Rainfall Regime and Optimal Root Distribution in the Australian Perennial Grass, *Austrodanthonia caespitosa* (Gaudich.)

A thesis submitted in fulfilment of the degree of Doctor of Philosophy in the School of Earth and Environmental Sciences at the University of Adelaide.

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2007

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ABSTRACT

This study aimed to determine whether rainfall regime has driven differentiation in the Australian perennial grass, *Austrodanthonia caespitosa*, resulting in local ecotypes possessing characters, such as deep rootedness or summer activity, that may be particularly useful in reducing deep drainage for salinity mitigation, or whether the species shows a plastic response in root growth to soil water distribution.Rainfall regime varies within a given annual rainfall because size and ditribution of rainfall event vary. This can have an important effect on soil water distribution, both spatially and temporally. This study investigates the relationship between rainfall regime and the structure of root systems in local populations of *Austrodanthonia caespitosa* (Gaudich.), Firstly, it examined a number of indices useful in quantifying variation in small-scale rainfall regime, including seasonal bias, event size, event frequency, and the clustering of events, as well as how rainfall event size may be changing over time across Australia. The variation in soil water distribution that results from different rainfall regimes is expected to interact with root distribution in plants, either acting as a selective force and driving genotypic differentiation in response to soil water availability, or through plasticity in root placement. The relationship between rainfall regime and root depth distribution was examined in *Austrodanthonia caespitosa* (Gaudich.), or white-top wallaby grass, a perennial grass common across southern Australia.

Growth and reproductive traits of plants grown from seeds collected from across the range of this species under a single rainfall regime were compared and correlated with

the rainfall indices and soil type in order to establish possible abiotic explanations for trait variability. Phenological characters were found to be particularly variable between ecotypes, but high local variation between ecotypes suggested factors operating on a spatial scale smaller than the rainfall gradients are responsible for population differentiation.

In order to investigate the interaction between rainfall event size and root depth, an experiment was conducted to investigate plant response to watering pulse size and frequency, with plants grown under a range of controlled watering regimes, and root depth distribution compared. The primary response in root growth was plastic, with shallow roots being developed under small, frequent events, and deep roots developed under large, infrequent waterings. Differences between ecotypes were less important, and there was no interaction between ecotype and watering treatment, indicating the same degree of plasticity in all ecotypes.

Plants from a range of populations were grown under a controlled climate, first under winter conditions, then under summer conditions, with summer water withheld from half the plants, in order to determine the response to summer watering and summer drought. Plants that were watered over summer showed a strong growth response, increasing shoot biomass significantly. This effect was particularly strong in South Australian populations, which was unexpected as they originate from a region with low, unpredictable summer rainfall. Root depth was not strongly influenced by summer watering treatment.

Finally, an evolutionary algorithm model was constructed in order to examine optimal

plant traits under a variety of rainfall regimes. The model highlighted the importance of the interaction between rainfall regime and soil type in determining optimal root placement. Variable root cost with depth was also found to be an important trade-off to be considered, with high root loss in the surface soil layers, due to high temperatures, making a shallow rooted strategy less efficient than if root costs were equal throughout the root system.

Overall, no ecotypes of *A.caespitosa* could be identified that had characters particularly suited to deep drainage reduction, as the drought tolerant nature of the species, and the dormancy during times of drought, may lead to low overall water use. However, it may be a useful native component in pasture systems, due to its strong growth response to summer rainfall, a characteristic found to be particularly strong in a number of South Australian ecotypes.

3. STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

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4. ACKNOWLEDGEMENTS

I would like to acknowledge the advice and guidance of my two supervisors, Associate Professor José M. Facelli, and Professor Victor O. Sadras. I would like to thank Dr. Jennifer Watling for assistance with the ecophysiological experiments. A number of fellow students, past and present, in the department offered outstanding assistance and support, including Dr. Tanja Lenz, Dr. Mansour Mohammadian, Dr. Martin Escoto-Rodriguez, Gregory Hay, Alice Dewar, Gael Fogarty, and Susan Gehrig.

I would like to thank SARDI for providing access to greenhouse and root washing facilities, and the CSIRO for providing top-up funding to support this project.

A number of local and shire councils were extremely helpful in allowing me access to collect specimens from roadsides and other public areas. These include the councils of Naracoorte and Lucindale, Tatiara, Karoonda East Murray, Southern Mallee, Mount Barker, Yankalilla, Victor Harbour, Goyder, Clare and Gilbert Valleys, Copper Coast, Moira, West Wimmera, Buloke, Northern Grampians, Loddon, Berrigan, Jerilderie, Carrathool, Hay, Deniliquin and Bland.

Thankyou to Cathy Waters, the LIGULE project, and Blackwood Seeds for supplying seeds for use in the pilot study. I would also like to thank my wife Skye for her support during this project and on field trips.

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6. INTRODUCTION

The effect of climate on plant growth is complex. In arid and semi-arid systems, as found in Southern Australia, water is the primary limiting resource for many plant species (Noy-Meir 1973), and the processes that drive and are driven by water in these systems require further study. The water balance of a system takes into account inputs from precipitation and run-on, and losses from run-off, deep drainage, and evaporation. The concept of water balance highlights the water and energy coupling in an ecosystem, describing how much plant evaporative demand is not being met by available water, or how much water is unusable excess (Stephenson 1990). A wide variety of factors affect water input to the soil; total rainfall amount, rainfall seasonality, rainfall event size, variability in rainfall over various time scales, as well as topography, soil type, and land use can determine the availability of soil water at various depths. Plants ultimately access the soil water through the roots, which can vary in distribution and architecture in order to optimise the utilisation of water. Variations in rainfall variables that influence the availability of soil water at different depths may result in different plant root distributions, due to genotypic change or phenotypic plasticity (Yanagisawa and Fujita 1999). Understanding the relative importance of climatic factors and soil type on plant root distribution may aid in the selection and breeding of plant accessions for specific purposes, such as salinity mitigation.

6.1. *Climate*

Different locations experience different rainfall regimes not only in the most commonly reported variable, total annual rainfall, but also in the distribution of that rainfall throughout the year. In Australia, monsoonal summer rain dominates the tropical north of the continent, while in the south winter rainfall is generated by frontal systems (Gentilli 1971). The southeast of Australia tends to experience a Mediterranean climate with most rain falling in winter, and the proportion of summer rainfall increasing northwards towards an equitable regime (Johnston, Clifton *et al.* 1999). Unlike other continents, such as Africa, a true rain-free desert does not separate the summer- and winter-dominated areas in Australia. Instead, there is a region where rainfall is aseasonal, and a histogram of monthly rainfall averages shows no peak (Walter 1971d). It should be noted, however, that this flat distribution does not necessarily equate to constant rainfall. Rather, in inland areas rainfall is highly variable and unpredictable, and can occur with equal probability in any month within this zone (Walter 1971a) leading to a long-term average displaying equitable rainfall.

Walsh and Lawler (1981) developed an index of seasonality of rainfall, which measures contrasts in rainfall amounts across the year as a coefficient of variation. A value of 0 represents a completely equitable regime, while the maximum value of 1.83 indicates all precipitation occurs within a single month. The index has a weakness in that two opposing regimes, dominated by summer and winter rainfall for instance, could have similar index values. However the index is useful in comparing seasonality of precipitation between years, and quantifying long-term trends of seasonality. It is also

useful for mapping gradients in seasonality across large areas. Seasonality index contours have been plotted for England and southern Africa (Walsh and Lawler 1981). Variation in rainfall seasonality index has also been calculated for transects across southern Australia (Sadras 2003), but there may be some value in producing a more detailed contour map of the index for the continent. The vector seasonality index (Markham 1970) is another means of examining rainfall seasonality across a region. This index provides more information than the Walsh and Lawler index, in that it indicates the timing of rainfall, but can be affected by bimodal rainfall regimes, where rain has two peaks during the year.

The seasonality of rainfall is important because it reveals the correlation between the amount of rainfall and other factors, such as evaporation, that will influence the effectiveness of rainfall to plants. Plant formations may be better correlated with evapotranspiration and water deficit, which are results of seasonality, than with total rainfall and energy (Stephenson 1990). The interaction between precipitation and evapotranspiration means that plants experience alternating periods of "stress time" and "growth time" (Neilson 1986). Summer rain is also considered less effective than winter rain (Noy-Meir 1973). In the arid and semi-arid regions of Australia, more summer rainfall than winter rainfall is required to produce the same amount of plant growth, as summer rainfall is subject to greater evaporation (Austin, Williams *et al.* 1981; Gentilli 1971). Similar results have been found in arid regions in North America, with a study in the Chihuahuan Desert finding winter rain more effective than summer rain due in part to higher evaporation in the summer (Kemp 1983).

Differential evaporation throughout the year can cause rainfall from different seasons may penetrate to different soil depths. A modeling study of the Patagonian steppe found that summer rain, and winter rain during dry years, only wet the surface layers. Deeper soil layers were only recharged during particularly wet winters (Paruelo and Sala 1995). In a modeling study of the Chihuahuan desert, it was found that the penetration of summer rain was limited by evaporation, removing water from the top 10cm of the soil. Recharge of deeper layers was possible with lower annual rainfall if winter rainfall was dominant due to lower evaporation in the cool season (Reynolds, Kemp *et al.* 2000). However, the increased dominance of winter rainfall may result in other water losses from the system, as an increase in cool-season rainfall has been found to result in more water lost to run-off and deep drainage (Clifton 1995; Keating, Gaydon *et al.* 2002; Paruelo, Sala *et al.* 2000) while increased summer rainfall results in greater loss to evaporation (Keating, Gaydon *et al.* 2002). In Australia, differences in climate seasonality, which produces a coupling or uncoupling of rainfall and evaporative demand, can strongly influence the species composition (Garden and Dowling 1995).

As well as varying in seasonal concentration, different areas experience differences in rainfall event size in terms of rainfall rate and clustering of rain days. Rainfall is a chain dependent process (Katz 1977), governed by Markov chains, so individual events are not independent but related by probabilities. The occurrence of rainfall on one day is influenced by whether rainfall occurred on the previous day, resulting in a clustering pattern of “wet days” that differs between locations.

Rainfall has been found to be a self-organised, critical process, governed by power laws that describe the relationship between event size and frequency (Peters, Hertlein *et al.* 2002). Radar on the Baltic coast was used to measure the frequency and size of rainfall events over an extended period (Peters and Christensen 2002). The radar was capable of measuring event sizes much smaller than is detectable by a conventional rainfall gauge. The workers found that rainfall event size is scale free; there was no typical event size, and the frequency of occurrence of an event was inversely proportional to the event size raised to the power of 1.4 over a number of orders of magnitude. The power law broke down below 10 minutes, a time related to the formation and release of water droplets from clouds, and above three days, a time related to the passage of frontal systems. The scaling power can vary in different locations and at different times of the year (Sadras 2003). A small value represents a bias towards large events, while a large value represents a bias towards smaller events. While "small" events in ecological studies are often considered those less than 5mm in size (Sala and Lauenroth 1982), the scale-free nature of rainfall events indicates that such a distinction is arbitrary, and the terms "small events" and "large events" are used in relative terms in this study.

This parameter has also been calculated for a number of locations across Australia, and in the south east of the continent was found to decrease from west to east (Sadras 2003). More detailed mapping of this parameter across Australia may be of use. Gentilli (1971) documented some of the variation in rainfall event size and duration in Australia. Northern Australia experiences heavy rainfall from tropical cyclones and

monsoonal events, while in the south, frontal systems produce rain that is lighter but more persistent. A number of workers have proposed that small rainfall event size may be one factor that leads to accumulation of salt in the soil (Gentilli 1971; Prescott 1931), while others have suggested large events are associated with recharge and upward flow of saline water (Sadras 2003).

One of the most important impacts of rainfall event size on plant growth is the penetration of water into the soil profile. There has been some argument regarding the importance of small events in water-limited systems. Noy-Meir (1973) considered small rainfall events unimportant as they are subject to greater evaporative loss than larger events. Small rainfall events would only benefit surface cryptogams, and only relatively rare large events would benefit higher plants. In contrast, Sala and Lauenroth (1982) found that small rainfall events, less than 5mm in size, account for a large proportion of precipitation in arid and semi-arid regions. Small events wet top layers, while large events penetrate to deeper layers. Plants need fast response times and shallow roots to take advantage of the small events.

A number of studies have examined the relationship between event size and water availability in the soil profile. In terms of water loss from the system, event sizes may be associated with different hydrological processes (Loik, D. *et al.* 2004). Large events may lead to deep drainage and runoff, while small events may be lost to evaporation from the surface layers (Sadras 2003). In Australia, run-off was found to be lower in southern locations that experienced less intense rainfall events (Keating, Gaydon *et al.* 2002). A study on the stony downs in the arid interior of Australia (Hunter and

Melville 1994) found that the effectiveness of small events was enhanced by run-on, as water that failed to penetrate stony soil was redirected to surrounding areas. In the arid south west of North America, it was found that winter precipitation penetrates to deep soil layers, while summer monsoon precipitation was restricted to surface layers, due to high evaporation and the short, intense nature of events, which increases run-off (Williams and Ehleringer 2000). In a study on the Patagonian Steppe, it was found that large rainfall events allowed water to penetrate to soil layers that are less affected by evaporation (Paruelo, Sala *et al.* 2000). A modelling study in the Chihuahuan Desert found that the 10-40cm soil layer was the most important to all plant functional types, as small rainfall events were able to penetrate to this depth, but the layer was deep enough to prevent evaporation (Reynolds, Kemp *et al.* 2000). However, the exact dimensions of this soil layer may have been an artefact of their model's construction. The authors suggest that there is a minimum threshold of rainfall below which no water will penetrate to deep drainage, and that this threshold is expected to be lower in winter-rainfall environments due to lower evaporative demand.

A number of authors have stressed the importance of investigating short-term, small-scale changes in the water balance that may be associated with individual rainfall events. In most systems, variability in soil moisture is temporal and short-term (Sultan and Bazzaz 1993b) indicating the importance of adaptive plasticity in utilising this resource. Stephenson (1990) considered it important to study the water balance over short periods rather than long term averages, particularly in responses to water deficits.

Soil type, and its interaction with rainfall variables, can influence the availability of

water to plants. An early account of soil formation and distribution in Australia suggests that climatic influences such as evaporation, rainfall event size and rainfall seasonality have influenced soil formation by controlling leaching and salt accumulation (Prescott 1931). In arid and semi-arid regions, sandy soil may allow greater availability of water than clay soil due to the inverse texture effect (Shreve 1942). Clay soils experience greater run-off than sandy soils, and water penetrates deeper into sandy soils than clay soils, escaping the greater evaporative losses from upper layers (Walter 1971c). Recharge of deeper soil layers and groundwater is greater in sandy soils (Petheram, Walker *et al.* 2002). The greater water availability in sandy soils can result in deeper rooting depths of plants in the community, an effect that has been found in a study of global root distribution (Schenk and Jackson 2002a).

6.2. Plant Root Distribution

Plants display a wide variation in root architecture, with depth and distribution varying both between species and within species, in response to soil type, climate and competition. A study of root distributions globally (Jackson, Canadell *et al.* 1996) found differences in rooting depth of different plant functional types. Grasses had a higher proportion of roots in the upper 10cm of the soil profile (44%) than shrubs (21%) and trees (26%). Differences in root architecture also exist within species, with studies on soybean (Raper Jr. and Barber 1970) and wheat (Oyanagi, Sato *et al.* 1991a) finding dramatically different root distributions among varieties.

Some species are able to obtain an advantage through dimorphic root systems

(Williams and Ehleringer 2000) enabling them to utilise water from both deep and shallow sources. The root distribution can affect the efficiency of resource extraction. A modelling study of root architecture (Ge, Rubio *et al.* 2000) found that plants with deeper root systems experienced greater inter-root competition, resulting in decreased efficiency of resource uptake per unit of root mass. Widespread, shallow root systems had less overlap of resource acquisition zones, but could experience greater mortality from soil drying in upper layers. The aboveground environment can also influence root depth, with defoliation of pasture species found to result in concentration of roots in upper soil layers (Pook and Costin 1971).

The presence of neighbouring plants can alter the shape of root systems. A study of soybean root distribution found that plants grown in individual pots displayed different root architecture than those grown in rows with other plants (Raper Jr. and Barber 1970). Row plants displayed significant downward root growth at a distance from the plant base, while individual plants continued horizontal root extension. In a study of the development of sunflower root systems, Sadras *et al.* (1989) found that high population density led to earlier exploration of deeper soil, and low population density resulted in continued root exploration until later in the growing season.

A short term or small-scale factor that can impact on plant root distribution is the proliferation of roots in response to brief or localised high resource availability. Berntson and Woodward (1992) found greater horizontal branching of roots and shorter link lengths in a treatment with high water availability. Bell and Sultan (1999) found plasticity in root growth and development in response to water availability in

Polygonum species; roots were longer per mass in dry soil compared to wet soil, and deployment of roots was to deep or shallow layers in dry or flooded treatments respectively. "Rain roots" which grow after soil wetting and are shed when the soil is dry are present in some species (Palta and Nobel 1989). These have a high hydraulic conductivity, and may help plants to take advantage of short periods of water availability.

Extensive research has shown that roots proliferate in zones of high nutrient availability. Drew (1975) found increased lateral branching and proliferation of roots of barley in a zone of high nitrogen concentration, with the increased nitrogen in that zone was able to compensate for low nitrogen in the rest of the soil. Robinson (1994) provided a review of studies on local root proliferation in zones of nutrient enrichment, and found that it was a common but not universal response, and the proliferation is not always substantial. Dense branching and reduction in specific root length is likely where the nutrient is a relatively immobile ion such as phosphate, although root diameter shows little plasticity (Hutchings and de Kroon 1994). Local proliferation and lateral branching of roots in response to high resource availability may be considered an example of morphological plasticity, compensating for variability, either temporal or spatial, in nutrient supply (Sultan and Bazzaz 1993a). In a study of arid-land grasses, Larigauderie and Richards (1994) found no differences in root proliferation response to nutrient enrichment between species of varying productivity and competitiveness. They concluded that all species were equally plastic in their response, but that the morphological plasticity did not alter the competitive ability or productivity of these

species. Plasticity will be covered in more detail in a later section.

Root distribution can be altered by differences in proportional allocation between roots and shoots, often as a response to resource availability in the environment. Studies have found greater proportional biomass allocation to roots in dry soil (Bell and Sultan 1999), and greater growth of new roots under drought stress (Hoogenboom, Huck *et al.* 1987). Schwinning and Ehleringer (2001) suggest that during periods of high resource availability, the proliferation of shallow roots may enable to plant to reduce its root:shoot ratio, producing more aboveground biomass. During drought periods, greater biomass needs to be allocated to deep roots, so the root:shoot ratio may rise.

Conventional studies of gravitropism and root growth direction have concentrated on simplistic positive, negative, and diagravitropic (horizontal) responses. Digby and Firn (1995) introduced the concept of a gravitropic set-point angle, a developmentally defined but environmentally alterable angle at which an organ will tend to grow. Plants need a mechanism to restore organs to a variety of angles, not just horizontal or vertical, as plant organs show a wide variety of angular variation, and studies of binary responses of seedling roots may be an inappropriate system in which to study gravitropism (Firn and Digby 1997). In research on Japanese wheat cultivars, it was found that a single dominant gene primarily controlled root growth angle (Oyanagi, Sato *et al.* 1991b), although the environment could also influence growth angle: some cultivars show a gravitropic response under conditions of low soil water potential (Oyanagi, Nakamoto *et al.* 1993a). These workers were able to establish a relationship between gravitropic set-point angle and vertical root distribution, and measure the

growth angle of roots growing through a buried colander to determine differences in rooting depth (Oyanagi 1994).

6.3. Root distribution, soil water and climate

A number of studies of global root distribution have found variation in rooting depth that correlated with the availability of water in different soil layers. Some ecosystems, such as deserts, savannahs, grassland and dry forest lack of roots in upper soil layers, as these layers were too dry for resource uptake during a large part of the growing season (Schenk and Jackson 2002a). Lateral root spread in upper layers is high in arid environments, allowing plants to utilize water that infiltrates only to shallow layers (Schenk and Jackson 2002b). The distribution of root is important in determining survival and competitive ability in systems where water is the most limiting resource (Coupland and Johnson 1965). It is important to remember, however, that the location of roots in the soil may not always be an accurate indicator of the zones of water acquisition (Dawson and Ehleringer 1991; Hurd 1974), as plants may have roots throughout the soil profile but switch between using different water sources when it provides an advantage (Ehleringer and Dawson 1992), and some roots may have a purely structural and support function, including nutrient acquisition in the upper soil zones. The structural support role of roots is demonstrated in the ability of trees with extensive root anchorage to withstand tropical storms (Basnet *et al.* 1992)

The importance (Sala and Lauenroth 1982) or unimportance (Noy-Meir 1973) of small rainfall events to soil water availability has been discussed above. A number of studies

have found that species respond to small rainfall events, and that the response to small events differs from that of large events. Ivans *et al.* (2003) found a rapid response in nitrogen utilisation in two arid species, *Artemisia tridentata* and *Agropyron desertorum*, to both small and large summer rainfall events. The size of the rainfall event changed the length of the response, and the species with more roots in upper soil layers showed a more rapid response to the water input. Small rainfall events were found to have an effect on the growth of grasses on the stony downs of Australia, with deeper-rooted grasses staying green for a longer period after the event, due to the deeper penetration of water. The response was greater and longer lasting on coarse soil than on clay soil. Franco and Nobel (1990) found rapid root growth after infrequent rainfall events in the desert succulent *Agave desertii*. *Bouteloua gracilis* was found to use water in the upper soil layers, taking advantage of small rainfall events (Dodd, Lauenroth *et al.* 1998).

In studies of pastures in Australia, it was proposed that annual pasture species are unable to take advantage of above average rainfall, as they only possess shallow roots and cannot access the excess water that penetrated deeper into the soil profile (Johnston, Clifton *et al.* 1999). Shallow-rooted species, however, are also seriously affected by years with below average rainfall, while deep-rooted perennials were expected to utilise a greater range of available soil water (Pook and Costin 1971). This suggests that shallow-rooted species may require a more predictable rainfall regime for survival. Williams and Ehleringer (2000) suggest that there may be a critical amount of rainfall above which it becomes profitable for plants to rely on shallow roots.

Numerous studies have also highlighted differences in plant water use from different

soil layers in response to the availability of the resource in these layers, revealing complex dynamics. In a study involving deuterium enriched irrigation, Schwinning *et al.* (2002) found different life forms used water from different soil layers, which indicated use of different initial water sources. Herbs, grasses and cacti used water from upper soil layers, wetted by recent rain showers, trees used stored water in lower layers, and shrubs used a mixture of both sources. They suggest that in the case of a rainfall event, shrubs may switch to utilizing water from upper layers and water in depper layers act as a a reserve during dry summer conditions. Bell and Sultan (1999) found differences in the root deployment to soil layers between species in flooded and dry treatments. *Polygonum persicaria* showed deployment to lower layers in dry conditions, and upper layers in wet conditions, while the response was slower and less pronounced in *Polygonum cespitosum*, indicating lower plasticity. A study of woody species in Japan found a lack of plasticity in species response to different soil water availability, but did find specific species with different rooting depths were growing at specific points along a slope where soil water availability varied (Yanagisawa and Fujita 1999). Species with shallow roots grew on the upper slope where they were able to access small rainfall events, and at the bottom of the slope where the soil was permanently wet. Deeper-rooted species grew where there was a deeper ground water supply. In an experiment on the Patagonian Steppe (Golluscio, Sala *et al.* 1998) shrubs were found to use water from deeper layers, while grasses used water from surface layers. Large summer rainfall events were always used by the grasses, but were only used by shrubs in dry years when deeper soil layers were dry. Kemp and Culvenor (1994) pointed out an additional explanation for shallow root production: concentration

of nutrients is generally higher in surface layers. Drying of surface layers may limit the availability of these nutrients to plants. Plants can survive by utilising deeper water, but may not grow unless they have access to the shallow nutrients.

Overall water availability can have an effect on root growth in different soil layers, and production of deeper or shallower roots has found to be associated with dry and moist conditions in a wide variety of species. Bennett and Doss (1960) studied the effect of soil moisture on rooting depth in forage species, and found that rooting depth decreased with an increase in soil moisture. Rainfall was frequent, and most extraction occurred from shallow rather than deep soil layers. A study on lupins found that soil water deficit resulted in an increase in root length density, resulting in an increased efficiency of water uptake by individuals experiencing drought conditions (Rodrigues, Pacheco *et al.* 1995). Similar results were found in a study of soybean root distribution, where normal low-rainfall treatment resulted in faster growth of deep roots, and the treatment receiving additional irrigation had more surface roots (Hoogenboom, Huck *et al.* 1987). Plants under drought stress allocated proportionally more biomass to new roots. Oyanagi and Sato (1991a) suggested that deep roots might provide drought resistance in wheat, with varietal differences in root depth associated with locations experiencing dry or moist conditions. A study on legumes found that the proliferation of deeper roots during periods of moisture stress improved the water status of the plants (Devries, Bennett *et al.* 1989). Fitter (1986) found differences in the topology of roots of *Trifolium pratense* under different watering conditions. At intermediate watering conditions, roots branched in a random structure. In low water conditions, the roots

grew in a deep, herringbone structure, while in high water conditions the roots grew in a dichotomous structure with increased branching.

The ability of plants to quickly respond to differences in soil water availability, as a result of rainfall inputs, is an expression of phenotypic plasticity. Sultan (2003) considered the root growth response of *Polygonum* spp. to soil moisture (Bell and Sultan 1999) an example of plasticity. One species displayed a greater capacity to respond, and a faster response, to changes in the spatial availability of the resource. Sultan and Bazzaz (1993a) suggest that plasticity may evolve due to the fitness advantage of being able to quickly respond to small pulses of resources.

Some species of plants may be moisture-pattern specialists, developing root architectures that reflect the precise spatial and temporal resource environment they experience, rather than simply contant low- or high-resources. A modelling study designed to determine optimum phenotypes for root distribution in pulse-driven ecosystems (Schwinning and Ehleringer 2001) found differences in the optimum root distribution for accessing pulse water and deep water. If pulse water is more important in the system than deep water, the optimal phenotype leans towards low root:shoot ratios and shallow roots, while if deep water is more important, the optimal phenotype involves high root:shoot ratios and deep roots. Increasing the number of roots in shallow soil improves the plant water status during pulse events, and shallow roots may be coupled with lower root:shoot ratios to maximise carbon gain during the events. Williamson (2002) found, in a modelling study of arid ephemeral plants, that plants may show a complex growth response to different rainfall event sizes and frequencies,

with the response curve reflecting the relative amount of roots at various depths in the soil profile. A specific root distribution, therefore, had one or more "optimal" rainfall event sizes that produced maximum growth. In this model, however, rooting depth followed a fixed development pattern, and did not display a plastic response to soil water availability.

Plants may also alter the sources they extract water from seasonally, and plants with different root structures may access different seasonal rainfall events. There are differences in the seasonal water use by different life forms. For instance, Schwinning *et al.* (2002) found that shrub and grass species on the Colorado Plateau switched to using pulse water in summer as the deeper winter water dried up. In contrast, a study of trees in Western Australia found that the plants used water from upper layers in winter when rainfall was frequent, and only extracted deeper groundwater during the summer. In a study of desert plants, Ehleringer *et al.* (1991) found that annuals and succulents were dependent on summer rainfall, woody and herbaceous perennials used both summer and winter rainfall, and deep-rooted perennials failed to utilize summer rainfall. A number of studies have found that contrasts in the rooting depths of grasses and shrubs lead to different seasonal water usage. In a modelling study based on the Patagonian Steppe, Paruelo and Sala (1995) found that spring and summer rain that only reached the surface layers was utilised by the grasses, while deeper water was used by shrubs. This was confirmed in an experiment in the same system (Golluscio, Sala *et al.* 1998), with shrubs using deeper water and only using summer rainfall in extremely dry years, while grasses used water from large summer rainfall events from the shallow

soil layers. A study in Colorado found that grasses were using spring and summer rainfall from upper layers, while trees used deeper groundwater (Dodd, Lauenroth *et al.* 1998).

Plants may change root growth at different depths in the soil throughout the year in response to seasonal rainfall inputs. Fernandez and Caldwell (1975) found phasing of root growth throughout the year in semi-desert shrubs in Utah. As the year progressed and the soil became drier, the zone of root growth moved down the soil profile. The deepest roots continued to grow in the driest season. A study on snakeweed on the North American steppe found that the root deployment pattern of the species was altered by variations in seasonal rainfall (Wan, Yilmaz *et al.* 2002). Root extension into deeper soil was increased by winter precipitation.

Some authors believe there may be a threshold level of rainfall beyond which it becomes profitable for plants to produce shallow roots. In winter rainfall dominated areas, plants may have no shallow roots, but as the proportion of summer rainfall increases it may be predictable enough for investment in shallow or dimorphic root systems (Ehleringer and Dawson 1992). However, it will be costly for plants to maintain shallow roots if rainfall is unpredictable, and the production of shallow roots at a threshold level of predictable rainfall may be a plastic response in individual plants, or a selective force acting on genotypes (Williams and Ehleringer 2000).

A number of workers have suggested that differential seasonal water use by plants allows for the coexistence of different species in the ecosystem. In reference to summer-rainfall savannahs, Walter (1971d) suggests that winter rain may favour

deep-rooted plants, such as woody shrubs, while summer rainfall is used by actively transpiring grasses. Grasses are dormant in the winter in this system, so additional precipitation in this season will not promote grass growth, but will be available for use by shrubs. Similarly, Weltzin and McPherson (1997) suggest that bimodal patterns of precipitation could allow for species coexistence. Shallow rooted grasses may utilise growing season precipitation, while deeper-rooted plants use water that percolated to deeper layers when grasses are dormant. Increased summer precipitation will favour the shallow rooted plants. In contrast, in Australian grasses summer activity may be linked to deep root systems, with the upper soil layers being dried out by annual species during the spring (Clifton 1995). Some systems have particularly shallow soil, and a two-layer water partitioning explanation is inappropriate. In these systems, seasonal water partitioning may occur through differences in phenology, with different species showing a growth response at different times of the year to access the rainfall that occurs at that time (Reynolds, Kemp *et al.* 2000).

Seasonal associations between precipitation and temperature result in differential use of water by plant species with different photosynthetic pathways. C_4 species are favoured where the winters are dry and the summers wet, while C_3 species are favoured by wet winters (Neilson 1986). Where these species coexist, the result may be utilisation of different seasonal events by different photosynthetic groups. C_4 species will use water from convectional summer storms, while C_3 and CAM plants will utilise frontal winter rainfall (Kemp 1983).

The above research demonstrates that plant root system architecture is intrinsically

linked to the availability of water at varying depths in the soil profile. This in turn is controlled by climatic factors such as rainfall event size, frequency, seasonality and evaporation. Therefore, one may expect variation in plant rooting depth with gradients in climatic factors, either through short-term plasticity or natural selection. For variation within an individual species, genotypic differences in rooting depth were correlated with a dry or flooded climate in Japanese wheat cultivars (Oyanagi, Sato *et al.* 1991a). Schenk and Jackson's (2002a) global survey of root distribution found a number of correlations between climatic factors and root distribution. There is a lack of shallow roots between 20 and 32 degrees latitude, representing particularly dry ecosystems. Roots in grasslands and shrublands showed a strong correlation with climatic factors, in particular with the length of the dry season. In non-forest vegetation rooting depth decreased with higher annual precipitation, while in forest vegetation it increased. They found that much variation in root depth correlated with climate variables that are associated with water supply and evaporative demand. The authors suggest in a related paper (Schenk and Jackson 2002b) that rooting depth is more correlated with annual precipitation than evaporation, but some variance is likely to be due to seasonal variability in precipitation, as infiltration depth of the water will vary. Understanding the differentiation in the ability of plants to utilise rainfall events of different timing, intensity and duration may be vital to understanding the dynamics of arid and semi-arid systems (Schwinning, Davis *et al.* 2002). Further research in this area is necessary to examine correlations between root architecture and climatic factors other than annual rainfall and evaporation, so as to take into account factors that influence water availability on a shorter time scale. It is also important to determine

whether climatic variation has acted as a selective force on populations, resulting in the evolution of genotypes optimised for a specific location, or if the plant in question is able to exhibit a plastic response to soil water availability.

6.4. Plasticity

Plasticity is variation of phenotypic response in response to environmental conditions. Plants with the same genotype may exhibit several different phenotypes, for instance leaf size or rooting depth, depending on the environment they face. Schlichting (1993) believes that there may be specific genes for plasticity, with plasticity being exhibited either through regulatory genes that trigger other genes along an environmental gradient or past a certain threshold, or through differential allelic sensitivity to environmental conditions. This hypothesis is rejected by Via (1993), who suggests plasticity itself is not the target of selection, nor controlled by specific genes. Taxa with different degrees of plasticity have simply experienced different ranges of environmental variation in the past, and have had different selection on trait values. The author believes environment-specific gene expression is a mechanism for plasticity, and the existence of regulatory genes for plasticity is unnecessary. Moran (1992) stresses that plasticity involves detecting an environmental cue, then exhibiting a response appropriate for the expected environment. For instance, cold temperatures may be the cue for triggering a response for oncoming wet winter conditions. The connection between cue and response, and the timing of the response, is vital for the plastic response to be advantageous, and in the cast of response to rainfall events, the response has to occur within a time frame that allows for utilisation of the water input (Moran

1992; Sultan 2003). Moran suggests that while temporal variation can promote polyphenism, the selection for plasticity is favoured if the event is predictable and the cue is appropriate for the response.

The sessile nature of plants means that plasticity is important for the plant to be able to survive changing environmental conditions (Schlichting 1986), and the developmental and regenerative capacity of the meristem allows plants to be highly plastic in their morphology (Trewavas 1981). The importance and expression of plasticity may be different between different plant functional types (Grime, Crick *et al.* 1986). For instance, in annual plants, the plastic response will need to sustain reproduction under stress. In perennial plants, plasticity should allow for altering biomass allocation and timing reproduction to ensure survival. In productive habitats, morphological plasticity may be part of the foraging mechanism, while in unproductive habitats it will relate to utilisation of brief resource pulses (Sultan and Bazzaz 1993a).

As plasticity allows a genotype to grow and reproduce in several different environments, many generalist and colonising species show high plasticity (Sultan 2003). Populations of a species may display different capacity for plastic response (Sultan 2003). Sultan and Bazzaz (1993a) believe plasticity may be common in annual species, allowing them to utilise resources quickly when available and ensure reproductive success, while perennials may have a more complex series of responses to transient environmental stresses. They found that the greatest variation in response between genotypes to environmental conditions occurred in favourable conditions rather than limiting conditions, suggesting that genotypes may vary in their ability to

exploit good conditions more than their ability to tolerate poor conditions. In most systems, variation in soil moisture is primarily temporal and short-term, so adaptive plasticity is important (Sultan and Bazzaz 1993b), and the ability to switch water sources quickly as conditions change is advantageous (Ehleringer and Dawson 1992). More competitive species and species from richer soils display higher levels of root plasticity, as they actively forage to take advantage of the soil resources (Hutchings and de Kroon 1994).

Several studies have identified plasticity in root deployment in response to rainfall input and soil water. Bell and Sultan (1999) found that two species of *Polygonum* varied in the strength and speed of changes in root deployment to different soil layers under a variety of water regimes, indicating a different degree of plasticity between the species (Sultan 2003). Heathcote *et al.* (1987) studied the production of roots of *Carex flacca* plants in response to flooding, and found a plastic response rather than genotypic differentiation. They stress that both plasticity and genotypic differentiation are important in achieving ecological amplitude, but that often only genotypic differentiation is reported in the literature. In a study of *Festuca pallescens*, differences in the morphology of mainly above ground parts was found to correlate with environmental variation (Oliva, Martínez *et al.* 1993). However, when plants were transplanted and grown under identical conditions, differences in morphology disappeared, indicating differences were due to plasticity rather than genotypic variation. Further analysis and identification of the causes of phenotypic variation, plastic or genotypic, is vital to increasing our understanding of the dynamics of

ecosystems.

6.5. Australian Grasses

Australian grasses evolved under a dry and fluctuating climate, experiencing high fire frequency, low soil fertility, and low grazing pressure (Garden and Dowling 1995; Lodge 1994; Whalley 1990). They are able to survive periods of moisture stress (Lodge 1994), but are often incapable of responding to increased soil nutrient concentrations (Whalley 1990). There is a shift in native grassland species composition at 29 to 32 degrees latitude, from tropical to temperate affinities (Roberts 1990), and within these regions, species are considered to vary with soil properties such as acidity, total rainfall, and seasonality of rainfall (Garden and Dowling 1995; Mitchell 1990).

In Australia, C_4 grasses are more numerous where the summer is hot and wet, and decline with decreasing temperature and summer rainfall, while C_3 grasses are more common where spring is cool and wet and decline with decreasing spring rainfall and increasing temperature (Hattersley 1983).

Winter-dominant rainfall pattern does not favour Australian native grasses under the present agricultural regime, as heavy grazing occurs during the summer months when there is a water deficit, leading to the death of native grasses (Mitchell 1990). Current agricultural practices and grazing tend to shift the species composition from tall tufted species towards shorter species that can remain green all year, such as *Austrodanthonia caespitosa* and *Microlaena stipoides* (Garden, Jones *et al.* 1996). A survey in the Goulburn district of New South Wales found that cultivation reduces the abundance of

Austrodanthonia species, but does not reduce the abundance of *Microlaena* species (Munnich, Simpson *et al.* 1991). *Austrodanthonia* spp. were found to be negatively associated with annual grasses. On the New England tablelands, factors that influenced the distribution and abundance of *Austrodanthonia* spp. included the time since last cultivation, altitude, drainage, soil type and texture, and phosphorous in soil. Lenz and Facelli (2006) also found a negative association between annual grasses and perennial grasses such as *Austrodanthonia caespitosa* in the grasslands in the mid-north of South Australia, but the abiotic factors determining perennial grass abundance were less clear, although extreme rainfall events were considered a possible driving force.

Annual grasses avoid summer drought by setting seed and dying, while perennials survive drought by relying on underground organs and rapid response to rainfall (Kemp and Culvenor 1994). The replacement of native perennial species with summer dormant, annual pasture species, has resulted in water being left over in autumn, leading to lower autumn soil water deficit and greater deep drainage in winter (Johnston, Clifton *et al.* 1999).

Austrodanthonia caespitosa persists over a large area of southern Australia, over a wide range of climates. This grass also displays a high degree of genetic variation among populations, a fact highlighted in a series of studies. Hodgkinson and Quinn (1976) found no difference in temperature optima across a north-south transect for *Austrodanthonia caespitosa*, but did find that northern populations were faster growing, and had a lower root:shoot ratio. They believed the faster growth enabled the plants to increase biomass and set seed before the drought period. The high morphological and

physiological variation present in these species was noted. In another study it was found that reproduction in this species was modulated by day length and temperature in southern populations, while the control was relaxed in more northern, arid populations to allow for opportunistic reproduction (Hodgkinson and Quinn 1978). These differences were due to genotypic variation between populations. Reproductive characters of *Austrodanthonia caespitosa* were assessed and seed size and weight were found to be highly variable, although no selective cause could be identified (Quinn and Hodgkinson 1984). The effect of planting density and temperature on tillering, plant height and leaf dimensions, as well as the degree of phenotypic plasticity present, was also found to vary between populations (Quinn and Hodgkinson 1983). Scott and Whalley (1984) found differences between populations of *Austrodanthonia caespitosa* on a more local scale, comparing sheep-camp populations to nearby populations that experienced lower grazing pressure. The selective pressure faced by the heavily grazed plants resulted in shorter tillers, more tillers, later flowering time and greater seed set. They suggest that prostrate character may begin as a plastic, phenotypic response to grazing pressure, but is heritable in the long term.

Although grazing may alter the phenotype of *Austrodanthonia caespitosa*, the species as a whole appears unresponsive to grazing (Austin, Williams *et al.* 1981), but responsive to seasonal rainfall variation, with the timing of the first effective rains important in seedling establishment. The authors considered seedling establishment to be of greater importance than adult growth, which they expected to be similar from year to year. Williams (1974) considered the species an opportunist that is able to respond to

rainfall in all seasons, although survival is decreased in the presence of annuals as they compete for water in winter. *Austrodanthonia caespitosa* in northern regions is subject to low and erratic rainfall, imposing high density-dependent mortality (Quinn and Hodgkinson 1984), resulting in selection for rapid growth and quick seed set (Hodgkinson and Quinn 1976), a response facilitated by opportunistic reproductive triggering in these populations (Hodgkinson and Quinn 1978).

Garden *et al.* (2001) found that the distribution of *Austrodanthonia* spp. was not greatly influenced by soil type, and the species is moderately acid tolerant (Lodge 1994), and tolerant to aluminium in the soil (Mitchell 1993), a potentially important characteristic given the influence of aluminium on root growth (Crawford and Wilkens 1998).

6.6. Use of Australian grasses in salinity control

Clearing of native vegetation has resulted in increased soil salinity in non-irrigated systems. Greater amounts of water percolate into the deeper layers that contain saline water, and salts accumulate in higher layers due to evaporation of water that has moved up the profile by capillary action (Peck 1978). Deep-rooted perennials transpire all year round, and continue to remove soil water in summer, while the annual grasses that have replaced them only transpire in winter. Recharge is therefore generally higher under shallow rooted annual vegetation than deep-rooted perennial vegetation. Recharge is also greater under sandy soils, with the structure of clay soils having a greater impact on recharge than total rainfall (Petheram, Walker *et al.* 2002). Native perennial grasses are likely to have roots that are deeper than annual species, therefore

utilising deeper water, reducing acidification and deep drainage (Garden, Dowling *et al.* 2001). Deep-rooted perennial grasses are also able to respond to rainfall whenever it occurs, for instance, during the summer months, therefore drying the soil and reducing winter recharge (Wilson 1996). There is a need to develop land practices that result in water balances similar to those experienced under native vegetation, relying on transpiration to remove excess water from the soil. Using native perennial pastures may allow to achieve this (Carbon, Roberts *et al.* 1982; Dyson 1993), if used in conjunction with suitable grazing practices, as defoliation can result in a concentration of roots in surface soil layers and a reduction of the usefulness of the deep rooted character (Pook and Costin 1971).

In order to combat salinity by reducing deep drainage, well adapted, summer-active perennial grasses with deep roots are required (Lodge 1994). Replacement of native perennials with summer-dormant annuals has resulted in water remaining in the soil profile after summer, increasing deep drainage in winter (Johnston, Clifton *et al.* 1999), therefore promoting species that are capable of using summer rainfall will dry the soil and reduce winter recharge. More water is lost as run-off and drainage with increasing winter rainfall, so these areas should benefit from a management regime that includes plants capable of using rainfall over a longer period and of drying the soil in summer, so as to accommodate the high winter rainfall (Clifton 1995). Planting summer active grasses may also help reduce soil acidification by promoting year-round legume growth (Munnich, Simpson *et al.* 1991).

Another form of salinity, which may be potentially more damaging than salinity from

shallow water tables, is transient salinity (Rengasamy 2002). Water infiltration is slow in sodic subsoils, and waterlogging may result in the formation of a perched watertable. Salts migrate to the saturated zone, and high summer evaporation leads to the accumulation of salts in this layer. Ameliorating transient salinity requires different methods than those used to control salinity due to shallow water tables; in particular, modifying the soil to permit greater drainage and leaching of salts below the root zone. However, selection of plants of deep-rooted character, able to access subsoil nutrients, is still advantageous.

Further advantages of planting native perennial grasses include reducing erosion, and sediment and nutrient transport (Prosser and Hairsine 1995). Summer active grasses may also be useful in preventing nitrogen leaching, as this process peaks in late summer and autumn when annuals are dormant (Mitchell, Waterhouse *et al.* 1993). Finally, native grasses may be useful in landscaping projects, as they are low maintenance, aesthetically pleasing, and allow the reestablishment of woody species due to their bunched nature and the gaps between bunches (Lodge and Groves 1990).

The influence of rainfall event size on salinity in Australian soils has been known for some time, with Prescott (1931) noting that small rainfall amounts per day are associated with salt accumulation. Gentilli (1971) considered rainfall intensity below 5mm per day likely to cause accumulation of salt in the soil. Sadras (2003) highlights the importance of considering the different processes associated with small and large rainfall events in land management. Climate change may also have an impact on deep drainage, with workers finding that increased CO_2 concentration leads to increased

deep drainage, particularly when the rainfall is concentrated in a few large events (Jackson, Sala *et al.* 1998), as plants are able to maintain a high photosynthetic rate with reduced transpiration and stomatal conductance, reducing water usage.

A paradox arises in the selection of species suitable for use in salinity mitigation in Australia. In order to prevent recharge, species that are able to utilise all available water, even in summer, are required. However, many Australian grass species may in fact be drought tolerant, a characteristic that may lead to die back, reduction in water use during dry periods, and growth only when water is readily available (Kemp and Culvenor 1994). Inefficient water users may utilise and remove excess groundwater, but may not persist in dry conditions, while efficient water users may be drought tolerant, but use water too slowly to be of use for preventing recharge (Johnston and Shoemark 1993). Some native grasses may be water saving rather than water using, making them unsuitable for salinity mitigation (Mitchell 1993).

6.7. Aims

This study aims to apply a number of rainfall indices, measuring seasonal rainfall bias, event size, clustering and drought length, across Australia, in order to correlate rainfall with productivity and traits of native grasses. Aspects of the drought tolerance and summer activity of *Austrodanthonia caespitosa* will be investigated, in particular variation in growth traits that may make this a useful pasture species, between ecotypes sourced from across the range of the species in southern Australia. Given the climatic influence on both plant rooting depth and soil salinity, and the requirement for summer

active, deep rooted plants for salinity control, a number of questions regarding the usefulness of Australian grass species are raised. This study aims to investigate variability in rooting architecture in the species *Austrodanthonia caespitosa*, to determine the contribution of plasticity and genotypic differentiation on root distribution, and to see how root distribution is correlated with factors such as rainfall seasonality, event size and soil type that may also influence salinity. In addition, I aim to use optimality modelling is utilized to examine theoretical optimal plant biomass allocation and root distribution under a variety of rainfall regimes and soil types.

There is an association between rainfall event size with both salinity and root distribution, although both small and large events considered to be associated with salinity though different processes, while deep rooted plants are considered useful in combating salinity. Similarly, areas with a high bias towards winter rainfall are considered at a risk of salt accumulation due to greater winter recharge, but there is conflicting evidence of associations between rainfall seasonality and rooting depth. This study aims to determine the feasibility of using plants of non-local provenance to combat salinity, if the plants show characteristics such as deep roots and summer water use that are useful in reducing winter recharge.

7. RAINFALL PATTERNS IN AUSTRALIA

7.1. Introduction

Historically, description of rainfall regime across Australia and other regions has focused on average annual and monthly rainfall totals, with some consideration of inter-annual variation (Australian Bureau of Meteorology 1988; Gentilli 1971). However, intra-annual rainfall regime can be an important driver of both natural ecosystems and agricultural biological systems, though the impact of factors such as rainfall event size, the spacing between rainfall events, the clustering of rainfall events, and the seasonal bias of rainfall.

Precipitation event size, which may vary, for instance, with whether the region of influence receives the majority of its rain from frontal systems or large storm events, can have an impact on the infiltration depth of water into the soil profile (Reynolds, Kemp *et al.* 2004). Small precipitation events may only penetrate into the surface of the soil (Kemp 1983) while larger events may be expected to wet the soil profile to a deeper depth, assuming infiltration is not limiting. Schwinning and Sala (2004) suggest that different sizes of rainfall events will trigger different ecological processes, from nitrogen mineralisation, though to plant growth and seed germination. In terms of total rainfall and productivity in the system, large events can contribute a disproportionate amount to rainfall. Golluscio (1998), in a study on the Patagonian steppe, found that the number of small events varied little from year to year, but there was high variability in the number of extreme events, explaining the variation in total rainfall between years.

Similarly, in a study examining the effect of crop stubble on soil moisture in Walepup, Australia and Balcarce, Argentina, it was found that in high rainfall years, large events contribute a greater proportion of total rainfall (Monzon, Sadras *et al.* 2006). Event-size can influence the hydrological pathway of water in the system (Loik, D. *et al.* 2004), with water from small rainfall events tending to be lost through evaporation and canopy interception (Sadras and Baldock 2003), while large events contribute to run-off and deep-drainage (Reynolds *et. al.* 2004).

The gap between precipitation events, also termed the interpulse, also has important implications for ecosystem functioning. Evaporative loss is the main factor driving soil water dynamics during the interpulse period (Loik *et. al.* 2004), with the potential to lead to plant stress in water limited systems. The drought tolerance of plants in a system may be related to the typical interpulse length experienced in that system, with long interpulse periods resulting in plant mortality (Lundholm and Larson 2004) especially in the seedling stage (Veenendaal, Ernst *et al.* 1996). Drying of the surface soil during the interpulse can be particularly important for grasses, as the majority of the roots are in the surface 20cm of the soil (Huang and Fu 2000), and therefore interpulse length and changes in rainfall regime may profoundly influence grassland and pasture dynamics.

Event clustering and the timing of individual events throughout a growing season also have a strong influence on ecological communities (Schreiber and Sutter 1972). Consecutive days of small events may have the same impact on a system as a single large precipitation event (Reynolds, Kemp *et al.* 2004), and in arid systems, some

consider many small events to be more useful to plants than a single large event (Sala and Lauenroth 1982) although others have disregarded the importance of precipitation events less than 5mm in size in arid systems (Noy-Meir 1973). Germination, again, may be strongly influenced by the clustering of rainfall events and the influence of multiple small rainfall events (Veenendaal, Ernst *et al.* 1996). A study (du Plessis 2001) suggests that in an arid environment, single large events may not be sufficient to trigger germination, but multiple small events may be.

The seasonal distribution of rainfall is important because it influences the interaction between precipitation and evaporation, and the water balance of the system. Stephenson (1990) highlights the importance of considering ecosystems and soil water in terms of the water balance, taking into account the seasonal coupling of water and energy input into a system. Systems with similar rainfall and temperature profiles on an annual scale may have vastly different water balances and deficits due to the seasonal timing of rainfall and evaporation. Rainfall is less effective in the summer due to higher evaporation rates (Reynolds, Kemp *et al.* 2004), which can influence plant growing season and root distribution, given the low water availability in surface soil. There may be a threshold amount of summer rainfall before it becomes useful for plants grow roots in the surface soil to utilize it (Ehleringer and Dawson 1992). In contrast, environments with a strong bias towards winter rainfall tend to experience greater deep drainage and run-off (Seyfried, Schwinning *et al.* 2005).

There is clearly strong and significant variation in factors such as rainfall event size, interpulse length, clustering and seasonality across Australia. Regions such as the

northern tropics and the Mediterranean-climate southern coastal regions have strong biases towards summer and winter precipitation respectively. In addition, different weather systems in different regions of the continent influence the small-scale rainfall regime, including event size and spacing. For instance, southern regions that experience frontal systems may have strong clustering of small events, while more arid, central regions tend to get intense rainfall events from isolated storm events. This variation, and any change in rainfall pattern over time, may be expected to influence productivity across geographical areas (Henkin, Seligman *et al.* 1998; Le Houérou, Bingham *et al.* 1988; Paruelo and Lauenroth 1995), plant characters such as root distribution and allometry (Fay, Carlisle *et al.* 2003)**,** and species assemblages (Weltzin, Loik *et al.* 2003)**.** Event and interpulse size may have an influence on competitive interactions, providing a diverse range of niches for plant water acquisition and contributing to species diversity (Goldberg and Novoplansky 1997; Goldberg and Miller 1990).

This study aims to apply a number of established and novel indices to precipitation regime in Australia. As both agricultural and native plant species may be expected to response to small-scale rainfall influences in water limited systems, it may be useful to have a greater understanding, and simple descriptors, of intra-annual variation in rainfall across the continent. For example, species traits and ecosystem function may be strongly affected by drought length, particularly in grasslands (Fay, Carlisle *et al.* 2000), or by the clustering of rainfall events (Veenendaal, Ernst *et al.* 1996). Climate change may also be expected to modify rainfall regime on a scale smaller than annual

rainfall totals, for instance, by resulting on more extreme rainfall events (Hughes 2003), and new indices may be useful in quantifying climate change.

Rainfall seasonality is analysed with the Walsh and Lawler (1981) seasonality index, and the vector seasonality index (Markham 1970), two methods that enable monthly rainfall distribution to be summarised in small numbers of variables, in order to map small-scale gradients in seasonality across the continent. Event-size is described using an index that makes use of the scale-independent nature of rainfall events to measure the bias towards small or large events independent of annual rainfall totals, a method that can also be applied to interpulse length. Event clustering is described using Markov-chain analysis of rain days, a method contrasted with the average rain-period length. The potential for these indices to be used in climate-change analysis is explored, with an analysis of changes in rainfall event-size bias over time across Australia. Changes in rainfall regime, particularly changes in event size and frequency, are expected to have significant impacts on terrestrial ecosystems (Weltzin, Loik *et al.* 2003).

7.2. Methods

Data Sources and Maps

Rainfall data for the indices were obtained from the Australian Bureau of Meteorology. Different stations and datasets were used for different indices, (listed in Table 1). Stations were selected to give good coverage of the continent, with a particular emphasis on the south-east. Daily rainfall data was obtained from the patched point

dataset (PPD). In addition, 189 rainfall stations identified as being high quality (Lavery, Kariko *et al.* 1992) were used in the analysis of temporal trends in τ. These stations had the most complete records, with the fewest missing days and most consistent, accurate recording of rainfall. These stations, however, did not necessarily provide as even coverage as the larger set used in production of the static maps. Indices were smoothed using the Kriging method and plotted as contour maps over Australia using Golden Software Surfer 8.

Table 1- Rainfall data used in calculation of indices.

Index	**Data Type**	**Number of Stations**	**Record Length (years)**
Walsh and Lawler	Monthly Averages	969	25-150
Vector	Monthly Averages	969	25-150
τ statistic	Daily PPD	350	100
τ statistic change	Daily High Quality PPD	189	80
G statistic	Daily PPD	433	80-100
Markov	Daily PPD	247	100

Event Length	Daily PPD	247	100

Walsh and Lawler seasonality index

The Walsh and Lawler seasonality index (Walsh and Lawler 1981) calculates a single value indicating the strength of seasonality of rainfall regime, with no regard to the direction or distribution of the seasonality. It is calculated as the sum of deviations of monthly rainfall from the expected monthly rainfall if rain was equal in all months, as expressed in equation 1.

$$SI = \frac{1}{R}\sum_{n=1}^{n=12}\left|\bar{x}_n - \bar{R}/12\right| \qquad \text{Equation 1}$$

Where $\bar{x}_n$ = mean rainfall of month *n* and $\bar{R}$ = mean annual rainfall.

The period of time over which monthly averages were calculated varied from 25 to 150 years, but the large number of stations utilized is expected to smooth variation caused by short rainfall records at some stations.

Vector seasonality

Seasonality vectors provide information on both the intensity of seasonality in rainfall regime, and the direction of seasonality, that is, the time of year in which most rainfall occurs (Markham 1970). The average amount of rainfall occurring in a month determines the length of that month's vector, and the vector's direction is determined by an angle associated with the month. For instance, January has a vector angle of 15

degrees, February has a vector angle of 45 degrees, and so on, increasing by 30 degrees for each month. Monthly vectors are added to generate an overall rainfall vector.

