Author:
Du Plessis, Sarah J

Title:
Plastic Responses of Root System Architecture to Drought and Temperature Stress

Plastic Responses of Root System Architecture to Drought and Temperature Stress

Sarah du Plessis

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Masters by Research in the Faculty of Life Sciences, School of Biological Sciences.

March 2020

Word count: 13 227

Abstract

A knowledge of how genotypes, populations and species respond to environmental variation is important for understanding the likely impacts of future climate change. Plasticity is the environment-specific response of a genotype to environmental variation within a generation and can be adaptive or maladaptive. Plants are ideal organisms to study plasticity, for example using field transplants, however root systems are difficult to study in the field. Therefore, intensive laboratory experiments on root systems compliment large field studies of plasticity.

This study used a species of ragwort, *Senecio chrysanthemifolius,* to assess the plasticity and specific root trait responses of genotypes to drought and temperature stress. Field experiments identified two types of responses to changing environments, showing higher and lower relative fitness or flower number, outside the species' home range. Twenty-four genotypes (12 of each type) were identified and used in the current study. To test the plasticity of root systems, I used a fully-crossed experimental design of low and high drought and temperature treatments. I used multiple cuttings of each genotype, grown in vertical agar plates, allowing assessments of root architecture for the same genotype in multiple environments.

The 12 genotypes identified as showing increased relative fitness outside the range in the field, possessed smaller shoots and larger root:shoot ratio, relative to the other genotypes. This suggests that genotypes with larger root:shoot ratios may show an increase in relative fitness outside their home range. Increased temperature resulted in increased root diameters, and deep, narrow root systems, whereas high drought stress resulted in overall smaller root systems. Plasticity was observed in root traits across treatments, and genotype-by-environment response among all 24 genotypes was not detected. However, a lack of GxE interactions in the plastic response of root traits suggests strong stabilising selection for the observed response, and therefore observed differences in fitness in the field are unlikely to be due to plasticity in root traits.

Dedication and Acknowledgements

I would like to thank my supervisor Jon Bridle, for the opportunity to be a part of the larger project in Sicily, and his support throughout this Masters project. I would also like to thank Greg Walter for his help getting to grips with the project in Sicily, and for his patience and support through the analysis and writing-up process. I would also like to thank everyone who worked on the field experiment, from which the seeds and cuttings I used in Bristol came from, especially Delia Terranova. Also, for making my time in Sicily so enjoyable.

In Bristol, I am incredibly grateful to Beth Eldridge and Lucia Primavesi for their suggestions and patience throughout the pilots and experimental design phases. Tom Pitman and the GroDome team were also incredibly helpful throughout the process, not only with advice, but the growing, care and maintenance of the genotypes from Sicily. Also, to Gemma Glasscock for her help running the experiment, and moral support throughout.

Finally, I am incredible grateful to my family and friends in Bristol for their unrelenting support and encouragement from day one.

Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: Sarah du Plessis DATE: 24/03/2020

Contents

List of Figures

List of Tables

Thesis Outline

Chapter 1 provides a broad, general review of current literature relevant to this thesis, covering background theory and knowledge gaps in this field.

Chapter 2 is a data chapter, assessing the impact of increased temperature and drought stress on *S. chrysanthemifolius* root system architecture.

Chapter 3 provides a general discussion of these findings, in the broader context provided in Chapter 1.

1 Chapter 1: General Introduction

Understanding how genotypes, populations and species respond in the short- and long-term to changes in the environment is important when considering the effects of ongoing and future climate change.

Global average temperatures have increased by 0.6 °C in the past 100 years (Walther *et al.*, 2002) and are predicted to increase by a further 1.5 °C in the next 100 years, unevenly across land, ocean, Arctic and Antarctic regions (Collins *et al.*, 2013). This will lead to an increase in precipitation by 1 to 3 % per °C of warming (also unevenly based on latitude), and changes to atmospheric circulation, the water cycle, a reduction in the cryosphere and an increase in ocean temperatures (Collins *et al.*, 2013). Although many species have experienced substantial changes in climate over evolutionary history, current warming is occurring more rapidly, and therefore there is uncertainty in whether species can respond and adapt quickly enough (Root *et al.*, 2003).

The impacts and responses of organisms to current climate change can be grouped into four main categories, describing the different levels of responses from individuals, through species, communities and ecosystems (Walther *et al.*, 2002). Phenological and physiological responses are most commonly recorded (Walther *et al.*, 2002). This was demonstrated by a meta-analysis by Parmesan and Yohe (2003), which identified an average advancement of spring events by 2.3 days per decade, based on 677 species from plant, bird, insect, amphibian and fish taxa. The same meta-analysis also identified changes in species' range and distributions as an average range shift of 6.1 km per decade towards the poles (Parmesan and Yohe, 2003). Another effect of climate change includes changes to community composition and interactions among species, with disturbances to communities amplified by climatic disturbances via the downstream effects on species interactions across trophic levels (Walther *et al.*, 2002). For example, Brown *et al.* (1997) observed a substantial shift in species composition of the arid Chihuahuan Desert ecosystem following an increase in winter precipitation (Brown *et al.*, 1997). Recent changes to global climate have already impacted a range of taxa and ecosystems, and therefore the predicted, future changes will require rapid, adaptive responses to enable species persistence.

1.1 Plasticity

Phenotypic plasticity is the ability of a single genotype to express different phenotypes in different environments (West-Eberhard, 1989, DeWitt *et al.*, 1998). When studying the impact of a change in environment on single individuals, plasticity is the first response as it occurs within a single generation (Snell-Rood *et al.*, 2018). For clones of a genotype grown across an environmental

gradient (e.g. temperature), the change in phenotype reflects the plastic response to the environmental variation, and is termed the 'reaction norm', where a flat reaction norm would show no plasticity, and steeper reaction norms reflects stronger plastic responses to the environmental gradient (Chevin *et al.*, 2013). Although plasticity involves no change in genotype, there is still an underlying genetic difference between genotypes which are more or less plastic, and therefore plasticity itself has the potential to evolve (Ashander *et al.*, 2016).

Depending on the specific phenotypic trait, the accuracy of the environmental change as a cue, and the combination of these which maximise fitness, phenotypic plasticity may be strong or weak, adaptive or maladaptive and maintain population fitness in response to environmental variation. For example, high levels of plasticity in the timing of reproduction as a response to average spring temperature was shown to be adaptive in a Dutch population of great tits (*Parus major*), as this acts as an environmental cue of earlier hatching times and faster growth rates of caterpillars, and therefore earlier prey availability for nestlings (Nussey *et al.*, 2005). If day length was the environmental cue for great tits, as the environment changes, in this case warms, although day length and spring temperature may have been identical through evolutionary history, day length would become an inaccurate timing cue for great tit reproduction timing.

By contrast, plasticity may be maladaptive for several reasons: if there is a delay between an environmental cue and the phenotypic response (for example between development and later life), if the cost of plasticity is higher than the benefit of the phenotype generated, or if the selected reaction norm is detrimental in environments beyond those that the species has experienced before (Via *et al.*, 1995, Chevin *et al.*, 2012). Understanding the circumstances which lead to adaptive or maladaptive plasticity will enable us to predict how species will respond to environmental changes, and therefore their resilience to climate change.

1.2 Genotype-by-Environment Interactions

In assessing phenotypic plasticity within a population, genotypes may vary in their response to environmental variation, creating genotype-by-environment interactions (GxE) (Anderson *et al.*, 2011, Josephs, 2018). The presence of GxE interactions in a population means that there exists genetic variation in the strength and direction of plasticity in response to the same environmental variation and provides the potential for plasticity to evolve by selection for certain genotypes that have adaptive plastic responses. By contrast, a lack of GxE interactions suggests strong stabilising selection may have removed variation among genotypes in their plasticity, and the population will

have limited capacity to evolve adaptive plasticity in response to future environmental variation (Oostra *et al.*, 2018).

1.3 Evolutionary Rescue

Evolutionary rescue is the recovery of a population following a sharp decline in population size caused by a change in the environment that imposes novel stressful conditions (Bell and Gonzalez, 2009, Carlson *et al.*, 2014). Specifically, if evolutionary rescue is to maintain population persistence, then population recovery must be accompanied by a corresponding change in allele frequency that promotes rapid adaptation to the new conditions (Bell and Gonzalez, 2009, Carlson *et al.*, 2014). Although this increase in population size has an underlying genetic factor, if the selection pressure is reduced (e.g. the environment returns to its original state) allele frequencies may return to values before the environmental perturbation (Lenormand *et al.*, 1999). This was observed as an annual cycle by Lenormand *et al.* (1999) in *Culex pipiens* mosquitos, as an increase in pesticide resistant allele frequencies in response to summer insecticide application, followed by insecticide application stopping and pesticide resistance allele frequencies decreasing.

1.4 Building Our Understanding of Positive Responses to Environmental Change

Although both plasticity and evolutionary rescue are responses to environmental change, there are important differences between them. Plasticity is a non-genetic response, enabling a change in phenotype without a change in genotype and therefore providing an immediate (within a generation), potentially adaptive change in phenotype (Charmantier *et al.*, 2008). By contrast, evolutionary rescue occurs solely through selection on genetic variation that is beneficial in novel environments, which occurs across generations, and therefore provides a slower adaptive change in phenotype than plasticity (Charmantier *et al.*, 2008).

Since plasticity and evolutionary rescue are both phenotypic responses to changes in the environment, albeit through different mechanisms, it is important to understand whether these two mechanisms for responding to environmental change interact, and whether they enable or inhibit one another. Ghalambor *et al.* (2007) discuss how plasticity has challenged the traditional perspective of selection acting on a phenotype, and therefore on a genotype, suggesting that plasticity 'shields' the genotype from the effects of selection on the phenotype by changing the phenotype while preventing selection from acting on the genotype. More broadly acknowledged is the perspective that plasticity can increase survival in a novel environment in the short-term,

maintaining population size and providing time for selection to occur, therefore maximising population persistence (West-Eberhard, 1989, Chevin and Lande, 2010, Ashander *et al.*, 2016). Lande (2009) modelled phenotypic plasticity under a sudden environment change, predicting that it would initially move the mean phenotype of a population towards the optimum of the new environment, enabling adaptation and slow genetic assimilation of the phenotype and reduced plasticity. Based on this theory, it has been suggested that plasticity facilitates evolutionary rescue in novel extreme environments (Merila and Hendry, 2014), invasive species (Davidson *et al.*, 2011) and anthropogenic disturbances (Crispo *et al.*, 2010), highlighting the importance in understanding how plasticity and evolutionary rescue together determine whether population persistence will occur (Ashander *et al.*, 2016).

1.5 Testing for Genetic Variation in Reaction Norms

Quantitative genetics is the study of the genetics underlying continuous traits in a population, in order to study the evolutionary processes shaping them (Dudley, 1997, Lynch and Walsh, 1998). Applying Mendelian principles to polygenic traits, quantitative genetic estimates are calculated by comparing phenotypes of individuals with known relatedness, and partitioning how much variance in a phenotype is determined by shared genes, and how much is from environmental effects (Lynch and Walsh, 1998). Depending on the breeding design, phenotypic variance can be partitioned into sire or additive genetic variance, dam or maternal effects, and sire-dam interaction or non-additive genetic variance (Lynch and Walsh, 1998). Where additive genetic variance of a trait represents its narrow sense heritability, maternal effects describe the influence of the dam (for example variation in seed investment), and non-additive genetic variance describes phenotypic variation arising from differing allelic combinations (or sire-dam genetic interactions) at a given locus (dominance) or among loci (epistasis) (Lynch and Walsh, 1998). Broad-sense heritability can also be calculated as the ratio of genetic variance (additive and non-additive) to phenotypic variance (Lynch and Walsh, 1998). Traditionally used in animal and plant breeding (to improve economically valuable traits such as yield under artificial selection), quantitative genetics is also applied to the study of genetic variance and covariance of traits to understand evolutionary change in quantitative traits (Dudley, 1997, Lynch and Walsh, 1998, McGlothlin *et al.*, 2018, Walter *et al.*, 2018). Such approaches can be especially powerful when used in field experiments to assess how trait variation relates to fitness, as well as how heritabilities change in different environments. A field study using a breeding design allows you to record phenotypic variance of offspring, partition this variance, and therefore calculate the heritability of traits in the field, and across environmental gradients (Via *et al.*, 1995,

Lynch and Walsh, 1998). Therefore, quantitative genetics can identify whether rapid evolutionary responses to environmental variation are possible.

1.6 Root System Architecture

Plants can be ideal study organisms for evolutionary ecology experiments, partially due to the ease of using clonal propagation in many species (Anderson *et al.*, 2011, Walter *et al.*, 2020), which allows genotypes to be replicated, and tested in multiple environmental conditions.

Root systems are of crucial importance to variation in plant fitness through their role in water and nutrient uptake, but also as mechanical support of the shoot (Lynch, 1995). The term root system architecture is used to describe the spatial configuration (shape, structure etc.) of a root system in soil, or an artificial substrate (de Dorlodot *et al.*, 2007). Dicotyledons generally maintain a primary root and several orders of lateral roots branching off of these, leading to a tap rooted structure, whereas monocotyledons have primary and lateral roots during seedling development, and shoot-borne, axial roots become important after this stage, leading to a fibrous structure (Koevoets *et al.*, 2016). However, there is a huge range in root system structures beyond tap rooted and fibrous, for example adventitious and tuberous roots.

