated into the same survival analysis to explore their relative effects. The results of this analysis are then compared to more simple analyses of the effects of each variable in turn.

## Methods

### *Species*

Seedlings from 16 species across three families were studied (Asteraceae, Fabaceae and Poaceae, see Appendix 1 for details). The species were selected to have a wide range of seed sizes within each family and to have non-dormant seeds. The average seed mass was calculated using a sample of 50 seeds from each species.

### *Growth Environment*

The seedlings were grown under controlled conditions in the Arthur Willis Environment Centre, Sheffield, UK. The daytime temperature was 22°C, with a minimum light intensity of 200µmol and the temperature at night was 15°C. The experiment was repeated in two separate time periods, due to resource limitations. The species were equally represented across each treatment.

Seeds were germinated in petri dishes lined with damp filter paper, timed to standardise the germination date across all species. The seedlings were then transferred into individual inserts in seed trays (l = 38cm, w = 24cm, d = 4.8cm), with 12 seedlings in each tray (randomly allocated within each treatment). This was conducted in two sets, so that when the treatments were applied, seedlings were at two ages: 7 and 14 days post-potting. This increased the range of biomasses for each species on the day the treatment was applied. The seedlings were grown in Levington's M3 (high-nutrient) compost and initially watered every 1-2 days as required.

The longest leaf length and number of leaves were measured for each individual every 3-4 days from the day of potting until the treatment day. The seedlings were allocated to one of three experimental procedures, 14 days after the older group of seedlings were planted into the compost: drought, defoliation or the control. A fourth group of seedlings were grown for a series of destructive harvests, in order to estimate seedling biomass.

### *Mortality Experiment*

There were 14 individuals per species per age in each treatment across the whole experiment, giving a total of 1344 plants (i.e. 84 individuals per species). On the day the treatments were initiated, leaf height and number were measured per seedling, before: they were defoliated (cut to 5mm above soil level for grasses and stem-based species, or cut to 5mm long from the centre of the rosette for the other species); a drought regime was initiated (water was completely withheld) or they were left as the control (high light and high water). Defoliation was repeated every 3-4 days after the initial treatment. Mortality was recorded daily.

### *Destructive Harvests*

The destructive harvests were conducted to provide an estimate of seedling biomass in the mortality experiment. In each repeat of the experiment, 10 seedlings per species were harvested every 3-4 days over a period of 14 days - from the potting of the first set of seedlings until treatments were applied to the experimental seedlings. This produced harvests over 5 time intervals. Leaf height and number were recorded for each seedling at each date. The harvested seedlings were cleaned, dried in an oven at 68°C for 48 hours and then weighed. The data set from the destructive harvests was then used to predict the biomass of the treatment seedlings.

## Statistics

### *Destructive Harvests*

The destructive harvest data set was used to produce a regression model of biomass, which was then used to predict biomass in the treatment seedlings using the ‘predict’ function in R (R Development Core Team, 2012).

### *Mortality Experiment*

SGR was calculated by fitting a non-linear mixed-effects model to the individual growth curves (Paine *et al.*, 2012, Rose *et al.*, 2009) using the self-starting four parameter logistic (SSfpl) model in the 'nlme' package (Pinheiro *et al.*, 2012). 'Species' was added as a fixed effect (lowering the AIC by 714.516 and the BIC by 568.498, compared to the initial model without species). SGR was calculated at the smallest size common to all species to minimise the effects of resource limitation. The other explanatory variables were seedling mass just before the application of the treatments, and seed mass.

A 'complex', multiple variable survival analysis was then performed for the defoliation and drought treatment, using an accelerated failure time survival model (AFT) in the 'survival' package (Therneau and Lumley, 2011). The control had 0% mortality after the application of treatments to the other groups, so was not included in this analysis. The model selection process began with a basic model, then added explanatory variables and interactions (see Appendix 2 for the model selection process). To visualise the effects of the different explanatory variables we plot the predictions of the model for each variable, with the other variables set to their mean values. For example, the effect of SGR on survival was assessed by plotting the model predictions against SGR, having set seedling and seed mass to their average values. Due to the complex nature of the model, standard errors and rugs (to show the data distribution) were plotted to allow a visual assessment of the effect of each variable.

To allow a comparison of the effect of studying multiple factors influencing seedling survival, a second, 'simple', single variable analysis of the data set was conducted, using each of the explanatory variables in turn (see Appendix 3 for the model selection process).

## Results

### *Destructive Harvest*

The harvest seedlings were grown to model the biomass of the treatment seedlings. A linear regression of biomass, log transformed, including the following variables: longest leaf length, leaf number, species and the day of measurement, accounted for 85% of the variance in biomass (linear regression: F = 142.3, d.f. = 19, 494, $p<0.001$, $R^2 = 0.85$).

***Explanatory Variables***

SGR ranged from 0.071 to 0.209 g $g^{-1}$ $day^{-1}$. There are significant positive regressions between: SGR and seed mass; seedling and seed mass and between SGR and seedling biomass (Figure 3.1). Therefore, seedlings with a larger seed mass have higher SGRs and biomasses than seedlings with a low seed mass, and as SGR increases so does seedling biomass.

The SGR and biomass data from just before the treatments were applied were used in an AFT (accelerated failure time) survival model, along with family and seed mass, to quantify the factors impacting survival time. A survival analysis was produced for each treatment, including all the explanatory variables. The final model for both treatments had interactions between most or all of the explanatory variables (SGR, biomass and seed mass) and family (survival after drought was dependent on: Family * SGR * biomass * seed mass whereas survival in the defoliation treatment was dependent on: family * (SGR + biomass) * seed mass). The results varied between families and treatments. Generally, within the defoliation treatment, survival increased with lower seed masses, increased with biomass or was non-significant (depending on the family) and showed evidence of both increasing and decreasing with SGR, depending on the family (Figure 3.2). Similarly, in the drought treatment, survival was typically higher with low SGR, high biomass and low seed mass (Figure 3.3). There was only one average recorded seed mass and SGR value per species, so there were less available data for the model than for biomass. There was a significant four-way interaction between family, SGR, biomass and seed mass in the drought treatment, which suggests that survival depends on complex interactions.

The simple single variable survival analyses, which modelled each explanatory variable in turn, yielded some differing results (Figure 3.4). In the defoliation treatment, there was no effect of any of the variables (SGR, biomass or seed mass) on survival, although survival varied with family in all cases (Figure 3.4). In the complex model, there were also some examples of no interaction between the explanatory variable and survival. However, in the complex model of defoliation, there was an overall negative relationship between survival and seed mass and the varying effects of SGR observed in the complex model (Figure 3.2) were also not present in the simple model.

In the simple drought treatment models, there was also no effect of SGR on survival, while species with a high biomass and generally species with a high seed mass survived for longer than species with low biomasses and seed masses (Figure 3.4). The two sets of drought models find the same positive effect of biomass on survival, but the results for SGR and seed mass differ.

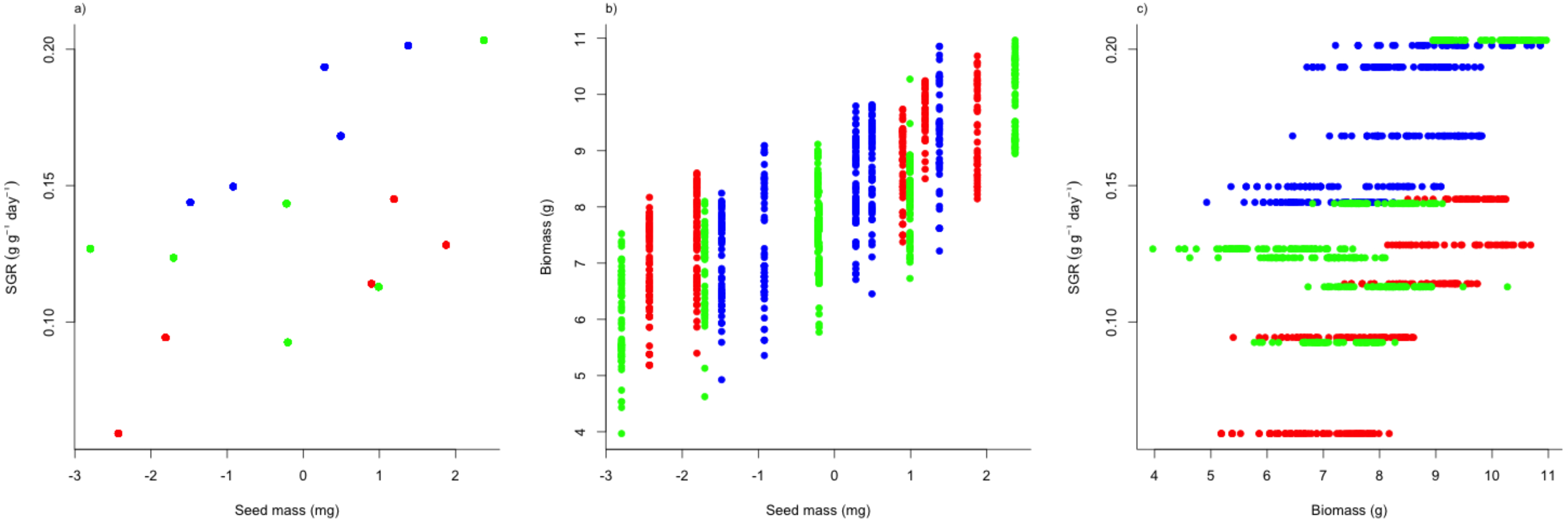

Figure 3:1: Regressions of the explanatory variables. a) the regression between SGR and seed mass (linear regression: F = 15930, d.f. = 4, 1100, $R^2$ = 0.983, $p < 0.001$); b) the regression between biomass and seed mass (linear regression: F = 28080, d.f. = 4, 1100, $R^2$ = 0.990, $p < 0.001$); and c) the correlation between SGR and biomass (Spearman's rank correlation: S = 345776269, rho = -0.542, $p < 0.001$). Red points = Asteraceae, blue points = Fabaceae and green points = Poaceae. All explanatory variables (SGR, biomass and seed mass) have been log-transformed.

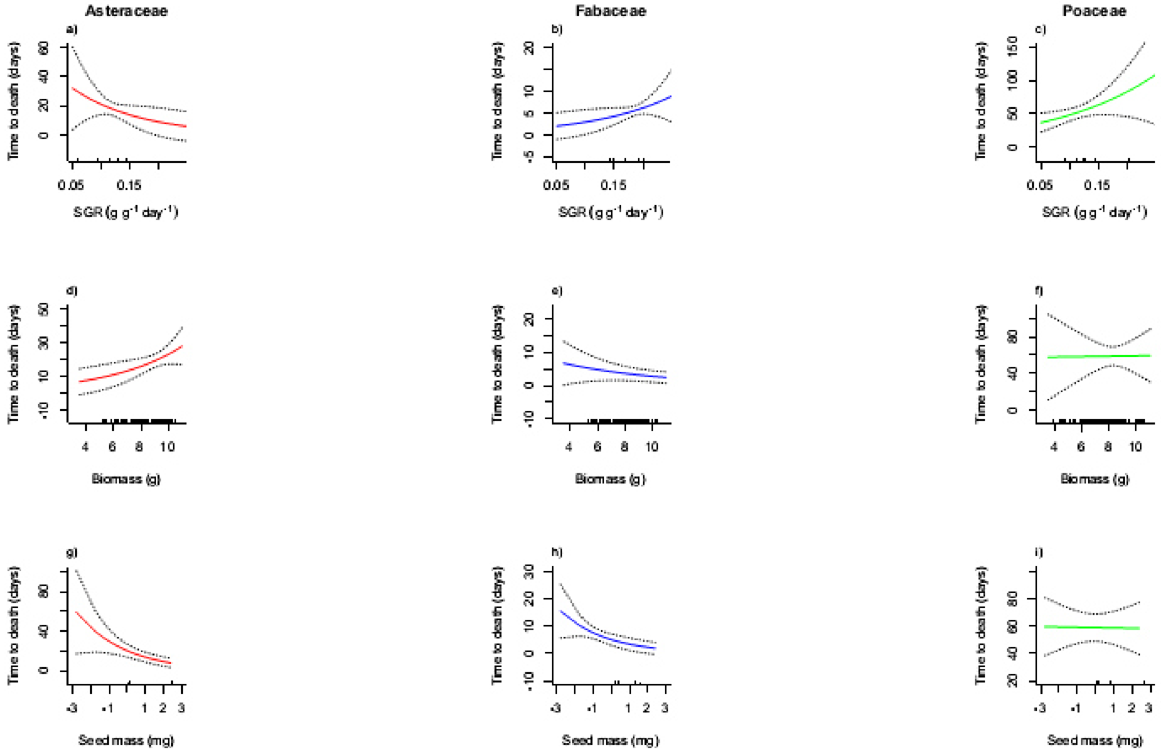

Figure 3.2: Modelled response from the original model incorporating all the variables of plant survival in the defoliation treatment. a) the effect of SGR on Asteraceae survival, b) the effect of SGR on Fabaceae survival, c) the effect of SGR on Poaceae survival, d) the effect of biomass on Asteraceae survival, e) the effect of biomass on Fabaceae survival, f) the effect of biomass on Poaceae survival, g) the effect of seed mass on Asteraceae survival, h) the effect of seed mass on Fabaceae survival and h) the effect of seed mass on Poaceae survival. Red lines = Asteraceae, blue lines = Fabaceae and green lines = Poaceae. Dotted lines = standard error. Black lines along the x-axis show the data distribution (rug). All explanatory variables (SGR, biomass and seed mass) have been log-transformed.