Vectors were calculated using a Python program. First, monthly rainfall averages ($\bar{x}_n$) and month-angles (A) were converted to Cartesian coordinates for each month (Equations 2a and 2b).

$$X_n = \bar{x}_n \cos(A_n) \qquad \text{Equation 2a}$$

$$Y_n = \bar{x}_n \sin(A_n) \qquad \text{Equation 2b}$$

Cartesian coordinates were then summed to calculate a summary coordinate, the displacement of which from 0,0 indicates the strength and directionality of rainfall (equations 3a and 3b).

$$x_{tot} = \sum_{m=1}^{m=12} x_m \qquad \text{Equation 3a}$$

$$y_{tot} = \sum_{m=1}^{m=12} y_m \qquad \text{Equation 3b}$$

Angle and magnitude of the vector from 0,0 were then calculated. Magnitude was divided by the average annual rainfall for the site to normalize vector magnitudes between sites with different annual rainfall averages (equations 4a and 4b).

$$Angle = \arctan\left(\frac{x_{tot}}{y_{tot}}\right)$$ Equation 4a

$$Magnitude = \frac{\sqrt{x_{tot}^2 + y_{tot}^2}}{\sum_{m=1}^{m=12} R_m}$$ Equation 4b

The angles above were calculated in radians, and then converted to degrees. In addition, the angle was converted to "compass" degrees through the following process:

If $x_{tot} > 0$: Corrected Angle = 90 – Angle

If $x_{tot} <= 0$: Corrected Angle = (90 – Angle) + 180

In the calculations, the vector for January was taken as 15°, representing the average for the month and therefore the middle day of the month. Therefore, 0°, or "North" on the map, represents January 1st.

τ (Tau) event-size index

τ is defined as the slope of the regression of log(rainfall event size) vs log (event size frequency). As rainfall event distribution is expected to follow a power law (Peters and Christensen 2002; Sadras and Baldock 2003), τ could be calculated at any time or event size scale. For the purposes of this study, a daily time scale was used, as daily rainfall records were most easily available.

Events sizes were divided into 5mm-wide classes. For instance, counts were made in the data of the number of rainfall events less than or equal to 5mm in size, greater than 5mm but less than or equal to 10mm in size etc. In the regression, the upper limit of each event size classed was used as the x value. Both event counts and size classes were log-transformed and a linear regression was performed, with the slope reported as the τ statistic. Separate regressions were performed for the summer and winter subsets of the rainfall data, with "summer" defined as the months from October to March, and "winter" the months from April to September.

For the analysis of temporal change in τ, data from the years 1920 to 2000 were used, as this was the period over which the data sets were most complete. τ was calculated for intervals of 1-year, 5-years and 10-years between the years 1920 and 2000, with annual, summer and winter values reported. In cases where there were less than three rainfall events during a calculation period, the point was discarded as a meaningful regression was unable to be performed. This situation arose quite frequently while calculating summer τ values at one-year intervals. If a rainfall station had regression slopes of the same sign (positive or negative) for all three sampling resolutions, the site is marked with a + or – on the map. Positive marks indicate sites where there has been an increase in rainfall event size bias, while negative marks indicate a decrease in rainfall event size bias. Sites with regressions that did not have consistent slope directions at different sampling scales are marked with circles, to indicate uncertainty of trend.

Gap-size index

The distribution of sizes of gap (drought length) between rainfall events is also expected to follow a power law (Peters, Hertlein *et al.* 2002). A Python program was written to analyse daily rainfall records, and record a distribution of drought lengths versus the number of drought events of that length. Drought lengths of zero, that is, consecutive days of rain, were ignored, and drought lengths of up to 1,000 days were recorded. Records of drought length distribution were also calculated for summer and winter months. Drought length and number of drought events in each class were both log-transformed, and a linear regression performed, with the slope being recorded as the gap-size index, G. Separate regressions were performed for the summer and winter subsets of the rainfall data, with "summer" defined as the months from October to March, and "winter" the months from April to September.

Markov probability and event length

Categorizing rainfall data into "event" and "non-event" days allows rainfall to be viewed as a Markov-chain process. Daily rainfall events were analysed to determine the (1,1) Markov probabilities in a 1st-order chain, that is, the probability that if it is raining on day t, it will also be raining on day t+1. This statistic gives a measure of how grouped rainfall events are; low values indicate a regime where rainfall events are isolated and occur on single days, while high values indicate a regime where rainfall tends to occur for several days in a row. It may therefore help delineate regions where rainfall is dominated by storm events, and regions where rainfall is dominated by

frontal systems that take several days to pass over.

Another way of examining rainfall event clustering is by examining the number of days rain typically experienced in succession, termed the pulse length. The average pulse length was determined by considering successive days of rainfall, surrounded by days with no rainfall occurrence, as rainfall pulses. The average number of days per rainfall pulse throughout the data set was recorded as the P value. Both the Markov and the pulse length index have only been calculated for the entire year at present, rather than for summer and winter seasons.

7.3. Results

Walsh & Lawler Seasonality Index

The rainfall regime is highly seasonal in the tropical north, the west coast, and coastal South Australia (Figure 1). In the tropics, rainfall is biased strongly towards the summer months, with almost no rainfall in the dry season. Highly seasonal areas in southern Australia are those with a Mediterranean climate – South Australia and southwestern Western Australia. In these areas, rainfall is biased strongly towards the winter months. The Great Dividing Range also appears to influence rainfall seasonality, with an increase in seasonality along its range, particularly in northern New South Wales.

Much of New South Wales, Victoria and Tasmania experience an equitable rainfall regime, as do arid inland areas. Southern Western Australia has a winter dominated

rainfall regime, and the north of the state has a summer dominated rainfall regime. However, the seasonality index remains equally strong along the coastline from south to north, unlike the transition from winter to summer rainfall in other parts of Australia.

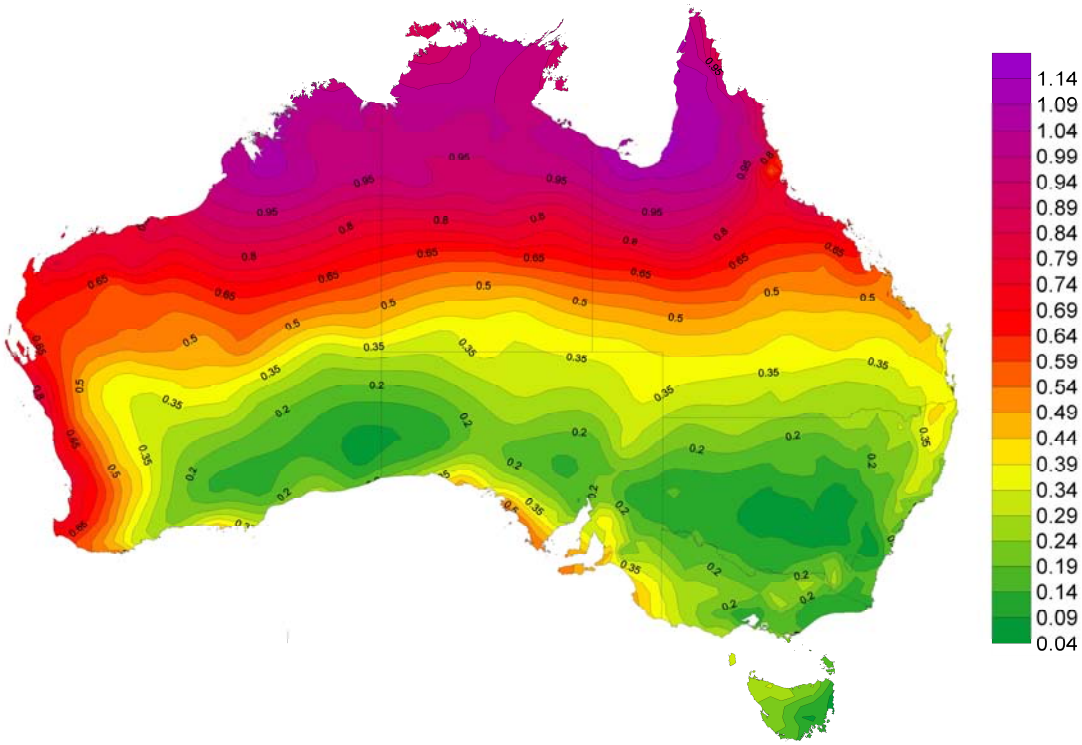

Figure 1- Walsh and Lawler seasonality index across Australia

Vector Seasonality

The bias towards summer rainfall in the north and winter rainfall in the south is clear from the orientation of the vectors (Figure 2). Smaller scale variations in seasonal timing of rainfall is apparent, with Victoria having a bias towards rainfall earlier in the year than South Australia, and the coast of New South Wales having a bias towards spring rain, compared to summer rain inland of the Great Diving Range. Arid and

aseasonal areas show low magnitude vectors with at times random direction.

Along the Western Australian coast, the vector seasonality index shows shift in time of rainfall concentration from winter rain in the south, to spring rain around the Exmouth Gulf, to summer rain in the tropical north.

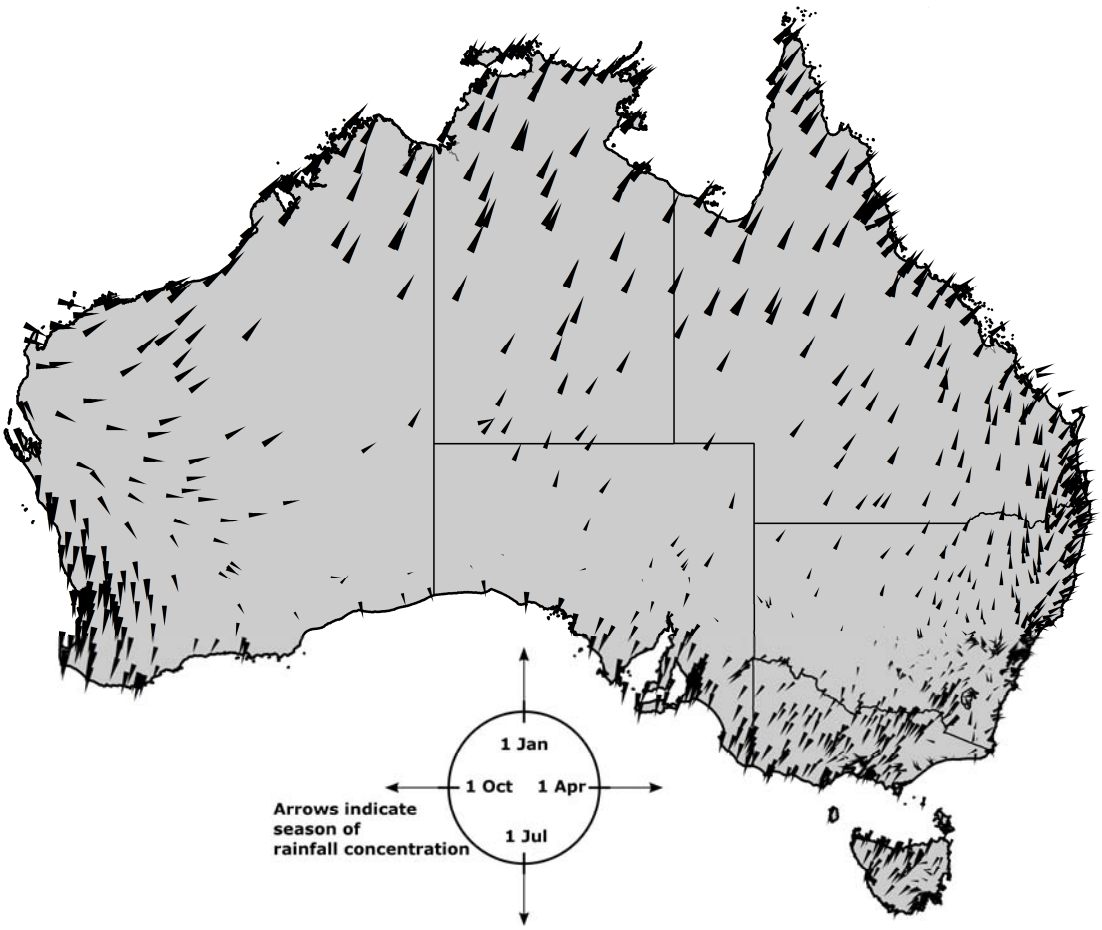

Figure 2 - Vector seasonality index across Australia

In order to test the validity of the vector seasonality index compared to the Walsh and Lawler seasonality index, a linear regression was performed (Figure 3) between vector magnitude and Walsh and Lawler value. The r^2 value of 0.988 (n=1137) indicates an excellent correlation between the two indices indicating strength of seasonality, and it appears that in Australia errors caused by multi-modal rainfall distributions are small.

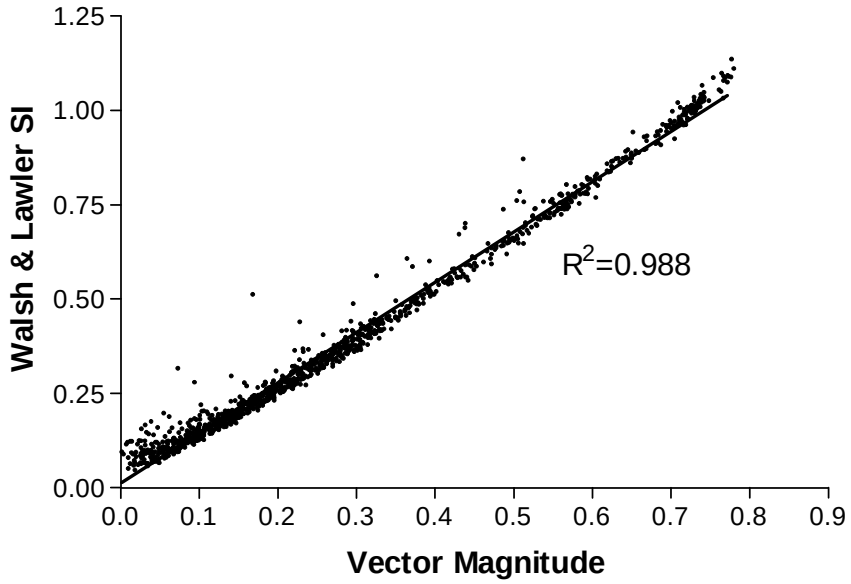

Figure 3 - Correlation between Walsh and Lawler index and vector magnitude index. Both indices are dimensionless.

τ (Tau) event-size index

Large values of the index indicate a bias towards smaller rainfall events, while small values indicate larger events. Large rainfall events dominate much of northern Australia

(Figure 4). In the northern tropics, the bias towards large events may be due to the intense nature of tropical monsoon rain, while in more arid regions a large proportion of the rainfall comes in infrequent, intense storm events.

The southern coastline of Australia, on the other hand, is dominated by smaller rainfall events. Rainfall in this region is dominated by frontal systems. In particular, the southeast of South Australia, coastal Victoria and western Tasmania experience a bias towards small showers. Small spatial scale variations in rainfall event size can be seen along the east coast, with coastal regions experiencing larger rainfall events than sites further inland beyond the Great Dividing Range. Rainfall event size in Tasmania decreases from east to west.

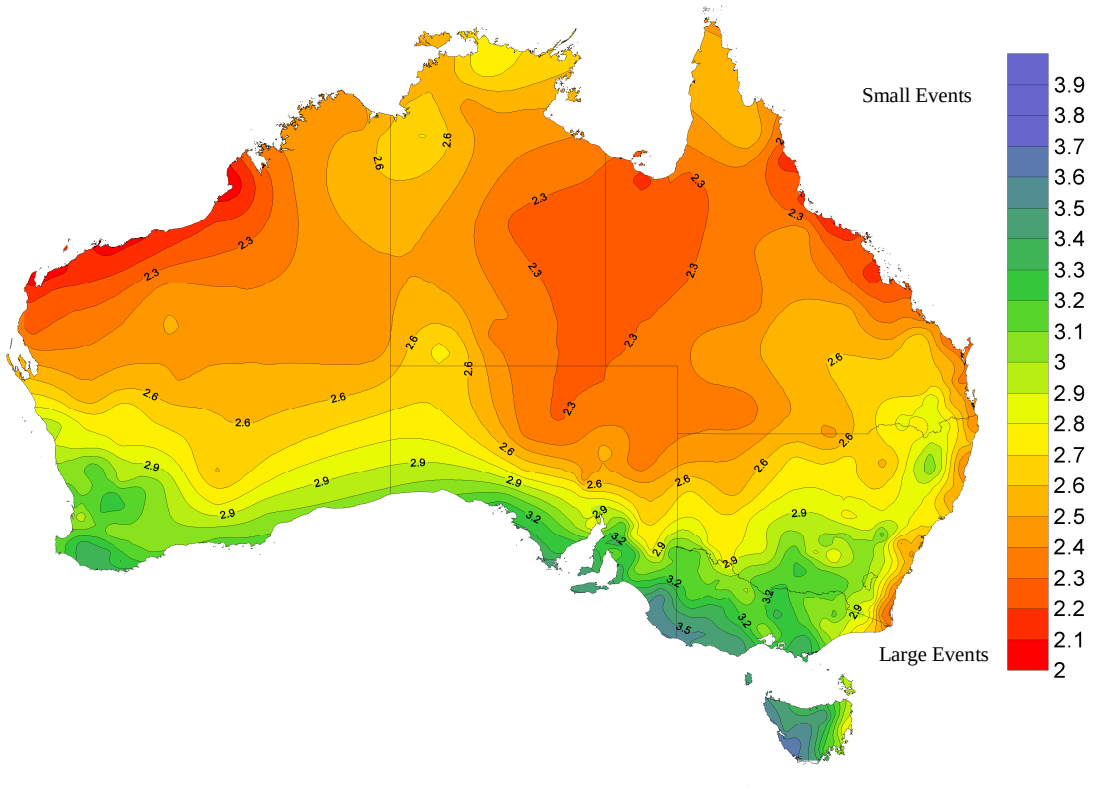

Figure 4 - t (Tau) event-size index across Australia

In summer, small rainfall events dominate the southern coast, Tasmania, Victoria and the Great Dividing Range (Figure 5). There also appears to be a bias towards smaller events in the tropical north of the Northern Territory. Large summer events dominate in the arid interior, where most rainfall arrives as intense storm events, and coastal Queensland. Summer rainfall event size in Tasmania decreases from east to west.

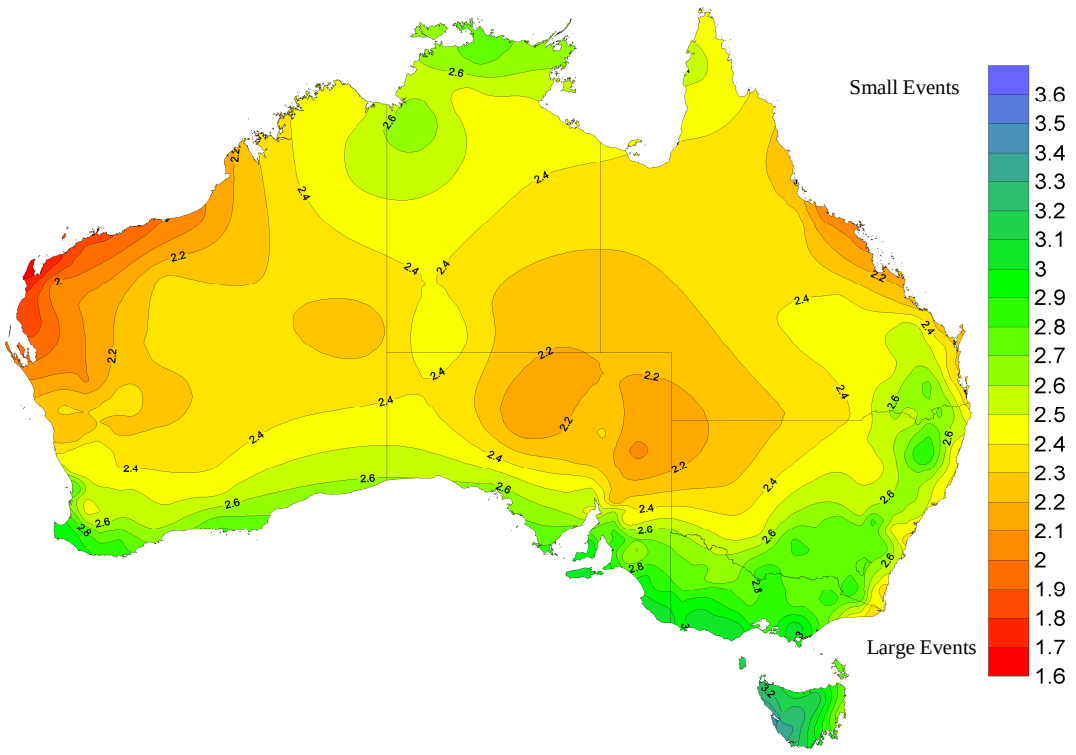

Figure 5 - t (Tau) event-size index across Australia for the summer half year

Overall, southern Australia experiences a bias towards small rainfall events in winter, while northern Australia experiences a bias towards large rainfall events (Figure 6). Southern Australia is dominated by frontal systems bringing showers, while during the winter months, the only rainfall much of northern Australia receives is in the form of occasional intense storms.

Again, the Great Dividing Range through New South Wales has a clear impact on rainfall patterns, with larger winter events on the coast, and smaller events inland. Winter rainfall event size in Tasmania decreases from east to west.

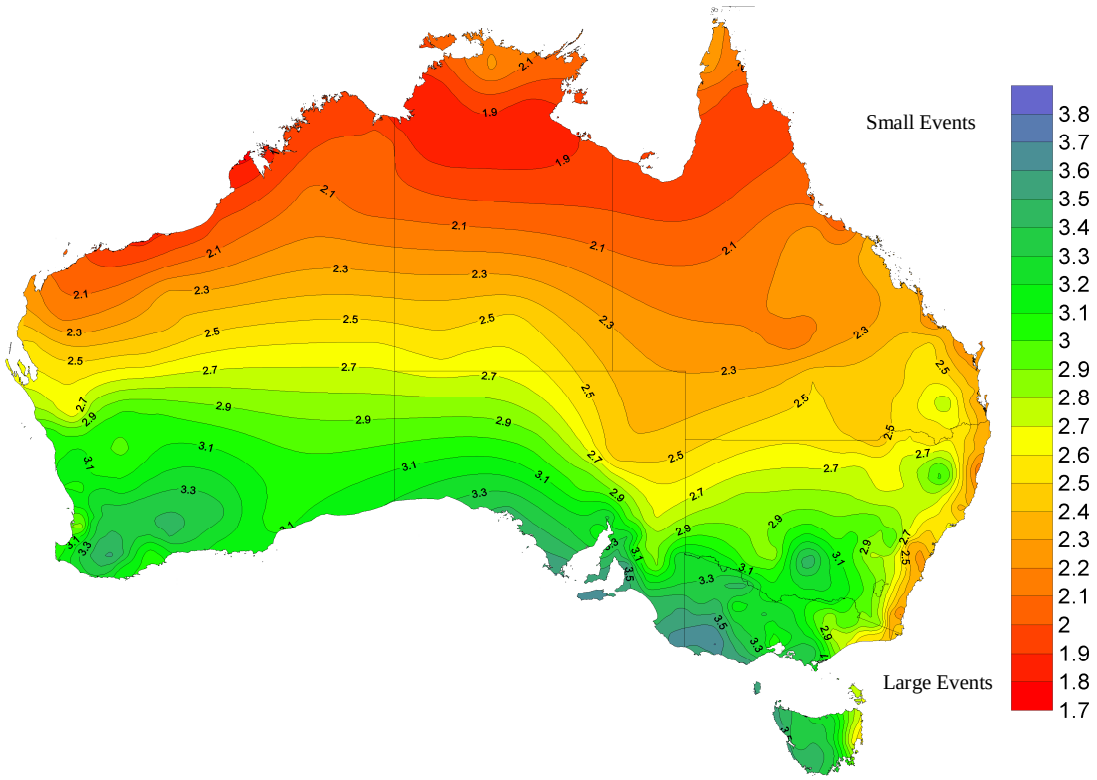

Figure 6 - t (Tau) event-size index across Australia for the winter half year

Gap-size index

High values of the gap-size index indicate areas with a bias towards small gaps between rainfall events, while low values of the index indicate large gaps between rainfall events. Most of arid central and northern Australia experiences a rainfall regime with long droughts between rainfall events (Figure 7). In tropical areas, the low value of the index reflects the dry season, where rainfall may not occur for several months. An anomaly is apparent on the north Queensland Pacific coast, between Cairns and Townsville, where higher values of the index occur. This region, centred on Innisfail, receives one of the highest annual rainfall totals in Australia, as the Great Dividing

Range meets the ocean and uplift of moist oceanic air deposits near constant precipitation. Rainfall in this region is very frequent compared to other sites at the same latitude.

Coastal New South Wales, Victoria and parts of South Australia and southern Western Australia have relatively high values of this index, indicating frequent rainfall events. Tasmania, in particular, experiences relatively small gaps between rainfall events, particularly on the west coast.

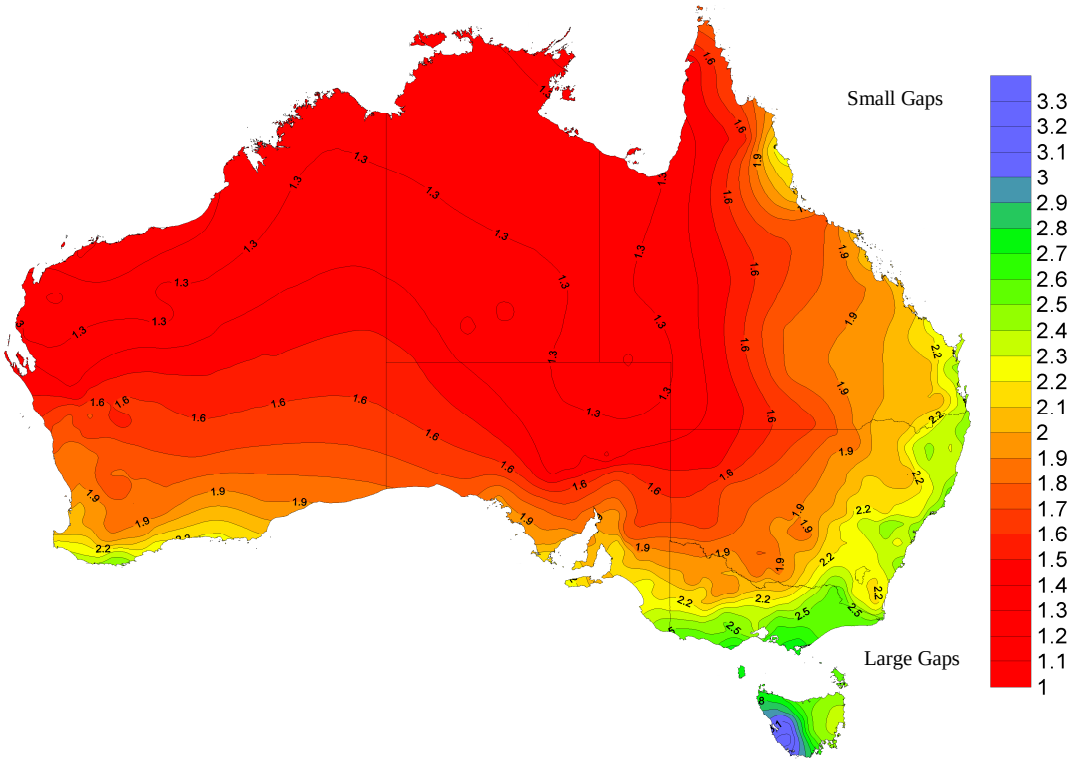

Figure 7 - Gap-size index across Australia

The east coast of Australia, from Victoria through to north Queensland experiences frequent rainfall events during the summer, as does the south coast of Western

Australia, with drought lengths increasing as one moves inland (Figure 8). Summer gap-size index decreases heading into South Australia, where summer rainfall is less frequent. Again, the local effect around Innisfail in north Queensland is clear, with very frequent events compared to other sites at the same latitude. Unexpectedly, the arid regions and the tropical north experience longer gaps between rainfall events in summer.

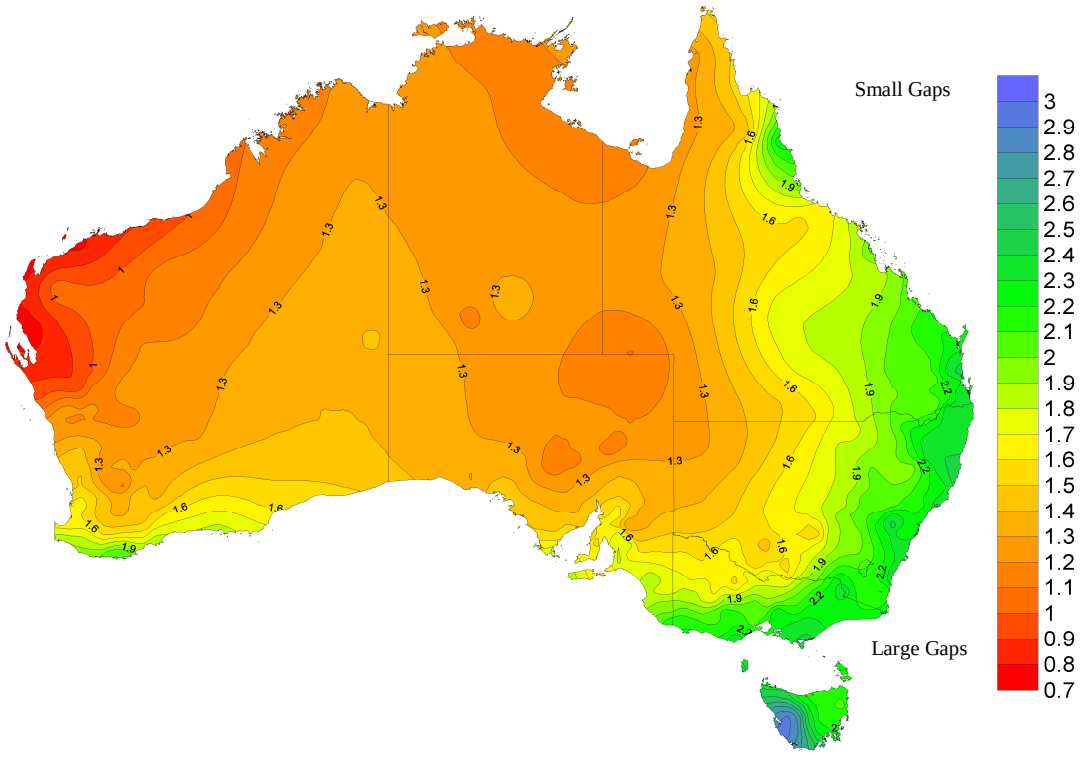

Figure 8 - Gap-size index across Australia for the summer half year

A clear difference can be seen between the winter gap-size and the summer gap-size map along the east coast, particularly in Queensland, where events have become much

less frequent during winter (Figure 9). In contrast, rainfall events in winter are more frequent in South Australia and southwest Western Australia.

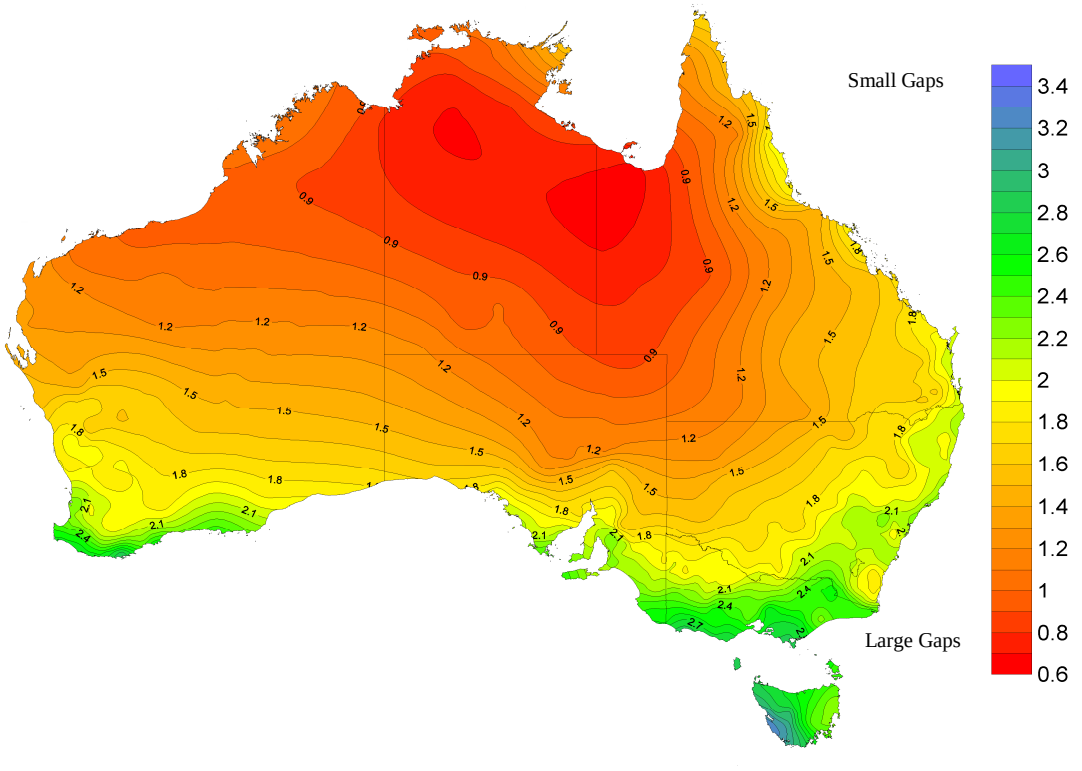

Figure 9 - Gap-size index across Australia for the winter half year

Markov (1,1) probability and event length

The arid inland has low probability of consecutive days of rain, as most rain in these regions occurs as intense, isolated storm events (Figure 10). By contrast, most coastal areas have a higher probability of consecutive days of rainfall. In the south, rainfall is dominated by frontal systems that can take several days to pass over and deliver rain, hence a high probability of several days consecutive rain. High probability values arise in northern Australia due to the constant daily rainfall occurring during the tropical

monsoon. Western Tasmania stands out as having a very high probability of consecutive rainfall days, a reflection of the high number of rain days per annum in that region.

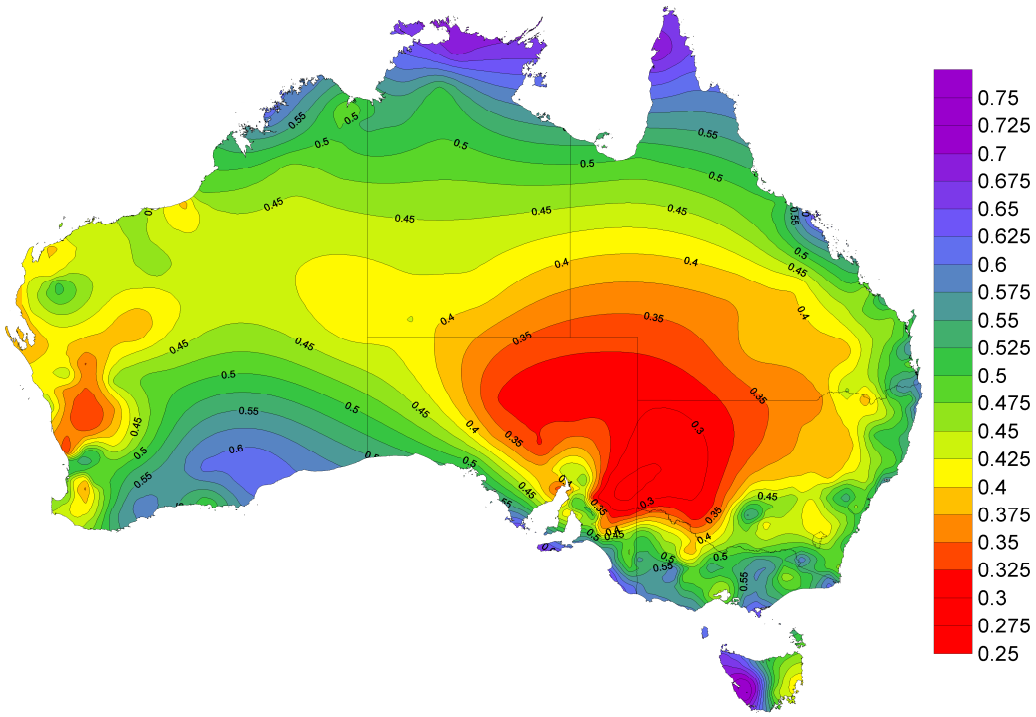

Figure 10 - Markov (1,1) probability across Australia.

For the summer half-year, the high probability of consecutive days rain in the tropical regions, and the high rainfall east coast is clear, with southern and western areas, and the arid interior showing a low probability of consecutive rainfall (Figure 11).

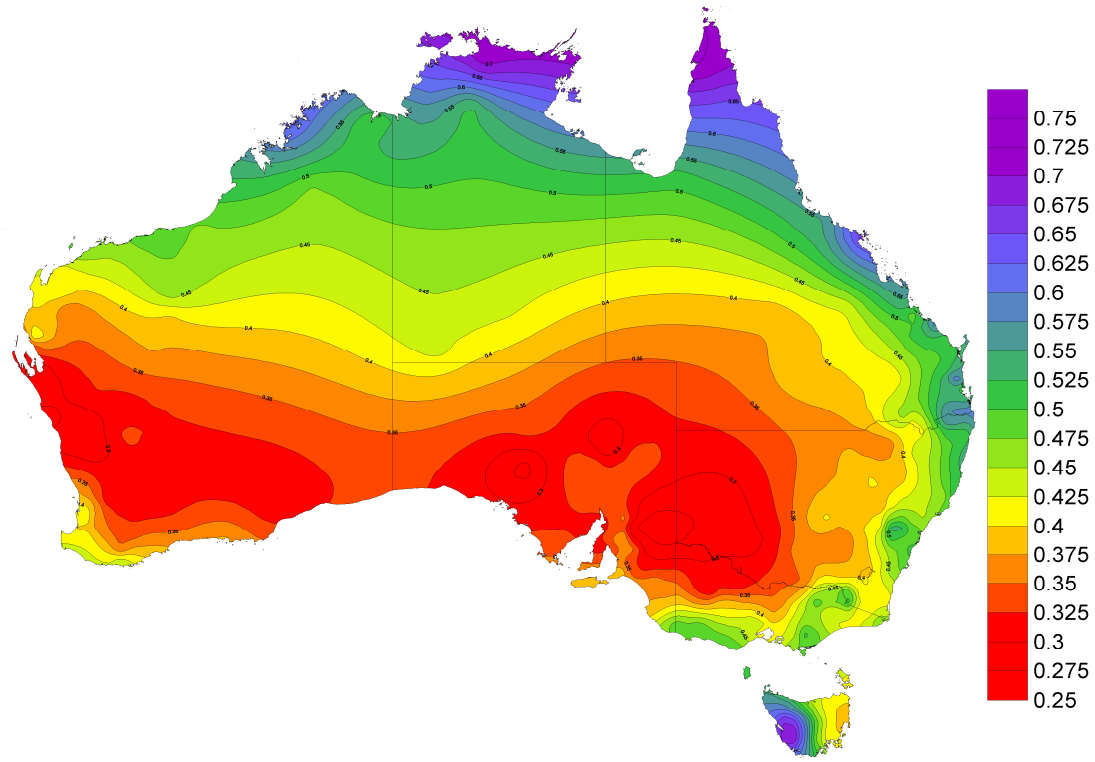

Figure 11 - Markov (1,1) probability across Australia for the summer half-year.

Compared to the summer, consecutive days of rainfall are much less common in the tropics during the winter, and the inland area where multiple days of rainfall occur with a low probability has expanded northwards (Figure 12). Southern areas, around the coast of South Australia, Victoria, and south western Western Australia, areas dominated by frontal systems in winter, show a high probability of consecutive rainfall days.

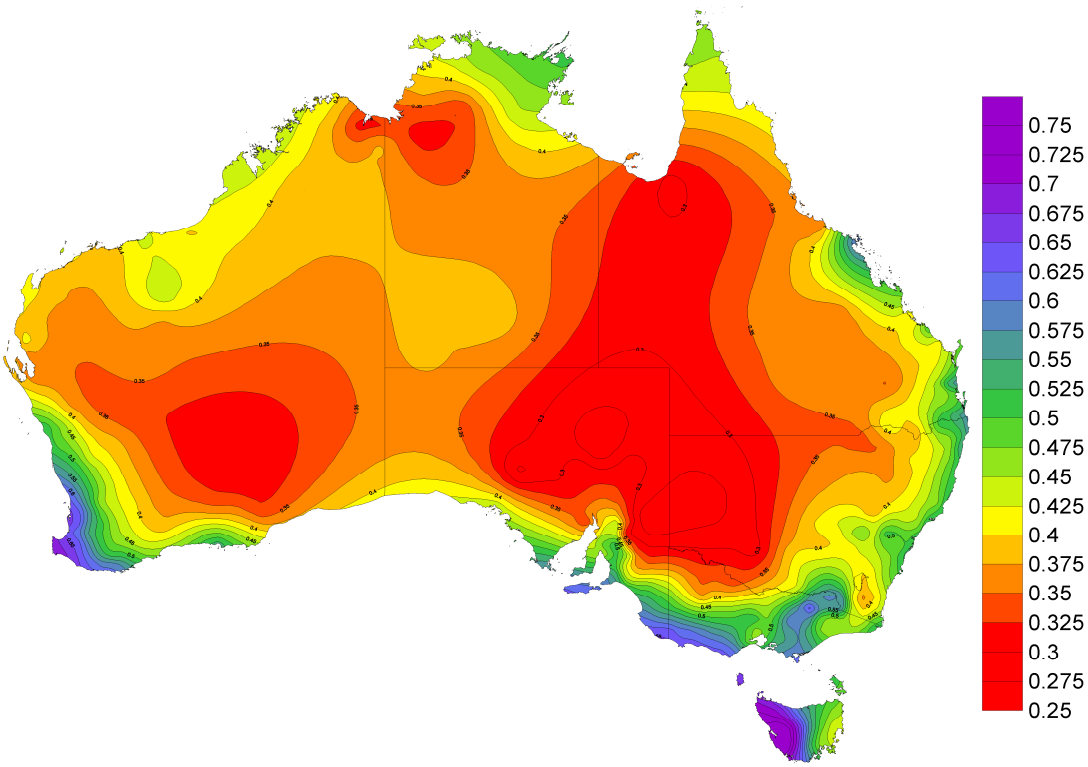

Figure 12 - Markov (1,1) probability across Australia for the winter half-year.

There are a number of similarities between the map of average days per rainfall events (Figure 13) and the Markov probability map, in particular, the tendency for short periods of rain in the arid centre, and longer periods around the coast and in Tasmania.

Arid areas generally experience rainfall events of only 1 to 2 days. Frontal dominated southern Western Australia, South Australia and Victoria tend to have rainfall events lasting 2-3 days. The west coast of Tasmania has an average of greater than three consecutive days of rain per event. The tropical north also has a high average event length, reflecting the near daily rain during the summer monsoon season.

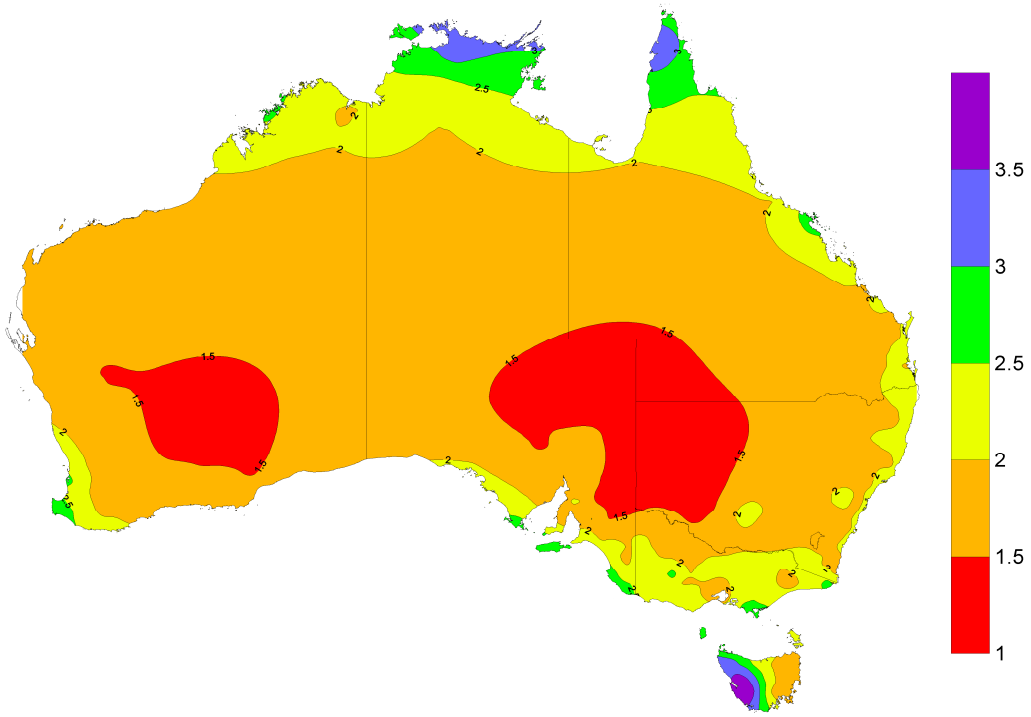

Figure 13 - Average pulse length (days) across Australia

τ event-size index change over time

Areas with a visually consistent trend towards larger rainfall events include inland south-western Western Australia, southern South Australia and parts of Victoria (Figure 14). Areas with a consistent trend towards smaller rainfall events include far, coastal south-western Western Australia, northern South Australia, New South Wales, Queensland and the Northern Territory.

Figure 14 - Trend in τ-statistic across Australia, 1920 – 2000, indicating change in rainfall event size bias. + = trend towards larger events, - = trend towards smaller events, O = no significant change.

The regression analysis was repeated using rainfall data for the summer (Figure 15) and winter (Figure 16) half year. Summer event size shows a trend towards smaller events at a few stations in south-western Western Australia, but with a trend towards larger events further inland. A trend towards smaller events is evident in most of South Australia, inland New South Wales, central Queensland, and the Northern Territory.

Figure 15 - Trend in t during summer across Australia, 1920 – 2000

Areas with a trend towards increasing winter rainfall event size include inland south-western Western Australia, semi-arid South Australia, south-eastern Queensland, and parts of western Victoria. Areas with a trend towards smaller winter rainfall events include south-western Western Australia, coastal south-eastern South Australia, western Queensland, the Northern Territory, and Tasmania.

Figure 16 - Trend in τ during winter across Australia, 1920 – 2000

Significant correlations of τ event-size with time

τ index was highly variable for year to year, leading to few significant correlations with time, hence the choice of a conservative approach to reporting directions of change in the above maps. Table 2 shows rainfall stations with significant correlations for the 1-year sampling period for the annual data set, Table 3 shows the summer data set and Table 4 shows the winter data set.

Table 2- Significant correlations in t index for annual records.

Regression significant p<0.05	Regression significant p<0.01	Regression significant p<0.005
Annual + Beechworth (082001) - Branxton (061014) + Canary Island (080004) + Cape Grim (091011) + Gabo Island (084016) + Merredin (010092) + Nyerilup (010541) + Orroroo (019032) - Pardelup (009591) - Peppermint Grove (009594) - Pine Creek (014933) - Rylstone (062026) + St. Arnaud (080009)	+ Appila (019001) - Boulia Airport (038003) - Burketown PO (029004) - Casino Airport (058063) - Roebourne (004035) - Tempe Downs (015557) - Toorourrong Reservoir (086117) + Wickliffe (089033) + Wilsons Promontory (085096) - Yolla (091109)	- Barraba PO (054003) - Bingara PO (054004) + Blinman (017014) + Cape Naturaliste (009519) - Clarence Town (061010) - Fairymead Sugar Mill (039037) + Hopetoun (009557) - Kalamia Estate (033035) - Katherine (014902) + Leslie Manor (090053) - Lindenow (085050) - Macknade Sugar Mill (032032) + Meredith (087043) + Mt Brisbane (040140) - Mudgee (062021) - Murgon PO (040152) - Natimuk (079036) + Wallangra (054036) - Westbourne (009616) - White Cliffs PO (046042)

Table 3 - Significant correlations in t index for summer records.

Regression significant p<0.05	Regression significant p<0.01	Regression significant p<0.005
Summer - Bathurst (063005) + Boolardy (007007) + Broomehill (010525) - Burketown PO (029004) + Canary Island (080004) - Clarence Town (061010) - Collarenebri (048031) - Doctors Creek (041024) - Fingal (092012) - Kalamia Estate (033035) - Kaniva (078078) + Kilmore College (088034) - Marree (017031) + Mt Brisbane (040140) - Mulga Downs (044054) - Natimuk (079036) + Nyerilup (010541) - Orbost (084030) - Tempe Downs (015557) - Westbourne (009616) + Wickliffe (089033) - Yolla (091109)	+ Blinman (017014) - Casino Airport (058063) + Gabo Island (084016) + Leslie Manor (090053) + St Arnaud (080009) - Urandangi (037043)	+ Arthur River (010505) - Barraba PO (054003) - Bingara PO (054004) - Boulia Airport (038003) + Cape Schanck (086017) + Casuarina Vale (010024) - Fairymead Sugar Mill (039037) - Hay (075035) + Hopetoun (009557) - Katherine (014902) - Kondoolka (016022) - Lindenow (085050) - Macknade Sugar Mill (032032) + Meckering (010091) + Meredith (087043) - Mudgee (062021) - Murgon PO (040152) - Rylstone (062026) - Toorourrong Reservoir (086117) + Walk Walkin (010133) + Wallangra (054036) - White Cliffs PO (046042) + Wydgee (007090)

Table 4 - Significant correlations in t index for winter records.

Regression significant p<0.05	Regression significant p<0.01	Regression significant p<0.005
Winter - Arthur River (010505) - Beaufort (089005) + Canary Island (080004) + Cape Naturaliste (009519) - Casino Airport (058063) - Clarence Town(018065) + Cleve (018065) + Hay (075035) - Kalamia Estate (033035) - Murgon PO (040152) + Rainbow (077036) - Roebourne (004035) - Sheringa (018045) + St Arnaud (080009) + Twin Peaks (006048) + Wickliffe (089033) + Yardea (016055) - Yolla (091109) + Yoweragabbie (007095)	+ Appila (019001) - Barraba PO (054003) + Casuarina Vale (010024) - Farleigh Co-Op Sugar Mill (033023) + Hallett (021028) + Hopetoun (009557) + Minlaton (022009) + Orroroo (019032)	- Bauple (040013) - Birdsville (038002) + Blinman (017014) - Canning Downs (041013) - Fairymead Sugar Mill (039037) + Lake Carmody (010670) + Leslie Manor (090053) + Mt Brisbane (040140) - Pardelup (009591) - Tempe Downs (015557) + Wallangra (054036) + Wallaroo (022020) + Wentworth PO (047053) - Westbourne (009616) - White Cliffs PO (046042) + Whyalla (018058) + Wilsons Promontory (085096)

7.4. Discussion

The Walsh and Lawler seasonality index described both the strong summer-biased precipitation regime in the tropical north, and the winter-biased regime in southern Western Australia and South Australia. Arid regions, and areas of inland New South Wales and Victoria have a low seasonality index. It should be noted that as this index

is calculated from long-term averages, this indicates an equal probability of rainfall in all months, rather than constant rainfall, as in arid regions rainfall events may be very unpredictable and widely spaced, and vary from year to year. The resolution of this index allows changes in seasonality along the Great Dividing Range to be discerned, with a stronger seasonal bias on the range compared to coastal and inland areas, especially in northern New South Wales. A failing of this index is revealed along the Western Australian coastline, where a strongly seasonal rainfall regime is observed along the coast from the winter-dominated regime in the south to the summer-dominated regime in the north. In not providing any information about the direction of the seasonal bias, the Walsh and Lawler seasonality index fails to explain the lack of an equitable regime between the two seasonal extremes as found in eastern Australia.

The vector seasonality index, in contrast, gives a clear indication of bias towards different months of the year, with indications of summer rainfall in the north and winter rainfall in the south. This index also gives an indication of small shifts in the seasonal timing of rainfall across short gradients, for instance, winter rain occurs earlier in the year in Victoria than South Australia, and coastal New South Wales has a bias towards spring rain, while inland areas have a stronger bias towards summer rain. Random vector directions in arid regions confirm the highly variable, unpredictable rainfall in the region, with even averages over many decades resulting in recordings of different seasonal rainfall biases in closely situated rainfall stations. The vector seasonality index also reveals the reason for equal strength of seasonal bias along the Western Australian coastline. There is indeed a steady shift in time of rainfall concentration

from winter rain in the south, to spring rain around the Exmouth Gulf, to summer rain in the tropical north, with no equitable regime in the middle.

One potential weakness of the vector seasonality index in comparison to the Walsh and Lawler index is the difficulty in dealing with multi-modal rainfall regimes, that is, areas where there is more than one annual rainfall peak. The Walsh and Lawler index is based upon absolute deviations from an average rainfall, with no regard to the actual time of year in which rainfall peaks occur, and will give the same index value even if months are shuffled. The vector seasonality index, by contrast, relies on the timing of rainfall. If the rainfall occurs in two peaks, the vector direction will lie in the period between the peaks, when in reality it may be a period of low rainfall. If peaks are separated by six months, the vector magnitude will be zero despite a high degree of monthly inequality. The regression of vector strength against Walsh and Lawler index indicate a near-linear relationship with few outliers, indicating that these two indices are close to equivalent and there are no multi-modal rainfall regimes in Australia, which would have caused a disagreement between the two indices.

The τ- statistic described large rainfall events in the tropics, from monsoonal rain, and in arid areas influenced by occasional storm events, while the southern coasts, dominated by frontal systems, had a bias towards smaller rainfall events. Again, differences were visible between coastal and inland New South Wales, with large rainfall events along the coast and smaller events inland. Dividing rainfall event size bias into summer and winter half-years provided more information about the seasonal rainfall regime, and the usefulness and limitations of this index. In the summer

half-year, the extreme tropical north actually had a bias towards small events, despite the strong monsoonal rain at this time of the year. It should be remembered that this index describes the distribution of rainfall event sizes for a particular location, so although Darwin may experience very large events compared to other areas, within the scope of Darwin's rainfall regime at this time of the year extreme events may be rare. For the winter half-year, the τ- statistic describes the small events from the frontal-dominated system in coastal southern Australia, while at this time of the year northern Australia has a bias towards large events, as during the “dry” season, what rain does occur is from infrequent large storm events.

The gap-size index shows a bias towards small interpulse lengths in southern Australia, dominated by frontal systems. In contrast, the tropics and arid interior have a bias towards large interpulse lengths. While this is expected in the arid regions, due to low and infrequent precipitation, the tropical regions experience very frequent rain during a period of the year, and in this region the index may be affected by the very long interpulse periods during the dry season and may not adequately describe annual rainfall regime. The region around Innisfail and Cairns on the north Queensland coast is an exception to the trend towards large interpulse lengths in the tropics, due to local topographic effects of the Bartle Frere range. Dividing the gap index into summer and winter half years provides more information, with Mediterranean-climate regions of southern Australia having a bias towards frequent rain in the winter and larger interpulse lengths in the summer. The bias towards longer gaps in the Northern Territory is unexpected, as rainfall occurs very frequently during the monsoon season.