Roots are extremely plastic in their responses to the environment at a very local scale, for example based on soil type, nutrient and water availability and distribution (Lynch, 1995, Fitz Gerald *et al.*, 2006, de Dorlodot *et al.*, 2007). Gradients in resources are common in soil, and often present trade-offs for root systems, for example nutrients are often denser in topsoil, but given that these soils are more exposed, they often contain less water and are subject to extreme temperatures (Lynch, 1995).

Understanding root system architecture has important implications for agriculture, land erosion and water retention, but also in the resilience of populations and ecological communities to a changing environment. The role of roots in reducing soil erosion, and how root system architecture affects this has been extensively studied (De Baets *et al.*, 2007, Burylo *et al.*, 2012), driven by its importance in agriculture. This has led to the development of high throughput phenotyping methods (Chen *et al.*, 2015, Akhtar *et al.*, 2018), which can be valuable in the application of large scale quantitative genetic studies in the laboratory. However, given their size and intimate interactions with the soil (and organisms within), root systems are difficult to measure in the field. Using destructive methods overlooks some traits (e.g. lateral root angle) and lacks accuracy in others (e.g. root system width), while non-destructive methods are often prohibitively expensive (Chen *et al.*, 2015). Laboratory experiments that complement large-scale quantitative genetic field experiments

provide an opportunity to accurately phenotype and compare root system architecture for given genotypes under environmental variation in controlled conditions (Shi *et al.*, 2013).

1.7 Plasticity in Root System Architecture

Average global temperatures and precipitation are expected to increase, with more intense downpours and longer dry periods between rain events, leading to droughts (Collins *et al.*, 2013). Alongside the many impacts this will have on the natural environment, this poses a serious threat to crop production and agriculture (Wang *et al.*, 2003). Due to the world's growing human population and associated food demand, research has historically focussed on crop varieties which maximise yield (Koevoets *et al.*, 2016). However, in practice, suboptimal growing conditions lead to a gap between potential and realised yield, causing research to shift towards more robust, tolerant and plastic varieties which maintain a lower yield across a range of abiotic stresses (Koevoets *et al.*, 2016). Recent research into plant responses to predicted climate change (increased drought, salinity and extreme temperatures) has focussed on biotechnology, genetic engineering in particular, as a strategy for improving stress tolerance in crops (Wang *et al.*, 2003). However, abiotic stress affects a range of processes within plants, including gene expression, cell processes, and morphology. The complexity of such responses means that individual genetic determinants of drought resistance are difficult to isolate and identify (Wang *et al.*, 2003, Yue *et al.*, 2006, Bernier *et al.*, 2009). The majority of research into drought and high temperature responses of plants aims to map loci which influence variation in a trait, known as Quantitative Trait Loci (QTLs) (Courtois *et al.*, 2003, Fitz Gerald *et al.*, 2006, Yue *et al.*, 2006, de Dorlodot *et al.*, 2007, Bernier *et al.*, 2009, Tardieu and Tuberosa, 2010). Identification of such loci may be achieved by growing a range of genotypes under abiotic stress, phenotyping for resistance and then identifying QTLs associated with that resistant response (Bernier *et al.*, 2009). Marker-assisted selection can then be used to create genotypes containing alleles at multiple QTLs that increase resistance (Bernier *et al.*, 2009).

1.7.1 Temperature

Several studies have assessed the effects of increasing or decreasing temperature on root system architecture of *Arabidopsis thaliana* and common crop species such as rice (*Oryza sativa*) and maize (*Zea mays*) (Koevoets *et al.*, 2016). Field recordings show how temperature naturally varies with soil depth and diurnal oscillations (Walter *et al.*, 2009), such that deeper soils experience delayed and smaller changes in temperature (Koevoets *et al.*, 2016).

In general, higher temperatures lead to decreased primary root length, lateral root density (in sunflower, *Helianthus,* genotypes) and angle of lateral roots growing from primary roots (in maize) (Seiler, 1998, Nagel *et al.*, 2009, Koevoets *et al.*, 2016). Functionally, decreased primary root length reduces the rate of water acquisition and anchoring, decreased lateral root density reduces drought and herbivore resistance (Garnier *et al.*, 2015), and a smaller angle of lateral root growth reduces the volume of soil accessible to the roots (Nagel *et al.*, 2009). Higher than optimal temperatures also lead to increased root diameters in lettuce (*Lactuca sativa*) (Qin *et al.*, 2007), increasing storage and water transport, but decreasing the rate of nutrient acquisition (Garnier *et al.*, 2015).

Root:shoot ratios are affected by non-optimal temperatures, however whether these are increased or decreased in response to high or low temperatures appears to be species specific, for example, an increase in temperature leads to a decrease in root:shoot ratio in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), but an increase in maize (Engels, 1994, Fullner *et al.*, 2012). An increased root:shoot ratio in response to increased temperatures may be an adaptive response to increased evapotranspiration at higher temperatures, however root:shoot ratio is also affected by the amount of carbon and nitrogen within the plant (Engels, 1994, Koevoets *et al.*, 2016).

The mechanism of most of these effects is the severe reduction in the elongation rate of root cells, due to a decrease in extensibility of the cell wall (Pritchard *et al.*, 1990, Pardales *et al.*, 1992). Cold stress in *Arabidopsis* inhibits auxin transport out of the base of cells, leading to increased auxin concentrations which no longer promote, but instead inhibit root elongation (Koevoets *et al.*, 2016). By comparison, the mechanism of how plants respond to high temperatures are not as well studied, but thought to involve ethylene levels (Qin *et al.*, 2007).

Notably, temperatures were uniform across the root and shoot system in the majority of these studies, which does not reflect the natural soil environment, and root system architecture is significantly different when grown under a temperature gradient (Fullner *et al.*, 2012).

1.7.2 Drought

Broadly, in dicotyledons, drought stress leads to a decrease in the angle of growth, number and length of lateral roots, and an increase in the length of the primary root (Koevoets *et al.*, 2016). These responses arise from a shift in investment from lateral to primary roots, as an adaptive response for accessing water and anchoring the plant (Garnier *et al.*, 2015, Koevoets *et al.*, 2016). The model organism of plants, *Arabidopsis thaliana,* has demonstrated this when grown on agar medium containing an osmotica to mimic osmotic stress (Deak and Malamy, 2005), and these traits

(investment in primary root growth, and limiting lateral root growth) have been used to identify drought tolerant mutants in *Arabidopsis* (Xiong *et al.*, 2006). The mechanism behind these responses is the inhibition of lateral root formation from lateral root primordia (and not preventing lateral root initiation), through the presence of abscisic acid (ABA) (Deak and Malamy, 2005).

Senecio vulgaris root system architecture has been studied under elevated carbon dioxide and drought stress, which caused no change in the density of roots, but reduced branching and total root length under drought stress (Berntson and Woodward, 1992). Interestingly, similar root system architecture was identified in high carbon dioxide and low water, as ambient carbon dioxide and high water, suggesting a trade-off or common pathways to stress response in root system architecture changes (Berntson and Woodward, 1992).

Hydrotropism is the growth of roots towards water sources, and has been shown to be common in a range of plant species (Takahashi *et al.*, 2009, Cassab *et al.*, 2013). Gravitropism is the growth of a plant in response to gravity, for example roots towards gravity and shoots away from gravity (Cassab *et al.*, 2013). Gravitropism and hydrotropism can interfere with one another, leading to species specific responses when contradictory (Takahashi *et al.*, 2009), or differing root system architecture associated with drought stress based on this gravitropism-hydrotropism interaction. This highlights the less visible factors influencing root system architecture, and how the interactions among these factors can be complex and little understood.

1.8 Study Species and System

The species used in this study are closely related ragwort species, from the large, and globally widespread *Senecio* genus, within the Asteraceae or daisy family, and the Asterales order. *S. aethnensis* and *S. chrysanthemifolius* are found on Mt Etna, Sicily. *S. aethnensis* is a high-altitude specialist, found above 2 000 m above sea level (a.s.l.) on recent lava flows, and is exposed to higher levels of UV and lower temperatures than lower altitudes (Brennan *et al.*, 2009, Osborne *et al.*, 2013). *S. chrysanthemifolius* is found below 1 000 m a.s.l. on agricultural and waste land, exposed to lower levels of UV, but higher temperatures than higher altitudes (Brennan *et al.*, 2009, Osborne *et al.*, 2013). Where *S. aethnensis* produces entire, thicker leaves, and larger flower heads, *S. chrysanthemifolius* has highly dissected, thinner leaves and smaller flower heads (Brennan *et al.*, 2016). Divergence between these species is likely to have occurred around 150 000 years ago, coinciding with the maximum elevation of Mt Etna increasing above *S. chrysanthemifolius*' current range (Osborne *et al.*, 2013). This has led to a stable hybrid zone between the two species' ranges (1

000 to 2 000 m a.s.l.), in which intermediate phenotypes are observed and selected against, suggesting strong divergent selection in relation to elevation (Wong *et al.*, 2019).

Both species are dicotyledons, showing tap rooted root structures, but otherwise their root systems are relatively unstudied. A previous study (von Celsing, 2017) compared root morphology between *S. aethnensis* and *S. chrysanthemifolius*, suggesting no significant difference in root:shoot ratio between species, but that *S. aethnensis* had a faster growth, greater root biomass and lateral root length. However, seeds were grown in non-sterile rooting pouches, for 21 or 27 days, with only a few reaching 10 cm root depths. This may suggest the species is difficult to grow under laboratory conditions for root phenotyping (von Celsing, 2017).

1.9 Knowledge Gaps

The fitness of plant genotypes in novel environments is studied to better understand adaptive and maladaptive responses, however since these are usually large-scale field experiments, little is known about the role of root systems in these responses.

Root systems are less studied than above ground components of plants. Current root phenotyping methods are generally expensive (and therefore low-throughput) or inaccurate (Chen *et al.*, 2015). Although there are some established associations between above and below ground traits (Garnier *et al.*, 2015), using above ground traits as proxies for root traits loses accuracy. As methods become more widely used, they are likely to become less expensive and therefore accessible for high-throughput studies.

When assessing questions about root responses in the wild it is important to reflect the natural environment in laboratory studies, in order to applying findings to wild populations. Factors such as temperature gradients with depth (Fullner *et al.*, 2012), light differences above and below ground (Xu *et al.*, 2013), and within and between day variation affect the complex plasticity observed in root systems.

The genetic pathways and molecular mechanisms of how root system architecture responds to multiple abiotic stressors are also not fully understood. For example, although a lot is known about the mechanism of inhibiting root elongation under low temperatures (Koevoets *et al.*, 2016), far less is known about the mechanism under high temperatures (Qin *et al.*, 2007).

Although a lot is known about yield and abiotic stress tolerance in crop species, applying this knowledge to understand how both natural populations and crop species will be affected by predicted climate change is relatively rare.

Although work has been done on *S. vulgaris* root systems in response to elevated CO_2 and drought stress (Berntson and Woodward, 1992), little is known about *S. chrysanthemifolius* root systems and responses to abiotic stress. A comparison of two closely related, and well-studied (above-ground) species also allows the incorporation of evolutionary history into the interpretation of any findings, for example the recent speciation between species.

1.9 References

Akhtar, J., Galloway, A. F., Nikolopoulos, G., Field, K. J. & Knox, P. 2018. A quantitative method for the high throughput screening for the soil adhesion properties of plant and microbial polysaccharides and exudates. *Plant and Soil,* 428**,** 57-65.

Anderson, J. T., Willis, J. H. & Mitchell-Olds, T. 2011. Evolutionary genetics of plant adaptation. *Trends in Genetics,* 27**,** 258-266.

Ashander, J., Chevin, L. M. & Baskett, M. L. 2016. Predicting evolutionary rescue via evolving plasticity in stochastic environments. *Proceedings of the Royal Society B-Biological Sciences,* 283**,** 10.

Bell, G. & Gonzalez, A. 2009. Evolutionary rescue can prevent extinction following environmental change. *Ecology Letters,* 12**,** 942-948.

Bernier, J., Serraj, R., Kumar, A., Venuprasad, R., Impa, S., Gowda, R. P. V., Oane, R., Spaner, D. & Atlin, G. 2009. The large-effect drought-resistance QTL qtl12.1 increases water uptake in upland rice. *Field Crops Research,* 110**,** 139-146.

Berntson, G. M. & Woodward, F. I. 1992. The root-system architecture and development of Senecio vulgaris in elevated CO2 and drought. *Functional Ecology,* 6**,** 324-333.

Brennan, A. C., Bridle, J. R., Wang, A. L., Hiscock, S. J. & Abbott, R. J. 2009. Adaptation and selection in the Senecio (Asteraceae) hybrid zone on Mount Etna, Sicily. *New Phytologist,* 183**,** 702-717.

Brennan, A. C., Hiscock, S. J. & Abbott, R. J. 2016. Genomic architecture of phenotypic divergence between two hybridizing plant species along an elevational gradient. *Aob Plants,* 8**,** 13.

Brown, J. H., Valone, T. J. & Curtin, C. G. 1997. Reorganization of an arid ecosystem in response to recent climate change. *Proceedings of the National Academy of Sciences of the United States of America,* 94**,** 9729-9733.

Burylo, M., Rey, F., Mathys, N. & Dutoit, T. 2012. Plant root traits affecting the resistance of soils to concentrated flow erosion. *Earth Surface Processes and Landforms,* 37**,** 1463-1470.

