Germination success and drought response in *Erica coccinea*

Jenny Leonard

Thesis presented for the degree of Master of Science

in the Department of Biological Sciences

University of Cape Town

December, 2014

Supervisors: Dr. Adam West and Dr. Fernando Ojeda

Plagiarism declaration

I know the meaning of plagiarism and declare that all of the work in the thesis, save for that which is properly acknowledged, is my own.

Abstract

Resprouters and seeders are two common phenotypes found in fire-prone ecosystems. Although the distribution of the two forms is usually attributed to fire frequency, it has been proposed that the distribution of resprouter and seeder *Erica* in the Cape Floristic Region (CFR) of South Africa is determined more by water availability. *Erica* seeders are predicted to withstand the mild droughts of the southwest CFR better than *Erica* resprouters, which would account for the abundance of seeders in this region. This thesis tested the assumptions that 1) seeders germinate more quickly and successfully than resprouters and 2) seeders survive mild drought better than resprouters. A germination experiment (Chapter 2) and a drought experiment (Chapter 3) were conducted using *Erica coccinea*, a common *Erica* species in the CFR, which contains both a resprouter and a seeder form. Germination success was also tested for a third form of *E. coccinea* found only in fire refugia. I predicted that this form would not require smoke as a cue for germination. Results indicated that (1) resprouters germinated faster than seeders in the presence of smoke, (2) seeders had better germination success than resprouters in the absence of smoke, (3) the "pyrofuge" form did not require smoke to germinate and (4) seeders had lower survival than resprouters during drought. Overall, these results refuted the proposition that *E. coccinea* seeders have improved germination and drought tolerance. However, variation between populations within the seeder form indicated that more populations should be tested to verify that these results represent the species as a whole. Due to the lack of variation between populations of the "pyrofuges", it is clear that this form has adapted to its' fire-free environment by allowing for germination in the absence of fire.

Acknowledgements

It is strange to me that acknowledgements, like abstracts, are presented at the beginning of a thesis. To me, they are the ultimate conclusion. You may only acknowledge, understand, recognize and admit at the end of a process.

At times, I was a fairly unwilling passenger of my thesis. Perhaps it was a simple mismatch between project and personality. Perhaps if things had been different, we would have matched. Perhaps everyone experiences this with their thesis at some point. For the sake of acknowledgement, I would like to admit that this was hard.

It was the people in my life who helped me find my way through and showed me that I could enjoy myself. The two big names were my supervisors, Dr. Adam West (University of Cape Town) and Dr. Fernando Ojeda (University of Cadiz). Their enthusiasm and excitement for the project fed my interest and confidence. There were also the numerous support staff members that, although it often wasn't their job, went out of their way to make sure I had everything I needed. I would like to thank Des for providing space for the germination and drought experiments, for being patient with my when my time lines changed, and for playing awesome music in the soils lab. I would like to thank Dawood for being reliable and for understanding that I learn best by discussing the task at hand and for happily walking me through the steps for the soluble sugars and starch analyses. And last, but definitely not least, I would like to thank Zide for spending countless hours helping me propagate my plants and being such good company during that process.

I would like to thank my field assistant friends: Rebecca, Amy and Andy for the epic trip to De Hoop; Michelle for the disastrous Stellenbosch trip in which the car broke down and for the many, many other adventures over the fantastic years of our

friendship; and Taariq for being the keenest matric student I have ever met and the best help at isolating root material. You will go far.

I would like to thank my family and friends for keeping me sane, fed and happy. Thank you to my friends Cathy, Eleanor, Kate, Nick, Leila, Justin, everyone in Game of Zones and UCT Ultimate, and my colleagues at the Writing Centre and CHED. A special thanks to my darling housemate Sandy who was the only other person besides my supervisors to read and comment on the whole thing. Thanks Sandy! Thank you to my family Dean, Kim, Katie, June, Steve, JJ, Alexis, Hanlie, Neil, Colin and my grandparents for words of encouragement, for believing in me, and for always being ready to distract me from my thesis by asking about the wedding plans.

I would like to thank Thomas, my fiancé, for his patience, compassion and love. I do not know where or what I would be without you. Thank you for always being willing to provide macaroons and tea and for your firm belief (always) that everything will be okay.

And where would I be without my examiners? Reading a thesis can be a thankless task – I am so grateful for your time and would like to thank you for committing to reading this document. You deserve your favourite beverage and a snack before you continue. I hope you enjoy the read.

Table of Contents

Chapter 1: Introduction to resprouters and seeders

1.1 Adaptation to disturbance

Disturbance is one of the many factors that shapes ecosystems. Not only does it physically change the ecosystem (e.g. landslides, windstorms and floods), but it also causes a secondary change in the plant life through the plant's ability to adapt. Plants have developed a variety of different mechanisms to withstand different kinds of disturbance. Evidence of these adaptations can be found all over the world. In Amazonian floodplains, the seeds of several tree species have a greater longevity in a submerged state than a dry state (Parolin *et al.*, 2004). In California, Monterey pines have thicker bark in areas with historically frequent fires (Stephens & Libby, 2006). In South Africa, many species in the fynbos biome flower or germinate in response to fire (Le Maitre & Midgley, 1992). These are only a few examples of plant traits adapted to disturbance-prone environments.

Fire is a common disturbance in some ecosystems. It is able to act as a driving force of change in ecosystems that have oxygen, fuel and a source of ignition (Bond & Keeley, 2005). In their review of the history of fire, Pausas & Keeley (2009) lay out a timeline that marks the origin of fire to be at least 440 million years ago. The spread of C4 grasses (Keeley & Rundel, 2005) and angiosperms in general (Bond & Scott, 2010) have been attributed to fire, as well as the tremendous diversification of terrestrial vegetation in the late Paleozoic (Scott & Glasspool, 2006).

Although fire initially makes the landscape appear destroyed, plants have adapted to propagate under and withstand these conditions. Resprouters and seeders are two main regeneration forms found in fire-prone environments (Wells, 1969; Keeley & Zedler, 1978; Bond & van Wilgen, 1996; Verdú, 2000; Pausas & Keeley, 2014). They each have distinct strategies for post-disturbance reestablishment. Resprouters are able to survive and grow new material after a disturbance by resprouting from dormant subterranean or basal buds, or occasionally from horizontal rhizomes or roots (Bond & van Wilgen, 1996; Pausas & Keeley, 2014). Basal buds can be found on a swollen lignotuber in some plants and are a common feature in *Eucalyptus* and some Proteaceae*,* Rosaceae and Ericaceae. Resprouters invest carbon resources into resprouting structures in addition to investing in seeds for regeneration (Bond & van Wilgen, 1996). Please note that in this thesis, the term "resprouter" is synonymous with "facultative seeder" (e.g. Pausas & Keeley, 2014) or "facultative resprouter" (e.g. Thomas *et al.*, 2010), a description used for plants with seeds that germinate after a fire as opposed to an "obligative resprouter" which has seeds that do not survive fire (Pausas & Keeley, 2014).

Fire-cued seeders ("obligate seeders", sensu Pausas & Keeley, 2014) lack the ability to resprout and divert their resources to creating a seed bank and investing in above-ground biomass instead of a resprouting structure (Bond & van Wilgen, 1996). It is suspected that seeders are derived from resprouters (Wells, 1969; Bond & van Wilgen, 1996; Verdaguer & Ojeda, 2005; Pausas & Keeley, 2014), though this has not been determined for all species (Pausas & Keeley, 2014). For seeders, fire destroys the adult plant, but also triggers germination of the seed bank (Keeley & Bond, 1997; Bell, 2001). Seeders generally produce more seeds than resprouters, having traded the ability to resprout for the production of more seeds (Bellingham & Sparrow, 2000) as well as more above ground growth and leaf production (Verdú, 2000). Seeder populations are usually even-aged due to the adults dying by fire and the subsequent germination of the seed bank (Wells, 1969; Bond & van Wilgen, 1996; Pausas & Keeley, 2014).

1.2 Distribution of resprouters and seeders

Resprouter and seeder forms across many genera can be found in fire-prone ecosystems in California, Australia, the Mediterranean basin and South Africa (e.g.

Le Maitre & Midgley, 1992; Brown, 1993; Keeley & Bond, 1997; Bell, 2001; Pausas *et al.*, 2006; Keeley *et al.*, 2011). If seeders and resprouters were equally adapted to fire, their numbers would be fairly uniform in the places that they are found. However, the balance is unequal among regions and genera. In southwestern Western Australia, 49-75% of plants will be resprouters, depending on the community (Bell, 2001). The resprouter percentage in California is 44-50% (Bell, 2001). Other Mediterranean-type climates lack reseeding forms almost entirely and are instead dominated by resprouting evergreen trees and shrubs (Bell, 2001). In the Cape Floristic Region (CFR) of South Africa, woody species are primarily seeders (>80% in several genera; Le Maitre & Midgley, 1992). An example of this is the species-rich *Erica* genus, where only 10% of species are resprouters while the rest are seeders (Le Maitre & Midgley, 1992; Ojeda, 1998). Even within the ericas of the CFR, the pattern is not homogeneous. For instance, there are more resprouter species in the northwest CFR and more seeders in the southwest CFR (Ojeda, 1998).

1.3 Hypotheses for these distributions

Variation in distribution shows that these phenotypes are differentially adapted to their environments. The main factor that is evoked to explain these differences is differences in fire regime, particularly ‘fire frequency’. If fires are too frequent, seeders may not reach maturity in time to set seed before the next fire (van Wilgen *et al.*, 1992). This would lead to more resprouters in the community. If fires are infrequent, seeders would outcompete resprouters because of their higher allocation to seed production (Bellingham & Sparrow, 2000), leading to more seeders in the community.

The relationship between resprouter and seeder distribution and the frequency of fire has also been interpreted in terms of the 'available gaps' (Keeley & Zedler, 1978). If fires are more frequent, fires will be less intense due to a shorter duration of fuel accumulation. In this scenario, fewer resprouters will die in the fire, creating fewer gaps for seedings. However, if fires are less frequent, resprouters might die naturally before fires, reducing the potential for resprouting. Because of the accumulated biomass (both from dead plants and from the extended inter-fire growth of live plants), infrequent fires will burn more intensely and more resprouters will die in the fire. This results in larger gaps for seedlings to occupy. If seeders are able to produce more seeds than resprouters, seeders would dominate in post-fire environments where fires occur less frequently (Keeley & Zedler, 1978).

Although the 'available gaps' hypothesis and the ‘fire frequency’ hypothesis are slightly different, their end result is the same; resprouters are expected to have higher success under high-frequency fire regimes, and seeders are expected to have higher success under low-frequency fire regimes. This explanation holds for some resprouter and seeder patterns, but not all. For instance, it does not explain the distribution of the resprouter and seeder *Erica* of the CFR (Ojeda, 1998). Although there are alternative hypotheses that emphasise factors besides fire (e.g. Bond & van Wilgen, 1996; Midgley, 1996), the only hypothesis available that might explain the distribution of *Erica* in the CFR relates to seedling recruitment and the pressures of summer drought (Ojeda, 1998; Ojeda *et al.*, 2005).

Ojeda (1998) constructed a geographical distribution of ericas in South Africa and, within that, the CFR. Although the CFR ericas are predominately seeders, he found that resprouters can co-exist with the seeders in the northwest and east of the CFR, whereas resprouters become uncommon in the southwest (Figure 1.1). Ojeda (1998) suggested that physiological differences between resprouters and seeders at a seedling stage could be the reason for differential success in these areas instead of fire, which is fairly uniform throughout the region and has an average fire return interval of 10-13 years (Van Wilgen *et al.*, 2010) with most fires occurring in the summer (Le Maitre & Midgley, 1992). It is predicted that seeder seedlings would invest more material in aboveground biomass (e.g. Paula & Pausas, 2006) than resprouter seedlings, which would use resources to develop belowground resprouting organs instead. Resprouters spend energy on swollen underground structures such as root-crowns and burls to house dormant buds as well as organs for starch storage that act as an energy reserve to promote growth after a disturbance (Verdaguer & Ojeda, 2002). These organs are considered to be metabolically expensive to maintain (see Verdaguer & Ojeda, 2002). The balanced allocation of above and below ground growth allows seeder seedlings to be more resilient than resprouter seedlings during the first summer of water stress. The improved drought response of seeders has been tested and shown in other studies (e.g. Vilagrosa *et al.*, 2014).

Ojeda (1998) specifically predicted that severe droughts, which are most likely to occur in the northwest, would hinder recruitment in both forms, allowing resprouters to persist by resprouting after drought. In the east, aseasonal rainfall is predicted to favour overall recruitment, which allows the coexistence of seeders and resprouters. However, the southwest, an area with a mild summer drought, would favour the seeders over the storage-oriented resprouter. A simple simulation model confirmed that the seeder form can invade and replace the resprouter form under moderate summer drought in the CFR (Ojeda *et al.*, 2005).

Another contributing factor to the improved survival predicted for seeders under drought is the possibility of early emergence in seeder seedlings. The high generational turnover of the seeder would allow natural selection to act more frequently and thereby speed up evolution (Wells, 1969; Pausas & Keeley, 2014). Early emergence has been linked to a long-term increase in fitness benefits such as growth, survival and fecundity (de Luis *et al.*, 2008). In the case of the CFR ericas, it

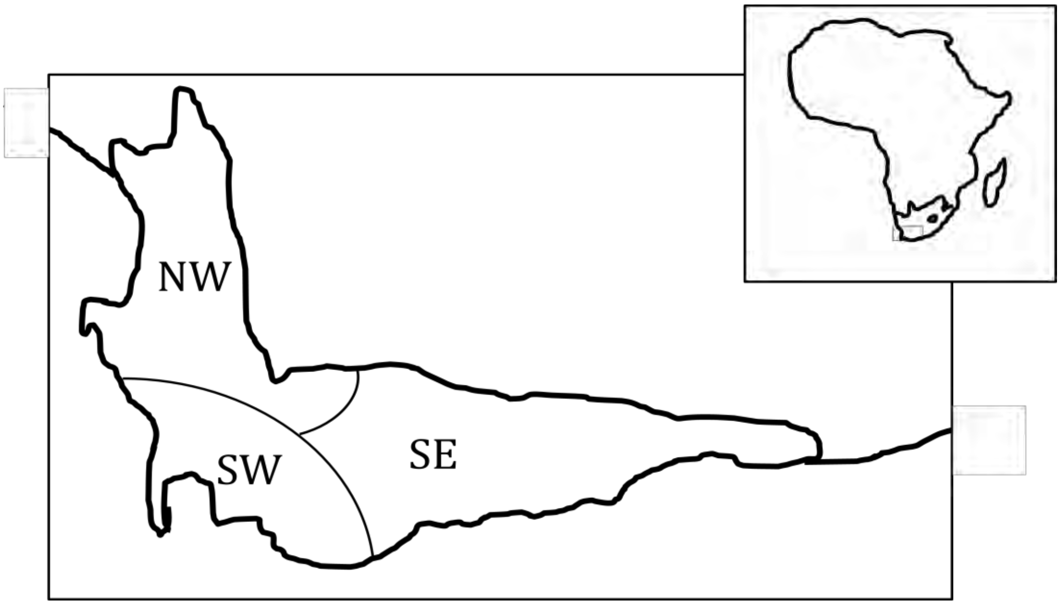

Figure 1.1: Outline of the Cape Floristic Region (CFR). The northwest is more arid and experiences more severe droughts, the east is aseasonal and the southwest has a mild summer drought. *Erica* resprouters co-exist with seeders in the northwest and eastern CFR, but are less common in the southwestern CFR. Adapted from Ojeda *et al.*, 2005.

is possible that seeders have adapted to respond more quickly to a fire cue. If they are able to germinate more quickly after a fire, they would be larger and more established during the first drought season. This could give them an additional competitive advantage over resprouters in a mild drought scenario.

1.4 How do we evaluate these predictions?

In order to test the hypothesis (Ojeda, 1998) and the subsequent model (Ojeda *et al.*, 2005) that predicts mild drought as the reason for resprouter and seeder distributions in CFR ericas, it is important to find closely related species to minimize the interaction of other variables (e.g. Schwilk & Ackerly, 2005). There are several ericas from the CFR that contain both the resprouter and seeder forms within the species. *Erica coccinea* is one such species (Figure 1.2). It also contains an additional third form, which will be discussed in more detail in Chapter 2. Besides having both forms, *E. coccinea* is an ideal model for this study because a solid foundation of knowledge has already been built from previous studies involving this species.

Erica coccinea is a widespread *Erica* species that grows to be 1-2 meters high (Schumann & Kirsten, 1992) on south-facing slopes in the CFR (Malan, 2013). Flowers are usually red or yellow (with some populations offering orange, pink and greenish) and are pollinated by sunbirds (Schumann & Kirsten, 1992; Malan, 2013). Flowering and seed dispersal times are segregated between forms (Malan, 2013) with seeders flowering mostly in winter-spring and resprouters flowering in summer-autumn. *Erica coccinea* has a symbiotic association with mycorrhizae, a preference for nutrient-poor soils characteristic of the fynbos, and improved germination in the presence of smoke (N. Zide, pers. com).

The seeder form of *E. coccinea* is found throughout the southwestern CFR, with resprouters only found in higher altitude coastal areas that receive reliable rainfall (Ojeda, 1998). *Erica coccinea* is a resprouter in the northern CFR (characterized by moderate to severe summers), the southern Langeberg (aseasonal) and the eastern CFR (aseasonal; Ojeda, 1998). ‘Mixed’ populations (i.e. containing both seeder and

Figure 1.2: *Erica coccinea* seeder adults at the end of their flowering season from the population in Potberg (De Hoop Nature Reserve). Photo credit: Rebecca Karpul.

resprouter individuals) have been found in the southwestern CFR (Ojeda, 1998; Malan, 2013). Although both forms can occur in the same population, the forms are genetically determined rather than a result of phenotypic plasticity (Verdaguer & Ojeda, 2002). Adult resprouter individuals have a higher starch content than seeders in their roots as well as more specialized starch-storing organs, i.e. major and minor parenchymatic rays (Bell & Ojeda, 1999). This has also been found at a very early stage in seedlings (see Verdaguer and Ojeda 2002). Verdaguer & Ojeda (2005) discovered that *E. coccinea* resprouters have active basal buds while seeders only have atrophied buds. The presence of atrophied buds indicates that seeders are most likely the derived state, as is the case with other seeder lineages (Pausas & Keeley, 2014).