However, this index is calculated for six months of the year, which may also take in relatively dry periods with large gaps between the events, significantly altering the slope of the regression in this highly seasonal region.

The Markov clustering index indicates a low probability of consecutive days of rain in the arid interior, which is as expected in a region where most precipitation comes in the form of isolated single-day storm events. A higher probability of clustered events occurs along the coasts, and in southern Australia, where frontal systems can deliver rain over several days, and clustered events are more common. Interestingly, there is a discrepancy between the information provided by the gap-statistic index, and the Markov index for tropical northern Australia. The Markov index indicates a high probability of consecutive days of rainfall during the, as influenced by daily rainfall during the wet season. However, the gap-statistic index indicates a bias towards large interpulse length, due to long drought periods in the dry season. This is true even when comparing these indices for the summer half year only. The Markov index for the summer half year indicates a very high probability of consecutive days of rain, while the gap-statistic index for the same period show a bias towards large interpulse lengths. A possible explanation for this is that the regression the gap-statistic is based on is biased by including a few large drought lengths during the end of the dry season, which would be included by looking at this six-month period. The gap-statistic index may not be appropriate in highly seasonal climates**,** because the regression used to calculate the index appears to be strongly influenced by seasonal droughts rather at the expense of day-to-day interpulse lengths, and Sadras and Baldock (2003) suggest the τ- statistic

may face similar limitations. There was a strong similarity between the information provided by the Markov index and the average event length index, although the Markov index appears to provide a higher resolution in detecting differences across small spatial scales.

Analysis of change in the τ- statistic over time was intended to be conservative, as few sites showed significant trends in the index over time, and the index proved to be highly variable when calculated from only a few years' rainfall data. The clearest regional trend was for a shift towards smaller events and fewer extreme events in the southwest of Western Australia, a phenomenon observed by others (Haylock and Nicholls 2000; Hughes 2003; Li, Cai *et al.* 2005). This trend is particularly strong in the winter half-year. A number of stations across Australia show significant regressions with the τ-statistic calculated at multiple time scales, but apart from Western Australia no regional trends can be identified, with in some cases quite close rainfall stations show trends in opposite directions. There is a potential for rainfall records at a station to change over time with, for instance, the removal of vegetation, the construction of buildings, or slights shifts in the location of the rain gauge (Lavery, Kariko *et al.* 1992). However, stations used in this analysis were from a data set filtered from these influences. Possibly, the calculation of this index from relatively short periods of rainfall data into order to track changes through time results in too much noise for conclusions about changes in rainfall regime to be drawn.

7.5. Conclusion

Both the Walsh and Lawler seasonality index and the vector seasonality index provide high resolution descriptions of gradients in seasonal rainfall bias across Australia. The strong linear relationship between the Walsh and Lawler index and the vector length index indicates a lack of bimodal rainfall regimes in Australia that may distort the vector direction index's description of the seasonal timing of rainfall. Therefore, the vector index is close to equivalent to the Walsh and Lawler index for Australia, but provides more information on gradients in the timing of rainfall during the year. The τ-statistic and G-statistic also provide a useful description of small scale variation in event size bias and gap length bias. However, applying them to regions with a very strong seasonal bias in rainfall regime, such as the tropics, may be inappropriate due to a conflict between processes operating on different time scales, for instance, annual seasonal drought and interpulse periods between rainfall events during the wet season. The Markov (1,1) rainfall clustering index and the event length index provide similar information, with a greater resolution in the Markov (1,1) index, although again this index provides a conflicting description to the G-statistic index in tropical areas due to highly seasonal rainfall. Conservative analysis of change in the τ- statistic over time revealed few regional trends in rainfall event size bias over time, with geographically close stations often showing opposite trend directions. However, there was a consistent trend towards smaller rainfall events, particularly during winter, in the southwest of Western Australia.

Overall, it appears that for Australia, the vector seasonality index gives a useful description of rainfall seasonality, and the τ- statistic can also be applied across the continent to describe event size bias. Indices that measure temporal distribution of rainfall days, including the G-statistic and the Markov probability statistic, appear to be influenced by seasonal factors on a scale longer than daily rainfall, and therefore their use should be restricted to well defined seasonal subsets of annual rainfall data.

8. NATURAL RAIN POPULATION COMPARISON

8.1. Introduction

Shifts in allele frequencies and differentiation in genotypically controlled plant characters are influenced by abiotic factors acting as selective forces on plants. There is a potential for climatic influences, for instance temperature averages and extremes, frost occurrence or rainfall regime, to drive genotypic differentiation in plants (Patterson, Paull *et al.* 1978; Raper Jr. and Barber 1970; Slatyer and Morrow 1977). Rainfall, in particular, may be expected to drive selection in water limited systems, as the size, spacing and seasonal distribution of rainfall has an impact on the spatial and temporal availability of water in the soil profile, leading to the potential for a variety of strategies for root placement and water uptake.

The size of individual rainfall events can influence the depth of infiltration of water in a system, and heterogeneity in soil water availability can lead to a trade-off in the optimal placement of roots (Williams and Ehleringer 2000). Different sizes rainfall events, which can differ between regions, may therefore result in different plant root niches (Schwinning and Sala 2004). Small rainfall events may only infiltrate to surface soil layers (Kemp 1983), leading to an optimal strategy of shallow root placement, while larger events penetrate deeper (Paruelo, Sala *et al.* 2000), leading to deeper root growth. Small rainfall events in semi-arid systems are considered useful by some workers (Sala and Lauenroth 1982), triggering ecosystem responses such as nitrogen mineralization (Schwinning and Sala 2004) but there may be a threshold size of rainfall events before

shallow roots become useful for extracting the water. Water from small rainfall events tends to suffer greater loss to evaporation (Loik, D. *et al.* 2004), and harsh, high temperature conditions in surface soil layers can lead to high costs of root maintenance (Davidson 1969a). Noy-Meir (Noy-Meir 1973) suggested that, in arid systems, rainfall events of less than 5mm are not useful, and plants may instead rely on deeper water.

The spacing of rainfall events, or the interpulse length, may also have an influence on plant characters, as differences in plant morphology and physiology affect the drought tolerance of plants, and the ability to survive at low soil water potentials. Drought tolerance in plants has been found to be linked to root depth and proliferation, with deep roots correlated with drought tolerance in grasses (Garwood and Sinclair 1979), and Bell and Sultan (1999)finding that more roots in dry soil can aid soil moisture capture. Deep roots aid drought survival if they are able to access deep, stable soil water storage during the dry interpulse period. While growth and competition are the main plant processes affected by pulse events, plant survival and death become dominant during the interpulse (Goldberg and Novoplansky 2001), which highlights the potential importance of interpulse length as a selective force.

The seasonal distribution of rainfall throughout the year may also act as a selective force on plant growth and root characters, as the interaction and timing of rainfall input and evaporative demand results in different soil water availability patterns (Stephenson 1990). Summer rain, for instance, is considered less effective than winter rain (Reynolds, Kemp *et al.* 2004), meaning seasonal rainfall distribution is potentially as important as annual rainfall averages in describing total available water in a system.

There are also relationships between the seasonality of rainfall and depth distribution of water in the soil, and therefore the distribution of roots. The high availability of water during the winter in winter-biased rainfall regimes may keep the surface soil constantly moist, due to low evaporation, leading to a shallow rooting habit (Schwinning and Sala 2004). However, in winter-biased rainfall regimes plants experience very dry summers, where shallow roots can become damaged, and are expensive to maintain. Globally, deep roots have been found to be associated with winter-biased rainfall regimes (Schenk and Jackson 2002b). In a study of snakeweed root dynamics, Wan (2002) found a regime of winter watering and summer drought resulted in deeper roots than the opposite. It seems more likely that a winter rainfall regime would result in a deeper root distribution, which could make use of deeper penetrating water in the winter (Schwinning and Ehleringer 2001), but maintain extraction from deep reserves during dry summers. There may be a threshold amount of summer rain before there is an advantage to deploying shallow roots to make use of summer rainfall events, which tend to result in shallow water infiltration and only short-term water availability (Williams and Ehleringer 2000). Plants in areas where seasonal rainfall is predictable, whether summer or winter biased, may face a stronger selective force than plants in more variable environments, such as the arid regions of Australia where rain is rare, but may happen at any time of the year. Under these unpredictable conditions with random seasonality, strategies of deep root deployment that utilize groundwater, followed by rapid growth of fine roots that utilize rain after an event, may be optimal. A short life history strategy may also be a means of making use of unpredictable rainfall, with much of the biomass in these regions composed of short-lived ephemerals after large

rainfall events. Flowering phenology may also be driven by rainfall seasonality, with some perennial plants showing a more annual-like strategy, with strong winter growth and early flowering, in regions with unpredictable summer rainfall (Hodgkinson and Quinn 1976).

Austrodanthonia caespitosa is a perennial grass common across southern Australia, covering a wide range of average annual rainfall and inter-annual rainfall variability. Previous studies have found a high degree of variation in this species across its range, including variation in flowering time with latitude (Hodgkinson and Quinn 1978), variation in growth rate over the winter growing season (Quinn and Hodgkinson 1984), and variation in growth habit over small spatial scales (Scott and Whalley 1984). The high degree of variability over small spatial scales make it a useful candidate for selection and improvement (Robinson and Archer 1988), with variability likely to be the result of differentiation to local niche environments (Wilson 1996). We may expect to see a correlation between relevant plant traits and climate variables across the range of this species if climate has acted as a selective force. In this study, populations of *Austrodanthonia caespitosa* were sampled from a range extending from the northern Yorke Peninsula in South Australia, through the Mount Lofty Ranges and eastern South Australia, through Victoria to southern New South Wales at Wyalong. This covers an area of approximately equal annual rainfall totals, between 400-500mm, but the range varies in seasonality, from a strongly winter-biased rainfall regime in South Australia, to an equitable regime in central New South Wales. There is also some variation in rainfall event spacing, with populations in southern Victoria and coastal South

Australia experiencing short gaps between rainfall days during winter, with longer gaps experienced in New South Wales. Complex variation also exists across this range in the event size bias, with coastal and southern populations experiencing a bias towards small rainfall events, while northern and inland populations experience a bias towards larger rainfall events. Soil texture also varies locally between collection sites.

Grasses have been found to be sensitive to change in climate regime (Fay, Carlisle *et al.* 2002), suggesting that rainfall regime may act as a selective force in these species. *Austrodanthonia spp.* have been found to be responsive to changes in seasonal rainfall (Austin, Williams *et al.* 1981), and the high degree of variation between populations indicates they may be a useful model species to investigate differentiation in response to rainfall regime. In terms of characters that may be influenced by rainfall regime, root depth appears an ideal candidate. Rainfall regime results in different heterogenous water distributions in the soil, making the distribution of roots important in water acquisition in water-limited environments (Yanagisawa and Fujita 1999), and trade-offs between deep and shallow roots may result in variation within a species in response to rainfall regime (Williams and Ehleringer 2000).

The aim of this study is to investigate differences between a range of populations of *Austrodanthonia caespitosa* from southern Australia in morphology, growth rate, phenology, and root depth distribution. I attempt to assess the degree of variability in these characters across a gradient in rainfall regime, and between different soil types, in order to determine which environmental factors may explain population differentiation.

8.2. Methods

A growth experiment was set up to measure intraspecific variation in *Austrodanthonia caespitosa* under natural meteorological conditions, rather than a fixed watering schedule in a glass house. Polythene planting tubes (Poly Products, Regency Park) with a height of 25cm and a width of 8cm were filled with sandy loam soil (Jeffries, Wingfield) and placed on the roof of a building at the North Terrace campus of the University of Adelaide (34.91 S 138.60 E).

On June 28 2004, seeds from a range of populations of *Austrodanthonia caespitosa* (Table 5) were germinated on moist filter paper in petri dishes. A map of the locations of all populations can be found in Appendix A. On July 7 2004, germinated seeds were sown, and a week, seedlings that had died were replaced with new seedlings.

Table 5 - Populations of *Austrodanthonia caespitosa* used in the natural rain population comparison

Population	Latitude	Longitude	Locality
SA-002	33.97 S	137.75 E	Kadina
SA-006	33.55 S	138.95 E	Mokota
SA-009	33.91 S	138.58 E	Spring Gully
SA-010	36.04 S	140.31 E	Keith
SA-012	36.35 S	140.67 E	Mundulla

SA-021	35.16 S	139.01 E	Red Creek
SA-023	35.62 S	138.11 E	Cape Jervis
VIC-004	36.29 S	143.09 E	Donald
VIC-006	36.11 S	143.83 E	Boort
VIC-007	35.91 S	145.49 E	Strathmorten
NSW-001	35.57 S	144.98 E	Deniliquin
NSW-004	36.16 S	145.94 E	Jerilderie
NSW-005	33.89 S	147.12 E	West Wyalong
NSW-007	33.84 S	146.27 E	Rankin Springs

Plants were monitored several times a week. The date of flowering of each plant was recorded during late spring and early summer, and the date of death, defined as the point when no green biomass existed on the plant, was recorded over summer. When a plant was considered dead, a Theta probe was used to measure deep soil moisture, by inserting it through the polythene tube 3cm above the base of the planting tube. All plants had died by December 2004.

Dead plants had their aboveground organs trimmed at the soil surface. Material was placed in paper envelopes and dried at 100°C for two days before being weighed with

an electronic balance. Remaining soil cores were moved to a cool room at 2°C for storage until roots could be washed.

Soil cores were cut at the middle of the soil column, 12cm from the soil surface. Upper and lower soil cores were washed over a 1mm sieve, and roots were washed further to remove remaining soil and organic particles. Roots were then placed in paper envelopes and dried at 100°C for two days before being weighed with an electronic balance.

Experimental data was analysed using ANOVA to detect differences between populations. In the case of phenological data, where populations did not flower or had too few flowering plants to be suitable for analysis using ANOVA, those populations were excluded from the analysis. A non-metric multidimensional scaling (NMS) ordination was performed using PCORD version 4 software. Measured plant data, including flowering time, lifespan, and root and shoot masses in the first matrix, and environmental variables in the second matric. These environmental variables included data measured at the collection sites; latitude, longitude, soil depth to rock, soil clay percentage and soil bulk density, and interpolated data obtained from the rainfall analysis; seasonality index, τ-statistic, winter τ-statistic, G-statistic, summer and winter G-statistic.

8.3. Results

Phenology

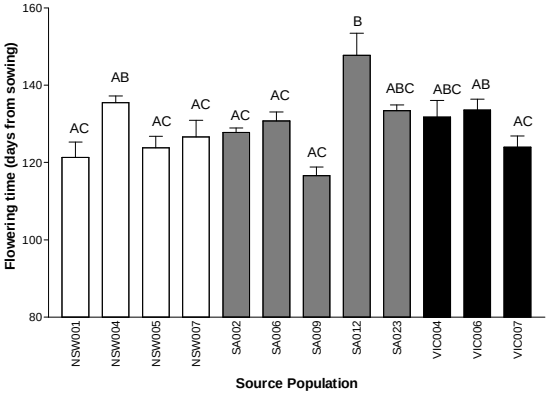

Figure 17- Number of days after planting when first flowering was observed for populations in the natural rainfall experiment*. .

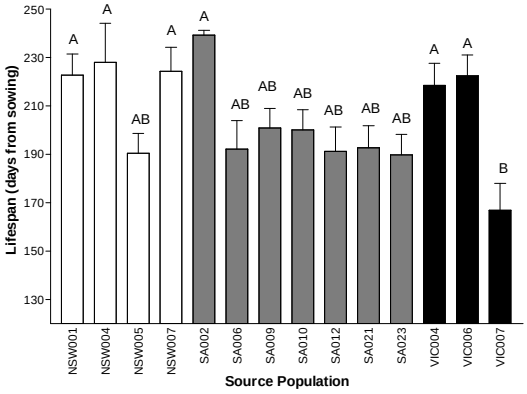

Figure 18 - Lifespan of plants from day of planting for populations in the natural rainfall experiment*.

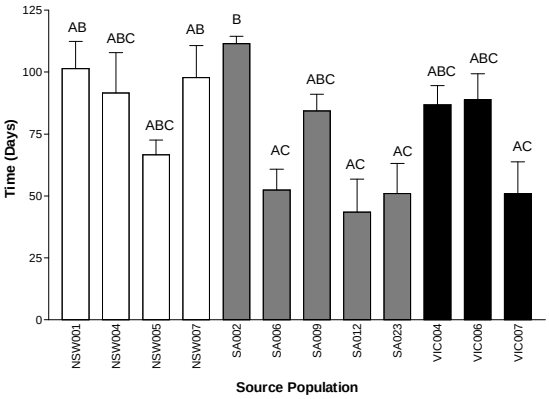

Figure 19 - Number of days between flowering and death for populations in the natural rainfall experiment*.

* Bars indicates standard errors and letter indicate significant idfferences.

In examining phenological traits, the analysis revealed significant differences in flowering time (df=11, p<0.0001, Figure 17), length of from planting to dormancy (df=13, p<0.0001, Figure 18), and the length of time between flowering and dormancy (df=11, p<0.0001, Figure 19) between populations. In some cases, there appear to be regional trends, such as a tendency for shorter life spans in South Australian ecotypes compared to New South Wales, but significant local differentiation within regions is the main source of variation.

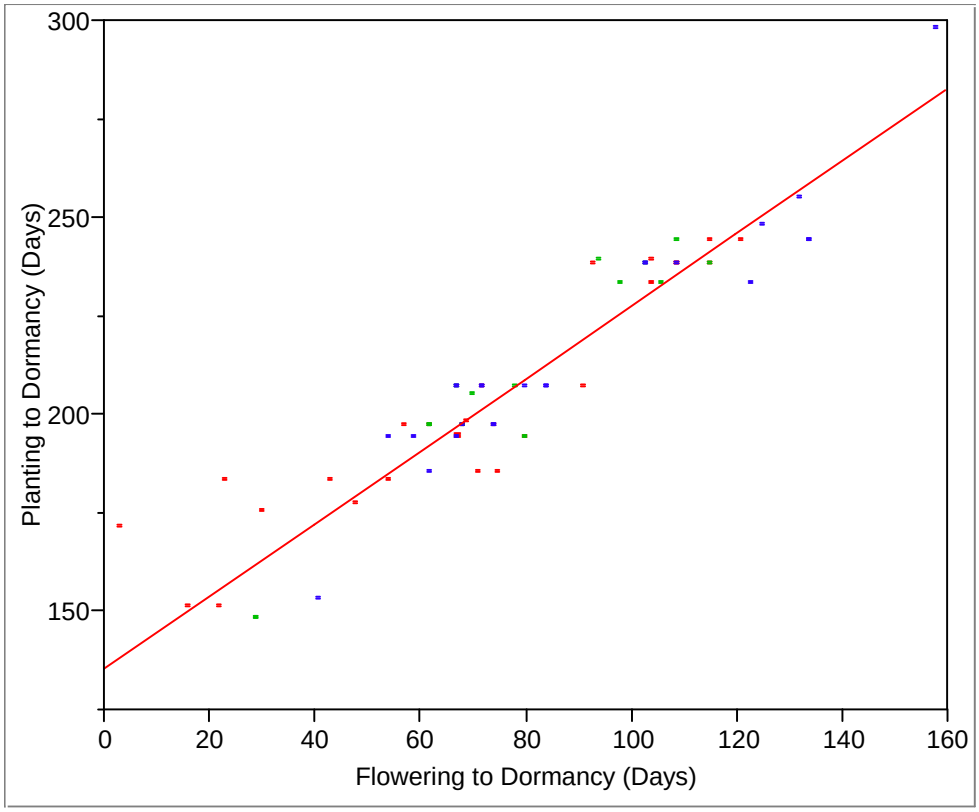

Figure 20 - Linear regression of time from flowering to dormancy versus time from planting to dormancy.

There was a strong positive correlation between the time from flowering to dormancy,

and the time from planting to dormancy (Figure 20, $r^2 = 0.89$, n = 65), indicating that the length of the survival period after flowering explained most of the variation in lifespan.

Growth

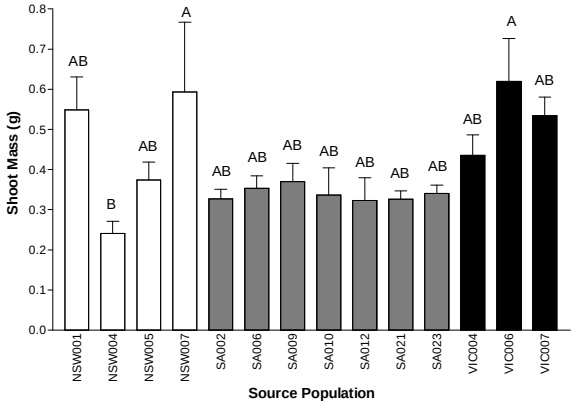

Figure 21 - Dry shoot mass for populations in the natural rainfall experiment*.

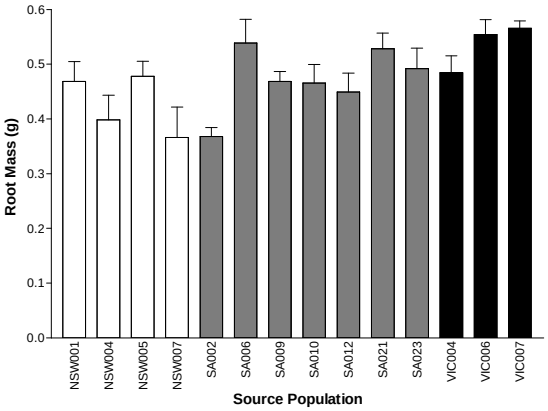

Figure 22 - Total dry root mass for populations in the natural rainfall experiment*.

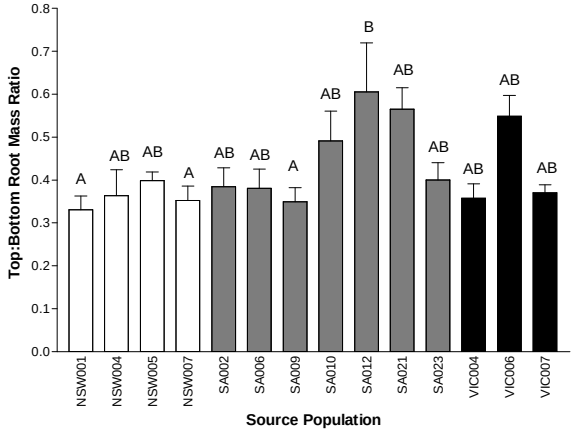

Figure 23 - Top:bottom root mass ratio for populations in the natural rainfall experiment*.

* Bars indicate standard error and letters indicate significant differences. Letters not included in Figure 22 due to the complexity of the pair-wise comparisons.

There were significant difference in dry shoot mass between populations (df=13, p=0.0032, Figure 21) with a Tukey HSD test revealing that populations NSW007 and VIC006 had particularly high shoot biomass, and population NSW004 having low shoot biomass. Total dry root mass also had significant differences between populations (df=13, p=0.0008, Figure 22). Populations NSW004, NSW007 and SA002 had particularly low root mass, while populations VIC006 and VIC007 had particularly high root mass.There were significant differences in the ratio of roots in the top and bottom soil layers (df=13, p=0.0013, Figure 23). A Tukey HSD test showed population SA012 to have a particularly high top:bottom ratio, that is, shallow roots, compared to populations NSW001, NSW007 and SA009.

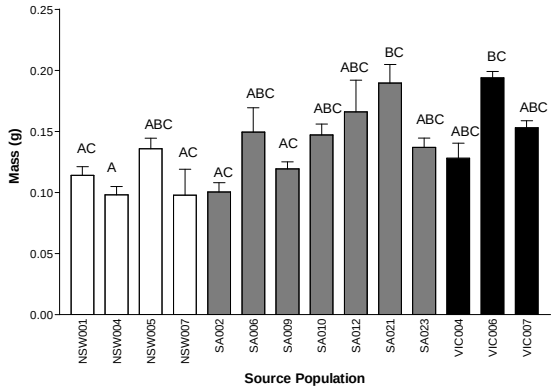

Figure 24 - Root mass in top soil layer for populations in the natural rainfall experiment*.

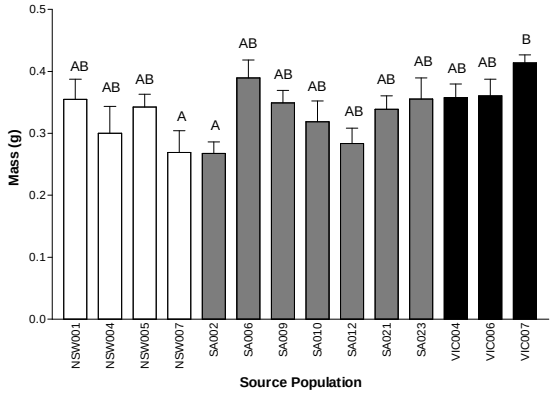

Figure 25 - Root mass in bottom soil layer for populations in the natural rainfall experiment*.

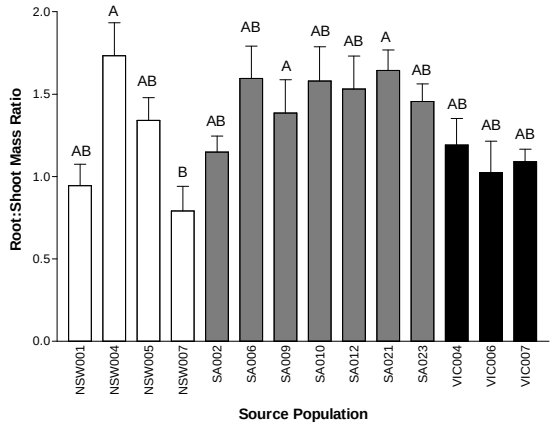

Figure 26 - Root:shoot mass ratio for populations in the natural rainfall experiment*.

* Bars indicate standard error and letters indicate significant differences.

Root mass in the top and bottom layers was also considered separately, with differences found between populations in both shallow (df=13, $p<0.0001$, Figure 24) and deep (df=13, $p=0.0135$, Figure 25) soil layers.Root:shoot mass ratio was significantly different between populations (df=13, $p=0.0009$. Figure 26) with a Tukey HSD test

showing that population NSW007 had a low root:shoot ratio, indicating a bias towards shoots, compared to populations NSW004, SA009 and SA021.

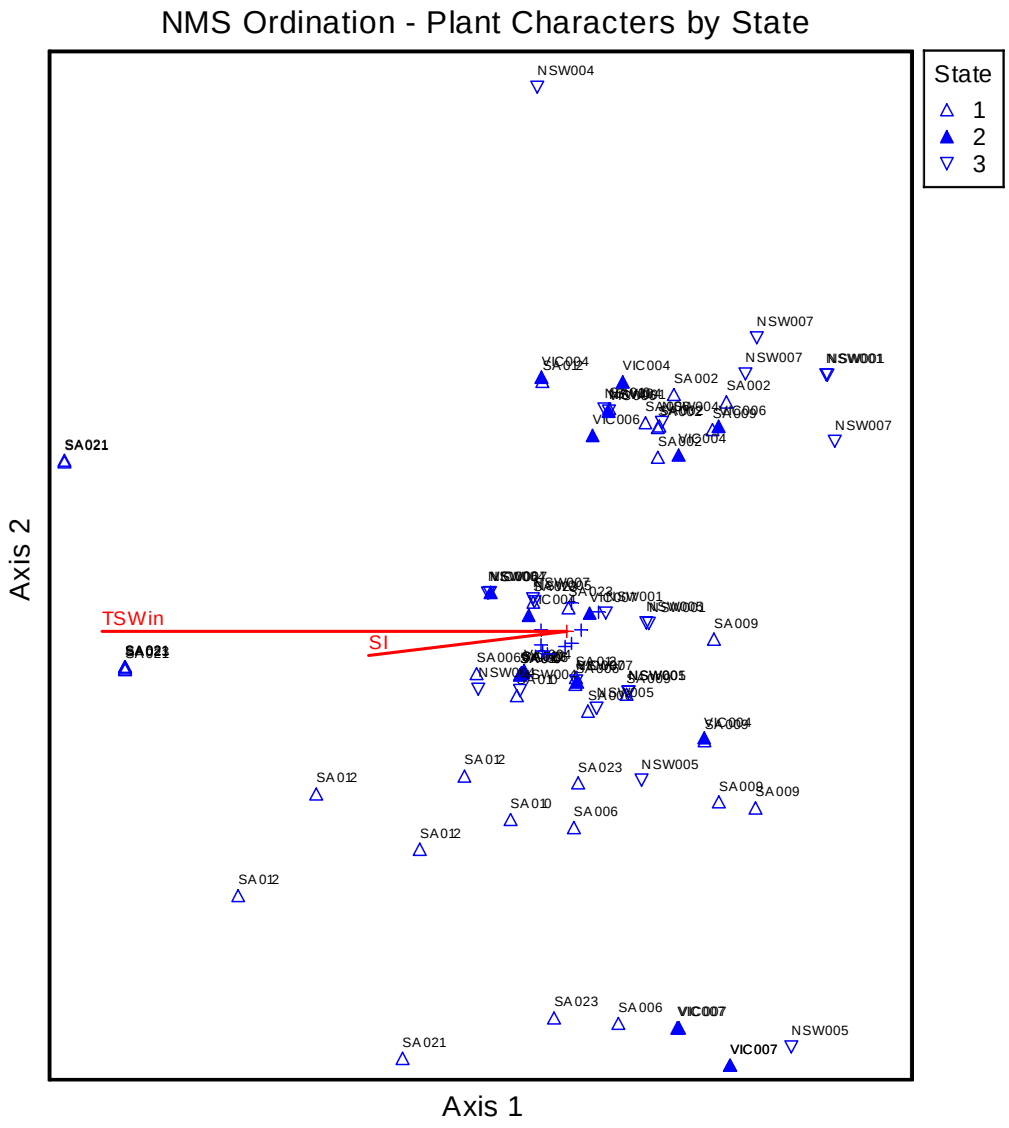

Figure 27 - NMS Ordination of measured plant characters, overload with joint plot of environmental variables. Stress = 6.64. State 1 = SA, 2 = Vic, 3 = NSW.

The NMS ordination presented in two dimension (Figure 27), had a stress value of 13.1, and shows that plants could not clearly grouped into states based on similar characters in the ordination. Time to death and time to flowering provided the greatest discrimination among samples. Seasonality index, and τ- statistic during the winter half-year were the climatic variables with the most explanatory power, although the cut-off r^2 value for the joint plot in the figure was low, at 0.1, indicating these variables explained very little of the variation.

8.4. Discussion

Significant differences were found between populations in a number of morphological and phenological characters, although some of the clearest differences were flowering time and time to dormancy. These two factors explained the majority of the variation in both axes of the NMS ordination. However, there were few significant differences between populations or regions for time to flowering, and within-population variation in this character was high, indicating some degree of plasticity. A previous study (Hodgkinson and Quinn 1978) has identified extensive variability within this species in phenology, with southern populations in Victoria and Tasmania having a set flowering time, and northern populations from NSW having flowering time controlled by water availability. Life span before senescence was also variable, with a number of significant differences found between populations. Again, however, trends were not clear, with all three states having short and long-lived populations. A higher number of New South Wales populations had long life spans, approaching 230 days from planting, while the majority of South Australian populations had life spans of around 200 days,

with the exception of population SA002 which was significantly longer. Although it may be considered unusual that South Australian populations lasted a shorter time when grown in a climate most similar to that of source compared to New South Wales populations, the longer lifespan of most New South Wales populations may indicate a genotypically controlled adaptive phenology. If New South Wales is considered as a high summer rainfall zone, plants from this region may maintain growth longer before dormancy, as compared to South Australian plants were rainfall after October is low and erratic. A study on other Australian grasses linked shorter life span to a more unpredictable climate (Cox and Conran 1996). Previous studies (Hodgkinson and Quinn 1976; Quinn and Hodgkinson 1984), in regards to *Austrodanthonia caespitosa* populations further north in New South Wales, have considered this end of the species range to experience a hot, dry summer, where short life spans, high growth rates, and early reproduction are favoured, although this may not apply in southern New South Wales where my populations were collected. In addressing the question of whether dormancy is genotypically controlled or whether it occurs when soil moisture reaches a lower threshold, one must remember that an active plant requires the presence of water in the soil. Longer living plants still had access to water later in the season, suggesting either a strategy of water conservation by these populations allowing maintenance of green tissue for longer, or equal water availability in all populations, but the early senescence of some populations while a water store still remained. Laude (1953) noted the variety of strategies grasses may employ in managing dormancy, with some species remaining active only if water was supplied, others becoming dormant even with watering. Species that did not use water availability as a cue for summer dormancy did

not respond with growth when further water was added, suggesting species that respond to summer water, as *A.caespitosa* does, may not require low soil water availability to trigger dormancy. Measurements of deep soil water were taken with a Theta probe upon dormancy, but measurements were highly variable and affected strongly by any rain that occurred on the previous day, and are not presented here. However, the strong apparent influence of individual large rain events during spring-summer growth period may explain some of the variability found in time to dormancy. The plants that showed longer life spans may have, by chance, been able to remain active long enough to receive a further rainfall event, the utilization of which enabled a significantly longer time to dormancy. This may be supported by the measurements of time between flowering and dormancy, where both inter- and intra-population variability is high.

Shoot mass also showed significant variability between populations, although this character varied very little between the South Australian populations included. As detailed above, previous studies (Hodgkinson and Quinn 1976; Hodgkinson and Quinn 1978; Quinn and Hodgkinson 1984) have suggested that high productivity and short time to flowering and dormancy may reflect an adaptation to an unpredictable environment. Although some eastern populations showed significantly greater shoot biomass than South Australian populations, this was not consistent. Indeed, some of the populations with highest shoot mass also showed high intra-population variability, which may indicate the maintenance high genetic diversity within the populations as an adaptation to an unpredictable climate, although this is difficult to confirm with the low replicate numbers. Higher shoot production in New South Wales and Victorian

populations in this experiment may also reflect the higher winter rainfall received in Adelaide compared to population sources. Non-South Australian plants may be adapted to more limiting water availability during winter. A further explanation may be maternal effects, with seeds collected from the wild being grown directly in this experiment without a generation in-between. Indeed, seed size and weight was quite variable between populations, which may have impacted particularly on early seedling growth, a phase which has been noted to often be slow in this species (Jones 1996). However, no significant correlations were found between average population seed weight and any growth variable (results not shown).

Root distribution, as described by the top:bottom root mass ratio, was also quite variable, with a few South Australian and Victorian populations having notably shallower roots. Again, intra- populations variability was high, for top:bottom root ratio and root mass partitioned into the top and bottom soil layers. Visually, there appears to be a bias towards more shallow roots in many South Australian and Victorian populations, which may be expected in these regions that experience a higher proportion of winter rain, and smaller rainfall events. High winter rainfall can keep the surface soil layers moist, with low evaporation, favouring root placement in the surface soil, and small rainfall events only penetrate to shallow soil layers (Loik, D. *et al.* 2004).

There were no strong correlations between any of the environmental variables and measured growth variables, in either the linear regressions or multivariate analysis, although seasonality index and longitude had the highest explanatory power. The

multivariate analysis also failed to discriminate between plants by state or source soil type. Overall, this suggests a lack of relationship between the genotypically determined plant characters and the environmental variables included in the analysis, indicating no selective influence of factors such as rainfall regime. However, there were clear and significant differences between populations in characters such as productivity, root depth, and biomass allocation, indicating a significant degree of diversity between, and sometimes within populations. There are two possible explanations for this variation; selection by factors not examined in this study, or random gene shifts and high genetic diversity overall. There appeared to be little evidence of gradual gradients of change in plant characters across the sampling range, suggesting any selective force acting on populations is operating at a smaller spatial scale. Indeed, populations collected in superficially similar environments only tens of kilometres apart often had quite distinct characteristics. Local environmental effects such as soil type are a strong possibility, as soil type has been found to cause genotypic shifts over very small spatial scales (Snaydon and Davies 1982), although once again no correlation was found between growth characters and the soil variables included in this study. It should be remembered that soil measurements included here were derived from a coarse national dataset rather than local samples, although this may not be important given that *A.caespitosa* is wind pollinated and populations will integrate genetic material from plants growing over a wide area. Measurements of soil depth, rockiness, pH, nutrients and other structural features may provide better explanation of genotypic variation in this species. Grazing pressure has also been found to result in genotypic shifts in *Austrodanthonia sp.* (Scott and Whalley 1984) over small spatial scales, although seeds

in my study were collected mainly from roadsides rather than grazed pastures.

Conclusion

There were clear differences in morphology and phenology between populations of *A.caespitosa*, with particularly strong differences in time to flowering and dormancy. As all plants experienced the same climatic conditions, including temperature and day length, this indicates genotypic differentiation in phenology between populations. NSW populations appeared to survive longer before dormancy, which contrasts with other studies indicating a shorter lifespan for NSW populations adapted to harsh summer conditions. These longer lived plants may possess a more conservative water use pattern. South Australian and Victorian populations appeared to trend towards shallower roots, which may be expected in regions dominated by high winter rainfall, due to a constantly moist surface soil layer. There was high intra-population variability in some characters, suggesting strong genetic diversity, which may provide populations with the ability to survive in an unpredictable climate. Overall, no strong correlations were found between plant characters and rainfall regime indices, and the strong differences between plants from closely situated collection sites suggests a more local effect, such as soil type, drives population differentiation.

9. PULSE-SIZE GLASSHOUSE EXPERIMENT

9.1. Introduction

As with the seasonality of rainfall, the size and spacing of individual rainfall events can have an important impact on ecosystems, in terms of depth of water infiltration, length of water availability, and length of water-limited periods between rainfall events. Given the important impact of these factors on resource availability to plants, small-scale rainfall regime may be expected to act as a selective force on the evolution of plant characters, or, alternatively, plants should have phenotypic plasticity in characters related to water acquisition, such as root depth or root:shoot allocation. Size and spacing of rainfall events may determine whether plants develop a strategy of using shallow or deep water, with events of a low size or frequency being below a threshold of usability (Schwinning and Ehleringer 2001).

The size of individual rainfall events affects the depth of penetration of water into the soil profile (Reynolds, Kemp *et al.* 2004). Smaller rainfall pulses may only wet the surface soil layers (Kemp 1983; Sala and Lauenroth 1982; Yanagisawa and Fujita 1999), while larger events may be expected to penetrate deeper and increase water availability for longer (Paruelo, Sala *et al.* 2000). The ultimate hydrological pathway of water in a system may be determined by the event size, with Loik et al. (2004) suggesting water from small events is disproportionably lost to evaporation and canopy interception, and water from large events is lost to run-off and deep-drainage. Soil evaporation is greater in the surface of the soil profile (Maestre, Cortina *et al.*

2003), so small events may be less useful to plants due to high evaporative loss (Sadras and Baldock 2003) while large events that penetrate deeper into the profile can escape evaporation (Paruelo, Sala *et al.* 2000). On the community-scale, Schwinning and Sala (2004) suggest that there may be a hierarchy of biological responses to different pulse sizes, with small events promoting bacterial activity and nitrogen mineralization, while larger pulses may be transpired by plants, and in some systems extreme rainfall events are required for seed germination.

The length of time between rainfall events, or interpulse length, is also important for the growth and survival of plants in water-limited systems. Evaporation and drying of the soil profile, particularly the surface soil, is an important process during the interpulse period (Loik, D. *et al.* 2004), and plants may differ in their ability to survive low soil water potential and their strategies to maximize use of water when available. Mortality is potentially a more important process than growth during the interpulse period (Sher, Goldberg *et al.* 2004), particularly in seedlings, and this stress may result in interpulse length being an important selective force in water-limited systems. Grasslands are characterised by the prevalence of shallow roots (Jackson, Canadell *et al.* 1996), and event spacing may have a strong influence on the productivity and composition of these systems, due to soil drying from the surface down. There is also the potential for an interaction between event size and gap size, for instance Sher et al. (2004) found that under high water availability conditions, peak survival of *Vulpia* and *Erodium spp.* was found with small, frequent rainfall events, but under lower total water, peak survival occurred at an intermediate interpulse length, indicating that

frequent events may not be useful if they are too small.

The variation in soil water availability with depth caused by different rainfall regimes is expected to have an influence on root distribution. Yanagisawa and Fujita (1999) suggest that the root distribution of plants is an important character when the soil water distribution is heterogeneous. Assuming that small rainfall events are biologically useful (Sala and Lauenroth 1982), a region with a bias towards small rainfall events may result in evolution of shallow root depth of plants, while large events may promote evolution of deeper roots that make use of deeper penetrating, longer lasting water, although the possibility of phenotypic response to rainfall event size must also be considered.

A number of studies have found evidence of plant use of small rainfall events, for example, forage grasses using water from the surface soil under small, frequent rainfall events, (Bennett and Doss 1960), a leaf water potential response in *Boutuloua gracilis* following watering events of 5mm (Sala and Lauenroth 1982), arid C_3 plants using water from frontal systems where the rainfall only wet the surface soil(Kemp 1983), and shallow rooted grasses making use of small rainfall events on the Colorado Plateau (Schwinning, Davis *et al.* 2002).On the other hand, small rainfall events may not be biologically useful in a system due to low penetration and high evaporative loss from the surface soil. Noy-Meir (1973) considered events smaller than 5mm not to be useful in arid and semi-arid systems. The water from such a rainfall event would wet just the top 5 cm of soil, and would evaporate rather quickly. Furthermore, placement of roots

in the surface soil in order to access water from small rainfall events may not be useful due to the high cost of shallow roots. Roots in the surface soil are subject to hot, dry conditions, resulting in high root respiration rates (Atkin, Bruhn *et al.* 2005) and a greater root loss through desiccation (Davidson 1969a), particularly in hotter, water-limited environments. Forbes et al. (1997) found decreases in root longevity at higher temperatures in *Lolium perenne*. Constant loss of shallow roots entails rapid growth of replacement roots when precipitation occurs (Chesson, Gebauer *et al.* 2004), and given the short length of time water remains available in surface soil, this may not be an optimal strategy. Therefore, below a threshold of event size or frequency, a better strategy of root placement may be a bias towards deeper roots to utilize groundwater or deeper water from occasional large events. Although nutrient dynamics is not considered here, it is important to consider the advantage of shallow roots in the acquisition of mineral nutrients, particularly phosphorous (Ge, Rubio *et al.* 2000).

While root distribution may result from phenotypic plasticity, for example, the proliferation of roots in response to high soil moisture availability (Pregitzer, Hendrick *et al.* 1993), rainfall regime may also act as a selective force, resulting in genotypically determined differences between populations in root distribution, although in reality plants tend to lie between the extremes of purely fixed and purely plastic phenotypes (Aphalo and Ballaré 1995). Previous studies with varieties of wheat (Oyanagi, Sato *et al.* 1991a) have found genotypic differentiation in response to soil moisture regime, with root growth angle appearing to be under simple genetic control (Oyanagi, Sato *et al.* 1991b). Genotypic selection is more likely where the variation is spatial but locally

fixed (Snaydon and Davies 1972), while plastic responses to environmental variation are expected where there is pulsing and temporal variation in the environment (Moran 1992; Sultan 2003) although plasticity requires accurate prediction and response to the environment. For example, Hutchings and de Kroon (1994) suggest that low plasticity in root growth may be expected in a strongly pulsed environment, as maintaining a constant root distribution may be more effective than quickly growing new roots in response to a short-lasting pulse. However, if root proliferation and water extraction are able to keep up with changing patterns of water availability in the soil profile, then prediction and response to rainfall regime may be beneficial. Plasticity in response to rainfall regime might therefore be expected in a grass in a water-limited environment, although there may be differences in plasticity between populations (Schlichting 1986) across a gradient in rainfall regime and predictability, with ecotypes experiencing less heterogeneity in resources showing less plasticity in root distribution (Fitter 1991).

Austrodanthonia caespitosa, or white-top wallaby grass, is a perennial C_3 grass found throughout southern Australia. It grows across regions experiencing a wide range of annual rainfall totals and intra-annual rainfall regimes, including differences in rainfall event and interpulse sizes. This species displays a high degree of genotypic variation between populations (Quinn and Hodgkinson 1983), which is thought to reflect selection and differentiation to local environmental niches (Wilson 1996). The high genotypic and phenotypic diversity in this species across its range indicates the potential for local adaptation to environment. Grasses are considered highly sensitive to changes in climate and rainfall regime, including factors such as pulse and interpulse

size (Fay, Carlisle *et al.* 2002), and rainfall regime has the potential to act as a selective force in shaping characters in this species, such as root depth and root:shoot ratio. As a result of adaptation to local climate, specific ecotypes may be found to have a particularly deep rooting habit, even under different rainfall regimes. Presence of deep roots in grasses has been found to correlate well with drought tolerance and the ability to extract all the available water from the soil (Garwood and Sinclair 1979), reducing deep drainage, and deep-rooted ecotypes may be particularly efficient at making use of the large rainfall events that contribute to deep drainage (Seyfried, Schwinning *et al.* 2005).

The aim of this experiment was to investigate variation between *A. caespitosa* individuals collected across a climate gradient from South Australia to New South Wales, where annual rainfall totals are approximately equal, but rainfall event size distribution changes. A range of watering regimes, simulating different pulse and interpulse sizes, were applied to plants in a glasshouse experiment in order to determine whether (a) plants show a plastic response in root depth to water availability, (b) plants show a fixed root depth response that reflects the rainfall regime in the source environment, or (c) plants show variation in plastic response to watering, revealed by an interaction between watering treatment and source population.

9.2. Methods

An experiment was set up to compare the growth and biomass allocation of populations of *Austrodanthonia caespitosa* under different water pulse regimes. The three watering

treatments applied were:

Small Pulse – 5mm (1 min) twice a week.

Medium Pulse – 10mm (2 mins) once a week.

Large Pulse – 20mm (4 mins) once a fortnight.

An electronically controlled watering system was set up in a glasshouse, under part shade with temperature maintained at 23.9°C. The watering system timer was an Orbit WaterMaster model 57114. Three sprinkler systems were set up on three tables. Each system consisted of seven "mister" microsprinklers set on risers at a height of 40cm above the surface of the table, that is, 15cm above the surface of the planting tubes. Each system was controlled by solenoid valves. Spray rate was calculated at an average of 5mm of rainfall per minute of operation by placing beakers at a number of locations under the sprinklers to catch the water. Flow rate was not adjusted during the course of the experiment.

A number of 25cm high x 8cm wide polythene planting tubes (Poly Products, Regency Park) were filled with sandy loam soil (Jeffries, Wingfield) to 1cm below the surface. After filling, the height of the soil column was 24cm. Tubes were arranged underneath the watering system, with no space between tubes.

Seeds from ten populations of *Austrodanthonia caespitosa* (Table 6) were germinated on damp filter paper in Petri dishes. Populations were selected for this experiment if they had a large number of seeds available, and covered the sampling range well.

Table 6 - Populations of *Austrodanthonia caespitosa* used in pulse-size experiment

Population	Latitude	Longitude	Locality
NSW004	35.16 S	145.94 E	Jerilderie
NSW005	33.89 S	147.12 E	West Wyalong
SA002	33.97 S	137.75 E	Kadina
SA010	36.04 S	140.31 E	Keith
SA020	35.35 S	139.12 E	Langhorne Creek
SA022	35.51 S	138.70 E	Middleton
SA023	35.62 S	138.11 E	Cape Jervis
VIC003	36.36 S	142.84 E	Litchfield
VIC006	36.11 S	143.83 E	Boort
VIC007	35.91 S	145.49 E	Strathmorten

Seedlings were planted on April 24, 2004. Ten seedlings were planted per population per watering treatment, giving a total of 300 planting tubes. For the first three days 5mm of water was applied to all treatments, after which experimental watering treatments begun. After one week, a number of seedlings had died (29 in treatment A, 26 in treatment B, 12 in treatment C) and these were replaced with fresh seedlings. No

further replacements of dead plants were made after this time.

On June 30, it was discovered that the watering system had failed, and had saturated all treatments with water overnight. Planting tubes were immediately moved to a freezer at -20°C to prevent further growth and to preserve root distribution at the time of experimental failure.

Plants were harvested according to the following protocol:

> Frozen planting tubes were removed from the freezer, and shoots were trimmed at the soil surface, placed in paper envelopes, and dried in an oven at 100°C before being weighed on an electronic balance.
>
> The remaining frozen soil core was sliced with a rotary masonry saw at a depth of 12cm below the surface of the soil. Because a number of tubes had lost soil from the bottom during saturation, it was decided to slice at a point 12cm from the top, assuming that soil would have been lost from the bottom, and any roots in the bottom half of the soil would be compressed into that space.
>
> Frozen soil slices were stored in plastic freezer bags and returned to the freezer until ready for root washing.
>
> Frozen slices were washed over a 2mm sieve with warm water. Roots were collected and placed in sealed Petri dishes.
>
> Roots were washed further in RO water, dipped into a solution of methyl violet stain (0.5g L^{-1}), then washed again, before being spread out in petri dishes filled

with water.

The water in the petri dishes was left to evaporate, leaving dried, spread-out, stained roots in the dish.

Roots were then sealed in transparent plastic slides constructed from overhead transparency film. Slides were scanned at 300dpi and the images stored for later analysis.

Dried roots were then removed from the slides and weighed on an electronic balance.

Root measurements, including length, surface area, volume and fractal dimension were determined by processing the scanned root images in Regent Instruments WinRhizo software. The fractal dimension of the root system is a measurement obtained by analysis of images of root system branching, giving an indication of the complexity of the root branching pattern (Fitter and Strickland 1992; Walk, van Erp *et al.* 2004).

Two-way ANOVAS were performed to determine significant differences for a variety of plant measurements, with population source, watering treatment and the interaction of these terms as effects. Tukey HSD tests were used to determine the direction of differences between treatments.

9.3. Results

There were significant differences in dry shoot mass between treatments (df=29, p=0.0085). Dry shoot mass differed between populations (df=9, p<0.0001,

Figure 28), but the watering treatment (df=2, p=0.75) and interaction (df=18, p=0.79) were not significantly different.

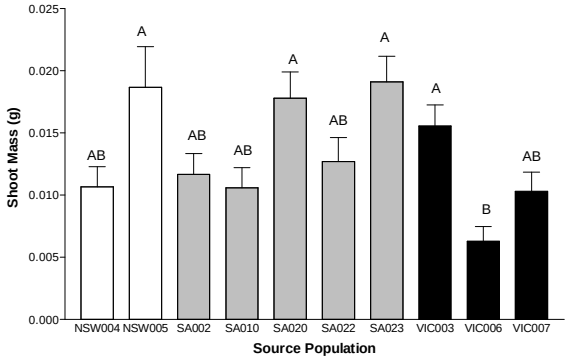

Figure 28 - Differences in dry shoot mass between populations for pulse-size experiment*.

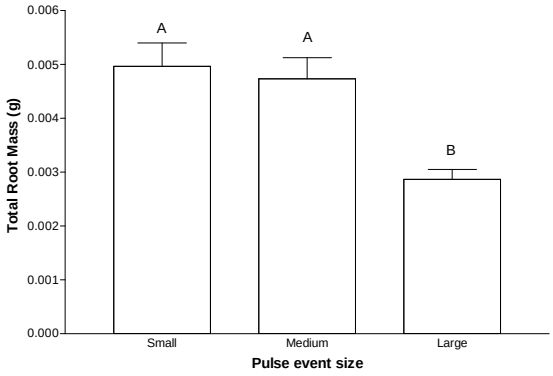

Figure 29 - Difference in total dry root mass between watering treatments for the pulse-size experiment*.

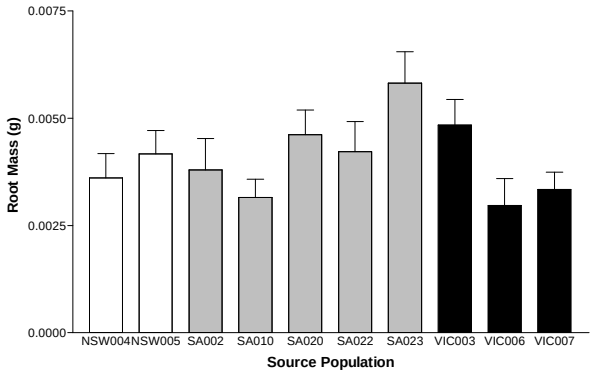

Figure 30 - Difference in total dry root mass between populations for the pulse-size experiment*.

* Error bars indicate standard error, and letters indicate significant differences as determined by Tukey HSD test. Letters have been ommitted in Figure 30 due to the complexity of the pair-wise comparisons.

There were significant differences in total dry root mass between treatments (df=29, p=0.004). Both watering treatment (df=2, p<0.0001, Figure 29) and source population (df=9, p=0.038, Figure 30) had significant effects on root mass. The large/infrequent treatment resulted in lower total root mass than the more frequent treatments, but no differences were found between populations in the Tukey HSD test, despite the significant ANOVA result. The interaction term for total root mass was not significant (df=18, p=0.617).

There were significant differences in total dry biomass between treatments (df=29, p=0.0061). The effect of watering treatment was not significant (df=2, p=0.1459), but there were significant difference in total biomass between populations (df=9, p=0.0003, Figure 31). The interaction term was not significant (df=18, p=0.5184).

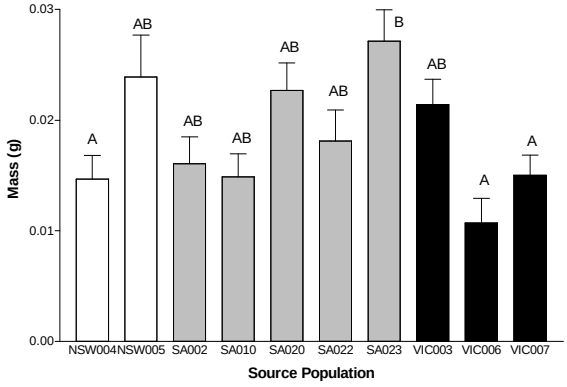

Figure 31 - Difference in total dry biomass between populations for the pulse-size experiment*.

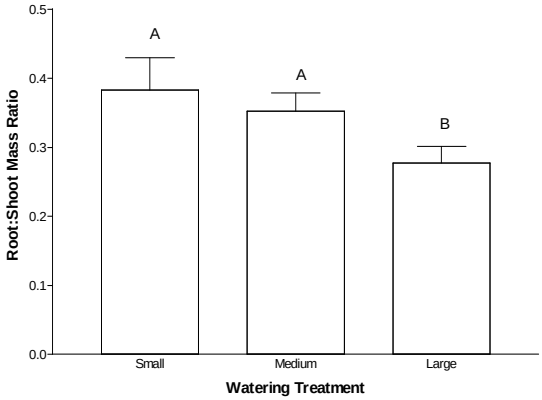

Figure 32 - Difference in root:shoot ratio between watering treatments for the pulse-size experiment*.