Carlson, S. M., Cunningham, C. J. & Westley, P. A. H. 2014. Evolutionary rescue in a changing world. *Trends in Ecology & Evolution,* 29**,** 521-530.

Cassab, G. I., Eapen, D. & Campos, M. E. 2013. Root hydrotropism: An update. *American Journal of Botany,* 100**,** 14-24.

Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E. B. & Sheldon, B. C. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science,* 320**,** 800-803.

Chen, Y. L., Djalovic, I. & Rengel, Z. 2015. Phenotyping for root traits. *In:* Kumar, J., Pratap, A. & Kumar, S. (eds.) *Phenomics in Crop Plants: Trends, Options and Limitations.* 1 ed. India: Springer.

Chevin, L. M., Collins, S. & Lefevre, F. 2013. Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. *Functional Ecology,* 27**,** 966-979.

Chevin, L. M., Gallet, R., Gomulkiewicz, R., Holt, R. D. & Fellous, S. 2012. Phenotypic plasticity in evolutionary rescue experiments. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 368**,** 12.

Chevin, L. M. & Lande, R. 2010. When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? *Evolution,* 64**,** 1143-1150.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W. J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A. J. & Wehner, M. 2013. Long-term Climate Change: Projections, Commitments and Irreversibility. *In:* Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, UK: Cambridge University Press.

Courtois, B., Shen, L., Petalcorin, W., Carandang, S., Mauleon, R. & Li, Z. 2003. Locating QTLs controlling constitutive root traits in the rice population IAC 165 x Co39. *Euphytica,* 134**,** 335-345.

Crispo, E., DiBattista, J. D., Correa, C., Thibert-Plante, X., McKellar, A. E., Schwartz, A. K., Berner, D., De Leon, L. F. & Hendry, A. P. 2010. The evolution of phenotypic plasticity in response to anthropogenic disturbance. *Evolutionary Ecology Research,* 12**,** 47-66.

Davidson, A. M., Jennions, M. & Nicotra, A. B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters,* 14**,** 419-431.

De Baets, S., Poesen, J., Knapen, A. & Galindo, P. 2007. Impact of root architecture on the erosion-reducing potential of roots during concentrated flow. *Earth Surface Processes and Landforms,* 32**,** 1323-1345.

de Dorlodot, S., Forster, B., Pages, L., Price, A., Tuberosa, R. & Draye, X. 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends in Plant Science,* 12**,** 474-481.

Deak, K. I. & Malamy, J. 2005. Osmotic regulation of root system architecture. *Plant Journal,* 43**,** 17-28.

DeWitt, T. J., Sih, A. & Wilson, D. S. 1998. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution,* 13**,** 77-81.

Dudley, J. W. 1997. Quantitative genetics and plant breeding. *Advances in Agronomy, Vol 59,* 59**,** 1-23.

Engels, C. 1994. Effect of root and shoot meristem temperature on shoot to root dry-matter partitioning and the internal concentrations of nitrogen and carbohydrates in maize and wheat. *Annals of Botany,* 73**,** 211-219.

Fitz Gerald, J. N., Lehti-Shiu, M. D., Ingram, P. A., Deak, K. I., Biesiada, T. & Malamy, J. E. 2006. Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. *Genetics,* 172**,** 485-498.

Fullner, K., Temperton, V. M., Rascher, U., Jahnke, S., Rist, R., Schurr, U. & Kuhn, A. J. 2012. Vertical gradient in soil temperature stimulates development and increases biomass accumulation in barley. *Plant Cell and Environment,* 35**,** 884-892.

Garnier, E., Garnier, E., Navas, M.-L., Navas, M. L., Grigulis, K. & Grigulis, K. 2015. *Plant Functional Diversity: Organism traits, community structure, and ecosystem properties*, Oxford University Press.

Ghalambor, C. K., McKay, J. K., Carroll, S. P. & Reznick, D. N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology,* 21**,** 394-407.

Josephs, E. B. 2018. Determining the evolutionary forces shaping G x E. *New Phytologist,* 219**,** 31-36.

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M. & Testerink, C. 2016. Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science,* 7**,** 19.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology,* 22**,** 1435-1446.

Lenormand, T., Bourguet, D., Guillemaud, T. & Raymond, M. 1999. Tracking the evolution of insecticide resistance in the mosquito Culex pipiens. *Nature,* 400**,** 861-864.

Lynch, J. 1995. Root architecture and plant productivity. *Plant Physiology,* 109**,** 7-13.

Lynch, M. & Walsh, B. 1998. *Genetics and analysis of quantitative traits,* Sunderland, Mass., Sunderland, Mass. : Sinauer.

McGlothlin, J. W., Kobiela, M. E., Wright, H. V., Mahler, D. L., Kolbe, J. J., Losos, J. B. & Brodie, E. D. 2018. Adaptive radiation along a deeply conserved genetic line of least resistance in Anolis lizards. *Evolution Letters,* 2**,** 310-322.

Merila, J. & Hendry, A. P. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications,* 7**,** 1-14.

Nagel, K. A., Kastenholz, B., Jahnke, S., Van Dusschoten, D., Aach, T., Muhlich, M., Truhn, D., Scharr, H., Terjung, S., Walter, A. & Schurr, U. 2009. Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. *Functional Plant Biology,* 36**,** 947-959.

Nussey, D. H., Postma, E., Gienapp, P. & Visser, M. E. 2005. Selection on heritable phenotypic plasticity in a wild bird population. *Science,* 310**,** 304-306.

Oostra, V., Saastamoinen, M., Zwaan, B. J. & Wheat, C. W. 2018. Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications,* 9**,** 11.

Osborne, O. G., Batstone, T. E., Hiscock, S. J. & Filatov, D. A. 2013. Rapid speciation with gene flow following the formation of Mt. Etna. *Genome Biology and Evolution,* 5**,** 1704-1715.

Pardales, J. R., Kono, Y. & Yamauchi, A. 1992. Epidermal-cell elongation in sorghym seminal roots exposed to high root-zone temperature. *Plant Science,* 81**,** 143-146.

Parmesan, C. & Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature,* 421**,** 37-42.

Pritchard, J., Barlow, P. W., Adam, J. S. & Tomos, A. D. 1990. Biophysics of the inhibition of the growth of maize roots by lowered temperature. *Plant Physiology,* 93**,** 222-230.

Qin, L., He, J., Lee, S. K. & Dodd, I. C. 2007. An assessment of the role of ethylene in mediating lettuce (Lactuca sativa) root growth at high temperatures. *Journal of Experimental Botany,* 58**,** 3017-3024.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C. & Pounds, J. A. 2003. Fingerprints of global warming on wild animals and plants. *Nature,* 421**,** 57-60.

Seiler, G. J. 1998. Influence of temperature on primary and lateral root growth of sunflower seedlings. *Environmental and Experimental Botany,* 40**,** 135-146.

Shi, L., Shi, T. X., Broadley, M. R., White, P. J., Long, Y., Meng, J. L., Xu, F. S. & Hammond, J. P. 2013. High-throughput root phenotyping screens identify genetic loci associated with root architectural traits in Brassica napus under contrasting phosphate availabilities. *Annals of Botany,* 112**,** 381-389.

Snell-Rood, E. C., Kobiela, M. E., Sikkink, K. L. & Shephard, A. M. 2018. Mechanisms of plastic rescue in novel environments. *Annual Review of Ecology, Evolution, and Systematics,* 49**,** 331-354.

Takahashi, H., Miyazawa, Y. & Fujii, N. 2009. Hormonal interactions during root tropic growth: hydrotropism versus gravitropism. *Plant Molecular Biology,* 69**,** 489-502.

Tardieu, F. & Tuberosa, R. 2010. Dissection and modelling of abiotic stress tolerance in plants. *Current Opinion in Plant Biology,* 13**,** 206-212.

Via, S., Gomulkiewicz, R., Dejong, G., Scheiner, S. M., Schlichting, C. D. & Vantienderen, P. H. 1995. Adaptive Phenotypic Plasticity - Consensus and Controversy. *Trends in Ecology & Evolution,* 10**,** 212-217.

von Celsing, C. 2017. *Investigating the relative root to shoot investment and root morphology of two endemic Sicilian Senecio species in relation to ecology.* MSci Thesis, University of Bristol.

Walter, A., Silk, W. K. & Schurr, U. 2009. Environmental effects on spatial and temporal patterns of leaf and root growth. *Annual Review of Plant Biology.* Palo Alto: Annual Reviews.

Walter, G. M., Abbott, R. J., Brennan, A. C., Bridle, J. R., Chapman, M., Clark, J., Filatov, D., Nevado, B., Ortiz-Barrientos, D. & Hiscock, S. J. 2020. Senecio as a model system for integrating studies of genotype, phenotype and fitness. *New Phytologist,* 226**,** 326-344.

Walter, G. M., Aguirre, J. D., Blows, M. W. & Ortiz-Barrientos, D. 2018. Evolution of genetic variance during adaptive radiation. *American Naturalist,* 191**,** E108-E128.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J. M., Hoegh-Guldberg, O. & Bairlein, F. 2002. Ecological responses to recent climate change. *Nature,* 416**,** 389-395.

Wang, W. X., Vinocur, B. & Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* 218**,** 1-14.

West-Eberhard, M. J. 1989. Phenotypic Plasticity and the Origins of Diversity. *Annual Review of Ecology and Systematics,* 20**,** 249-278.

Wong, E. L. Y., Nevado, B., Osborne, O. G., Papadopulos, A. S. T., Bridle, J. R., Hiscock, S. J. & Filatov, D. A. 2019. Strong divergent selection at multiple loci in two closely related species of ragworts adapted to high and low elevations on Mount Etna. *Molecular Ecology***,** 19.

Xiong, L. M., Wang, R. G., Mao, G. H. & Koczan, J. M. 2006. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiology,* 142**,** 1065-1074.

Xu, W. F., Ding, G. C., Yokawa, K., Baluska, F., Li, Q. F., Liu, Y. G., Shi, W. M., Liang, J. S. & Zhang, J. H. 2013. An improved agar-plate method for studying root growth and response of Arabidopsis thaliana. *Scientific Reports,* 3**,** 7.

Yue, B., Xue, W. Y., Xiong, L. Z., Yu, X. Q., Luo, L. J., Cui, K. H., Jin, D. M., Xing, Y. Z. & Zhang, Q. F. 2006. Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. *Genetics,* 172**,** 1213-1228.

2 Chapter 2: Testing the Response of Root Traits to Drought and Temperature Stress

2.1 Introduction

Understanding how genotypes, populations, species, communities and ecosystems respond to environmental change is crucial under future climate change predictions. Plasticity and evolutionary rescue (see Chapter 1) are both important mechanisms in how organisms respond to environmental change and understanding how plasticity and evolutionary rescue interact is important in predicting the resilience of populations.

Root systems are difficult to accurately study in the field, and therefore laboratory experiments to study root response compliment field studies in above-ground responses of plants to environmental change. Together laboratory and field experiments ensure more aspects of a plants response are being factored in. Quantitative genetics breeding designs allow the heritability of traits to be assessed in the field, and parallel laboratory studies into the plasticity of root system architecture can reveal the importance of root plasticity in determining fitness variation in the field experiments.

Root systems are important in land management for agriculture to reduce land erosion and increase water retention of soils. Equally, root systems in plant breeding for agriculture is important to improve plant anchoring and optimise water and nutrient uptake. Increased temperatures generally lead to decreased primary root length, lateral root density and lateral root branching angle, meaning a smaller volume of soil is accessible to the root system (Koevoets *et al.*, 2016). Increased drought stress generally causes root investment to shift from lateral to the primary root, leading to increased root system depth and anchoring (Koevoets *et al.*, 2016).

Although two closely related species (*S. chrysanthemifolius* and *S. aethnensis*) are found in study system, only *S. chrysanthemifolius* is used in this laboratory experiment. *S. chrysanthemifolius*, a low-altitude species with a tap-rooted root system, found throughout Sicily, including on Mt Etna.

2.1.1 Field Tests for Genetic Variation in Plasticity

Walter *et al.* (in preparation) conducted a field study investigating genetic variation in plasticity and its effects on evolutionary rescue in *S. aethnensis* and *S. chrysanthemifolius* on Mt Etna, Sicily. Walter *et al.* used a breeding design to produce *S. chrysanthemifolius* offspring of known families, and planted clones of the offspring across an elevational range on Mt Etna, Sicily. Conveniently this range in altitudes spans known and novel environmental conditions and

geographic locations for this species, therefore from now on both the environmental and geographic range will be referred to the 'range'.

Plant height, leaf morphology, chlorophyll fluorescence and fitness (estimated as the number of flowers produced over 5 months) were recorded, allowing plasticity in these traits (and their interactions) to be calculated. The crossing design enabled total variance in fitness to be partitioned based on how much variation could be attributed to which source (see section 1.3). For example, the amount of variation in phenotypic fitness which comes from the sire, can then be used to find the additive genetic mean in fitness for each sire (or relative fitness), and the variance for all sires. Fitness decreased outside the species' range for all genotypes, but Figure 1 shows relative fitness within and outside the species' range.