Using microsatellite markers (developed by Segarra-Moragues *et al.*, 2009), it was found that there is more genetic diversity among and within seeder populations than resprouter populations (Segarra-Moragues & Ojeda, 2010). This is attributed to higher population turnover rates in seeder populations during post-fire recruitment (Wells, 1969; Segarra-Moragues & Ojeda, 2010; Pausas & Keeley, 2014). Average genetic variation across resprouter and seeder groups is only three percent, which makes the possibility of these groups being cryptic taxa unlikely (Segarra-Moragues & Ojeda, 2010).

1.5 Aims and thesis outline

This thesis tested the hypothesis (Ojeda, 1998) and the subsequent model (Ojeda *et al.*, 2005) that predicts the physiological advantage of *Erica* seeder seedlings over *Erica* resprouter seedlings in a mild drought scenario in CFR using *E. coccinea* as a model organism. I explored two possible mechanisms for differential success between *E. coccinea* seeders and resprouters: germination success cued by fire and season (Chapter 2) and physiological responses of seedlings to mild water stress in terms of plant water potential, stomatal conductance, photosynthetic rate, biomass allocation and carbohydrate allocation (Chapter 3). The implications of these findings were applied outwards to resprouters and seeders in CFR ericas and other fire-prone ecosystems around the world (Chapter 4).

1.6 References

Bell DT. 2001. Resprouters versus reseeders ecological response syndromes in the flora of southwestern Western Australia : fire resprouters versus reseeders. *The Botanical Review* **67**: 417–440.

Bell TL, Ojeda F. 1999. Underground starch storage in *Erica* species of the Cape Floristic Region - differences between seeders and resprouters. *New Phytologist* **144**: 143–152.

Bellingham PJ, Sparrow AD. 2000. Resprouting as a life history strategy in woody plant communities. *Oikos* **89**: 409–416.

Bond WJ, Keeley JE. 2005. Fire as a global "herbivore": the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution* **20**: 387–94.

Bond WJ, Scott AC. 2010. Fire and the spread of flowering plants in the Cretaceous. *New Phytologist* **188**: 1137–1150.

Bond WJ, van Wilgen BW. 1996. *Fire and Plants*. London, UK: Chapman and Hall.

Brown NAC. 1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**: 575–583.

Keeley JE, Bond WJ. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**: 153–167.

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**: 406–11.

Keeley JE, Rundel PW. 2005. Fire and the Miocene expansion of C4 grasslands. *Ecology Letters* **8**: 683–690.

Keeley JE, Zedler PH. 1978. Reproduction of Chaparral shrubs after fire : a comparison of sprouting and seeding strategies. *American Midland Naturalist* **99**: 142–161.

De Luis M, Verdú M, Raventós J. 2008. Early to rise makes a plant healthy, wealthy, and wise. *Ecology* **89**: 3061–3071.

Le Maitre D, Midgley J. 1992. Plant reproductive ecology. In: Cowling RM, ed. *The Ecology of Fynbos: Nutrients, Fire and Diversity*. Cape Town, South Africa: Oxford University Press, 135–174.

Malan M. 2013. *Intraspecific variation in Erica coccinea.* Master's thesis, University of Cape Town, Cape Town, South Africa.

Midgley JJ. 1996. Why the world's vegetation is not totally dominated by resprouting plants; because resprouters are shorter than reseeders. *Ecography* **19**: 92–95.

Ojeda F. 1998. Biogeography of seeder and resprouter *Erica* species in the Cape Floristic Region-Where are the resprouters? *Biological Journal of the Linnean Society* **63**: 331–347.

Ojeda F, Brun FG, Vergara JJ. 2005. Fire, rain and the selection of seeder and resprouter life-histories in fire-recruiting, woody plants. *The New Phytologist* **168**: 155–65.

Parolin P, De Simone O, Haase K, Waldhoff D, Rottenberger S, Kuhn U, Kesselmeier J, Kleiss B, Schmidt W, Piedade MTF, *et al.* 2004. Central Amazonian floodplain forests : tree adaptations in a pulsing system. *The Botanical Review* **70**: 357–380.

Paula S, Pausas JG. 2006. Leaf traits and resprouting ability in the Mediterranean basin. *Functional Ecology* **20**: 941–947.

Pausas JG, Keeley JE. 2009. A burning story: the role of fire in the history of life. *BioScience* **59**: 593–601.

Pausas JG, Keeley JE. 2014. Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist* **204**: 55–65.

Pausas JG, Keeley JE, Verdú M. 2006. Inferring differential evolutionary processes of plant persistence traits in Northern Hemisphere Mediterranean fire-prone ecosystems. *Journal of Ecology* **94**: 31–39.

Schumann D, Kirsten G. 1992. *Ericas of South Africa*. Vlaeberg, South Africa: Fernwood Press.

Schwilk DW, Ackerly DD. 2005. Is there a cost to resprouting? Seedling growth rate and drought tolerance in sprouting and nonsprouting *Ceanothus* (Rhamnaceae). *American Journal of Botany* **92**: 404–410.

Scott AC, Glasspool IJ. 2006. The diversification of Paleozoic fire systems and fluctuations in atmospheric oxygen concentration. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 10861–5.

Segarra-Moragues JG, Donat-Caerols S, Ojeda F. 2009. Isolation and characterization of microsatellite loci in the Cape fynbos heath *Erica coccinea* (Ericaceae). *Conservation Genetics* **10**: 1815–1819.

Segarra-Moragues JG, Ojeda F. 2010. Postfire response and genetic diversity in *Erica coccinea*: connecting population dynamics and diversification in a biodiversity hotspot. *Evolution; International Journal of Organic Evolution* **64**: 3511–24.

Stephens SL, Libby WJ. 2006. Anthropogenic fire and bark thickness in coastal and island pine populations from Alta and Baja California. *Journal of Biogeography* **33**: 648–652.

Thomas PB, Morris EC, Auld TD, Haigh AM. 2010. The interaction of temperature, water availability and fire cues regulates seed germination in a fire-prone landscape. *Oecologia* **162**: 293–302.

Verdaguer D, Ojeda F. 2002. Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape *Erica* (Ericaceae) species. *American Journal of Botany* **89**: 1189–1196.

Verdaguer D, Ojeda F. 2005. Evolutionary transition from resprouter to seeder life history in two *Erica* (Ericaceae) species: insights from seedling axillary buds. *Annals of Botany* **95**: 593–9.

Verdú M. 2000. Ecological and evolutionary differences between Mediterranean seeders and resprouters. *Journal of Vegetation Science* **11**: 265–268.

Vilagrosa A, Hernández EI, Luis VC, Cochard H, Pausas JG. 2014. Physiological differences explain the co-existence of different regeneration strategies in Mediterranean ecosystems. *The New Phytologist* **201**: 1277–88.

Wells P V. 1969. The relation between mode of reproduction and extent of speciation in woody genera of the California Chaparral. *Evolution* **23**: 264–267.

Van Wilgen BW, Bond WJ, Richardson DM. 1992. Ecosystem management. In: Cowling RM, ed. *The ecology of fynbos: nutrients, fire and diversity*. Cape Town: Oxford University Press, 345–371.

Van Wilgen BW, Forsyth GG, De Klerk H, Das S, Khuluse S, Schmitz P. 2010. Fire management in Mediterranean-climate shrublands: a case study from the Cape fynbos, South Africa. *Journal of Applied Ecology* **47**: 631–638.

Chapter 2: Germination cues in resprouter, seeder and 'pyrofuge' forms of *Erica coccinea*

2.1 Introduction

Germination is a complex process controlled by dormancy. A seed must be fully mature and in a non-dormant state in order to germinate and grow (Baskin & Baskin, 1998; Finch-Savage & Leubner-Metzger, 2006). To break dormancy, seeds rely on a multitude of cues ranging from seasonal fluctuations in temperature and water availability to the presence of organic and inorganic chemicals (Baskin & Baskin, 1998; Finch-Savage & Leubner-Metzger, 2006). These cues are necessary because without some level of dormancy, germination can occur at any time whether or not conditions are suitable for seedling growth. Dormancy has been described as a "bet-hedging strategy" (Donohue *et al.*, 2010), allowing seeds to hold out for conditions that are suitable to maximize a seedling's potential to reach adulthood.

A key to this "bet-hedging strategy" is recognizing environmental factors that indicate favourable conditions. The most ubiquitous environmental factor that can cause changes in dormancy state is temperature (Baskin & Baskin, 1998). Many plants are programmed to germinate in specific ranges of temperature or after cold stratification (e.g. Walck *et al.*, 1997). This temperature often relates to a season that is appropriate for growth and will support the plant, such as temperatures that correspond with a season of high rainfall (Ooi *et al.*, 2004; Quintana *et al.*, 2004). In Mediterranean ecosystems, seasonal rainfall corresponds with late autumn to early spring temperatures (Walck *et al.*, 1997).

Temperature is becoming a less reliable way of predicting appropriate seasonal conditions. Rainfall patterns and temperatures are becoming increasingly variable, and extreme weather events are becoming more frequent (IPCC, 2007). In a review of the effect of climate change on plant regeneration from seed, Walck *et al.* (2011) show that changes in temperature may enhance germination in some cases, but delay or preclude germination in others. Even if seeds are able to germinate under changing conditions, they may do so at a time that will not support their needs to reach adulthood. For example, if seeds germinate at a specific temperature that matches the mean temperature during a typical rainy season but rain occurs later in the year or in insufficient quantities, this could lead to individual mortality at best or local extinctions in the worst circumstances (Walck *et al.*, 2011).

However, many plants are safeguarded against germinating during false temperature fluctuations by requiring additional cues to break dormancy. Temperature may only raise a seed out of total dormancy and into a state of conditional dormancy, where germination can then be triggered by an additional cue (Baskin & Baskin, 1998). For some plants, additional cues can include moisture, exposure to light (or complete darkness) or organic and inorganic chemicals (Baskin & Baskin, 1998). In some cases, these cues can be quite general. Gibberellin is a general plant hormone that promotes germination in many plants (Penfield & King, 2009). Other cues can be highly specific to the plant's surroundings. For example, *Lilaea scilloides* and *Pilularia americana*, two species found in temporary wetlands of the western United States, respond strongly to a temperature cue but also require inundation to germinate (Bliss & Zedler, 1998). Submergence requirements are also found in flood-disturbed environments such as the Central Amazonian Floodplain Forests (Parolin *et al.*, 2004).

In ecosystems that are disturbed by fire, a seed must recognize fire in order to establish successfully in the post-fire environment (Paula & Pausas, 2008). Post-fire environments are ideal places for new seedlings to establish because of the lack of competition with previously-established plants. If seeds can remain dormant until after a fire has occurred, competition for resources is reduced, there is more light, and available nutrients increases briefly (Buhk *et al.*, 2007). If seeds can’t remain dormant, they risk germinating between fires. In this case, seedlings may not reach maturity before the next fire, thereby minimizing reproductive potential and diminishing the population's ability to propagate (Holmes & Newton, 2004).

Smoke is an important cue for facilitating germination in a post-fire environment (Staden *et al.*, 2000). Dixon *et al.* (1995) discovered 45 species from Western Australia that responded positively to smoke, 23 of which had been reported as impossible to germinate under conventional methods. Smoke has also been found to improve germination in: common sagebrush-steppe species in the western United States (Blank & Young, 1998); shrubs, herbaceous perennials and annuals in the Californian chaparral and coastal sage scrub (Keeley & Bond, 1997; Keeley & Fotheringham, 1998); and a number of different families in the Cape Floristic Region (CFR) of South Africa (Brown, 1993; Brown *et al.*, 2003).

Examples of smoke stimulated germination have been found in both resprouter and seeder phenotypes. Resprouters invest their carbon in belowground organs (such as rhizomes, lignotubers and/or thick roots) that facilitate the process of resprouting after a fire (Vesk & Westoby, 2004). As a trade-off for this ability, resprouters generally have a lower seed set than species that do not resprout (Bell, 2001). Seeders, which do not have adult plants that survive fire, rely only on seeds to ensure the establishment of a new generation. Regardless of these two strategies, there are species of both forms that show improved germination with smoke (Brown, 1993; Brown *et al.*, 2003) as they both produce seeds that must compete in the post-fire environment. However, since seeders experience a whole generational turnover after every fire, this could cause them to adapt more quickly to their local environment (Wells, 1969; Keeley & Zedler, 1978; Pausas & Keeley, 2014). This in turn could cause them to have a faster, more complete response to smoke.

The evolutionary time frame under which fire can act as a selective pressure on plant traits like smoke-stimulated germination is contested. Fire-selected traits are evident in areas where hominid-controlled fires have been present for about 780,000 years (Ne'eman *et al.*, 2004). Other studies indicate that these pressures can occur over an even smaller time frame (e.g. Måren *et al.*, 2010). Some research indicates a possible selection timeframe of approximately 6000 years, or 60-600 generations (Gómez-González *et al.*, 2011).

If plants can so quickly evolve under the selective pressures of fire, it may also be possible for those adaptations to be reversed in the absence of fire. Although breaking dormancy in the absence of fire would be risky, there are places within fire-prone ecosystems where fire does not reach. Plants that are restricted to germinating only in post-fire environments would be at a disadvantage in such microhabitats. For example, survival, growth, fecundity and establishment of *Hypericum cumulicola* declined in extended fire-free intervals (Quintana-Ascencio & Morales-Hernandez, 1997). It is suspected that long fire-free intervals have led to local extinctions of *H. cumulicola* (Quintana-Ascencio *et al.*, 1998). Plants such as these would not be able to establish in a fire regufia.

These "fire refugia" should not be confused with "climate-change refugia" (Ashcroft, 2010; Keppel *et al.*, 2012), which are defined as areas where a species retreats to and potentially expands from. The fire refugia described here resemble the vegetation islands described by Bond (1988) or, more precisely, the *inselbergs* or rocky outcrops in Australia, which exist free of fire within a fire-prone vegetation matrix (Clarke, 2002; Tapper *et al.*, 2014). Whether plants in fire refugia have adapted to their fire-free state by increasing germination in the absence of fire has, to my knowledge, not yet been studied.

In this chapter, I investigated temperature-driven and smoke-stimulated germination in resprouters, seeders and plants from populations found in fire refugia. The experiment was designed using *Erica coccinea*, a woody fynbos plant endemic to the CFR. *Erica coccinea* has a resprouter form, a seeder form, and a form similar to the seeder found only in fire refugia, which has been dubbed a 'pyrofuge' form. Smoke-treated and untreated seeds from each form were incubated under three different temperature ranges (8-15°C, 13-20°C and 18-25°C). Two populations of each form were tested to determine whether findings were true to the form or were instead because of interpopulation variation. For resprouters and seeders, higher germination success was predicted for smoke-treated seeds than untreated seeds. I expected higher germination success in all three forms with lower temperatures that correspond with winter temperatures of the CFR, which has a winter rainfall/summer drought regime. For the smoke treatment, higher germination success and rate were expected in seeders over resprouters because of the seeders' more frequent generational turnover (capacity to adapt more quickly to change) and reliance on recruitment (stronger selective pressures). However, for the pyrofuge form, I predicted no effect of smoke on germination success and a higher sensitivity to temperature as a cue for germination. This would indicate a reversal of dependency on fire-stimulated germination and an increased dependence on temperature, which could be problematic in the face of climate change.

2.2 Methods

2.2.1 Study species

Erica coccinea is a woody shrub found in the Cape Floristic Region. It has three growth forms: a resprouter, a seeder and a 'pyrofuge'. Seed germination is stimulated by smoke in both the resprouter and seeder forms (N. Zide, pers. comm.). The resprouter form has a woody lignotuber and active buds at the base of the stem that can give rise to multi-stemmed plants (Bell & Ojeda, 1999). The seeder form has no lignotuber but it does have buds at the base of the stem, though these buds are atrophied (Verdaguer & Ojeda, 2005). The presence of atrophied buds suggests that the seeder form is derived from the resprouter form (Verdaguer & Ojeda, 2005), which is suspected in many other plants (Wells, 1969; Bond & van Wilgen, 1996; Verdaguer & Ojeda, 2005; Pausas & Keeley, 2014). Seeder populations of *E. coccinea* are even-aged as a result of adult death during fire and seed germination after fire (F. Ojeda, pers. obs.). Resprouters and seeders (and pyrofuges) produce numerous tiny seeds per seed pod. No study has been conducted to compare seed production or viability between these two types.

The 'pyrofuge' form is found in areas such as rocky outcrops where fire is unlikely to penetrate. Like normal seeders, this form has no lignotuber and does not produce viable basal buds (Malan, 2013). There is field evidence that recruitment occurs even without a fire cue, since populations have a broad age structure and seedlings can be observed in fire free stands (F. Ojeda, pers. obs.).

2.2.2 Collection and preservation

Seeds were collected from two distinct populations of each form (Table 2.1). Ripe capsules were taken from at least 20 individuals during the natural dispersal period of each population during 2011 or 2012 (Table 2.1). Seeds were separated from their seedpods under a microscope. They were then visually evaluated for viability as they were too small and solid to be tested through a tetrazolium test or cut test. Seeds were deemed viable if they were ovular, opaque and glossy. Seeds that were shrivelled, transparent or dull were not used in the experiment.

Seeds were pooled for each population and were stored at ambient temperature (~25°C) in paper bags. The bags were kept in a sealed container filled with silica beads to ensure a dry environment. These conditions were maintained until the experiment commenced in October, 2013.

2.2.3 Treatments

Treatment (smoke-treated and untreated) and temperature (8-15°C, 13-20°C and 18-25°C in 12:12 hour periods) were combined factorially to generate six different conditions. Eight replicates of 25 seeds from each of the six populations were then exposed to these different conditions. For conditions requiring smoke, seeds were placed in open petri dishes in a cardboard box. Smoke generated from green and dry fynbos litter was pumped into the box using a bee smoker. The material in the smoker was replaced several times to ensure a constant stream of smoke into the box. After several applications, the box was sealed and left to sit for two hours.