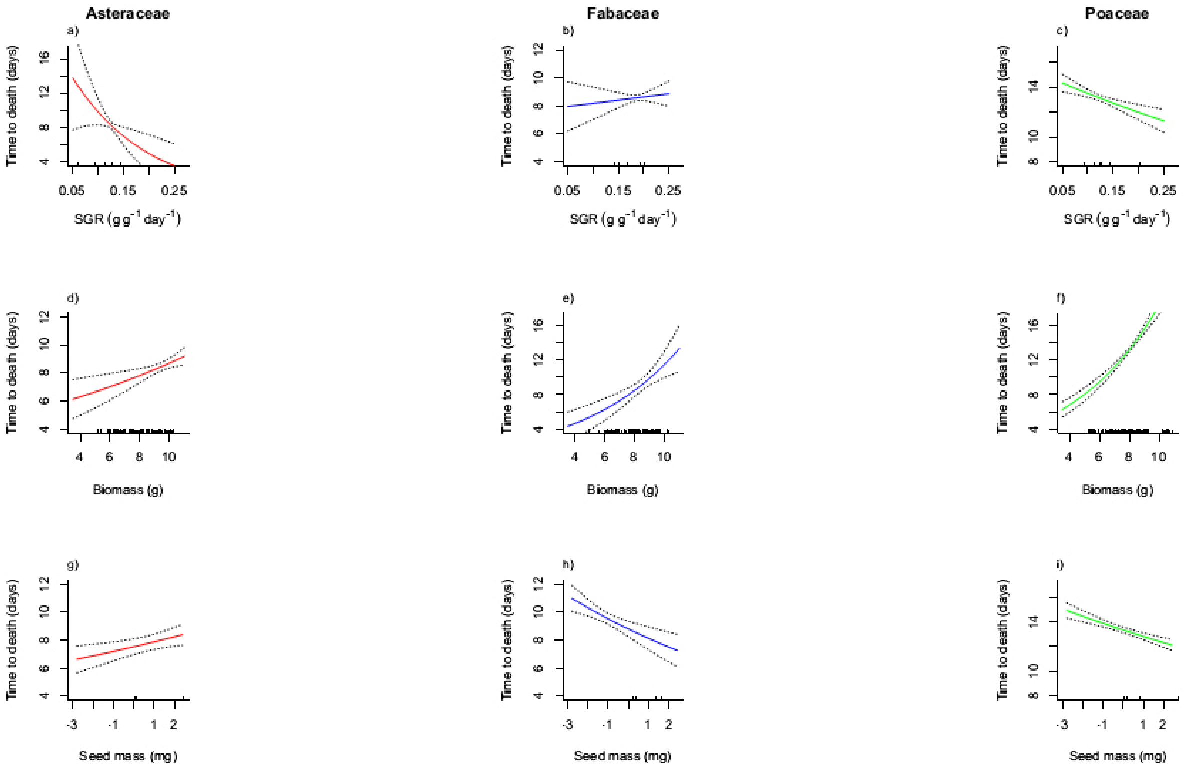

Figure 3.3: Modelled response from the original model incorporating all the variables of plant survival in the drought treatment. a) the effect of SGR on Asteraceae survival, b) the effect of SGR on Fabaceae survival, c) the effect of SGR on Poaceae survival, d) the effect of biomass on Asteraceae survival, e) the effect of biomass on Fabaceae survival, f) the effect of biomass on Poaceae survival, g) the effect of seed mass on Asteraceae survival, h) the effect of seed mass on Fabaceae survival and h) the effect of seed mass on Poaceae survival. Red lines = Asteraceae, blue lines = Fabaceae and green lines = Poaceae. Dotted lines = standard error. Black lines along the x-axis show the data distribution (rug). All explanatory variables (SGR, biomass and seed mass) have been log-transformed. Note that the scale of the y-axis differs from Figure 2.

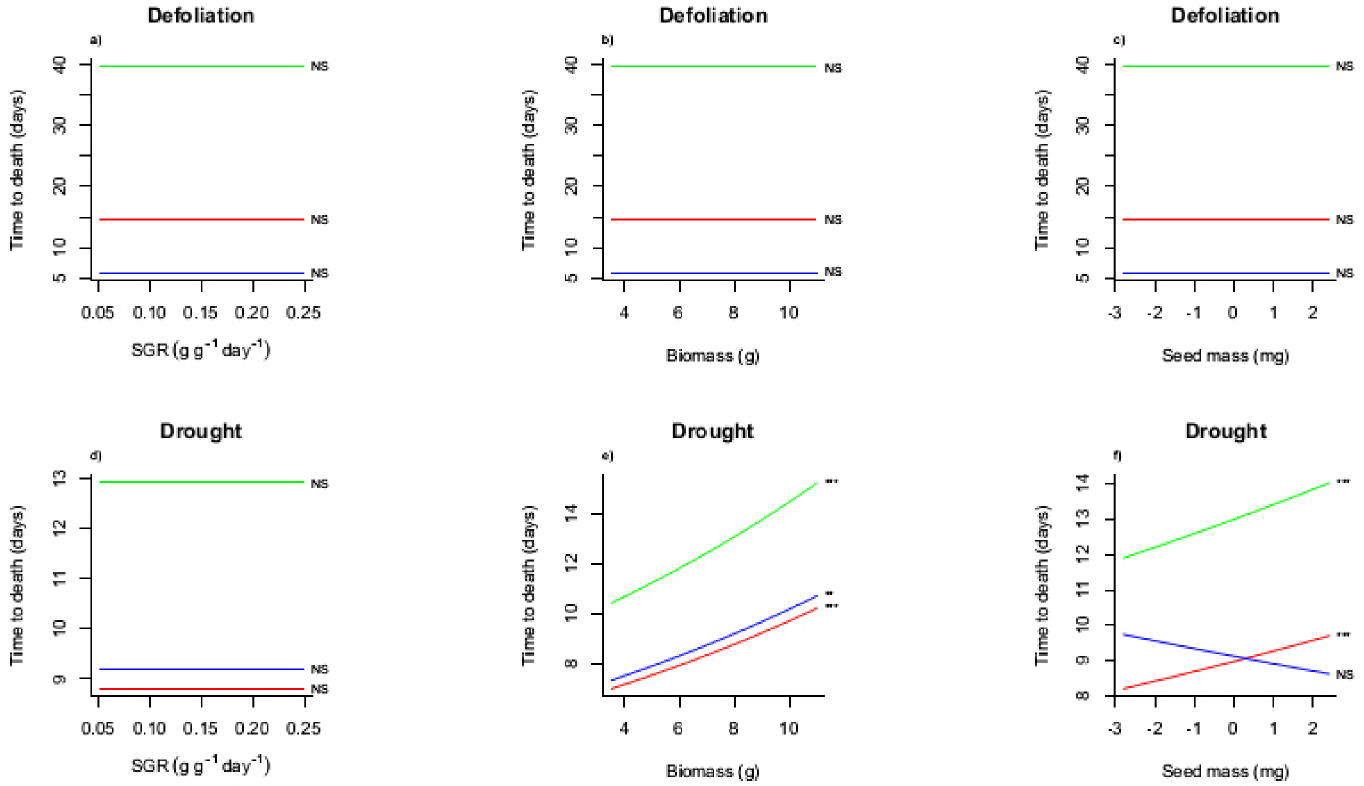

Figure 3.4: Modelled response from models with isolated variables of plant survival in the treatments a) the effect of SGR on survival of defoliation, b) the effect of biomass on survival of defoliation, c) the effect of seed mass on survival of defoliation, d) the effect of SGR on drought survival, e) the effect of biomass on drought survival and f) the effect of seed mass on survival of drought. Red lines = Asteraceae, blue lines = Fabaceae and green lines = Poaceae. NS = non-significant effect of the variable per family in the group of models accounting for each variable in turn, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. All explanatory variables (SGR, biomass and seed mass) have been log-transformed. Note the graphs are drawn to different scales.

## Discussion

Seedlings from small seeds grow slowly and so are small (Figure 3.1a & b). Slow growth and large biomass are associated with high survival, which creates an inconsistency: seedlings from small-seeded species have a survival advantage through slow growth, but a disadvantage due to their small size. Previous studies on the effect of seed mass on seedling survival are inconsistent, with some authors suggesting that species with large seed masses have greater survival (Moles and Westoby, 2004b, Leishman *et al.*, 2000), whereas others find no effect (Moles *et al.*, 2003, Moles and Westoby, 2004a, Augspurger, 1984). However, this previous work has explored the effect of seed mass in isolation, and so its effect on seedling survival will have been confounded with changes in seedling size and growth rate.

In the simple, single variable analysis, survival was independent of seedling traits in 13 of the 18 regressions (across treatments, family and explanatory variables, Figure 3.4). This lack of effect on survival includes every variable in the defoliation treatment. The exceptions were an increase in survival in the drought treatment as biomass and seed mass increase (except for seed mass in Fabaceae, Figure 3.4). Thus the simple, single variable analyses are in agreement with the literature, where the effects of seed size on seedling survival are inconsistent, and often not significant (4 out of 6 family – treatment combinations, Figure 3.4c & f).

The results from the complex, multiple variable analyses suggest a very different pattern, with 14 of the 18 regressions indicating that the seed and seedling traits do have an important effect on seedling survival (Figure 3.2 & 3.3). The final survival model for defoliation contained a three-way interaction (family x (SGR + biomass) x seed mass), while drought model had a four-way interaction (family x SGR x biomass x seed mass). This highlights the complex nature of the relationship between seedling survival and seed / seedling traits.

The effects of the explanatory variables differed between the two analyses. For example, in the simple analysis, seed mass either had no effect or improved seedling survival, whereas in the complex analysis, in 4 out of 6 regressions, small seed mass was beneficial for seedling survival (with one of the others showing a positive relationship and the other no association with survival, Figure 3.2 & 3.3). This advantage of having a small seed could be a consequence of the seedlings growing slowly (low SGR), as previous studies have identified survival costs of fast growth (Rose *et al.*, 2009).

In the simple analyses, large seedlings survive better in the drought treatment, but there was no effect of seedling size on survival after defoliation (Figure 3.4b & e). The method of defoliation may partially explain this, as a greater proportion of mass was removed from the large seedlings. Therefore large seedlings, and hence large seeded species which produce large seedlings, were at a disadvantage. This method was chosen because a preliminary study demonstrated that all the species were highly resilient to defoliation, and required extensive damage to produce any mortality. Additionally, cutting the seedlings to a standard height may also be more representative of grazing on seedlings in the field. In this case the results of the complex analysis were similar (Figure 3.2 & 3.3).

In the simple analyses, SGR had no effect on seedling survival in either treatment. In the complex analysis the results for SGR were highly variable. In the drought treatment, two of the families exhibit the expected decrease in survival as SGR increases, and the third family (Fabaceae) shows no effect of SGR on survival (Figure 3.3). The results for defoliation treatment were similar for the Asteraceae, with survival highest in slow-growing species. The higher survival in slow-growing species supports the theory of the fast-slow continuum (Franco and Silvertown, 1996). In the Poaceae however, fast growth was associated with improved survival following defoliation (Figure 3.2c). This could again be associated with the method of defoliation, as fast-growing species can recover more between periods of defoliation.

There were significant differences in the responses to the treatments between the three families. The Poaceae (grasses) had a consistently higher survival rate than the other families (Figure 3.2 – 3.4). One reason for this could be their physical structures. The thin leaves of grasses may reduce transpiration, and therefore possibly increase survival in the drought treatment. The evolutionary history of the grasses (providing traits such as basal meristems and fast-growth) may explain their high survival rates in the defoliation treatment (Coughenour, 1985). Grasses have adapted to many stressful conditions, abiotic (for example buffelgrass in arid deserts, Ward *et al.*, 2006) and biotic (for example through high tolerance to grazing, Harper, 1978, cited in Kemp and Culvenor, 1994), so they are able to respond to stressful environments.

## Conclusion

Previous research, showing inconsistent effects of seed and seedling traits on seedling survival we believe may be misleading, as a consequence of covariation between seed mass, and seedling size and growth rate (SGR). Large seedling size is clearly advantageous, but the mechanism by which it is achieved, namely fast growth, is not, making it essential to separate their effects. This suggests that the interactions between the traits need to be accounted for in order to observe their effects on survival.

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# Appendix 1

## *List of Species*

Table S3.1: List of species used in the experiment, originating family and average seed mass

| Family | Species | Seed mass (mg) |
|---|---|---|
| Asteraceae | Achillea millefolium | 0.088 |
| Asteraceae | Centaurea nigra | 2.450 |
| Asteraceae | Centaurea scabiosa | 6.528 |
| Asteraceae | Cirsium vulgare | 3.289 |
| Asteraceae | Sonchus asper | 0.164 |
| Fabaceae | Medicago lupulina | 1.323 |
| Fabaceae | Melilotus altissima | 3.969 |
| Fabaceae | Trifolium dubium | 0.227 |
| Fabaceae | Trifolium pratense | 1.636 |
| Fabaceae | Trifolium repens | 0.398 |
| Poaceae | Agrostis stolonifera | 0.061 |
| Poaceae | Brachypodium pinnatum | 2.694 |
| Poaceae | Dactylis glomerata | 0.803 |
| Poaceae | Festuca ovina | 0.817 |
| Poaceae | Hordeum murinum | 10.725 |
| Poaceae | Poa trivialis | 0.182 |

# Appendix 2

## *Survival Model Selection Process for the Complex Multiple Variable Models*

### A. Defoliated Plants

Table S3.2: Model selection process of the survival analysis for the defoliated plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -1073.584 | 2149.167 |
| Family | -995.646 | 1997.292 |
| Family + SGR | -995.561 | 1999.121 |
| Family + Biomass | -995.625 | 1999.25 |
| Family + Seed mass | -995.619 | 1999.238 |
| Family + SGR + Biomass + Seed mass | -995.252 | 2002.504 |
| Family * SGR + Biomass + Seed mass | -990.943 | 1997.885 |
| **Family * (SGR + Biomass) * Seed mass** | **-976.178** | **1972.355** |
| Family + SGR * Biomass * Seed mass | -982.961 | 1991.921 |
| Family * SGR * Biomass + Seed mass | -981.780 | 1999.559 |
| Family * SGR * Biomass * Seed mass | -973.472 | 1994.943 |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models.