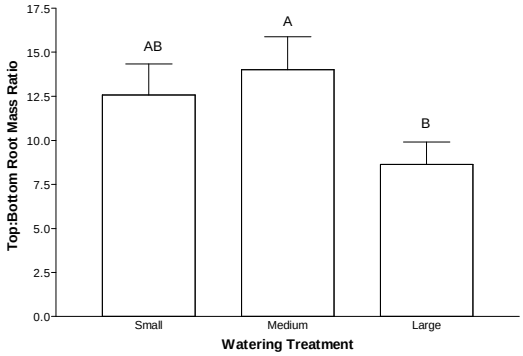

Figure 33 - Difference in top:bottom root mass ratio between watering treatments in the pulse-size experiment*.

* Error bars indicate standard error, and letters indicate significant differences as determined by Tukey HSD test.

For the whole model ANOVA, root:shoot ratio was not significant (df=29, p=0.1755). However, the effect of watering treatment alone was significant (df=2, p=0.0268, Figure 32), with a lower root:shoot ratio in the large/infrequent watering treatment, that is, there was a bias towards more shoot biomass and less root biomass.

For the whole model ANOVA, root mass ratio in the top versus bottom soil layers was not significant (df=29, p=0.498). However, there was an effect of watering treatment (df=2, p=0.0453, Figure 33) with the large/infrequent treatment having a lower top:bottom ratio than the medium treatment, with lower ratio indicating a bias towards deeper roots. The small watering treatment treatment was intermediate.

Due to the very low root masses, there were concerns about the accuracies of the weights measured, and the top:bottom root ratio was repeated using root lengths. Whole model ANOVA for top:bottom root length ratio was not significant (df=29, p=0.081). However, there was a significant difference between watering treatments (df=2, p=0.0023, Figure 34) with the large/infrequent treatment resulting in a lower top:bottom ratio than the other treatments, indicating a bias towards deeper roots.

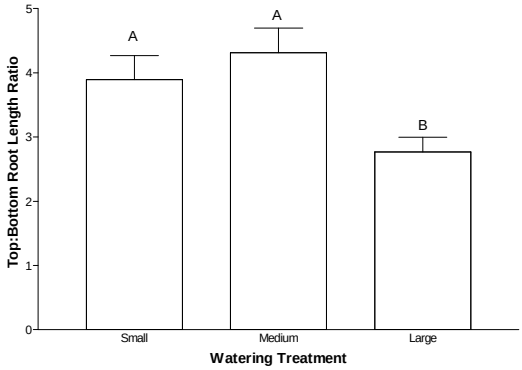

Figure 34 - Difference in top:bottom root length ratio between watering treatments in the pulse-size experiment*.

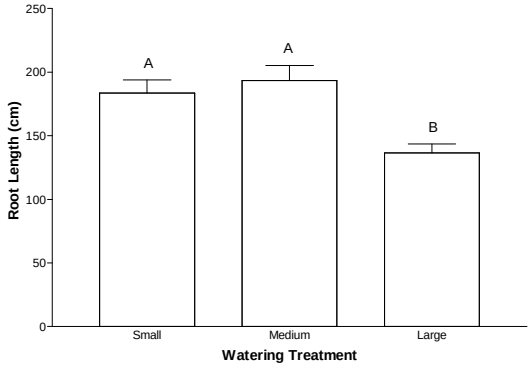

Figure 35 - Difference in total root length between watering treatments in the pulse-size experiment*.

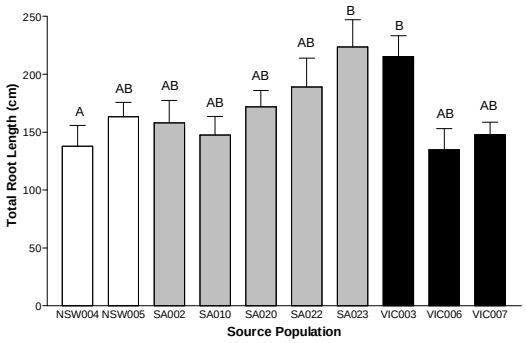

Figure 36 - Difference in total root length between populations in the pulse-size experiment*.

* Error bars indicate standard error, and letters indicate significant differences as determined by Tukey HSD test.

There were significant differences in total root length between treatments (df=29, p=0.0026). Watering treatment had a significant effect (df=2, p<0.0001, Figure 35) with the large/infrequent treatment resulting in lower total root length compared to the more frequent watering treatments. Population source also had a significant effect (df=9, p=0.0032,

Figure 36) but the interaction term was not significant (df=18, p=0.889).

Scanning of roots enabled calculation of the fractal dimension of roots, indicating the degree of branching and complexity in root structure. There were significant differences in fractal dimension of roots in the top layer between treatments (df=29, p=0.0002). Watering treatment had a significant effect (df=2, p=0.0142, Figure 37) with roots in the top layer in the large/infrequent watering treatment having a lower fractal dimension than the more frequent watering treatments. Population source also had a significant effect (df=8, p<0.0001, Figure 38) but the interaction term was not significant (df=18, p=0.4944).

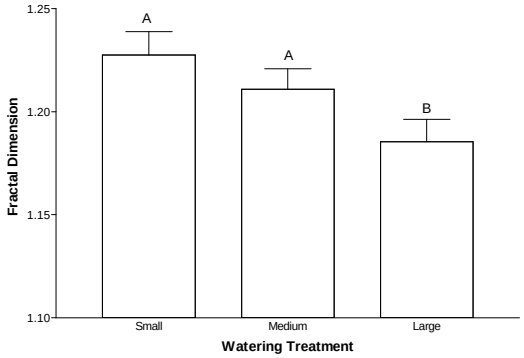

Figure 37 - Difference in fractal dimension between watering treatments for roots in the top soil layer in the pulse-size experiment*.

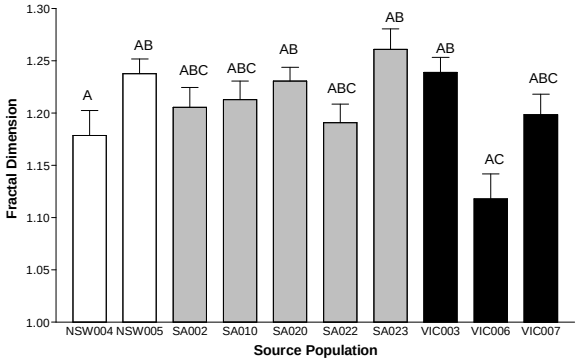

Figure 38 - Difference in fractal dimension between populations for roots in the top soil layer in the pulse-size experiment*.

* Error bars indicate standard error, and letters indicate significant differences as determined by Tukey HSD test.

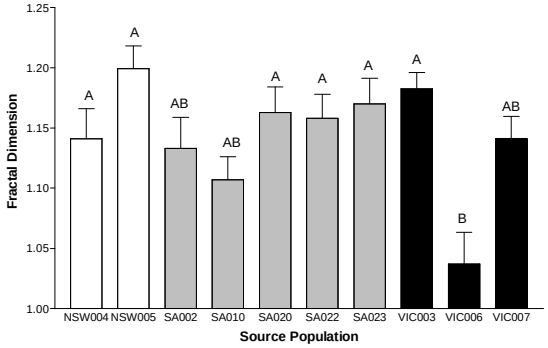

Figure 39 - Difference in fractal dimension between populations for roots in the bottom soil layer in the pulse-size experiment*.

There were also significant differences in the fractal dimension of roots in the bottom soil layer between treatments (df=29, p=0.0127). As with roots in the top soil layer, there were significant differences in fractal dimension between populations for deep roots (df=8, p=0.0003, Figure 39), but watering treatment did not have a significant effect for deep roots (df=2, p=0.969). The interaction term was not significant (df=18,

p=0.576). For both shallow and deep roots, population VIC006 appears to have a particularly low fractal dimension.

Fractal dimension was regressed against root length in the top (Figure 40) and bottom (Figure 41) soil layers. There was a positive linear relationship between fractal dimension and root length in the top (r^2=0.49, n=272) and bottom (r^2=0.21, n=272) layers, although the relationship appears stronger in the top layer, and the relationship was not strictly linear, with a wider range of root lengths with higher fractal dimensions.

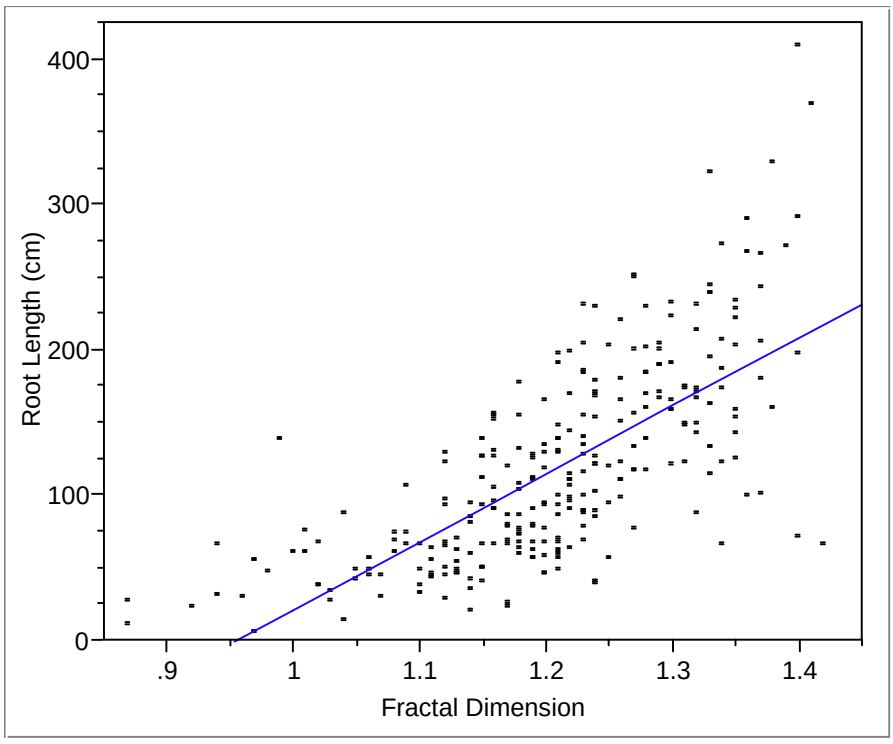

Figure 40 - Root length versus fractal dimension in the top soil layer.

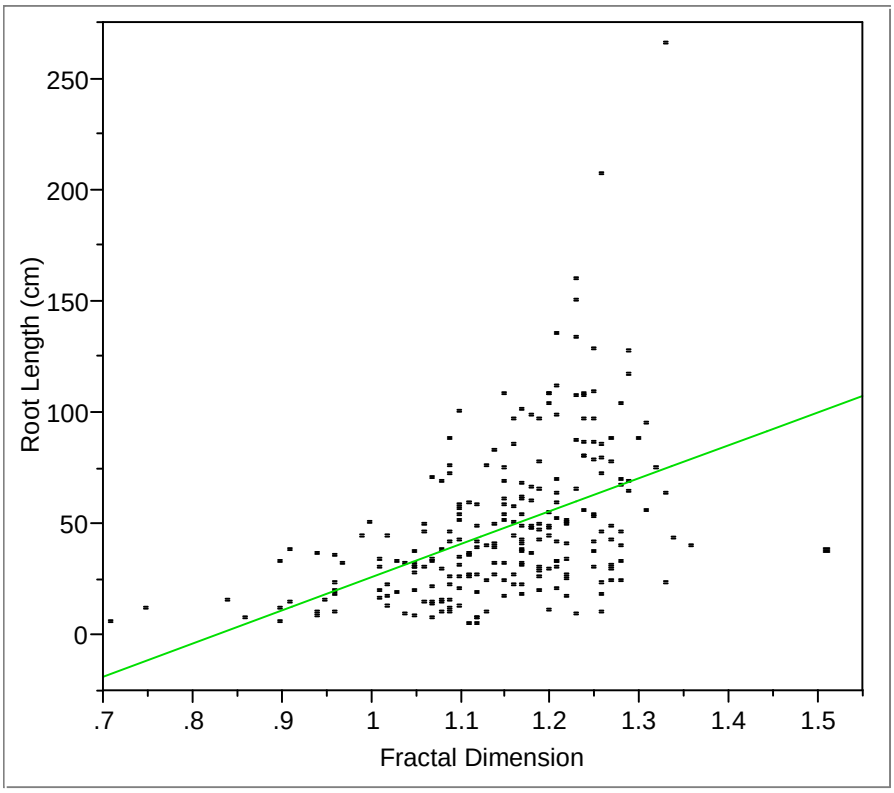

Figure 41 - Root length versus fractal dimension in the bottom soil layer.

9.4. Discussion

Variations in a variety of root measurements in this experiment were found between watering pulse treatments rather than between populations. This indicates that the species displays a high degree of plasticity in root growth in response to soil water availability, with no significant interaction indicating differences in plasticity between populations. Consistent variations in root measurements between populations, which would have indicated genotypic differentiation and possibly different selection histories

for root growth, were not found.

The ratio between roots in the top soil layer and the bottom soil layer, measured through both root mass and root length, showed a relationship with watering regime that indicated that plants utilized small events in the surface layer by deploying roots preferentially in this layer. Large, infrequent pulses were associated with a bias towards deeper roots, indicating a plastic response of root deployment to deeper soil wet by larger watering events. Such plastic responses in root deployment under different water availabilities may be common, although many studies have, in the past, focused on identifying genotypic differences (Heathcote, Davies *et al.* 1987) Small rainfall events are considered only able to wet the surface soil layers (Loik, D. *et al.* 2004), while larger events penetrate deeper. Previous workers have debated the usefulness of small rainfall events of less than 5mm in water limited ecosystems (Noy-Meir 1973; Sala and Lauenroth 1982), but the fact that shallower roots were found in the smallest, most frequent watering treatment indicates that pulse events of this size in this study were biologically useful enough for shallow root deployment to be beneficial.. The most useful way to view the effect of rainfall event size in water limited systems is as a hierarchy of responses to event size (Schwinning and Sala 2004), with very small events triggering microbial activity, small pulses contributing to transpiration, and large events triggering seed germination. Had the small, frequent events proved too small to be biologically useful to the plant, an alternate strategy may have been to deploy deep roots and make use of more stable deep water supplies, water present in the soil at the beginning of the experiment. The optimal strategy is

controlled a threshold of either event size or frequency, which determines whether to rely on deep water storage, or whether to invest in shallow roots and take advantage of small pulses(Schwinning and Ehleringer 2001). It should be noted, however, that the young age of the plants during the experiment might have made surface water use unavoidable. Seedlings, particularly those of grasses without a tap root, must rely on moist surface soil conditions as deeper roots grow, therefore responsiveness to small watering events during this life stage is to be expected. The significant difference in top:bottom root ratio with watering treatment over the short experiment period, however, indicates the presence of plasticity even in the early development of the root system.

Total root length and root mass were found to be significantly different between watering treatments, with less total root length found in the treatment with large, infrequent watering events, and more root length in the intermediate and small-pulse treatments. Previous studies have reported root proliferation in response to a plentiful resource, both nutrients (Larigauderie and Richards 1994) and water (Loomis and Ewan 1936; Pregitzer, Hendrick *et al.* 1993), and alternatively increases in root length in regions where a resource is limiting, as a means of increasing availability and acquisition of the limiting resource (Sultan 2003). It appears that changes in growth and proliferation within an organ group, such as roots, may result in proliferation in response to high resource levels, but changes in allometry between organ groups result in increased biomass allocation to the organ acquiring the most limiting resource. If root proliferation reflects effort directed into acquiring a limiting resource, the results

indicate that water from the large, infrequent events are less limiting to the plants than the small, frequent watering treatment. The pathway of large events in a system differs from that of small events; a larger proportion of water from large events is lost to run-off and deep drainage, but a smaller proportion is lost to evaporation (Loik, D. *et al.* 2004). In the confines of planting tubes, where run-off is not possible and watering events were still too small to result in loss to drainage, large, infrequent watering events may provide more usable water than small events due to lower evaporative loss, provided the plants can survive the prolonged drought period between waterings, which *A. caespitosa* appears to be able to do (Bolger, Rivelli *et al.* 2005). It is important to consider that while root length is a better indicator of resource uptake than root mass (Sun, Coffin *et al.* 1997), measurements of root activity and actual patterns of water uptake are the best measures of plant response to soil resource availability (Ehleringer and Dawson 1992) although they are difficult to obtain for grasses.

An alternative explanation to differences in root mass or root length may be differences in plant size resulting from higher growth rates. Indeed, differences found in root mass between populations reflect the differences in total biomass between populations. However, no differences in total biomass were found between watering treatments while differences in root mass and root length between watering treatments were significant, indicating differences in the amount of roots may reflect something other than absolute plant sizes. Examination of the root:shoot ratio provides more information on the relative allocation of assimilates to above- or below-ground organs, with biomass expected to be greater in the region where resources are most limiting

(Sultan 2003). A number of studies have found high root:shoot ratio is related to low water availability, at both the global (Schenk and Jackson 2002b) and local scale. For instance Rodrigues et al. (1995) found an increase in root:shoot ratio in lupins under drought conditions, and Bell and Sultan. (1999) found that while less root mass in total was found in *Polygonum spp*. exposed to dry conditions, a higher proportion of plant mass was in roots in the dry treatment. Fischer and Turner (1978) also noted that root:shoot ratio increases with low soil water content. Limitations in nutrients can also result in a shift in biomass allocation towards the roots (Reynolds and D'Antonio 1996), and Wilson (1988) proposed that water would have a similar affect to mineral nutrients in determining root:shoot ratio. Root:shoot ratio is lower in the large, infrequent watering treatment, indicting less root mass relative to shoot biomass. This again indicates that water is less limiting to plants receiving the large, infrequent pulses, with more biomass being allocated to above-ground organs for light acquisition rather than below-ground for water acquisition. This is in contrast to the findings of Fay et al.(2003) in a study in a C_4 grassland, where larger, less frequent pulses resulted in a higher root:shoot ratio, indicating large pulse events are more limiting in that system. In using root:shoot ratio as an indicator of plant response to resource limitation, it is important to recognise that allometry can also change with absolute plant size, and throughout the life of the plant (Fernández and Reynolds 2000). In this experiment, however, no significant differences were found in total plant mass between watering treatments, only biomass allocation, and all plants were the same age upon harvesting.

Simple root systems will have a fractal dimension close to one, while complex root

systems with branching at a variety of scales will have fractal dimensions approaching two. Branching pattern may give an indication of root proliferation in response to locally high resource availability. Berntson and Woodward (1992) found longer inter-branch lengths in *Senecio vulgaris* under dry conditions, and denser branching under moist conditions. However, a study of the fractal dimension of the root systems of a range of sorghum varieties found higher fractal dimension in more drought tolerant varieties (Masi and Maranville 1998), suggesting that complex branching patterns may be a way of increasing water acquisition from dry soil. The drought tolerant sorghum varieties in this study were also found to have a deeper rooting habit, which may have also contributed to their drought tolerance. In the top soil layer in this experiment, the large, infrequent watering treatment resulted in a significantly lower fractal dimension than the intermediate and small, frequent watering treatments. A lower fractal dimension indicates a less complex root architecture that might be expected when soil has a lower water availability, and roots explore with long interbranch lengths in order to extend into zones of higher water availability. The higher fractal dimensions found in the top soil layer of the treatments receiving smaller watering pulses may indicate root proliferation in response to the higher water availability in the shallow soil in these treatments. No significant difference in fractal dimension of the root system between watering treatments was found in the deeper soil layer, possibly indicating a more stable water environment with fewer differences in water dynamics between treatments than in the surface soil. Differences in total root lengths in the top and bottom soil layers appear to reflect differences in fractal dimension, with lower root length in the top soil layer of the large, infrequent treatment, but no differences in root length in the

bottom soil layer. If we assume that root proliferation is associated with capturing resources in zones of high resource concentration, this again indicates differences between treatments in the surface layer, with higher water availability at the surface with smaller, more frequent pulse events.

It appears that small watering events resulted in plants responding plastically by producing more roots in the surface soil. Large events penetrated deeper into the soil profile, leaving the surface soil to dry out during the inter-pulse period resulting in low root deployment in the surface layer. However, the lower root:shoot ratio found in the large, infrequent watering treatment indicates large events result in greater water availability, possibly through deep water being less affected by evaporation. Chesson et al.(2004) suggest that in natural systems, while recharge of deep layers is infrequent, a higher average water content is maintained, indicating large events and deep water may be less limiting water sources than small, shallow pulses.

While no significant differences were found between populations in top:bottom root ratio, or root:shoot ratio, variables measured that reflected overall productivity did show significant variation between populations. Total biomass was highly variable, although no trend across the range from South Australia to New South Wales was evident. Root mass and root length were similarly variable, but with no trend reflecting the selective influence of climatic gradients. Previous studies have related the timing of flowering in different populations of *A. caespitosa* to productivity (Hodgkinson and Quinn 1976; Quinn and Hodgkinson 1984), concluding that plants at the northern limit of the range of the species, in northern New South Wales, showed a higher growth rate

in order to flower earlier before the onset of hot summer conditions. In my treatment of rainfall seasonality across the gradient from South Australia to New South Wales, I regard New South Wales as experiencing higher summer rainfall than the western end of the gradient. . Despite the higher proportion of summer rain in this area, the rain may not be very effective due to the higher average summer temperatures and evaporation rates. Total biomass measurements do not, however, indicate higher growth in the New South Wales populations, and most variation between populations in growth variables indicates a few "stand out" populations, with genotypically determined productivity produced by an influence occurring at a finer scale than the climatic gradient examined here. Once again, the young age of the plants at harvest lends caution to interpreting final biomass as representing productivity or growth rate. Rather, it may reflect seedling growth rate, which in this species has been found to be low (Jones 1996), with higher and more representative growth differences likely to be found in older plants, although clearly differences do exist in growth rates and root distribution even in young plants, which can enable vulnerable seedlings to survive sub-optimal establishment conditions.

9.5. Conclusion

Austrodanthonia caespitosa plants displayed plastic root growth response to watering regime, with no clear evidence of genotypic differentiation, or differences in plasticity between populations. There was a trend towards shallower root deployment under small/frequent and intermediate watering regimes, and a trend towards deeper roots under large/infrequent watering events. Root mass and root:shoot ratio differences

between watering treatments indicated large/infrequent events were less limiting in this experiment, possibly due to lower evaporative loss compared to small/frequent events. The infrequent watering regime did not result in plant mortality, indicating the ability of this species to survive long interpulse periods. Root system proliferation and complexity, as measured by the root fractal dimension, also indicated the less limiting nature of large/infrequent events, and the differences in root response in the surface soil between watering treatments. While no evidence was found of particular populations having a genotypically fixed root depth distribution, the plasticity of the species in response to water pulse regime indicates a mechanism for drought tolerance, with the ability to develop a root system in response to immediate water availability.

10. SEASONAL WATERING COMPARISON

10.1. Introduction

Historically, ecological analysis of the effects of precipitation in ecosystems has focused on relating annual rainfall to primary productivity (Le Houérou, Bingham *et al.* 1988). These studies suggest that the relationship between annual rainfall and annual productivity is particularly strong in grasslands. Knapp and Smith (2001) found a strong relationship in North American grasslands, and suggested that deserts don't have enough plant density to make use of extraordinary rainfall events, while forests tend not to be water limited.

In addition to the importance of inter-annual variation, intra-annual rainfall variation, such as the seasonal timing of rainfall, can have important influences on plant growth and community structure. (Stephenson 1990) emphasised the importance of understanding the water balance of ecosystems. Energy supply and water supply are coupled, with energy required to extract water, and water required to use energy for growth. The seasonal timing of rainfall, as related to times of high evaporative demand, needs to be taken into consideration. Rainfall is considered less effective in summer due to high evaporation rates {Reynolds, 2004). Winter rain, while more effective in temperate climates where green biomass is maintained, tends to have a higher proportion of water lost to deep drainage and run-off from a saturated soil profile (Seyfried, Schwinning *et al.* 2005).

Seasonality of precipitation also affects the infiltration depth of water into the soil, and heterogeneity in soil water availability may result in different optimal strategies for plant root depth (Williams and Ehleringer 2000). Winter rain tends to penetrate deeper into an already wet soil profile. In an evolutionary algorithm modelling study, Schwinning and Ehleringer (2001) found that a higher proportion of the annual rainfall falling during the winter resulted in increased deep soil recharge and deeper roots, while winter drought resulted in shallower roots. Schenk and Jackson (2002b) found a relationship between winter rainfall bias and deep roots at the global level. In a study of Snakeweed (*Gutierrezia sarothrae*), Wan et al. (2002) found a regime of winter rainfall and summer drought resulted in deeper roots than the reverse. In comparison, summer rainfall, entering a soil profile dried by high evaporation, may only wet the surface soil layers (Paruelo, Sala *et al.* 2000; Weltzin, Loik *et al.* 2003) and a high density of roots in the soil surface may be necessary to extract water before it is lost to evaporation (Fischer and Turner 1978). Therefore, an optimal root distribution strategy may favour shallow roots in summer-rainfall areas, and deep roots in winter-rainfall areas.

However, due to low evaporation rates in winter, surface soil layers may remain moist longer (Schwinning and Sala 2004) and utilization of surface water with shallow roots may be advantageous to plants, with the additional advantage of the extraction of nutrients concentrated in the top soil, such as phosphorous (Ge, Rubio *et al.* 2000). Summer rain, on the other hand, may only be available in the surface soil for short periods due to high evaporation rates during this time of the year, reducing the

usefulness of surface roots (Schenk and Jackson 2002a). Veneklaas and Poot (2003) found that shrubs and forbs in south-west Western Australia experienced stress in summer when they only possessed shallow roots. Furthermore, it may be energetically expensive to maintain roots in shallow soil during summer, due to high temperatures and extended periods of low water availability. High soil temperatures can lead to higher carbohydrate loss (Davidson 1969a), short root life spans (Forbes, Black *et al.* 1997) and root desiccation, meaning roots have to be replaced constantly in order to utilize summer rainfall events. Several workers have noted that there many be a threshold amount of summer rainfall above which it becomes optimal for plants to allocate resources to roots that can extract water from surface layers (Ehleringer and Dawson 1992). This predicts that there should be intra-specific variation in root depth along a gradient in rainfall seasonality.

Rainfall seasonality also affects hydrological processes, such as deep drainage. Deep drainage is of concern in Australia, as the replacement of deep-rooted perennial vegetation with annual crops throughout much of the agricultural zone has resulted in increased drainage, leading to rising water tables and dryland salinity (Peck 1978; Petheram, Walker *et al.* 2002). Higher amounts of deep drainage are found in areas with a strong bias towards winter rainfall, and on coarse soils (Dyson 1993; Keating, Gaydon *et al.* 2002; Seyfried, Schwinning *et al.* 2005). Reducing deep drainage requires plants that can remain active over summer (Hatton and Nulsen 1999), continuing to dry the soil profile, in order to provide greater water storage capacity for winter. Deep rooting habit and the ability to continue to transpire at low soil water

content are also characters for deep drainage control (Seyfried, Schwinning *et al.* 2005). Native perennial grasses have the potential to be useful for reducing deep drainage, provided they are drought tolerant and can remain summer active (Johnston, Clifton *et al.* 1999; Lodge 1994).

One species with the potential to fulfil this role is *Austrodanthonia caespitosa* (white-top wallaby grass), a perennial C_3 grass common over southern Australia. Its range covers a wide variation in annual total rainfall, and seasonal distribution of rainfall. This study aims to investigate the responses of a range of collections of this species, obtained from sites with rainfall ranging from strong winter precipitation in South Australia, through to an even distribution of rainfall in southern New South Wales. This species is capable of remaining active over summer if water is available (Robinson and Archer 1988), with Williams(1961) noting that it appears to thrive on late spring and early summer rain. Austin et al. (1981) noted that *Austrodanthonia spp.* are responsive to differences in seasonal rainfall, and Williams(1968) found small-scale rainfall regime to have an impact on population dynamics in the species. A high degree of morphological and physiological diversity has been found in this species across its range, including variation in characters related to rainfall seasonality and predictability. For example, populations from northern New South Wales, in a climate with low and unpredictable rainfall and low summer rainfall usefulness, were found to display rapid winter growth and early flowering, compared to more southern populations (Hodgkinson and Quinn 1976). Over the range sampled in this study, the South Australian collections experience very low, infrequent rainfall during the summer

months, while the proportion of rainfall falling in the summer in New South Wales is higher, but evaporation rate and average temperature are also higher. We might expect to see a stronger response to summer water availability in ecotypes from an environment with higher or more predictable summer rain, while plants sourced from environments with low summer rain may show greater growth during winter and less responsiveness to summer rain. Plants may demonstrate different strategies for surviving seasonal drought (Kemp and Culvenor 1994), for instance, maintaining transpiration at a reduced rate, relying on deep water reserves, or becoming dormant and then responding when large rainfall events occurs. Bolger et al. (2005) reported that *A. caespitosa* shows high drought tolerance compared to other Australian and exotic perennial grasses, with leaves surviving 40 days in stage III drought conditions, the point at which stomata reach minimal conductance (Sinclair and Ludlow 1986). The aim of this experiment is:

(a) To investigate the capacity of *A. caespitosa* to survive summer drought, and to determine if responsiveness to summer rainfall varies between ecotypes. It is hypothesized that plants from New South Wales may display a stronger response to summer water than South Australian populations, as summer rainfall proportion is higher in New South Wales, and the plants may show adaptation to that seasonal regime.

(b) To investigate differences in root depth distribution between populations and the summer water/summer drought watering treatments, to determine the strategy of root placement to survive drought, and to utilize larger summer watering events.

10.2. Methods

I set up an experiment to measure the responsiveness in growth and biomass allocation of different populations of *Austrodanthonia caespitosa* exposed to different seasonal watering regimes. Two treatments were imposed, both receiving equal amounts of water in winter, but one of the treatment was watered over summer while the other was droughted. On October 30, 2004 seeds from five populations of *Austrodanthonia caespitosa,* as listed in Table 7, were germinated on moist filter paper in Petri dishes in a controlled temperature room, then transplanted to seedling trays filled with sandy loam soil (Jeffries, Wingfield) three days after germination. Seed populations for this experiment were selected to reflect a contrast between Mediterranean climate plants from South Australia, and equal-rainfall regime plants from New South Wales. Seedlings were grown in a controlled temperature room for three weeks at 20°C with a 12 hour day/night cycle.

Table 7 - Populations of Austrodanthonia caespitosa used in the seasonal watering experiment.

Population	**Latitude**	**Longitude**	**Locality**
SA020	35.35 S	139.12 E	Langhorne Creek
SA021	35.16 S	139.01 E	Red Creek
SA023	35.62 S	138.11 E	Cape Jervis
NSW004	35.16 S	145.94 E	Jerilderie
NSW005	33.89 S	147.12 E	West Wyalong

Polythene planting tubes (Poly Products, Regency Park), 50cm high and 10 cm in diameter, were filled with Mt Compass Sand (Jeffries, Wingfield), a loamy sand soil. On November 19, 16 seedlings of each population were planted in the planting tubes, and eight plants of each population were allocated to the summer watering, and eight to the summer drought treatments. The tubes were placed in a controlled climate room, with a night-time temperature of 10°C and a day-time temperature of 18°C. The controlled climate room was set to a 12-hour day/night cycle, with 6 hours of the day providing 60% sunlight, and 6 hours providing 100% sunlight. Plants were watered with 20mm equivalent of water on the first two days to aid establishment.

Two hundred mL of water was applied on November 30, and following that, 200mL were added every two weeks. On December 23, the controlled climate room broke down, and plants were moved to a different controlled climate room, set to a day/night temperature of 12°C/22°C, 10 hours of light, 14 hours of dark. On March 1, 2005, the day watering was due, photosynthetic activity of all plants was measured with a Pulse Amplitude Modulated Chlorophyll Fluorometer (Mini-PAM, Heinz Walz GmbH, Germany). One mature leaf on each plant was measured under ambient light conditions. A further 200mL of water was then applied to all pots, and photosynthetic activity was measured again the following day.

On March 16, 2005, the “summer” treatment was initiated, and plants were moved from the growth cabinet to a glasshouse at the Roseworthy Campus of the University of Adelaide. Glasshouse temperature was set to 30°C. Volumetric soil water content in

upper and lower soil was measured with a Theta probe inserted through the polythene tube 5cm below the surface of the soil, and 5cm above the base of the planting tube.

Shoot growth before and after the summer watering treatments were imposed was examined separately by considering leaves under the 15cm trimming level to be biomass produced during the winter phase of the experiment, and leaves trimmed above 15cm to be summer growth. On March 30 2005 all biomass above 15cm was trimmed, placed in paper envelopes and dried in an oven at 80°C for a week before being weighed . Watering of summer treatments occurred at 2-week intervals, and trimming of biomass above 15cm occurred at 4-week intervals.

On May 18, 2005, plants were harvested. Biomass above 15cm was trimmed and placed in paper bags for drying, and the remaining shoots were trimmed and stored separately for drying. Planting tubes were cut in half at a height of 22cm below the soil surface, and roots in each layer were separated from the soil by shaking on a 2mm-mesh sieve. The remaining soil was washed from the roots in a sink, and the washed roots were placed in paper envelopes for drying. Above-15cm shoots, lower shoots, and roots were dried at 100°C for two days before being weighted with an electronic balance. A series of 2-way ANOVAs were used to examine differences between populations and watering treatments in the seasonal watering comparison experiment.

10.3. Results

Population SA020 showed a significantly higher total shoot mass than populations NSW004 and NSW005 according to the ANOVA (df=4, p=0.0005, Figure 42), and summer-watered plants also produced more total shoot mass than droughted plants (df=1, p<0.0001), but the interaction term was not significant (df=4, p=0.4475).

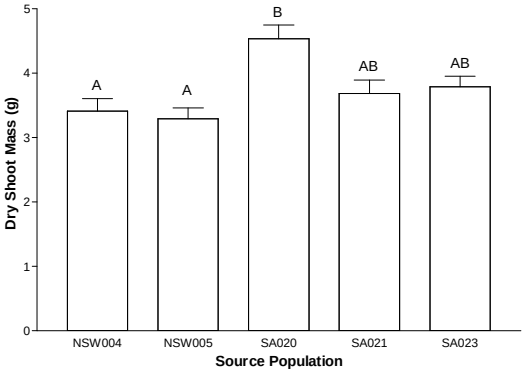

Figure 42 - Dry shoot mass at harvest by population in the seasonal watering experiment*.

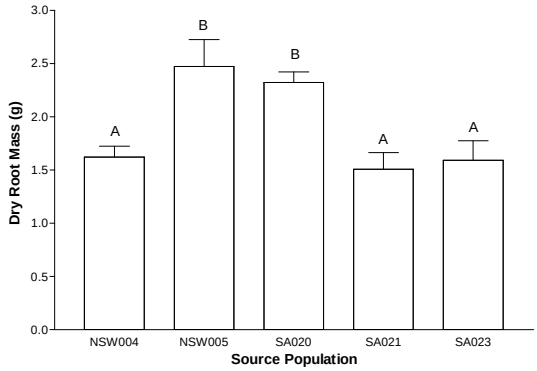

Figure 43 - Dry root mass by population in the seasonal watering experiment*.

* Error bars indicate standard error, and letters indicate significant differences as detected by Tukey HSD test.

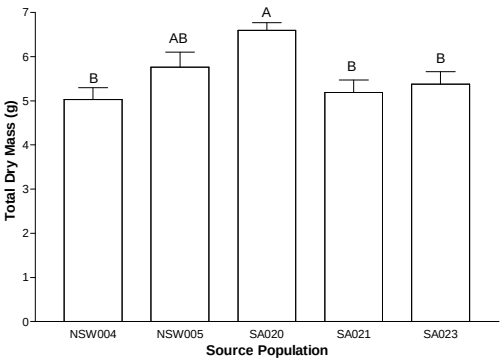

Figure 44 - Total dry mass by population in the seasonal watering experiment*.

While watering treatment did not alter total root mass according to the ANOVA (df=1, p=0.359), populations NSW005 and SA020 did have higher root mass than the other populations (df=4, p<0.0001, Figure 43), and there was no interaction between the

effects (df=4, p=0.133). Summer-watered plants produced more total biomass (df=1, p=0.0192) than droughted plants, and population SA020 also had significantly higher total biomass than populations NSW004, SA021 and SA023 (df=4, p=0.001, Figure 44). The interaction term was not significant (df=4, p=0.288).

Summer-watered plants were found to have a significantly lower root:shoot ratio than summer-droughted plants (df=1, p=0.0022), and population NSW005 was found to have a higher root:shoot ratio than the other populations There was a marginally non-significant interaction term, (ANOVA df=4, p=0.0544, Figure 45),with population NSW005 having a particularly high root:shoot ratio in the summer drought treatment. Although this interaction was not significant at a 5% level, the implications should be considered as ignoring an interaction is potentially more serious than accepting a non-significant interaction (Fowler 1990, Facelli and Facelli 2002).

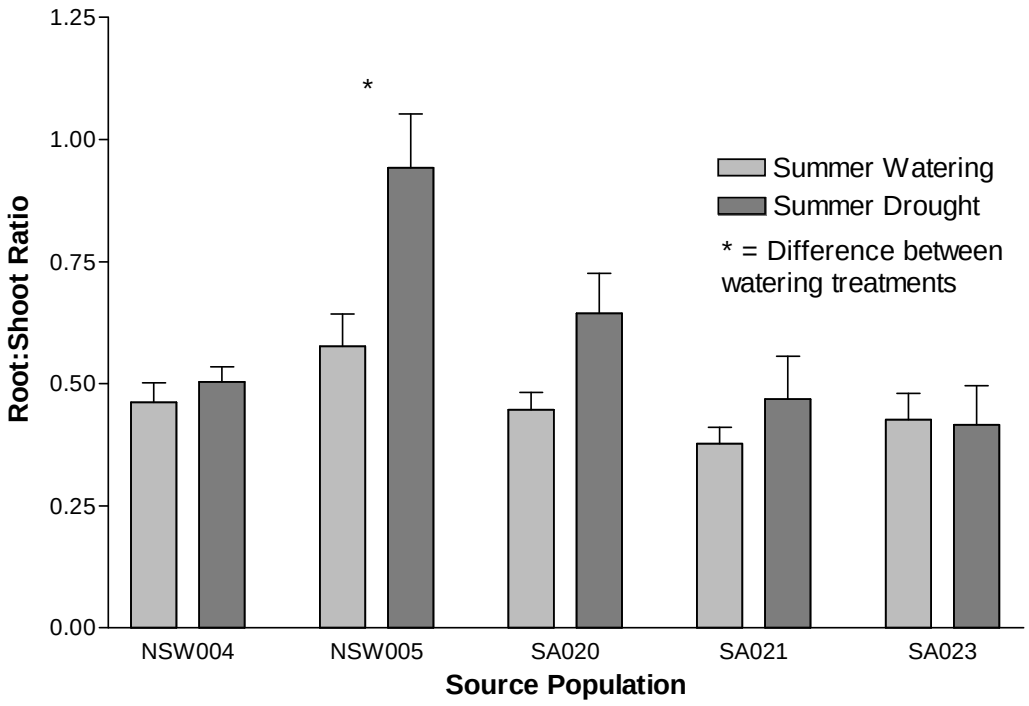

Figure 45 - Root:shoot ratio for watering treatments and populations in seasonal watering experiment. Error bars indicate standard error, and asterisk indicates a difference between watering treatments for the same population as indicated by a Tukey HSD test.

Summer-watered plants showed a slightly lower top:bottom root ratio, that is, more deep roots, than the summer-droughted plants, with the ANOVA showing a marginally non-significant effect (df=1, p=0.062, Figure 46) Population SA020 had a higher top:bottom root ratio, that is, more shallow roots, than populations NSW004 and NSW005 (df=4, p=0.0047, Figure 47), but the interaction term was not significant (df=4, p=0.282).

Figure 46 - Top:bottom root ratio by watering treatment in the seasonal watering experiment*.

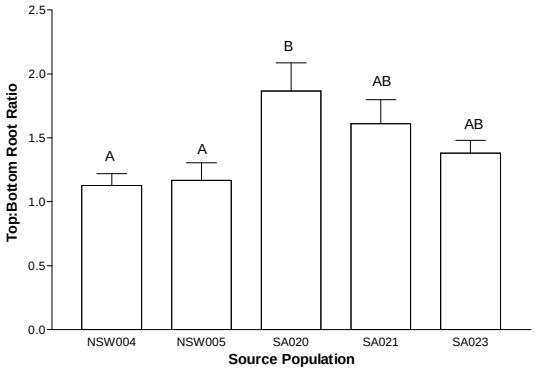

Figure 47 - Top:bottom root ratio by population in the seasonal watering experiment*.

* Error bars indicate standard error, and letters indicate significant differences as detected by Tukey HSD test.

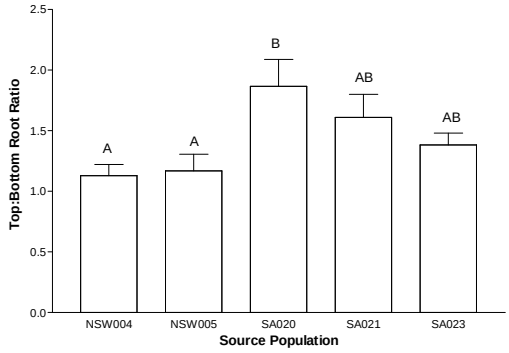

Figure 48 - Winter shoot dry mass by population in the seasonal watering experiment*.

Watering treatment did not affect below 15-cm shoot mass (df=1, p=0.1608) confirming leaf biomass below 15cm was not affected by summer watering treatment. However, population SA020 had higher shoot production during winter than the other populations. (df=4, p=0.0003, Figure 48) The interaction term was not significant (df=4, p=0.652).

As expected, summer-watered plants produced significantly more 15cm trimmed shoot mass than summer droughted plants. (df=1, p<0.0001) with summer-watered plants producing more shoot mass than summer-droughted plants. Populations SA020, SA021 and SA023 also had significantly higher summer shoot growth than

population NSW005 (df=4, p=0.001) The interaction between watering treatment and population was significant (df=4, p=0.0089, Figure 49). A Tukey HSD test on all treatment combinations showed that there were no significant differences between watering treatments for the New South Wales populations, while there were for the South Australian populations, suggesting the South Australian populations were more responsive to summer water addition. There were no significant differences between populations in the droughted treatment.

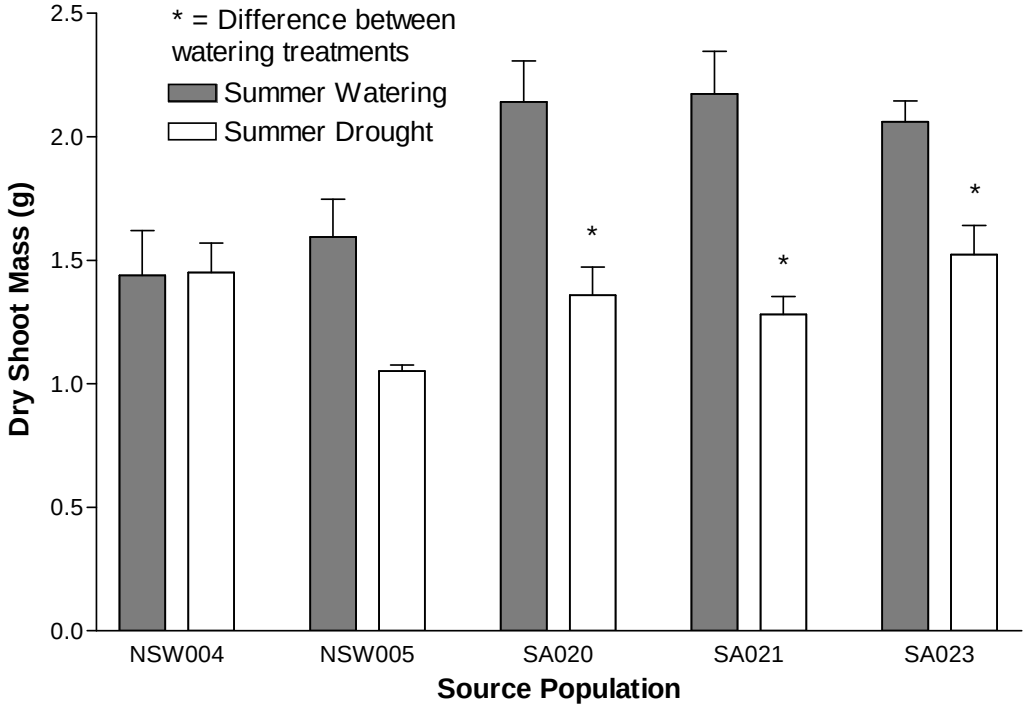

Figure 49 - Summer shoot growth for watering treatments and populations in seasonal watering experiment. Error bars indicate standard error, and asterisks indicate a difference between watering treatments for the same population as indicated by a Tukey HSD test.

In examining summer shoot growth as a proportion of total shoot biomass, with summer-watered plants producing a higher proportion of their growth in summer than summer-droughted plants (df=1, $p<0.0001$). Populations SA021 and SA023 also produced a higher proportion of their growth in summer than populations NSW005 and SA002 (df=4, p=0.0004) The interaction term was also significant (df=4, p=0.0032) and the results are graphed in Figure 50. Again, there appears to be a tendency for

some South Australian populations to be more responsive to summer rainfall than New South Wales populations.

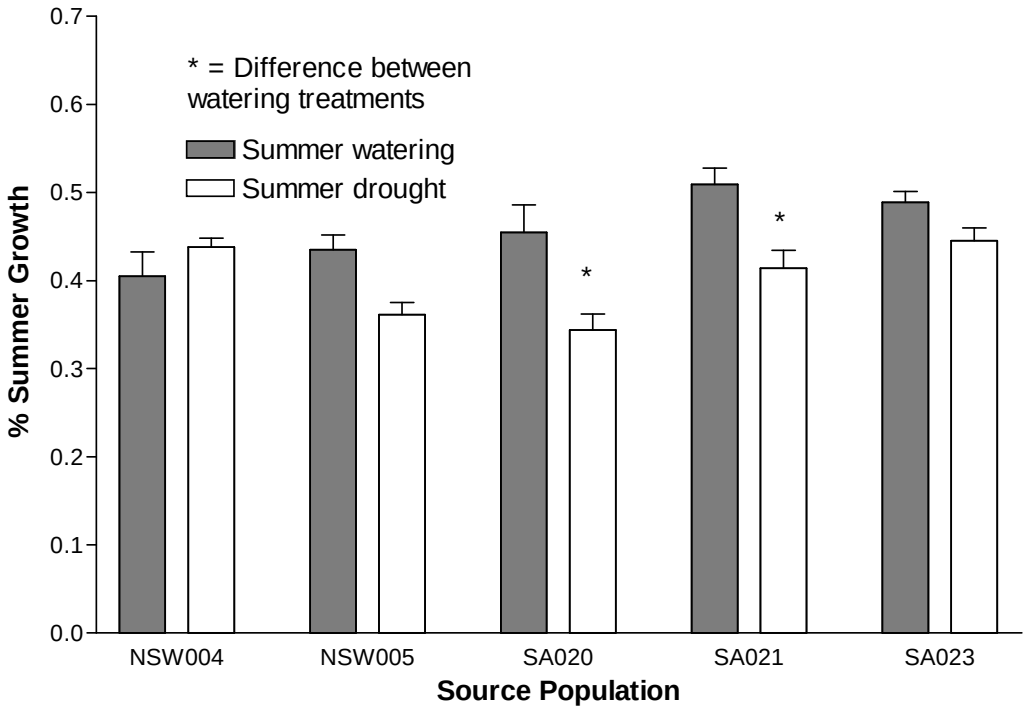

Figure 50 - Summer shoot growth as percentage of total for watering treatments and populations in the seasonal watering experiment. Error bars indicate standard error, and asterisks indicate a difference between watering treatments for the same population as indicated by a Tukey HSD test.

PSII quantum efficiency (Fq'/Fm') was higher after watering than before watering (df=1, p<0.0001, Figure 51) with Fq'/Fm' higher after watering than before watering. Population also had a significant effect (df=4, p=0.0029, Figure 52) with population SA020 having a significantly higher Fq'/Fm' than population NSW004. The interaction term was not significant (df=4, p=0.205).

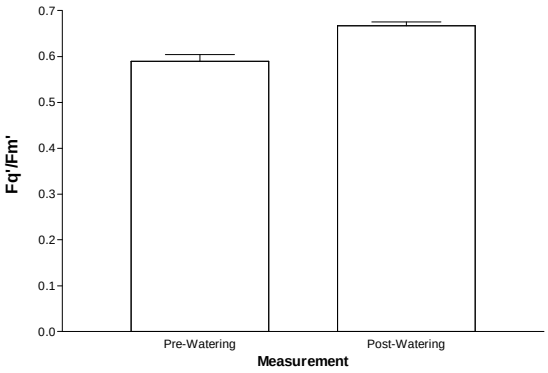

Figure 51 - PSII quantum efficiency (Fq'/Fm') before and after a watering event in the seasonal watering experiment*.

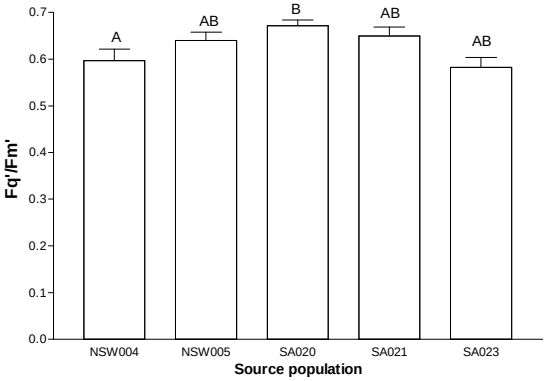

Figure 52 - PSII quantum efficiency (Fq'/Fm') for populations in the seasonal watering experiment*.

Figure 53 - Total dry mass per mL of water supplied for watering treatments in the seasonal watering experiment*.

* Error bars indicate standard error, and letters indicate significant differences as detected by Tukey HSD test.

With water as a limiting resource, total biomass is expected to correlate with total water added. In this experiment, the two seasonal treatments received different total watering amounts, which alone may explain differences in total biomass between watering treatments. Therefore, an ANOVA was performed to compare total biomass per mL of water added. Summer-watered treatments had less biomass per unit water than summer droughted treatments ($df=1$, $p<0.0001$, Figure 53). This indicates more growth was achieved with less water addition during winter. There were also differences between populations ($df=4$, $p=0.0014$) as would be expected as this comparison is equivalent to

the total dry weight comparison. The interaction term was not significant (df=4, p=0.181).

10.4. Discussion

As might be expected, additional summer watering resulted in more shoot biomass production than the summer drought treatment, confirming that water was a limiting resource in the summer-drought treatment, and that *A. ceaspitosa* plants remain active over summer and are able to make use of additional water in this period. The significantly reduced growth per unit of water added in the summer-watered plants confirms the lower effectiveness of rain occurring during summer. There were, however, no significant differences between watering treatments in below-ground biomass, which is important given the effect watering had on shoot biomass. An increase in root biomass in the summer-watered plants might have been expected as assimilates are invested in continued expansion of the root system over summer. Alternatively, imposing drought and reducing soil water availability may have resulted in a shift in root:shoot ratio towards root growth in the droughted treatment. Shifts in root:shoot ratio are expected to be in the direction of the organs that capture the most limiting resource (Davidson 1969b; Reynolds and D'Antonio 1996). Kemp and Culvenor (1994) list three possible plant responses to drought; escape, such as dormancy or persistence in the system as seed, adjustment, such as reduction in leaf area or stomatal control of water use, and recovery, such as low growth during drought then response to the return of water availability. A shift towards root growth in the summer-drought treatment would have indicated a strategy of drought adjustment by these plants, as a means of maintaining transpiration at low water availability. Bell and

Sultan (1999) found higher relative root biomass in droughted treatments in *Polygonum sp.*, a possible adaptive response to increase water acquisition ability during drought. Rodrigues et al. (1995) also found an increased root:shoot ratio under drought in *Lupinus albus.* However, the lack of difference in root biomass between treatments here, the significantly greater shoot growth with summer watering, and the significantly lower root:shoot ratio in the summer-watered plants combine to indicate that changes in allocation to above- and below-ground organs with summer watering are due to change in shoot biomass rather than root biomass. Other studies have found that water stress tends to affect shoots more than roots (Busso, Fernandez *et al.* 1998). For *A. caespitosa* in this study, dormancy was taken to mean a lack of measured growth, but in plants affected by summer drought this was accompanied by a lack of green leaf biomass. In this experiment, summer-droughted plants tend towards slowing of growth and dormancy, while summer-watered plants respond to watering events with the rapid production of aboveground biomass. The species therefore shows a strong growth response to summer water addition, presumably coupled with high transpiration rate and water usage to maintain the growth, indicating a "recovery" strategy. This has important consequences for the use of this species as a component of pastures, and for deep drainage reduction. A species that is capable of utilizing large summer rainfall events to produce green biomass, rather than remaining dormant, can make a useful contribution to forage in grazing systems, and reduce soil water content (Holmes and Rice 1996). Large summer rainfall events may be lost to evaporation in dormant pastures, while species capable of rapid growth in response to rain use the available water more efficiently for production. Utility of the species for deep drainage reduction

is more questionable. Reduction of deep drainage requires transpiration over summer, leaving the soil profile dry by the beginning of autumn and capable of storing high winter rainfall amounts (Johnston, Clifton *et al.* 1999; Singh, Bird *et al.* 2003). Soil profiles that are still wet at the end of summer are more likely to experience deep drainage during winter. Given the results of this experiment, unless large summer rainfall events occur, the species appears to become dormant and does not produce more root biomass to continue water acquisition. Therefore, *A. caespitosa* may not contribute significantly to drying the soil profile over summer, instead responding to and transpiring only summer rainfall events, rather than deeper water left over from the previous winter and spring.

The height of the planting tubes used in this experiment, 50cm, reflected a typical root depth obtained in this species, but not the maximum possible depth, which is expected to be significantly deeper. Root depth, as indicated by the top:bottom root mass ratio, showed a slight, marginally significant difference between watering treatments, with the summer-watered plants having deeper roots. This measured difference may not reflect an actual, repeatable difference in root depth with season, especially given that no difference in total root mass was observed between treatments. However, deeper roots in summer-watered plants should be expected. High temperatures reached in the surface soil may result in high respiration rates and root death in this layer, making reliance on surface roots costly. For instance, Davidson (1969a) reported high rates of carbohydrate loss under high soil temperatures, which had an influence on root:shoot ratio. Forbes et al. (1997) found decreases in root longevity at higher temperatures in

Lolium perenne, while Williams and Ehleringer (2000) suggest periods of drought in the surface soil can result in cavitation and damage to roots. Additionally, high evaporation rates that render water available for only a short time after a pulse may make root placement in this layer less useful in summer than in winter. The large watering events applied in the experiment are expected to have penetrated into the bottom soil layer, and a higher root investment in this layer would be expected given the unavailability of water in the surface layer.

Focusing on growth partitioned into seasonal components, winter above-ground production was calculated as above-ground weight at harvest, minus additional growth trimmed over summer. The lack of effect of watering treatment on this parameter confirms the suitability of using leaf-mass below trim level as a measurement of growth during winter, given that different watering treatment were not imposed during the winter growth period. Summer growth was determined from trimmings of leaves over summer, and the difference in shoot production between the two watering treatments becomes clear when summer growth is examined in isolation. As reflected in total biomass, summer shoot growth was greater, and summer shoot growth as a proportion of total was greater in the plants that were watered over summer. Interestingly, however, this effect was not found for all populations. South Australian populations showed a substantial shoot production over summer, which was not observed in New South Wales populations. Interestingly, Williams (1961) found that *A. caespitosa* showed a strong response to large late-spring/early-summer rainfall events in a population at Deniliquin in New South Wales, while my experiment showed little

responsiveness of New South Wales populations to summer water but a strong response in South Australian populations.