Smoke-treated and untreated seeds were then sown into petri dishes filled with agar and incubated at the three incubation temperatures. Weather station data from areas near *E. coccinea* populations were used to choose incubation temperatures that resembled approximate summer temperatures (25°C during the day and 18°C at night), autumn temperatures (20°C during the day and 13°C at night) and late autumn/winter temperatures (15°C during the day and 8°C at night). Diurnal

Table 2.1: Location for each population of Erica coccinea used in this thesis and the years seeds were collected. The superscript after the population indicates which populations were used for Chapter 2 and Chapter 3.

Type	Population	Latitude	Longitude	Year
Resprouter	Swellendam (A)^{2}	33°59'56"S	20°26'47"E	2011
	Stellenbosch (B)^{2}	33°59'56"S	18°57'48"E	2012
	Blackburn Ravine (C)^{3}	34°03'21"S	18°22'19"E	2011
Seeder	Napier (A)$^{2\&3}$	34°31'41"S	19°53'17"E	2011
	Potberg (B)^{2}	34°22'19"S	20°32'33"E	2012
Pyrofuge	Olifantsbos (A)^{2}	34°04'59''S	18°21'43''E	2011
	Chapman's Peak (B)^{2}	34°16'03''S	18°23'40''E	2011

fluctuations have been shown to improve germination responses in many cases (Pierce & Moll, 1994; Baskin & Baskin, 1998). Incubation chambers were programmed for 12-hour diurnal temperature fluctuations. The chambers were kept dark except for the brief exposure to light when germination was being recorded. Germination was scored once a week for eight weeks. Seeds with emerged radicles were recorded as germinated and discarded from the petri dish. If empty or damaged seeds were discovered during this process, they were removed from the overall count. If mould was present in the dish, seeds were transplanted into a fresh dish. No antimicrobial agents were used in the agar. If less than 10% germination was found for all conditions in a single population, the population was deemed unfit. Under these parameters, one resprouter population (B) was discarded and not used in the analysis.

2.2.4 Statistics

Total cumulative germination and time to 50% germination was recorded for each petri dish. An analysis of cumulative germination and time to 50% germination for the different forms (resprouter, seeder and pyrofuge) under the different treatments (smoke-treated or untreated) and temperatures (8-15°C, 13-20°C and 18-25°C) was conducted using a full factorial ANOVA (STATISTICA version 12, StatSoft, Tulsa, USA). All assumptions were met. For a population level analysis, a full factorial ANOVA was conducted with the five viable populations under the different temperatures and treatments (STATISTICA version 12, StatSoft, Tulsa, USA). All assumptions were met. Due to low yields under 18-25°C, this temperature treatment was excluded from analyses investigating population variation in germination success and rate. A nested ANOVA was not used because there were an unequal number of populations per form due to the failure of the resprouter population B. For all ANOVAs, means that were significantly different were separated by a Duncan's post-hoc test.

2.3 Results

2.3.1 Resprouter and seeder germination success

Resprouters and seeders had high germination success for smoke-treated seeds at both of the lower temperatures (Table 2.2). The only form that had reasonable success at the highest temperature (18-25°C) was the resprouter, which yielded nearly 50% germination for seeds treated with smoke. Both other forms and untreated resprouter seeds had close to 0% germination success at the highest temperature (18-25°C).

When comparing smoke-treated resprouters and seeders at the lower temperatures, the only significant difference was at 13-20°C, where resprouters and seeders achieved 90.4% and 77.6% germination success respectively (Table 2.2). There was no difference in germination success at 8-15°C. Resprouters and seeders achieved 82.0% and 83.0% germination success respectively at this temperature (Table 2.2). When seeds were left untreated, neither form had very high germination success, though seeders were significantly more successful than resprouters (Table 2.2). Untreated seeder seeds had 18.1% success at 13-20°C and 24.6% success at 8-15°C whereas untreated resprouter seeds had 4.0% success at 13-20°C and 8.1% success at 8-15°C.

Table 2.2: Mean cumulative percent germination (± SE) of resprouter, seeder and pyrofuge *Erica coccinea* seeds under three incubation temperatures (8-15°C, 13-20°C and 18-25°C) and two smoke treatments (smoke-treated and untreated). Analysis was performed using a full factorial ANOVA ($F_{4,222} = 13.57$; $p < 0.0001$). Different letters indicate significant differences (Duncan's post-hoc test).

		Germination (% ± SE)	
Temperature	Form	Untreated	Smoke
18-25°C	Seeder	0e	1.0 ± 0.4e
	Resprouter	1.0 ± 0.7e	46.9 ± 4.4c
	Pyrofuge	0e	0e
13-20°C	Seeder	18.1 ± 2.9d	77.6 ± 2.8b
	Resprouter	4.0 ± 1.3e	90.4 ± 2.0a
	Pyrofuge	16.8 ± 2.7d	39.3 ± 5.2c
8-15°C	Seeder	24.6 ± 5.3d	83.0 ± 3.3ab
	Resprouter	8.1 ± 1.3e	82.0 ± 3.3ab
	Pyrofuge	85.7 ± 2.1ab	87.7 ± 2.3a

2.3.2 Pyrofuge germination success

At the lowest temperature, pyrofuge seeds achieved 87.7% success with smoke and 85.7% success without smoke (Table 2.2). These values were not significantly different from each other or from the success achieved by smoke-treated resprouters and seeders at 8-15°C (Table 2.2). Pyrofuge seeds had no germination at the highest temperature and minimal germination at 13-20°C, where smoke-treated seeds yielded 39.3% success and untreated seeds yielded a significantly lower 16.8% success (Table 2.2). The smoke-treated pyrofuge seeds at 13-20°C were significantly less successful than smoke-treated resprouter and seeder seeds at this temperature (Table 2.2). However, untreated pyrofuge seeds had similar germination to untreated seeder seeds and greater germination than resprouter seeds at 13-20°C (Table 2.2).

2.3.3 Germination rates

Smoke-treated resprouter seeds germinated significantly faster than smoke-treated seeder seeds at 13-20°C, reaching 50% germination 7 days earlier (Table 2.3).

Table 2.3: Mean germination rates (T50) in number of days (± SE) for resprouter, seeder and pyrofuge *Erica coccinea* seeds under two smoke treatments (smoke-treated and untreated) and the two high-yield incubation temperatures (8-15°C° and 13-20°C). Conditions that did not yield 50% germination were not included in the analysis (NA). Analysis was performed using a full factorial ANOVA ($F_{2,148} = 4.88$; $P < 0.0001$). Different letters indicate significant differences (Duncan's post-hoc test).

		T50 (days ± SE)	
Temperature	Form	Untreated	Smoke
13-20°C	Seeder	NA	18.0 ± 0.8b
	Resprouter	NA	11.2 ± 0.2d
	Pyrofuge	NA	NA
8-15°C	Seeder	NA	13.4 ± 0.6c
	Resprouter	NA	12.5 ± 0.6cd
	Pyrofuge	21.4 ± 0.8a	18.4 ± 1.1b

Germination rates were similar at 8-15°C for the resprouters and seeders, with both forms reaching 50% germination after about 13 days (Table 2.3). Pyrofuge seeds geminated significantly more slowly than the resprouter and seeder seeds and only reached 50% germination at 8-15°C (Table 2.3). Smoke-treated pyrofuge seeds germinated faster than untreated pyrofuge seeds, reaching 50% germination at 18 days and 21 days respectively (Table 2.3). Pyrofuge seeds were the only seeds to reach 50% germination in the absence of smoke (Table 2.3). Germination rates could not be compared at 13-20°C for the pyrofuge seeds, at 18-25°C for all forms, or in untreated seeder and resprouter seeds because 50% germination was not attained for under these conditions.

2.3.4 Population variation in germination success and rate

Both seeder populations had similar germination success with smoke for the lower temperatures (Figure 2.1). However, the success of untreated Seeder B seeds was much higher than the success of untreated Seeder A seeds (Table 2.4). Seeder A only had 5.5% success at 8-15°C and 9.5% success 13-20°C while Seeder B had 43.7% success at 8-15°C and 26.7% success 13-20°C. Seeder B had significantly higher germination success at 8-15°C than at 13-20°C while there was no significant difference in germination success between those two temperatures for Seeder A. Seeder populations had similar germination rates, with a slightly faster rate observed at 8-15°C than 13-20°C for smoke-treated seeds for both populations (Table 2.5).

Population level variation could not be analysed between resprouter populations due to the failure of one of the populations (B). However, when the single Resprouter A population was compared to the two seeder populations individually, there was no significant difference between the success of the untreated Seeder A seeds and the untreated Resprouter A seeds (Table 2.4; Figure 2.2). Untreated Seeder B seeds had significantly higher germination success than both Seeder A and Resprouter A. On the other hand, smoke-treated Resprouter A seeds maintained a significantly faster germination rate at 13-20°C against both seeder populations (Table 2.5). There was no difference in rates among these three populations at 8-15°C, and there was no discernible pattern to indicate higher germination success under smoke because success differed between the two temperatures (Table 2.4).

Both pyrofuge populations had high germination success for smoke-treated and untreated seeds at 8-15°C, with germination success of 80-92% (Figure 2.3). At 13-

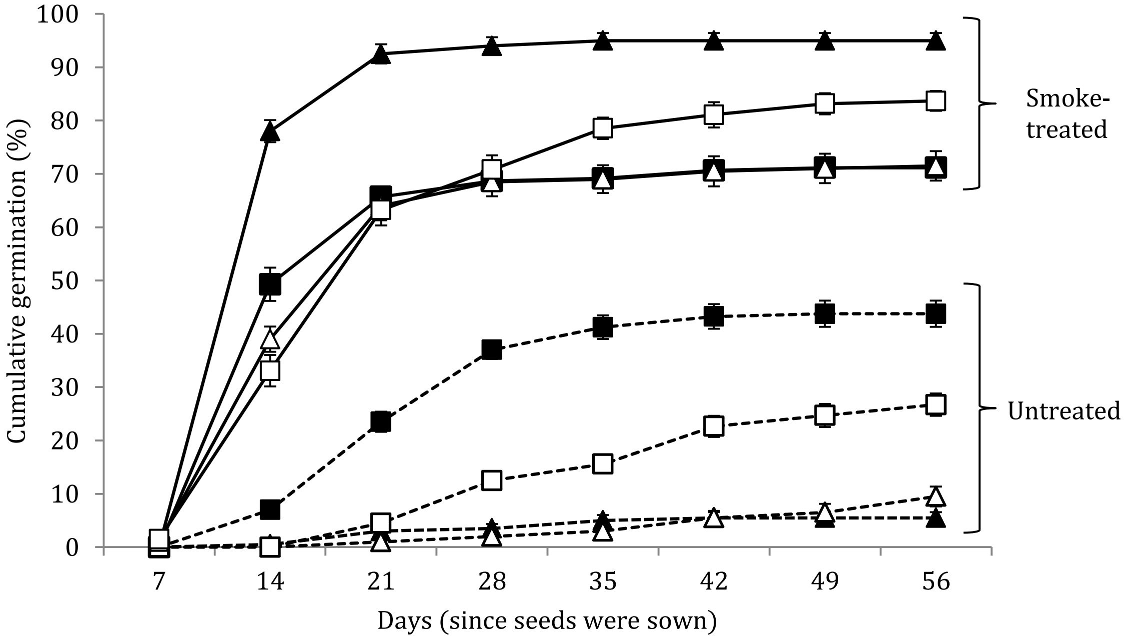

Figure 2.1: Cumulative percent germination over time for untreated (dashed line) and smoke-treated (solid line) *Erica coccinea* seeder populations from Seeder A (triangle) and Seeder B (square) under diurnal incubation temperatures 8-15°C (filled) and 13-20°C (unfilled).

Table 2.4: Mean cumulative percent germination (± SE) for each population of resprouter, seeder and pyrofuge *Erica coccinea* under two smoke treatments (smoke-treated and untreated) and the two high-yield incubation temperatures (8-15°C and 13-20°C). Analysis was performed using a full factorial ANOVA ($F_{4,140} = 18.52$; $p < 0.0001$). Different letters indicate significant differences (Duncan's post-hoc test).

		Germination (% ± SE)	
Temperature	Population	Untreated	Smoke
13-20°C	Seeder A	9.5 ± 2.6h	71.5 ± 3.9d
	Seeder B	26.7 ± 3.0g	83.7 ± 2.6bc
	Resprouter A	4.0 ± 1.3h	90.4 ± 2.0ab
	Pyrofuge A	9.1 ± 2.0h	20.6 ± 1.4g
	Pyrofuge B	24.5 ± 3.1g	58.0 ± 3.9e
8-15°C	Seeder A	5.5 ± 1.5h	95.0 ± 2.0a
	Seeder B	43.7 ± 3.4f	71.2 ± 1.4d
	Resprouter A	8.1 ± 1.3h	82.0 ± 3.3c
	Pyrofuge A	90.5 ± 2.6ab	83.4 ± 3.2bc
	Pyrofuge B	80.9 ± 2.5c	92.0 ± 2.5a

Table 2.5: Mean germination rates (T50) in number of days (± SE) for each population of resprouter, seeder and pyrofuge *Erica coccinea* under two smoke treatments (smoke-treated and untreated) and the two high-yield incubation temperatures (8-15°C and 13-20°C). Conditions that did not yield 50% germination were not included in the analysis (NA). Analysis was performed using a full factorial ANOVA ($F_{4,140} = 6.22$; $p < 0.0001$). Different letters indicate significant differences (Duncan's post-hoc test).

		T50 (days ± SE)	
Temperature	Population	Untreated	Smoke
13-20°C	Seeder A	NA	17.2 ± 0.9c
	Seeder B	NA	18.8 ± 1.2bc
	Resprouter A	NA	11.2 ± 0.2d
	Pyrofuge A	NA	NA
	Pyrofuge B	NA	NA
8-15°C	Seeder A	NA	11.5 ± 0.2d
	Seeder B	NA	15.3 ± 0.8cd
	Resprouter A	NA	12.5 ± 0.6d
	Pyrofuge A	24.1 ± 0.5a	22.0 ± 0.8ab
	Pyrofuge B	18.7 ± 0.5bc	14.8 ± 1.0cd

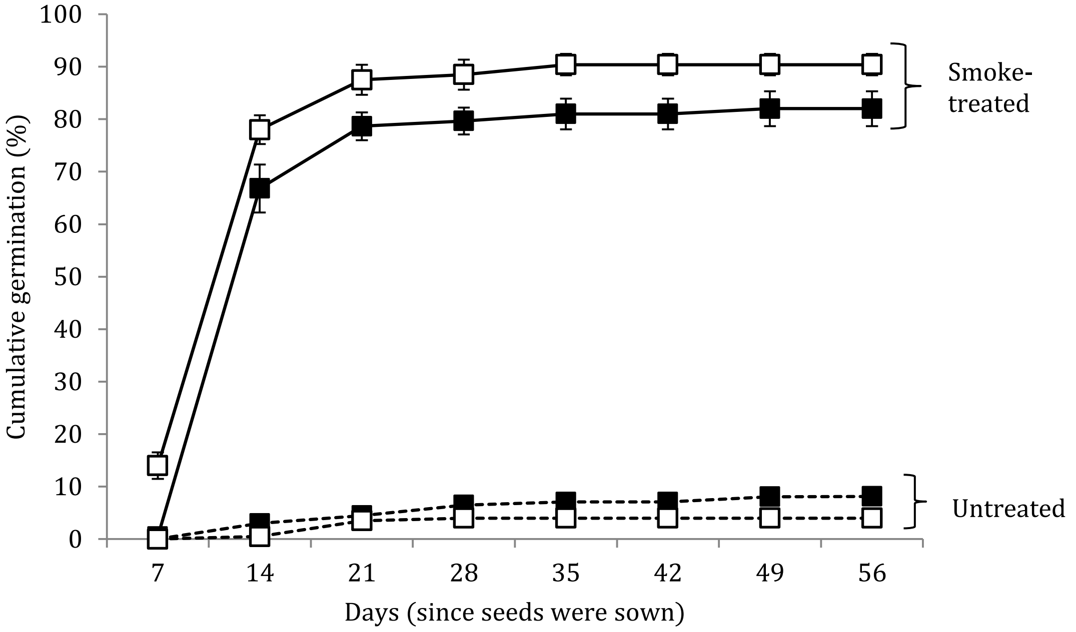

Figure 2.2: Cumulative percent germination over time for untreated (dashed line) and smoke-treated (solid line) *Erica coccinea* resprouter population from Resprouter A under diurnal incubation temperatures 8-15°C (filled) and 13-20°C (unfilled).

20°C, Pyrofuge A had significantly higher germination success for both smoke-treated seeds (58.0%) and untreated seeds (24.5%) compared to the Pyrofuge B smoke-treated seeds (20.6%) and untreated seeds (9.1%; Table 2.4). These results show a significantly higher success in smoke-treated than untreated seeds for both populations at 13-20°C. At the lowest temperature (8-15°C), Pyrofuge A seeds germinated more quickly than Pyrofuge B seeds for both untreated and smoke-treatment (Table 2.5). Pyrofuge B germination rates were also significantly slower than the seeder and resprouter populations, while Pyrofuge A germination rates did not differ from the other populations under a smoke treatment at 8-15°C.