From the 314 individuals used in the field study, 24 individuals (hereafter, 'genotypes') were selected for this experiment based on their fitness outside the home range (Figure 1). Chosen genotypes included Reaction Norm Type 1 (RNT1), which were genotypes that showed the greatest relative fitness outside their range. Reaction Norm Type 2 (RNT2) genotypes were selected as those that showed the lowest relative fitness outside their range. Notably, these genotypes show a change in rank from within the range, 500 m site and outside the range, 2 000 m site, where RNT1 genotypes ranked low in the 500 m site and high in the 2 000 m site, whereas RNT2 genotypes ranked high in the 500 m site and low in the 2 000 m site.

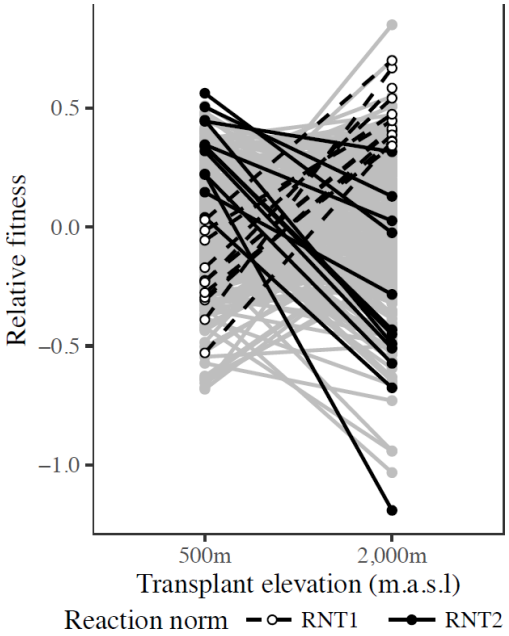

Figure 1. Results of the Walter *et al.* (in preparation) field transplant experiment, highlighting genotypes selected for this experiment. Relative fitness within the range (500 m a.s.l.) and outside the species range (2 000 m a.s.l.), for all genotypes used in the study (grey lines), and genotypes selected for this experiment (black lines), with those grouped as Reaction Norm Type 1 identified with a dashed line and Reaction Norm Type 2 with a solid line.

2.1.2 Aims

The aim of this study is to investigate whether the chosen genotypes (reflecting differences in fitness variation) respond to a change in environment in the laboratory by changing root system architecture.

The first aim of this chapter is to test, at the species level, for plasticity in root system architecture under drought and temperature stress, in *S. chrysanthemifolius* genotypes. Based on previous studies (Engels, 1994, Seiler, 1998, Qin *et al.*, 2007, Fullner *et al.*, 2012, Koevoets *et al.*, 2016), I hypothesise that:

- All genotypes will show plasticity in root system architecture in response to temperature and drought stress
- High temperatures will lead to decreased root depth and root:shoot ratio, and increased root diameter
- High drought stress will lead to increased root depth, decreased root system width

The second aim of this study is to test for specific root traits associated with genotypes grouped by reaction norm type in the field (RNT1 and RNT2) based on the change in relative fitness of a genotype across altitudes. It is likely that genotypes which showed an increase in relative fitness outside of their range (RNT1) are more likely to show adaptive responses under environmental change. Therefore, since root system architecture is known to be important in overall plant fitness, and based on previous studies (Engels, 1994, Qin *et al.*, 2007, Garnier *et al.*, 2015, Koevoets *et al.*, 2016), I hypothesise that:

- RNT1 and RNT2 will show differences in root system architecture
- RNT1 will show adaptive responses relative to RNT2, across increased temperature and drought stress, as increased root:shoot ratio, increased root diameter

2.2 Methods

2.2.1 Field Experiment and Genotype Selection

To quantify genetic variance in relative fitness across an elevation gradient, Walter *et al.* (in preparation) used a quantitative genetic breeding design. Crossing was aided by the study species, *S. chrysanthemifolius*, being a hermaphroditic and self-incompatible ragwort native to Sicily.

Cuttings were sampled from 72 individuals across five, separate, low-altitude sites from wild populations on Mt Etna, Sicily, and grown in glasshouses. Half the individuals were randomly allocated as dams, and the remaining, sires. A full-sibling, half-sibling breeding design was used, in which each of the 36 sires was crossed with three dams, producing 108 families. Three seeds from each family were germinated and then grown in the glasshouse (now referred to as genotypes, N = 312). Multiple cuttings were taken from each plant, the cut section dipped in a growth hormone, placed in trays containing peat moss and left for two weeks to produce roots. Once cuttings produced roots, 7-9 cuttings of each genotype were transplanted into each transplant site (500 m, 1000 m, 1500 m and 2000 m a.s.l.) on Mt Etna (n = 2700 cuttings per site, N = 8149 cuttings total). Cuttings were watered daily for one month until they established, and then watered every 3-4 days afterwards.

After six months of growth, fitness was estimated by collecting and counting the number of flowers from each individual. Relative fitness was calculated using a Bayesian Generalised Linear Mixed Model, specifically R package MCMCglmm, with the number of flowers as a poisson-distributed response variable (Hadfield, 2010). The fitness reaction norms for each genotype were then calculated as the change in relative fitness from their natural environment (500 m a.s.l.) to the edge (1500 m a.s.l.) and outside their range (2000 m a.s.l.). All genotypes showed lower fitness at 2000 m a.s.l., but genotypes varied in their relative fitness. From the variation among genotypes (Figure 1), we selected 12 which showed increased relative fitness outside their range and grouped them as Reaction Norm Type 1 (RNT1). Similarly, we selected 12 genotypes which showed lower relative fitness outside their range and grouped them as Reaction Norm Type 2 (RNT2). In the analysis, whether a genotype was identified as RNT1 or RNT2 in the field is a factor referred to as its 'reaction norm'.

For each of the 24 genotypes, 3-4 cuttings were taken from the field and transported to Bristol University. Cuttings were transferred into a 75 % Levington F2 compost and 25 % perlite mix and grown under a propagation lid. After 21 days, cuttings which had produced roots were moved into larger pots containing all-purpose compost (Sinclair) with 0.4 % Osmocote granular long-term

fertiliser and 0.04 % Exemptor granular insecticide and cut back when appropriate (approximately after eight weeks). The cuttings which had initially failed to produce roots were kept under the propagation lid until they produced roots. Throughout this process, cuttings and plants were kept in controlled conditions at a constant 20 °C, on a 16:8 hour light:dark cycle.

2.2.2 Laboratory Experiment

I used a nested, fully-factorial design with two variables, drought stress and temperature, with two levels of each variable (Figure 2). These conditions were selected to represent a combination of extreme environments possible under future climate change predictions. Weather data collected in the field were used to guide treatment levels with the intentions of mirroring differences between 500 m and 2 000 m sites (in the knowledge that it would not be possible to incorporate daily or seasonal fluctuations under laboratory conditions). However, after extensive pilot experiments the level of the treatment and the differences between low and high treatments were adjusted to balance the likelihood of cuttings producing roots (increased under high temperatures and humidity) and therefore observing enough phenotypic variance between low and high treatments and genotypes.

		Temperature	
		20 °C	25 °C
Drought stress	0.5 % sorbitol	Treatment 1	Treatment 2
	5.0 % sorbitol	Treatment 3	Treatment 4

Figure 2. Nested design of temperature and drought stress, creating 4 treatments.

For each of the 24 genotypes, 6 cuttings were taken for each treatment (n = 24 cuttings per genotype, N = 576 cuttings in total, Figure 3). Due to the scale of the experiment, two observers set up the full experiment in batches over 3 days. For each batch (n = 24), one observer would take and prepare a cutting from each genotype, therefore randomising genotypes between observers.

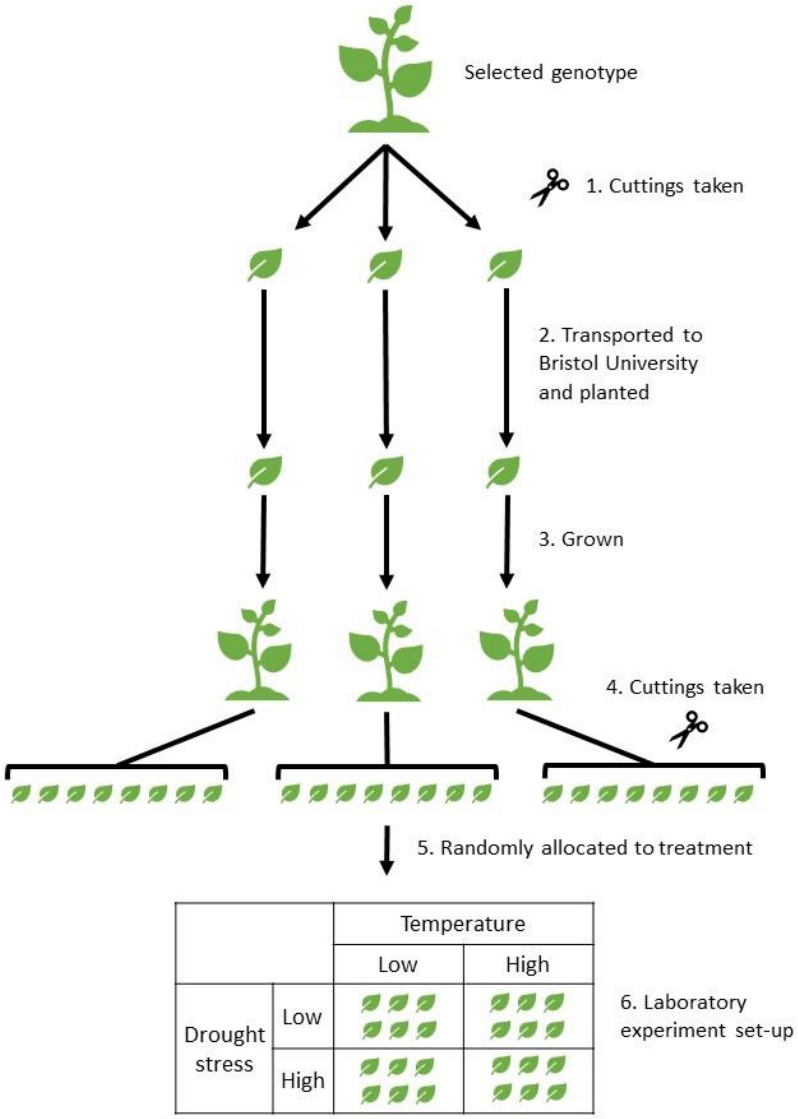

Figure 3. Schematic from field to experimental set-up for 1 genotype. Each leaf represents cuttings of the same genotype, and this whole process was replicated for all 24 genotypes. From 3 cuttings being taken from an individual in the field (step 1), transported to Bristol University and planted (step 2), grown (step 3), a further 8 cuttings taken per plant (step 4), and randomly allocated to a treatment (step 5) for the laboratory experiment set-up (step 6). Leaves represent cuttings of the same genotype.

The growth of seeds on vertical agar plates for root phenotyping is relatively common, with a range of protocols available (Bengough *et al.*, 2004, Hargreaves *et al.*, 2009, Iyer-Pascuzzi *et al.*, 2010, Chen *et al.*, 2015). The Grierson group (University of Bristol) study root-soil cohesion and advised an adaptation of one of these protocols. In a pilot study, this protocol was used to grow *S. chrysanthemifolius* seeds with a germination rate of 83 %, and therefore was chosen to develop for the growth of cuttings of the same species. Growing cuttings on agar is mentioned in the literature (Woo *et al.*, 1997, Nguyen *et al.*, 1999, Mandal *et al.*, 2000), with varying levels of success and often

with the intention of propagating individuals, rather than phenotyping traits. For this study, extensive pilot experiments were used to develop a method of growing and phenotyping root traits from cuttings, based on the method of growing seeds on agar.

Cuttings were cut down to include at least two nodes (approximately 1-4 cm) and leaves trimmed to reduce water requirements and maximise likelihood of successfully producing roots. Before being assigned to treatments, cuttings were weighed using analytical scales (Sartorius ME5), and anti-static weighing boats, allowing the measurement of proportional change in biomass.

The remainder of the method was conducted under sterile conditions to minimise the risk of infecting the agar plate during the experiment. The base Murashige and Skoog (MS) agar media was prepared as 1 % agar (Sigma-Aldrich A4675) and 0.22 % MS (Sigma-Aldrich M5524), and the pH adjusted to 5.7 using KOH. For the drought stress treatments, before adjusting the pH D-sorbitol (Sigma-Aldrich S1876) was added as 0.5 % in the low drought stress treatment and 5 % in the high drought stress treatment. Following autoclaving, 80 ml media was poured into each 12 cm square plate (Greiner Bio-One 688102).

Cuttings were sterilised by submerging in 10 % bleach solution for 10 minutes, and then submerged in water to remove the bleach solution. A small hole was made in the agar using sterile forceps, along the central line of the plate (6 cm from either side of the plate) and 2 cm from the top of the plate, using a template. The sterilised cutting was placed in the hole in the agar, and the plate closed, labelled and sealed using microporous tape. Cuttings were taken, weighed, plated and sealed in the agar plates in batches of 24 to ensure consistent and minimal time between the cutting being taken and plated. Once sealed, plates were moved to one of the two High Specification Plant Growth Chambers (Snijders Labs) depending on the treatment to which it was allocated. Plates were stood near vertical (approximately 60 °) to promote downward root growth along the surface, or within the agar, producing a 2-D root structure. Plates were placed in groups of 9, totalling 32 groups within each growth chamber and genotypes were randomised between groups using a Latin square design.