Across the transect from South Australia to New South Wales from which populations were sampled, seasonal bias decreases, from concentrated winter rain in South Australia, to even rainfall year-round in central New South Wales. If rainfall seasonality has acted as a selective force on plant summer growth strategy, we should expect a higher degree of dormancy in South Australian populations originating from a region of low summer rainfall, and more summer activity in New South Wales populations originating from a region where summer rainfall makes up a higher proportion of the annual total. However, the results show significant shoot growth in most summer-watered South Australian populations, but no significant shoot growth in the summer-watered New South Wales populations. The interpretation of New South Wales as being a high summer rainfall region may be mistaken, given that higher summer rainfall is combined with higher average temperatures and evaporation rates (Australian Bureau of Meteorology 2006). Previous work examining population differences in *A.caespitosa* along a gradient from Tasmania to northern New South Wales considered populations from northern New South Wales, well inside the summer rainfall zone, to be experiencing a "hot, dry" summer climate, where summer rainfall was less reliable and useful (Quinn and Hodgkinson 1984) compared to the southern end of the range. This is certainly true, in a comparison of northern New South Wales with the more temperate summer climate of southern Victoria and Tasmania. Williams(1968), in describing the climate experienced by a population of *A.caespitosa*

near Deniliquin, close to the eastern end of the collection range in my study, considered winter rain in this region to be more effective than summer rain. If summer shoot growth is a reflection of the populations' responsiveness to summer rainfall effectiveness, the results of this experiment indicate that this might also be true in comparing Mediterranean South Australia to the even-rainfall zone of New South Wales. Despite the differences in summer growth found between these two regions, there was no correlation between summer growth and rainfall indices, including Walsh and Lawler Seasonality Index (Walsh and Lawler 1981) and τ-statistic, in linear regressions (results not shown). This confirms that effects other than rainfall, such as temperature, may drive the adaptation to the different strategies found in the experiment.. In a study comparing the responses of Mediterranean and desert species of *Vulpia* and *Erodium* to rainfall pulsing regime, Sher et al. (2004) found that Mediterranean plants, from a region with higher effective rainfall, were more plastic and were better able to respond to additional watering than plants from more arid regions. South Australian populations of this species appear more able to use the large, infrequent summer rainfall events experienced in that region, while the New South Wales populations appear dormant. Previous studies (Hodgkinson and Quinn 1976) have suggested that northern New South Wales populations of this species display a life-history strategy closer to that of an annual grass than a perennial, including high winter growth and early flowering before the dormant, dry summer period, while studies of Mediterranean-climate grasslands have found an annual life-history strategy is more likely where summers are dry and hot (Jackson and Roy 1986). Similar dormancy was found in this experiment, but winter growth was not found to be

significantly higher in the New South Wales populations. Indeed, the only population showing significantly higher winter growth than any other was South Australian in origin. It would have been useful to include populations from the southern end of the range of the species in the experiment. In this way, New South Wales populations in which summer growth is limited due to the low effectiveness of rainfall events, and South Australian populations in which summer growth occurs opportunistically after large rainfall events, could be compared with populations from a region where summers are both wet and mild, and water may be less limiting and available more continuously.

Besides the differences between populations in summer shoot growth, there were a few genotypically controlled differences between populations evident in other plant variables measured. Population SA020, grown from seed collected near Langhorne Creek on the lower Murray River, showed a number of significant differences from other populations used in the experiment. It showed higher growth during winter compared to all other populations, high productivity overall, high shoot biomass, and high photosynthetic efficiency as measured by chlorophyll fluorescence. It also showed significantly shallower roots than other populations, and a high root mass overall. It is possible that the dense, shallow-rooted phenotype of this population contributed to its higher growth rate during winter. Winter rain is expected to be more available in the surface soil than summer rain, as lower evaporation rates during this season allow rainfall pulses to keep the surface soil moist for a longer period. This population may display a strategy of high utilization of winter rain from a frequently wet surface soil layer. The total root mass increasing total water acquisition may be

more important than the depth of roots in this case, however, as all populations experienced the same watering regime, with no loss to drainage, during winter, so root depth distribution may have had little influence during the winter growth period. In addition, summer growth in this population was high, despite the cost of shallow roots during this period.

Some other significant differences were found between populations in regards to top:bottom root mass ratio, with New South Wales populations having significantly deeper roots than population SA020, and the other South Australian populations also appearing to possess shallower roots. As detailed below, shallow roots may be useful in an environment with high winter rainfall, such as South Australia, where low evaporation rates lead to water lasting longer in the surface soil (Schwinning and Sala 2004). Examination of growth per unit water supplied in this experiment confirms the greater effectiveness of winter rainfall, a result confirmed by others (Reynolds, Kemp *et al.* 2004; Reynolds, Kemp *et al.* 2000) less total rain is required in winter-rain dominated regions compared to summer-rain biased regions for the same productivity. Despite the shallower roots that appeared to be present in South Australian populations, summer growth was strong, while the deeper roots possessed by New South Wales populations did not assist them in making use of more stable, deep soil water resources over summer to maintain growth. Within the parameters of this experiment, relative root depth, while variable, may not have had a significant impact on transpiration and growth. Partitioning of root depth distribution during winter and summer by means of multiple harvests or rhizotron would provide more information about the interaction

between seasonal watering availability and genotypically influenced root distribution.

10.5. Conclusion

Austrodanthonia caespitosa plants showed a tendency towards dormancy under summer drought conditions, but showed significant shoot production when watered over summer, a "drought recovery" strategy, indicating the potential of this species to provide useful forage production over summer by taking advantage of large rainfall events. However, the dormancy and lack of deep root growth during summer drought conditions suggests the species may not be useful in drying the soil profile and reducing deep drainage during drier summers, although additional research into soil water dynamics during summer watering and drought would be useful. Despite being sourced from a region with strong winter rainfall, and infrequent summer rainfall, South Australian populations appeared more responsive to summer watering than New South Wales populations, by producing more shoot biomass. Summer water availability to this species may actually be greater or more reliable in South Australia due to lower evaporation rates and cooler temperatures than central New South Wales during summer. There were no trends in winter above-ground growth differences between New South Wales and South Australian population that may have indicated different life history strategies and growth rates,. South Australian populations did tend to have shallower roots, which may be useful in maximising water acquisition in winter when evaporative loss from the surface soil is low.

11. EVOLUTIONARY ALGORITHM MODEL

11.1. Introduction

Organisms that have faced natural selection and evolved at a particular location may be expected to possess traits suited to the local resource dynamics, allowing the most efficient, or optimal, utilization of the resources available. In any arid, semi-arid and temperate dryland systems, soil water is the main resource limiting growth, and hence soil water regime may act as a strong selective force on the morphology and development of water acquiring organs. In the past, total annual precipitation has been related to productivity (Le Houérou, Bingham *et al.* 1988; Paruelo and Sala 1995), particularly in grasslands (Knapp and Smith 2001). Traits such as root depth has also been related to total annual precipitation (Schenk and Jackson 2002b), with increased root depth associated with more total annual precipitation (Schenk and Jackson 2002a).

At a finer scale, however, soil water distribution and the temporal dynamics of soil water availability are more strongly linked to intraannual rainfall regime than annual rainfall averages. Aspects of smaller-scale rainfall regime that may impact on soil water availability include rainfall event or pulse size, spacing between events or interpulse length, and the seasonal bias of the rainfall. Event size is variable between locations depending on the prevalence of, for instance, frontal, storm or monsoonal weather systems. The size of individual rainfall events can affect the penetration depth of the water in the soil profile (Reynolds, Kemp *et al.* 2004), with small events only wetting surface layers (Kemp 1983), while larger event penetrate deeper and last longer

at depth (Paruelo, Sala *et al.* 2000). The pathway of water through the system is also influenced by event size, with large events contributing more to run-off and deep drainage (Loik, D. *et al.* 2004) but escaping more soil evaporation, and small events contributing a greater proportion of water to soil evaporation (Sadras and Baldock 2003). The interpulse length, representing the spacing between rainfall events, is also variable across the landscape, with some sites receiving frequent rainfall, and others being subject to only occasional storm events. Interpulse period and rainfall frequency can have an important influence on vegetation due to high evaporation and low soil water content during drought periods (Loik, D. *et al.* 2004), resulting in mortality (Goldberg and Novoplansky 1997) and strong selective pressure. Pulse frequency may be a particularly important driver in grassland systems (Fay, Carlisle *et al.* 2000), as drying of the surface soil can have rapid negative impacts on shallow-rooted grasses. The seasonal timing of rainfall also has an important influence on the distribution and availability of water in the soil profile. The coupling or decoupling of rainfall timing with energy input into a system can result in the same annual rainfall total being more or less useful to plants in a system (Stephenson 1990). For example, summer rainfall is considered less effective than winter rainfall, as a greater proportion of summer rainfall is lost to evaporation in the hot conditions (Reynolds, Kemp *et al.* 2004), and water use efficiency is a negative function of vapour pressure deficit (Kemanian, Stöckle *et al.* 2005). Rainfall seasonality can also determine the depth of water penetration, with summer rain entering a dry soil profile only wetting the surface layers (Paruelo, Sala *et al.* 2000; Weltzin, Loik *et al.* 2003). Winter rainfall penetrating a moist profile can recharge deeper soil layers (Schwinning and Ehleringer 2001), but may also face

greater losses to run-off and deep drainage (Paruelo, Sala *et al.* 2000; Weltzin, Loik *et al.* 2003). Soil texture can also have an important influence on the soil water dynamics, with deeper penetration of water in coarse soils (Cody 1986; Sun, Coffin *et al.* 1997), due to greater infiltration capacity. In water limited systems, evaporative loss from sandy soil may also be less due to the inverse-texture effect (Noy-Meir 1973; Shreve 1942).

As water is the primary limiting resource in arid and semi-arid system, plants may be expected to display morphological adaptations to a particular soil water distribution and regime. Root depth distribution is one trait that may show significant variation in response to soil water distribution, with root depth expected to show greater correlation with intra-annual rainfall regime than long-term climate averages (Schenk and Jackson 2002a). The optimal root distribution based on event size is unclear. Some authors (Sala and Lauenroth 1982) consider small rainfall events useful, leading to the expectation of a shallow root distribution for acquiring small rainfall events, while large events penetrate to deep soil layers (Reynolds, Kemp *et al.* 2000), leading to a deep root distribution being optimal. However, other workers consider small events not to be useful, particularly in arid and semi-arid regions (Noy-Meir 1973), due to low penetration and rapid evaporative loss. Therefore, there is the possibility that roots accessing more stable deep water supplies may be optimal in a location experiencing small rainfall events, and there may be a threshold of event size or frequency before shallow roots to take advantage of this rainfall are optimal. Pulse frequency, or interpulse length, may also influence optimal root depth, particularly in the stability of

surface water availability. Infrequent rainfall events can result in prolonged periods of low water availability in the surface soil, leading to selection for deeper roots independent of the size of rainfall events or total rainfall.

Similarly, there are two possibilities of optimal root placement with different seasonal rainfall bias. Deep roots may be expected with climates biased towards winter rainfall, due to the deeper penetration of winter rain, while summer rain only reaches surface layers, so shallow roots may be optimal. A number of studies have found an association between deep roots and winter rain (Schwinning and Ehleringer 2001), including on a global scale (Schenk and Jackson 2002b). In a study of snakeweed root dynamics, Wan et al. (2002) found a plastic response where deeper roots were developed under winter rainfall and summer drought, and shallow roots were found under the opposite rainfall conditions. However, high winter rainfall, coupled with low evaporation during this time of the year, can also keep the surface layers constantly moist (Schwinning and Sala 2004), potentially favouring a shallow root distribution with high winter rainfall. Plants may also be unable to make use of summer rainfall events below a particular threshold of size, frequency or predictability (Williams and Ehleringer 2000), relying on deep stable water supplies rather than making use of shallow penetrating summer events. Finally, optimal root depth may be related to soil texture, with some evidence of an association between coarse, sandy soil and deep roots (Schenk and Jackson 2002a; Seyfried, Schwinning *et al.* 2005), although the reduced evaporation rates in sandy soil (Noy-Meir 1973) suggests there may be conditions where shallow roots are favoured in this soil.

It should be noted that in reality, resources other than water may influence adaptation in root distribution, although in water limited systems it should be important. Physical constraints, such as impenetrable soil layers, can reduce the actual range of root depths available. Nutrient that are spatially heterogenous the soil, such as the shallow distribution of phosphorous (Ho, McCannon *et al.* 2004), can also create trade-offs in root depth distribution.

Root:shoot ratio is another plant growth trait that determines the ability of a plant to utilize resource, and which is often affected by resource limitation, with greater relative growth of organs expected in the region with the most limiting resource (Sultan 2003), and water deficit has been found to reduce shoot growth more than root growth (Busso, Fernandez *et al.* 1998). As such, different rainfall regimes can result in different water availability and limitation, so differences in root:shoot ratio may be expected. In a grassland system, Fay et al.(2003) found more root growth with large, infrequent rainfall events compared to small, frequent pulses, while Fernandez and Caldwell(1975) found a shift towards more root mass with high winter rain in desert shrubs. There appears to be a complex relationship between rainfall regime and root:shoot allocation, as both large events, and winter rainfall, are in some circumstances considered more effective, so one would imagine water to be less limiting, and hence more shoot allocation might be expected instead. Rainfall regime, particularly seasonality, can also have an affect on optimal life history strategy in grasses, in the determination of an annual or perennial strategy, and the continuation of growth over summer. Perennial grasses do not survive as well when the summer is dry

(Jackson and Roy 1986), leading to an annual strategy being more common. There is evidence of high winter growth rates and early flowering in perennials that escape summer drought (Kemp and Culvenor 1994), requiring high winter water availability, and life cycles may be shortened as a reaction to an unpredictable climate (Cox and Conran 1996).

Evolutionary models offer a tool to examine strategies for plants in a complex and heterogenous environment. Rather than attempting to correlate plant traits with the abiotic environment, which requires carefully selected descriptors of the environmental effects of interest, we attempt to determine what the "ideal" plant for a given environment would look like. This involves introducing variability in traits into the individuals in the model, using a heterogenous simulated environment to trigger selection and adaptation (Bousquet and Le Page 2004) then identifying which individuals did well and poorly under those conditions. Including temporal variation in resources and environment improves the accuracy of models (Metcalf, Rose *et al.* 2003), which is particularly important when dealing with small-scale resource fluctuations such as daily rainfall input and evapotranspiration. Modelled plants possess a genome, with individual genes controlling the expression of a particular trait, for instance root depth, root:shoot allocation, or germination requirements. These modelled genes need not reflect actual plant genetics, but should reflect traits that are under some form of genotypic control. Individual plants in the model grow in response to the simulated environment, within defined parameters and based on traits controlled by the model genes. At the end of a generation of the model, a subset of the modelled

plants are selected as being the fittest individuals from that population. Fitness can be defined in order to answer the question of interest; for instance, fit plants may be those with the most total biomass, or those that have transpired the most water, or those that achieve greatest reproductive allocation. The fittest plants in a generation undergo genetic recombination with other selected plants, and a new population of plants is created containing the fittest genes from the previous population. Genes may also under go some random mutation, in order to ensure complete exploration of all possible trait combinations. After a number of generations, genes tend towards the optimal trait values for the environment to which the population was exposed.

In defining the plant model and the variability allowed in trait values, it is important to consider trade-offs, so optimal outcomes remain biologically realistic. For example, in the case of roots, although the surface soil may be wet more frequently than deeper soil layers, it is important to consider the high cost of shallow roots in grasses, as these roots may experience high respiration rates and root desiccation in the hot surface soil (Williams and Ehleringer 2000), reducing their value. Other factors, such as water use efficiency and photosynthetic rate, can run to a maximum if not constrained by realistic biological trade-offs, as an increase in these variables will always lead to an increase in growth. Plant characteristics returned by evolutionary algorithm models are only optimal within the constraints and trade-offs defined in the model, and are unlikely reflect realized adaptations in real plants. Farnsworth and Niklas (1995) note evolution may not be a case of optimality, but simple of more or less efficient designs under the environmental constraints imposed, and that optimal adaptations are likely to change

during the life of an organism under different environmental circumstances. Such a capacity for changeable characteristics, for example plasticity, is not included in this model. Despite the output of evolutionary algorithm models not reflecting true biological optima, the term “optimal traits” will be used in this chapter for simplicity.

A number of workers have used evolutionary algorithm models to examine optimum plant traits, with particular reference to biomass allocation and root distribution. Schwinning and Ehleringer (2001) used an evolutionary algorithm model to determine optimal plant functional type in an arid system, in response to a particular water pulse regime. This model was based on the hydraulic properties of plant organs, such as stem conductance, root allocation to deep or shallow water sources, and stem water storage. The model produced optimal plant functional types that correlated well with actual plant strategies in arid systems. van Wijk and Bouten(2001) developed an evolutionary algorithm model to examine optimal root placement of trees in different soil profiles. This model based plant fitness on the amount of water removed from the soil profile, included the costs associated with different rooting depths, and identified optimal strategies of root placement in different soil types and competitive environments. The model developed in this paper extends on previous plant morphology evolutionary algorithm models by focusing on root distribution in multiple soil layers, rather than a two-layer soil model, and by modelling the growth of a grass with fitness determined by growth, rather than tree roots with, with fitness based on water extraction (van Wijk and Bouten 2001).

Austrodanthonia caespitosa is an Australian perennial grass that is common across southern Australia. It is highly variable across its range, with variations found in flowering time with latitude (Hodgkinson and Quinn 1978), and more northern populations showing a faster growing, more annual-like life cycle (Quinn and Hodgkinson 1984). Local differences have also been identified, with sheep grazing resulting in a shorter habit than nearby ungrazed populations (Scott and Whalley 1984). These local differences in plant genotype may be the result of selective pressures in the local environment (Wilson 1996), with plant traits reflecting adaptation to resource dynamics, for instance. Rainfall regime is highly variable across the range of the species, in annual averages, seasonality, event size and event frequency, as detailed in the previous chapter on rainfall regime in Australia. The species also grows in a wide range of soil types. Based on experimental knowledge of the extent of variation in this species, and important trade-offs in morphology that may be present, an evolutionary algorithm model may be useful in exploring adaptive plant traits, and the environmental factors behind variation in root depth and root:shoot ratio. There is some evidence of climate change in Australia, particularly changes in total annual rainfall, and event intensity, which may have an influence on traits such as root depth. Identifying adaptations to different rainfall regimes may be useful in determining ecosystem response and species survival in the face of climate change.

The model discussed here has been developed to explore the effects of small scale moisture regime and soil type on adaptive root:shoot allocation and root distribution of *Austrodanthonia caespitosa*. The model aims to determine what environmental factors,

such as soil type and rainfall regime, are important in influencing the placement of deep or shallow root, and which combination of soil type and rainfall regime result in the least limiting soil water availability, and greatest plant growth. Gradients in event size, frequency, seasonality, and total water are simulated in order to identify the existence of thresholds beyond which water acquisition strategy may shift. Real daily climate data from a number of meteorological stations across southern Australia were also used as model input, in order to examine the potential for rainfall regime to act as a selective force. Finally, the model is used to examine the importance of the high cost of surface roots in determining water acquisition strategy, and the potential for plant density to influence root distribution.

11.2. Methods

Model structure

An evolutionary algorithm model was constructed to explore optimal biomass allocation and root depth strategies under a variety of rainfall regimes, climates and soil types. Individual components of the model, such as the soil water dynamics, and plant biomass assimilation, have been informed by previous models, but the coupling and evolutionary optimisation components of this model have been developed for this study. A simplified flux diagram of major operations of the water and photosynthesis sub models can be found in Figure 54. The model operates on a daily time step, and consists of an individualistic “big leaf” plant growth model with a number of growth parameters under genetic control, and a soil moisture sub-model that calculates rainfall

infiltration and evaporation in three soil layers. The plant growth model is coupled to an evolutionary algorithm routine that selects plants according to a fitness function at the end of each generation of model execution, and recombines genes to generate a new population of plants for the subsequent generation. After a number of generations, the model outputs gene values that represent the optimum plant traits for the climate and soil data that acted as model input input. Competition between individual plants is not explicitly modelled, but density dependent effects can be simulated by modifying the soil surface area occupied by the plant.

Rainfall input is defined as a quantity (mm) delivered at a set frequency (days), partitioned into summer and winter half-years if required. Alternatively, rainfall data can be read from a text file containing daily rainfall records from a rainfall station. Other climate inputs are based on monthly averages, and include daily maximum temperature, daily 3pm relative humidity, and daily pan evaporation. Soil type is defined by the volumetric wilting point and field capacity, and by the rate of diffusion of water between the layers. Each soil layer is represented by a volume, defined by the layer height and the soil surface area being considered. The daily solar flux integral is calculated from the latitude of the site. A list of all model parameters can be found in Table 8.

Table 8 - Model parameters.

Parameter	Value/Units	Source
Specific Leaf Area	220 cm^2 g^{-1}	Based a value obtained for *Austrodanthona* from a study of drought and defolation in perennial Australian grass species. (Bruce 2001)
Photosynthetic Efficiency	0.0092 μmol CO_2 μmol $photons^{-1}$	Derived from experiment using a Cirrus 2 portable photosynthesis system - See Appendix B
Water Use Efficiency	Range, 0.000402 g mL^{-1} to 0.000539 g mL^{-1}	Derived from experiment, based on a regression of plant dry weight against water supplied - See Appendix B

Water Extraction Rate per Root Length	Range, 0.02 mL cm^{-1} day^{-1} to 0.04 mL cm^{-1} day^{-1}	Based on a previous simulation model of root growth dynamics and water use. (Adiku, Braddock *et al.* 1996)
Specific Root Length	10,000 cm g^{-1}	An approximation based on average values obtained in the Pulse Size Experiment
Soil Water Conductivity (K)	10%	Approximation of the amount of water that diffuses between soil layers as a percentage of water content difference in unsaturated soils.
Loss of mass due to respiration based, on temperature	Based on $R = ae^{bT}$. a = 0.000625 b = 0.0693	Based on typical values discussed in Atkin, Bruhn et al. 2005.

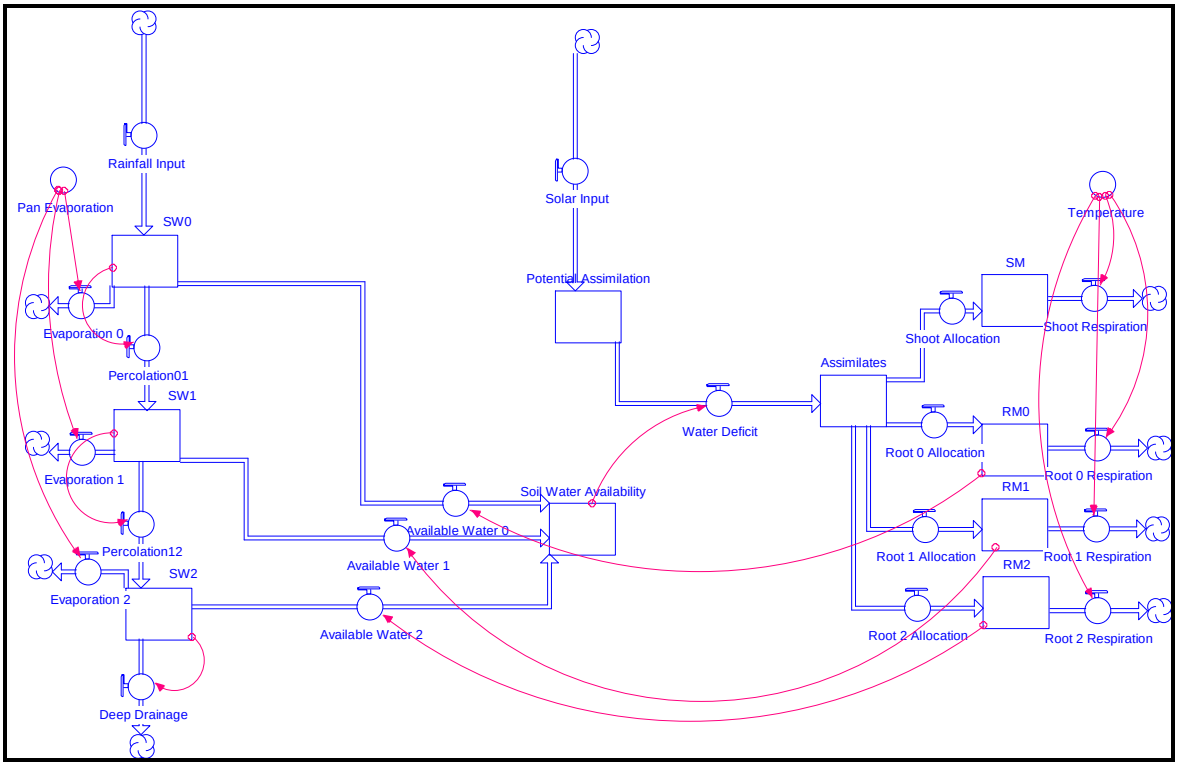

Figure 54 - Simplified flux diagram of water and photosynthesis model, with soil water storage on the left, and plant biomass allocation on the right.

Daily plant growth loop

Each generation in the model grows for two calendar years, a timeframe chosen to allow model plants to experience both winter and summer growing conditions. Model trials were run for 150 generations, adequate to result in stable gene output. Each model generation beings with 300 seeds, which must meet appropriate germination requirements before growth begins. The model runs on a daily time step, and during each day calculations are carried out in the following order.

The daily environmental variables are calculated, including rainfall input, temperature, relative humidity, and solar integral.

The cascading soil water infiltration function is called, to introduce the daily rainfall into the soil and calculate any deep drainage.

The soil water diffusion function is called to calculate further water redistribution in the soil along moisture gradients.

The respiration function is called to remove biomass from plant roots and shoots as a function of temperature.

The plant growth function is called, which includes calculation of photosynthesis, soil water acquisition, biomass allocation and seed germination.

The soil water evaporation function is called.

At the end of two years growth, the evolutionary algorithm sub-model determines the fittest plants, and recombines genes to produce the next generation of plants. The model outputs utilized here are average gene values and plant growth variables from the population at the end of the final generation in the simulation, reflecting the optimum plant traits. In addition, the model can output soil moisture data, and deep drainage quantities. Also, the software can be set to report gene averages at the end of each generation, in order to track shifts in gene value over time, or to report plant growth and soil water content daily for individual plants in the model.

Water infiltration

Water infiltration is modelled primarily through a cascading infiltration model. This is a simple but hydrologically realistic model of water penetration in pulse events (Schwinning and Sala 2004), and has been utilized in a number of recent plant models (Eitzinger, Trnka *et al.* 2004; Reynolds, Kemp *et al.* 2004). As seed collection sites for the other associated experiments were flat, and rainfall was low, run-off was not included in the model and was assumed to be zero. The model has three soil layers, the top two being 10cm in depth each, and the bottom layer being 20cm in depth, to simulate a large, deep water store. This gives a 40cm soil column, comparable to the pot size used in associated plant growth experiments. Drainage below the deepest layer is recorded as a model output, and there is assumed to be no negative effect of waterlogging on plant growth or root deveopment. Water is added to the top soil layer until saturation point is reached, then the remaining water is added to the next layer, and so forth. Any water still remaining after the third layer has been filled is recorded

as deep drainage. This algorithm models both the vertical movement of water from the surface to deep layers, and allows particularly dry layers to absorb a higher proportion of the rainfall.

Soil water diffusion

In addition to the cascading infiltration function, a diffusion function is included to model diffusion of water from moist to drier layers, smoothing gradients in soil water and increasing the penetration of water pulses (Schwinning and Sala 2004). For each pair of layers, the difference in soil water content is calculated, and a percentage of that difference is exchanged between the pair of layers, from the wetter layer to the drier layer, ignoring drainage below the three modelled soil layers. This simple diffusion approximation allows small amounts of water to be transferred between layers with similar water contents, and larger amounts to be transferred when the gradient is large. The conductivity parameter, K, allows the modelling of different soil textures.

Respiration

Respiration rate R, the proportion of biomass respired, is calculated according to the first-order exponential equation:

$$R = ae^{bT}$$

Where T is the temperature in Celsius, *a* is the respiration rate at 0°C, and *b* is a parameter that describes the rate of increase in respiration with temperature. While no respiration measurements were taken on *Austrodanthonia caespitosa,* this equation was

fitted to an exponential curve with a respiration rate of 0.25% at 20°C, within the realistic range of 30%-80% of average daily assimilation (Atkin, Bruhn *et al.* 2005), and a Q_{10} value of 2, representing a doubling of respiration rate with every 10°C increase in temperature. Values of 0.000625 and 0.0693 were obtained for parameters *a* and *b* by fitting the respiration equation to the Q_{10} curve calculated from that respiration rate.

R indicates the proportion of biomass respired and removed from the organ. Three different respiration sub-models are available; the first intends to simulate the lower cost of roots in deeper soil layers by reducing the temperature in the respiration function, in the 2nd layer by 5°C and in the 3rd layer by 10°C. The second respiration function has equal temperatures, and respiration rates for shoots and all root layers. The third respiration function simulates a lag in temperature deeper in the soil, with the 2nd layer experiencing temperature of the previous month, and the 3rd layer experiencing temperature from two months ago, in an effort to simulate the heat storage capacity of the soil. In the winter, deeper soil layers may be warmer than the surface, while in summer, deeper soil layers may be cooler.

Photosynthesis and assimilation

Photosynthesis in the model uses the "big leaf" approach, with all aboveground biomass able to photosynthesise, and no self-shading taken into consideration. This is a reasonable approach to take for a short, open species such as *A.caespitosa*. Photosynthetic leaf area is calculated as follows:

$LA = SLA * DM$

Where LA is leaf area in cm^2, SLA is specific leaf area $cm^2\ g^{-1}$ and DM is dry mass g. Shipley and Vu (2002) reported a SLA of 227.1 $cm^2\ g^{-1}$ for *Danthonia spicata*, while Bruce(2001) reported a SLA value of 220 $cm^2\ g^{-1}$ for the Australian species *Austrodanthonia auriculata*. This later value was used in the model. SLA remained constant throughout the life of the plants, and no account was made for changes with ontogeny or water supply.

Potential daily assimilation is then calculated as follows:

$A = LA * P_{eff} * Flux * C_m$

Where A is assimilated carbon (g), LA is leaf area (cm^2), P_{eff} is photosynthetic efficiency in μmol CO_2 μmol $photons^{-1}$, Flux is daily solar flux integral in μmol photons cm^2, and C_m is the molecular weight of carbon. P_{eff} was calculated from measurements with a CIRRUS 2 photosynthesis system as part of the water use efficiency experiment, described in appendix B. A value of 0.0092 μmol CO_2 μmol $photons^{-1}$ was obtained from this experiment for use in the model.

Daily solar flux integral is estimated numerically from an assumed PAR of 1500 μmol $m^{-2}\ s^{-1}$ when the sun is at the zenith, and calculations of the hourly altitude of the sun above the horizon, with the flux reduced with lower altitude. Solar altitude is calculated following Carruthers et al. (1990). Solar angles could be calculated at any interval required to give a more accurate estimate of the daily solar integral, but hourly

calculations were considered accurate enough for the model.

The photosynthesis function returns a potential daily assimilation amount (g) which is then modified based on water availability before being allocated to organs.

Water availability and usage

An important aim of this model is to explore the relationship between soil water and growth. This necessitates a mechanism for letting the amount of soil water available moderate growth. More specifically, there needed to be multiple soil water stores which, through interaction with roots, reduced growth when there was a soil water deficit, and allowed maximum growth when soil water was plentiful and available to roots.

A simpler linear approach was taken in this model, where growth is moderated by processes of supply and demand, although other methods, including hydrological models based on water potentials and conductances, may also be suitable (Schwinning and Ehleringer 2001). Demand, W_{plant}, is determined by the potential maximum assimilation for the day, calculated by the photosynthesis function, and the water use efficiency, that is, the amount of water that would be transpired if that amount of photosynthesis took place. Integrated water use efficiency for *A.caespitosa* was calculated in a variable watering growth experiment, by a regression of dry weight against total water added. Two populations were used in this experiment, populations SA023 and NSW005, which returned WUE values of 0.000539 g mL^{-1} and 0.000402 g mL^{-1} respectively. These WUE values are of the same order of magnitude although

slightly lower than other measures in perennial grasses from Mediterranean climates (see for example Marais 2006). The low values may reflect the measurements being based on individual potted plants rather than a large scale canopy. As the model is also based on individual plants growing over a defined area of bare soil, these WUE values were considered realistic in the context. WUE in the model was under genetic control, in order to determine if there were any circumstances under which WUE values lower than the maximum possible were ever optimal, and these two values were used as the extremes of the phenotypic range of this parameter. Daily WUE is adjusted by the vapour pressure deficit calculated from average monthly humidity and temperature, to decrease WUE during the warmer months.

Supply is determined by the amount of water in the three soil layers, and the mass of roots in each of the soil layers. W_{tot} is the ideal maximum amount of water required to support the photosynthesis for that day, calculated from total assimilation *A* and water use efficiency WUE. W_{prop}, the maximum amount of this water to be extracted from each layer, is determined by the proportion of root mass in each layer. Following that, the maximum water able to be extracted, W_{max}, is determined by the length of roots in each layer, a product of the root mass and the specific root length, and the maximum water extraction rate per root length. Specific root length was calculated from plants in the pulse-size experiment, taking an average root length per mass. An approximate value of 10,000 cm g^{-1} was obtained, and this is a fixed parameter in the model. Maximum water extraction rate was included as early versions of the model allowed plants with relatively few roots to dry the soil profile in a day under high

photosynthesis conditions, leading to a bias towards consistently low root mass allocation. The CERES-wheat model uses a maximum water extraction rate of 0.03024 mL cm^{-1} day^{-1} of root length (Adiku, Braddock *et al.* 1996). Water extraction rate per root area was made variable and under genetic control in the model to examine if any water conserving strategies that reduced water uptake rate were optimal, and the value of this parameter was allowed to vary between 0.02 and 0.04 mL cm^{-1} day^{-1}, centred on the value used in the CERES model. The amount of water roots in a layer will attempt to remove is the lesser of W_{prop} and W_{max} for each layer, allowing extraction limited by either root mass or length. Water is then subtracted from the soil in each soil layer, to the wilting point. The difference between the total water wanted, W_{tot} and the water actually removed, W_{ex} is added to the water deficit W_{def}.

The difference between the water deficit W_{def} and the total water wanted, W_{tot}, determines how much growth actually takes place. For instance, if only 50% of the wanted water is available, assimilation will be reduced by 50%.

This algorithm allows larger plants to place greater demand on the soil water supply, allows soil water supply to control growth, and allows root distribution to modify where water is extracted from the soil profile.

Assimilation and Biomass Allocation

Once the daily amount of assimilate is determined, based on photosynthesis and water availability, biomass is allocated to organs under the control of three genes, RS (Root:Shoot ratio), RD0 (Root Determinant 0) and RD1 (Root Determinant 1). RS

determines the allocation between shoots and roots, with the maximum genetic value of 255 indicating a 75% allocation to roots, and the minimum genetic value of 0 indicating a 25% allocation to roots. RD0 determines the proportion of allocation to the surface layer, compared to the two deeper layers, with a maximum value of 255 representing 100% of root biomass allocated in this layer, and a minimum value of 0 representing no biomass allocation in this layer, and allocation to the deeper layers instead. While an allocation of no roots to the surface layer is of course biologically impossible, the model results show that in no case did plants allocate no surface roots, so this did not affect the outcome of simulations. RD1 is similar to RD0, in allocating the remaining root biomass left after the surface layer to the second and third layers. A maximum RD1 value of 255 indicates allocation of 100% of biomass to roots in the second layer, while a minimum RD1 value of 0 results in allocation of all remaining root biomass to the third layer.

Evaporation

Modelling soil evaporation in isolation of plant evaporation is difficult, especially when considering the evaporative contribution of different soil layers. Some models rely on a two-phase soil evaporation model, moderating evaporation by soil hydraulic conductivity (Paruelo, Sala *et al.* 2000), while other models restrict soil evaporation to a single 10cm surface layer (Reynolds, Kemp *et al.* 2000).

A simple algorithm was implemented that was designed to enable evaporation to vary throughout the year, based on measured tank evaporation value rather than climatic

variables such as vapour pressure deficit and temperature. The model also allowed different amounts of water to be evaporated from deep and shallow soil layers, and allowed less evaporation to occur when the soil had a lower water content, a method used in other models (Dunbabin, Diggle *et al.* 2002). Daily evaporation from each layer was calculated as follows:

$$E_{layer} = E_{prop} * W_{PS}$$

Where E_{layer} is the amount of water to be removed from the layer, E_{prop} is the percentage of tank evaporation to be removed from that layer, set at 70% for the top layer, 5% for the middle layer, and 0.5% for the bottom layer, and W_{PS} is the percentage saturation by volume of the soil layer. These evaporation percentage values are approximations aimed to allow for greater evaporation from surface layers, and to allow a proportion of water to remain for transpiration after soil evaporation, as evaporation and transpiration are not coupled in this model, with transpiration instead being a function of growth and soil water availability. For example, in the surface soil 30% of the total evaporative demand is reserved for potential plant transpiration, while in the next layer, 95% of total evaporative demand is potential transpiration.

Plant reproduction

Evolutionary algorithm models, by their nature, utilize fitness, genetic recombination and reproduction as core processes in the model design. Using the evolutionary algorithm technique to model actual natural selection in a biological organism offered an opportunity to more closely model the processes of reproduction. An early version

of the model aimed to do this by incorporating more realistic biological processes into the fitness, genetic recombination and reproduction functions. This version of the model operated with a continuous timeline, no intrinsic population size, and overlapping generations. Plant flowering was triggered by a genotypically determined temperature, allowing pollen transfer, genetic exchange and seed production, with the fitness function determining the relative contribution of flowering plants to the gene pool.

This simple simulation of flowering and reproduction was used so that phenological traits themselves could come under genetic control. For instance, genes coded for critical water content and temperature for germination, time until maximum seed germinability, seed life span, critical temperature for flowering, and flower life span. There was potential for many of these phenological traits to be under selection under different climatic and rainfall regimes in this species. For instance, Hodgkinson and Quinn(1978) found differences in flowering timing between southern and northern populations of *A.caespitosa*, with southern populations having predictable flowering time, and northern populations showing a relationship between flowering and rainfall. Strategies for seed germination may also be important; plants experiencing small rainfall events or summer drought may delay germination until later in the season when soil water storage is higher.

Runs of this model version, however, showed little stabilising selection for phenological traits, and the algorithms used proved inefficient in reporting the optimum genotype for plant growth traits. Lack of strong control of population size meant a

sizable soil seed bank built up, slowing down model processing while adding little improvement to model output. Adding a carrying capacity to limit seed germination improved model speed at the expense of randomly deleting seeds carrying favourable genotypes. The fertilization function relied too much on random chance, with the potential for optimum genotypes to be ignored, and sub-optimal genotypes to be selected by chance. While the model still tended towards the optimal genotype, progress was much slower. Often, several thousand years of model simulation was required before gene shift stabilised, so this form of the model was abandoned.

In the second model revision, a more traditional evolutionary algorithm approach was used. The model is broken down into non-overlapping generations, each lasting two years. The two year life span was chosen to allow for plants to experience summer conditions as a mature plant, to experience how summer water usage may alter autumn and winter water availability, and to allow integration over multiple years of rainfall when using real rainfall data.

Each generation starts with a set number of 300 plants, as seeds. At the end of the two years of modelling, plants are sorted according to the fitness function of choice, for instance, total biomass. The top 10% of plants are selected as the “best” plants, and genetic recombination occurs among these plants to produce the next generation, including mutation at a rate of 3% across the genome, with a mutation involving the insertion of a randomly selected 1 or 0 at a random point in the genome. Mutation rate can have an influence on model output, with low mutation rates resulting in poor exploration of the fitness landscape, while high mutation rates can result in accidental

loss of optimal genotypes. Recombination involves selecting two plants at random, and for each gene in the offspring plant randomly selecting one of the parental genes.

This method ensures that the best plants are always selected for, and the worst plants are not, greatly enhancing the speed at which the optimal genotype is determined. Using this method, and depending on the size of the plant population and the rate of mutation, gene shift stabilizes at around 150 generations. The fitness function, however, is binary in effect, with genotypes either making it into the next generation, or not. A bias could be added to allow those at the top of the chosen 10% to contribute a greater proportion to the gene pool than those at the bottom, but the current algorithm ensures a reasonable degree of genetic diversity is transferred from generation to generation, while maintaining fast evolution rates.

Soils

Three soil textures were used in the simulations, defined by wilting point and field capacity, as outlined in Table 1. The surface area of the soil volume was set to 100cm^2, and the top, middle and bottom soil layers had depths of 10, 10 and 20cm respectively. The soil conductivity parameter K was set to 0.1.

Table 9 Soil texture inputs for model.

Soil	***Wilting Point (%)***	***Field Capacity (%)***	***Storage Capacity (%)***
Sand	7.0	15.0	15.0
Loam	14.0	30.0	30.0
Clay	21.0	40.0	40.0

Simulation Experiments

A range of simulations was run with the model, with different rainfall and soil inputs, listed in Table 11. All models used monthly climate data as input for temperature and evaporation, while rainfall occurred on a daily time step, either simulated or derived from a 50 year daily rainfall data set for the simulation location.

Firstly, the model was run with output set to report gene values for each generation in order to examine gene shifts over time. The model was also run with soil water content of the three soil layers reported daily for a year, under a range of rainfall regimes, including 10mm per 7 days, 20mm per 14 days, strong winter bias, and actual daily rainfall data from seed collection sites SA002 and NSW005.

The pulse size simulation aimed to examine the effects of rainfall event size and frequency on adaptive plant traits, in the three soil types. The model was run across a

gradient in pulse event size and frequency, with the total rainfall held constant, across the three soil types. Pulses occurring at frequencies of 2, 5, 7, 10, 12, 15, 17, 20, 22 and 25 day were used, with the total rainfall amount held constant at 10mm per 7 days, or 520mm $year^{-1}$. There were six replicates of each simulation, and each replicate was run for 150 generations. Climate data for Adelaide was used for the humidity, evaporation and temperature inputs, the variable-with-depth respiration model was used.

The seasonality simulation aimed to examine the effects of rainfall seasonality, ranging from mild summer bias through to strong winter bias, on adaptive plant traits in the three soil types. The model was run across a gradient in seasonal bias in rainfall, from slight summer bias to large winter bias, for the three soil types. Rainfall frequency was constant at an interval of seven days, but amount of rainfall per event in each season varied, from 12mm in summer / 8mm in winter, to 4mm in summer / 16mm in winter at increments of 1mm. Total rainfall amount per year was held constant at 10mm per 7 days, or 520mm $year^{-1}$. There were eight replicates of each simulation, and each replicate was run for 150 generations. Climate data for Adelaide was used for the humidity, evaporation and temperature inputs, the variable-with-depth respiration model was used.

The total rainfall simulation examined plant adaptive response to different total rainfall amounts, with a set rainfall frequency. The model was run across a gradient in total

annual rainfall for the loam soil. Rainfall event frequency was held constant at an interval of seven days, but the amount of rain per event ranged from 4 to 15mm at increments of 1mm. This corresponded to a variation in annual rainfall from 208mm to 780mm. There were six replicates of each simulation, and each replicate was run for 150 generations. Climate data for Adelaide was used for the humidity, evaporation and temperature inputs, the variable-with-depth respiration model was used.

A simulation was also conducted using real daily rainfall records from seed collection locations, in order to determine potential plant adaptation to a real rainfall regime. The model was run using daily rainfall data from stations located near each seed collection site, with the exception of populations SA005, SA011 and VIC002, for which the nearest rainfall recording station was shared by another population in the model. Fifty years of daily rainfall data, from the period 1950 to 2000, was obtained for each station from the Australian Bureau of Meteorology's Patched Point Dataset. Monthly average maximum temperature, 3pm relative humidity and tank evaporation data were also obtained from the Bureau of Meteorology, for the closest station to each collection site that maintained this data. Temperature, humidity and evaporation data were shared by several sites in the model. Weather stations used for each climate variable for each population are listed in Table 10.

Table 10 - Populations and climate stations used in daily rainfall data model runs.

Population	***Rainfall***	***Temperature***	***Humidity***	***Evaporation***
SA001	Pt. Clinton (22022)	Price (22015)	Price (22015)	Price (22015)
SA002	Kadina (22006)	Kadina (22006)	Kadina (22006)	Price (22015)
SA003	Moonta (22011)	Kadina (22006)	Kadina (22006)	Price (22015)
SA004	Manoora (23310)	Bundaleer (21008)	Bundaleer (21008)	Bundaleer (21008)
SA006	Mount Bryan (21034)	Bundaleer (21008)	Bundaleer (21008)	Bundaleer (21008)
SA007	Spalding (21047)	Clare (21014)	Clare (21014)	Bundaleer (21008)
SA008	Blyth (21003)	Clare (21014)	Clare (21014)	Bundaleer (21008)
SA009	Clare (21014)	Clare (21014)	Clare (21014)	Bundaleer (21008)

Population	***Rainfall***	***Temperature***	***Humidity***	***Evaporation***
SA010	Keith (25507)	Keith (25507)	Keith (25507)	Padthaway (26089)
SA012	Mundulla (25510)	Serviceton (78034)	Serviceton (78034)	Padthaway (26089)
SA013	Wolseley (25519)	Serviceton (78034)	Serviceton (78034)	Padthaway (26089)
SA020	Langhorne Creek (24515)	Murray Bridge (24521)	Murray Bridge (24521)	Adelaide (23000)
SA021	Callington (24508)	Strathalbyn (23747)	Strathalbyn (23747)	Adelaide (23000)
SA022	Goolwa (23718)	Victor Harbour (23751)	Victor Harbour (23751)	Adelaide (23000)
SA023	Cape Jervis (23773)	Victor Harbour (23751)	Victor Harbour (23751)	Adelaide (23000)
VIC001	Glenorchy (79015)	Stawell (79080)	Stawell (79080)	Stawell (79080)
VIC003	Litchfield (78027)	Donald (78072)	Donald (78072)	Stawell (79080)

Population	***Rainfall***	***Temperature***	***Humidity***	***Evaporation***
VIC004	Donald (78072)	Donald (78072)	Donald (78072)	Stawell (79080)
VIC005	Narrewillock (80094)	Wycheproof (78042)	Donald (78072)	Stawell (79080)
VIC006	Boort (80002)	Boort (80002)	Boort (80002)	Stawell (79080)
VIC007	Cobram (80007)	Numurka (80101)	Boort (80002)	Stawell (79080)
NSW001	Deniliquin (74128)	Deniliquin (74128)	Deniliquin (74128)	Deniliquin (74128)
NSW002	Finley (74042)	Berrigan (74009)	Berrigan (74009)	Deniliquin (74128)
NSW003	Berrigan (74009)	Berrigan (74009)	Berrigan (74009)	Deniliquin (74128)
NSW004	Jerilderie (74055)	Berrigan (74009)	Berrigan (74009)	Deniliquin (74128)
NSW005	West Wyalong (50044)	Wyalong (73054)	Wyalong (73054)	Deniliquin (74128)

Population	***Rainfall***	***Temperature***	***Humidity***	***Evaporation***
NSW006	Yalgogrin North (50045)	Wyalong (73054)	Wyalong (73054)	Deniliquin (74128)
NSW007	Rankins Springs (75057)	Wyalong (73054)	Wyalong (73054)	Deniliquin (74128)

Each location was modelled for 8 replicates of 150 generations. For each generation, lasting two years, a random pair of two consecutive years was selected from the 50 years of rainfall data for that location for model input. This allowed plants to experience a broad range of rainfall regimes recorded at a particular location, in order to drive selection with long-term rainfall patterns rather than a fixed and possibly unrepresentative shorter period. However, due to the strong influence of total annual rainfall on productivity and drainage and the variability in annual rainfall from year to year, results for absolute biomass was not comparable between locations, and the analysis focuses on long-term gene shifts and proportional allocation.

The respiration function simulation aimed to determine the importance of high cost shallow roots in influencing optimal root distribution under a range of rainfall event sizes. The three different soil respiration sub-models were tested, depth-variable, constant respiration and time-lag. The model was run with each respiration method

over a range of pulse frequencies, including watering events at frequencies of 2 days, 7 days, 12 days and 20 days. Total rainfall amount over the year was held constant at 10mm per 7 days, or 520mm year^{-1}. There were six replicates of each simulation, and each replicate was run for 150 generations. Climate data for Adelaide was used for the humidity, evaporation and temperature inputs.

Finally, the soil surface area simulation aimed to mimic plant density effects by reducing the total soil water volume plants had access to, in order to determine plant adaptive response to density and competition. The model was run with a range of soil surface areas per plant, determining total water volume accessible to the roots, as a means of representing plant density and competition. Total rainfall amount over the year was held constant at 10mm per 7 days, or 520mm year^{-1}. There were 8 replicates of each simulation, and each replicate was run for 150 generations. Climate data for Adelaide was used for the humidity, evaporation and temperature inputs.

Table 11 - Summary of simulation experiments.

Simulation	**Rainfall Data**	**Soil**	**Number of Runs**	**Other Notes**
Pulse size	520mm per year, but delivered at frequencies from 2 to 25 days.	Sand, Clay and Loam	6 replicates x 150 generations	Adelaide climate data, depth variable respiration.
Seasonality	520mm per year, delivered every 7 days. With a seasonal bias ranging from 40% winter rain to 80% winter rain.	Sand, Clay and Loam	8 replicates x 150 generations	Adelaide climate data, depth variable respiration.

Total water	Range 208mm to 780 mm annually, with rainfall every 7 days.	Loam	6 replicates x 150 generations	Adelaide climate data, depth variable respiration.
Daily Rainfall Record	Each model year used one year of rainfall data, randomly chosen from 50 years of data for seed collection locations.	Loam	8 replicates x 150 generations	Local climate data, depth variable respiration.
Respiration	520mm per year, but delivered at frequencies from 2 to 25 days.	Loam	6 replicates x 150 generations	Adelaide climate data, depth variable, lag and fixed respiration functions.

Soil Surface Area	520mm per year, 10mm per 7 days.	Loam	8 replicates x 150 generations	Adelaide climate data, soil surface area ranging from 25cm^2 to 150cm^2.

11.3. Results

The outputs of the evolutionary algorithm model are qualitatively validated and compared with experimental results in the overall thesis discussion. Due to the model dealing with carbon weight, compared with dry biomass in the experiments, and the lack of inclusion of additional factors that influence absolute growth such as nutrients, growth output of the model cannot be directly compared with experimental results. The model results aim to concentrate on gene selection rather than growth.

Gene shifts and selection

The model was set to output average gene values for each generation in order to track shifts in gene value during the process of selection. The model was run with a 10mm watering event every seven days, with the depth-variable respiration function and monthly averate of Adelaide climate data.

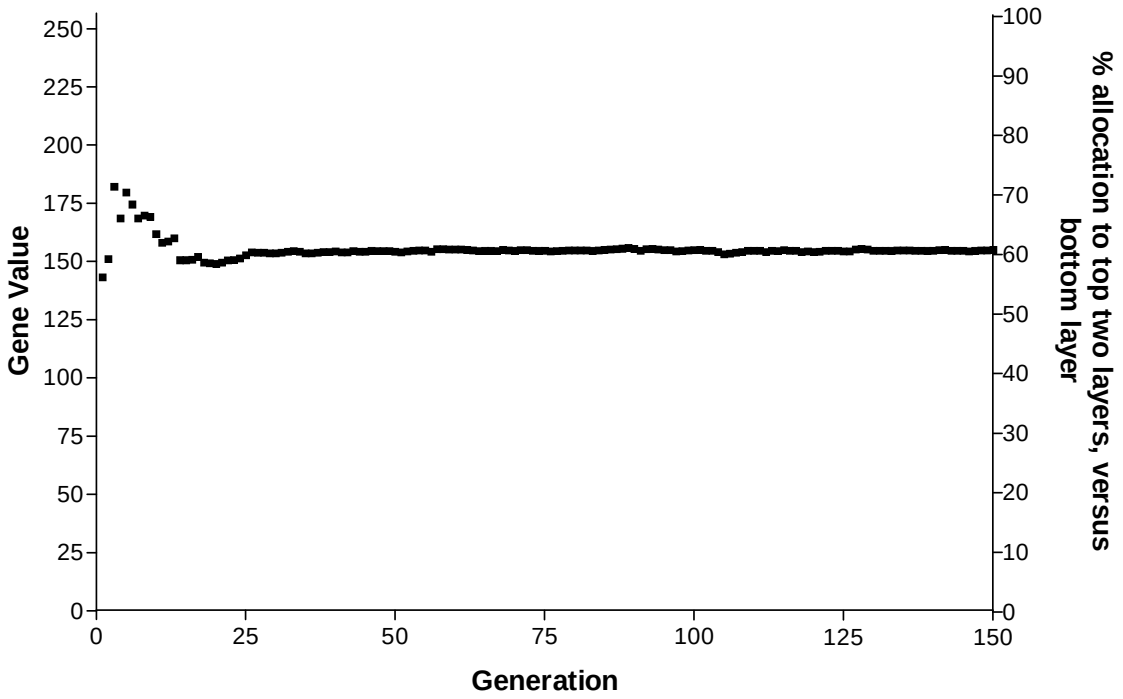

Figure 55 - Shift in value of RD1 gene, a gene under strong selective pressure, over 150 model generations.

Figure 55 shows the shift in the average value of the gene RD1 over 150 generations. This gene undergoes strong selection, and after an initial shift in average gene value, the optimal value of approximately 150, indicating 58% allocation to the upper layer, is reached by generation 30. After this point, variation in the average gene value is primarily due to random mutation.

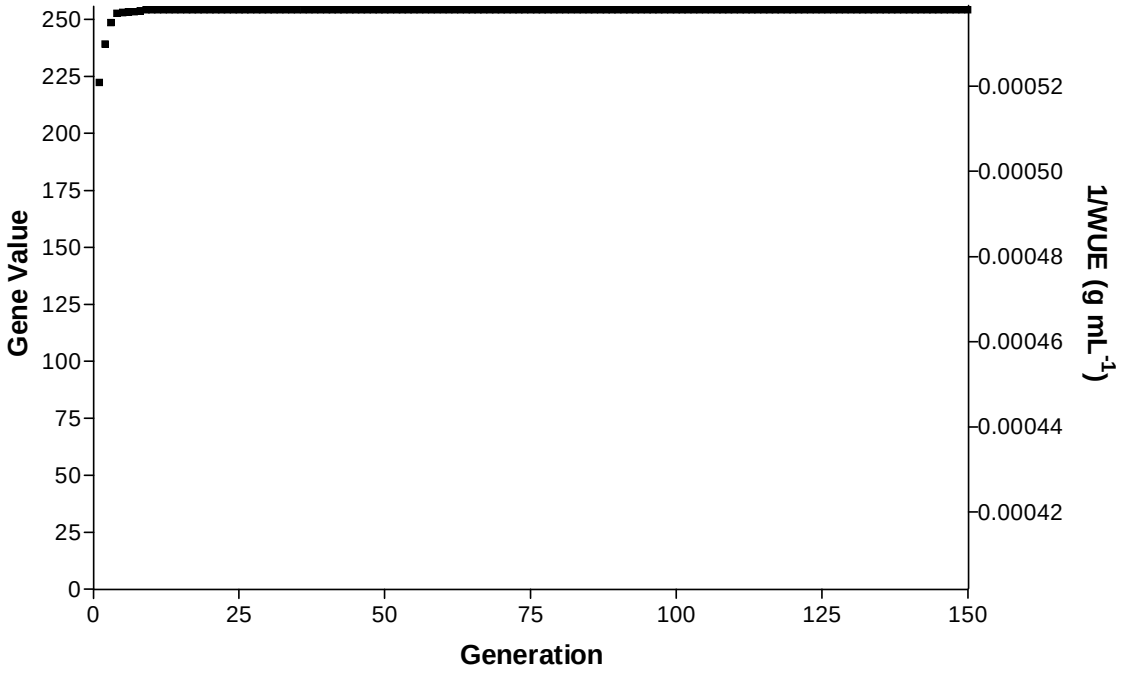

Figure 56 - Shift in value of WUE gene, a gene where higher values always produce greater growth, over 150 model generations.