2.4 Discussion

2.4.1 Trade-offs in germination success

Smoke was the most important germination cue for *Erica coccinea* resprouters and seeders. Both forms had drastically improved germination in the presence of smoke

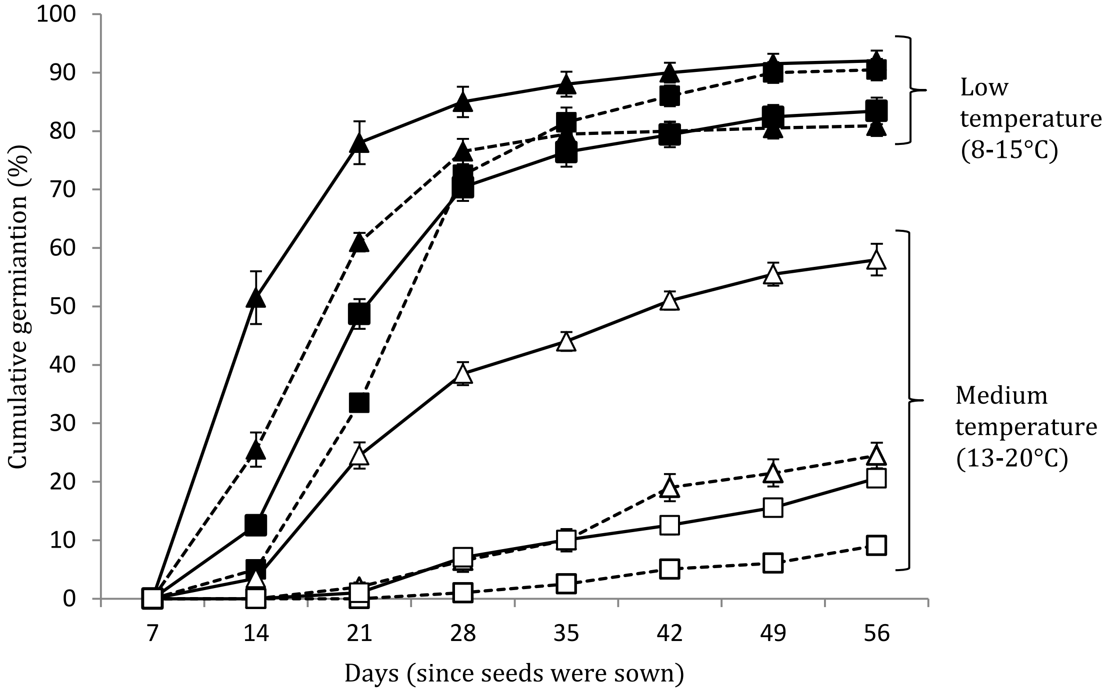

Figure 2.3: Cumulative percent germination over time for untreated (dashed line) and smoke-treated (solid line) *Erica coccinea* pyrofuge populations from Pyrofuge A (triangle) and Pyrofuge B (square) under diurnal incubation temperatures 8-15°C (filled) and 13-20°C (unfilled).

(Table 2.2 & 2.4, Figures 2.1 & 2.2). They also responded to temperature as a cue. Minimal germination success occurred at the highest set of temperatures regardless of whether seeds were smoke-treated or untreated (Table 2.2). This indicates that resprouters and seeders require a temperature cue as well as a smoke cue in order to germinate. Ooi *et al.* (2004) found similar results for seedling emergence of *Leucopogon* species in south-eastern Australia. They observed a delay in post-fire seedling emergence until late-autumn or early winter. They concluded that fire primes seeds for germination but that season determines when germination occurs.

There were some overall differences between seeders and resprouters in terms of germination success and rate. Seeders had slightly higher germination success than resprouters in the absence of smoke (Table 2.2). This could mean that seeders have the ability to germinate between fires, though there is no evidence of this in the field (Ojeda pers. obs.). Resprouters on the other hand were able to achieve some level of germination at the highest temperature (18-25°C, Table 2.2) in the presence of smoke as well as a faster germination rate than seeders at 13-20°C (Table 2.3). This could mean that they are able to germinate faster and earlier in the season after a fire, which could give them a competitive edge over seeders. Alternatively, germinating earlier and more rapidly may simply allow resprouters to compete with the more numerous seeders, since resprouters are expected to produce fewer seeds than seeders (Bell, 2001). However, at this time there is no seed production data available for this species.

These observed differences between seeders and resprouters become complicated by population-level differences are examined. The relative success of untreated seeder seeds was due entirely to the Seeder B population, with no significant difference in germination between untreated Seeder A and Resprouter A (Table 2.4). The conclusion that resprouters are able to germinate faster and earlier in the season after a fire is then thrown into question, as the analyses only included data from one population (Table 2.5). With such high variability between the seeder populations, there could also be differences between resprouter populations.

There have been cases where higher variability has been observed between populations rather than species. For instance, *Erica australis*, a resprouter species found in the Mediterranean Basin, had high interpopulation variability in terms of its germination success (Cruz et al., 2003). In the case of E. coccinea, selective pressures unique to each population could be driving population level differences between forms. However, local selection is not always a strong selective force. Moreira *et al.* (2010) showed that *Cistus salviifolius* and *Lavandula stoechas* germination differed in ways that were specific to their species but not to their region. By surveying more extensively, one could answer whether there is more variability between forms or between locations for *E. coccinea* resprouters and seeders.

It is also worthwhile pointing out that Seeder B seeds were collected in 2012, Seeder A seeds were collected in 2011, and the study was conducted in 2013 (Table 2.1). Baskin & Baskin (1998) recommend that germination studies should start 7-10 days after collection or there is risk of the seeds becoming unviable. This could account for differences in germination success between the two populations (Table 2.4, Figure 2.1). However, both populations were able to reach similarly high levels of success even with the difference in collection time. Therefore, this is an unlikely cause for the population variability observed.

2.4.2 The curious case of the pyrofuge

Erica coccinea pyrofuge seeds do not need smoke to reach maximum germination success. In fact, they had the same germination success at 8-15°C as smoke-treated resprouter and seeder seeds regardless of whether they had been treated with smoke (Table 2.2). Even variation between the pyrofuge populations did not negate the overall pattern that emerged from these findings. This suggests that the absence of fire has led to germination traits that allow populations in fire-refugia to persist within a fire matrix. For this to be apparent within a species is a remarkable discovery.

Although pyrofuge seeds do not need smoke for germination, they did benefit from the presence of smoke at higher temperatures. Smoke increased germination success in both pyrofuge populations by approximately two-fold at 13-20°C (Table 2.4). This result adds fuel to the ongoing debate about the origin of fire-adapted traits. It has been shown that some plants (e.g. succulents from fire-free habitats in South Africa; Pierce *et al.*, 1995) respond to a smoke cue even though they would rarely encounter smoke in their natural environments. This is one of the reasons why Bradshaw *et al.* (2011) have proposed that fire-adaptations such as smoke-stimulated germination are exapted traits rather than adapted traits. This would mean that although plants do well because of a particular trait, the trait might not have been a direct adaptation for that particular cue. Flematti *et al.* (2013) support this view in their compilation of the main discoveries around the chemicals present in smoke. They propose that these chemicals have an endogenous origin; because these chemicals have existed for a long time and are even produced by plants, plants have ‘adopted’ certain chemicals as indicators of a post-fire environment.

However, dormancy itself is by definition an adaptation (Finch-Savage & Leubner-Metzger, 2006; Keeley *et al.*, 2011). To claim that a dormancy-breaking cue is not an adaptation may be a moot point. However, Keeley *et al.* (2011) do concede that the origin of this adaptation could have been in response to something other than fire, although the classification would remain an adaptation rather than an exaptation.

The *E. coccinea* pyrofuge brings a caveat to this argument. The pyrofuge form has adapted to germinate in a fire-free environment. It becomes easy to infer that smoke-stimulated germination has in turn adapted as a response to fire. This may well be the case for resprouter and seeder *E. coccinea*, though it would not be appropriate to extend this conclusion to all plants (such as the succulents from fire-free habitats; Pierce *et al.*, 1995). However, the pyrofuge's improved germination as a response to smoke at the intermediate temperature (Table 2.2) could be used to show that smoke-stimulated germination must have evolved in response to something other than fire, since pyrofuges exist outside of the fire matrix. However, this could be explained away by describing the pyrofuge as an emerging species that has not fully broken away from seeder and resprouter *E. coccinea,* which require smoke for germination.

Instead of smoke, pyrofuges rely primarily on temperature as a cue. This is the opposite of the cue requirements of seeders and resprouters. While resprouters and seeders reached approximately 80% germination for smoke treated seeds at 13-20°C and 8-15°C, pyrofuges only attained that level of germination at 8-15°C (regardless of whether smoke was present, Table 2.2). This may make the pyrofuge form more sensitive to climate change than the resprouter or seeder form. Climate is predicted to become warmer and drier in the CFR (Yates *et al.*, 2010). If temperatures remain warmer later in the year, pyrofuges may suffer from late germination and depreciated germination rates. This could lead to smaller and fewer individuals that would then need to survive the temperatures and water scarcity of summer.

In this case, pyrofuges might need to migrate in order to survive. Increases in temperature and change in rainfall patterns may alter the distribution ranges for a variety of species (Walck *et al.*, 2011). Climate change is predicted to shift species ranges southwestward and to higher elevations in the CFR (Yates *et al.*, 2010). Habitat fragmentation (whether natural or anthropogenic) and dispersal abilities may hinder species ability to track climate change by way of migration (Walck *et al.*, 2011). In the case of the pyrofuge, migration of any kind will be difficult as this would entail moving off of their rocky outcrop "islands". If they migrate away from the fire refugia, they would be vulnerable to germination between fires. If the area burned before they were able to reach reproductive maturity, a local extinction could occur.

2.5 Conclusion

These results show that there is little difference between resprouter and seeder forms in *Erica coccinea* with regards to smoke-stimulated germination. However, pyrofuges rely primarily on temperature to break dormancy and do not rely on smoke for germination. I propose that this form has "lost" the adaptation to use smoke as a germination cue, the loss of which is in fact an adaptation in itself. I worry about the pyrofuge’s future due to climate change and hope that more attention is paid to conserving and understanding plant communities occurring in fire refugia within fire-prone ecosystems.

2.6 References

Ashcroft MB. 2010. Identifying refugia from climate change. *Journal of Biogeography* **37**: 1407–1413.

Baskin CC, Baskin JM. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, California: Academic Press.

Bell DT. 2001. Resprouters versus reseeders ecological response syndromes in the flora of southwestern Western Australia : fire resprouters versus reseeders. *The Botanical Review* **67**: 417–440.

Bell TL, Ojeda F. 1999. Underground starch storage in *Erica* species of the Cape Floristic Region - differences between seeders and resprouters. *New Phytologist* **144**: 143–152.

Blank RR, Young JA. 1998. Heated substrate and smoke: Influence on seed emergence and plant growth. *Journal of Range Management* **51**: 577–583.

Bliss SA, Zedler PH. 1998. The germination process in vernal pools: sensitivity to environmentalt conditions and effects on community structure. *Oecologia* **113**: 67–73.

Bond WJ, Midgley J, Vlok J. 1988. When is an island not an island? Insular effects and their causes in fynbos shrublands. *Oecologia* **77**: 515–521.

Bond WJ, van Wilgen BW. 1996. *Fire and Plants*. London, UK: Chapman and Hall.

Bradshaw SD, Dixon KW, Hopper SD, Lambers H, Turner SR. 2011. Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science* **16**: 69–76.

Brown NAC. 1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**: 575–583.

Brown NAC, van Staden J, Johnson TA, Daws MI. 2003. A summary of patterns in the seed germination response to smoke in plants from the Cape Floral Region. In: Smith RD, ed. *Seed conservation: turning science into practice*. Royal Botanic Gardens, Kew, 1023.

Buhk C, Meyn A, Jentsch A. 2007. The challenge of plant regeneration after fire in the Mediterranean Basin: scientific gaps in our knowledge on plant strategies and evolution of traits. *Plant Ecology* **192**: 1–19.

Clarke PJ. 2002. Habitat islands in fire-prone vegetation: do landscape features influence community composition? *Journal of Biogeography* **29**: 677–684.

Cruz A, Pérez B, Velasco A, Moreno JM. 2003. Variability in seed germination at the interpopulation , intrapopulation and intraindividual levels of the shrub *Erica australis* in response to fire-related cues. *Plant Ecology* **169**: 93–103.

Dixon KW, Roche S, Pate JS. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **101**: 185–192.

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. 2010. Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics* **41**: 293–319.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *The New Phytologist* **171**: 501–23.

Flematti GR, Waters MT, Scaffidi A, Merritt DJ, Ghisalberti EL, Dixon KW, Smith SM. 2013. Karrikin and cyanohydrin smoke signals provide clues to new endogenous plant signaling compounds. *Molecular Plant* **6**: 29–37.

Gómez-González S, Torres-Díaz C, Bustos-Schindler C, Gianoli E. 2011. Anthropogenic fire drives the evolution of seed traits. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 18743–18747.

Holmes PM, Newton RJ. 2004. Patterns of seed persistence in South African fynbos. *Plant Ecology* **172**: 143–158.

IPCC. 2007. *Climate change 2007: synthesis report*. Geneva, Switzerland: IPCC.

Keeley JE, Bond WJ. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**: 153–167.

Keeley JE, Fotheringham CJ. 1998. Smoke-induced seed germination in California chaparral. *Ecological Society of America* **79**: 2320–2336.

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**: 406–11.

Keeley JE, Zedler PH. 1978. Reproduction of Chaparral shrubs after fire: a comparison of sprouting and seeding strategies. *American Midland Naturalist* **99**: 142–161.

Keppel G, Niel KP Van, Wardell-johnson GW, Yates CJ, Byrne M, Mucina L, Schut AGT, Hopper SD, Franklin SE. 2012. Refugia: identifying and understanding safe havens for biodiversity under climate change. *Global Ecology and Biogeography* **21**: 393–404.

Malan M. 2013. *Intraspecific variation in Erica coccinea.* Master's thesis, University of Cape Town, Cape Town, South Africa.

Måren IE, Janovský Z, Spindelböck JP, Daws MI, Kaland PE, Vandvik V. 2010. Prescribed burning of northern heathlands: *Calluna vulgaris* germination cues and seed-bank dynamics. *Plant Ecology* **207**: 245–256.

Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**: 627–35.

Ne'eman G, Goubitz S, Nathan R. 2004. Reproductive traits of *Pinus halepensis* in the light of fire: a critical review. *Plant Ecology* **171**: 69–79.

Ooi MKJ, Auld TD, Whelan RJ. 2004. Delayed post-fire seedling emergence linked to season : a case study with *Leucopogon* species (Epacridaceae). *Plant Ecology* **174**: 183–196.

Parolin P, De Simone O, Haase K, Waldhoff D, Rottenberger S, Kuhn U, Kesselmeier J, Kleiss B, Schmidt W, Piedade MTF, *et al.* 2004. Central Amazonian floodplain forests : tree adaptations in a pulsing system. *The Botanical Review* **70**: 357–380.

Paula S, Pausas JG. 2008. Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology* **96**: 543–552.

Pausas JG, Keeley JE. 2014. Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist* **204**: 55–65.

Penfield S, King J. 2009. Towards a systems biology approach seed dormancy to understanding and germination. *Biological Sciences* **276**: 3561–3569.

Pico FX, Quintana-Ascencio PF, Menges ES, Lopez-Barrera F. 2003. Recruitment rates exhibit high elasticity and high temporal variation in populations of a short-lived perennial herb. *Oikos* **103**: 69–74.

Pierce ASM, Esler K, Cowling RM. 1995. Smoke-induced germination of succulents (Mesembryanthemaceae) from fire-prone and fire-free habitats in South Africa. *Oecologia* **102**: 520–522.

Pierce SM, Moll EJ. 1994. Germination ecology of six shrubs in fire-prone Cape fynbos. *Vegetatio* **110**: 25–41.

Quintana JR, Cruz A, Fernández-Gonzalez F, Moreno JM. 2004. Time of germination and establishment success after fire of three obligate seeders in a Mediterranean shrubland of central Spain. *Journal of Biogeography* **31**: 241–249.

Quintana-Ascencio PF, Dolan RW, Menges ES. 1998. *Hypericum cumulicola* demography in unoccupied and occupied Florida scrub patches with different time-since-fire. *Journal of Ecology* **86**: 640–651.

Quintana-Ascencio PF, Morales-Hernandez M. 1997. Fire-mediated effects of shrubs, lichens and herbs on the demography of *Hypericum cumulicola* in patchy Florida scrub. *Oecologia* **112**: 263–271.

Staden JVAN, Brown NAC, Jäger AK, Johnson TA. 2000. Smoke as a germination cue. *Plant Species Biology* **15**: 167–178.

Tapper S-L, Byrne M, Yates CJ, Keppel G, Hopper SD, Van Niel K, Schut AGT, Mucina L, Wardell-Johnson GW. 2014. Isolated with persistence or dynamically connected? Genetic patterns in a common granite outcrop endemic. *Diversity and Distributions* **20**: 987–1001.

Verdaguer D, Ojeda F. 2005. Evolutionary transition from resprouter to seeder life history in two *Erica* (Ericaceae) species: insights from seedling axillary buds. *Annals of Botany* **95**: 593–9.

Vesk PA, Westoby M. 2004. Sprouting ability across diverse disturbances and vegetation types worldwide. *Journal of Ecology* **92**: 310–320.

Walck JL, Baskin CC, Baskin JM. 1997. Comparative achene germination requirements of the rockhouse endemic *Ageratina luciae-brauniae* and its widespread close relative *A. altissima* (Asteraceae). *American Midland Naturalist* **137**: 1–12.

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P. 2011. Climate change and plant regeneration from seed. *Global Change Biology* **17**: 2145–2161.

Wells P V. 1969. The relation between mode of reproduction and extent of speciation in woody genera of the California Chaparral. *Evolution* **23**: 264–267.

Yates CJ, Elith J, Latimer AM, Le Maitre D, Midgley GF, Schurr FM, West AG. 2010. Projecting climate change impacts on species distributions in megadiverse South African Cape and Southwest Australian Floristic Regions: Opportunities and challenges. *Austral Ecology* **35**: 374–391.

Chapter 3: Drought tolerance and mortality in resprouter and seeder *Erica coccinea* seedlings

3.1 Introduction

Resprouter and seeder phenotypes are found in many fire-prone ecosystems around the world (e.g. Le Maitre & Midgley, 1992; Brown, 1993; Keeley & Bond, 1997; Bell, 2001; Pausas *et al.*, 2006; Keeley *et al.*, 2011). In the Cape Floristic Region (CFR) of South Africa, the species-rich *Erica* genus contains only 10% resprouters while the rest are seeders (Ojeda, 1998). The few resprouter *Erica* species are found mostly in the northwest and eastern CFR while seeders are more abundant in the southwest CFR (Ojeda, 1998).

Fire frequency is expected to be the main driver of resprouter and seeder proportions in an environment; resprouters are expected to have higher success under high-frequency fire regimes, and seeders are expected to have higher success under low-frequency fire regimes (Keeley & Zedler, 1978; van Wilgen *et al.*, 1992; Bellingham & Sparrow, 2000; Altwegg *et al.*, 2014). However, fires in the CFR occur at fairly regular intervals, with no clear frequency differences between the northwest CFR and the southwest CFR (Ojeda, 1998; Ojeda *et al.*, 2005; Van Wilgen *et al.*, 2010).