Both growth chambers were set to a constant 70 % humidity, on a 15:9 hour light:dark cycle, however one was set to 20 °C and the other 25 °C, as the low and high temperature treatment. Light meter readings were taken in both growth chambers at six different but consistent locations (Skye SpectroSense2 light meter). The same model growth chambers were used and initially run on the same settings of light levels, however light meter readings taken before the experiment started showed significant difference in light levels between chambers (paired $t(5) = -7.189$, $p < 0.001$). Settings were adjusted to be different for each chamber, and subsequent light meter readings showed no significant difference in light levels between chambers (paired $t(5) = -1.693$, $p = 0.151$).

The time at which plates were put in the growth chambers was recorded and each batch of plates removed for phenotyping after exactly 12 days.

2.2.3 Data Collection

After 12 days, cuttings were recorded as having successfully produced roots or not (binary variable). Plates were then removed from the growth chamber and photographed using a Canon EOS 200D camera. Each plate was placed within the corner of a fixed frame and photographed from above using a fixed camera arm, to ensure the camera position relative to the plates remained constant (approximately 30 cm between camera and plate). A black background ensured maximum possible contrast between the roots and background, and a fixed ruler provided scale. The effect on the phenotyping software's ability to distinguish roots from background was compared across a range of lighting conditions, for example using an angled lamp. However, the set-up which provided the greatest contrast between roots and the background was direct overhead lighting in a windowless room. The frame was not moved throughout the experiment and photos were captured remotely to remove the risk of shadows or variation in lighting. Fresh biomass was measured following the same protocol for weighing the cuttings before the experiment, however roots were cut away from the original cutting or 'shoot' and weighed separately, providing independent measurements of shoot and root.

Images of the plates were labelled and imported into the computer program, Gia Roots (Galkovskyi *et al.*, 2012). Images were rotated and the scale was set for all images. Manual cropping of individual images ensured only the root systems were being analysed. Threshold parameters were adjusted to ensure most accurate differentiation between the roots and background across a random subset of images, assessed by eye (example images in Figure S1). Once selected, the configuration of threshold parameters was applied to all images, and all images processed. The output included 20 quantitative, numerical traits and five diagnostic images. All images were visually checked and re-processed where background or imperfections in the plate were identified as roots.

Table 1. Description of traits , including those manually measured and produced by Gia Roots adapted from (Galkovskyi *et al.*, 2012). Traits highlighted in bold are those selected for further analyses (see Data Analysis, Multivariate Dimension Reduction).

Trait	Source	Trait explanation
Root.weight	Manually measured	Root weight (mg)
Shoot.weight	Manually measured	Shoot weight (mg)
Weight.change	Manually measured	Starting weight divided by total (root and shoot) end weight
R.S	Manually measured	Root weight divided by shoot weight (R:S ratio)
Diameter	GiaRoots	Root width averaged over entire root system (cm)
Bushiness	GiaRoots	The root system is split using multiple horizontal lines, and the number of roots crossing each horizontal line estimated. 'Bushiness' is the ratio of the maximum to the median number of roots crossing each horizontal line for the root system
Depth	GiaRoots	Vertical depth of the total root network (limited by 12 cm depth of the agar plates, cm)
Axis.ratio	GiaRoots	Of the best fitting ellipse of the root system, the ratio of the minor to major axis (major and minor axes are always at a right angle)
Length.distribution	GiaRoots	When the network is split into the top third, and lower two thirds based on network depth, this is the proportion of the root system found in the lower two thirds
Major.ellipse.axis	GiaRoots	Of the best fitting ellipse of the root system, the length of the major axis (cm)
Width	GiaRoots	Horizontal width of the total root network (limited by 12 cm width of the plates, cm)
Minor.ellipse.axis	GiaRoots	Of the best fitting ellipse of the root system, the length of the minor axis (cm)
Area	GiaRoots	Total area of root network (cm^2)
Convex.area	GiaRoots	Area of the convex hull encompassing the root network (cm^2)
Perimeter	GiaRoots	Total length of the perimeter of the root system (cm)
Solidity	GiaRoots	Total network area divided by the network convex area
Specific.root.length	GiaRoots	Total root length divided by root system volume (cm^2)
Surface.area	GiaRoots	Assuming a tubular shape, uses the radius of the roots to calculate the surface area at each point along the network skeleton and totals (cm^2)
Length	GiaRoots	Length of all root branches summed (cm)
Volume	GiaRoots	Assuming a tubular shape, uses the radius of the roots to calculate the volume at each point along the network skeleton and totals (cm^3)
W.D	GiaRoots	Network width divided by depth (W:D ratio)

2.2.4 Data Analysis

All statistical analyses were conducted in R version 3.6.1 (R Core Team, 2019).

2.2.4.1 Proportion of Cuttings That Produced Roots

Six cuttings from each genotype were grown under each treatment, however the treatments affected the proportion of these six cuttings that produced roots, affecting sample size for trait analysis. For this reason, a generalised linear mixed model was used to model the proportion of cuttings that produced roots, against the fixed effects of temperature, drought and reaction norm, accounting for the random effect of genotype. The proportion of cuttings which produced roots was a binary response, producing a binomial distribution and therefore the 'glmer' command in the R package 'lme4' (Bates *et al.*, 2015) was used to fit a generalised linear mixed model allowing for a binomial distribution of errors. Specifying a distribution of errors enables p-values to be calculated for a mixed model, as this provides information for the distribution of the test statistic. Therefore, when reporting these results, I have included the effect size estimate, standard error, 95 % confidence intervals, p-values and z-values, which specify the distance and direction of the estimate from the mean, standardised across variables by standard deviation.

To test for a significant genotype-by-envrionment interaction (GxE), two models were compared with varying random effects structures (Figure 4). Firstly, a random intercept but fixed slope model, modelling all genotypes with the same slope across environments, but with differing intercepts (Figure 4a). Secondly, a random intercept and random slope model, allowing genotypes to be modelled with differing intercepts and slopes across environments (Figure 4b). A likelihood ratio test evaluates the contribution of a single factor (or factor structure) to a model, by comparing the fit of the model with and without the factor (or between varying factor structures), and if there is no significant difference, the simplest model would be selected (Bolker *et al.*, 2009). If the second model (random intercept and random slope) fits the data significantly better than the first, this suggests including random slopes describes significant variation, and therefore there is significant GxE. However no significant difference or the first model fitting the data better than the second suggests no significant GxE.

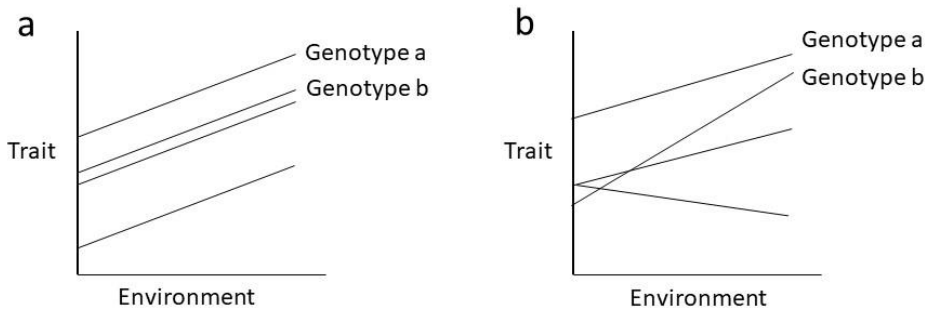

Figure 4. Conceptual relationship between a trait and an environmental gradient for four different genotypes , modelled using different random effects structures to assess genotype-by-environment interaction in a dataset. Using a fixed slope and random intercept model (a) constrains all genotypes to respond in the same way (i.e. no GxE). Using a random slope and random intercept model (b) allows genotypes to have differing responses to environmental change (i.e. GxE). Comparing how well models using the different random effects structures fit the data determines whether there is significant genotype-by-environment interaction.

To test for significant interactions between the main effects, I sequentially removed interaction terms and used likelihood ratio tests to compare models after each term was removed. If there was no significant difference between models after a single interaction term was removed, it suggests that the term did not explain significant variation in the model and could be removed.

To verify the results of the mixed effects models on the principal components scores, I used a MANOVA on the same 7 traits with temperature, drought, reaction norm and their interaction as fixed effects.

2.2.4.2 Multivariate Dimension Reduction

To analyse differences among treatments for the 21 traits, multivariate tests (Multivariate Analysis of Variance [MANOVA]) or dimension reduction (Principal Component Analysis [PCA]) could be used. However, for 21 traits, MANOVA would not have enough degrees of freedom, and PCA becomes complex to interpret, usually resulting in lots of traits contributing very small amounts of variance. Therefore, I used a combination of correlation plots, PCA and Linear Discriminant Analysis (LDA) to reduce the dataset to seven biologically important and independent traits for further analysis. Initially, I used a correlation matrix to identify traits that were highly correlated, retaining only one of the highly correlated traits (Figure S2). I then used a PCA to identify traits with low loadings across the first 3 principal components (PC1 to PC3 represent 78% of the variance in the dataset) as having little contribution to the largest axes of variance in the dataset (Table S1). I then

used LDA to identify the axes that best separated temperature, drought and treatments respectively (Table S2). Traits with high coefficients were identified as traits contributing to differences in root responses to temperature or drought or both. Using a combination of the above methods I chose seven traits to analyse differences among treatments; root weight, shoot weight, root:shoot, diameter, depth, area and width:depth (highlighted in bold in Table 1). To check for substantial changes in variance between the original (21 traits) and reduced (7 traits) datasets, I visually compared PCA biplots (Figure S3).

2.2.4.3 Multivariate Trait Responses

To test for multivariate differences among treatments I first conducted a PCA on these 7 traits to reduce dimensions to the first three principal components. I then used a linear mixed model to quantify changes in PC1 across treatments, using the fixed effects of temperature, drought and reaction norm. Significant interaction terms among the main effects were tested for using sequential removal and model comparisons using likelihood ratio tests (as explained above). Genotype was modelled as a random effect, and two effects structures were compared using likelihood ratio tests to identify significant GxE (as explained above). This was then repeated to identify the best fitting models for PC2 and PC3, separately.

Since the principal component scores are not binomial (as in the previously described mixed model), error distributions are not specified and the distribution of the test statistic for a mixed model with several factors is less accurate. Therefore p-values are more complex to calculate and provide less information than in simple linear models, or where error distributions have been specified. To fit a linear mixed-effects model to the PC1, PC2 and PC3 responses, the 'lmer' function was used in the 'lme4' package (Bates *et al.*, 2015). Instead I have reported the effect size estimate, standard error, 95 % confidence intervals and t-values, which describe the effect size of a variable relative to variation in the dataset.

2.3 Results

2.3.1 Proportion of Cuttings That Produced Roots

The proportion of cuttings that produced roots was significantly higher in the low drought stress treatment, and significantly higher in the high temperature treatment, with no significant difference between different reaction norm groups (Table 2 and Figure 5).

Table 2. Generalized linear mixed model means (Estimate), standard error (SE), 95% confidence intervals (95% CI), Z-value and p-value, for the fixed effects (Parameter) on proportion of cuttings that produced roots. Significant p-values highlighted in bold.

Parameter	Estimate	SE	95% CI		Z-value	p-value
			Lower	Upper		
Intercept	1.158	0.303	0.659	1.678	3.823	**< 0.001**
Reaction norm	0.468	0.377	-0.169	1.114	1.241	0.215
Drought	-2.385	0.224	-2.764	-2.024	-10.640	**< 0.001**
Temperature	1.045	0.209	0.705	1.394	4.999	**< 0.001**

Figure 5. Differences in proportion of cuttings that produced roots between treatments and reaction norms, predicted using a generalized linear mixed model.

Similarly, likelihood ratio tests were used to compare models including differing interaction terms, suggesting the best fitting model includes no interaction terms and only the fixed effects (Table 3).

Table 3. Likelihood ratio tests comparing interaction terms when modelling the proportion of cuttings that produced roots against the fixed effects of reaction norm (RN), drought and temperature, modelling genotype with a random intercept.

Model structure	Compared to	χ^2	df	p-value
RN:drought:temp + RN:drought + RN:temp + drought:temp + RN + drought + temp	Removed RN:drought:temp	1.15	1	0.284
RN:drought + RN:temp + drought:temp + RN + drought + temp	Removed RN:drought	1.37	1	0.243
RN:temp + drought:temp + RN + drought + temp	Removed RN:temp	0.39	1	0.532
drought:temp + RN + drought + temp	Removed drought:temp	1.34	1	0.246

The best fitting random effects structure included a random intercept only, suggesting no GxE (Table 4).

Table 4. Likelihood ratio tests comparing random effects structures when modelling the proportion of cuttings that produced roots against the fixed effects of reaction norm, drought and temperature, and all possible interaction terms. (1|gen) specifies that each genotype has a different, random intercept, where (temp*drought||gen) specifies that each genotype has a different, random intercept and slope, representing GxE in the model.

Random effects structure	Compared to	χ^2	df	p-value
(1|gen)	(temp*drought||gen)	8.52	16	0.932

2.3.2 Multivariate Dimension Reduction

The PCA using the seven selected traits is presented in Table 5. PC1 is the axis of greatest variance, representing 56.6% of total variance, and showed a large contribution of root weight, depth and area in the same direction, suggesting this axis represents variance in overall size. PC2 described 19.1% of the total variance, and showed a large positive loading for diameter, but a large negative loading for width:depth. PC2 therefore represents variation in root systems from a large root diameter and small width:depth (i.e. deep, narrow root systems, with large root diameters), to small root diameters with large width:depth (i.e. wide, shallow root systems, with small root diameters). PC3 described 11.1% of total variance and showed a large negative contribution from shoot weight and a large positive contribution from root:shoot, suggesting this axis represents actual shoot weight, and shoot weight relative to root weight.