Figure 56 shows the shift in the average value of the WUE gene, a gene for which higher values appear to always create fitter plants; in this case water use efficiency is always beneficial at a maximum. This gene value quickly shifts to the maximum possible value, 255, after only a few generations, reflecting the lack of trade-off in this character in the model.

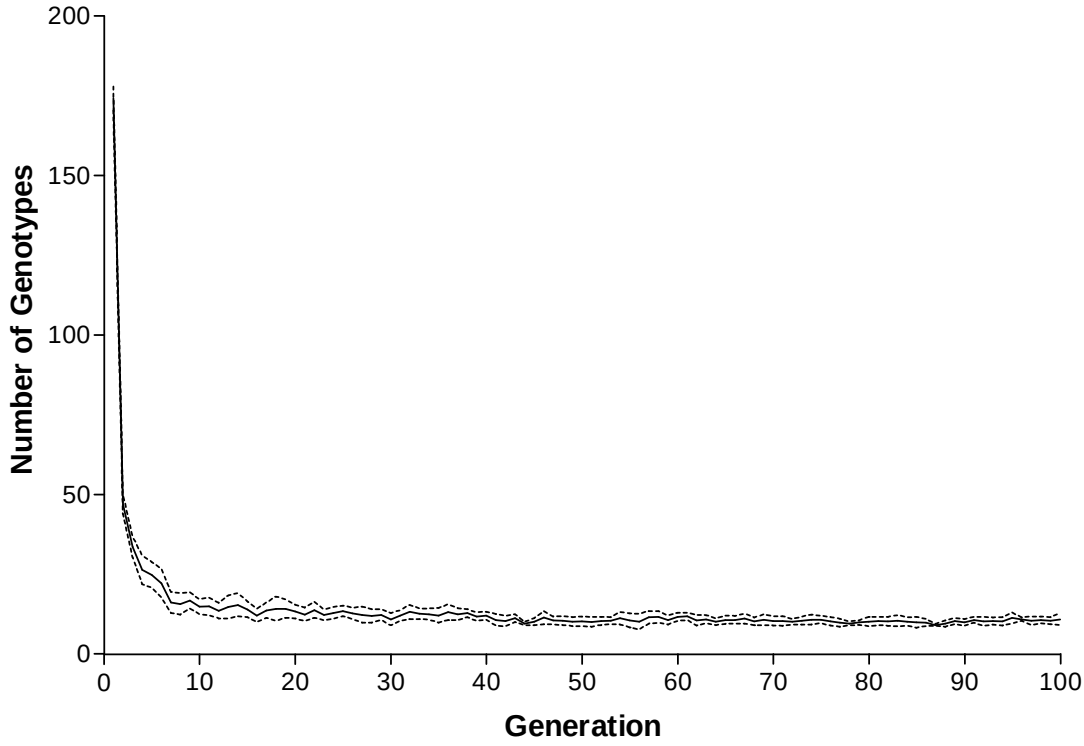

Figure 57 - Change in the number of different genotypes of the RD0 gene in the population over 100 generations. Dashed lines indicate standard error.

Figure 57 shows a count of discrete genotypes, representing the allelic diversity, for the RD0 gene over 100 generations of modelling, run with 10 simulation replicates. Genetic diversity rapidly decreases in the first 10 to 20 generations, as unfit alleles are purged. However, diversity is maintained at approximately 10 alleles after this point, due to random mutations occurring at a frequency of 3% in a population of 300 plants.

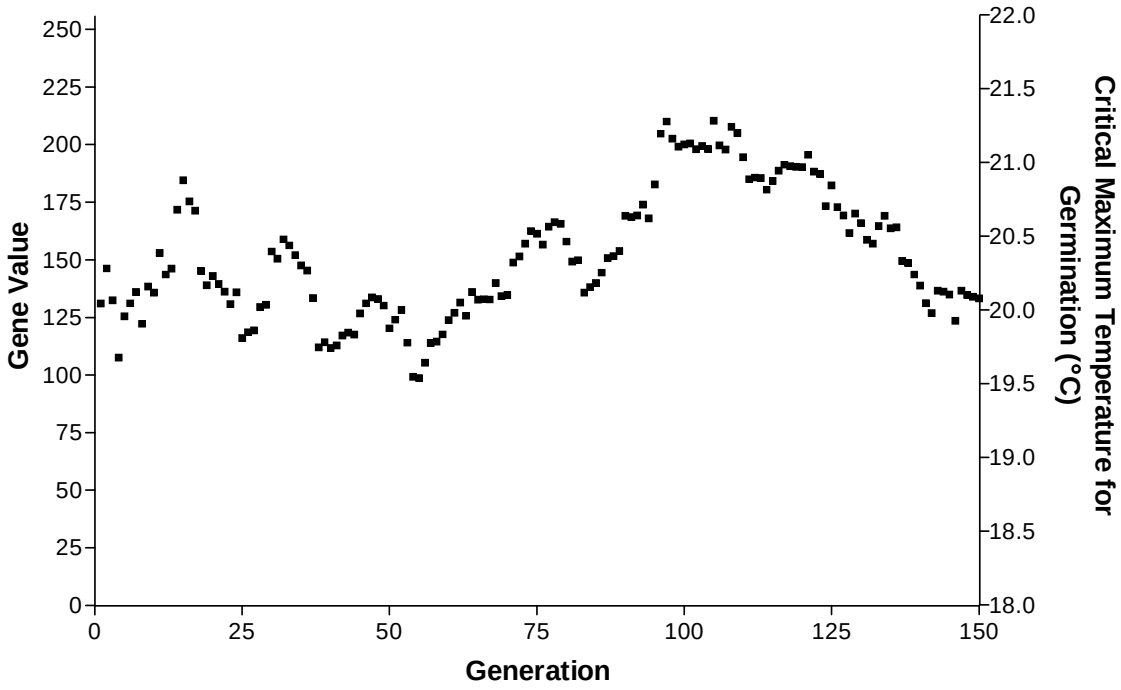

Figure 58 - Shift in value of Germ_T gene, a gene under no selective pressure, over 150 model generations.

Figure 58 shows the shift in average gene value of the Germ_T gene, defining minimum temperature for germination. This gene did not contribute to the fitness of the plants, and did not face any selective pressure, as indicated by the random walk of average gene value over 150 generations.

Soil moisture regime

The model was set to output the soil water content of each soil layer over one year of execution, under a variety rainfall regimes, including 10mm per 7 days, 14mm per 20 days, strong winter rainfall bias, and using real daily rainfall records from two locations.

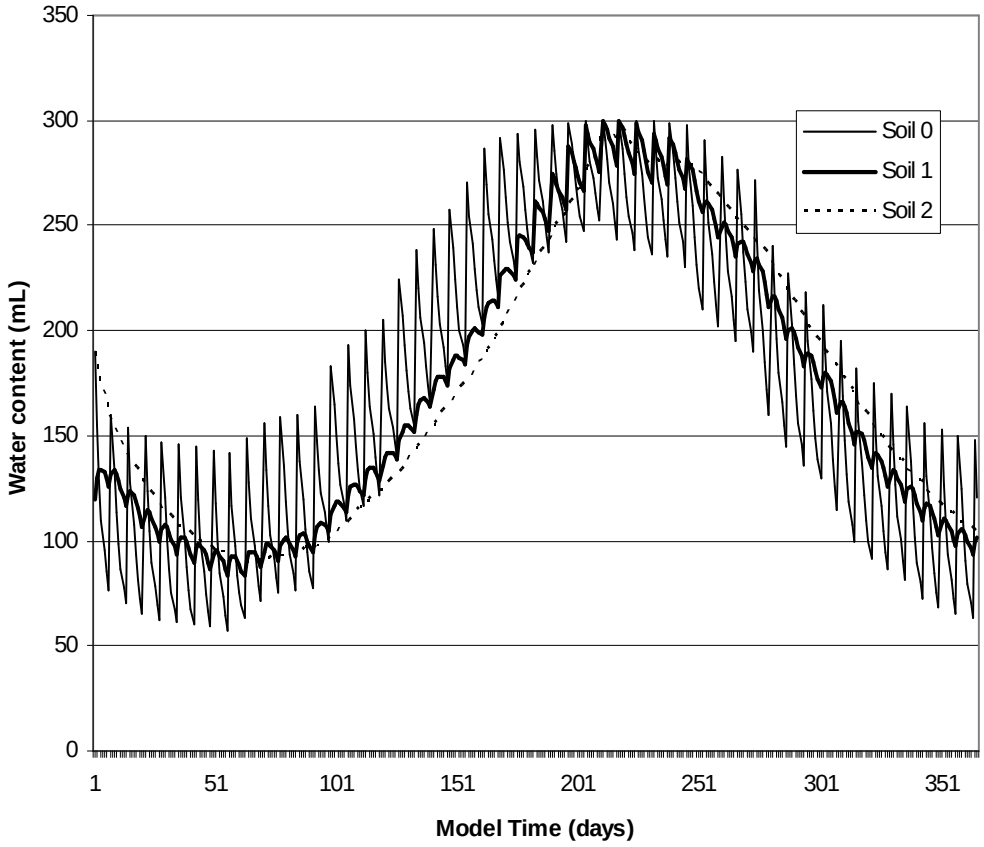

Figure 59 - Soil water content in three soil layers over one year of model execution, with a 10mm rainfall event every seven days

Figure 59 sows the soil water content in the three soil layers, over one year, with a 10mm rainfall event supplied once a week. The surface soil layer undergoes greater

variation in soil water content than the deeper layers, and deeper soil layers display a delayed response to rainfall input. There is a peak in soil water content in winter, with low soil water content in summer, due to different evaporative demand.

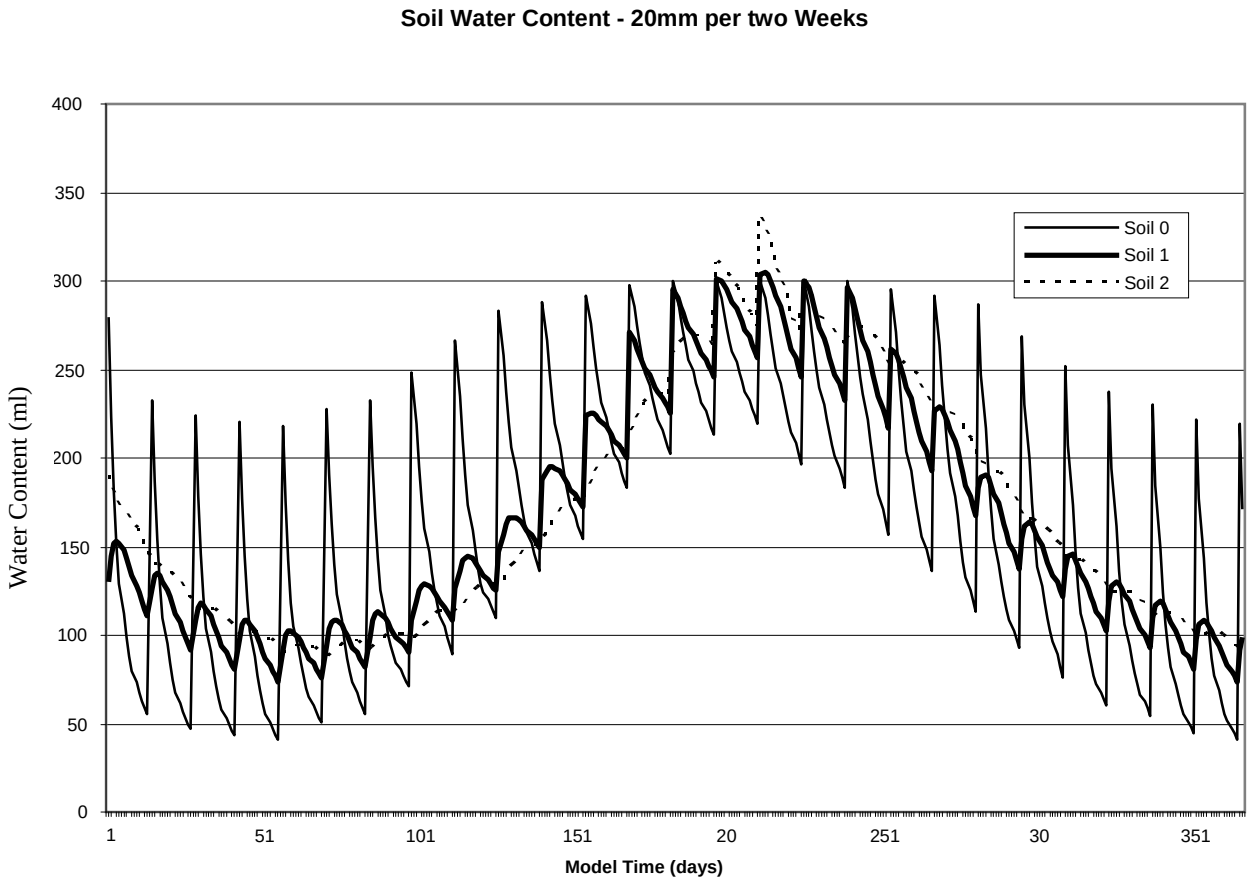

Figure 60 - Soil water content in three soil layers over one year of model execution, with a 20mm rainfall event every 14 days.

Figure 60 shows the soil water content in the three soil layers, over one year, with a 20mm rainfall event supplied every two weeks. Variation in soil water content is greater than with the smaller, more frequent pulses, with even the deepest soil layer showing an oscillating pattern of soil moisture. The larger events result in deeper

penetration of water, resulting in greater water content in the deeper soil layers, particularly in winter when soil evaporation is low.

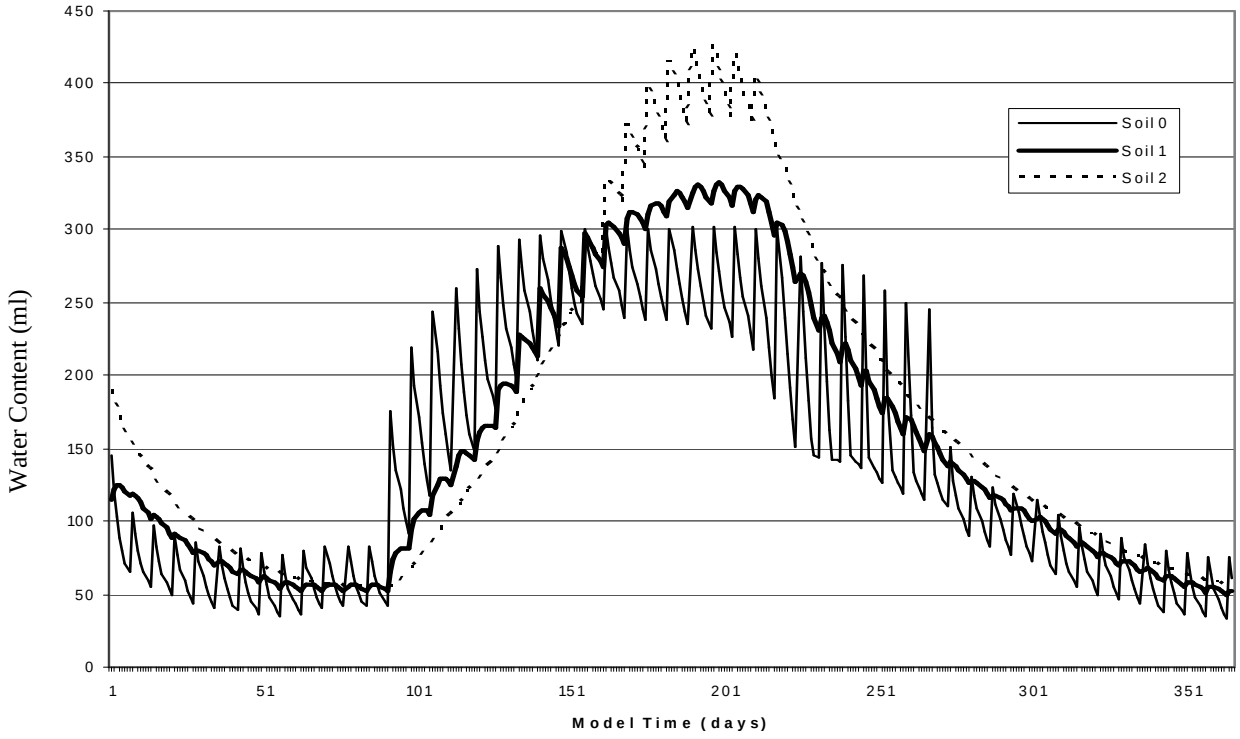

Figure 61 - Soil water content in three soil layers over one year of model execution, 5mm per seven days in the summer half-year, and 15mm per seven days in the winter half-year.

Figure 61 shows soil water content over a year, under a rainfall regime biased strongly towards winter rainfall, with 15mm per week during the winter half-year, and 5mm per week delivered during the summer. Winter rainfall bias leads to high soil water content in winter, especially in the deeper soil layers due to penetration of large rainfall events, while soil water content during summer is low, and decreases throughout the season.

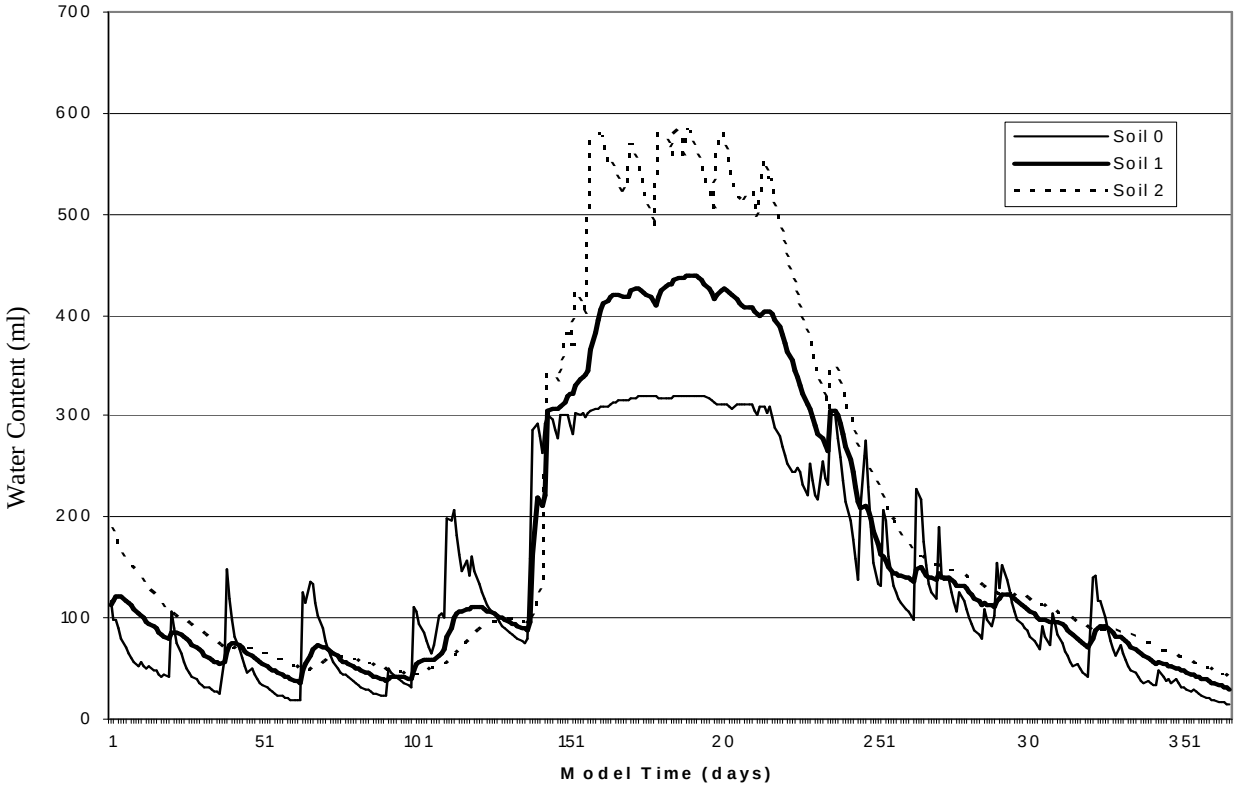

Figure 62 - Soil water content in three soil layers over one year of model execution, using daily rainfall data from site SA002, year 1956.

Figure 62 shows modelled soil water content using real rainfall data from the seed collection location SA002, near Kadina on the Yorke Peninsula in South Australia. The year 1956 was chosen randomly from the dataset to produce this graph. This is a strongly winter-biased rainfall regime, with small, frequent rainfall events. Soil water content is high during the winter growing season, especially in deep soil layers, while soil water content during the summer is low, with occasional rainfall events that only wet surface soil layers.

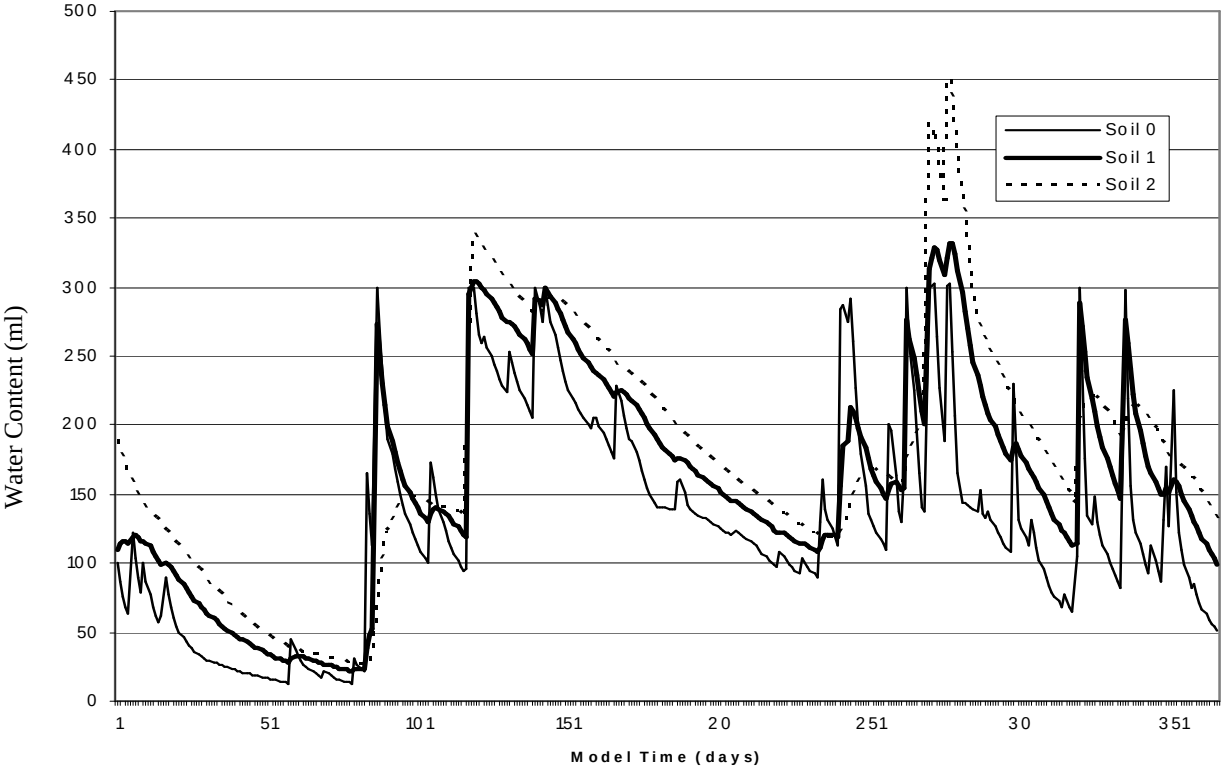

Figure 63 - Soil water content in three soil layers over one year of model execution, using daily rainfall data from site NSW005, year 1970.

Figure 63 shows modelled soil water content using real rainfall data from the seed collection location NSW005, near West Wyalong in southern New South Wales. The year 1970 was chosen randomly from the dataset to produce this graph. There is no bias towards rainfall during a particular season at this location, and the site receives a greater proportion of large rainfall events. There is no seasonal winter hump in soil water content visible at this location, rather a series of infrequent, large events provide the greatest influence over soil water content. Rainfall penetration to deeper layers depends on the water content of shallower layers at the time of a large rainfall event, as can be seen comparing the peaks at day 90 and day 120. Soil water content during

summer has the potential to be high during periods after large rainfall events.

Pulse Size and Interval, and Soil Texture

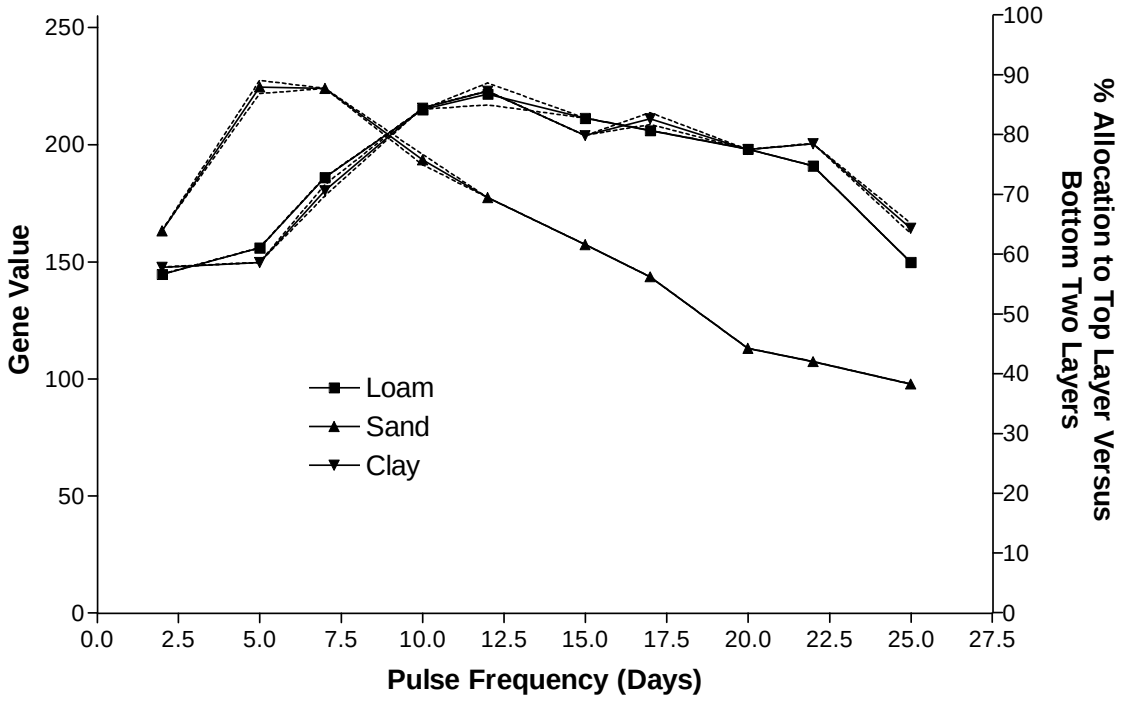

Figure 64 - Optimal RD0 gene value across a gradient in water pulse frequency, for loam, sand, and clay soils.

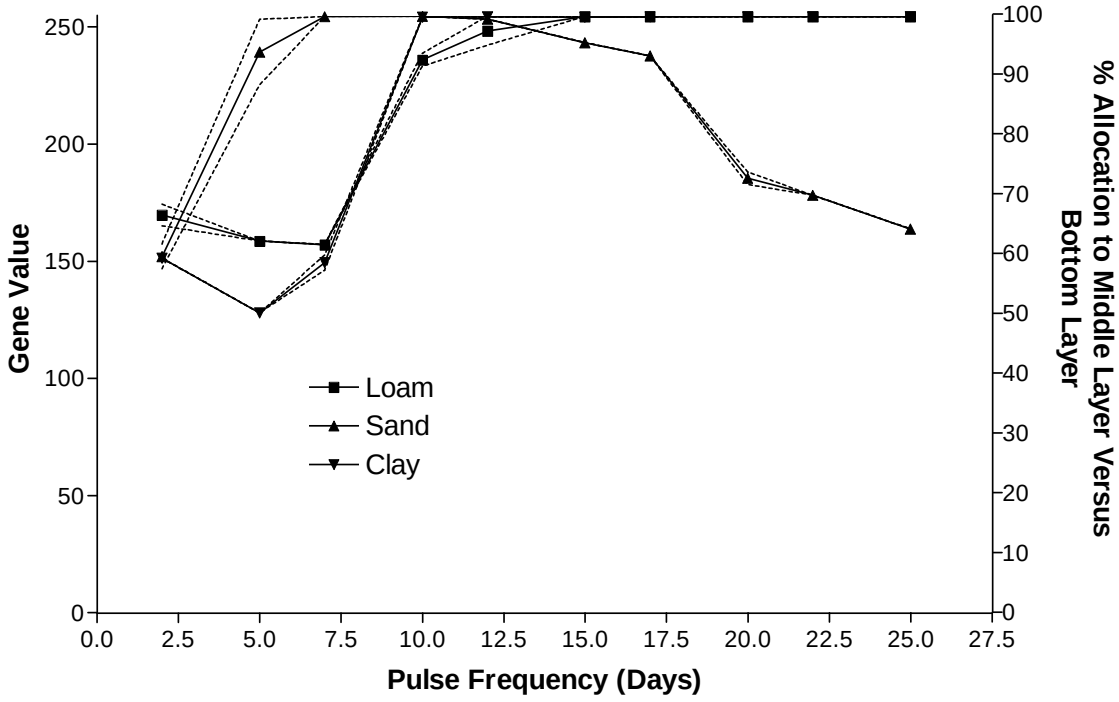

Figure 65 - Optimal RD1 gene value across a gradient in water pulse frequency, for loam, sand, and clay soils.

Figure 64 and Figure 65 show the shift in optimal values of the RD0 gene, determining biomass partitioning between the top and middle soil layers, and the RD1 gene, determining biomass partitioning between the middle and bottom soil layers, across a gradient in watering frequency. Clay and loam soil appear to have similar patterns of root depth distribution, with the most roots in the surface layer found at a watering frequency of 12 days. Deepest roots were present with small, frequent pulses, but were lost as watering frequency decreased. Sand soil displayed a different pattern, with a peak in shallow roots at a more frequent pulse interval, around 5 days. In the allocation

between the middle and deepest soil layers, sand soil showed the opposite trend to the other two soil types, with shallow roots with frequent pulses, and deeper roots with infrequent pulses.

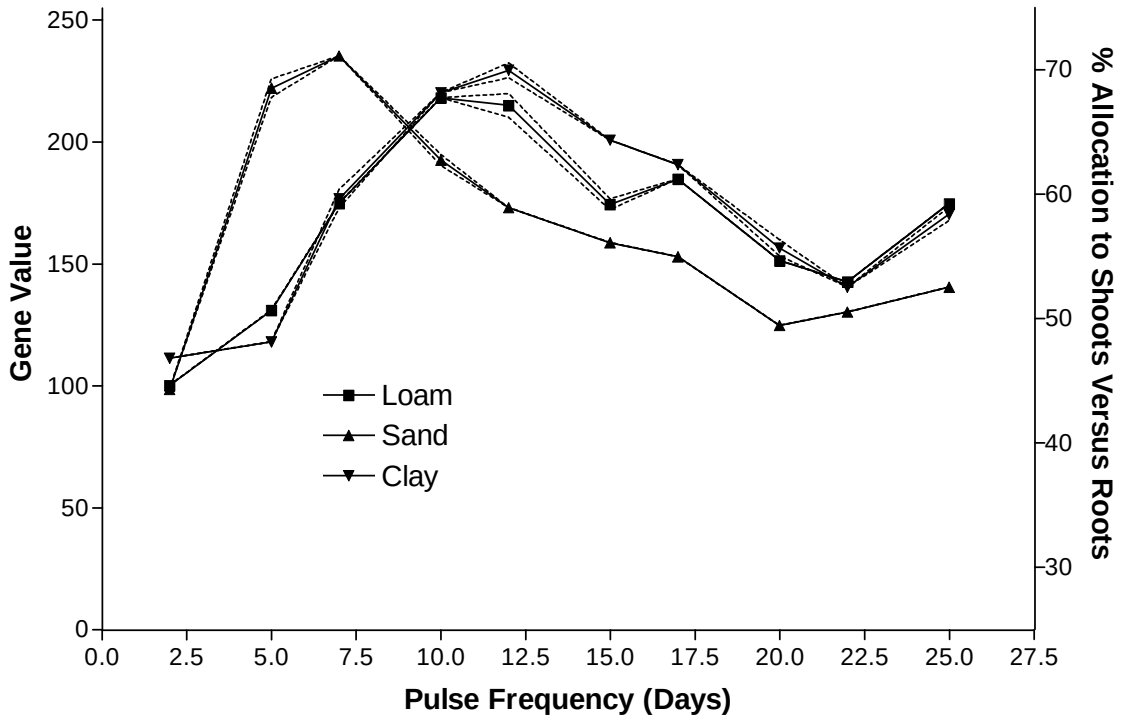

Figure 66 - Optimal RS gene value across a gradient in water pulse frequency, for loam, sand, and clay soils. Dashed line indicates standard error.

Figure 66 shows the optimal RS gene value for the three soil types across a gradient in watering frequency. The RS gene represents the shoot:root ratio, with higher values indicating more shoot allocation, and lower values indicating more root allocation. All soil types showed a similar trend in optimal allocation, with a peak in shoot allocation at intermediate pulse frequencies, although the peak RS gene value for sand soil

occurred at a higher watering frequency than the other soil types.

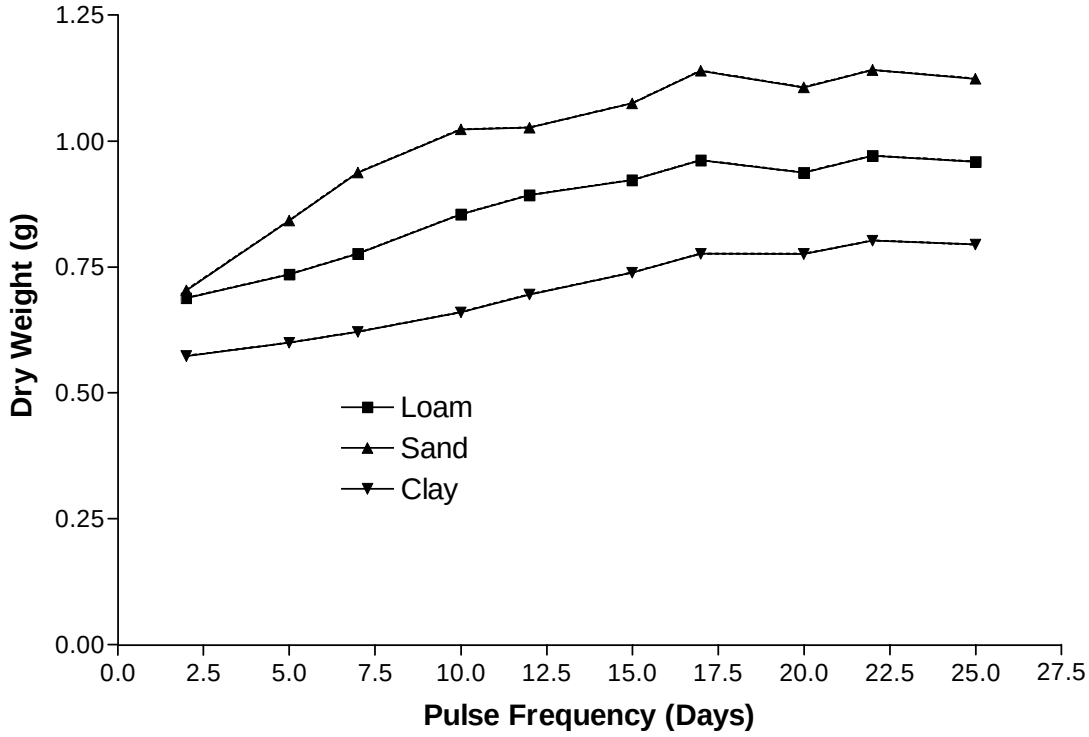

Figure 67 - Total dry weight across a gradient in water pulse frequency, for loam, sand, and clay soils.

Figure 67 shows the average total dry weight of plants at the end of model execution, for the three soil types over a gradient in watering frequency. All three soil types show a similar trend, with an increase in biomass with large, infrequent watering events. However, overall biomass was greater with coarser soils.

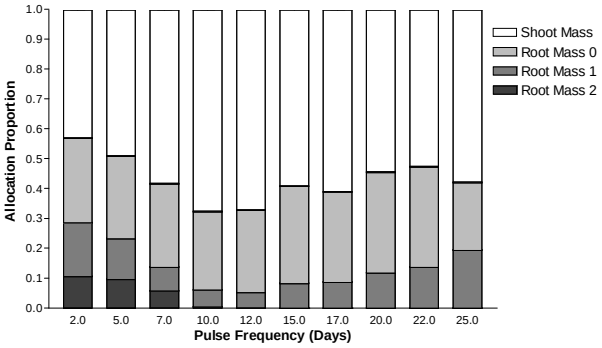

Figure 68 - Proportion of biomass allocated to shoot and root layers across a gradient in water pulse frequency, for loam soil.

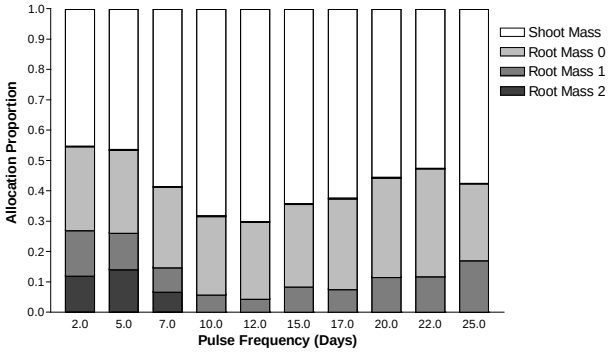

Figure 69 - Proportion of biomass allocated to shoot and root layers across a gradient in water pulse frequency, for clay soil.

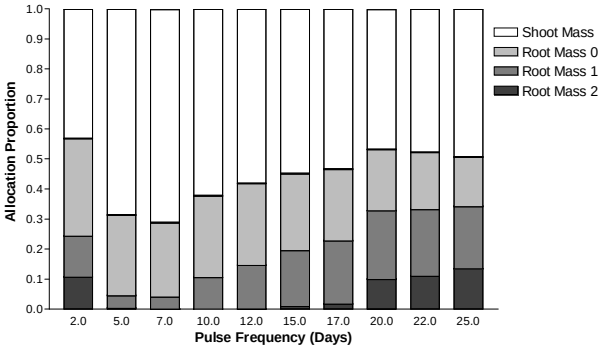

Figure 70 - Proportion of biomass allocated to shoot and root layers across a gradient in water pulse frequency, for sand soil.

Figure 68, Figure 69, and Figure 70 show the proportion of biomass allocation to shoots and roots, for loam, clay and sand soil, across a gradient in watering interval. Loam and clay show similar patterns of biomass allocation, with the deepest roots only optimal with small, frequent pulses. Shoot allocation is greatest at intermediate pulse frequencies, with larger, less frequent watering events resulting in greater root mass, and greater root allocation in the middle soil layers. Sand soil also shows a peak in

shoot biomass at intermediate watering interval, but deep roots are optimal with both small, frequent watering events, and with decreasing pulse frequency.

Seasonal Rainfall Bias and Soil Texture

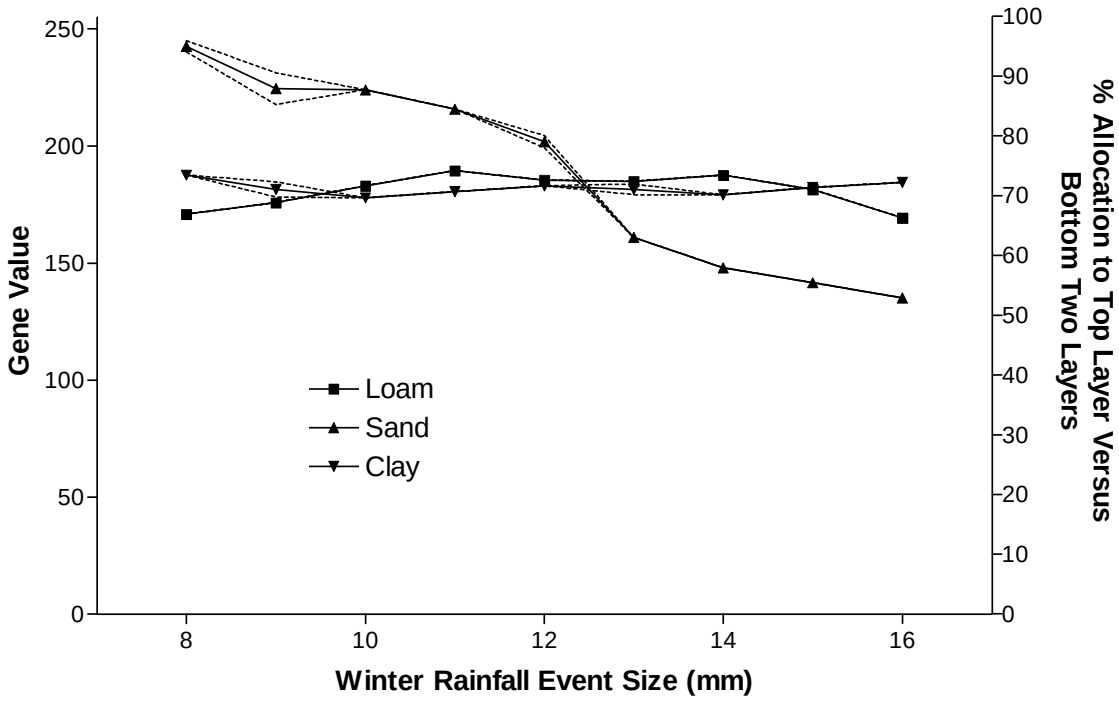

Figure 71 - Optimal RD0 gene value across a gradient in seasonal rainfall bias, for loam, sand, and clay soils.

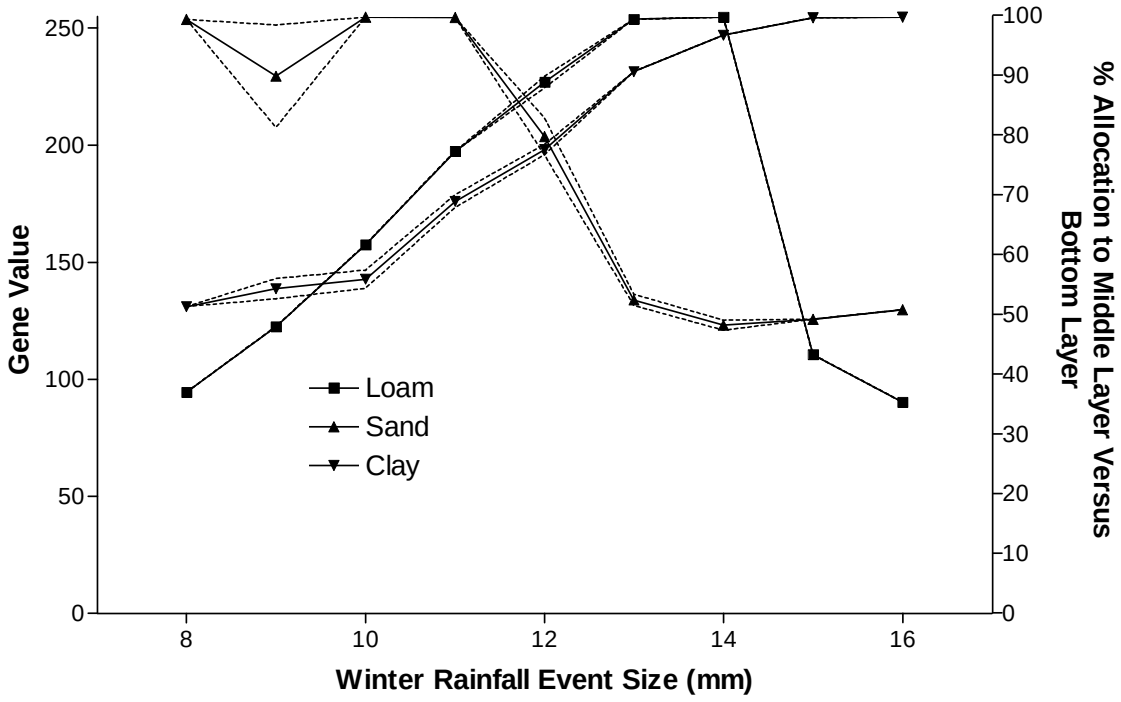

Figure 72 - Optimal RD1 gene value across a gradient in seasonal rainfall bias, for loam, sand, and clay soils.

Figure 71 and Figure 72 show the optimal RD0 and RD1 gene values across a gradient in seasonal rainfall bias for the three soil types. Root allocation between the surface and middle layers, determined by RD0, remains stable for loam and clay soil, while for sand soil, more surface roots are optimal with low winter rainfall, with allocation to the middle layer increasing as winter rainfall increases. Root allocation between the middle and deepest soil layers, as determined by RD1, varies greatly between soil types, with sand soil showing deeper roots with increasing winter rainfall, clay soil showing shallower roots with increasing winter rainfall, and loam soil showing allocation to deep roots with both low and high winter rainfall, and shallower roots at

intermediate seasonal rainfall bias.

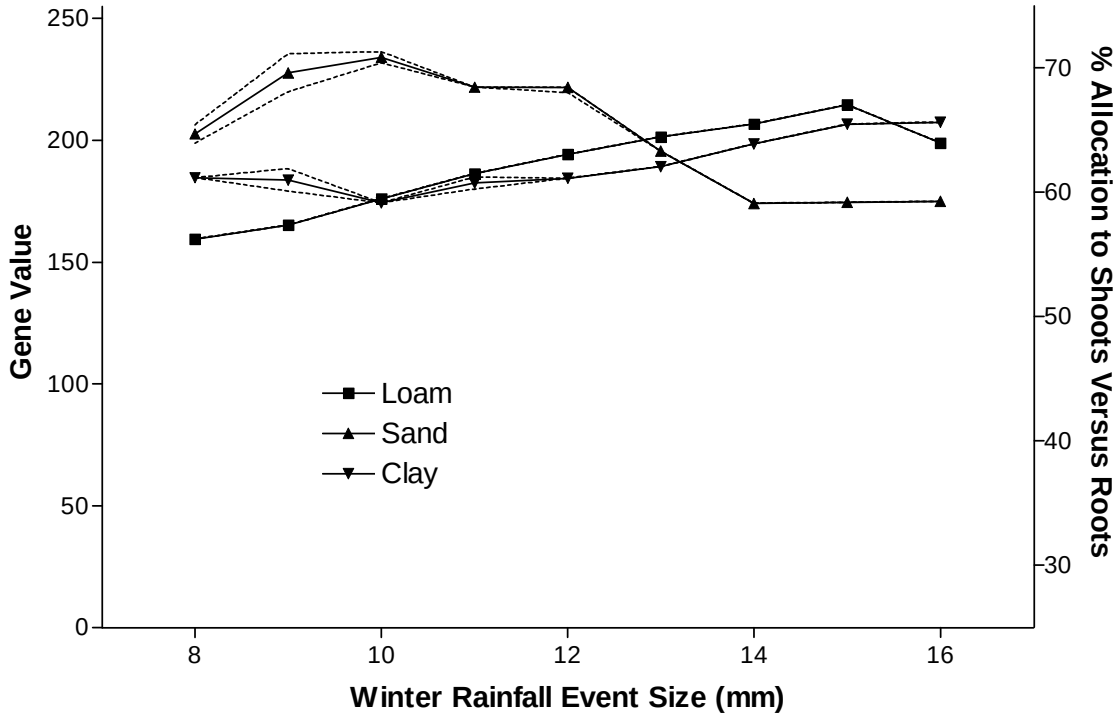

Figure 73 - Optimal RS gene value across a gradient in seasonal rainfall bias, for loam, sand, and clay soils.

Figure 73 shows the optimal RS gene value, reflecting shoot:root ratio, across a gradient in seasonal rainfall for the three soil types. Clay and loam soil show a slight trend towards increasing shoot biomass with increasing winter rainfall, while sand soil shows the opposite trend, with greater shoot biomass under and equitable or summer rainfall regime.

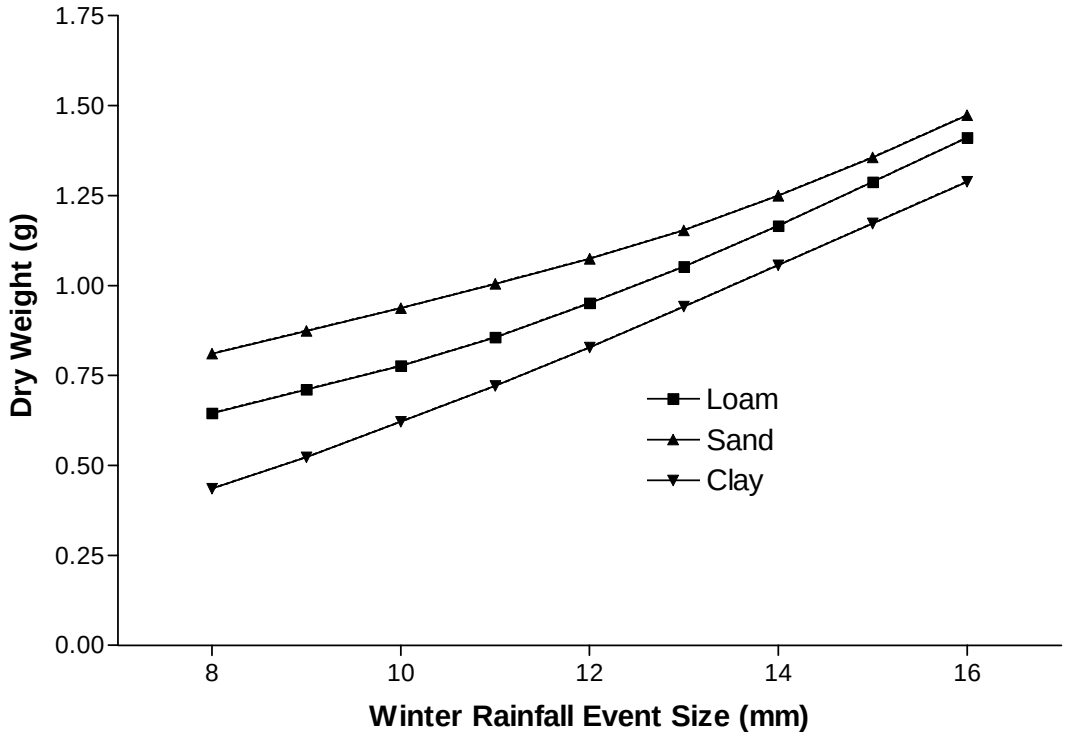

Figure 74 - Total dry weight across a gradient in seasonal rainfall bias, for loam, sand, and clay soils.

Figure 74 shows shift in total dry weight of plants across a gradient in seasonal water bias for the three soil types. For all three soil types, total biomass increases with increasing winter rainfall. Seasonal rainfall proportion in the model relies on delivering larger events in one season than the other, so the potential exists for trends in total dry weight to be a result of event size rather than seasonal bias. However, winter rainfall event sizes below 10mm were modelled by having larger summer rainfall events than winter, and these values still resulted in low biomass, suggesting the trend is the result of increased efficiency of winter water, rather than an effect of large watering events. Again, as with the model of pulse frequency, sand soil results in the greatest dry

weight, while clay soil produces the least.

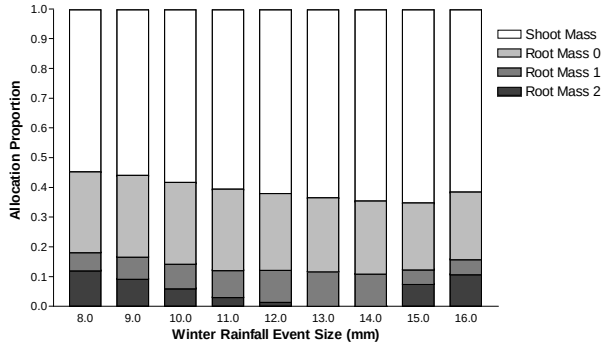

Figure 75 - Proportion of biomass allocated to shoot and root layers across a gradient in seasonal rainfall bias, for loam soil.

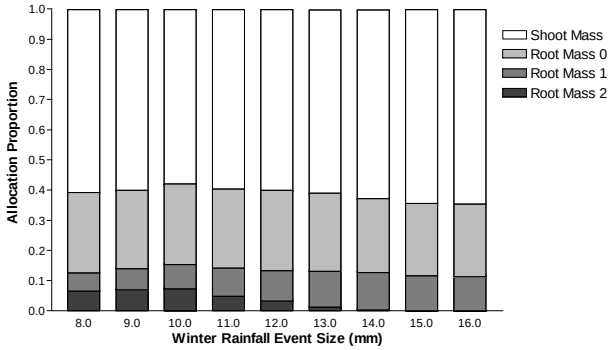

Figure 76 - Proportion of biomass allocated to shoot and root layers across a gradient in seasonal rainfall bias, for clay soil.

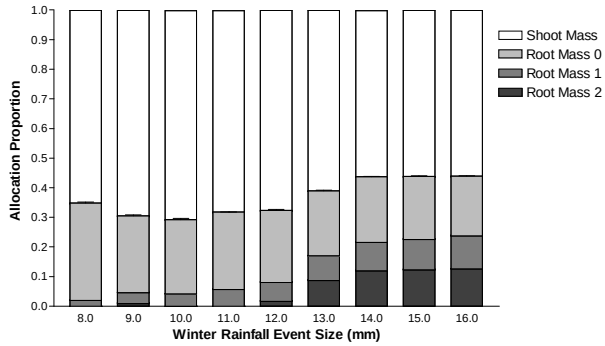

Figure 77 - Proportion of biomass allocated to shoot and root layers across a gradient in seasonal rainfall bias, for sand soil.