The only hypothesis available that might explain the distribution of *Erica* species in the CFR relates to seedling recruitment and pressures of summer drought (Ojeda, 1998; Ojeda *et al.*, 2005). At a seedling level, seeder seedlings are expected to suffer less from drought than resprouter seedlings. Seeder seedlings should invest more material in above-ground biomass (Verdú, 2000), while resprouters should invest in below-ground resprouting organs (lignotuber and thick roots) over above-ground biomass. Seeders benefit from allocation to above-ground biomass because they are able to invest energy into the creation of tougher, more drought tolerant leaves, a trait called sclerophylly (Paula & Pausas, 2006). Additionally, it is assumed that the rapid establishment of seeder seedlings after fire will lead to bigger, more mature seedlings when the first drought comes (Cowling *et al.*, 2005; Pratt *et al.*, 2010). Seeder seedlings have also been found to maintain higher hydraulic conductance in both leaf and stem xylem (Vilagrosa *et al.*, 2014). This is suspected to be an indication of carbon efficiency. Whatever the reason, the improved drought response of seeders has been tested and shown in numerous studies (e.g. Paula & Pausas, 2006; Pratt *et al.*, 2008; Vilagrosa *et al.*, 2014).

Ojeda (1998) used the predicted drought tolerance of seeder seedlings to explain the distribution of resprouter and seeder ericas in the CFR. Ojeda (1998) suggests that there are more seeders in the CFR because the mild summer droughts inhibit seedling success in resprouters since they must invest in below-ground carbon reserves instead of above-ground production and root extension (Verdaguer & Ojeda, 2002; Schwilk & Ackerly, 2005). Seeders will have an advantage of added recruitment success and resource use to devote to above-ground biomass and more extensive (rather that storage-oriented) root mass (Bell & Ojeda, 1999; Verdaguer & Ojeda, 2002; Pratt *et al.*, 2010). This assumption was used in a simple simulation model which confirmed that seeders can replace resprouters under moderate drought (Ojeda *et al.*, 2005), which may explain the high percentage of *Erica* seeders in the CFR.

Recently, there has been an increasing need to understand the mechanisms behind drought-induced plant mortality. The need is primarily due to an increase in large-scale plant die-backs (e.g. forests; Allen *et al.*, 2010). Die-backs have been occurring more frequently in recent years due to increasing temperatures and recurrent droughts. Although the main goal of this thesis is to explore the possible mechanisms for differential success between *E. coccinea* seeders and resprouters, collecting data that indicates the mechanism responsible for death during a drought could provide further insight into the currently relevant concerns around drought-induced die-backs.

Two main physiological mechanisms have been proposed as causing plant mortality in response to drought: carbon starvation and hydraulic failure. A seminal paper by McDowell *et al.* (2008) defines and explains these mechanisms. Hydraulic failure occurs when a plant becomes irreversibly dehydrated. This leads to cavitation, where the water column breaks up and develops air pockets. Hydraulic failure is expected when droughts are short and severe. Carbon starvation on the other hand occurs when a plant maintains its hydraulic function by closing stomata and reducing photosynthesis. It is proposed that the reduction in photosynthesis leads to the plant running out of carbon reserves to maintain basic metabolism. In addition to this, the plant may produce ethanol and other volatiles to maintain cell function under these conditions. Biotic agents, such as insects, might recognise these cues and be able to target stressed individuals and inflict further damage, thereby contributing to mortality (McDowell *et al.*, 2008). The combination of carbon reserve depletion and biotic agent outbreak has been observed in several instances (e.g. Aakala *et al.*, 2011).

The hydraulic failure hypothesis is broadly supported in the literature as a plausible mechanism for explaining plant mortality during drought. Failure of plant hydraulic systems have been correlated to mortality in California chaparral (Pratt *et al.*, 2008), Australian conifers (Brodribb & Cochard, 2009) and Amazonian rainforest trees (da Costa *et al.*, 2010). Although this correlation has been made in these cases and many others, it is difficult to prove the causality of plant death by hydraulic failure (Sala *et al.*, 2010). Even so, the hypothesis is widely accepted as a probable explanation for plant death in drought conditions.

Conversely, the carbon starvation hypothesis as a mechanism for mortality is contested in the literature. While several studies have been conducted in support of the hypothesis (e.g. Adams *et al.*, 2009; Breshears *et al.*, 2009), there have been questions, doubts, and even direct criticism of the studies (Leuzinger *et al.*, 2009) and of the hypothesis itself (Sala *et al.*, 2010). The main criticisms revolve around the lack of a useful method to definitively prove that carbon depletion causes death. Carbon starvation is assumed to be the mechanism for mortality without excluding other possible variables (Leuzinger *et al.*, 2009; Sala *et al.*, 2010). In an attempt to address this criticism, Sevanto *et al.* (2014) showed that plants kept in complete darkness but in fully saturated conditions experience severe carbon depletion and eventual death. Although this proves that there are circumstances under which carbon starvation may be observed, experiments such as these do not help us understand when carbon starvation might occur naturally. As such, the validity of the carbon starvation hypothesis remains unclear and requires further interrogation.

In this chapter, the hypothesis that mild summer droughts limit resprouter recruitment (Ojeda, 1998) was tested by subjecting resprouter and seeder *Erica coccinea* seedlings to a mild water stress and measuring various physiological parameters. I also investigated which hypothesis, carbon starvation or hydraulic failure, most likely explained the mortality mechanism in resprouters and seeders. Resprouter and seeder *E. coccinea* seedlings were exposed to a mild but prolonged drought and changes in photosynthetic rate, stomatal conductance, and water potential were measured over time, as well as total non-structural carbohydrate content at the end of the drought.

3.2 Methods

3.2.1 Study species

Erica coccinea is a common species of *Erica* in the fynbos biome. The species contains resprouter and seeder forms. The resprouter form is characterized by a lignotuber and active basal buds that make resprouting possible (Bell & Ojeda, 1999). The seeder form does not have a lignotuber and the basal buds are atrophied (Verdaguer & Ojeda, 2005). The forms usually occur in discrete populations, although some populations are comprised of both resprouter and seeder individuals. The resprouter population in Blackburn Ravine and the seeder population in Napier were used in this study (Table 2.1, see Chapter 2). I attempted to use more than one population of each form to account for possible interpopulation variation, but the other populations yielded seedling numbers that were well below what was required for the experiment.

3.2.2 Collection and preservation

Ripe capsules were harvested from at least 20 individuals during the population's dispersal period in 2011. Seeds were separated from capsules and stored at ambient temperature (~25°C) in paper bags in an air-tight container filled with silica beads to ensure a dry environment. These conditions were maintained until planting commenced in February 2013.

3.2.3 Planting and transplanting

Approximately a thousand seeds from each population (were scattered across a tray filled with acidic fynbos soil (pH ≈ 5; Figure 3.1A). Trays received an additional layer of soil and were placed inside a sealed tent. Green and dry fynbos litter was burnt in a drum and the smoke was pumped into the tent using a generator (Figure 3.1B). Smoke was pumped continuously into the tent for an hour. Trays were left inside the sealed tent for an additional 2 hours. They were then moved to a glasshouse in the Kirstenbosch Botanical Gardens where they were kept under fully hydrated, ambient glasshouse conditions.

Seedlings began to emerge in April, 2013 (Figure 3.1C). After five months of growth, seeds were transplanted into individual 100cc pots (Figure 3.1D). No *Erica*-specific mycorrhizae was added to the pots after transplanting, even though ericas are known to have specific mycorrhizae that assist with the uptake of nutrients (Ojeda, 1998). However, mycorrhizae require carbon from the plant (Kozlowski, 1992), and this could have complicated non-structural carbohydrate measurements. Instead, seedlings were fertilized with liquid manure (generated and used by the Kirstenbosch Botanical Gardens nursery) as needed (about once a month).

3.2.4 Drought conditions and plant response measurements

One year after emergence, seedlings were transported to a greenhouse facility at the University of Cape Town. Healthy seedlings from both populations were randomly selected for the drought treatment and the control. Although individuals were the same age, they were of a variety of sizes ranging from 3cm to 9cm. Size matching

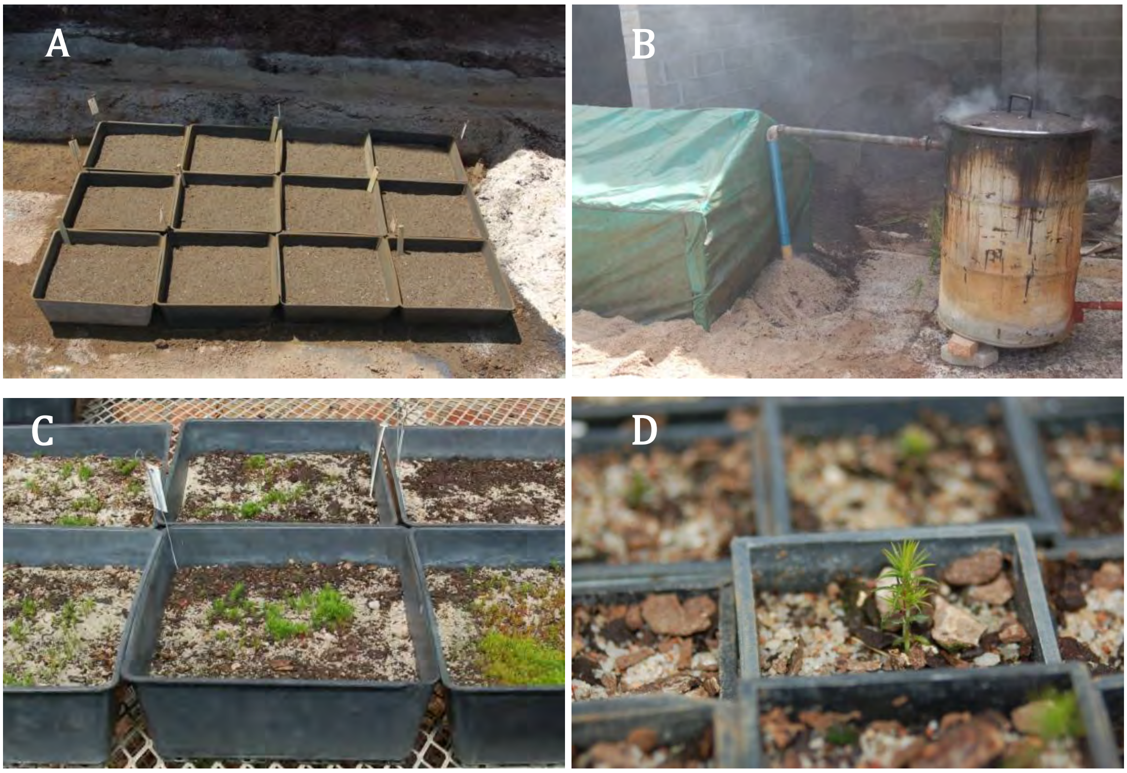

Figure 3.1: *Erica coccinea* seedlings were sown into large trays (A) in February, 2013 and sealed in a tent that was then filled with smoke (B). Seedlings emerged in April (C). In September, seedlings were transplanted into individual pots (D). Drought commenced in March, 2014.

was not possible as it would have caused many of the available plants to be discarded. The control plants were kept at field capacity and the droughted plants received 80% of their daily water loss. Daily water loss was calculated by weighing the pot and replacing 80% of whatever weight was lost after 24 hours. Plants were kept in the same temperature-controlled chamber with a 12-hour diurnal light/dark fluctuation (daytime light of 1000 umol m^{-2} s^{-1}) and temperature fluctuations of 22°C during the day and 15°C at night.

A mortality curve was generated by calculating the proportion of dead seedlings from the remaining plants on each measurement day. Death was affirmed if plants were dry, colourless and crispy. This was confirmed by a separate re-watering experiment where ten resprouter seedlings and ten seeder seedlings that were assumed dead received ample water for the following three months. None of the plants were revived.

Plant response measurements were taken for eight different plants from each of the forms (resprouter and seeder) and treatments (control and drought) nine times throughout the droughting period. Because plants were destructively harvested to measure water potential, each measurement day contained a different group of eight plants per form and treatment. A Li-Cor 6400 infrared gas analyser (Li-Cor BioSciences, Lincoln) was used to measure midmorning photosynthetic rate and stomatal conductance (between 9am and 11am). Light in the chamber was set to 800 μmol m^{-2} s^{-1}. CO_2 concentrations were kept at 400 ppm, flow rate was set to 400 μmol s^{-1} and humidity was maintained slightly below ambient. Shoot water potential was measured at midday using a Scholander pressure chamber (PMS instruments). Photosynthetic rate, stomatal conductance and water potential were measured at day zero to determine if there were any significant differences between groups (one-way ANOVA). The only difference observed was a slightly lower water potential of seeder seedlings in the drought group than the resprouters in the drought group and seeders in the control group ($F_{3,28} = 3.08$; $P = 0.043$).

Towards the end of the experiment, dead plants sometimes occurred within the randomly selected replicates of eight plants for a given measurement period. In this case, these plants were not measured as they were prone to crumbling inside the measurement chamber which both damaged the chamber and risked biomass loss for subsequent biomass, soluble sugars and starch measurements. Instead, these plants were assigned photosynthetic rate and stomatal conductance values of zero and water potential values of -6 MPa, a rough estimate generated from the P_{88} values (water potential at 88% loss of conductivity) of two *Erica* species (*E. monsoniana* and *E. plukenetii*; Skelton, 2014). An alternative approach would have been to exclude these dead plants from the dataset entirely. However, this would have biased the results towards healthy plants and failed to adequately capture the real drought response of these groups. While a zero value for photosynthetic rate and stomatal conductance is well justified, the -6 MPa value for the water potential is somewhat arbitrary, as real values could have approached infinity for completely desiccated plants. However, I feel that the approximate P_{88} value of -6 MPa was an accurate reflection of a lethal water potential for this species, without imposing overly negative values that would have skewed the dataset.

3.2.5 Biomass, soluble sugars and starch

Plants from the last two measurement days (days 32 and 40) were used for the analysis of biomass, soluble sugars and starch. These two days were combined and treated as one "endpoint" (i.e. >32 days drought) in order to generate a more robust sample size (n=16, 8 plants from each day). Root and shoot dry weights were measured for each of these plants, from which root:shoot ratios were calculated.

Plants from the "endpoint" were then paired (n=8) to generate enough biomass for the soluble sugars and starch analysis. Analysis was conducted using the method in Alcoverro *et al.* (1999). To briefly summarize, soluble sugars were extracted using methanol, after which the methanol was evaporated and the remaining sugars were dissolved in water. Each sugar sample and a sucrose standard were treated with resorcinol and hydrocloric acid and incubated at 80°C for 15 minutes. The dehydrated ketoses reacted with resorcinol to produce a reddish colour. Absorbance of each sample and the standard was measured at 486nm using a 96-well microplate spectrophotometer (Thermo Scientific Multiskan Spectrum, SkanIT software 2.4.2, 2004-2007).

The pellet left over from the soluble sugar extraction was treated with sodium hydroxide and left to sit at room temperature for 18 hours to break the starch into glucose. After centrifugation, the supernatant and a sucrose standard were treated with anthrone reagent and sulfuric acid and then incubated at 100°C for 15 minutes. Glucose and sulfuric yielded furfural which reacted with anthrone reagent to produce a greenish colour. The absorbance of each sample and the standard was measured at 486nm using a 96-well microplate spectrophotometer (Thermo Scientific Multiskan Spectrum, SkanIT software 2.4.2, 2004-2007). All soluble sugar and starch data are expressed as a percentage of dry biomass.

3.2.6 Statistical analysis

Mortality curves for each form (resprouter and seeder) and treatment (control and drought) were analysed using a log rank test for Kaplan–Meier curves. An analysis of plant response (photosynthetic rate, stomatal conductance and water potential) for different forms (resprouter and seeder) under the different treatments (control and drought) was conducted using a full factorial ANOVA for each measurement day (STATISTICA version 9, StatSoft, Tulsa, USA). All assumptions were met. Means were separated using a Duncan's post-hoc test. A t-test was then used to determine whether the differences between control and drought (control minus drought) on each day for each physiological response were different between resprouters and seeders.

Root:shoot biomass ratios for different forms (resprouter and seeder) and treatments (control and drought) at the endpoint (n=16, days 32 and 40) were analysed using a full factorial ANOVA. Data were log transformed to meet the assumptions for the ANOVA. Soluble sugars and starch content for different forms (resprouter and seeder), treatments (control and drought) and biomass types (above-ground, below-ground) at the endpoint (n=8, paired plants from days 32 and 40) were also analysed using a full factorial ANOVA. These data were log transformed and means for all ANOVAs were separated using Duncan's post-hoc tests.

3.3 Results

3.3.1 Plant response

Erica coccinea seeder seedlings died significantly sooner than *Erica coccinea* resprouter seedlings under moderate drought conditions ($\chi^2_{(1, 20)}=24.74$, $P<0.01$; Figure 3.2). Both forms began to die on day 25. By day 32, seeders had experienced 30% mortality and resprouters had experienced 18% mortality. By day 40, the end of the experiment, seeders reached 65% mortality and resprouters had reached 57% mortality. This separation did not extend to day 50, when both forms reached 95% mortality.