### B. Drought Plants

Table S3.3: Model selection process of the survival analysis for the drought plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -818.412 | 1638.823 |
| Family | -678.083 | 1362.165 |
| Family + SGR | -676.476 | 1360.951 |
| Family + Biomass | -646.221 | 1300.441 |
| Family + Seed mass | -668.110 | 1344.219 |
| Family + SGR + Biomass + Seed mass | -630.963 | 1273.926 |
| Family * SGR + Biomass + Seed mass | -627.264 | 1270.527 |
| Family + SGR * Biomass * Seed mass | -621.820 | 1263.639 |
| Family * SGR * Biomass + Seed mass | -610.199 | 1246.398 |
| Family * (SGR + Biomass) * Seed mass | -610.361 | 1256.721 |
| **Family * SGR * Biomass * Seed mass** | **-596.259** | **1240.517** |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models and there was no significant difference between the final two models ($p = 0.142$), so the less complex model was chosen.

# Appendix 3

### *Survival Model Selection Process for the Simple Single Variable Models*

Table S3.4: The survival analysis model selection process for SGR of the defoliated plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -1073.584 | 2149.167 |
| **Family** | **-995.646** | **1997.292** |
| Family + SGR | -995.561 | 1999.121 |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models and there was no significant difference between the family and family * SGR model (p = 0.219), so the less complex model was chosen.

Table S3.5: The survival analysis model selection process for biomass of the defoliated plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -1073.584 | 2149.167 |
| **Family** | **-995.646** | **1997.292** |
| Family + Biomass | -995.625 | 1999.25 |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models.

Table S3.6: The survival analysis model selection process for seed mass of the defoliated plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -1073.584 | 2149.167 |
| **Family** | **-995.646** | **1997.292** |
| Family + Seed mass | -995.646 | 1999.292 |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models and there was no significant difference between the family and family * seed mass model (p = 0.189), so the less complex model was chosen.

Table S3.7: The survival analysis model selection process for SGR of the drought plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -818.412 | 1638.823 |
| Family | -678.083 | 1362.165 |
| **Family + SGR** | **-676.476** | **1360.951** |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models and there was only a slightly significant difference between the family + SGR and family * SGR model (p = 0.021), so the less complex model was chosen.

Table S3.8: The survival analysis model selection process for biomass of the drought plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -818.412 | 1638.823 |
| Family | -678.083 | 1362.165 |
| **Family + Biomass** | **-646.221** | **1300.441** |
| Family * Biomass | -642.401 | 1296.802 |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models and there was only a slightly significant difference between the family + biomass and family * biomass model (p = 0.022), so the less complex model was chosen.

Table S3.9: The survival analysis model selection process for seed mass of the drought plants

| Terms | Log-lik | AIC |
|---|---|---|
| ~1 | -818.412 | 1638.823 |
| Family | -678.083 | 1362.165 |
| Family + Seed mass | -668.110 | 1344.219 |
| **Family * Seed mass** | **-662.813** | **1337.626** |

Model in bold is the final model used. Log-lik represents the log-likelihood. All models use a log-normal distribution. AIC was calculated as 2k – 2*log-lik, where k = number of parameters. ANOVA was also used to compare similar models.

# Chapter 4

# Do Seedling Traits and the Initial Growth Environment Affect Survival?

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## Introduction

Growth rates vary between species, growth forms and environments. This variance occurs because different species growing in different conditions have different strategies and different requirements. These differences can be explained using the fast-slow growth continuum (Franco & Silvertown, 1996). It suggests that slow-growing species invest more resources in storage and preparation for stress events, while fast-growing species direct more of their resources to growth, and aim to avoid stressors during their shorter lifespans.

When a stress event occurs, slow-growing species typically have a higher survival rate than fast-growing species (Rose *et al.*, 2009; This thesis, Chapter 3). Slow-growing species are typically found in unproductive environments, whereas fast growing species tend to grow in productive environments. When slow-growing species are placed in productive environments from the beginning of their life-cycle (due to dispersal or experimental set ups), they remain slow-growing (Mahmoud & Grime, 1976). However, when fast-growing plants are grown in mild-moderately stressful conditions, their growth is reduced, and becomes more similar to that of slow-growing species (Mahmoud & Grime, 1976), indicating that plants have some ability to respond to the environment.

Seedling biomass also varies with environmental conditions. Where water or nutrients are limiting, a greater proportion of the biomass can be found in the roots, whereas a greater proportion of the biomass is found above-ground when light is the limiting factor (Poorter & Nagel, 2000). Total biomass is generally a response to the environment, with plants in more stressful conditions growing to a smaller biomass than plants grown in optimal conditions (Chapter 3).

While there is some variation in results, large seed mass generally increases the likelihood of seedling survival (Moles & Westoby, 2004). This may be due to a large seed mass increasing seedling survival because there are more reserves in the seed, which the seedling can use to survive when the environment initially becomes more stressful.

Plant characteristics in response to variable environments have previously been investigated, and it has been shown that there is some plasticity in growth between fertile and infertile environments (Vijver *et al.*, 1993). In these cases, it is assumed that changes are due to adaptation and plasticity. In adapting to one environment, a seedling could either be increasing or decreasing its chance of surviving a change in the environment in the future.

This experiment is unusual because of its 2 tiers of stressor. Most experiments consider the effect of 1 stressor on one plant, but in the wild, plants need to be adapted to face multiple stressors in the same season. Here, we grow seedlings from 5 families in different levels of sub-optimal conditions (low light, low nutrients or a combination), which aimed to stress the plants, and then impose a drought until death, to investigate the effects of the treatments on survival.

## Methods

### *Species*

29 species from six families were used: Asteraceae, Fabaceae, Poaceae, Juncaceae, Lamiaceae and Caryophyllaceae (see Appendix 1 for more details).

### *Germination*

Seeds were germinated in petri dishes lined with filter paper. Placement in the petri dishes was staggered to ensure that all the species germinated at the same time. Germination was also staggered so that plants were either 10 or 21 days post-germination when the drought treatment began. This increased the variance in size within species at the point of treatment. Some species were scarified with sandpaper. Post-germination, seedlings were transferred into individual pots within a seed tray (l = 38cm, w = 24cm, d = 4.8cm). The substrate was 50% sand and 50% vermiculite: sand was chosen as a low-nutrient alternative to soil, and supplemented with vermiculite to increase water and air retention.

### *Treatments*

The plants within each species were randomly assigned to a treatment. The treatments were:

- High light (minimum of 200 μmols) and high nutrients (half-strength Rorison's solution, Hewitt (1966), applied weekly while watering)
- High light and low nutrients (no added nutrients)
- Low light (grown under 60% shade netting) and high nutrients
- Low light and low nutrients

The sub-optimal treatments (from the latter three bullet points) were intended to provide a stressful environment, but not to produce plant mortality. After three weeks, half the plants in each treatment were subjected to a drought, where water was completely withheld (creating a total of eight treatment groups). Plant height (the length of the longest leaf for rosette based species) and the total number of leaves were measured per plant every 3-4 days for the first three weeks (i.e. until the drought treatment was added) and once the drought began, mortality was recorded daily. 28 individuals of 29 species were grown across the eight treatment groups, providing a total of 6496 plants (including plants that died during establishment). After the drought started individuals in the shade treatment continued in the shade, however no nutrients were applied to any plants as the nutrient treatment was applied in solution.

Due to the large number of plants and the associated resource limitations, four greenhouse rooms were used and the experiment was repeated across two consecutive time-periods. Every room and experimental half contained all the species across all the environments, as a precaution against room and experimental half effects. Due to variable germination, a few species were not studied in both halves of the experiment.

### ***Destructive harvests***

A further set of plants were grown and destructively harvested to provide a proxy of the biomass of the main set of plants. 2016 plants were grown in both halves of the experiment in the treatment conditions, with plants being harvested throughout the first 3 weeks of the experiment (i.e. until the drought treatment began). As with the main experiment, plants were measured every 3-4 days. After measuring, three individuals per species per treatment were harvested. Their roots were cleaned and the plants were placed in a drying oven at 68°C for 48 hours. The leaf, stem and root biomass were then weighed. There were six harvests, with the final harvest occurring on the first day of the drought treatment.

### ***Statistics***

The destructive harvest data was used to predict the biomass of the treatment plants. The model used incorporated room, planting date, experimental half, height, leaf number, species, shade and nutrient effects and was selected using a stepwise procedure, and the ‘predict’ function in R (R Development Core Team, 2011) was then used to obtain individual plant biomass.

Over recent years, the importance of understanding the pattern of growth over time has been emphasised, with the size-corrected relative growth rate (SGR) recommended when growth is non-linear (Paine *et al.*, 2012, Paul-Victor *et al.*, 2010, Rees *et al.*, 2010, Rose *et al.*, 2009, Taylor *et al.*, 2010). Analysis of the predicted biomasses suggested that individual plant growth could be described by a simple quadratic function, which was fitted using lmer (lme4, Bates *et al*., 2012). The model included an individual-specific random effect, the fitted model was:

lmer(biomass ~ species + species/day + species/I(day^2) -1 + (1|indiv)

where biomass is log10 biomass, day is time from planting and indiv is the individual-specific random effect. As we were interested in comparing the effects of growing under different conditions (shade/nutrients) we fitted the model to each of the four treatment combinations separately.

Separating the effects of different seed and seedling traits is difficult as there are problems with colinearity, for example seedling biomass just before the drought was applied can be written as:

biomass ≈ seed mass * seedling RGR * age

To deal with this problem we performed analyses on seedling biomass and its components separately. The components of seedling biomass analysed were seed mass, initial seedling relative growth rate (the linear coefficients from the lmer model), and age (either 10 or 21 days post-germination before the drought treatment began).

Survival was modelled using a Cox-mixed-effects model (coxme, Therneau, 2012) and the effect of different variables (seedling biomass just before drought, growth rate, seed mass and age) were visualised using rank-hazard plots (Karvanen and Harrell 2009). To produce a rank-hazard plot, we first ranked the covariate then calculated the hazard (chance of dying) relative to the median of the covariate. The relative hazard for covariate $y$ is given as:

$$\exp(\beta * (y - \text{median}(y)))$$

where β is the appropriate coefficient from the coxme model.

Two models were developed to understand how:
a) Initial shade/nutrient treatment and seedling biomass just before the drought started affect survival and
b) Initial shade/nutrient treatment and the components of seedling biomass (growth rate, seed mass and age) affect survival of drought. In this model all three effects were fitted simultaneously.

All models included room and experimental half effects, and a random effect for species nested within family.

## Results

The Cox mixed effects models strongly suggested that the effect of seedling biomass interacted with both the shade and nutrient treatments (Figure 4.1, see Appendix 2). Across all seedlings, the likelihood of mortality varied approximately 20 fold compared with the median seedling mass (Figure 4.1). In both the shade and low nutrient treatments the effect of seedling mass was amplified, particularly in the shade treatment (Figure 4.1a).

In the second model, which included all three components of seedling mass, we found interactions with shade for seed mass, seedling RGR and seedling age, and a nutrient by age interaction (Figure 4.2). There was a highly significant effect of seedling RGR in both the low and high light treatments, with slow growing plants experiencing higher mortality relative to the median. This effect was particularly pronounced in the shade treatment (Figure 4.2a). Seed mass was only marginally significant in the shade treatment and did not effect survival in high light (Figure 4.2b). Older plants experienced reduced mortality rates particularly in the high light and low nutrient treatments (Figure 4.2c & d).

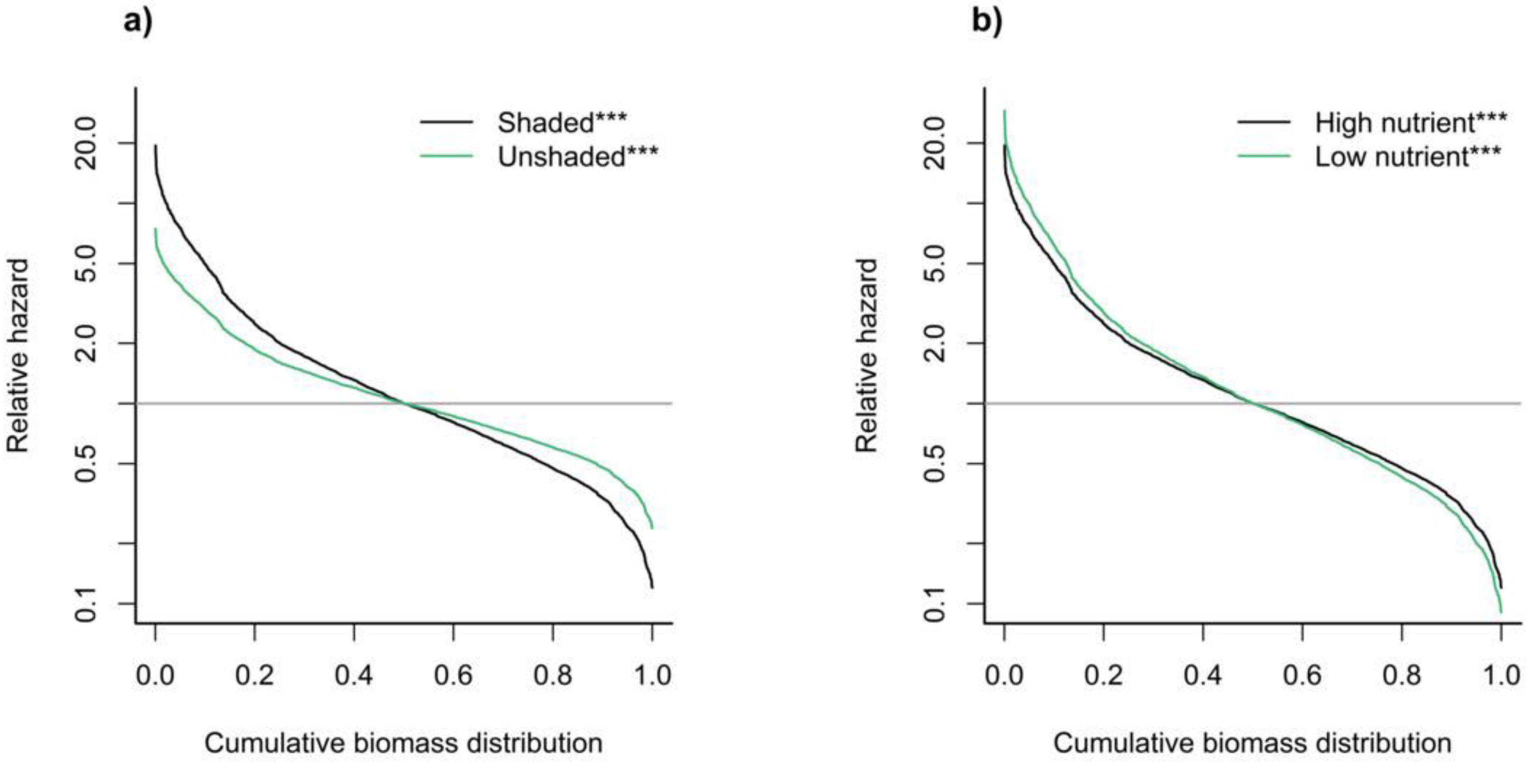

Figure 4.1: The effect of biomass on survival of the drought treatment in a) varying light levels and b) varying nutrient levels. The higher the relative hazard, the higher the likelihood of death. The grey line represents relative hazard = 1, and represents an equal chance of dying at all biomasses. * = significant at $p \leq 0.05$, ** = significant at $p \leq 0.01$ and *** = significant at $p \leq 0.001$.