Table 5. Principal component analysis eigenvalues and eigenvector loadings for final 7 variables, for principal components 1 to 3, including the proportion of total variance in the dataset which they represent. Eigenvector loadings with an absolute value greater than 0.25 are in bold.

	PC1	PC2	PC3
Proportion of variance	0.5661	0.1910	0.1114
Cumulative proportion of variance	0.5661	0.7571	0.8685
Eigenvalues	1.9907	1.1563	0.8830
Eigenvector loadings:			
Root.weight	**0.4679**	-0.2309	0.0110
Shoot.weight	**0.3412**	-0.1773	**-0.7739**
R.S	**0.3940**	-0.1453	**0.6249**
Diameter	**0.2632**	**0.5484**	-0.0793
Depth	**0.4568**	0.1777	0.0620
Area	**0.4721**	-0.1437	0.0137
W.D	-0.0926	**-0.7357**	0.0129

Plotting PC scores averaged across replicates shows that drought treatments differ along the PC1 axis, suggesting high drought stress was associated with smaller root systems (Figure 6). Although less visible, temperature treatments could be distinguished along PC2 (Figure 6a), suggesting increased temperature leads to increased root diameters and smaller width:depth or, deep, narrow root systems. Grouping all treatments together, but separating the two types of

reaction norms (Figure 6b) showed that RNT1 genotypes had higher values for PC3 across treatments, suggesting smaller shoot weights and larger root:shoot ratio, or greater relative root weight.

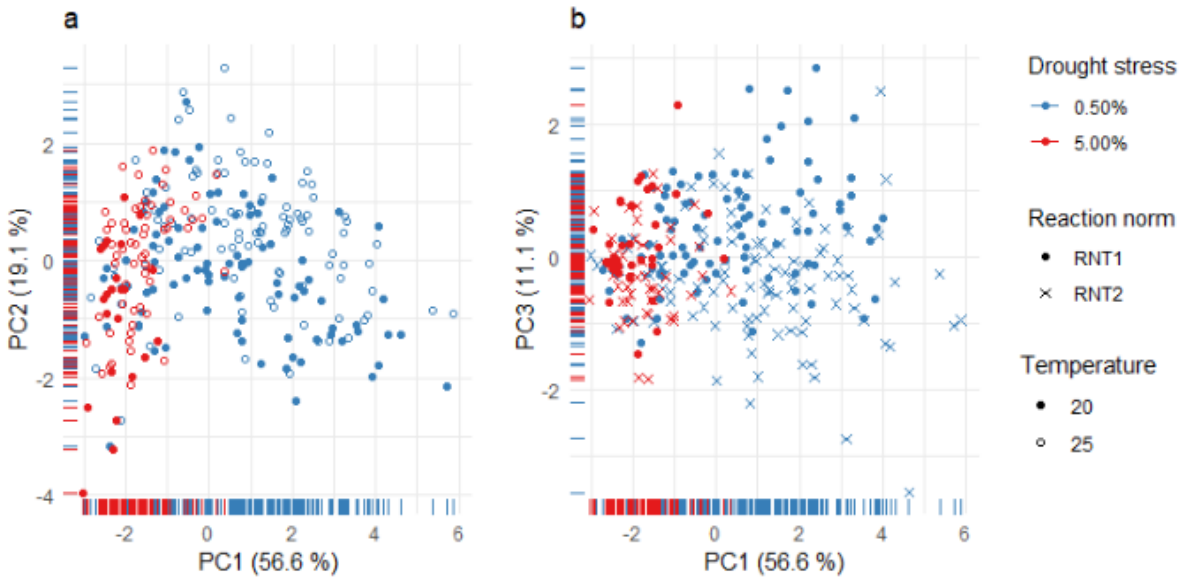

Figure 6. Principal component scores, averaged across each genotype replicate to give a point per genotype per treatment for Principal Components 1 and 2 (a) and Principal Components 1 and 3 (b).

2.3.3 Multivariate Trait Responses

I used linear mixed models to assess the fixed effects of temperature, drought and reaction norm, any interactions among these, and the random effect of genotype on PC1, PC2 and PC3 seperately.

The associations identified using linear mixed models match the associations between treatments and PC scores (e.g. PC1 and drought stress), and therefore root traits as described above. Specifically, PC1 was greater at higher drought stress, suggesting smaller root systems. PC2 was greater at higher temperatures, suggesting increased root diameters and smaller width:depth, or deep, narrow root systems. PC3 was higher in RNT1 genotypes suggesting genotypes which show higher relative fitness outside the range have smaller shoot weights and larger root:shoot ratio, or higher relative root weight.

Parameters with a large effect relative to variation in the dataset are identified by large t-values (Table 6), and predictions based on the results are plotted in Figure 7.

Table 6. Output of linear mixed models implemented on the three principal components. For each fixed effect, the mean (Estimate), standard error (SE), 95% confidence intervals (95% CI), z-value and t-value. The largest t-value for a main effect in each model is presented in bold.

Model	Parameter	Estimate	SE	95% CI		t-value
				Lower	Upper	
PC1	Intercept	0.265	0.218	-0.087	0.617	1.214
	Temperature	0.483	0.195	0.162	0.802	2.475
	Drought	-2.662	0.212	-3.007	-2.310	**-12.541**
	Reaction norm	0.386	0.253	-0.025	0.795	1.523
PC2	Intercept	-0.292	0.159	-0.548	-0.036	-1.839
	Temperature	0.715	0.132	0.498	0.932	**5.405**
	Drought	-0.449	0.144	-0.687	-0.214	-3.115
	Reaction norm	0.034	0.189	-0.273	0.339	0.179
PC3	Intercept	0.268	0.129	0.058	0.477	2.068
	Temperature	0.173	0.095	0.017	0.329	1.825
	Drought	-0.124	0.103	-0.293	0.047	-1.200
	Reaction norm	-0.680	0.161	-0.939	-0.419	**-4.229**

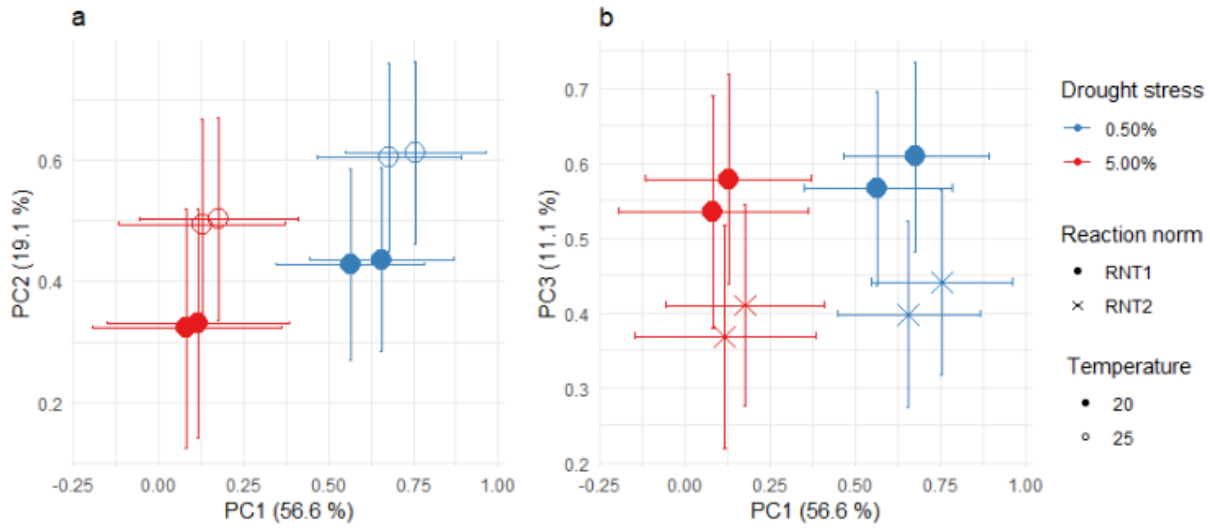

Figure 7. Linear mixed model predictions of principal component values, for all treatment combinations (two levels for drought stress, temperature and reaction norms) with standard error bars. Plotted by principal components 1 and 2 (a) and principal components 1 and 3 (b)

Interaction terms among the main effects were tested by sequential removal and models compared using likelihood ratio tests. There were no significant interactions between main effects (Table 7).

Table 7. Likelihood ratio tests comparing interaction terms when modelling PC1, PC2 and PC3 separately, against the fixed effects of reaction norm (RN), drought and temperature, modelling genotype with a random intercept.

Model structure	Compared to	PC1			PC2			PC3		
		χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value
RN:drought:temp + RN:drought + RN:temp + drought:temp + RN + drought + temp	Removed RN:drought:temp	0.57	1	0.452	0.31	1	0.575	0.15	1	0.696
RN:drought + RN:temp + drought:temp + RN + drought + temp	Removed RN:drought	0.82	1	0.366	0.02	1	0.882	2.39	1	0.122
RN:temp + drought:temp + RN + drought + temp	Removed RN:temp	0.68	1	0.408	1.54	1	0.214	0.01	1	0.932
drought:temp + RN + drought + temp	Removed drought:temp	0.07	1	0.789	1.33	1	0.249	0.04	1	0.838

I compared two linear mixed models with differing random effects structures (modelling genotype) using likelihood ratio tests, to test for GxE. I found no significant improvement in modelling PC1, PC2 or PC3, when genotypes were modelled with differing slopes, therefore no significant GxE (Table 8).

Table 8. Likelihood ratio tests comparing random effects structures when modelling PC1, PC2 and PC3 separately, against the fixed effects of reaction norm, drought and temperature, and all possible interaction terms. (1|gen) specifies that each genotype has a different, random intercept, where (temp*drought||gen) specifies that each genotype has a different, random intercept and slope, representing GxE in the model.

Random effects structure	Compared to	PC1			PC2			PC3		
		χ^2	df	p-value	χ^2	df	p-value	χ^2	df	p-value
(1|gen)	(temp*drought||gen)	10.30	16	0.851	7.79	16	0.955	2.54	16	0.999

I used a MANOVA to verify the PCA analysis. Although random effects cannot be accounted for in the MANOVA framework, there was no improvement of the model by incorporating genotype as an error term. Similar to the linear models, the main effects were significant, while no interaction terms were significant, with the exception of temperature:drought interaction which was significant in the MANOVA, but not in the linear mixed model (Table 4). This suggests there are subtle multivariate interaction effects identified using the multivariate approach, which may have been lost in the PCA or linear models.

Table 9. MANOVA of 7 traits against variables and interaction terms . Significant p-values highlighted in bold.

Variables	Approx. F-value	df	p-value
Temperature	8.109	7	**< 0.001**
Drought	28.352	7	**< 0.001**
Reaction norm	9.864	7	**< 0.001**
Temperature:drought	3.804	7	**< 0.001**
Temperature:reaction norm	0.589	7	0.765
Drought:reaction norm	1.438	7	0.190
Temperature:drought:reaction norm	0.394	7	0.905

2.4 Discussion

2.4.1 Root System Architecture in Response to High Temperature and Drought Stress

I hypothesised that high temperatures would lead to decreased root depth and root:shoot ratio, and increased root diameter. Although higher temperatures did lead to increased root diameters, it also led to deeper, narrower root systems. Even though this was not predicted, it may be an adaptive response to increased demand and decreased availability of water associated with higher temperatures. Shifting investment from lateral to the primary root is likely to increase water acquisition and anchoring, and increased root diameters improves water transport within the root system (Garnier *et al.*, 2015).

Drought stress was predicted to cause increased root depth, and decreased root system width. However, I found it led to overall smaller root systems (root weight, shoot weight, depth, diameter and area), with little effect on width:depth ratio. Reduced growth rate of roots (leading to overall smaller root systems) may be an adaptive response, because it reduces the amount of water required by the plant, however expected adaptive responses would be increased tap root depth to increase water acquisition, and increased root diameter to increase water transport in the root system (Garnier *et al.*, 2015).

All genotypes showed plasticity in root system architecture in response to temperature and drought stress, however it is not possible to differentiate between adaptive and maladaptive responses without fitness of the genotypes being recorded in this experiment.

The observed responses may be an example of a maladaptive plastic response. As previously mentioned (see Chapter 1.1 Plasticity) there are many possible scenarios that may lead to a maladaptive plastic response (Via *et al.*, 1995, Chevin *et al.*, 2012). Due to the experimental design, it is unlikely that any maladaptive plasticity is caused by a delay between the environmental cue and phenotype response. It is possible that root systems grown from seed (and not cuttings) would show a more adaptive response. It is also possible that the level of drought stress was higher than the species has been exposed to over evolutionary history, and therefore the adaptive reaction norm led to a maladaptive phenotype outside the experienced levels of abiotic stress. For example, smaller root systems may be an adaptive plastic response under lower levels of drought stress. Both low and high temperature treatments are temperatures the species experiences in the field, as recorded by dataloggers, and this experiment suggests root systems show adaptive responses to these temperatures. In contrast, drought stress treatments (based on concentrations and osmotic

potential calculations) are more difficult to equate to field recordings of humidity and precipitation, and therefore it is more likely that these treatments are beyond what the species experiences in the field, leading to the maladaptive response of root systems. It is also possible that the cost of an adaptive response to drought, under these specific conditions, is much greater and therefore the fitness increase from reaching the optimal phenotype is outweighed by the fitness cost of getting there, and therefore this response becomes maladaptive.