Mortality differences between seeder and resprouter seedlings were not reflected in the physiological differences. For the results of the full factorial ANOVAs and post-hoc tests, see Appendices 1 and 2. Both forms had similar photosynthetic rates,

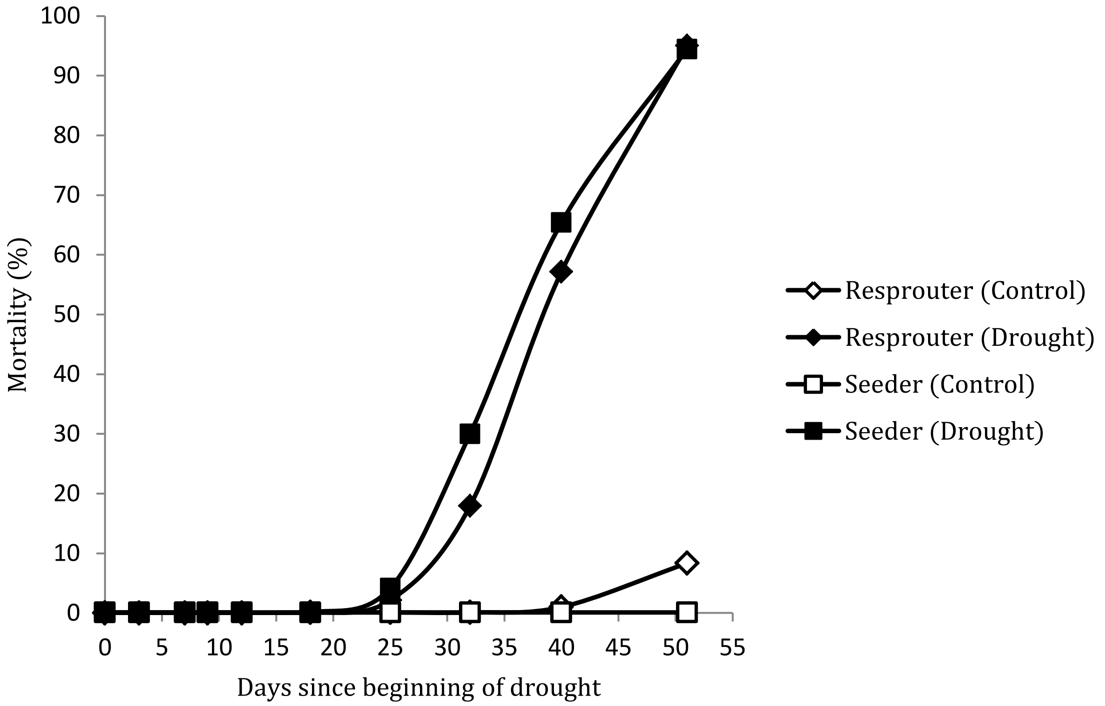

Figure 3.2: Mortality curves of seeder and resprouter *Erica coccinea* seedlings under a moderate drought treatment and a control treatment (non-droughted). Seeders had higher mortality rates than resprouters (Kaplan–Meier log rank test; $\chi^2_{(1,20)}=24.74$, $P<0.01$).

stomatal conductance and water potential for the duration of the experiment (Figures 3.3, 3.4 & 3.5, Appendix 1 & 2). Droughted seedlings had significantly lower photosynthetic rate than control seedlings on days 3, 25 and 40 ($P<0.05$; see Appendix 1 & 2), but there was no significant interaction between form (seeder and resprouter) and treatment (drought and control). Stomatal conductance was significantly lower in droughted seedlings compared to control seedlings from day 9 through day 40 ($P<0.05$, see Appendix 1 & 2), but there was no significant interaction between form and treatment. The resprouters had lower water potential than the seeder seedlings at the beginning of the experiment ($P<0.05$, see Appendix 1 & 2) but droughted seedlings had lower water potential than control plants on day 25, 30 and 40 ($P<0.05$, see Appendix 1 & 2) with no significant interaction between form and treatment. Resprouter and seeder seedlings were also similar in terms of the differences (control minus drought) in physiological responses for each measurement day (Figures 3.3, 3.4, & 3.5; t-test results given in Appendix 3).

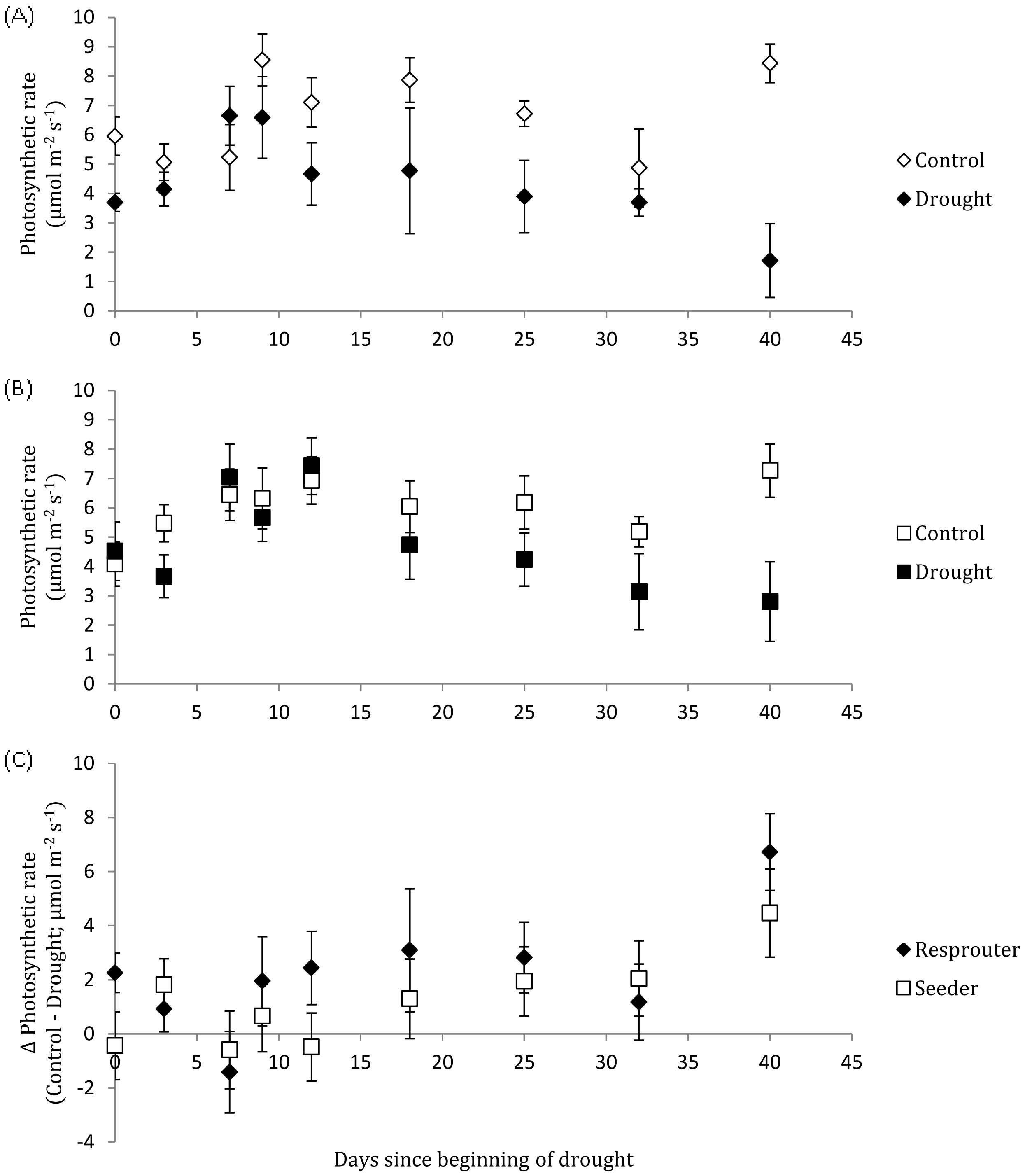

Figure 3.3: Photosynthetic rate (± SE) of resprouter (A) and seeder (B) *Erica coccinea* seedlings under drought and control treatments, and the difference (C) between these treatments for both forms. Analysis for each measurement day was performed using a full factorial ANOVA (see Appendix 1 & 2). A t-test was then used to analyse difference data (C).

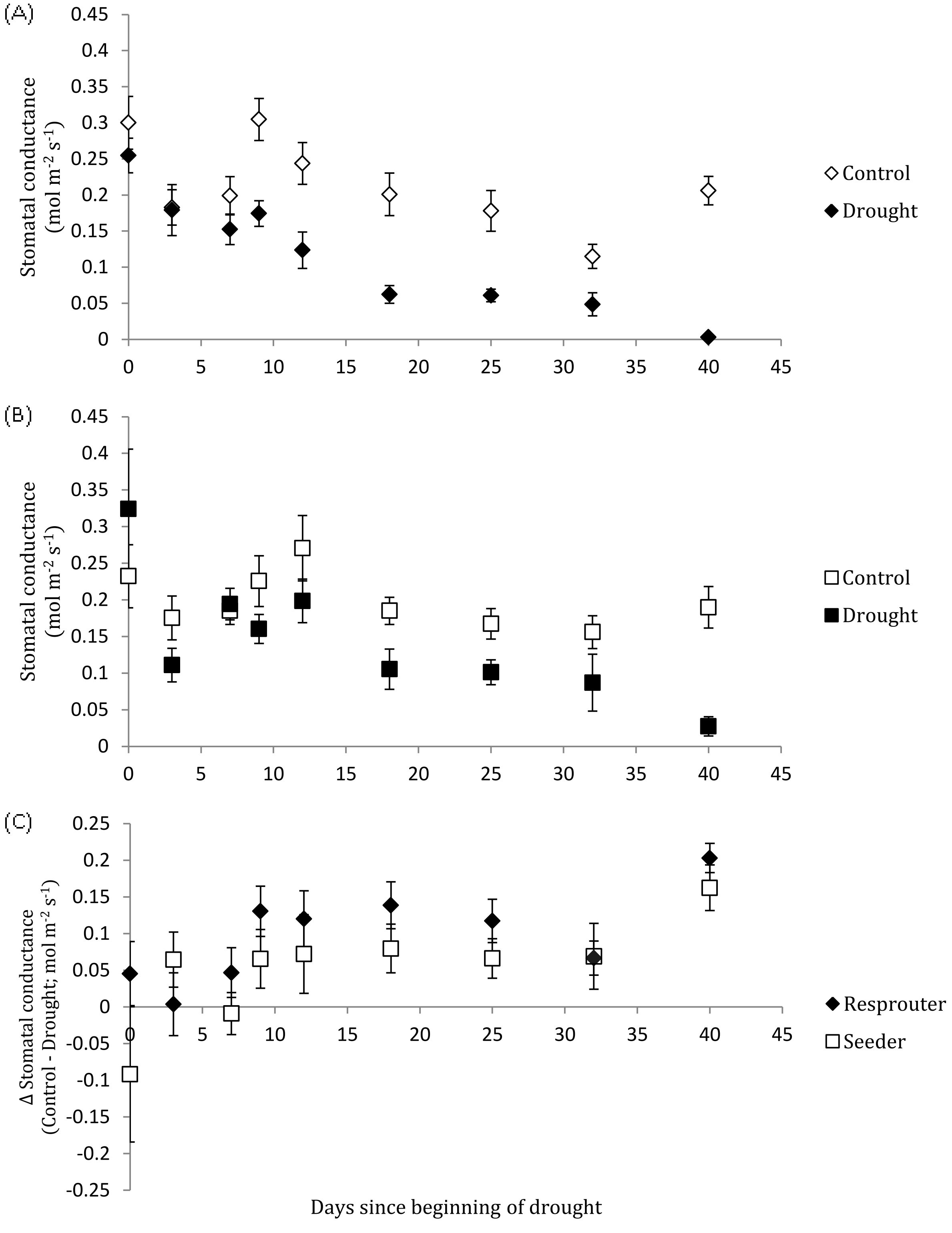

Figure 3.4: Stomatal conductance (± SE) of resprouter (A) and seeder (B) *Erica coccinea* seedlings under drought and control treatments, and the difference (C) between these treatments for both forms. Analysis for each measurement day was performed using a full factorial ANOVA (see Appendix 1 & 2). A t-test was then used to analyse difference data (C).

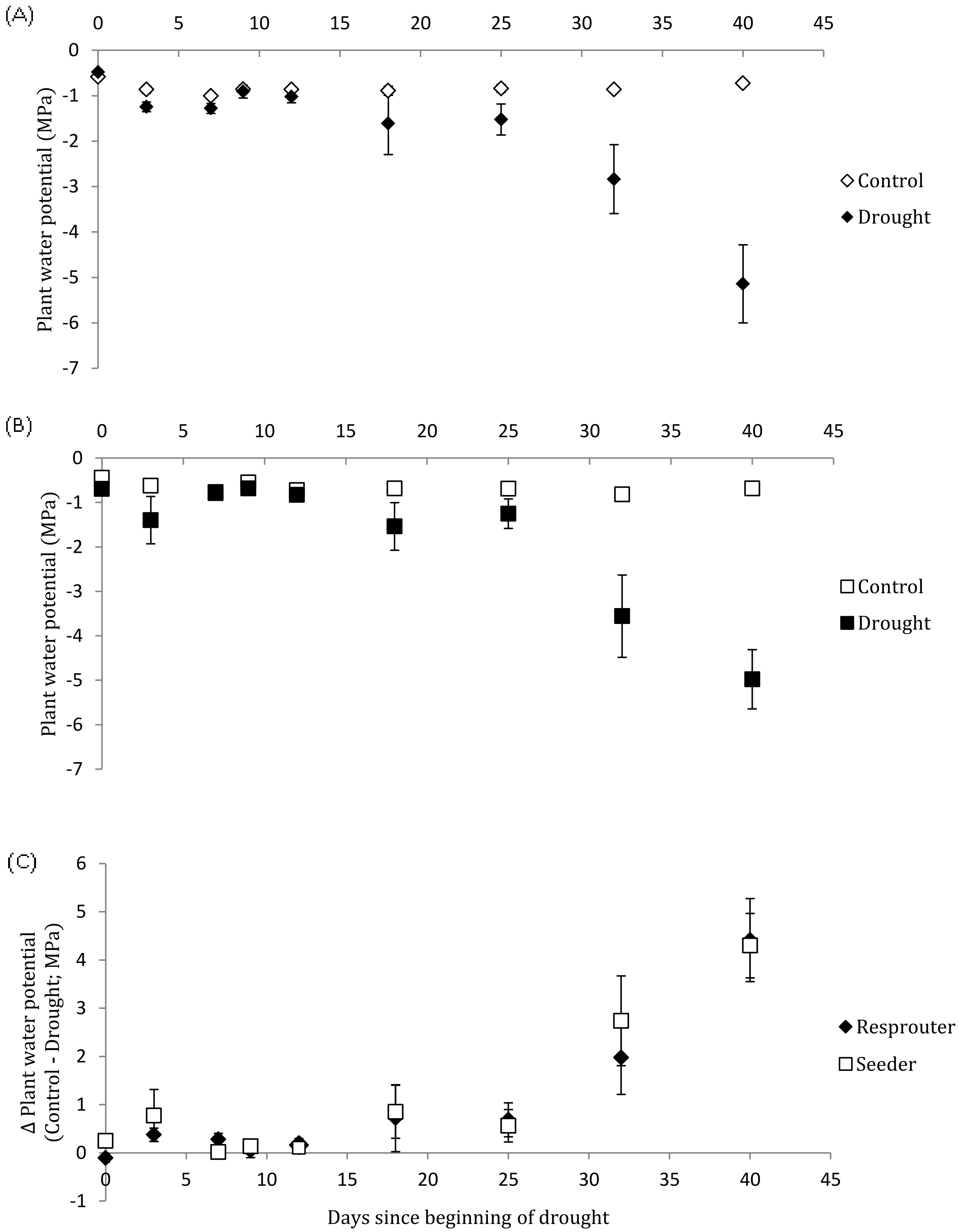

Figure 3.5: Plant water potential (± SE) of resprouter (A) and seeder (B) *Erica coccinea* seedlings under drought and control treatments, and the difference (C) between these treatments for both forms. Analysis for each measurement day was performed using a full factorial ANOVA (see Appendix 1 & 2). A t-test was then used to analyse difference data (C).

3.3.2 Biomass, soluble sugars and starch

There was a consistent decrease in root:shoot biomass ratio in the drought treatment for both resprouter and seeder seedlings (Figure 3.6), though the ratios for each treatment were similar between types. Drought caused an increase in below-ground soluble sugars for resprouters (Figure 3.7), but there was no change in soluble sugars between the control and drought treatments for the seeder. Overall, there was more soluble sugars above-ground than below-ground for both forms. Drought had no effect on starch for resprouters but it caused a decrease in above-ground starch for seeders. For results of the full factorial ANOVAs for root:shoot ratios and soluble sugars and starch content, see Appendix 4.

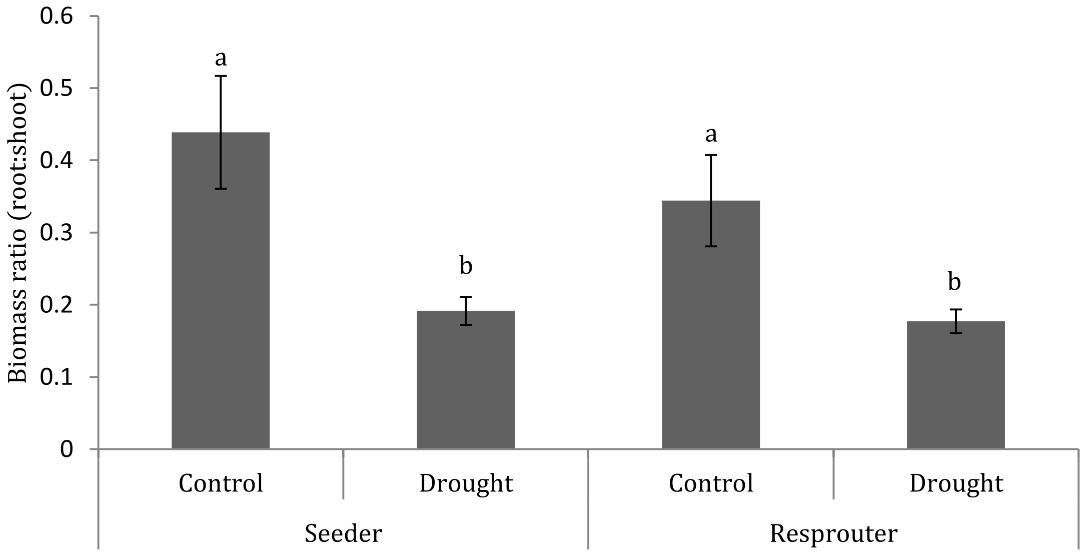

Figure3.6: Root:shoot biomass ratio (± SE, n=16)of seeder and resprouter *Erica coccinea* seedlings under drought and control treatments at the end of the experiment. Data were log transformed for analysis. Untransformed data is presented here because backtransformed means had a similar pattern. Analysis was performed using a full factorial ANOVA (see Appendix 4). Different letters indicate significant differences (Duncan's post-hoc test).