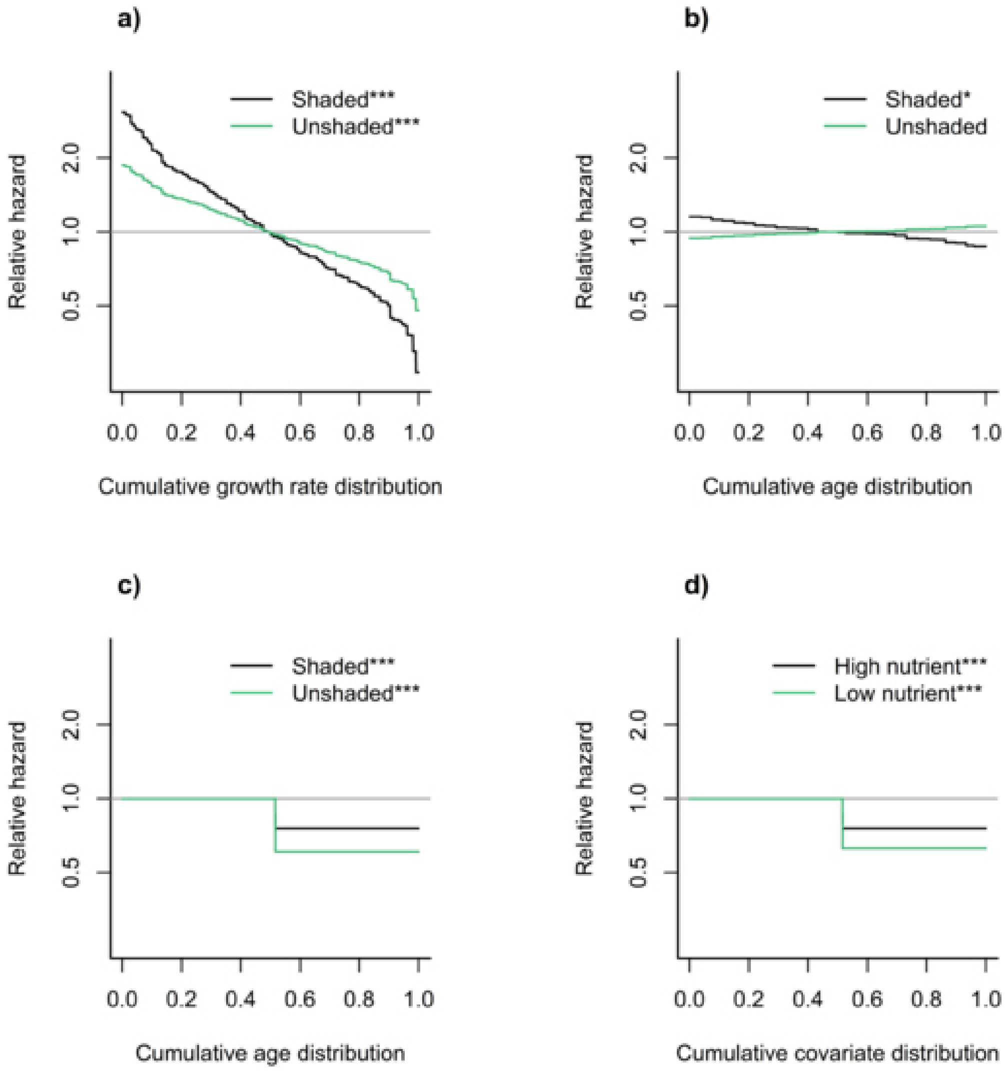

Figure 4.2: The effect of covariates on survival of the drought treatment in a) varying light levels and growth, b) varying light levels and seed mass, c) the 2 age groups and varying light levels and d) the 2 age groups and varying nutrient levels. The higher the relative hazard, the higher the likelihood of death. The grey line represents relative hazard = 1, and represents an equal chance of dying at all biomasses. * = significant at $p \leq 0.05$, ** = significant at $p \leq 0.01$ and *** = significant at $p \leq 0.001$.

## Discussion

Seedling biomass and all its components affected survival across different initial treatments. In the seedling biomass model survival was higher when biomass was larger for all the initial environments (high and low light and nutrient levels). These effects are very substantial with mortality rates being 20 times greater for the smallest seedlings compared with the median. A larger biomass provides a more extensive root system, which is advantageous in drought, as it will enable more water to be taken up. The advantage that is gained from large seedling size is amplified in the shade pre-treatment. This was probably a consequence of larger individuals being able to:

1) Access water from a larger volume due to their larger root systems
2) Reabsorb nutrient/water from a larger mass of tissues and
3) Store more carbon

In the low nutrient treatment the advantage of being a large seedling was also amplified, although the effect was smaller than the shade effect (Figure 4.2b) possibly because the plants stopped receiving nutrients after the beginning of the drought.

All three components of seedling size influenced the likelihood of survival and there were significant interactions with shade (Figure 4.2 a - c). Surprisingly, survival increased as seedlings grew slower, which would not be expected from the growth-survival trade-off (Franco & Silvertown, 1996; Rose *et al.*, 2009). However, there I another layer of complexity, as faster growth results in larger seedlings, which survive better in drought (Figure 4.1). It therefore seems that in this case, the costs of fast growth are outweighed by the increase in size, and so slow-growing seedlings experience greater mortality. Slow growth is particularly bad in the shade treatment, possibly because small seedlings that have been in the low light and drought treatment experience very high mortality rates.

Seed mass, which has previously been the focus of much research, was only weakly associated with seedling survival and only in the shade treatment, with small-seeded species having higher mortality in the shade. The effect size was however rather small, despite seed mass varying by more than 250-fold mortality rates increased by less than

50% relative to the median, compared with a more than 20-fold effect of seedling mass. This contradicts the majority of the literature, which suggests that a large seed mass can significantly improve seedling survival (including the meta-analysis by Moles & Westoby, 2004). The results from Chapter 3 suggest that analysing multiple traits in the same model can account for some of the effects of colinearity and produce different results to models with only 1 trait. However, Chapter 3 found that in drought (with prior optimal growth conditions), low seed mass led to higher survival. These two chapters differ in one major aspect – the initial growth conditions. In Chapter 3, the seedlings were initially grown in a near-optimal environment, which may have put the seedling under little pressure, and growth strategies may have been different, therefore changing the interaction between seed mass and survival.

As expected, being an old seedling increased a seeding's chances of survival (Figure 4.2c & d). Being old (21-day vs 10-days at the start of the drought treatment) reduced mortality rates by a larger amount in the high light and low nutrient treatments. The smaller effect of age in the shade treatment was unexpected, but could be related to the differences in seedling size between the two age classes. In the shade treatment, 21 day old seedlings were 2x larger than 10 day old seedlings, whereas in the unshaded treatment they were 2.7x larger (this difference being highly significant, contrast: $t=174$, $p<0.0001$). Because the effect of age on seedling mass is much larger in the high light treatment, age has a greater effect on seedling survival.

There was an interaction between age and nutrients (Figure 4.2d), with the effect of age (21-day vs 10-day plants) on the chance of dying being greater in the low nutrient treatment. In contrast, the effects of age and nutrient addition on seedling mass were additive. Older seedlings were larger, as expected, as were seedlings in high nutrient treatment but there was no interaction ($F=2.26$, $df=1,5659$, $p>0.1$). As a consequence plants derived a greater benefit from being older in the low nutrient treatment.

The low nutrient environment would have initially been a more stressful environment than the high nutrient environment, however, it may have encouraged the seedlings to adapt in a way that indirectly prepared them for survival of the drought. The balanced-growth hypothesis (see Shipley & Meziane, 2002), which suggests that in low nutrients, the seedling would have allocated most of its resources to the roots. The adaptation designed to increase nutrient absorption also increases water uptake in the drought environment and was therefore expected to increase survival. The effects of the low nutrient treatment were not as extreme as it had potential to be. None of the seedlings looked visibly deprived of nutrients. As the seedlings were still small and were likely to have remaining seed reserves, it is possible that the low nutrient environment was not as stressful as was initially intended. This would have led to less emphasis on root growth.

It should be noted that the shade treatment may provide an additional survival advantage: Due to the low light and therefore low evaporation and transpiration rates, the soil mixture remained moist for longer than it did in the high light treatment. Therefore, the seedlings potentially entered a ‘stressful’ environment slightly later than the seedlings grown in high light.

### Conclusion

This chapter demonstrates that a high biomass and an older seedling can increase the likelihood of surviving a stress event, a relationship which is impacted by the initial growth environment. Seed mass is only important in the shade treatment. This chapter also demonstrated an instance when a high RGR can increase survival. This also demonstrates some of the complexity of understanding a single seedling trait, as they are all inter-linked.

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## Appendix 1

### *Species List*

Table S4.1: List of species used in the experiment, family and average seed mass

| Family | Species | Seed mass (mg) |
|---|---|---|
| Asteraceae | Achillea millefolium | 0.0158 |
| Asteraceae | Arctium minus | 0.6138 |
| Asteraceae | Centaurea nigra | 0.2026 |
| Asteraceae | Centaurea scabiosa | 0.7189 |
| Asteraceae | Cirsium vulgare | 0.1715 |
| Asteraceae | Lactuca serriola | 0.0537 |
| Asteraceae | Sonchus asper | 0.0231 |
| Caryophyllaceae | Cerastium fontanum | 0.0051 |
| Caryophyllaceae | Silene dioica | 0.0700 |
| Caryophyllaceae | Silene latifolia | 0.0476 |
| Caryophyllaceae | Stellaria media | 0.0487 |
| Fabaceae | Medicago lupulina | 0.2052 |
| Fabaceae | Melilotus altissima | 0.3904 |
| Fabaceae | Trifolium dubium | 0.0240 |
| Fabaceae | Trifolium pratense | 0.1814 |
| Fabaceae | Trifolium repens | 0.0641 |
| Juncaceae | Juncus articulatus | 0.0028 |
| Juncaceae | Juncus conglomeratus | 0.0031 |
| Juncaceae | Juncus effusus | 0.0085 |
| Juncaceae | Juncus squarrosus | 0.0097 |
| Lamiaceae | Lamium purpureum | 0.0771 |
| Lamiaceae | Prunella vulgaris | 0.0648 |
| Lamiaceae | Thymus polytrichus | 0.0141 |
| Poaceae | Agrostis stolonifera | 0.0061 |
| Poaceae | Brachypodium pinnatum | 0.3504 |
| Poaceae | Dactylis glomerata | 0.0952 |
| Poaceae | Deschampsia flexuosa | 0.0290 |
| Poaceae | Festuca ovina | 0.0711 |
| Poaceae | Poa trivialis | 0.0210 |

**Appendix 2**

Table S4.2: Model selection process for the Coxme survival model incorporating biomass. Biomass was log transformed (base 10)

| No | Model | AIC | BIC |
|---|---|---|---|
| 1 | ~ (1\|Family) | 76.82 | 70.90 |
| 2 | ~ (1\|Family/Species) | 183.87 | 172.03 |
| 3 | ~ (1\|Species) | 181.19 | 175.27 |
| 4 | ~ Room + (1\|Family/Species) | 323.32 | 293.73 |
| 5 | ~ Room + faExp half +(1\| Family/Species) | 721.13 | 685.62 |
| 6 | ~ Room + Exp.half + Nutrients + (1\| Family/Species) | 732.09 | 690.65 |
| 7 | ~ Room + Exp.half + Nutrients + Shade + (1\| Family/Species) | 770.60 | 723.24 |
| 8 | ~ Room + Exp.half + Nutrients + Shade + Biomass + (1\| Family/Species) | 968.23 | 914 |
| 9 | ~ Room + Exp.half + Nutrients * Shade + Biomass + (1\| Family/Species) | 968.43 | 909.23 |
| 10 | ~ Room + Exp.half + Nutrients + Shade * Biomass + (1\| Family/Species) | 1021.78 | 962.58 |
| **11** | **~ Room + Exp.half + (Nutrients + Shade) * Biomass + (1\| Family/Species)** | **1030.82** | **965.7** |

The (1|Family/species) term specifies the random effects. The model in bold was chosen as the model which best explained the data, based on the AIC. With Coxme models, a high AIC and BIC signifies a better model.