It is also possible that the predicted changes in root system architecture associated with drought stress (increased root depth and decreased root width) are a result of hydrotropism. In a permeable, low-water environment, gravity will cause water to move towards the bottom of a growth medium, causing hydrotropism to be stronger and in the same direction as gravitropism. This experiment used a homogeneous growth medium, meaning a consistent water distribution surrounding the root system, and removing hydrotropism. This may have contributed to discrepancy between the predicted and realised root system architecture responses.

Interestingly, although the response to drought stress was not the predicted, adaptive change in structure (shifting investment from lateral to the primary root), this response was identified under increased temperatures. This suggests overlapping mechanisms of abiotic stress response, likely to occur because both higher temperatures and reduced osmotic potential of the growth medium led to reduced water availability for roots.

2.4.2 Genotype-by-Environment Response

No GxE was identified in the tests of likelihood of a cutting to root or any principal components of multiple root traits. This suggests stabilising selection has reduced the variation in how genotypes respond, and therefore genetic variation in response to drought and temperature stress. The field experiment identified GxE in the field, in fitness across altitude. It was not possible to measure fitness in this experiment, and the abiotic stressor and levels in this experiment are different to the conditions which varied with altitude, therefore GxE may either not be present in root system architecture or very difficult to detect. As demonstrated by the temperature and drought stress root system architecture responses, it is possible that the levels of stressors chosen for this experiment are higher than those which the species is typically exposed to, and therefore alongside maladaptive plastic responses, this may reduce visible signals of GxE.

Walter *et al.* (2020) assessed differences in gene expression between species and altitudes for *S. aethnensis* and *S. chrysanthemifolius* genotypes. Although there were similar differences in gene expression between environments and genotypes for each species, *S. chrysanthemifolius*

showed only 37 genes differentially expressed between genotypes and environments (i.e. gene expression indicating GxE), whereas *S. aethnensis* showed 464 genes differentially expressed (Walter *et al.*, 2020) . This suggests that *S. chrysanthemifolius* (relative to *S. aethnensis*) may show relatively low GxE in the field compared to *S. aethnensis* (Walter *et al.*, 2020) .

2.4.3 Reaction Norm Type Response to High Temperature and Drought Stress

This experiment found differences in root system architecture between RNT1 and RNT2 genotypes. Differences in root system architecture between RNT1 and RNT2 genotypes contrasts with the lack of GxE identified among all genotypes in this study. This is likely because the genotypes are not a random selection from the population, but specifically chosen based on the change in their fitness across altitudes in the field. Due to this non-random sampling, it is difficult to say whether the lack of GxE among genotypes in this experiment reflects a lack of GxE in root system architecture across the species. However, it does suggest that differences in root system architecture between RNT1 and RNT2 genotypes identified in this experiment, contributed to differences in fitness of these genotypes across altitudes in the field.

As predicted, RNT1 genotypes had larger root:shoot ratios, for example RNT1 average root:shoot ratio was 0.277 compared to 0.228 for RNT2. However, root diameter did not differ (as was predicted), but they were found to have smaller shoot biomass (which was not predicted). This could also be described as genotypes which favour smaller shoots (in biomass, and relative to root biomass) are more likely to show increased relative fitness at high altitudes in the field. This suggests that adaptive plasticity in novel environments in *Senecio*, demonstrated by an increase in relative fitness at high altitudes in the field, may be linked to larger root:shoot ratios and greater investment in root biomass. The higher relative fitness in RNT2 individuals at low altitude sites, suggests that a small root:shoot ratio and greater investment in shoot biomass increases fitness at low altitude, within home range sites.

Although the average Mediterranean climate leads to periods of drought through the summer months (Ross *et al.*, 2012), Walter *et al.* (2020) field study was conducted at a range of altitudes of Mt Etna, involving more complex variation in climate. The periods of drought experienced at low altitudes, contrast with the periods of frost at high altitudes, with data loggers at 1 500 m and 2 000 m sites regularly dropping below 0 °C, and at 500 m and 1 000 m sites regularly exceeding 40 °C (Walter *et al.*, 2020). There is also a gradient in soil type from nutrient rich agricultural land at low altitudes to fine, volcanic soil, and a reduction in organic material and total nitrogen at high altitudes (Walter *et al.*, 2020). Increased root:shoot ratio is likely to be adaptive at

high altitudes due to increased anchoring in unstable soil and increased nutrient acquisition in nutrient sparse soils relative to low altitudes (Garnier *et al.*, 2015). It is also known that nitrate deficiency (possible in high altitude soils) leads to increased primary root length, and decreased lateral root branching angle and number (Koevoets *et al.*, 2016), however these root system architecture responses were not observed and nitrate levels not altered in this experiment. It is thought that high altitude adaptations include rapid germination, growth and flowering between frosts to increase chances of survival into the following year, for example in *S. aethnensis* (Ross *et al.*, 2012), however growth rate was not recorded in this study. By contrast, increased shoot biomass as identified in RNT2 genotypes, is likely to be adaptive at low altitudes in order to outcompete neighbouring plants, to receive as much sunlight as possible and maximise photosynthetic potential.

It is important to note that comparisons are being made between two separate studies. One assessing fitness across altitude, and another assessing root traits under drought and temperature stress. Although associations can be made between genotype behaviour in both studies, we did not record how root traits responded in the field, nor their fitness in the laboratory.

2.5 References

Bates, D., Machler, M., Bolker, B. M. & Walker, S. C. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software,* 67**,** 1-48.

Bengough, A. G., Gordon, D. C., Al-Menaie, H., Ellis, R. P., Allan, D., Keith, R., Thomas, W. T. B. & Forster, B. P. 2004. Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant and Soil,* 262**,** 63-70.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. & White, J. S. S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution,* 24**,** 127-135.

Chen, Y. L., Djalovic, I. & Rengel, Z. 2015. Phenotyping for root traits. *In:* Kumar, J., Pratap, A. & Kumar, S. (eds.) *Phenomics in Crop Plants: Trends, Options and Limitations.* 1 ed. India: Springer.

Chevin, L. M., Gallet, R., Gomulkiewicz, R., Holt, R. D. & Fellous, S. 2012. Phenotypic plasticity in evolutionary rescue experiments. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 368**,** 12.

Engels, C. 1994. Effect of root and shoot meristem temperature on shoot to root dry-matter partitioning and the internal concentrations of nitrogen and carbohydrates in maize and wheat. *Annals of Botany,* 73**,** 211-219.

Fullner, K., Temperton, V. M., Rascher, U., Jahnke, S., Rist, R., Schurr, U. & Kuhn, A. J. 2012. Vertical gradient in soil temperature stimulates development and increases biomass accumulation in barley. *Plant Cell and Environment,* 35**,** 884-892.

Galkovskyi, T., Mileyko, Y., Bucksch, A., Moore, B., Symonova, O., Price, C. A., Topp, C. N., Iyer-Pascuzzi, A. S., Zurek, P. R., Fang, S. Q., Harer, J., Benfey, P. N. & Weitz, J. S. 2012. GiA Roots: software for the high throughput analysis of plant root system architecture. *BMC Plant Biology,* 12**,** 12.

Garnier, E., Garnier, E., Navas, M.-L., Navas, M. L., Grigulis, K. & Grigulis, K. 2015. *Plant Functional Diversity: Organism traits, community structure, and ecosystem properties*, Oxford University Press.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R Package. *Journal of Statistical Software,* 33**,** 1-22.

Hargreaves, C. E., Gregory, P. J. & Bengough, A. G. 2009. Measuring root traits in barley (Hordeum vulgare ssp vulgare and ssp spontaneum) seedlings using gel chambers, soil sacs and X-ray microtomography. *Plant and Soil,* 316**,** 285-297.

Iyer-Pascuzzi, A. S., Symonova, O., Mileyko, Y., Hao, Y. L., Belcher, H., Harer, J., Weitz, J. S. & Benfey, P. N. 2010. Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. *Plant Physiology,* 152**,** 1148-1157.

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M. & Testerink, C. 2016. Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science,* 7**,** 19.

Mandal, A. K. A., Chakrabarty, D. & Datta, S. K. 2000. In vitro isolation of solid novel flower colour mutants from induced chimeric ray florets of chrysanthemum. *Euphytica,* 114**,** 9-12.

Nguyen, Q. T., Kozai, T. & Van Nguyen, U. 1999. Effects of sucrose concentration, supporting material and number of air exchanges of the vessel on the growth of in vitro coffee plantlets. *Plant Cell Tissue and Organ Culture,* 58**,** 51-57.

Qin, L., He, J., Lee, S. K. & Dodd, I. C. 2007. An assessment of the role of ethylene in mediating lettuce (Lactuca sativa) root growth at high temperatures. *Journal of Experimental Botany,* 58**,** 3017-3024.

R Core Team 2019. R: A language and environment for statistical computing.

Ross, R. I. C., Agren, J. A. & Pannell, J. R. 2012. Exogenous selection shapes germination behaviour and seedling traits of populations at different altitudes in a Senecio hybrid zone. *Annals of Botany,* 110**,** 1439-1447.

Seiler, G. J. 1998. Influence of temperature on primary and lateral root growth of sunflower seedlings. *Environmental and Experimental Botany,* 40**,** 135-146.

Via, S., Gomulkiewicz, R., Dejong, G., Scheiner, S. M., Schlichting, C. D. & Vantienderen, P. H. 1995. Adaptive Phenotypic Plasticity - Consensus and Controversy. *Trends in Ecology & Evolution,* 10**,** 212-217.

Walter, G. M., Clark, J., Cristaudo, A., Nevado, B., Catara, S., Paunov, M., Velikova, V., Filatov, D., Cozzolino, S., Hiscock, S. J. & Bridle, J. R. 2020. Adaptation to contrasting habitats underlies distinct plastic responses to environmental variation in two closely related Senecio species. *bioRxiv*.

Walter, G. M., Clark, J., Terranova, D., la Spina, E., Giovanna Majorana, M., Pepe, G., Cristaudo, A., Cozzolino, S., Hiscock, S. J. & Bridle, J. R. in preparation. Adaptive plasticity and evolutionary rescue intertwined.

Woo, S. H., Park, J. M. & Yang, J. W. 1997. Induction of branch roots by cutting method in Hyoscyamus niger root culture. *Plant Cell Tissue and Organ Culture,* 48**,** 131-134.

2.6 Supplementary Material

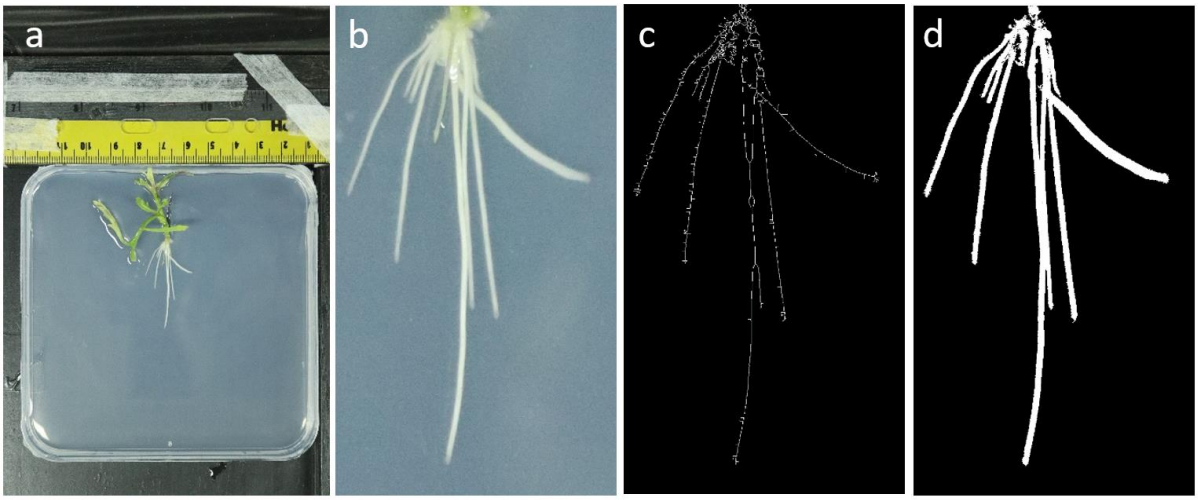

Figure S1. GiaRoots processing images. The input photograph of the cutting on the agar plate (a), cropped to remove the shoot (b), thinned (c), and once the threshold parameters have been applied (d).