3.4 Discussion

Summer drought patterns are expected to shape the distribution of resprouter and seeder *Erica* in the CFR (Ojeda, 1998). This rests on the expectation that seeder seedlings will survive drought better than resprouter seedlings (Ojeda, 1998; Vilagrosa *et al.*, 2014). The results presented in this chapter directly contradict this expectation by showing that *Erica coccinea* seeder seedlings died faster than resprouter seedlings under a mild drought (Figure 3.2), which is opposite to the bulk of the literature that has investigated resprouter and seeder responses to drought (Paula & Pausas, 2006; Pratt *et al.*, 2010; Vilagrosa *et al.*, 2014).

In light of these results, the hypothesis that mild drought drives *Erica* resprouter and seeder distribution must be re-examined. One of the reasons why this hypothesis was proposed is because fire-frequency is fairly uniform across the CFR (Le Maitre & Midgley, 1992; Van Wilgen *et al.*, 2010). However, the CFR has an average fire return

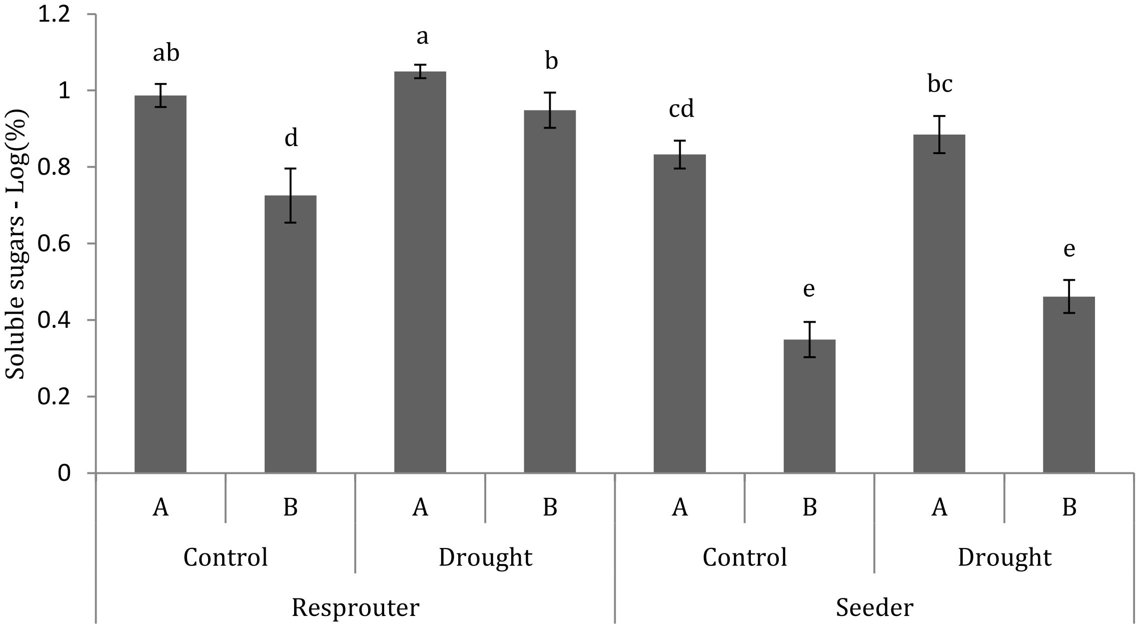

Figure3.7: Log of percent soluble sugars content of dry material (± SE, n=8) in above (A) and below (B) ground material in seeder and resprouter *Erica coccinea* seedlings under drought and control treatments at the end of the experiment. Analysis was performed using a full factorial ANOVA (see Appendix 4). Different letters indicate significant differences (Duncan's post-hoc test).

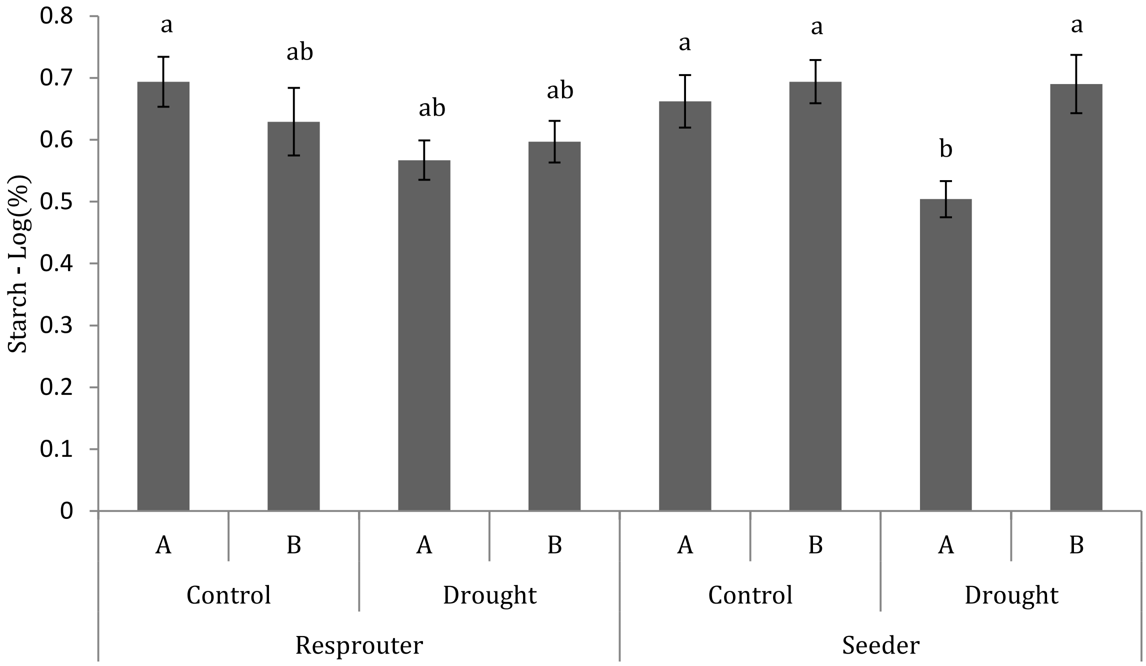

Figure 3.8: Log of percent starch content of dry material (± SE, n=8) in above (A) and below (B) ground material in seeder and resprouter *Erica coccinea* seedlings under drought and control treatments at the end of the experiment. Analysis was performed using a full factorial ANOVA (see Appendix 4). Different letters indicate significant differences (Duncan's post-hoc test).

interval of 10-13 years (Van Wilgen *et al.*, 2010). The ideal interval that supports *Proteaceae* seeders in South Africa is 9-13 years (Altwegg *et al.*, 2014). If this is true for *Erica* species, then fire could explain why there are so many *Erica* seeders. Although this still doesn't account for a higher presence of resprouters in the northwest and eastern CFR, the patchiness of fire in the fynbos might. Fires may have fairly regular return intervals, but they tend to be highly patchy (Tucker *et al.*, 2012). For example, in June 2012, the Greyton Nature Reserve burned (J. Leonard, pers. obs.). Most of the mountain and the surrounding hills had been burnt except for a small, south-facing slope. That one unburnt area contained a reprouter population of *E. coccinea* (Malan, 2013). Infrequent burns should surely select for the seeder form (Keeley & Zedler, 1978). While it is unclear what factors caused this area not to burn (e.g. fuel load, wind, higher moisture) and unlikely for the exact scenario to be replicated for all other populations of *E. coccinea* resprouters, it is important not to dismiss fire as a potential cause for *Erica* seeder and resprouter distribution.

The higher mortality of seeders in this experiment is not clearly reflected in the physiological responses. Photosynthetic rate and stomatal conductance were similar between droughted resprouter and seeder seedlings over the duration of the drought (Figure 3.3 & 3.4). Seeders initially had higher water potential than resprouters, but this difference disappeared by day 18 (Figure 3.5). It is likely that the drought was too severe to accurately capture differences in physiological responses. The watering regime (80% of daily water loss) for plants under the drought treatment was intended to induce a slow, gradual drought. However, almost all droughted plants died after 50 days (Figure 3.2). Had the drought been less severe, the initial separation in mortality rates between resprouters and seeders observed at days 32 and 40 may have been prolonged, allowing for more accurate detection between physiological responses over this time period.

Due to their rapid mortality and dried appearance at death, plants experienced hydraulic failure rather than carbon starvation. While the drought in this experiment was intended to be prolonged, its severity (plants died after 50 days) is grounds for assuming hydraulic failure. Hydraulic failure is generally expected in seedlings and is also expected in individuals or populations that experience short and severe drought (McDowell *et al.*, 2008). Carbon starvation on the other hand occurs during a prolonged drought, where the hydraulic system is able to maintain its function (by closing stomata, reducing photosynthetic rate) but the plant runs out of carbon reserves necessary for metabolism. For these seedlings, hydraulic system function was not maintained for either seeder or resprouter seedlings, and droughted plants died with levels of non-structural carbohydrates (NSCs) that were far from being exhausted (Figure 3.7 & 3.8).

The observed changes in carbon were consistent with predicted changes in carbon allocation during a drought (as summarized in Sala *et al.*, 2012). Carbon generated in the leaves moves to other parts of the plant under normal circumstances. Under drought, metabolism will slow down in order to conserve resources. If the metabolic sink becomes lower than the source, this could lead to a temporary increase in NSCs followed by a gradual decline as photosynthesis slows (McDowell *et al.*, 2011; Sala *et al.*, 2012). Resprouters may have died while NSCs were temporarily elevated, which could account for the higher levels of below-ground soluble sugars (Figure 3.7). The seeders on the other hand were harvested at a period of NSC decline, which would explain the decrease in below-ground starch. Although these results could fit with the prediction of carbon allocation change during drought, they should not be interpreted as evidence for carbon starvation in these seedlings.

As a side note, *E. coccinea* resprouter individuals are expected to have higher starch content than seeders in their roots, which has been found in adults (Bell & Ojeda, 1999) and very early seedling stages (Verdaguer & Ojeda, 2002). I did not find any difference between starch content below- or above-ground between resprouters and seeders in the control group (Figure 3.8). The method use by Verdaguer & Ojeda (2002) to quantify starch in seedlings was an image analysis of a cross section of a root fragment taken from 1 cm below the cotyledonary node. Although this may be true for that that section of root, these results indicate that this is not a complete reflection of total below-ground starch.

Although *E. coccinea* seeder seedlings died faster than *E. coccinea* resprouter seedlings, this does not mean that the trend will follow for all *Erica* species. The distribution hypothesis presented by Ojeda (1998) is based off of an assembly of over 400 species. My study focussed on a single, closely related pair of resprouter and seeder that even co-occur in some populations. Studies with additional species that are more genetically separated may reveal that *E. coccinea* is an exception, not a norm. To make inferences for the whole *Erica* genus based on such a closely related pair is ill-advised. A wider array of *Erica* species that are fully separated into resprouters and seeders should be used for future studies.

These results may also be because of interpopulation variation. In Chapter 2, I found significant variation in germination success between seeder populations. For this chapter, I was only able to use one population of each form. There have been examples of higher variability between populations rather than species (Cruz *et al.*, 2003; Moreira *et al.*, 2012) while other studies show species-specific differences outweigh regional variation (Moreira *et al.*, 2010). A previous study on *E. coccinea* found higher genetic diversity among and within seeder *E. coccinea* populations than among and within resprouter populations (Segarra-Moragues & Ojeda, 2010). I was unable to assess this in terms of germination success because of the failure of one of the resprouter populations. I was also unable to test for interpopulation variation in this chapter because only one population was used for each form. It is entirely possible that these populations of resprouter and seeder *E. coccinea* have a slightly different physiological response to drought than other populations. More studies are needed to determine if interpopulation differences are greater than interform differences for *E. coccinea*.

3.5 Conclusion

Our results show that year-old seeder *Erica coccinea* seedlings die faster than year-old resprouter seedlings during drought. Their mortality was due to hydraulic failure. This shows that the distribution of this species cannot be explained by the vulnerability of resprouter seedlings to drought. I suggest that future research should be conducted using a wider variety of more genetically separated seeder and resprouter *Erica* species in the CFR to test if *Erica* distribution is due to the vulnerability of resprouter seedlings to drought. It would also be worthwhile to investigate how important interpopulation variability is to drought tolerance in *E. coccinea* resprouters and seeders.

3.6 References

Aakala T, Kuuluvainen T, Wallenius T, Kauhanen H. 2011. Tree mortality episodes in the intact *Picea abies*-dominated taiga in the Arkhangelsk region of northern European Russia. *Journal of Vegetation Science* **22**: 322–333.

Adams HD, Guardiola-Claramonte M, Barron-Gafford G a, Villegas JC, Breshears DD, Zou CB, Troch P a, Huxman TE. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 7063–6.

Alcoverro T, Zimmerman RC, Kohrs DG, Alberte RS. 1999. Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. *Marine Ecology Progress Series* **187**: 121–131.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (Ted), ***et al.*** **2010**. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**: 660–684.

Altwegg R, De Klerk HM, Midgley GF. 2014. Fire-mediated disruptive selection can explain the reseeder-resprouter dichotomy in Mediterranean-type vegetation. *Oecologia* **Published** : 1–11.

Bell DT. 2001. Resprouters versus reseeders ecological response syndromes in the flora of southwestern Western Australia: fire resprouters versus reseeders. *The Botanical Review* **67**: 417–440.

Bell TL, Ojeda F. 1999. Underground starch storage in *Erica* species of the Cape Floristic Region - differences between seeders and resprouters. *New Phytologist* **144**: 143–152.

Bellingham PJ, Sparrow AD. 2000. Resprouting as a life history strategy in woody plant communities. *Oikos* **89**: 409–416.

Breshears DD, Myers OB, Meyer CW, Barnes FJ, Zou CB, Allen CD, McDowell NG, Pockman WT. 2009. Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment* **7**: 185–189.

Brodribb TJ, Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant physiology* **149**: 575–84.

Brown NAC. 1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**: 575–583.

Da Costa ACL, Galbraith D, Almeida S, Portela BTT, da Costa M, Silva Junior JDA, Braga AP, de Gonçalves PHL, de Oliveira A a R, Fisher R, ***et al.*** **2010**. Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. *The New Phytologist* **187**: 579–91.

Cowling RM, Ojeda F, Lamont BB, Rundel PW, Lechmere-oertel R. 2005. Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. *Global Ecology and Biogeography* **14**: 509–519.

Cruz A, Pérez B, Velasco A, Moreno JM. 2003. Variability in seed germination at the interpopulation , intrapopulation and intraindividual levels of the shrub *Erica australis* in response to fire-related cues. *Plant Ecology* **169**: 93–103.

Keeley JE, Bond WJ. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**: 153–167.

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**: 406–11.

Keeley JE, Zedler PH. 1978. Reproduction of Chaparral shrubs after fire: a comparison of sprouting and seeding strategies. *American Midland Naturalist* **99**: 142–161.

Kozlowski TT. 1992. Carbohydrate sources and sinks in woody plants. *Botanical Review* **58**: 107–222.

Leuzinger S, Bigler C, Wolf A, Körner C. 2009. Poor methodology for predicting large-scale tree die-off. *Proceedings of the National Academy of Sciences of the United States of America* **106**: E106; author reply E107.

Le Maitre D, Midgley J. 1992. Plant reproductive ecology. In: Cowling RM, ed. *The ecology of fynbos: nutrients, fire and diversity*. Cape Town: Oxford University Press, 135–174.

Malan M. 2013. *Intraspecific variation in Erica coccinea.* Master's thesis, University of Cape Town, Cape Town, South Africa.

McDowell NG, Beerling DJ, Breshears DD, Fisher R a, Raffa KF, Stitt M. 2011. The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in Ecology & Evolution* **26**: 523–32.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, *et al.* 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *The New Phytologist* **178**: 719–39.

Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**: 627–35.

Moreira B, Tormo J, Pausas JG. 2012. To resprout or not to resprout: factors driving intraspecific variability in resprouting. *Oikos* **121**: 1577–1584.

Ojeda F. 1998. Biogeography of seeder and resprouter *Erica* species in the Cape Floristic Region-Where are the resprouters? *Biological Journal of the Linnean Society* **63**: 331–347.

Ojeda F, Brun FG, Vergara JJ. 2005. Fire, rain and the selection of seeder and resprouter life-histories in fire-recruiting, woody plants. *The New Phytologist* **168**: 155–65.

Paula S, Pausas JG. 2006. Leaf traits and resprouting ability in the Mediterranean basin. *Functional Ecology* **20**: 941–947.

Pausas JG, Keeley JE, Verdú M. 2006. Inferring differential evolutionary processes of plant persistence traits in Northern Hemisphere Mediterranean fire-prone ecosystems. *Journal of Ecology* **94**: 31–39.

Pratt RB, Jacobsen a. L, Mohla R, Ewers FW, Davis SD. 2008. Linkage between water stress tolerance and life history type in seedlings of nine chaparral species (Rhamnaceae). *Journal of Ecology* **96**: 1252–1265.

Pratt RB, North GB, Jacobsen AL, Ewers FW, Davis SD. 2010. Xylem root and shoot hydraulics is linked to life history type in chaparral seedlings. *Functional Ecology* **24**: 70–81.

Sala A, Piper F, Hoch G. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *The New Phytologist* **186**: 274–81.

Sala A, Woodruff DR, Meinzer FC. 2012. Carbon dynamics in trees: feast or famine? *Tree physiology* **32**: 764–75.

Schwilk DW, Ackerly DD. 2005. Is there a cost to resprouting? Seedling growth rate and drought tolerance in sprouting and nonsprouting *Ceanothus* (Rhamnaceae). *American Journal of Botany* **92**: 404–410.

Segarra-Moragues JG, Ojeda F. 2010. Postfire response and genetic diversity in *Erica coccinea*: connecting population dynamics and diversification in a biodiversity hotspot. *Evolution; International Journal of Organic Evolution* **64**: 3511–24.

Sevanto S, McDowell NG, Dickman LT, Pangle R, Pockman WT. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell and Environmentell & environment* **37**: 153–61.