Figure 75 shows the proportion of biomass allocation to shoots and roots across a gradient in seasonal watering bias for loam soil. Deepest roots are developed in loam soil with both low and high winter rainfall bias. Figure 76 shows the proportion of biomass allocation to shoots and roots across a gradient in seasonal watering bias for

clay soil. Unlike the loam soil, deepest roots are only optimal under low winter rainfall, with high winter rainfall resulting in a shallower root distribution. Figure 77 shows the proportion of biomass allocation to shoots and roots across a gradient in seasonal watering bias for sand soil. Sand soil shows the opposite trend to clay soil, with a decrease in shallow root allocation and an increase in deep root allocation with increasing winter rainfall.

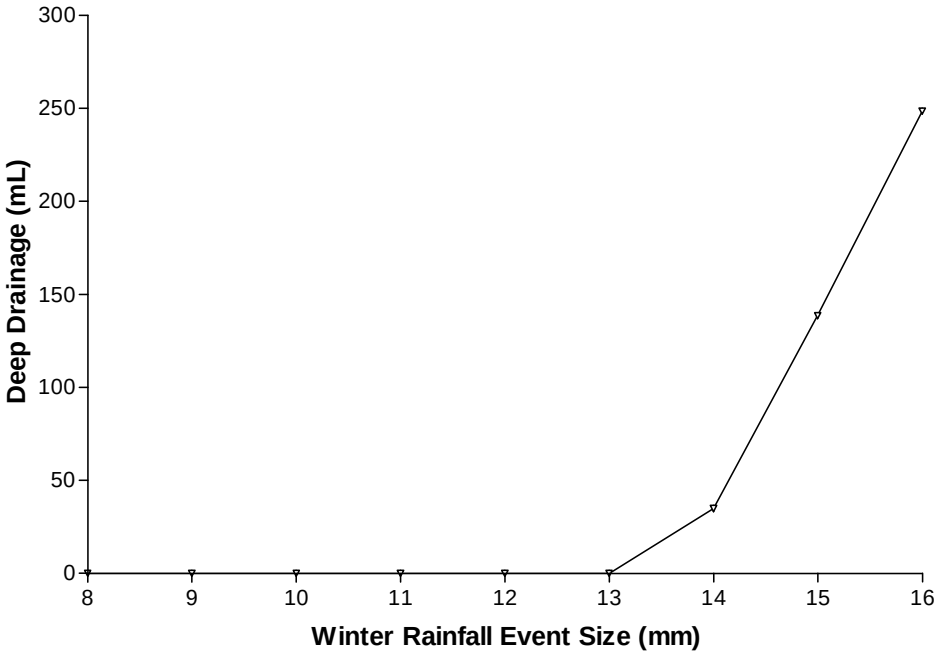

Figure 78 - Deep drainage loss across a gradient in winter rainfall bias for sand soil.

Deep drainage was only observed in sandy soil under a strongly winter biased rainfall regime, as shown in Figure 78.

Total Weekly Rainfall

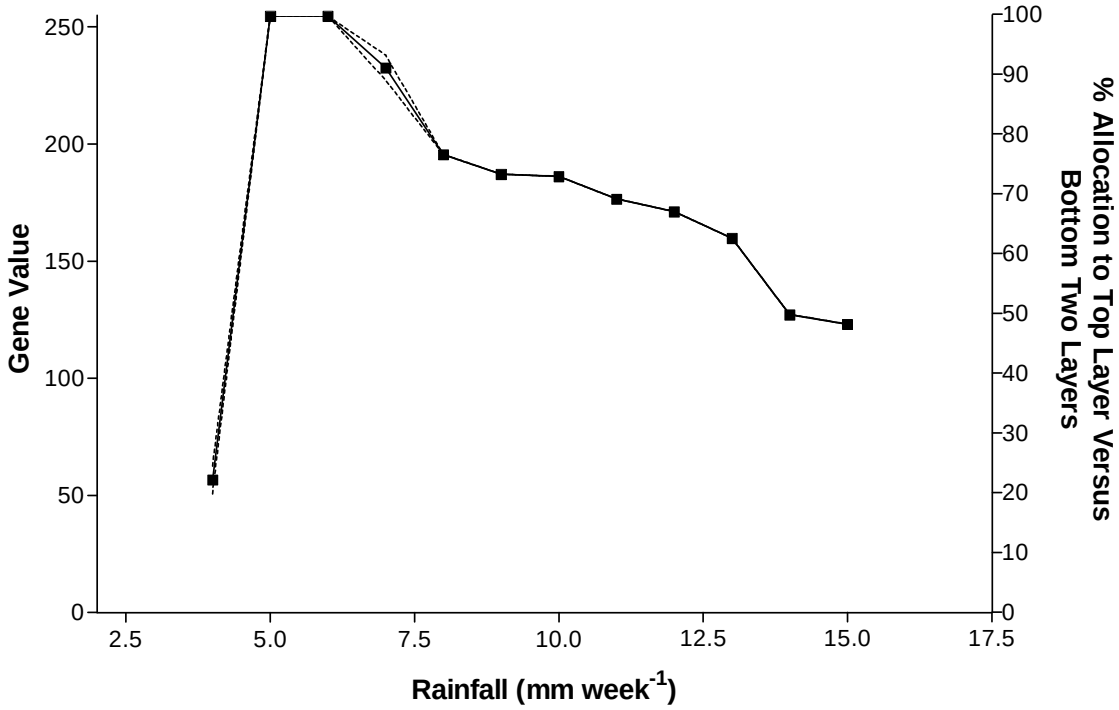

Figure 79 - Optimal RD0 gene value across a gradient in total rainfall, with a seven day pulse interval, for loam soil.

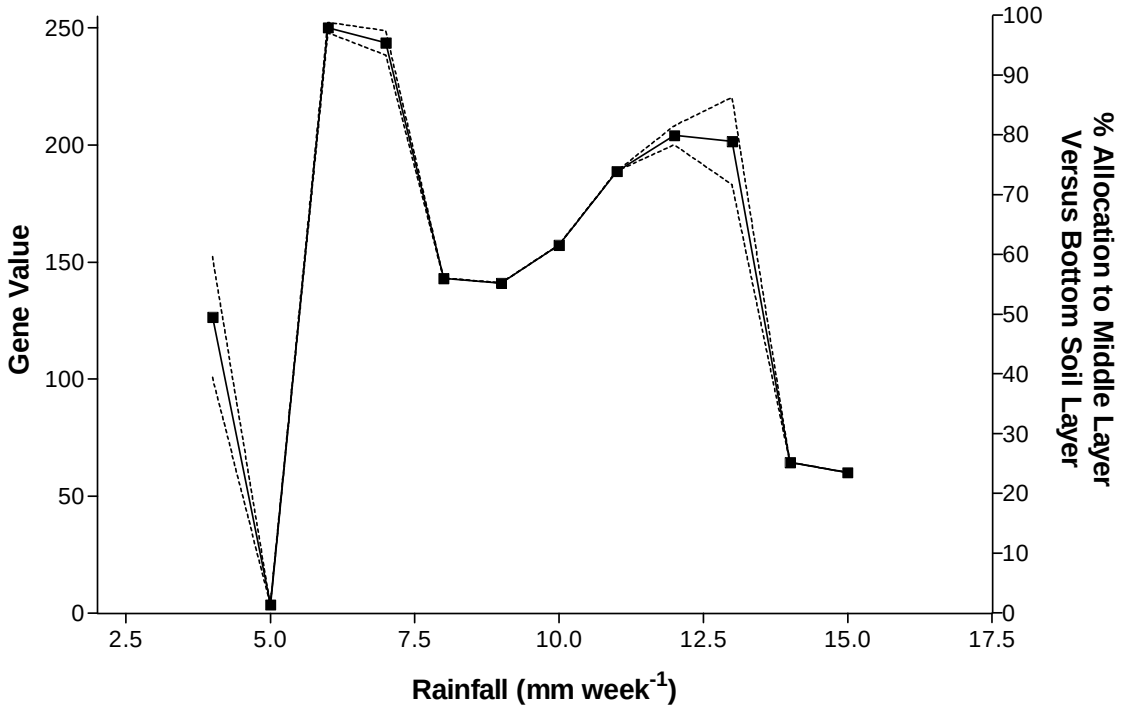

Figure 80 - Optimal RD1 gene value across a gradient in total rainfall, with a seven day pulse interval, for loam soil.

Figure 79 and Figure 80 show the shift in the optimal RD0 and RD1 gene values along a gradient in total weekly rainfall. Extremely low rainfall values produce optimal gene values that go against the trend seen at higher rainfall totals, due to the extremely low biomass totals produced with low rainfall (Figure 82). Roots in the top and middle layers, as defined by RD0, tend deeper as the rainfall amount increases. The RD1 gene, indicating root allocation to the deepest layer, shows a more complex pattern, possibly reflecting the trade-off between relying on deep water under low rainfall, and the deep

penetration of larger events.

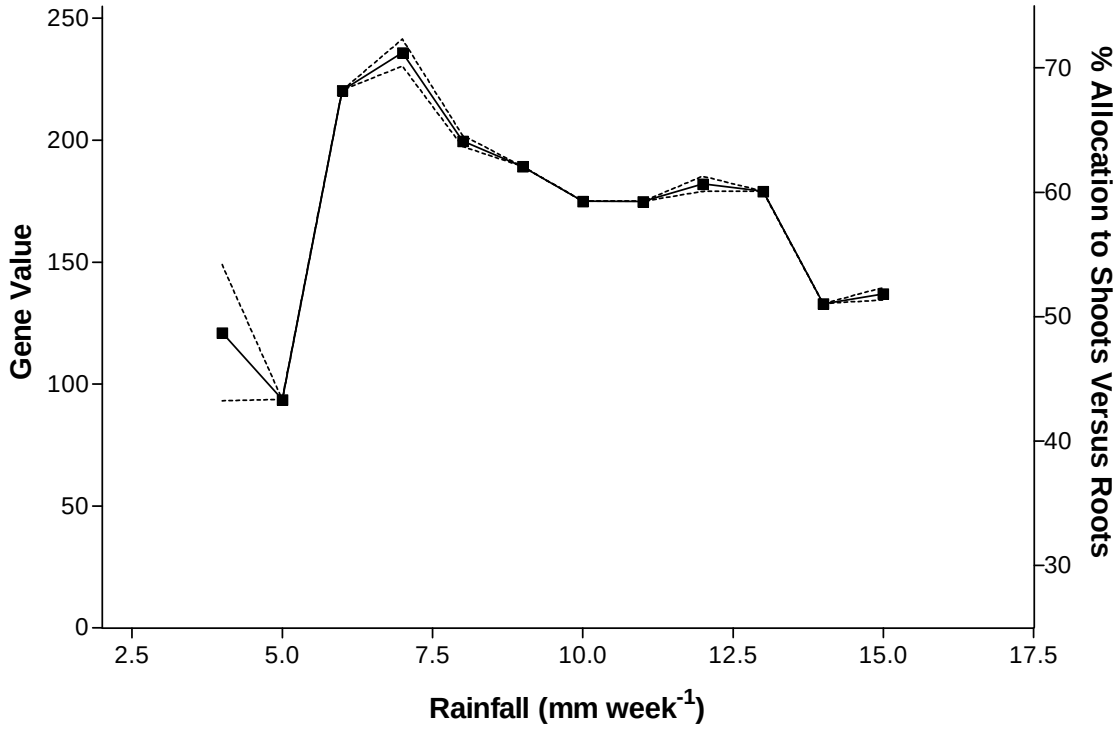

Figure 81 - Optimal RS gene value across a gradient in total rainfall, with a seven day pulse interval, for loam soil.

Figure 81 shows the shift in optimal RS gene value, indicating shoot:root allocation ratio, across a gradient in total weekly rainfall. Ignoring the two lowest rainfall amounts due to low total biomass, the trend is towards more root allocation and less shoot allocation with increasing weekly rainfall.

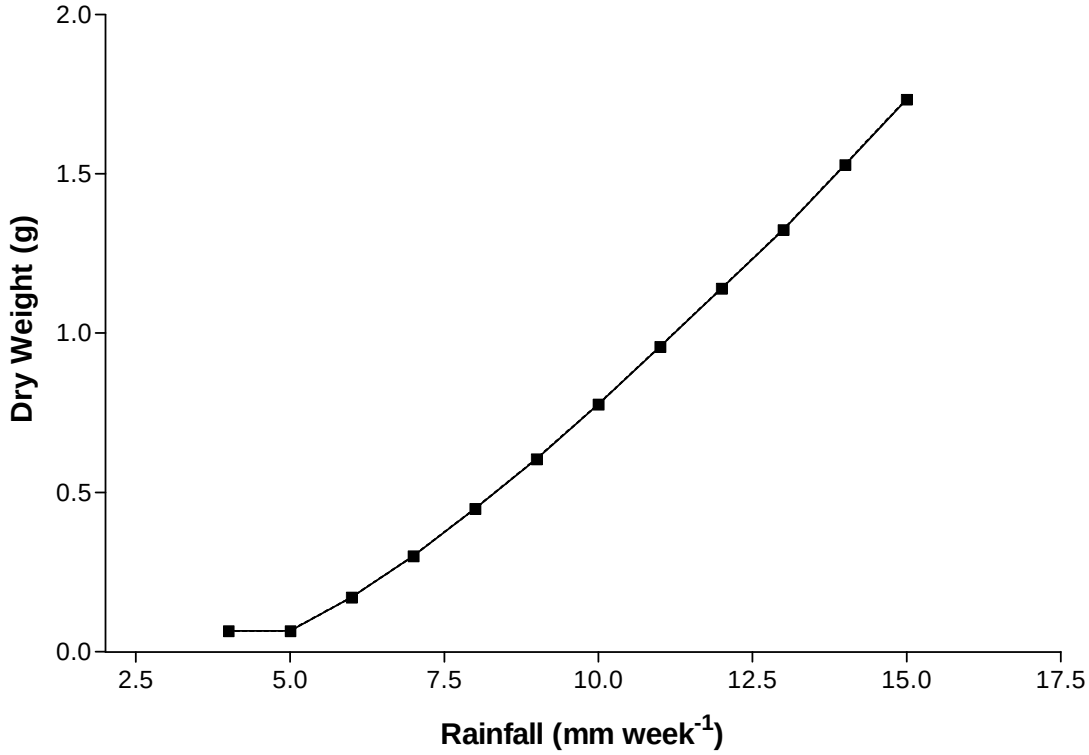

Figure 82 -Total dry weight across a gradient in total rainfall, with a seven day pulse interval, for loam soil.

Figure 82 shows the total dry weight across a gradient in total weekly rainfall. The relationship is close to linear, apart from the lowest two rainfall totals, where total biomass was close to zero. This represents a total annual rainfall of 200-250mm, below the minimum rainfall requirements of this species if the rainfall is not received mostly in winter. The linear increase in dry weight with rainfall does not reach an asymptote with high weekly rainfall, indicating water is still limiting, although higher weekly totals may result in a levelling off due to loss of water due to deep drainage, a limit on the root water extraction rate, or the meeting of leaf transpirational demand.

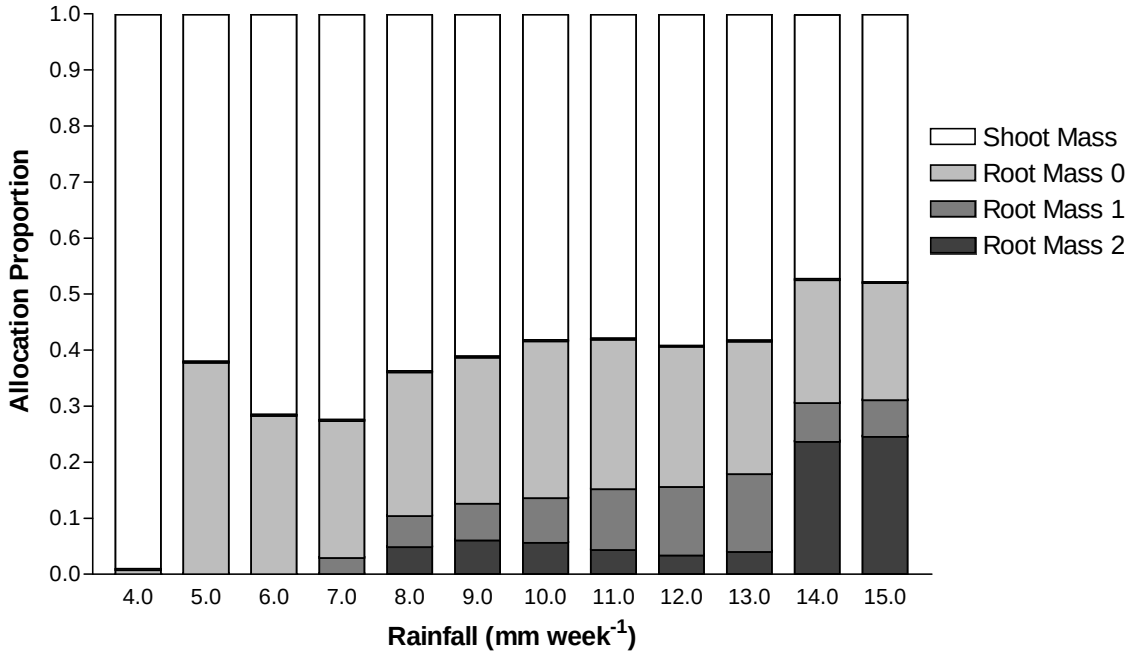

Figure 83 - Proportion of biomass allocated to shoot and root layers across a gradient in total rainfall per seven days, for loam soil.

Figure 83 shows the allocation of biomass to shoots and root layers across a gradient in total weekly rainfall. The shift towards roots, and deep roots in particular, with increasing rainfall is clear. The ability of larger rainfall events to penetrate to deeper soil layers explains the complex shift in RD1 gene optimum (Figure 80), with the appearance of thresholds of rainfall event size resulting in sudden shifts in allocation. For instance, 14mm events appear to regularly penetrate to the deepest soil layer, resulting in a sudden shift from allocation to the middle layer, to allocation to the deepest layer, beyond this point. Root allocation to the surface soil layer is fairly stable, as the plant extracts surface water from rainfall events of all sizes before loss to evaporation

Daily Rainfall Record Data

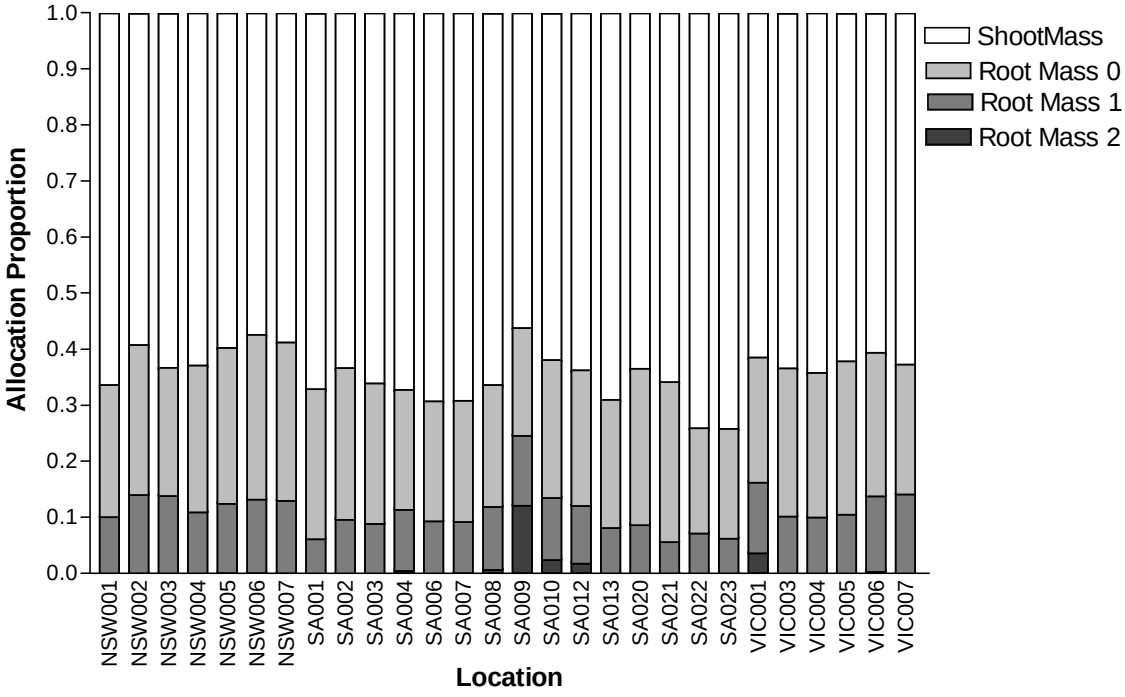

Figure 84 - Proportional biomass allocation to shoots and root layers, for rainfall records data for seed collection sites.

Figure 84 shows the proportional biomass allocation to shoots and root layers, for plants modelled under daily rainfall, temperature and evaporation data for a range of *Austrodanthonia caespitosa* seed collection sites. There is significant variation between sites, but no clear trends from state to state across the gradient in rainfall seasonality and event size. A few sites stand out for having particularly deep roots, including SA009 (Clare), SA010 (Keith), SA012 (Mundulla) and VIC001 (Serviceton). The later three are in the same region, of inland western Victoria and south-east South Australia. Sites SA022 and SA023 have particularly low root biomass and high shoot

biomass. Both these sites are located on the Fleurieu Peninsula in South Australia, which receives very high winter rainfall. Unlike the gene values which represent the combined selective effects of multiple years' rainfall, biomass totals for sites was closely related to annual rainfall total, which varies greatly from year to year in a real dataset. Therefore, it would not make sense to present total biomass data for this simulation. Doing so would require either an average of all the years of the simulation, with each year having a different rainfall total as well as biomass changing over time due to adaptation, or it would require reporting only the biomass for only the last year of the simulation, which would be unrepresentative of overall growth at the location.

Respiration functions

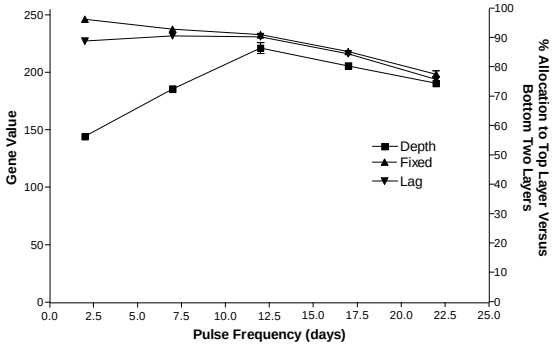

Figure 85 - Optimal RD0 gene value across a gradient in water pulse frequency, for different respiration functions in loam soil.

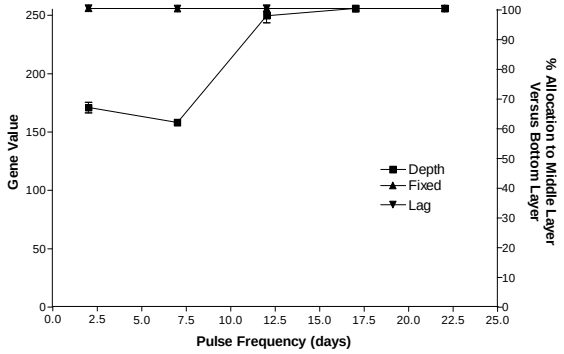

Figure 86 - Optimal RD1 gene value across a gradient in water pulse frequency, for different respiration functions in loam soil.

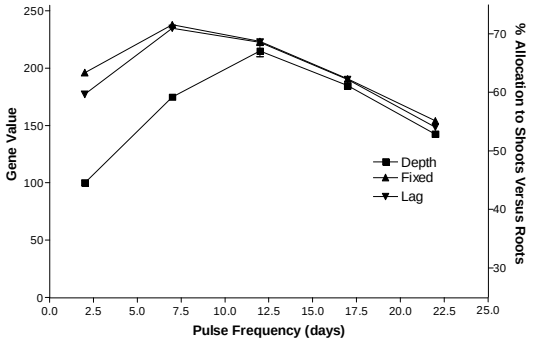

Figure 87 - Optimal RS gene value across a gradient in water pulse frequency, for different respiration functions in loam soil.

Figure 85 and Figure 86 show changes in the optimal RD0 and RD1 gene values across a gradient in pulse sizes and frequencies, for the three soil respiration functions. Fixed and lag respiration functions have similar trends for both genes, with deeper roots with larger, less frequent watering. Plants with these respiration functions developed no roots in the deepest layer, as reflected by the RD1 gene graph. Depth-variable respiration showed a peaked optimal gene graph, with shallow roots under

intermediate pulse regime, and deeper roots with small, frequent, and large, infrequent watering events. In the deeper soil profile, the depth variable respiration model resulted in deep roots with small, frequent events, and shallower roots with less frequent watering.

Figure 87 shows the trend in optimal values of the RS gene, representing shoot:root allocation. All three respiration functions have a similar trend, with a peak in shoot bias under an intermediate pulsing regime. However, with small, frequent pulse events, the depth variable respiration function produces greater root mass allocation.

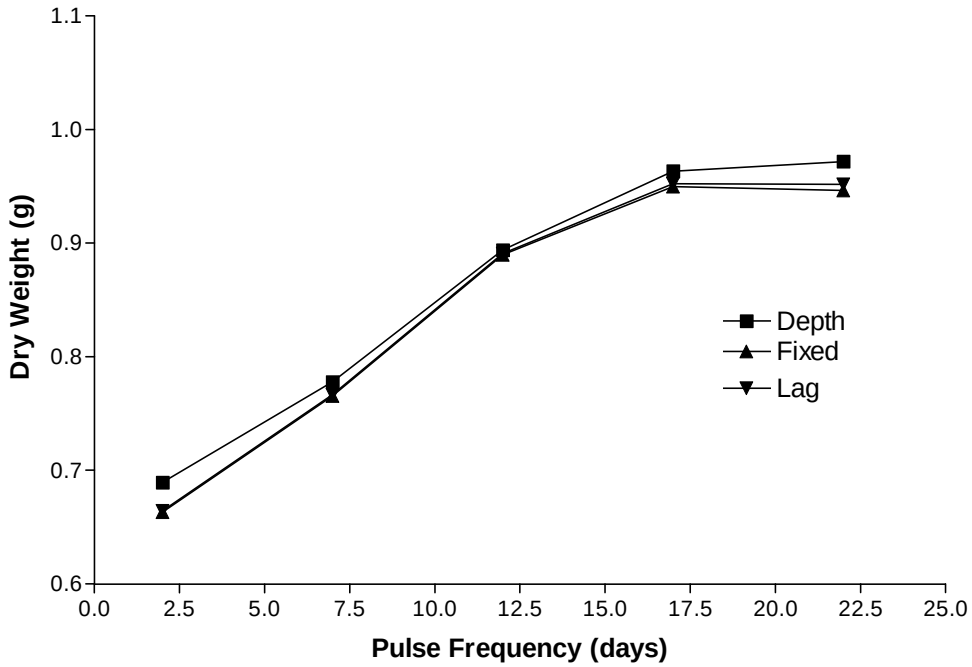

Figure 88 - Total dry weight across a gradient in water pulse frequency, for different respiration functions in loam soil.

Figure 88 shows the total dry weight of plants across a gradient in event size and frequency, under the three respiration functions. All three functions produced similar total biomass, with differences in root respiration having little effect on total biomass.

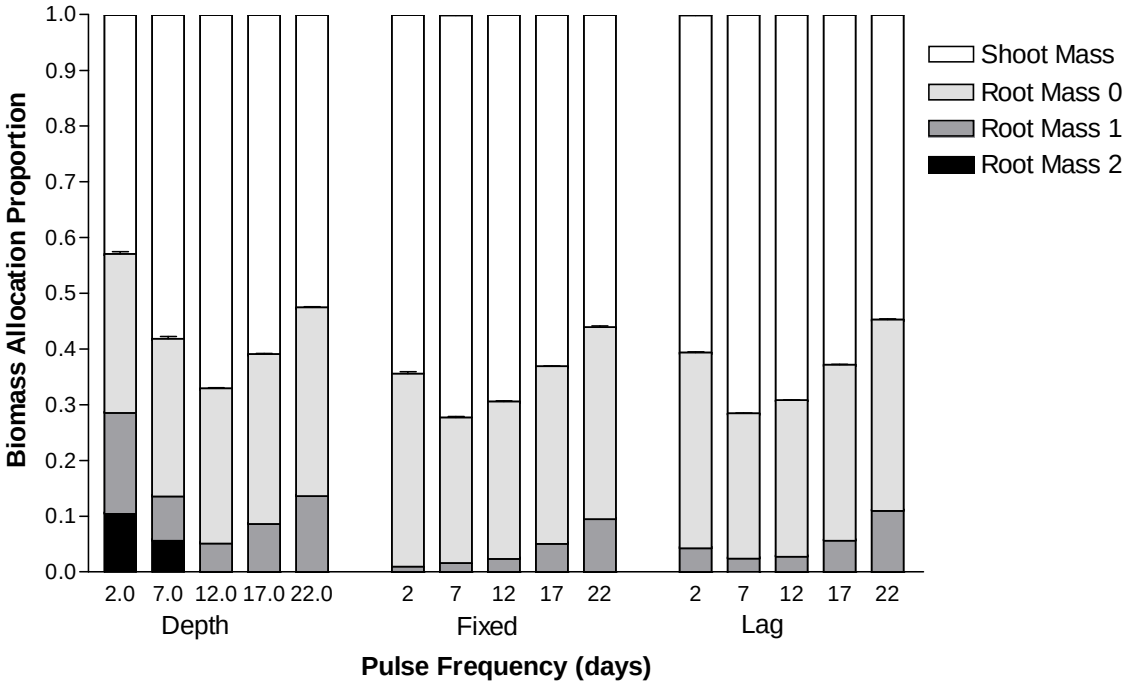

Figure 89 - Proportional biomass allocation across a gradient in water pulse frequency, for three respiration functions.

Figure 89 shows the proportional biomass allocation to shoots and root layers across a gradient in pulse size and frequency, for the three respiration functions. Again, fixed and time lag respiration functions have a similar response, with no root allocation to the deepest soil layer, while the depth variable function leads to the development of deep roots under small, frequent watering events.

Soil Surface Areas

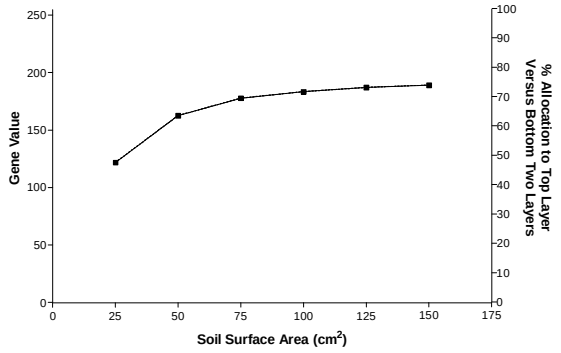

Figure 90 - Optimal RD0 gene value across a range of soil surface areas, in loam soil.

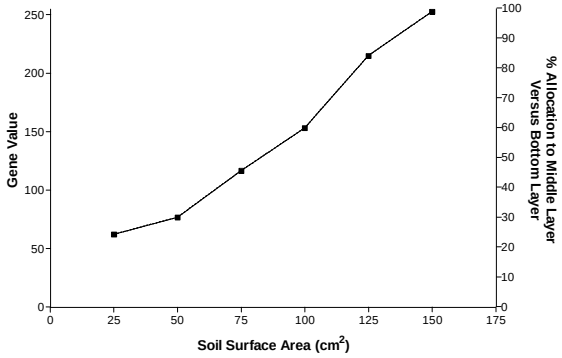

Figure 91 - Optimal RD1 gene value across a range of soil surface areas, in loam soil.

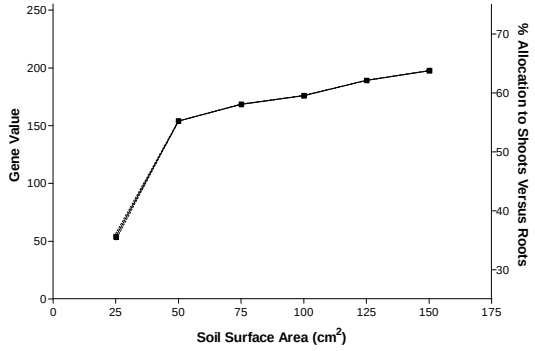

Figure 92 - Optimal RS gene value across a range of soil surface areas, in loam soil.

Figure 90 and Figure 91 show optimal values of RD0 and RD1 genes across a gradient in soil surface area and the volume of water available to the roots. Both genes show a shift towards shallower roots with increasing soil surface area, particularly in the middle and deepest soil layers.

Figure 92 shows the optimal RS gene value across a gradient in soil surface area, representing shoot:root allocation. There is greater allocation to shoots with increasing soil surface area.

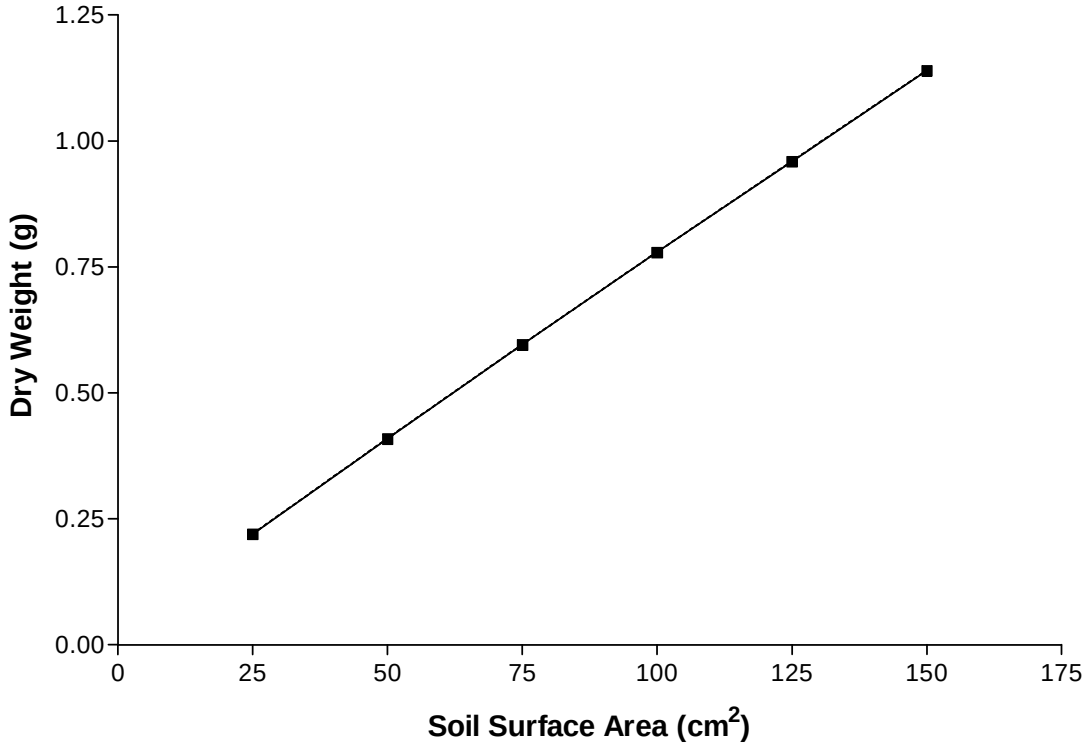

Figure 93 - Total dry weight across a range of soil surface areas, in loam soil.

Figure 93 shows the total dry weight of plants across a gradient in soil surface area. There is a simple linear relationship between soil surface area and dry weight, reflecting higher water availability with a larger soil volume.

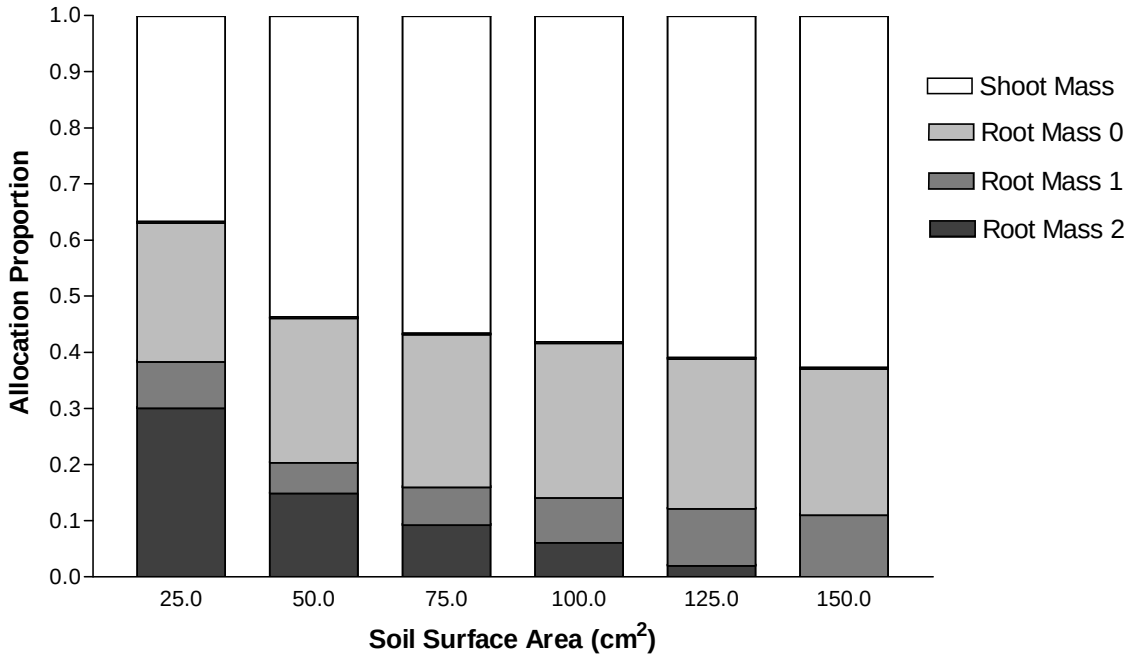

Figure 94 - Proportional biomass allocation to shoots and root layers, across a gradient in soil surface area for loam soil.

Figure 94 shows the proportional allocation to shoots and root layers across a gradient in soil surface area. There is a clear shift from deep roots, particularly in the deepest soil layer, to shallow roots with increasing soil surface area, and also a shift from shoot allocation to root allocation.

11.4. Discussion

Model Gene Shifts and Soil Moisture

Examination of shifts in gene values output by the model over a large number of generations indicates the three responses in optimal gene value; fixation, maximization, and randomness. The three genes on which the results focused, root distribution in the three soil layers, all became fixed at an intermediate value during the running of the model, indicating a strong selective pressure on these traits. RD0 is interpreted as explaining the importance of allocation to the surface soil layers, while RD1 is interpreted as explaining the importance of reliance on deeper soil water. The RS gene is interpreted as representing the amount of root mass required to support shoot transpiration, and hence how limiting soil moisture was to growth, with Sultan (2003) suggesting biomass is expected to be greater in the resource zone where resources are most limiting.

By contrast, the WUE gene representing water use efficiency, the amount of biomass gain per unit water transpired (Figure 56), did not stabilize to an intermediate optimum, and rather shifted to a maximum value, to achieve maximum biomass gain per unit of water. Such a value was obtained because, within the parameters of this model, higher water use efficiency always resulted in higher growth, and hence greater plant fitness according to the fitness function. In reality, water user efficiency is constrained by trade-offs such as stomatal conductance, and cannot increase indefinitely. Indeed, the maximum possible water use efficiency value in the model was obtained

experimentally from species growth and water use regressions. It would be possible to modify the fitness functions so that, for instance, plants allowing the lowest deep drainage were considered the most fit, in which case a plant with less efficient water use may be optimal. However, within the model trials presented here, deep drainage was rare and would not be suitable for selection. The PS gene, representing photosynthetic efficiency, the amount of biomass assimilated per unit radiation, also ran to a maximum, as higher photosynthesis would always lead to greater growth and greater plant fitness. Again, in real plants, photosynthesis is constrained by factors such as leaf chlorophyll concentration, and chemical reactions such as maximum rates of carboxylation, electron transport, triose-phosphate and CO_2 diffusion (Long and Bernacchi 2003). Unless trade-offs controlling these factors are included in the model, photosynthesis will be optimally at the experimentally determined maximum.

The three-layer soil water model appeared to provide a useful representation of soil water dynamics. Variation in event size led to differences primarily in the depth of water penetration, with smaller events increasing water content in the surface layer (

Figure 59), while large events (Figure 60) led to higher soil moisture in the deepest soil layer, consistent with expectations (Kemp 1983). Variation in event size and spacing affected the frequency at which surface soil water content oscillated, as well as the depth at which soil water content closely followed rainfall input. Ehleringer and Dawson(1992) suggested large oscillations in soil water content in the surface soil may be expected, due to rapid water loss though evaporation and transpiration, while deeper soil provides a more stable water source. In an experiment modifying watering event

size and frequency in a grassland system, Fay et al. (2003) found that large, infrequent events led to greater variability in soil moisture, longer dry periods, and 8% less water availability overall.

The soil water model also provided a reflection of the expected effects of seasonal rainfall bias on soil water distribution (Figure 61). Winter rain, both in the artificially generated rainfall model, and using real rainfall data from a winter rainfall site, resulted in a clear winter growing season with saturated soil, particularly in deep layers, followed by a dry summer with low soil water contents. Highly winter-biased rainfall is expected to result in penetration to deep soil layers (Schwinning and Ehleringer 2001), as well as maintaining moisture in the surface layers. In contrast, using daily rainfall data from a New South Wales site (Figure 63) where rainfall occurred with equal probability in all seasons, and with a bias towards large events, resulted in a contrasting pattern of soil water dynamics. Water was available at times throughout the year, after large rainfall events, as seen around day 90 in Figure 63, but the water was often short lasting, and there was no stable winter growing season with high soil water contents. The soil water model also demonstrates the importance of antecedent soil moisture, highlighting the importance of the timing of events (Schreiber and Sutter 1972). Depth of penetration of events can depend on the soil water content at the time rainfall occurs (Reynolds, Kemp *et al.* 2004). In the case of the model of New South Wales rainfall data, the penetration depth of large events depends on moisture in the surface layers, revealing the complex relationship between rainfall regime and distribution to soil water distribution. In arid systems, many rainfall events are small,

and recharge of deep layers relies on rare, large events (Reynolds, Kemp *et al.* 2000).

Pulse size / frequency

Across a gradient in rainfall event size and frequency, differences in biomass allocation between coarse and fine textured soil types were clear. In sand soil, deep root development was optimal with both small, frequent rainfall events (Figure 70), and large, infrequent rainfall events, with shallower roots optimal at intermediate pulse frequencies. Deep roots may be expected with small, frequent events if water from these events does not last long enough in the surface soils to be useful for the plant. These events have low penetration, and are subject to high evaporation rates (Sadras and Baldock 2003), leading plants to rely on deeper water reserves instead of investing roots in surface layers. Large, infrequent events appear to penetrate deep into the sandy soil (Loik, D. *et al.* 2004; Sun, Coffin *et al.* 1997), leading to the development of deeper roots (Schenk and Jackson 2002a) accessing the large amount of stable water in the deeper soil layers that can avoid evaporation (Paruelo, Sala *et al.* 2000). The presence of shallow roots at intermediate pulse frequencies may be explained by the presence of rainfall events large enough to last long enough to be useful to the plant in the surface layers before evaporative drying, but not large enough to penetrate to deeper layers. In contrast, the simulations run with clay (Figure 69) and loam (Figure 68) soil across the pulse gradient resulted in deep roots only with small, frequent pulses, with shallow roots only under large, infrequent watering events. Again, the development of deep roots with small events demonstrates an inability to use small events in surface layers efficiently. Small pulses may result in a strategy of maximizing carbon gain

between pulse by relying on deep soil water reserves (Schwinning and Ehleringer 2001) until a threshold of pulse usefulness is reached, when root strategy will shift to shallow roots. Unlike the sand soil, large, infrequent events of the size modelled here appear not penetrate to deep layers frequently enough in finer soils to warrant deep root development with this rainfall regime. A number of studies have found a relationship between coarse sandy soil and deep roots (Schenk and Jackson 2002a; Seyfried, Schwinning *et al.* 2005), and while the model found deep roots in sandy soil with both small and large events, compared to deep roots only with small events in finer soil, overall root depth was highly dependent on the rainfall regime rather than just soil texture.

The RS gene (Figure 66) indicates the bias towards allocation of biomass to above- or below-ground organs, and is interpreted as reflecting the region with the more limiting resource (Sultan 2003). All soil types showed a peak in allocation towards shoots at intermediate pulse frequencies, with more root allocation with small, frequent and large, infrequent events, suggesting water was least limiting at this intermediate watering regime. The peak in allocation to roots, particularly in the sandy soil, also occurred at the intermediate pulse frequencies which resulted in shallow roots. Previous optimality studies have found an association between shallow roots and high shoot allocation (Schwinning and Ehleringer 2001), suggesting intermediate pulse frequencies resulted in a stable, constant water source in the surface soil. The pulse size experiment in a previous chapter, examined the effect of pulse size and frequency on *A.caespitosa* root:shoot allocation, and found greater root allocation with small,

frequent events, while in a study of North American grassland species, Fay et al.(2003) found more root allocation with large, infrequent events. This may highlight the importance of conducting experiments over a wide range of pulse sizes and frequencies, as each of these experimental results may simply have represented a range of watering extremes more or less extreme than the peak in shoot allocation at intermediate pulse values. Sher et al. (2004) suggest that intermediate pulse frequencies may be most useful in low rainfall environments, as they exceed the threshold beyond which small events become useful, while small events may be more useful in high rainfall environments, where rain is more frequent.

Greatest total growth in the pulse size model was found in sandy soil (Figure 67). Cole and Metcalfe(2002) found that *Austrodanthonia spp.* are suited to sandy soil, and sandy soils have a more favourable water regime due to higher infiltration (Southgate, Masters *et al.* 1996) and lower evaporative loss (Noy-Meir 1973). Total growth also increased, in all soil types, with larger, less frequent events, despite shoot allocation being greater at intermediate pulse sizes. In terms of total growth, event size rather than frequency appears to be influencing the greater growth. Large events penetrate deeper into the soil profile, escaping evaporating and resulting in greater available water overall (Paruelo, Sala *et al.* 2000). This ignores potential loss of large events to run-off and deep drainage (Loik, D. *et al.* 2004), although run-off was not considered in this model, and deep drainage did not occur in the pulse size model run. It is important to consider that with infrequent events, mortality during the interpulse may be a more important outcome than plant growth (Goldberg and Novoplansky 1997), and that plant

mortality is not considered in this model. While low soil water content may have an impact on grass seedlings (Cox and Conran 1996), *A.caespitosa* has been shown to have a high tolerance to drought, with green tissue being maintained for up to 40 days (Bolger, Rivelli *et al.* 2005), a pulse frequency not reached in this simulation.

Seasonality

In the simulation across a gradient in seasonal rainfall timing, significant differences were again found between soil types in the root depth response to rainfall regime. In sandy soil (Figure 77), shallow roots were found to be optimal under high summer rainfall, while deep roots were developed with high winter rainfall. Summer events only penetrate to the surface layers, (Paruelo, Sala *et al.* 2000), which may explain the usefulness of shallow roots under high summer rainfall. However, summer rainfall is also subject to high evaporation rates (Reynolds, Kemp *et al.* 2004) and short water availability, which may make shallow roots inappropriate, leading to reliance on deep roots accessing a more stable water source. Evaporation from the soil surface may be lower in coarse textured soils (Loik, D. *et al.* 2004; Noy-Meir 1973), leading to a shallow roots strategy for summer rain in sandy soil in this model. This effect was accounted for in the model by the positive association between soil water content and evaporative rate, and the lower volumetric water content of sand soils. Deep roots may be expected in strongly winter-biased rainfall regimes, with a previous optimality study finding shallow roots associated with winter drought, and deep roots associated with deep recharge resulting from winter rain (Schwinning and Ehleringer 2001). A study of the root dynamics of snakeweed (Wan, Yilmaz *et al.* 2002) also found deeper roots

were developed under a regime of winter rain and summer drought.

In contrast, across a gradient in seasonal rainfall timing in clay soil (Figure 76), shallow roots were optimal with high winter rainfall, and deep roots were optimal high summer rainfall. The short lasting summer rainfall events were not utilized, and deeper soil water resources were used instead, possibly due to the greater evaporation and lower penetration depth of water in clay soil compared to sand soil. Shallow roots were developed with high winter rain, again due to the low penetration of large events in clay soil compared to sand soil, and the lower evaporation rates from the surface in winter. Plants modelled in loam soil were intermediate of sand and clay, with deep roots optimal with both low winter and high winter rainfall, and shallow roots at intermediate seasonality levels. Deep roots were developed with high summer rain due to the high surface evaporation of these events, and with high winter rain as the large events were able to penetrate to deeper soil layers than in clay soil, avoiding the development of more costly shallow roots. A number of previous studies have suggested there may be a threshold of summer rainfall amount or predictability before shallow roots become optimal for utilizing these events (Ehleringer and Dawson 1992; Williams and Ehleringer 2000), with plants relying on deeper water before this threshold is reached. The model results suggest that the threshold of summer rainfall amount required before use may be strongly dependent on soil texture. Plants simulated in sandy soil made use of both shallow summer and deep winter rainfall events, while plants simulated in clay soil relied on deep water with summer rainfall, indicating a higher threshold before summer water became useful in this soil.

The pulse size and frequency simulation appears to have a larger impact on root:shoot allocation than the seasonality gradient simulation, with only slight shifts in biomass allocation across the range of seasonality biases (Figure 73). Again, differences between soil types were evident, with a trend towards more shoot biomass with high winter rain in loam and clay soil, and a trend towards more roots with winter rain in sand soil. Winter rain is considered more effective than summer rain (Reynolds, Kemp *et al.* 2000), particularly in the region where this species occurs naturally (Williams 1968). As such, a shift towards more shoot biomass with winter rain, as observed in the finer textured soils, would be expected when water is a limiting resource. High root mass with winter rain, as found in the sandy soil, goes against this trend, and suggests that winter rainfall was more limiting in this system. Petheram et al. (2002) suggest there is higher deep drainage and recharge in sandy soils, and high winter rainfall is also more likely to be lost to drainage below the root zone than summer rainfall (Paruelo, Sala *et al.* 2000). Loss of water to drainage contributes to the inefficient use of rainfall (Sadras and Baldock 2003), and some water was lost to deep drainage in the high winter rainfall simulations in sandy soil (Figure 78). Increased root allocation and length is one strategy to extract water greater amounts of water from the soil, leading to less deep drainage loss and wasted resources.

Total dry weight was greater in all soil types with more winter rainfall (Figure 74), reflecting the greater effectiveness of winter rainfall compared to summer rainfall (Reynolds, Kemp *et al.* 2004), with water from winter rain penetrating deeper and escaping evaporation in the surface layers, combined with the lower vapour pressure

deficit. Again, higher growth was observed in the coarser soil, which may be a result of the deeper penetration of water in sand, reducing evaporative loss.

Total Weekly Rainfall

Under varying amounts of total rainfall, roots were, generally, deep with greater total water (Figure 83). A number of studies of global root distributions have found deeper roots with greater precipitation. Schenk and Jackson (2002a) found deeper roots with more rainfall, due to the increased penetration depth of large rainfall events, and Seyfried et al. (2005) also related greater root depth to higher annual precipitation. While relatively deeper roots for a given plant size are found in drier sites, absolute root depth tends to increase with rainfall (Schenk and Jackson 2002b). In contrast, Coupland and Johnson (1965) found shallower roots in moister regions, reflecting a more constant water availability in the upper soil layers, which highlights the importance of taking interpulse length and drought severity into account when examining the effects of rainfall totals. The pattern of root placement, particularly in lower layers, was not a linear function of rainfall total, however. Rather, there appeared to be thresholds beyond which root depth increased significantly. This is partly an artefact of the model, with soil layers being divided into rather coarse 10cm vertical blocks. In reality, rainfall events do not occur in such precisely measured amounts, and the variable size of rainfall events will result in a much less predictable relationship between annual precipitation and root depth, particularly if groundwater supplies a permanent water source (Singh, Milchunas *et al.* 1998), the effect of which

was not included in this model.

Root:shoot ratio has been considered as an indication of water limitation, with more roots expected with lower soil moisture. For instance, more root growth has been found in drought conditions in lupins (Rodrigues, Pacheco *et al.* 1995) and *Polygonum spp.* (Bell and Sultan 1999). However, in the simulation, root:shoot ratio tended towards root allocation with more total rainfall (Figure 81). There is a close association between total rainfall and productivity in arid and semi-arid systems (Seyfried, Schwinning *et al.* 2005). Total dry weight increases with increasing rainfall within the range simulated in this model, indicating that water is still limiting even at with high rainfall totals, as further water addition increases growth. In experimentally grown plants, root proliferation can occur in moist zones, resulting in high resource acquisition (Loomis and Ewan 1936). Bell (1999) found that while relative root mass was greater in droughted *Polygonum spp.*, there was greater absolute root length in the moist treatment, enabling efficient extraction of the large water store. Indeed, root plasticity and proliferation is usually reflected in an increase in root length rather than mass (Pregitzer, Hendrick *et al.* 1993), and in this model, root mass and length are directly linked through a fixed specific root length (SRL). Roots may be limited by the extraction rate per unit root length, but the only way to increase root length in the model is to allocate more mass. Including a genetically controlled term to define specific root mass, with associated hydraulic trade-offs, may provide the opportunity to better explore root response to zones of resource enrichment. While water remains limiting over the range of rainfall regimes simulated here, limitations may occur with

increased rainfall, due to increased deep drainage, limits to the rate of water extraction by roots, the negative effects of water logging, a particularly important factor in clay soil which is not included in this model.

Daily Rainfall Data

There were variations in root distribution and biomass allocation between simulations run with actual climate and daily rainfall data from locations across South Australia, Victoria and New South Wales (Figure 84), but no clear trends in plant structure were apparent between regions or across a gradient in rainfall regime. No significant correlations were found between optimal plant gene values and the seasonality, event size and gap size rainfall indices. Despite each generation in each replicate experiencing a different random pair of years of rainfall data, variance in optimum gene values for each location was extremely low, giving confidence in the biomass allocation for each location being the optimal response to the local climate regime. Local rainfall regime is clearly having an influence on optimal plant allocation, but, as with measurements on experimentally grown plants, differences between near-by populations may be greater than differences between populations in different regions. The climate indices used, despite being good descriptors of rainfall seasonality, event size bias and interpulse length bias, may not adequately reflect aspects of the rainfall regime that drive selection. It is noticeable, for instance, that few populations develop roots in the deepest soil layer under real rainfall conditions, while deep roots were developed under a variety of circumstances in the gradient models, although often at extreme rainfall regimes that may rarely exist in nature. Several sites with close

regional ties did develop deep roots, including sites near Clare in South Australian mid-north, and a number of sites in the southeast of South Australia and western Victoria. In the case of sites in the mid-north of South Australia, these sites are localized in a valley with a higher annual rainfall total than the surrounding areas, which may explain the greater water penetration in these areas. No rainfall descriptor clearly explains the deep roots found optimal in south-east South Australia and western Victoria, as closely located sites appear to have quite distinct biomass allocation, although the sites near Keith in South Australia do experience a rainfall regime strongly biased towards small events. A number of sites on the Fleurieu peninsula in South Australia also have lower root mass and higher shoot mass than other sites. This region receives very high winter rainfall, and higher annual rainfall over all, which may lead to soil water being less limiting, and high shoot production.