## Appendix 3

Table S4.3: Model selection process for the Coxme survival model with the components of biomass: growth rate, seed mass and seedling age. Growth rate and seed mass were log transformed (base 10)

| No | Model | AIC | BIC |
|---|---|---|---|
| 1 | ~ (1\|Family) | 76.82 | 70.90 |
| 2 | ~ (1\|Family/Species) | 183.87 | 172.03 |
| 3 | ~ (1\|Species) | 181.19 | 175.27 |
| 4 | ~ Room + (1\| Family/Species) | 323.32 | 293.73 |
| 5 | ~ Room + Exp.half +(1\| Family/Species) | 721.13 | 685.62 |
| 6 | ~ Room + Exp.half + Nutrients + (1\| Family/Species) | 732.09 | 690.65 |
| 7 | ~ Room + Exp.half + Nutrients + Shade + (1\| Family/Species) | 770.60 | 723.24 |
| 8 | ~ Room + Exp.half + Nutrients + Shade + factor(Age) + (1\| Family/Species) | 879.69 | 826.41 |
| 9 | ~ Room + Exp.half + Nutrients + Shade + factor(Age) + Growth + (1\| Family/Species) | 923.25 | 864.05 |
| 10 | ~ Room + Exp.half + Nutrients + Shade + factor(Age) + Growth + Seed size + (1\| Family/Species) | 921.32 | 856.21 |
| 11 | ~ Room + Exp.half + Nutrients * Shade + factor(Age) + Growth + (1\| Family/Species) | 921.73 | 856.62 |
| 12 | ~ Room + Exp.half + Nutrients + Shade * factor(Age) + Growth + (1\| Family/Species) | 930.40 | 865.28 |
| 13 | ~ Room + Exp.half + (Nutrients + Shade) * factor(Age) + Growth + (1\| Family/Species) | 934.79 | 863.75 |
| 14 | ~ Room + Exp.half + (Nutrients + Shade) * factor(Age) + Shade * Growth + (1\| Family/Species) | 939.45 | 862.49 |
| 15 | ~ Room + Exp.half + (Nutrients + Shade) * factor(Age) + (Nutrients + Shade) * Growth + (1\| Family/Species) | 938.74 | 855.87 |
| **16** | **~ Room + Exp.half + (Nutrients + Shade) * factor(Age) + Shade * Growth + Shade * Seed size + (1\| Family/Species)** | **973.20** | **884.40** |

| | | | |
|---|---|---|---|
| 17 | ~ Room + Exp.half + (Nutrients + Shade) * factor(Age) + Shade * Growth + (Nutrients + Shade) * Seed size + (1\| Family/Species) | 971.53 | 876.81 |

The (1|Family/species) term specifies the random effects. The model in bold was chosen as the model which best explained the data, based on the AIC. With Coxme models, a high AIC and BIC signifies a better model.

# Chapter 5

# Components of the relative growth rate under multiple environmental conditions

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## Introduction

Growth rates vary both within and between species (Poorter & Remkes, 1990; Li *et al.*, 1998) and plants are capable of regulating their growth in response to both the biotic and abiotic environment (Mcnaughton, 1983; Neumann, 2008). Seedling growth is calculated by the relative growth rate (RGR), which measures the increase in dry mass of an organism per unit of dry mass over time (and assumes that growth is exponential through time, eqn. 1, Turnbull *et al.*, 2008).

$$RGR = (ln W_1 - ln W_0) / (t_1 - t_0) \qquad \text{eqn.1}$$

where $W_0$ is the initial dry plant weight, $W_1$ is the final plant weight and t is the time interval over which growth is measured.

This method is slowly being superseded by a size-corrected RGR calculation (Metcalf *et al.* 2006; Turnbull *et al.* 2008; Rose *et al.* 2009; Paul-Victor *et al.* 2010; Rees *et al.* 2010; Paine *et al.* 2012; Turnbull *et al.* 2012), which does not assume that growth is exponential and calculates growth using non-linear models. RGR can also be studied by dividing it into components.

### *Components of the RGR*

RGR can be partitioned into two components: the net assimilation rate (NAR), which measures the increase in biomass per unit of leaf area over time and can be used as a proxy for photosynthetic rate (as suggested by Poorter & Van Der Werf, 1998); and the leaf area ratio (LAR), which calculates the leaf area per unit of plant mass (eqn. 2).

$$\text{RGR} = \text{NAR} \times \text{LAR} \qquad \text{eqn. 2}$$

LAR can be further partitioned into two more components: the leaf mass ratio (LMR), which is the ratio between the total leaf mass and the total plant mass; and the specific leaf area (SLA), which is the leaf area per unit of leaf (eqn. 3). In summary, NAR represents physiology, SLA represents leaf construction and LMR reflects allocation.

$$\text{RGR} = \text{NAR} \times \text{SLA} \times \text{LMR} \qquad \text{eqn. 3}$$

There has been extensive research on these growth components and how they can affect RGR. A large proportion of this research has focussed on identifying which component is the most important factor in growth (Poorter & Remkes, 1990; Shipley, 2002), with the ‘most important’ component being the one that accounts for the largest amount of variance in RGR. This question of importance has provided conflicting answers to the extent that multiple meta-analyses have been undertaken in an attempt to understand the overall pattern (Cornelissen *et al.*, 1998; Poorter & Van Der Werf, 1998; Veneklaas & Poorter, 1998; Shipley, 2006). However, even these contradict one another, with either SLA or NAR being described as the most important variable in describing changes in RGR.

### *Light Environment*

The results are also contradictory when the effects of environmental conditions are considered alongside the relative contributions of each of the RGR components. One variable that has been frequently studied for its effect on the partitioning of NAR, SLA and LMR is irradiance.

Three meta-analyses have brought together datasets from experiments with multiple light regimes. Poorter & Van der Werf (1998) found that LAR was the most important component in growth (relative to the other components), irrespective of light levels (Table 5.1). On the other hand, Veneklaas & Poorter (1998) and Shipley (2006) concluded that NAR was the most important component in high light, but not in low-light conditions (Table 5.1). Shipley (2006) proposed that the differences between his study and the other meta-analysis could be a result of his more recent meta-analysis consisting of a larger dataset, which also incorporated a larger range of species (herbaceous and woody, as opposed to solely herbaceous species, as studied by Poorter & Van der Werf (1998). This theory is supported by the results of the meta-analysis by Veneklaas & Poorter (1998), who analysed solely woody species, and found that NAR is the most important component in high light, but LAR is more important in low light (Table 5.1), similarly to Shipley (2006).

The greater contribution of NAR to RGR in high light has previously been explained by the diminishing importance of SLA with increasing light levels (Shipley, 2002). This suggestion stems from the theory that it is more important for plants in low-light environments to have a high SLA (i.e. thin leaves with a large surface area) to improve light absorption, which is a limiting factor to growth in low-light conditions.

### ***Water Environment***

There has been less research into the effects of drought on the components of RGR than light, and even less studying their relative contributions. Poorter & Nagel (2000) compiled seven papers to compare the relative effect of drought on NAR, SLA and LMR with that of a well-watered control. They found a decrease in NAR (there denoted as unit leaf rate, ULR) in drought conditions, but the change was not as extreme as that observed across other environmental gradients (irradiance and nutrient concentration).

There is also very little published research into the effect of excess water on RGR and its components, with a particular lack of research on the relative contribution of each component. This could possibly be due to NAR and LAR being studied in areas where flooding is not of importance, or a result of the difficulties of producing a consistent and robust experimental set-up for flooding plants. Simulated flooding as an experimental growth condition may also be seen as a waste of natural resources, particularly in drought-prone regions. Plants experiencing an over-abundance of water generally have lower RGRs, due to lower NARs, with no observed correlation between RGR and LAR (Nash & Graves, 1993; Blanch *et al.*, 1999).

The general pattern that emerges across the environmental conditions is that NAR is the main determinant of RGR in optimal growth conditions, but not in stressful conditions.

***The problem with NAR***

The equation for NAR (eqn. 4) incorporates the absolute growth rate (AGR; eqn. 5), which is therefore closely related to the RGR (see eqn. 1). However, AGR is strongly size-dependent (Rees *et al.*, 2010). Therefore, there is reason to suggest that using NAR to explain RGR may not be as biologically useful as previously assumed. Rees *et al.* (2010) suggest that NAR is strongly influenced by variation in plant size, whereas the other components of RGR are not (i.e. SLA and LMR). They found that as the variance in size increased, NAR accounted for more of the variance in RGR (which also experiences some size-bias, Turnbull *et al.*, 2008).

$$\mathrm{NAR} = (1 / \mathrm{La})\,(W_1 - W_0) / (t_1 - t_0) \qquad \text{eqn. 4}$$

$$\mathrm{AGR} = (W_1 - W_0) / (t_1 - t_0) \qquad \text{eqn. 5}$$

where La = leaf area.

This supports the theory that the amount of variation in RGR that NAR accounts for is related to variation in size within the population. This means that any treatment that changes the variance in plant size within a population will influence the role NAR plays in determining RGR. Studies on the relative components of RGR across different environments typically find that NAR is more important in optimal growth conditions, i.e. high light, nutrients and good water supply. However, the variance in seedling size is likely to vary with environmental conditions, and so the importance of variation in NAR will depend on the environment.

### ***Experiment***

This experiment aims to further explore the theory that the size-dependence of NAR and RGR causes the strong association between NAR and RGR, by investigating the components of RGR across different environmental conditions. This enables us to assess whether or not size-dependence is causing the different relative contributions of the components across environments. Seedlings were grown in low or high water or light treatments. The multiple conditions will produce sub-sets of seedlings with a range of variances in size allowing the theory to be tested across a larger range of growth rates than Rees *et al.* (2010). It was hypothesised that there would be a positive relationship between the variance in seedling size and NAR.

Table 5.1: Meta-analyses on the components of RGR in varying light levels

| Study | Species | Most important in low light | Most important in high light |
|---|---|---|---|
| Pooter & Van der Werf, 1998 | Herbaceous | LAR | LAR |
| Veneklaas & Poorter, 1998 | Woody | LAR | NAR |
| Shipley, 2006 | Herbaceous & woody | SLA | NAR |

## Methods

### *Species*

Seedlings from three families were grown: Poaceae (grasses), Fabaceae (legumes) and Asteraceae (the daisy family, previously Compositae). 30 species, which represent a range of seed masses, were selected from the University of Sheffield seed stores, of which 27 germinated (see Appendix 1 for a list of the species used in the study). The average weight of 50 seeds from each species was calculated to 0.1mg.

### *Growth Environment*

The seedlings were grown in the Arthur Willis Environment Centre at the University of Sheffield, UK. The greenhouse had a temperature of 22°C during the day, 15°C at night and had a minimum daytime light intensity of 200µmol.

### *Treatments*

Seedlings were grown under different light intensities and water regimes. The four treatments were:

- Control, where the seedlings were watered every 1-2 days, as required,
- Waterlogging, which consisted of a high-water treatment, with nowhere for the excess water to drain to (the seed tray had no holes and was constantly filled with water, which kept the soil saturated – preliminary work highlighted that seedlings often become dislodged from the soil and buried when over-watered from above, adding an additional size-dependent factor),
- Drought, in which seedlings were watered once a week and
- Deep shade, which consisted of a 5% light environment, created by two layers of black shade netting.

### *Experiment*

Due to resource limitations, the experiment was conducted across two time periods. The seeds were germinated in petri dishes lined with two layers of filter paper for 1 - 3 weeks (species-dependent), with seed placement in the petri dishes staggered to standardise the germination date. Once the seeds germinated, they were randomly potted in a seed tray (l = 38cm, w = 24cm, d = 4.8cm) with 24 compartments in Levington's M3 compost, and left for two days to adapt to their new environment. Seed trays were then allocated to one of the four treatments.

The first harvest was conducted one week after potting (i.e. five days after the treatments began) and further harvests were carried out every 2 - 4 days, with harvests closer together at the beginning of the experiment. There was a total of six harvest dates (at 5, 7, 9, 12, 16 & 19 days after the treatments began). At each harvest, three individuals per species per treatment (a total of 12 seedlings per species) were removed from the soil. The roots were washed, the leaves were scanned into a computer for the SLA analysis and the seedling was placed in a drying oven at 68°C for two days. The dry biomass of the leaves, shoots and roots of each seedling were recorded to 0.1mg. Mortality was recorded.

### ***Statistics***

The scanned leaves were used to calculate the leaf areas on ImageJ (Rasband, 1997 - 2012). SLA was then calculated using the leaf areas, following the convention of Hunt (1990).

NAR, SLA and LMR were plotted against variance in size. An explanation of the association between variance in size and SLA and LMR was then investigated further, using the mathematical theory presented by Rees et al (2010).

A size-standardised growth rate (SGR) calculation was planned, using a non-linear model of the growth curves (Paine *et al.*, 2012), to account for the decrease in growth over time. However, the seedlings were still in their exponential growth phase at the end of the experiment, so the slope of a linear regression was used to calculate RGR instead (which utilises a similar mathematical basis to eqn.1, however it enables the data from all the harvests to be used).

The biomass data for 12 days after the treatments began was lower than expected, probably due to the use of an inaccurate balance. Therefore, the data for that harvest was removed from the analysis.

## Results

The soil temperature in the shade treatment and the control were recorded at multiple times on different days, to assess additional changes to the growth environment caused by the shade netting. The soil in the shade treatment was on average 0.8°C warmer than the temperature of the control seedlings. Therefore, the shade treatment created additional environmental effects, but the disadvantage of the shade treatment prevented the higher temperature becoming a growth advantage for those seedlings. The seedlings in the shade treatment also required less watering, as the shade inhibited evaporation. These seedlings were monitored and watered when necessary, to maintain the soil at approximately the same moisture level as the other treatments (note: moisture levels were visually approximated).