Skelton, R. 2014. *The role of hydraulic strategies in understanding the response of fynbos to drought.* PhD thesis, University of Cape Town, Cape Town, South Africa.

Tucker CM, Rebelo AG, Manne LL. 2012. Contribution of disturbance to distribution and abundance in a fire-adapted system. *Ecography* **35**: 348–355.

Verdaguer D, Ojeda F. 2002. Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape *Erica* (Ericaceae) species. *American Journal of Botany* **89**: 1189–1196.

Verdaguer D, Ojeda F. 2005. Evolutionary transition from resprouter to seeder life history in two *Erica* (Ericaceae) species: insights from seedling axillary buds. *Annals of Botany* **95**: 593–9.

Verdú M. 2000. Ecological and evolutionary differences between Mediterranean seeders and resprouters. *Journal of Vegetation Science* **11**: 265–268.

Vilagrosa A, Hernández EI, Luis VC, Cochard H, Pausas JG. 2014. Physiological differences explain the co-existence of different regeneration strategies in Mediterranean ecosystems. *The New Phytologist* **201**: 1277–88.

Van Wilgen BW, Bond WJ, Richardson DM. 1992. Ecosystem management. In: Cowling RM, ed. *The ecology of fynbos: nutrients, fire and diversity*. Cape Town: Oxford University Press, 345–371.

Van Wilgen BW, Forsyth GG, De Klerk H, Das S, Khuluse S, Schmitz P. 2010. Fire management in Mediterranean-climate shrublands: a case study from the Cape fynbos, South Africa. *Journal of Applied Ecology* **47**: 631–638.

Chapter 4: Synthesis of findings on resprouter, seeder and ‘pyrofuge’ forms of *Erica coccinea*

“Science is no more than an investigation of a miracle we can never explain.”

— Ray Bradbury, The Martian Chronicles (1950)

4.1 Differences between resprouters and seeders

Erica coccinea resprouters and seeders are genetically distinct from one another (Verdaguer & Ojeda, 2002), which is actualized in their phenotypic differences. Resprouter adults have dormant basal buds which allow them to resprout after fire as well as a higher starch content than seeder adults in their roots (Bell & Ojeda, 1999). Seeders have atrophied basal buds (Verdaguer & Ojeda, 2005) but a higher seed set than resprouters (Malan, 2013).

This thesis adds to our understanding of *E. coccinea* resprouters and seeders by showing the similarities and difference in terms of germination success and response to drought. Chapter 2 showed that seeders had some level of germination when smoke was absent from the environment while resprouters had little germination in the absence of smoke. Resprouters germinated slightly faster than seeders and also had slightly higher success than seeders in the highest ambient temperature treatment. Chapter 3 showed that seeder seedlings died significantly faster than resprouter seedlings under a moderate drought.

These findings directly contradict previous studies and expectations. It was predicted that seeders would have faster and higher germination success due to their short generational times which should improve their ability to track their environment (see Chapter 2). It was also expected that they would have better drought resistance due to physiological benefits predicted for the seeder phenotype in general (see Chapter 3). There was no adaptive benefit detected in seeders in terms of germination or drought resistance. This goes against general expectations of the seeder phenotype (e.g. Pausas & Keeley, 2014; Vilagrosa *et al.*, 2014) and also questions the hypothesis presented by Ojeda (1998) that *Erica* distribution in the Cape Floristic Region (CFR) is due to the improved ability of seeder seedlings to withstand drought.

These findings may not be ubiquitous for all *E. coccinea* resprouters and seeders. As differentiated as these phenotypes are, there may be more variation between populations than between forms with regards to germination and drought resistance. There was evidence of interpopulation variation in the germination experiment (Chapter 2), in which the two seeder populations had different germination rates under smoke and different success in the absence of smoke. In fact, one seeder population was more similar to the resprouter population than it was to the other seeder population. It is possible that this variation may only be true for seeder populations. *Erica coccinea* populations have been found to have higher genetic diversity among and within seeder *E. coccinea* populations than resprouter populations (Segarra-Moragues & Ojeda, 2010). However, I was unable to determine interpopulation variation within the resprouters because of the failure of one population in the germination experiment and because only two populations yielded enough seedlings to be accurately assessed in the drought experiment. There are examples of higher variability between populations (Cruz *et al.*, 2003) although this is not always true (e.g. Moreira *et al.*, 2010). Further research should be dedicated to examining interpopulation variability in terms of germination and drought resistance in *E. coccinea* resprouters and seeders.

Using phenotypes within a species is recommended because it eliminates other possible variables (Schwilk & Ackerly, 2005). However, it may not be an accurate reflection of the phenotype in its fully differentiated form. The hypothesis behind the *Erica* distribution in the CFR was based on over 400 species (Ojeda, 1998). As it is unclear how interpopulation variation may have influenced the results of these two chapters, I would recommend using more distinct seeder and resprouter species within the *Erica* genus to provide more accurate information regarding the differential drought resistance of resprouters and seeders. As for *E. coccinea*, my conclusions that (1) resprouters germinated faster than seeders, (2) seeders had higher germination in the absence of smoke and (3) seeders died faster under a drought, are restricted to the populations used in this study until such time as inter-population variability in *E. coccinea* can be addressed.

4.2 Revisiting the curious case of the 'pyrofuge'

Erica coccinea has seeder-like populations found in fire refugia that have high germination success whether or not smoke is present. This discovery opens up a new avenue through which we can study fire-adapted traits in plants. Many studies referenced throughout this thesis have examined adaptations to fire, some of which have focused on how fast adaptations occur. Gómez-González *et al.* (2011) found that *Helenium aromaticum*, an annual herb native to Chile that has only been subjected to fire for 500 years, has seed traits that are associated with fire frequency. Here, we have the opportunity to reverse the typical approach to studying how traits adapt to fire and instead look at how fire-adapted traits adjust to the absence of fire. There are areas within fire-prone vegetation matrices that naturally lack fire (Bond *et al.*, 1988; Clarke, 2002; Tapper *et al.*, 2014) and also areas where fire regimes have changed more recently due to climate change or management practices (Walck *et al.*, 2011). Studying plants in fire refugia could improve our understanding of how plant traits change when fire is excluded from an environment.

4.3 Facilitating the study of plant trait evolution through conservation

The kind of research mentioned above is only possible if appropriate measures are taken to preserve and study fire refugia. Although refugia are often in isolated, out-of-the-way places, free from direct anthropogenic impacts, I suspect that they will be more sensitive to climate change. Chapter 2 showed that pyrofuges germinate at lower temperatures than resprouters and seeders. Additionally, these populations essentially live on islands in a sea of fire. As climate pushes the population's range off of these islands, pyrofuges will have to migrate into the fire and risk germinating in between fires, which could lead to the incineration of the population before it reaches maturity.

By conserving these small islands of unique vegetation, we are preserving our ability to study the evolutionary pressures that fire-adapted plants will experience in the absence of fire. We have a valuable snapshot of this species in terms of its evolutionary trajectory. Like Darwin and his observations of the finches, a moment in time has been captured when differentiation is noticeable. We only hold this single picture of what is to come. How the *Erica coccinea* pyrofuge will separate, differentiate and react in the future, however many millions of years from now (if they and we survive the Anthropocene) is for future scientists to observe and study. We are responsible to conserve species such as these, to protect them and give them a chance to show us the finer nuances of speciation and divergence.

4.3 References

Bell TL, Ojeda F. 1999. Underground starch storage in *Erica* species of the Cape Floristic Region - differences between seeders and resprouters. *New Phytologist* **144**: 143–152.

Bond WJ, Midgley J, Vlok J. 1988. When is an island not an island? Insular effects and their causes in fynbos shrublands. *Oecologia* **77**: 515–521.

Clarke PJ. 2002. Habitat islands in fire-prone vegetation: do landscape features influence community composition? *Journal of Biogeography* **29**: 677–684.

Cruz A, Pérez B, Velasco A, Moreno JM. 2003. Variability in seed germination at the interpopulation , intrapopulation and intraindividual levels of the shrub *Erica australis* in response to fire-related cues. *Plant Ecology* **169**: 93–103.

Gómez-González S, Torres-Díaz C, Bustos-Schindler C, Gianoli E. 2011. Anthropogenic fire drives the evolution of seed traits. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 18743–18747.

Malan M. 2013. *Intraspecific variation in Erica coccinea.* Master's thesis, University of Cape Town, Cape Town, South Africa.

Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**: 627–35.

Ojeda F. 1998. Biogeography of seeder and resprouter *Erica* species in the Cape Floristic Region-Where are the resprouters? *Biological Journal of the Linnean Society* **63**: 331–347.

Pausas JG, Keeley JE. 2014. Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist* **204**: 55–65.

Schwilk DW, Ackerly DD. 2005. Is there a cost to resprouting? Seedling growth rate and drought tolerance in sprouting and nonsprouting *Ceanothus* (Rhamnaceae). *American Journal of Botany* **92**: 404–410.

Segarra-Moragues JG, Ojeda F. 2010. Postfire response and genetic diversity in *Erica coccinea*: connecting population dynamics and diversification in a biodiversity hotspot. *Evolution; International Journal of Organic Evolution* **64**: 3511–24.

Tapper S-L, Byrne M, Yates CJ, Keppel G, Hopper SD, Van Niel K, Schut AGT, Mucina L, Wardell-Johnson GW. 2014. Isolated with persistence or dynamically connected? Genetic patterns in a common granite outcrop endemic. *Diversity and Distributions* **20**: 987–1001.

Verdaguer D, Ojeda F. 2002. Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape *Erica* (Ericaceae) species. *American Journal of Botany* **89**: 1189–1196.

Verdaguer D, Ojeda F. 2005. Evolutionary transition from resprouter to seeder life history in two *Erica* (Ericaceae) species: insights from seedling axillary buds. *Annals of Botany* **95**: 593–9.

Vilagrosa A, Hernández EI, Luis VC, Cochard H, Pausas JG. 2014. Physiological differences explain the co-existence of different regeneration strategies in Mediterranean ecosystems. *The New Phytologist* **201**: 1277–88.

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P. 2011. Climate change and plant regeneration from seed. *Global Change Biology* **17**: 2145–2161.

Appendix 1: Full factorial ANOVAs (F statistic and *P* value) of *Erica coccinea* seedling (n=8) responses including photosynthetic rate (a), conductance (b) and water potential (c) for different forms (resprouter and seeder) under the different treatments (control and drought) for each measurement day of the drought period. Significance is indicated by an asterisk.

a) Photosynthetic rate

Day	Form	Treatment	Interaction
0	0.6 (p=0.4458)	1.81 (p=0.1899)	3.97 (p=0.0561)
3	0 (p=0.9554)	**4.53 (p=0.0422)***	0.48 (p=0.4926)
7	0.93 (p=0.3436)	0.59 (p=0.4479)	0.16 (p=0.6936)
9	2.23 (p=0.1468)	1.53 (p=0.2264)	0.38 (p=0.5448)
12	1.94 (p=0.1748)	1.11 (p=0.3017)	2.49 (p=0.1255)
18	0.45 (p=0.5084)	2.5 (p=0.1255)	0.42 (p=0.5239)
25	0.01 (p=0.915)	**6.8 (p=0.0145)***	0.23 (p=0.6332)
32	0.01 (p=0.9044)	2.64 (p=0.1156)	0.19 (p=0.6654)
40	0 (p=0.9701)	**27.66 (p<0.0001)***	1.12 (p=0.2991)

b) Stomatal conductance

Day	Form	Treatment	Interaction
0	0 (p=0.9871)	0.23 (p=0.6376)	1.97 (p=0.1714)
3	1.74 (p=0.1975)	1.42 (p=0.2431)	1.14 (p=0.295)
7	0.4 (p=0.5301)	0.72 (p=0.4039)	1.57 (p=0.2202)
9	3.14 (p=0.087)	**13.89 (p=0.0009)***	1.52 (p=0.2272)
12	2.38 (p=0.134)	**8.51 (p=0.0069)***	0.53 (p=0.471)
18	0.33 (p=0.57)	**21.25 (p<0.0001)***	1.55 (p=0.2235)
25	0.55 (p=0.4641)	**21.11 (p<0.0001)***	1.65 (p=0.2097)
32	2.47 (p=0.1273)	**7.18 (p=0.0122)***	0 (p=0.961)
40	0.05 (p=0.8311)	**93.41 (p<0001)***	1.15 (p=0.2935)

c) Water potential

Day	Form	Treatment	Interaction
0	0.32 (p=0.5734)	1.25 (p=0.2723)	**7.67 (p=0.0098)***
3	0.03 (p=0.8712)	**4.28 (p=0.0479)***	0.5 (p=0.4833)
7	**18.1 (p=0.0002)***	2.99 (p=0.0947)	2.28 (p=0.1421)
9	**6.79 (p=0.0145)***	0.8 (p=0.3796)	0.17 (p=0.6848)
12	**4.45 (p=0.0439)***	2.76 (p=0.108)	0.13 (p=0.7238)
18	0.11 (p=0.7408)	3.46 (p=0.0737)	0.02 (p=0.8769)
25	0.75 (p=0.3949)	**6.59 (p=0.0159)***	0.06 (p=0.8029)
32	0.32 (p=0.5786)	**15.39 (p=0.0005)***	0.41 (p=0.5294)
40	0.08 (p=0.7836)	**133.66 (p<0.0001)***	0.02 (p=0.8809)

Appendix 2:

Duncan's post-hoc test for days yielding significantly different means from the full factorial ANOVAs of *Erica coccinea* seedling responses including (a) photosynthetic rate, (b) conductance and (c) water potential for different forms (resprouter and seeder) under the different treatments (control and drought) for measurement days during the drought period which indicated significant differences (Appendix B1). Different letters indicate significant differences within each day.

a) Photosynthesis

Day	Significant Categories	Mean
Day 3	Drought	3.91 ± 0.46^{b}
	Control	5.27 ± 0.42^{a}
Day 25	Drought	4.07 ± 0.48^{b}
	Control	6.45 ± 0.74^{a}
Day 40	Drought	2.22 ± 0.71^{b}
	Control	7.85 ± 0.76^{a}

b) Conductance

Day	Significant Categories	Mean
Day 9	Drought	0.167 ± 0.013^{b}
	Control	0.265 ± 0.024^{a}
Day 12	Drought	0.161 ± 0.021^{b}
	Control	0.257 ± 0.026^{a}
Day 18	Drought	0.085 ± 0.016^{b}
	Control	0.193 ± 0.017^{a}
Day 25	Drought	0.078 ± 0.011^{b}
	Control	0.173 ± 0.017^{a}
Day 32	Drought	0.072 ± 0.022^{b}
	Control	0.135 ± 0.015^{a}
Day 40	Drought	0.014 ± 0.007^{b}
	Control	0.198 ± 0.017^{a}

c) Water Potential

Day	Significant categories	Mean
Day 0	Seeder (Control)	-0.691 ± 0.073^{b}
	Resprouter (Control)	-0.584 ± 0.070^{ab}
	Seeder (Drought)	-0.479 ± 0.056^{a}
	Resprouter (Drought)	-0.444 ± 0.053^{a}
Day 3	Drought	-1.320 ± 0.264^{b}
	Control	-0.743 ± 0.065^{a}
Day 7	Resprouter	-1.141 ± 0.071^{b}
	Seeder	-0.777 ± 0.055^{a}
Day9	Resprouter	-0.884 ± 0.073^{b}
	Seeder	-0.614 ± 0.071^{a}
Day12	Resprouter	-0.943 ± 0.069^{b}
	Seeder	-0.773 ± 0.044^{a}
Day 25	Drought	-1.388 ± 0.224^{b}
	Control	-0.764 ± 0.054^{a}
Day 32	Drought	-3.196 ± 0.594^{b}
	Control	-0.840 ± 0.041^{a}
Day 40	Drought	-5.065 ± 0.373^{b}
	Control	-0.705 ± 0.044^{a}

Appendix 3: T-test for the difference between treatments (control minus drought) for both forms of *Erica coccinea* seedling responses including (a) photosynthetic rate, (b) stomatal conductance and (c) water potential for different forms (resprouter and seeder) under the different treatments (control and drought) for measurement days during the drought period which indicated significant differences (Appendix B1). Different letters indicate significant differences within each day.

a) Photosynthetic rate

Day	T-value	df	*P*-value
0	0.932	2	ns
3	0.348	2	ns
7	0.199	2	ns
9	0.306	2	ns
12	0.790	2	ns
18	0.333	2	ns
25	0.241	2	ns
32	0.219	2	ns
40	0.534	2	ns

b) Stomatal conductance

Day	T-value	df	*P*-value
0	0.672	2	ns
3	0.534	2	ns
7	0.627	2	ns
9	0.617	2	ns
12	0.365	2	ns
18	0.643	2	ns
25	0.642	2	ns
32	0.025	2	ns
40	0.551	2	ns

c) Water Potential

Day	T-value	df	*P*-value
0	1.355	2	ns
3	0.355	2	ns
7	0.755	2	ns
9	0.205	2	ns
12	0.178	2	ns
18	0.078	2	ns
25	0.126	2	ns
32	0.318	2	ns
40	0.054	2	ns

Appendix 4: Full factorial ANOVAs (F statistic and *P* value) of biomass, root:shoot ratios, soluble sugars content and starch content in *Erica coccinea* seedlings for different forms (resprouter and seeder) under the different treatments (control and drought) for above and below ground material. Duncan's post-hoc test was used to separate means (see Figures3.6-3.8 in Chapter 3).

	Root:shoot	Soluble sugars	Starch
Treatment	15.49 (*P*=0.0002)	**84.65 (p<0.0001)***	**7.73 (p=0.0075)***
Form	1.07 (*P*=0.3052)	**97.58 (p<0.0001)***	0.30 (p=0.5873)
Allocation	NA	**12.32 (p=0.0009)***	2.52 (p=0.1186)
Treatment*Form	0.58 (*P*=0.4492)	**17.91 (p<0.0001)***	0.01 (p=0.9782)
Treatment*Allocation	NA	0.89 (p=0.3486)	**4.65 (p=0.0356)***
Form*Allocation	NA	2.93 (p=0.0932)	**4.77 (p=0.0335)***
Treat.*Form*Allocation	NA	0.60 (p=0.4414)	0.27 (p=0.6066)


Erica coccinea