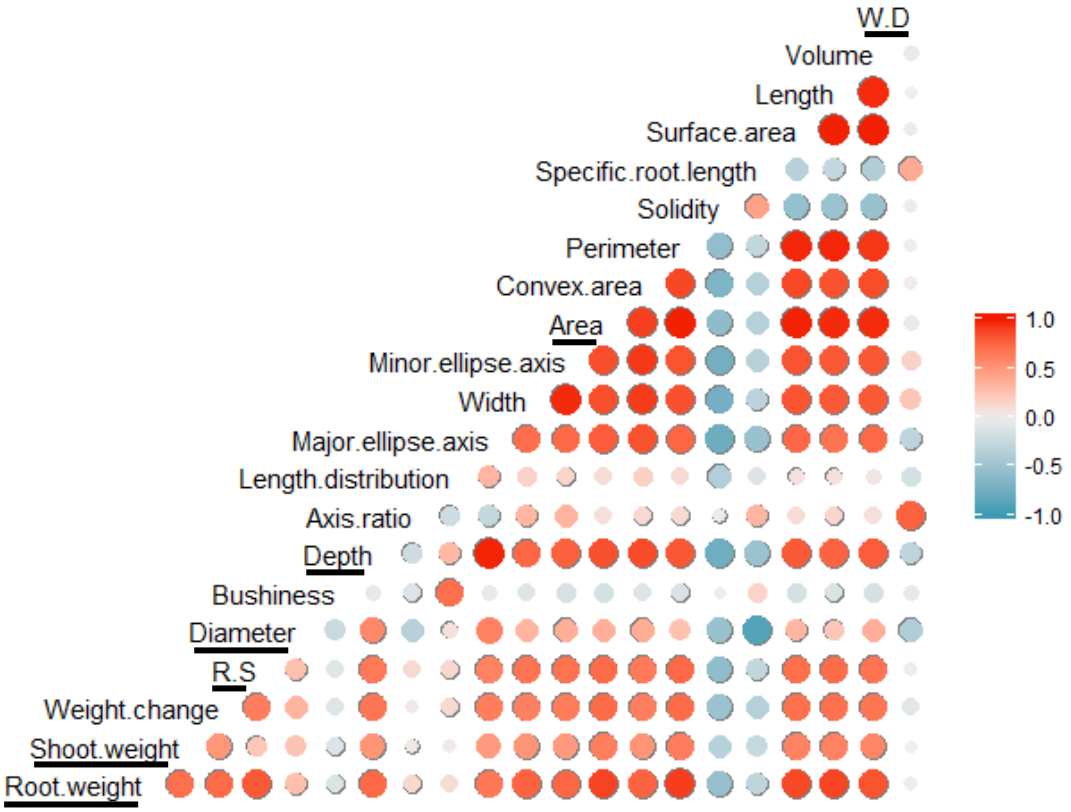

Figure S2. Correlation matrix of 21 variables , to identify highly correlated traits which could be removed from further analyses. Size and colour of circle represents strength and direction of correlation, as shown in the legend. Variables underlined are those selected for further analyses.

Table S1. Principal component analysis eigenvalues and eigenvector loadings for each variable , for principal components 1 to 3, including the proportion of total variance in the dataset which they represent. Loadings of more than 0.25 or less than -0.25 are indicted in bold. Variables with an asterisk are those selected for further analyses.

	PC1	PC2	PC3
Proportion of variance	0.568	0.135	0.086
Cumulative proportion of variance	0.568	0.703	0.789
Eigenvalues	3.453	1.684	1.343
Eigenvector loadings:			
Root.weight*	**-0.259**	-0.089	-0.007
Shoot.weight*	-0.181	-0.045	-0.081
Weight.change	-0.222	0.007	-0.014
R.S*	-0.222	-0.049	0.050
Diameter*	-0.137	**0.381**	**-0.256**
Bushiness	0.041	0.104	**0.656**
Depth*	**-0.263**	0.182	0.051
Axis.ratio	-0.017	**-0.504**	0.036
Length.distribution	-0.052	0.217	**0.627**
Major.ellipse.axis	**-0.251**	0.216	0.057
Width	**-0.259**	-0.141	0.075
Minor.ellipse.axis	**-0.262**	-0.121	0.043
Area*	**-0.281**	-0.062	-0.028
Convex.area	**-0.269**	-0.042	0.045
Perimeter	**-0.276**	-0.097	0.010
Solidity	0.212	-0.113	-0.150
Specific.root.length	0.134	**-0.367**	0.225
Surface.area	**-0.275**	-0.082	-0.038
Length	**-0.268**	-0.117	-0.005
Volume	**-0.270**	-0.062	-0.061
W.D*	0.019	**-0.478**	0.078

Table S2. Coefficients of linear discriminants from three linear discriminant analyses between temperature groups, drought stress groups, and treatments. Coefficients of more than 3 or less than -3 are indicted in bold. Variables with an asterisk are those selected for further analyses.

	LDA1: temp	LDA2: drought	LDA3: treatments		
			LD1	LD2	LD3
Proportion of trace			0.690	0.221	0.089
Root.weight*	-1.231	-0.523	-0.177	1.076	-0.768
Shoot.weight*	0.082	0.174	0.143	0.081	0.480
Weight.change	-0.254	-0.796	-0.723	0.154	-0.608
R.S*	0.437	0.162	0.031	-0.223	0.652
Diameter*	0.605	0.261	0.111	-0.881	-0.479
Bushiness	-0.109	0.313	0.351	-0.019	-0.198
Depth*	-1.709	-0.601	-0.101	1.144	-1.869
Axis.ratio	0.435	0.844	0.732	-0.515	0.216
Length.distribution	0.303	-0.240	-0.336	-0.091	0.462
Major.ellipse.axis	0.785	-0.008	-0.217	-0.906	-0.186
Width	-0.582	-0.531	-0.378	0.681	-0.061
Minor.ellipse.axis	-1.021	-0.761	-0.484	1.049	-0.391
Area*	**-7.455**	-1.614	0.391	**8.205**	0.104
Convex.area	1.527	1.033	0.622	-1.623	0.408
Perimeter	**5.892**	2.228	0.664	**-6.792**	-0.438
Solidity	-0.766	0.098	0.331	0.381	-1.009
Specific.root.length	0.394	0.374	0.287	-0.757	-0.673
Surface.area	**19.457**	**3.651**	-1.753	**-18.349**	**6.978**
Length	**-11.267**	-2.997	0.076	**11.581**	-2.074
Volume	**-5.713**	-0.701	0.925	**4.715**	**-3.537**
W.D*	-0.519	-0.372	-0.211	0.167	-1.086

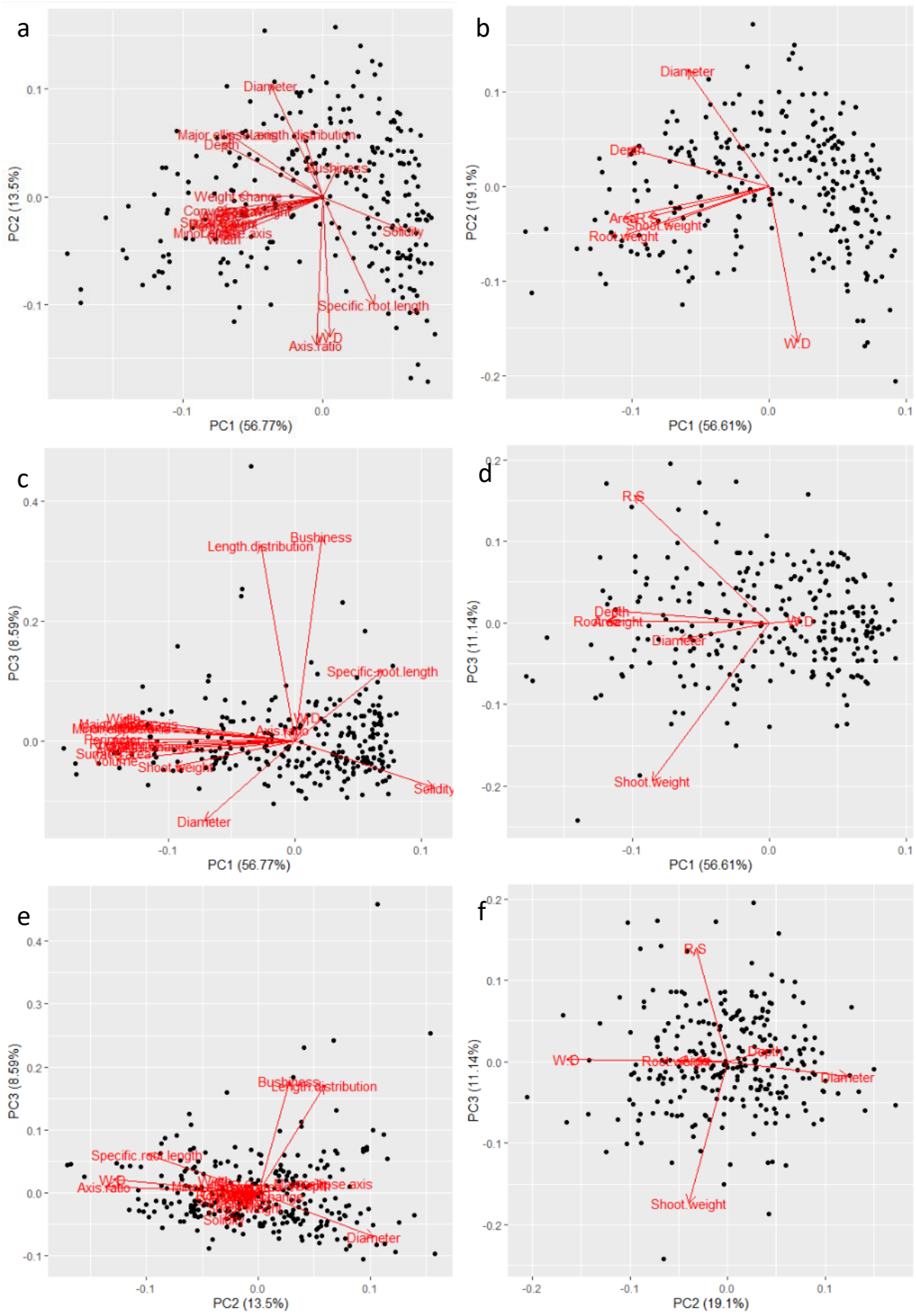

Figure S3. Principal component analysis biplots using 21 traits (a, c, e) compared to using 7 traits (b, d, f).

3 Chapter 3: General Discussion

Roots are highly complex systems, influenced by underlying genetic associations, and a broad range of environmental factors (for example, hydrotropism) leading to complex and interacting physiological responses. In order to understand each of these factors it is important to be able to isolate and observe the effects of each across a range of genotypes and species.

I assayed the sensitivity of root architecture to variation in temperature and drought using genotypes of *S. chrysanthemifolius*. Understanding the sensitivity of root systems is important for managing agriculture, land erosion and water retention. These are increasingly significant areas of research in the context of a growing population, with increasing food demands, and given climate change predictions of increased risk of floods and extreme weather events. In addition, understanding these interactions will enable us to use our knowledge of root system architecture in agriculture and crop species to maximise crop yield across a range of environments, building resilience to extreme weather events, and increasing soil stability and water retention. For example, QTL analysis has already been used to improve grain yield in rice under drought (Bernier *et al.*, 2009).

Using genotypes identified in the field study allows us to connect results of the root responses under controlled conditions to fitness responses across an altitude gradient in the field. Specifically, genotypes which showed increased relative fitness at high altitudes in the field, had larger root:shoot ratios and smaller shoot biomass in this study. This could represent an adaptive response to the unstable, nutrient sparse soils found at high altitudes. In contrast, genotypes that showed increased relative fitness at low altitudes in the field, had smaller root:shoot ratios and larger shoot biomass, possibly an adaptive response at low altitudes where soil is nutrient rich and therefore competition higher.

3.1 Future work

This study has emphasised the importance of understanding how plasticity affects a genotype's fitness to clarify when plasticity shifts from adaptive to maladaptive. As discussed in Section 1.3, plasticity has the potential to enable evolutionary rescue by moving a phenotype towards the new optimum after an environmental change, providing time for evolutionary rescue. This study has investigated plasticity of root systems, highlighting the role of adaptive plasticity in root system architecture in Walter *et al.* (2020) field study, in which root system architecture plasticity is likely to have played a minor role in moving phenotypes towards a novel optimum at high altitudes. Future work could use fitness measurements to assess whether observed responses

are adaptive or maladaptive, and using long-term studies assessing genetic changes will have the potential to identify at what point evolutionary rescue occurs.

For this study, methods were developed to sterilise and grow *S. chrysanthemifolius* cuttings in a transparent, 2D medium to enable high-throughput root phenotyping. Methods of imaging and processing these root systems were also developed for this work. However, for future studies this work could be improved by preventing light reaching the roots, allowing more substantial root (and shoot) growth, and producing a temperature gradient by depth, as would exist in a soil environment.

There is also more potential for unpicking the different factors influencing plastic responses of roots, including the quantitative genetics of roots traits (their heritabilities etc.) and molecular mechanisms of observed physiological responses. Equally, only drought and temperature stressors were assessed in this study, however phosphate and nitrate deficiency are common and known to strongly influence root system architecture.

3.2 References

Bernier, J., Serraj, R., Kumar, A., Venuprasad, R., Impa, S., Gowda, R. P. V., Oane, R., Spaner, D. & Atlin, G. 2009. The large-effect drought-resistance QTL qtl12.1 increases water uptake in upland rice. *Field Crops Research,* 110**,** 139-146.

Walter, G. M., Clark, J., Cristaudo, A., Nevado, B., Catara, S., Paunov, M., Velikova, V., Filatov, D., Cozzolino, S., Hiscock, S. J. & Bridle, J. R. 2020. Adaptation to contrasting habitats underlies distinct plastic responses to environmental variation in two closely related Senecio species. *bioRxiv*.