The treatments generated a wide range of plant sizes (Figure 5.1), with the largest plants occurring in the control treatment, the smallest in the shade, and waterlogged and drought plants being intermediate. The variances in plant mass (log transformed) were similar in the control and waterlogged treatments (0.374, 0.406 respectively), plants were least variable in size in the shade treatment ($\sigma^2$= 0.189) and slightly more variable in the drought ($\sigma^2$ = 0.273).

As the variance in size increases, NAR also increases (Figure 5.2), particularly within the control and waterlogging treatments. In the drought treatment, there is a lot of scatter. The graph begins fairly flat and NAR increases as the variance in size gets above 0.22 (seen in Figure 5.2 as above log10(-1.5)). The initial flat section of the graph is caused by the plants in the shade treatment. The positive correlation is also present when the families are compared separately (Figure 5.3).

The importance of NAR is consistent across all non-shade environments (accounting for 30% of the variance in RGR). In the shade treatment the importance of NAR drops to ≈15%, while the importance of SLA increases from ≈50% in non-shade to ≈75% in shade (Table 5.2). This supports the idea that a high leaf surface area to leaf mass ratio is important in low light levels.

Table 5.2: Average importance of the components of growth across the different environments

| | Control | Waterlogged | Drought | Shade |
|---|---|---|---|---|
| NAR | 0.308 | 0.302 | 0.312 | 0.148 |
| SLA | 0.547 | 0.436 | 0.509 | 0.759 |
| LMR | 0.145 | 0.262 | 0.179 | 0.093 |

There is no correlation between SLA or LMR and variance in size (Figures 5.4 & 5.5). This was investigated further to understand whether these components could also help to explain the contribution of NAR to the variance in size. The variation in SLA caused by variation in plant size was described by Rees *et al* (2010) as:

$$\mathrm{Var(SLA)} = (\beta_{1,a\mathrm{L}} - \beta_{1,m\mathrm{L}})^2\ \mathrm{Var}(m_\mathrm{p})$$

Where $m_\mathrm{p}$ is the total plant biomass (log scale), $a_\mathrm{L}$ is the leaf area, $m_\mathrm{L}$ is the leaf mass and $\beta_1$ is the fitted regression slope from the regressions ($a_\mathrm{L}$ – versus $\mathrm{m_p}$ and $m_\mathrm{L}$-versus=$m_\mathrm{p}$ – all variables log transformed).

Therefore variation in plant size will have little effect on variation in SLA if leaf area and leaf mass scale similarly with plant mass. Across the entire data set leaf mass is approximately isometric with plant mass ($\beta_{1,\mathrm{mL}}$= 0.997 ±0.005) whereas for leaf mass $\beta_{1,a\mathrm{L}}$= 0.895±0.010 (see also Figure 5.6). The difference between these two terms squared is ≈0.01, and so variance in plant size will have little effect on the variance in SLA.

LMR was studied in a similar manner. Rees et al. (2010) produced the following formula to describe the effects of size variation on LMR:

$$\mathrm{Var(LMR)} = (1 - \beta_{1,m\mathrm{L}})^2\ \mathrm{Var}(m_\mathrm{p}).$$

Again across the entire dataset $\beta_{1,\mathrm{mL}} = 0.996 \pm 0.005$ and so $(1 - \beta_{1,m\mathrm{L}})^2 \approx 0.000001$ making the variation in LMR almost independent of variation in plant mass.

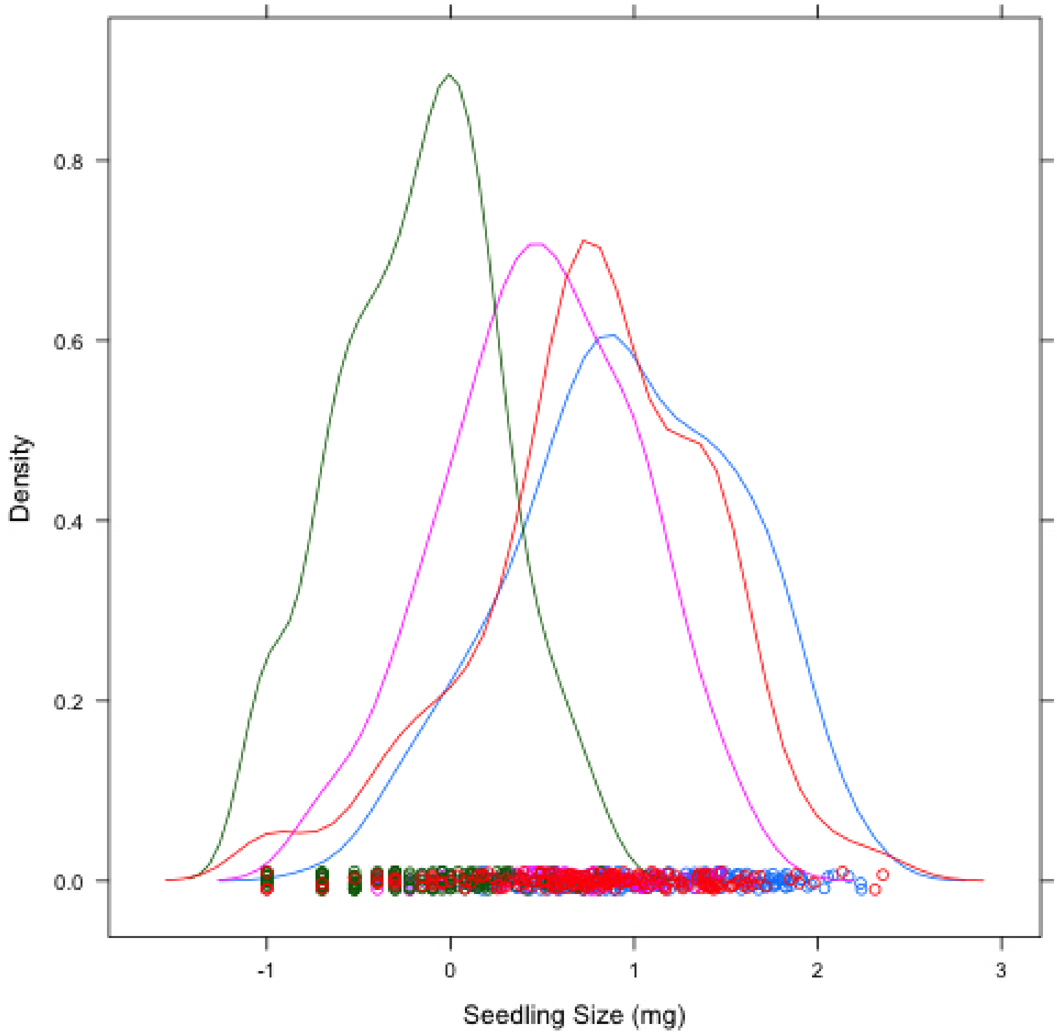

Figure 5.1: Density plot of seedling size cross the treatments. The colours represent the treatments; control = blue; drought = pink; shade = dark green and waterlogged = red. All variables are on the log10 scale.

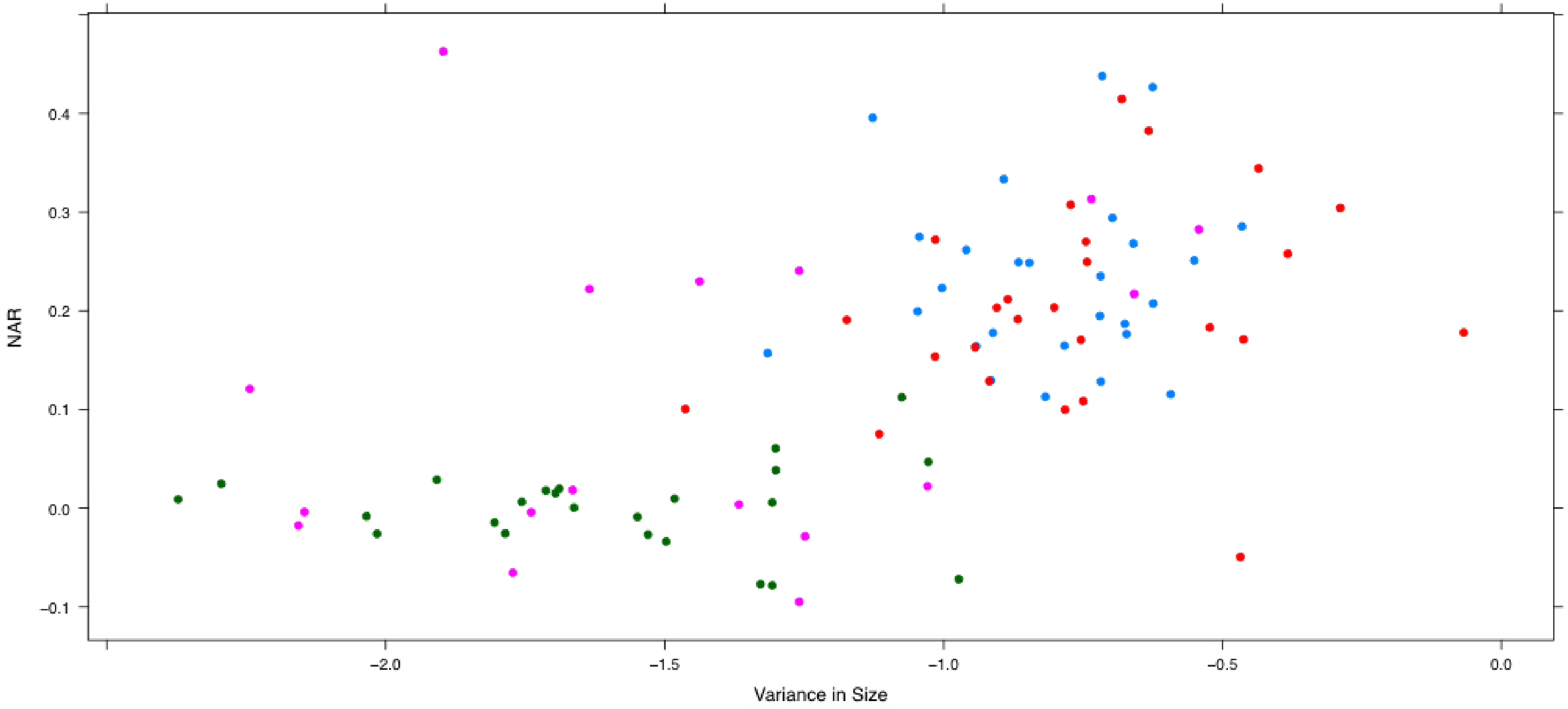

Figure 5.2: Changes in NAR with variance in size (Spearman's rank correlation: t = 6.826, df = 92, p <0.001, cor = 0.580). The colours represent the treatments; control = blue; drought = pink; shade = dark green and waterlogged = red. All variables are on the log10 scale.

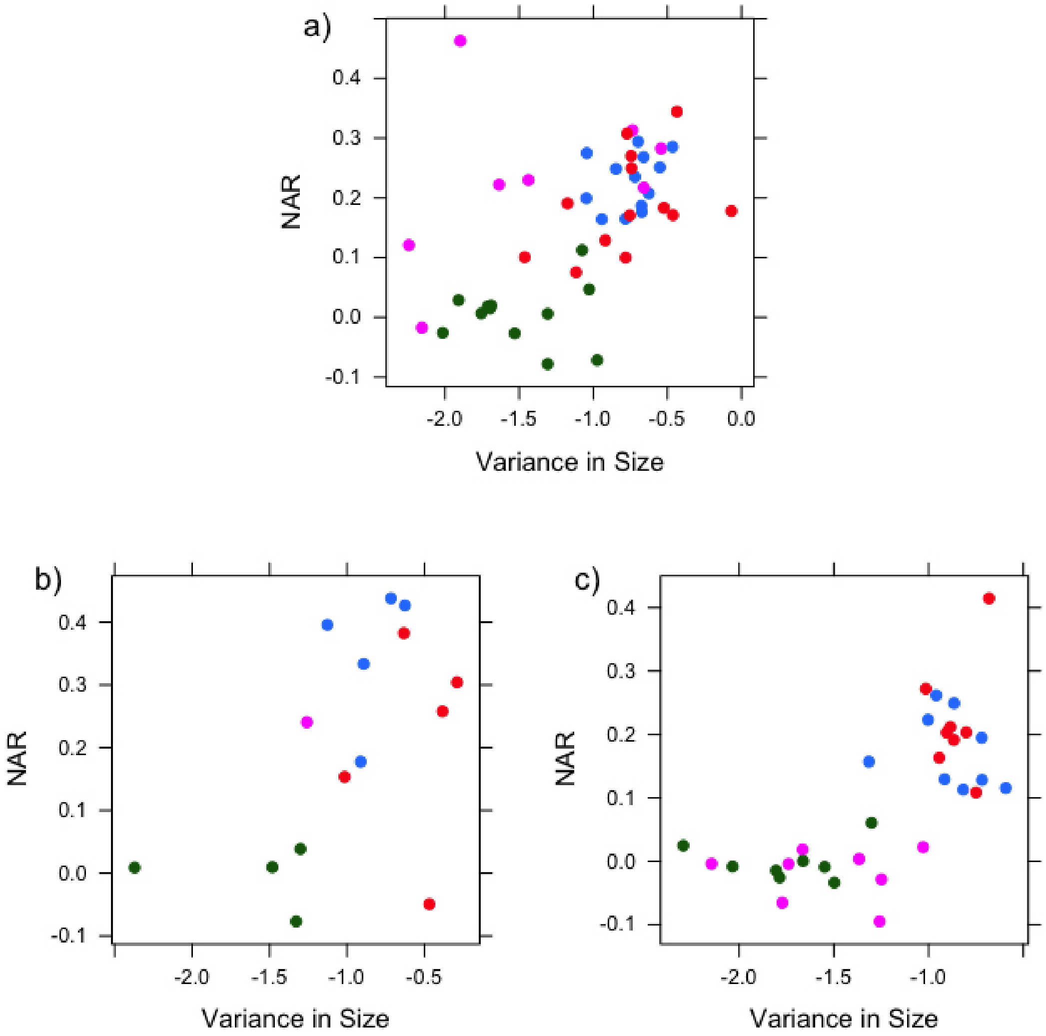

Figure 5.3: Changes in NAR with variance in size per family. a) Asteraceae (Spearman's rank correlation: t = 3.882, df = 44, p <0.001, cor = 0.505), b) Fabaceae (Spearman's rank correlation: t = 2.078, df = 13, p = 0.058, cor = 0.499), c) Poaceae (Spearman's rank correlation: t = 5.594, df = 31, p <0.001, cor = 0.709). The colours represent the treatments; control = blue; drought = pink; shade = dark green and waterlogged = red. All variables are on the log10 scale.

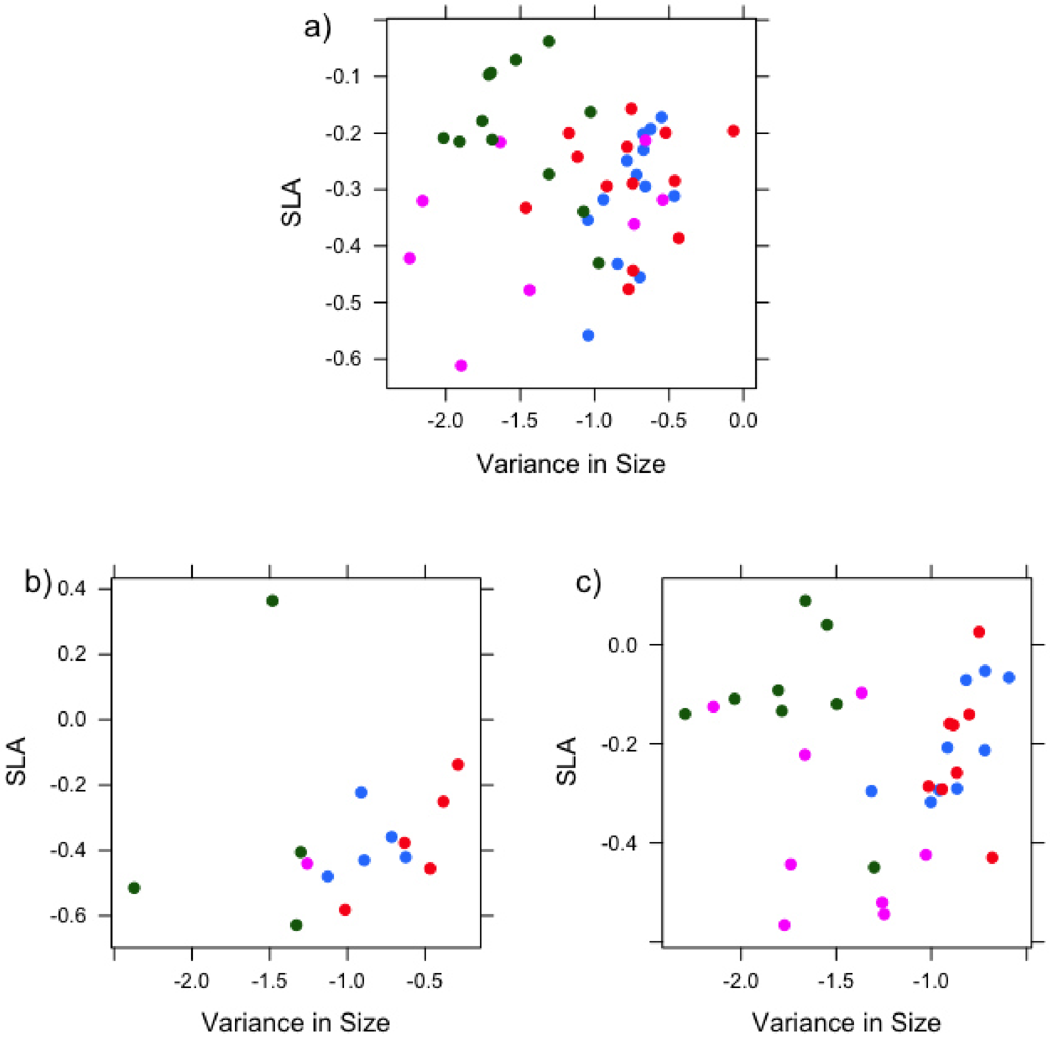

Figure 5.4: Changes in SLA with variance in size per family. a) Asteraceae (Spearman's rank correlation: t = -0.628, df = 44, p = 0.533, cor = 0.094), b) Fabaceae (Spearman's rank correlation: t = -0.395, df = 13, p = 0.699, cor = -0.109), c) Poaceae (Spearman's rank correlation: t = -0.48, df = 31, p = 0.635, cor = -0.086). The colours represent the treatments; control = blue; drought = pink; shade = dark green and waterlogged = red. All variables are on the log10 scale.

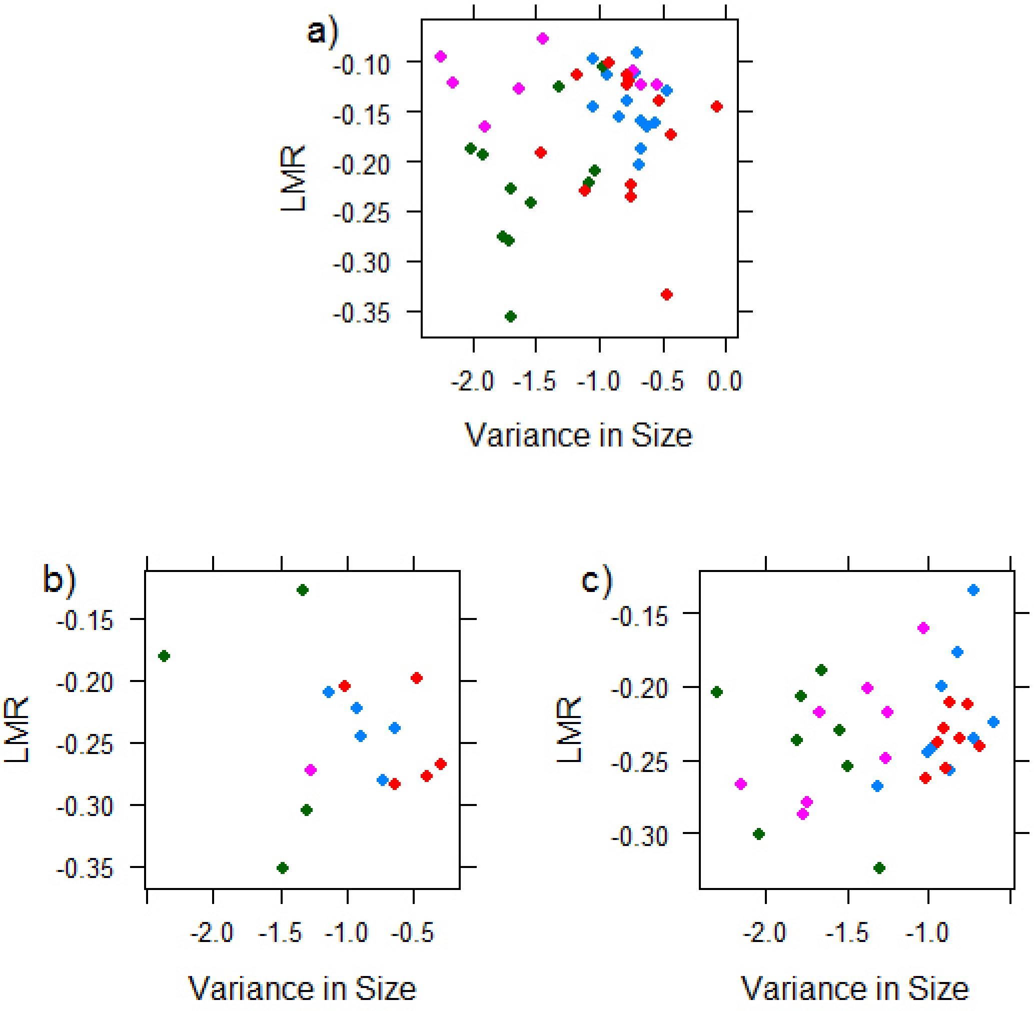

Figure 4.5: Changes in LMR with variance in size per family. a) Asteraceae (Spearman's rank correlation: t = 1.0846, df = 44, p = 0.284, cor = 0.161), b) Fabaceae (Spearman's rank correlation: t = -0.929, df = 13, p = 0.370, cor = -0.250), c) Poaceae (Spearman's rank correlation: t = 1.628, df = 31, p = 0.114, cor = 0.281). The colours represent the treatments; control = blue; drought = pink; shade = dark green and waterlogged = red. All variables are on the log10 scale.

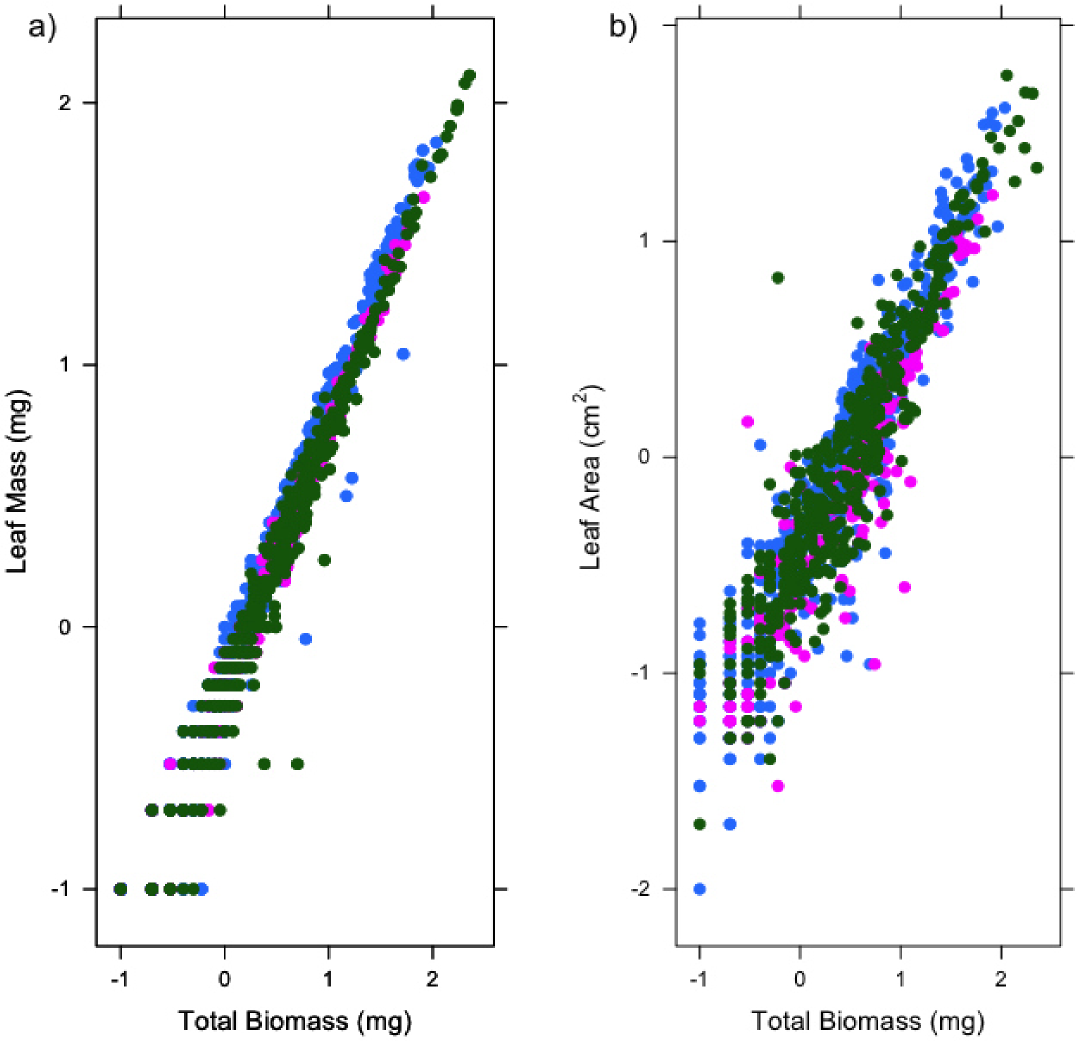

Figure 5.6: The relationship between total biomass and a) leaf mass (F = 9152.2, df = 53, 1131, $p < 0.001$, $R^2 = 0.98$) and b) leaf area (F = 7946, df = 1, 1183, $p < 0.001$, $R^2 = 0.87$). The colours represent the plant families; Asteraceae = blue; Fabaceae = pink and Poaceae = dark green. All variables are on the log10 scale.

## Discussion

### *Growth Components*

This paper set out primarily to investigate NAR, which is sometimes thought to be the most important factor in determining RGR. The positive relationship between NAR and the variance in size (Figure 5.2 & 5.3) supports the hypothesis that NAR will explain a high proportion of the variance in RGR when the variance in size is high. As the calculation for NAR incorporates the AGR (absolute growth rate, which only differs from RGR because it is not on the log scale), which is strongly size-dependent, NAR will be closely associated to RGR whenever there is substantial variation in plant size. Using NAR across seedlings of different sizes, particularly in situations where NAR and RGR are compared, may bias results. This situation is particularly likely to occur when the seedlings have been grown in different treatments; for example, in this experiment the shade treatment strongly reduced variance in seedling size.

The lack of a correlation between variance in size and both SLA and LMR was as expected. Both SLA (leaf area) and LMR (leaf mass) are largely consistent with seedling size, and so the variation in SLA is largely unaffected by variation in plant size (Figure 5.6). SLA and LMR were fairly consistent across the treatments, except in shade, where SLA was very important (see the section on ‘Treatments’, below).

The seedlings were still in their exponential growth phase, indicating that using the AGR within the NAR formula created size-dependency, however, RGR was not size-dependent in this case.

### *Treatments*

Overall, the treatments increased the amount of variance in size. As expected, the seedlings from the shade treatment exhibited the least variance in size. This may be a result of all the species using the same survival strategy of inhibiting growth whilst waiting for an increase in light levels. As such, the seedlings remained small and did not grow more leaves after being placed in deep shade. As highlighted above, the low variance in size will automatically produce a low NAR, and so boost the importance of SLA (Table 5.2). The alternative explanation is that plants growing in the shade are light-limited and therefore need to increase their light capture – by increasing their leaf area relative to their mass. SLA is therefore expected to be an important growth factor in the shade. These results concur with those of the previous meta-analyses, which agree that SLA/ LAR is the most important component contributing to the RGR in low light (Table 5.1).

There was a greater range of seedling growth responses to the drought treatment than the shade treatment, so the seedlings had slightly higher variation in size (Figure 5.1). Some species grew little, dried out and died very quickly (for example, *Inula conyza*, *Medicago lupulina*, *Poa trivialis*), and some briefly survived at a small size before dying (for example, *Lactuca serriola*, *Melilotus altissima* and *Hordeum murinum*). One *Lactuca serriola* seedling grew relatively very large, reaching a total dry biomass that was 31% bigger than the second largest individual in the drought treatment (note the wide range in seedling sizes for the waterlogged treatment in Figure 5.1). Although watered once a week, the drought treatment had the highest levels of mortality (only 40% of the seedlings survived between day 9 and 19 of the treatment). NAR was similar in the control, waterlogged and drought treatments, which contrasts with previous research, which showed that NAR decreases with drought (Blanch *et al.*, 1999; Poorter & Nagel, 2000).

Seedlings in the waterlogged treatment displayed the largest variance in size between treatments, with the control coming in second place (although the control seedlings had the largest average total biomass). The control seedlings probably grew to their optimum size in the time period permitted, and any observed variation could probably be attributed to inherent variation in the maximum RGR. However, in the waterlogging treatment, some seedlings were fast-growing (with a few *Hordeum murinum* individuals actually growing larger than in the control), whereas other seedlings struggled with the water stressor and grew slowly or died (e.g. *Trifolium dubium*, *Tanacetum vulgare*). The range of responses resulted in large variance across the treatment. Most of the variance in size between treatments and across the treatment and family combination was attributed to SLA. Previous studies have shown that NAR is reduced in flooding, compared to a control (Blanch *et al.*, 1999) or to a control and a drought treatment (Nash & Graves, 1993). This was not the case here, but it should be noted that there is a significant difference between a waterlogging and a flooding treatment. In a flooding, rather than a waterlogging treatment, where there is standing water on top of the soil, little growth would be expected and NAR may be less important, due to the high stress environment.

The findings of this study suggest that the inherent size-dependence in NAR could be causing or exaggerating the association between the quality of the environmental conditions and the proportion of growth attributed to NAR. More research needs to be conducted to understand whether this is a general trend. Repeating this study using seedlings that have finished their exponential growth phase may also impact the results. The theory presented here may have the potential to affect the perception of theories on biomass allocation, particularly across environmental gradients, so it is important to understand this. If NAR is size-dependent, further research could investigate the extent of the impact on ecological research, which makes assumptions about the relative contribution of the growth components.

Rees *et al.* (2010) highlighted a potential issue when NAR is used in association with RGR, which may be circumvented through comparing organisms at a common size. It does require more measurements and harvests though. This study had plants with log-linear growth and therefore cannot conclude whether or not the extra time, effort, space and study organisms required to calculate growth at a common size is necessary. Other areas of Ecology have found it important to compare results at a constant size to reduce size-dependence (Turnbull *et al.* 2008; Rose *et al.* 2009; Paul-Victor *et al.* 2010; Turnbull *et al.* 2012).

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## Appendix 1

List of species used in the experiment

Table S5.1: Species used in the experiment and their associated families

| Family | Species |
|---|---|
| Asteraceae | Achillea millefolium |
| Asteraceae | Centaurea nigra |
| Asteraceae | Centaurea scabiosa |
| Asteraceae | Cirsium vulgare |
| Asteraceae | Conyza canadensis |
| Asteraceae | Inula conyza |
| Asteraceae | Lactuca serriola |
| Asteraceae | Lapsana communis |
| Asteraceae | Leontodon hispidus |
| Asteraceae | Senecio jacobaea |
| Asteraceae | Sonchus asper |
| Asteraceae | Tanacetum vulgare |
| Asteraceae | Taraxacum officinale agg. |
| Fabaceae | Lathyrus pratensis |
| Fabaceae | Lotus corniculatus |
| Fabaceae | Medicago lupulina |
| Fabaceae | Melilotus altissima |
| Fabaceae | Trifolium pratense |
| Fabaceae | Trifolium repens |
| Fabaceae | Trifolium dubium |
| Poaceae | Anisantha sterilis |
| Poaceae | Brachypodium pinnatum |
| Poaceae | Dactylis glomerata |
| Poaceae | Festuca gigantea |
| Poaceae | Festuca ovina |
| Poaceae | Hordeum murinum |
| Poaceae | Poa annua |
| Poaceae | Poa trivialis |
| Poaceae | Agrostis stolonifera |

# Chapter 6

# Discussion

***Growth Rates***

This thesis principally focussed on growth rates, and found that:

- Chapter 2: Seed mass does not cause the variance in RGR between growth forms
- Chapter 3: SGR is a predictor of seedling survival, and needs to be modelled alongside other variables, such as biomass and seed mass
- Chapter 4: Both seedling traits and the initial growth environment affect survival
- Chapter 5: As NAR varies with size, comparing the components of the RGR between experiments may create bias

One recurring theme is that growth affects seedling survival (discussed in more detail in the “Variance in Growth” and “Environment” sections). The dependence of growth on other explanatory variables is also emphasized (Chapter 3 – 5), although variance in growth between growth forms is not dependent on seed mass. It is only once growth has been size-corrected that the co-dependent variables can be accurately modeled together.

Growth rates are of particular interest, due to changes in attitudes towards the methodology, which is becoming more prevalent throughout ecology. In this thesis, three data chapters required data collection, of which one group of plants were growing exponentially (Chapter 5), one was non-linear (Chapter 3), and one was mostly non-linear (Chapter 4). Therefore, there is a need to view growth curves, to allow the most relevant method to be applied (linear or non-linear model) in each situation and growth rates often need to be size-corrected across ecology. We cannot assume that growth always fits a certain pattern, but as with other topics requiring statistics, the shape of the data needs to be viewed, in order to choose the best method of calculating growth.

SGR is a valuable method of calculating plant growth. It can calculate growth independently of initial size (Turnbull *et al.*, 2008), which provides a more accurate recording of growth. However, more importantly, the differences in outcomes between using RGR and SGR can be significant (Turnbull *et al.* 2008; Rose *et al.* 2009; Rees *et al.* 2010; Turnbull *et al.* 2012), so the level of precision with which growth is calculated can impact the experimental results. As RGR is currently used across many areas of ecology, the question of how much SGR can change the ideas and theories in ecology potentially has a large-ranging response. Researchers currently using RGR in their fields of interest should take multiple harvests in future experiments and then apply the most relevant method of calculating growth, rather than assuming growth to be log-linear. In areas where SGR has not previously been studied, an approach similar to that of Turnbull *et al.* (2008; 2012) is recommended, where the both RGR and SGR are calculated, and any effect of the growth method can be recorded.

SGR has previously only been used on studies of seedlings. This is because the seedling stage is the part of the life cycle where SGR is most beneficial, as growth is variable, decreasing over time, whereas established plants have a more constant growth rate. It is therefore possible that SGR may not impact mature plants to the same extent as seedlings. This would mean that the extensive work that has labeled many species with an average RGR, such as Grime *et al.* (1988), the CSR theory (Grime, 1977) and other well established theories based on RGR may still be relevant and accurate under SGR.

We still need to investigate how far the effects of size-correction can cascade through the levels of organization (can it affect our understanding of plant populations and communities?).

### ***Variance in growth***

Growth is not static over time, within or between species, and changes in growth and differences between growth patterns can help us to understand plant physiology. This thesis found that variance in RGR between growth forms is not caused by seed mass, although no alternative explanations were found. As explained in Chapter 2, there could be associations with Tilman's theory (1988) and Grime's CSR theory (1977).

Variance in growth between species could be related to survival strategies. Slow-growing species survive longer in stressful environments than fast-growing species (whose fast-growth is an advantage in ambient conditions). This growth difference with survival was not always explicit when survival was simply modelled against growth, signifying again that growth is not a simple function, but is closely associated with other seed and seedling traits. Low growth, combined with a small seed mass and a large final size were beneficial for surviving stressful environments. However, small seeded species that grow slowly will produce relatively small seedlings. This suggests that there may be a trade-off to produce the most effective seedling for survival, and that other factors may be involved, particularly in the inter-linked trade-off between survival of stressful environments and increasing reproductive output in ambient environments. The different seed and seedling traits vary in importance with the stressor as well (see below).

### ***Environment***

The environment affects plant strategy. A large biomass is more important for surviving drought than herbivory. Fast-growing species are good competitors and fast-growing individuals can often reach the reproductive stage sooner than slow-growing ones. However, lowered survival of stressful conditions can be a cost of this fast-growth. Chapter 4 unexpectedly found that there are occasions when fast-growth can be more beneficial for survival, which is presumed to be due to an even greater benefit of large biomass in that situation.

Rees *et al.* (2010) found that the net assimilation rate (NAR) varies with plant size, which may impact the relative importance of the growth components across different environments. The results of Chapter 5 supported this, even though there was a much larger range of seedling sizes and RGR was used rather than SGR. Understanding the relative importance of the components of growth across different environments is useful for understanding plant physiology and seedling plasticity, as seedlings adapt to different environments. This would help us to understand the costs of different strategies, as higher plasticity reduces the costs of being in an environment that is not optimal for the preferred strategy.

We understand more about seedling physiology now, particularly growth. This is useful for understanding survival (and therefore is of use to conservation research or studies of environmental change, such as climate change) and can potentially enable us to predict future changes.

### ***Methods***

Researchers frequently only investigate the variables of direct interest to them, such as the effect of seed mass or growth rate on survival. This is understandable from an efficiency perspective – calculating the growth of a large number of plants is a seemingly long and pointless task when simply interested in seed mass. However, seed mass, growth and other variables are co-dependent, therefore, studying them independently will falsely bias any results. Chapter 3 found that studying the variables singularly sometimes produced different results to those where co-dependent variables were modelled together, most notably with a higher occurrence of non-significant results when variables were studied in isolation. To understand the true relationships between variables, all the co-dependent variables (even those which the investigator has no direct interest in) should be modelled together. The results may have more variation and therefore be less clear, but they will provide a more accurate representation of the true relationship between variables.

Comparative and meta-analyses are becoming more popular, with an increased focus on general trends. However, small experimental differences can have large impacts on results, particularly for a trait such as growth, which varies with subtle environmental changes. Standardising some aspects of greenhouse and growth chamber experimental set-ups may, in the long-term, lead to more useful combined analyses, with less noise in the data. A less radical alternative would require all authors to provide extensive details about the experimental set-up. This would provide the current freedom for researchers to produce an experiment that best suits their environment, plants and research aims. Additionally, the extra data would also provide a means of accounting for differences in experimental designs in future comparative and meta-analyses, rendering their results and implications more focussed, accurate and reliable.

### ***Greenhouse versus field experiments***

Whilst greenhouse experiments are thought to be more representative of nature than experiments conducted in growth chambers, the level of similarity has not been accurately quantified. The majority of experiments that develop our understanding of seedling growth have been conducted in growth chambers or greenhouses. Growth can vary considerably with the environment, so greenhouse experiments, where the number of variables can be limited, are appealing for studies investigating certain aspects of growth. It would be beneficial to replicate a growth experiment in growth chambers, greenhouses and in the field (see, for example, Paz & Martinez-Ramos, 2003), to compare the effects of location on growth and to understand the extent of the similarity between experiments in artificial environments and responses in the field.

### ***Future directions***

As previously mentioned, most studies of RGR and SGR have been conducted in controlled environments. One useful direction to take this work is to investigate growth physiology in the field. SGR would be more difficult (but not impossible) to calculate in the field, due to the requirements for frequent harvests.

Chapter 2 demonstrated that seed mass does not cause the difference in growth between growth forms. Further investigation into the cause of this difference is therefore required to understand the mechanisms of the fundamental differences between growth forms.

The effects of seed and seedling traits on seedling survival have been investigated, but the larger-scale implications of this are currently unknown. The long-term effects of seedling survival could be investigated, particularly in terms of species assembly within a community in response to the introduction of a new long-term stressor. Using this information, the effect of traits on survival across environments could be used to predict the presence and stability of plant populations in conjunction with other factors.

There is still more to learn about the extent of the impact SGR may have on ecology, as mentioned in the section titled "SGR". Further investigations into how SGR could impact other areas of ecology (e.g. studies of traits of invasive species/ asymmetric competition/ conservation) would be beneficial.

## Conclusion

The research produced for this thesis demonstrates that seed mass does not impact the differences in growth rates between growth forms; seedling survival of stress is based on a complex interaction of growth rate, biomass, seed mass, seedling age and probably additional factors not studied here, and hints that the high importance of NAR to describe variance in growth in good conditions may be due to size-dependence within the growth and NAR formulae. Growth varies considerably between species and environments and is therefore an interesting trait to study. However, it should be studied in relation to other co-dependent variables, such as seed mass and biomass. As with other areas of pure ecology, it is hoped that the knowledge found here will be of benefit to understanding wider areas of ecology through to the larger applied scale.

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