Respiration

Three different respiration functions were tried in order to examine the effects of giving surface roots a higher maintenance cost. In all simulations, the fixed respiration function and the lag respiration function responded similarly across a gradient in pulse size, while the depth variable respiration function, where shallow roots faced higher temperatures, showed a different pattern (Figure 89). Across a gradient in pulse size and frequency, no deep roots were developed with small rainfall events with the fixed and lag respiration functions, but deep roots did develop with large, infrequent watering due to deeper infiltration of large events. Under depth variable respiration, however, deep roots did develop with small, frequent events. This appears to be a clear reflection

of the effect of the cost of surface roots. With low-cost surface roots, experiencing the same respiration rate as deeper roots, surface roots are relied upon to acquire small, frequent events. However, with high-cost surface roots, the plant diverts allocation to deeper roots under a small, frequent waterings, to rely to more stable deep water supplies.

There was no significant difference in total biomass across the pulse size gradient for the three respiration functions, indicating the different rates of root biomass loss did not have a large effect on total plant biomass (Figure 88). Overall, this simulation shows the potential for shallow root costs to influence optimal allocation and root depth patterns, with high cost shallow roots leading to reliance on deep soil water rather than small surface events, and indicating that if shallow roots are used to acquire small rainfall events, the plant may incur a cost in maintaining those roots.

Soil Surface Area

The final simulation, with varying soil surface area, aimed to mimic at a simple level the effects of planting density, by manipulating the soil volume available to each plant, and hence the total water store. While the penetration of rainfall events and the relative soil water content after rain should be equal between all soil surface areas, the total amount of water available to the plant varies considerably, leading to different dynamics than the total water simulation. A smaller soil volume resulted in deep root development, while a larger soil volume resulted in a greater proportion of shallow roots (Figure 94), indicating a reliance on more stable deep soil water reserves when

total water in the surface layers is low, and quickly reduced to below wilting point by the combined effects of evaporation and transpiration. A study of the effects of planting density on root depth in cheatgrass (*Bromus tectorum* L.) found that higher planting densities resulted in a decrease in shallow root mass and an increase in deep root mass as a result of intraspecific competition (Sheley and Larson 1994). Similarly, in sunflowers (*Helianthus annus* L.) high planting density resulted in earlier production of deep roots, while at a low planting density roots continued to explore longer throughout the profile. Soybeans (*Glycine max* L.) were also found to develop more deep, vertical roots when grown in pots with other plants, compared to growing alone (Raper Jr. and Barber 1970). As expected, there was greater allocation to shoots and less to roots with increasing soil volume (Figure 92), reflecting the effect of greater total water per plant making water a less limiting resource. Total dry weight increased linearly with increasing soil volume, again reflecting the effect of greater total water availability on plant growth. There was no levelling off of total plant biomass at high soil volume values, which would have indicated water was no longer a limiting resource. However, it should be noted that the model does not take into account the costs of root density or horizontal root exploration. A given length of root in a layer is given equal access to the entire water store of that layer, independent of the spatial extent of that water store. Hence, inclusion of more realistic root depleting zones and root proliferation costs is needed to better account for the effects of soil volume size on plant growth.

Conclusion

The soil moisture model provided a useful representation of major aspects of soil water dynamics in relation to different rainfall regimes. Rainfall event size and frequency, seasonal rainfall distribution, total rainfall and soil volume all strongly influenced optimal plant allocation traits in this model. Soil texture also has an important influence on optimal plant traits, with coarse sandy soil allowing the penetration of large events and winter rainfall events, leading to the development of deep roots, while finer soils had lower infiltration, and relied on longer lasting surface water from large events and winter events. Similarly, plants in sand were able to make use of summer rainfall in surface layers, while in clay, high summer rainfall was less useful in the surface layers, and plants relied on stable deep water sources. The importance of the cost of surface roots is made clear by the reliance on surface water with low-cost surface roots, compared to reliance on deep water reserves when surface roots face relatively high respiration rates. Greatest bias towards shoot allocation is found at intermediate pulse intervals, as rainfall reaches a point of constant availability in the surface soil, while total dry weight increases with increasing event size and total rainfall, due to the penetration of large events beyond the zone of high evaporation. The shift of allocation towards deep roots with both small and large events highlights the need to examine plant responses across a broad gradient of pulse regimes. Although local rainfall regime resulted in different optimal plant phenotypes, there were no clear relationships between modelled optimal plant traits and the rainfall indices used, suggesting other aspects of intraannual rainfall regime need to be quantified.

12. DISCUSSION

The root distribution and allometry of *Austrodanthonia caespitosa* appears quite plastic, and highly sensitive to water and soil conditions. It also showsing a high degree of differentiation between the ecotypes examined in these experiments. This study reveals a complex array of factors that can influence root depth in this species, although a number of broad patterns in the water acquisition strategy of *A.caespitosa*, and the conditions under which this species may be useful in pastoral systems and for deep drainage reduction, are made clearer.

12.1. Plasticity and Genotypic Differentiation

Overall, there were no strongly significant correlations between experimentally measured plant traits and the rainfall indices calculated across south-eastern Australia, such as seasonality, event-size and gap-size. Although the model showed the potential for intraannual rainfall regime to have a strong impact on optimal root distribution, there were no correlations between the rainfall indices used and model output. This suggests that while the indices did provide useful information about small-scale variation in rainfall regime across the continent and the study region, they failed to take into account all the factors that act as a strong selective force on differentiation in *A.caespitosa*. One clear conclusion that may be drawn from genotypic differences observed in the plant growth experiment is the importance of factors operating at a local, rather than regional scale. Often, plants grown from seeds collected from neighbouring locations showed greater variation in measured plant traits than ecotypes

collected at opposite ends of the range. Therefore, local factors such as soil type, community composition and site history may have a more important influence on differentiation in this species than broad-scale factors such as climate. Wilson (1996) suggests differentiation to local niches may explain the high variability in this species. Neither there was any correlation between rainfall indices and optimal plant traits in the evolutionary algorithm model, where soil type was kept constant in the real rainfall simulation. Although there was variation in optimal traits between locations, and some sites showed strong differences in root depth, the lack of correlation with indices suggests the rainfall factors utilized, such as pulse size and seasonality, may not be the most important rainfall factors influencing root depth. The effects of modelling real rainfall data contrasted with the strong selective effects of a programmed, defined rainfall regime, suggesting that while in ideal conditions a particular root distribution may be optimal under a precisely defined rainfall regime, in reality rainfall is too variable and unpredictable to have as strong a selective effect on plants as is observed in the model. For instance, occasional large events will recharge deep layers, acting as a buffer (Singh, Milchunas *et al.* 1998) against the selective effects of small rainfall events. It is also important to recognize that the experiments showed a high degree of plasticity in root growth in this species, while the model did not allow for plasticity in root distribution, a factor that might well be introduced into the model as an additional genetically variable effect.

Genotypic differences between populations were examined most closely in the natural rainfall population comparison experiment. The greatest and most consistent difference

between populations was in phenological characters, time to flowering and time to dormancy. Some New South Wales populations appeared to be significantly longer lived before dormancy, which contrasts with previous research, which has found populations from northern New South Wales to have greater growth during winter and a shorter life-span (Hodgkinson and Quinn 1976). This was considered an adaptation to a hot, dry summer in a low rainfall environment, which may not be comparable to the southern New South Wales populations growth here. It is important to consider that phenological characters such as flowering and dormancy can be closely associated with climate, and there is evidence of phenology being controlled by day length in this species (Hodgkinson and Quinn 1978). Additionally, the results of the experiment indicate that factors other than low soil moisture may be triggering dormancy. As all ecotypes were grown under an Adelaide climate, the experiment may not give a true indication of the timing of life history stages in the collection location.

Multivariate analysis showed that none of the environmental variables included had strong explanatory power in discriminating between ecotypes. Seasonality index, the factor that had the strongest gradient across the collection range, had the strongest effect in the ordination, but the explanatory power was still very low. The ordination based on measured plant characters not only failed to discriminate between regions, for example states, but also failed to cluster plants within ecotypes. This, along with the high degree of variability within ecotypes that is apparent in ANOVA, shows there to be high intra-population genotypic variability as well as differentiation between populations. Although larger population samples sizes would be required to more

accurately examine intra- and inter-population genetic variability, high genetic diversity may be expected to allow populations to survive in an unpredictable environment (Mather 1943). For instance, where the end of the high rainfall season is unpredictable, and the summer drought sudden, variation in flowering time may be a means of ensuring some reproduction in all years. This bet-hedging strategy may manifest in, for example, variation in timing of flowering (Satake, Sasaki *et al.* 2001) or long seed dormancy times (Philippi 1993), or alternatively, environmental variation may prevent the fixation of a single genotype in the population.

In the pulse size and frequency experiment, plants showed a high degree of plasticity in root distribution, in both vertical placement of roots, and root proliferation as described by the fractal dimension, confirming the presence of root plasticity even in young plants of this species. Plasticity is considered a likely response to a strongly pulsed environment (Sultan 2003), and plasticity in root growth may help buffer plants against changes in soil moisture (Weltzin, Loik *et al.* 2003). Heathcote et al. (1987), in a study examining root plasticity and genotypic differentiation in response to flooding, suggests such plasticity may be common but is rarely reported, as genotypic differences are considered more important. Temporal variation, in particular, may be expected to produce plasticity (Moran 1992). Watering regime had little effect on above-ground biomass, which showed greater variability between ecotypes than watering treatment. Variation in productivity with ecotype was also found in the other growth experiments. Previous studies have associated differences in growth rate in *A.caespitosa* with the tendency towards shorter lifespans and time to flowering in populations that experience

a harsher, less predictable summer climate in northern New South Wales, although no clear regional trends in above-ground productivity were evident in the results from this experiment. Again, differences between closely situated ecotypes were often high. It is interesting to note that there was no interaction between watering treatment and population effects in this experiment, which would have indicated a varying degree of plasticity between ecotypes of this species, an effect that has been found in other species (Bell and Sultan 1999).

Clear, but unexpected, regional differences in response to summer rainfall were found in the seasonality experiment. South Australian ecotypes were found to have a strong leaf growth response to summer rainfall addition, while New South Wales ecotypes showed little additional growth. The growth response was primarily in above-ground biomass, with seasonal watering having no effect on root growth. The strong response of South Australian populations was unexpected, as this was considered a region of low summer rainfall, compared to New South Wales where rainfall during summer was more likely. However, the region of New South Wales sampled is in fact hotter in summer, and experiences higher evaporation rates, than coastal South Australia, reducing the effectiveness of what summer rain does occur, and possibly increasing the size of rainfall event required before a plant growth response would be observed. Another marginally significant genotypic effect observed in the seasonality experiment was a tendency towards deeper roots in New South Wales ecotypes, and shallower roots in South Australian ecotypes. High winter rainfall, as in South Australia, is expected to be correlated with a deep root distribution (Wan, Yilmaz *et al.* 2002) due to

deeper water penetration, but alternatively high winter rain may result in constant water availability in the surface soil during the winter. However, the lack of any clear regional trends in root depth in the other experiments mean this result may be interpreted as a product of the limited number of ecotypes examined in the seasonality experiment, and the high local variability between populations in characters such as root depth.

Given the evidence found of plasticity in root distribution in response to water regime, as highlighted by the pulse size experiment, and the strong growth response to summer watering, it is important to consider that the evolutionary algorithm model did not allow for any plasticity in plant growth. Root distribution in the model depended purely on genotypic control, and growth response was tied directly to water availability. It may be possible, and useful, to include plasticity in the model as a genotypically variable effect, with its own associated trade-offs (DeWitt, Sih *et al.* 1998). For instance, as well as being under genetic control, root distribution may, depending on the plasticity of the genotype, also respond to water availability in the soil layer. This may provide information on the optimal level of plasticity in root distribution under various resource regimes, and the influence plasticity has on plant growth and fitness, compared to a fixed strategy.

12.2. Rainfall Event Size

The τ- statistic described differences in event size bias across Australia, with particular contrasts between tropical and arid, inland regions, in which rainfall input was dominated by large storms, and coastal southern Australia, where small events were

more common. The index provided good spatial resolution in changes in event size bias across the continent, with small scale effects such as the influence of the Great Dividing Range made clear on the index charts. Seasonal variation in event size bias is also described well when this index is applied to different seasons. For instance, the index describes the relatively small rainfall events that dominate Mediterranean climate areas during winter, compared to larger events from storms that occur in this region during summer. Across the transect from South Australia to southern New South Wales, τ- statistic is variable, but within only a small range of the total variability found across the continent. Although there is, in general, a bias towards small events in coastal South Australia and large events in inland New South Wales, there is also significant local variation in this index, which adds noise to any gradient in event size across this range. This is expected, as although the seed collection sites were planned to be situated within a 400-500mm annual rainfall isohyet, this transect ranged from coastal to inland areas, as well as ranging over a range of altitudes and topographic features, including the Mt. Lofty Ranges and Grampians, which can greatly influence rainfall.

The pulse size and frequency experiment showed that root distribution response to rainfall event size was primarily plastic, with strong differences between watering regime, and weak differences between populations in root depth. Small rainfall events resulted in deployment to the surface soil layers, while large events resulted in deployment to the deeper soil, when measured both by root mass and root length. This

indicates the importance of watering event penetration depth, and confirms the importance of watering events of the order of 5mm in the context of this experiment (Sala and Lauenroth 1982).

The pulse-size experiment also revealed differences in biomass allocation to shoots and roots in response to watering treatments, with small, frequent events resulting in greater root length, and large, infrequent events resulting in less total root length. This effect was also reflected in differences in root:shoot mass ratio between watering treatments. If biomass allocation and organ proliferation within a resource region is regarded as a reflection of resource limitation (Sultan 2003), this suggests large rainfall events were less limiting in this system. Large events are expected to penetrate deeper into the soil profile, beyond the surface zone that is subject to high soil evaporation (Paruelo, Sala *et al.* 2000), therefore leading to higher total water availability under this rainfall regime. The structural reasons behind this root distribution and allocation is made cleared in the analysis of root fractal dimension, which gives a measure of root branching and proliferation. In particular, there was little root proliferation and branching in the surface soil under larger events, while there was with small rainfall events. Under the long interpulse lengths experienced in this treatment, the surface would have reached low water contents before rewetting. It appears that this led to a strategy of deep root deployment into a more stable water source, rather than growing a dense root system in the surface soil, where water was available for only short periods. A large, infrequent watering regime therefore appears to result in reduced water availability in the surface soil, but greater water availability overall under these experimental conditions.

The model revealed that these dynamics might change depending on the exact size and frequency of rainfall events, as well as with soil type, leading to difficulty in generalizing the strategy perennial grasses may employ under different pulse regimes. In sand soil, deep roots were found to be optimal with both small, frequent events, and large, infrequent events, with a shallower root distribution optimal at an intermediate watering regime. In fine soil, a response that contrasted with the results of the pulse experiment was found, with deep roots optimal with small events, and shallow roots optimal with large rainfall events. While clearly demonstrating the importance of soil type in determining plant responses to rainfall regime (Walter 1971b), there are a number of possible reasons for the disagreement between the model and the experiment. In the experiment, large gaps between watering events led to low water content in the surface soil, making a deep root distribution optimal. However, in the model, it appears the size rather than spacing of rainfall events was more important, with large events resulting in high surface water content, and shallow root development in fine soil where water penetration depth was lower. It should be noted that a fairly coarse loam soil was used in the pulse experiment, and there are similarities between the experimental results and the model results under coarse sandy soils. Indeed, the intermediate and large event watering regimes in the model and experiment give similar results, tending towards shallow and deep roots respectively. The main difference is found with small, frequent events. The experiment was conducted in a glasshouse, under semi-shade, while the model simulates full-sun evaporation. It may be that the

higher evaporation rates experienced in the model resulted in lower usefulness of very small events, and therefore a tendency to rely on deep water instead in these conditions, in contrast to the experiment where small events lasted long enough to be utilized by shallow roots.

The model showed a peak in shoot allocation at intermediate pulse frequencies and sizes, and more root allocation, taken to mean greater water limitation, with small or large rainfall events. This, again, contrasts with the pulse-size experiment, where root:shoot ratio decreased and water was found to be less limiting with large rainfall events. In the experiment, a potential explanation for greater water availability from large events was the depth of penetration of these events, beyond the zone of high evaporation. In the model, it was hypothesized that intermediate pulse sizes resulted in rainfall events large and frequent enough to maintain constant moisture in the soil profile, with larger, less frequent events resulting in some periods with dry soil. Again, differences in soil type and actual evaporation between model and experiment, as well as the lack of plastic biomass allocation in the model may explain this contrast. It should be noted that while root:shoot ratio was lowest at intermediate pulse frequencies in the model, total biomass, which also serves as a useful indicator of available water, was greatest with large events, agreeing with the experiment that large pulses may be most useful. The model, in its present "big leaf" form, may not provide an accurate association between allometry and resource limitation, with light controlling assimilation, but held constant and never actually limiting due to self-shading.

12.3. Interpulse Length and Event Frequency

Modifying rainfall event size while keeping rainfall total the same in the experiments and model necessitated changing the frequency of events, which can have important biological consequences. Under natural rainfall regimes, different locations can also have different characteristic rainfall frequencies and drought lengths, a factor the G-statistic attempts to describe. This index discriminates, again, between tropical and arid, and Mediterranean regions, with a bias towards large interpulse lengths in the tropics and more frequent rainfall events in Mediterranean area. However, the index does appear to be confounded by seasonal effects. In the tropics, large drought lengths occur during the dry season, but during the wet season rainfall occurs very frequently. Even dividing the index into winter and summer half years fails to give expected results, as the index calculated for the summer half-year still captures the long drought periods of the preceding dry season. Use of this index therefore requires accurate seasonal divisions, in order to recognize the differences in gaps between rainfall events, and seasonal droughts. This index did show a slight gradient along the transect from South Australia to New South Wales, with a trend towards frequent events in South Australia and coastal areas, and less frequent events in New South Wales, with the gradient appearing stronger in the winter half-year. Again, this transect covered only a small proportion of the scale of this index across the continent.

In the pulse-size experiment, interpulse length appeared to have an impact in the amount of time the surface soil was dry (Fay, Carlisle *et al.* 2000). Small rainfall

events, despite not being useful, at least appeared to keep the surface soil wet due to their high frequency of occurrence, leading to local root proliferation. In contrast, large rainfall events, despite being less limiting over all, resulted in extensive periods when the surface soil was dry due to their low frequency, leading to lack of root development in the surface soil. As discussed above, a related effect was observed in the model, with an intermediate watering regime resulting in high shoot allocation, as events were frequent enough to maintain moisture in the soil, while large, infrequent events resulted in a higher root:shoot ratio, possibly due to soil drying reducing water availability. However, event size appeared to have a greater influence than event frequency in the model, as total plant biomass continued to increase with larger, less frequent events. It should be noted that while the interpulse length is likely to be associated with plant mortality (Goldberg and Novoplansky 1997), mortality was not included in the model. This is realistic for the range of interpulse lengths utilized here. No mortality of established plants was observed in the pulse size experiment, under infrequent watering. A previous study has found *A.caespitosa* is able to maintain green leaf after 40 days of drought, while the experiment used a maximum of 14 days between waterings, and the model used a maximum of 25 days between waterings.

12.4. Seasonality

Seasonality of rainfall is an important factor influencing soil water availability and distribution in the soil, due to the interaction between rainfall input and evaporative loss (Stephenson 1990). Therefore, differences in rainfall seasonality across the landscape can be as important as differences in total annual rainfall. The two seasonality indices

utilized in this study, the Walsh & Lawler seasonality index (Walsh and Lawler 1981) and the vector seasonality index (Markham 1970) both provided useful information on gradients in rainfall seasonality at a high spatial resolution. Strong seasonality was evident in both Mediterranean areas, with a winter bias, and in tropical areas with a summer bias, while the arid interior showed an aseasonal rainfall regime. Of all the rainfall descriptors utilized, seasonality showed the strongest gradient across the population sampling transect, with a strong bias towards winter rainfall in coastal South Australia, through to aseasonal rainfall in New South Wales. The magnitude component of the vector seasonality index was close to equivalent to the Walsh and Lawler seasonality index, with a strong linear correlation between the two, indicating the lack of bimodal rainfall regimes in Australia. The vector seasonality index did provide additional information on the direction of seasonality, revealing, for example, a gradual shift in the timing of peak rainfall from south to north along the Western Australian coast.

The capacity of *Austrodanthonia caespitosa* to remain active over summer was confirmed in the seasonality experiment. However, in both the seasonality and the population comparison under natural rainfall, plants became dormant if water supply was inadequate. The seasonality experiment also showed the potential for South Australian populations to show a significant growth response to summer watering compared to New South Wales populations, which may require even larger rainfall quantities to break or avoid dormancy during summer. Importantly, the seasonality experiment revealed shoot growth in response to summer watering, rather than root

allocation as a means of continuing to transpire during drought, by increasing soil water extraction ability. Neither droughted nor watered plants showed a change in allocation to below-ground organs over summer, but summer watered plants did show a response in shoot growth. Kemp and Culvenor (1994) discuss a range of strategies relevant to perennial grasses facing drought. *A.caespitosa* appears to possess a "recovery" strategy of low, reduced growth during stressful periods, followed by a rapid growth when resources become available.

The evolutionary algorithm model did not include plant dormancy and regrowth, but as this trait appears to vary between populations, inclusion of dormancy in the model may be a useful future research direction. In implementing this, it would be necessary to consider the adaptive advantage of summer dormancy as a drought survival strategy (Kemp and Culvenor 1994), and the trade-offs associated with the amount of soil water required to maintain plant activity. For instance, lower water use efficiency in summer may lead plants to become dormant and stop transpiration, in order to make better use of the water in cooler conditions. Optimisation modelling of this may be implemented by including in the model genes that control onset of dormancy at either a fixed time, or at a critical soil water content, resulting in the loss of above-ground biomass. Genes may also be included to trigger resumption of growth, again at a set time or at a critical soil water content.

The seasonality experiment also showed a slight trend towards deeper root development with continued summer watering. This would be expected if plants that remain active during summer rely on deeply penetrating water from large rainfall events, as delivered

in this experiment, rather than rely on high-cost surface roots for water acquisition during this period. The evolutionary algorithm model highlighted the potential importance of soil texture in influencing root distribution strategy in relation to rainfall seasonality. In coarse, sandy soil, shallow roots were developed with summer rain, and deep roots were developed with winter rain. In clay soil, the trend was reversed, with deep roots with summer rain, and shallow roots with winter rain. This was related to the lower evaporative loss from sandy soils, hence the greater availability of water in the surface in summer compared to in clay soils, and the greater infiltration capacity of sandy soils, leading to the penetration of large winter rainfall events to the deepest soil layers in the coarse textured soil. In contrast, in clay soil summer rainfall was short lasting in the surface layers, leading to a reliance on deep water storage and the development of deep roots. The model results for clay soil are most similar to the experimental results, reflecting the low returns for investment in shallow roots in the surface to capture summer rainfall. There may be a threshold of summer rainfall amount, or event size, before it becomes useful in the surface soil (Ehleringer and Dawson 1992), and the model indicates that this threshold may be higher in fine soils. It should be noted that the experimental plants grew in a fairly coarse sandy loam soil, compared to the clay soil used in the model. The experimental plants also received larger watering events at two week intervals, compared to one week intervals used in the model, so the larger events are expected to have penetrated to deeper soil layers in the experiment than in the model run with coarse soil. Therefore, an additional process, deep water penetration, may have resulted in deep root development in the experiment as well as the high cost of surface roots that explain the model output. As expected,

both the model and the seasonality experiment showed greater growth per unit water in the winter than the summer.

12.5. Soil Type

The high degree of variation between closely situated ecotypes suggests that factors more localised than the rainfall gradients examined here are driving differentiation. Soil type is likely to have a local effect on selection, given its important influence on soil water distribution (Schenk and Jackson 2002a)**,** although past site history, including grazing history (Scott and Whalley 1984) and community composition may also be relevant factors. There were no significant correlations between the soil texture variables and plant traits in the natural rain population comparison, although this included the limited number of soil variables obtainable from the national dataset. Local soil collection and analysis for relevant data, as well as growing a second generation of plants to remove maternal effects may provide more power to determine the effects of soil on selection for plant traits.

The model confirmed the importance of soil texture in influencing temporal and spatial water availability, and in driving selection towards different optimal plant strategies. Across the gradients in pulse size and rainfall seasonality, soil texture changed the optimal root depth strategy under a particular rainfall regime. In general, coarse textured soils resulted in shallower roots when water availability was affected by evaporation, for instance with small events or during summer, due to lower evaporative loss from this soil type. Where water was available in larger quantities, under large events or winter rainfall, coarse sandy soil allowed greater infiltration to deeper layers

(Walter 1971b), and deep root development. Globally, deep roots have been found to be associated with sandy soil (Schenk and Jackson 2002a). In contrast, fine soil drives selection towards deep roots accessing stable deep water under limiting water conditions, but leads to the development of shallow roots where water is more plentiful, as the higher water storage capacity creates greater water availability in the surface soil. The model does not take into account the potential costs of waterlogging in clay soils under high rainfall, which may act as an additional tradeoff. In addition, many soil profiles in Australia are texture contrast soils or duplex soils (Chittleborough 1992), the effect of which can be easily simulated in the model, but which has not been explored here.

Soil texture in the model also had an affect on plant growth, which in the model was closely related to overall water availability. In particular, higher growth was observed consistently in coarse, sandy soils, despite the lower water storage capacity of this soil type. As detailed above, there tends to be less evaporative loss from sandy soils in semi-arid and arid systems (Shreve 1942), as water can percolate below the surface layers that are most affected by evaporation, leading to greater water availability overall. In addition, the lower wilting point of sand meant water was available to plants even after small rainfall events, while more water was required before plants were able to extract it from finer clay soil.

No experiments were performed comparing growth and root distribution of different ecotypes of *A.caespitosa* in different soil types, but this is a potential future research direction. Such a study may be necessary to confirm that soil texture is acting as a

selective force on plant traits, and to determine whether ecotypes sourced from one soil type are adapted to that soil, or whether they display plastic responses similar to those found in the pulse size experiment, allowing growth and survival in other soil types.

12.6. Deep Drainage Reduction and Utility

A primary aim of this project was to assess *A.caespitosa* for its usefulness in reducing deep drainage, and assisting in salinity mitigation. In particular, an effort was made to determine whether particular ecotypes displayed characters, such as a deep rooted habit and summer activity, that may be useful for this purpose, and to determine whether those characters are genotypically fixed or plastic.

The seasonal watering experiment confirmed characters that may make *A.caespitosa* a useful nature component of pastures. There was a strong shoot growth in response to summer rain, generating biomass from rainfall that may have been left unutilised in annual pastures. However, the ability to dry the soil over summer is less clear. While plants, particularly those from the South Australian ecotypes tested, utilized water from large summer watering events, those plants that were droughted showed no increased root growth response that may have increased soil drying under lower rainfall conditions. In order to make a significant impact in reducing deep drainage, the soil has to be dried over summer, leaving storage capacity for high winter rainfall (Johnston, Clifton *et al*. 1999). Plants that are drought tolerant, such as *A.caespitosa*, may have low year-round water use, and are therefore less useful for salinity mitigation

(Johnston and Shoemark 1993), and even perennial grasses with high water use are unlikely to reduce deep drainage in higher rainfall zones, approaching 700mm per annum (Ridley, White *et al*. 1997). The threshold of rainfall event size in summer that triggers growth may be too high for *A.caespitosa* to make a significant contribution to deep drainage reduction, and this threshold appears to vary between populations, a factor that warrants further investigation.

In comparing all the ecotypes under a natural rainfall regime, there was no evidence ecotypes from a certain climatic region had particularly deep roots that may be useful for deep drainage reduction. Rather, interpopulation variability had a strong local component, possibly driven by soil texture, as discussed above. There was some variation in phenology, with evidence New South Wales populations survived longer before dormancy, another useful character for increasing water use over summer. However, as phenology of some ecotypes of this species is strongly influenced by factors such as day length (Hodgkinson and Quinn 1978), the expression of this trait may depend on planting location.

The pulse size experiment revealed root depth to be quite plastic, with response to watering regime more important than genotypic differences between populations. No interaction was found between treatment and population here, indicating no difference in plasticity in plants collected across this range. This may limit the potential for the deep rooted character to be selected for in this species, as root distribution was both locally variable on a small spatial scale, and varied depending on soil water

distribution. However, this plasticity may go some way to explaining the survival of this species across the wide range of annual rainfall totals, distributions and soil types in which it is found in southern Australia.

The evolutionary algorithm model confirmed the conditions under which deep drainage is expected to be a concern, with deep drainage recorded in the model in sandy soil (Petheram, Walker *et al*. 2002) under high winter rainfall (Keating, Gaydon *et al*. 2002). However, these were also the conditions that lead to an optimum development of deep roots. If reflected as an adaptation in plants in their natural environment, or even as a plastic response in experimental plants, this character may help reduce deep drainage in those environmental conditions where it is expected to be worst.

12.7. Conclusion

Variation between populations in *A.caespitosa* in the characters examined here had a strong local component. This suggests factors operating on a spatial scale smaller than the rainfall gradients examined here, for instance soil type or site history, were driving population differentiation. However, the evolutionary algorithm model confirms the potential for differences in small-scale rainfall regime to lead to different temporal and spatial water availability in the soil profile, with different root distributions best suited to different rainfall regimes. The pulse size experiment revealed plasticity in root distribution to be an important characteristic of this species. Plants producing shallow roots with small, frequent watering events, and deep roots with large, infrequent events, rather than showing strong differentiation between populations, and large events

appeared to be less limiting. The evolutionary algorithm model also highlighted the importance of soil type in determining the optimal root distribution and biomass allocation response to rainfall pulse size, frequency and seasonality. It also concurred with the experimental results that indicate the species is able to efficiently utilize large rainfall events and survive long interpulse periods, and also confirms the importance of the high cost of shallow roots in influencing plant water acquisition strategy in the surface soil.

Overall, the perennial grass *A. caespitosa* has the potential to be a useful component of native pastures, with some South Australian ecotypes showing a strong growth response to summer rainfall. However, the utility of this species for deep drainage reduction is less clear, with no particularly deep-rooted genotypes identifiable. Summer activity and transpiration to dry the soil profile depend on large rainfall events, with drier conditions resulting in dormancy without increased root proliferation and water extraction.

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14. APPENDIX A – SEED COLLECTION

14.1. Austrodanthonia caespitosa Seed Collection

Potential collection sites for *Austrodanthonia caespitosa* in South Australia were identified from a range of biological and botanical surveys of regions within the state (Atkins 1994; Brandle 2000; Copley and Kemper 1992; Davies 1990; Diez and Foreman 1996; Forward and Robinson 1996; Graham, Opperman *et al.* 2001; Happy Valley Corporation, Mitcham Corporation *et al.* 1994; Hyde 1998; Hyde 2000; Hyde 1994; Hyde 1995; Hyde 1999; Kenny, Graham *et al.* 2000; Moore 1985a; Moore 1985b; Mowling 1979; Nature Conservation Society of South Australia 1977; Oppermann and Bates 1995; Owens 1995; Playfair and Robinson 1997; Robertson 1998; Robinson and Armstrong 1999; Robinson, Casperson *et al.* 1988; Rowett, Venning *et al.* 1981; Stewart 1996; Stokes 1996; Val, Foster *et al.* 2001; Young 1988; Young 1990). Eastings and northings for 280 sites were found, and the locations of these populations were plotted on a map of South Australia, so that potential sites within the 400-500mm rainfall band could be visited.

Detailed biological surveys for relevant regions of Victoria and New South Wales were unobtainable, so less precise surveys were relied upon. A survey of vegetation in the Boorowa Shire, New South Wales (NSW National Parks and Wildlife Service 2002) found that *A.caespitosa* was likely to be associated with red gum (*Eucalyptus camaldulensis*), yellow box (*Eucalyptus melliodora*) and white box (*Eucalyptus quadrangulata*) woodland. In the Riverina region of New South Wales, *A. caespitosa*

was associated with the Borree Woodland on the Hay Plains, as well as grassland communities around Jerilderie (Eardley 1999). Other potential collection ranges within the 400-500mm rainfall band in Victoria and New South Wales were identified from herbarium records in the Australian National Herbarium (http://www.anbg.gov.au/cpbr/herbarium/). A list of towns and conservation areas throughout New South Wales and Victoria where collection would be representative of the range of the species across the seasonality and event-size gradient was generated (Table 12). Wyalong was chosen as the northern limit of the collection effort, as to the north the climate tends to shift towards a summer-dominated rainfall regime. The Fleurieu and Yorke peninsulas in South Australia were chosen as the western limit of the survey as they showed the most extreme seasonality index values.

Table 12 – Towns and conservation areas in Victoria and New South Wales where *Austrodanthonia caespitosa* may be present.

Towns on Route for Roadside Collection	**Protected Areas Identified for Collection**
South Australia Yorketown, Ungarra, Maitland, Strathalbyn, Keith, Serviceton, Tintinara, Two Wells, Kimba, Wharminda, Orroroo, Murray Bridge, Clare, Burra, Riverton, Auburn, Kadina, Bordertown, Mundulla **Victoria** Serviceton, Nhill, Horsham, Warracknabeal, Watchem, Murrayville, Sea Lake, Swan Hill, Canary Island, Walpeup, Cohuna, Kerang, Ouyen, Waikerie,	**South Australia** Scott Conservation Park Spring Mount Conservation Park Deep Creek Conservation Park Waitpinga Conservation Park Innes National Park Warrenben Conservation Park Mokota Conservation Park Spring Gully Conservation Park Messent Conservation Park Mount Monster Conservation Park

New South Wales Deniliquin, Griffith, Wyalong, Barellan, Booligal, Hay, Rankin Springs,	**Victoria** Little Desert National Park Mount Arapiles Kooyoora State Park Leaghur State Park Kamarooka State Park Terrick Terrick National Park. **New South Wales** Jerilderie Nature Reserve Narrandera Nature Reserve Cocoparra National Park Buggigower Nature Reserve Cocopara Nature Reserve Charcoal Tank Nature Reserve

Council collection permits were obtained from the councils listed in Table 13. A permit to undertake scientific research was obtained from the South Australian Department for Environment and Heritage (Permit No. E24714) for collection of *Austrodanthonia caespitosa* seeds within protected areas in South Australia. Permits

for collection from protected areas in New South Wales and Victoria were also applied for, but were not approved before the collection trip, so in these states collection was limited to council land and roadsides.

Table 13 - Councils in South Australia, Victoria and New South Wales providing approval for roadside seed collection.

SA	Naracoorte and Lucindale Tatiara Karoonda East Murray Southern Mallee Mount Barker Yankalilla Victor Harbour Goyder Clare and Gilbert Valleys Copper Coast
Vic.	Moira West Wimmera Buloke Northern Grampians Loddon

NSW	Berrigan
	Jerilderie
	Carrathool
	Hay Shire
	Deniliquin
	Bland Shire

14.2. Collection and processing of Austrodanthonia caespitosa *seed*

Seed of *Austrodanthonia caespitosa* was collected on four trips between the 23rd of October and 17th of November, 2003. The first trip, from 23/10/2003 to 24/10/2003, encompassed the Yorke Peninsula from Port Clinton to Innes National Park. The second trip, from 30/10/2003 to 31/10/2003 encompassed the mid-north of South Australia including Riverton, Burra and Clare. The third trip, from 9/11/2003 to 15/11/2003 took in eastern South Australia from Tintinara to the Victorian border, and the listed towns through Victoria and New South Wales. The final trip, on 17/11/2003, collected in the lower Murray and Fleurieu Peninsula of South Australia.

Previously identified survey sites in South Australia were located using a GPS. Where survey sites were found to be on private land, or had no *Austrodanthonia caespitosa* present, nearby accessible locations were used instead. In Victoria and New South Wales, roadside populations of *Austrodanthonia spp* were identified while driving

around the chosen sampling districts in local councils that had provided collection permits. If populations were identified as *A. caespitosa* on closer observation, they were selected as collecting sites. All chosen collection sites had their location stored in the GPS, and a list of all collection sites is presented in Table 14 and as a map in Figure 95.

At each site, mature inflorescences were picked and stored in paper bags. Number of inflorescences collected was limited to not more than an estimated 5% of the total number of mature inflorescences at the site. A 5cm auger was used to take a soil sample from the 5-10cm soil depth at a random location within the sampling site. The soil sample was stored in a sealed plastic jar. The auger was also used to penetrate to a depth of 1m, and records were taken of the depth at which bedrock was reached or major changes in soil type occurred. Clay content, texture class and bulk density of each site was also estimated from the Australian Soil Resource Information System maps (http://www.clw.csiro.au/aclep/ASRIS2004.htm) for comparison with the collected samples.

Notes were also taken on the location of the site relative to the road and surrounding environment, and the presence of dominant species or overstory at each site.

In the laboratory, inflorescences were ground between corrugated rubber blocks to free the seeds. The mixture was then passed through a blower to separate heavy seeds from light hairs, glumes, lemmas and paleas. Total seed weight collected from each population was measured (Table 14). Seeds from each population were stored in small

plastic containers in the dark at room temperature until used.

Table 14 - Collection sites and seed amounts

Site Code	Latitude	Longitude	Near Town	Panicles	Seed Mass (g)
NSW001	-35.57173	144.97602	Deniliquin	100	1.175
NSW002	-35.63742	145.51416	Finley	100	0.234
NSW003	-35.6468	145.82392	Berrigan	110	0.497
NSW004	-35.15935	145.939	Jerilderie	110	1.036
NSW005	-33.89457	147.11525	West Wyalong	105	2.396
NSW006	-33.8482	146.86039	Yalgogrin	150	2.009
NSW007	-33.83705	146.27211	Rankin Springs	100	1.028
SA001	-34.06676	137.96029	Melton	50	0
SA002	-33.97242	137.74545	Kadina	100	0.2883
SA003	-34.0009	137.61681	Moonta	102	0.0883
SA004	-33.97123	138.83108	Manoora	101	0

SA005	-33.88859	138.97284	Emu Downs	111	0
SA006	-33.54945	138.95135	Mokota	50	0.09
SA007	-33.6658	138.59605	Clare	100	0.06
SA008	-33.89863	138.52217	Kybunga	200	0.081
SA009	-33.90711	138.57932	Spring Gully	50	0.311
SA010	-36.04462	140.31455	Keith	100	0.124
SA011	-36.19752	140.31763	Mount Monster	100	0.165
SA012	-36.34884	140.67023	Mundulla	110	0.069
SA013	-36.42349	140.90654	Wolselys	115	0
SA020	-35.3488	139.12117	Langhorne Creek	175	2.274
SA021	-35.16317	139.01097	Red Creek	130	3.701
SA022	-35.51145	138.69859	Middleton	130	2.6
SA023	-35.61616	138.10852	Cape Jervis	200	4.127
VIC001	-36.9135	142.65658	Glenorchy	100	0.164

VIC002	-36.82486	142.63127	Glenorchy	160	0.048
VIC003	-36.35539	142.84098	Litchfield	150	0.968
VIC004	-36.28921	143.09454	Donald	110	0.363
VIC005	-36.07691	143.52723	Boort	120	0.685
VIC006	-36.10892	143.83057	Boort	115	2.565
VIC007	-35.9081	145.49437	Strathmerton	50	0.821

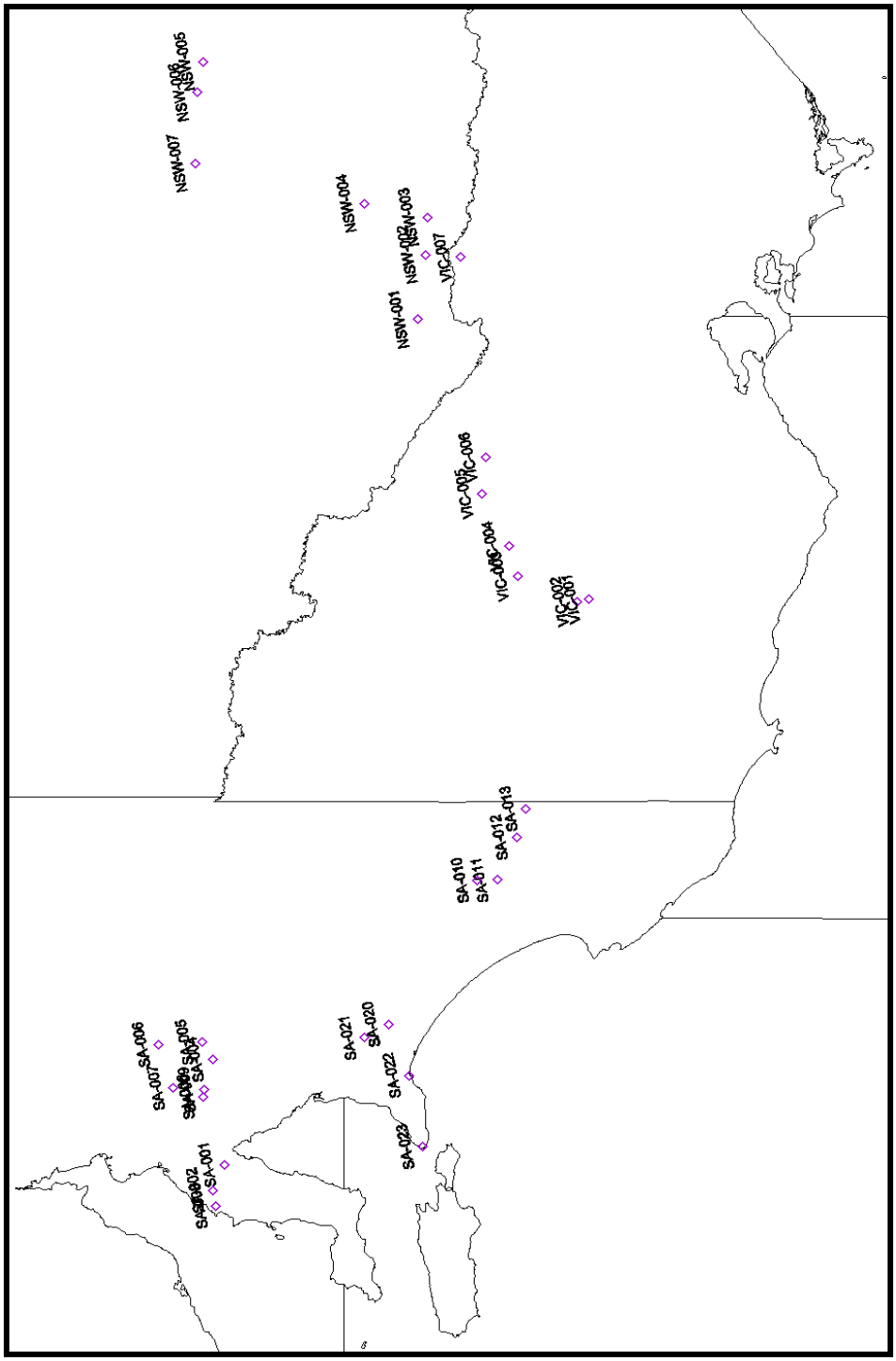

Figure 95- Seed collection locations in South Australia, Victoria and New South Wales.

15. APPENDIX B – PILOT STUDIES AND MINOR EXPERIMENTS

15.1. Pilot "Colander" study

Introduction

Variations in the distribution of roots in the soil profile is expected to be related to the distribution of water in the soil, particularly in water limited systems where the water is heterogeneously distributed in the soil (Yanagisawa and Fujita 1999). For example, we may expect proliferation of roots in areas of high resource availability (Pregitzer, Hendrick *et al.* 1993), with deep roots present with high availability of water deep in the soil profile, and shallow roots with high surface soil content. Variation in root depth may also be associated with degree of drought tolerance, with deep roots considered useful for surviving drought (Garwood and Sinclair 1979).

Soil water availability with depth is a result of climatic influences, such as rainfall regime and evaporative demand, and we may expect plants evolved under a particular climate to possess genotypically fixed root architecture, optimised to make best use of the local soil water dynamics. Variation in root architecture with local water availability has been identified in a number of species, for instance Oyanagi et al. (1991a) identified variation in the seminal root growth angle in wheat (*Triticum aestivum)* with rainfall regime in Japan. Variation in speed of deep root development has also been

15-310

identified between *Polygonum spp.* under different water regimes(Bell and Sultan 1999), although plasticity in root development was important. It has been hypothesized that roots may have a genetically controlled gravitropic set-point angle determining the angle, with respect to the vertical, at which roots grow (Digby and Firn 1995) and this angle may change over time to determine the shape of the root system. Nakamoto and Oyanagi (1994) developed a method of measuring the distribution of root growth angles in a species growing in pots under glasshouse conditions. This technique involves burying a colander, a perforated hemispherical bowl, in the soil, allowing plant roots to growth through the holes, then excavating the colander and determining the number of roots protruding from the colander at various angles around the sphere, from the horizontal to the vertical.

In this study, this technique is applied to plants of *Austrodanthonia caespitosa* and *Microlaena stipoides*, grown from seed collected from a range of rainfall regimes across southern Australia. These are two species of perennial grass that are known to be highly variable in morphological characters across their range. The aim of this experiment is to determine the suitability of the colander technique for measuring root angle distribution in these grasses, and to identify any variation in root angle between the plants.

Methods

A pilot study was initiated to determine the feasibility of using the colander method (Nakamoto and Oyanagi 1994; Oyanagi 1994; Oyanagi, Nakamoto *et al*. 1993b) to measure root growth angle distribution in *Austrodanthonia caespitosa*.

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Seeds of *Austrodanthonia caespitosa* and *Microlaena stipoides* were obtained from a commercial seed supplier (Blackwood Seeds), Cathy Waters and the LIGULE project. Seeds were germinated and grown in seedling trays for one month before transplanting. The sources of *A.caespitosa* used were "Auburn", "Florieton" , "South East" and "Dubbo" and the sources of *M.stipoides* were "Bradbury" and "LIG183".

Wire baskets were constructed out of hexagonal weave chicken wire. 300mm x 300mm squares of chicken wire were pressed into a 140mm diameter hemispherical mould and the edges were trimmed off. Baskets were painted with Wattyl brand "Killrust" epoxy enamel to prevent rusting. Plastic pots with a diameter of 200mm and a height of 180mm were filled to a depth of 100mm with Mount Compass loam soil (Jeffries Soils, Wingfield, SA). Baskets were placed on the soil surface, and more soil was added so that 10mm of basket was left exposed above the soil surface.

On August 10th 2003, four seedlings of each of the grass sources were planted in pots in the centre of the basket, and all pots were watered with 40mm of water to aid establishment. Watering events of 180mL, representing 5.6mm of rainfall, was applied two days a week throughout the experiment. Water was applied in two 90mm applications on each watering day to minimise soil disturbance.

After a week, it was noticed that several seedlings were in poor health (*M.s* Bradbury 3, *A.c* SE 1, *A.c* SE 3, *A.c.* Auburn 3) and they were replaced with new stock. After a month, plants in some pots had died (*A.c.* SE 1, *A.c.* SE 3, *A.c.* Auburn 3) and were not

15-312

replaced.

Plants were harvested on 10th October, 2003. In each pot, a plastic tie was tied around the exposed lip of the basket to indicate soil surface level. Pots were squeezed to loosen soil, and the pots were tipped on their side. Plant and basket were pulled out, with an effort made to avoid breaking roots radiating out from basket. A protractor was used to determine the line on the basket that was at a 45-degree angle to the soil surface, and this level was marked with further plastic ties. Counts were made of the number of roots protruding through the basket above and below the 45-degree line. Roots were removed from basket, roots and shoots separated and stored in paper bags. Shoots and roots were dried in an oven at 100 °C for two days, and then weighed to determine dry weight.

Results

One-way ANOVAs were used to examine differences between populations in deep root number, shallow root number, deep:shallow ratio, root mass, shoot mass, root:shoot ratio and total biomass.

There was no significant difference between populations for shallow root number ($df = 5$, $p=0.75$) or deep root number ($df=5$, $p=0.08$). Results are presented in Figure 96.

15-313

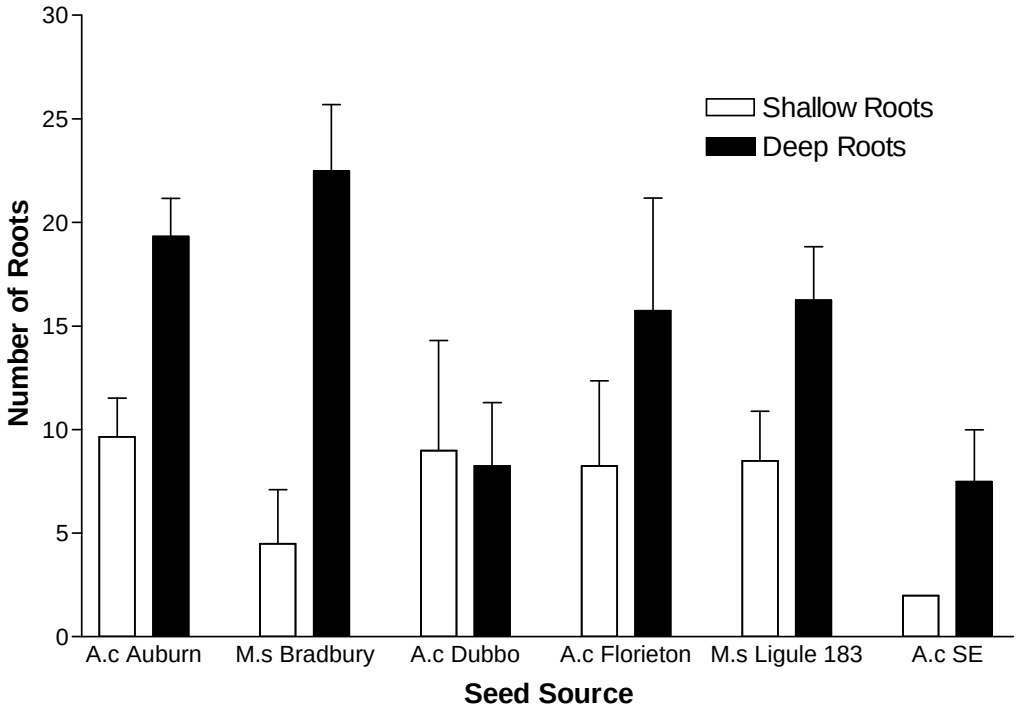

Figure 96 - Shallow and deep root counts for populations in colander pilot study.

Figure 97 shows shallow:deep root ratio for all populations. The ANOVA showed no significant difference between populations (df=5, p=0.26).

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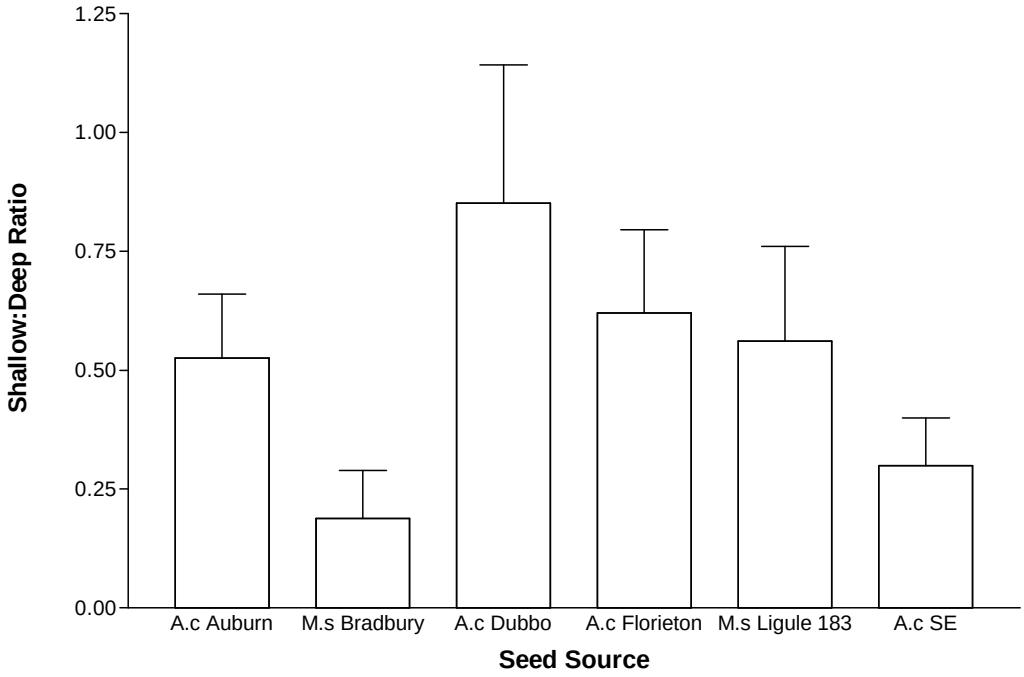

Figure 97 - Shallow:deep root ratio for populations in colander pilot study.

There was a significant difference in root mass between populations (df=5, p=0.025), as shown in Figure 98. A Tukey HSD test revealed that population *A.caespitosa* South-East was significantly different from the other populations.

15-315

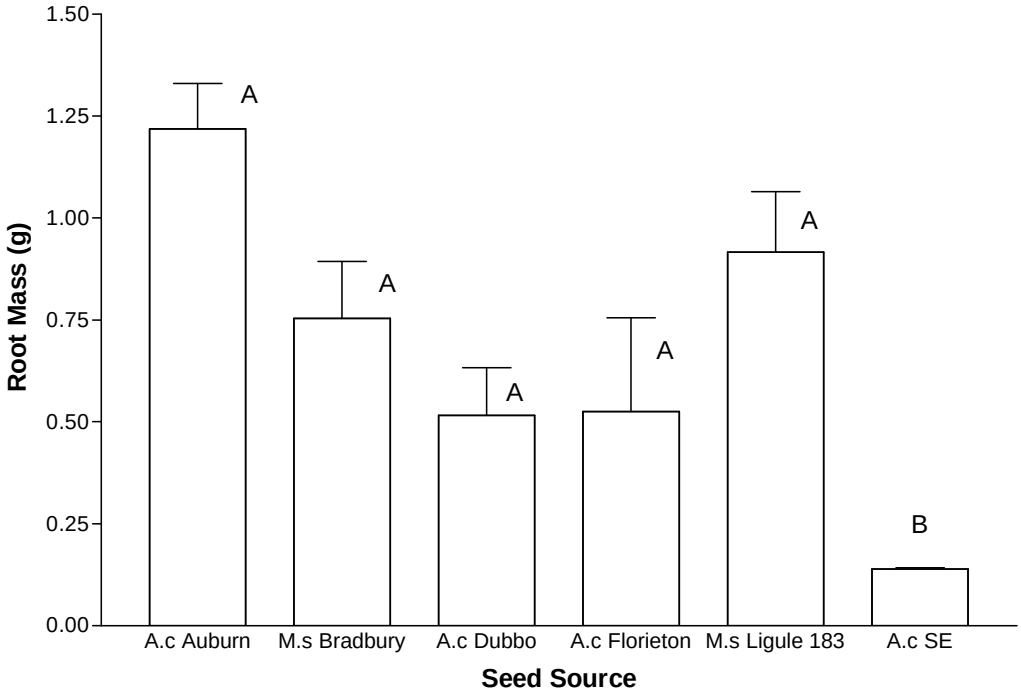

Figure 98 - Root mass for populations in colander pilot study.

No significant difference in shoot mass was found between populations (df=5, p=0.07), shown in Figure 99.

15-316

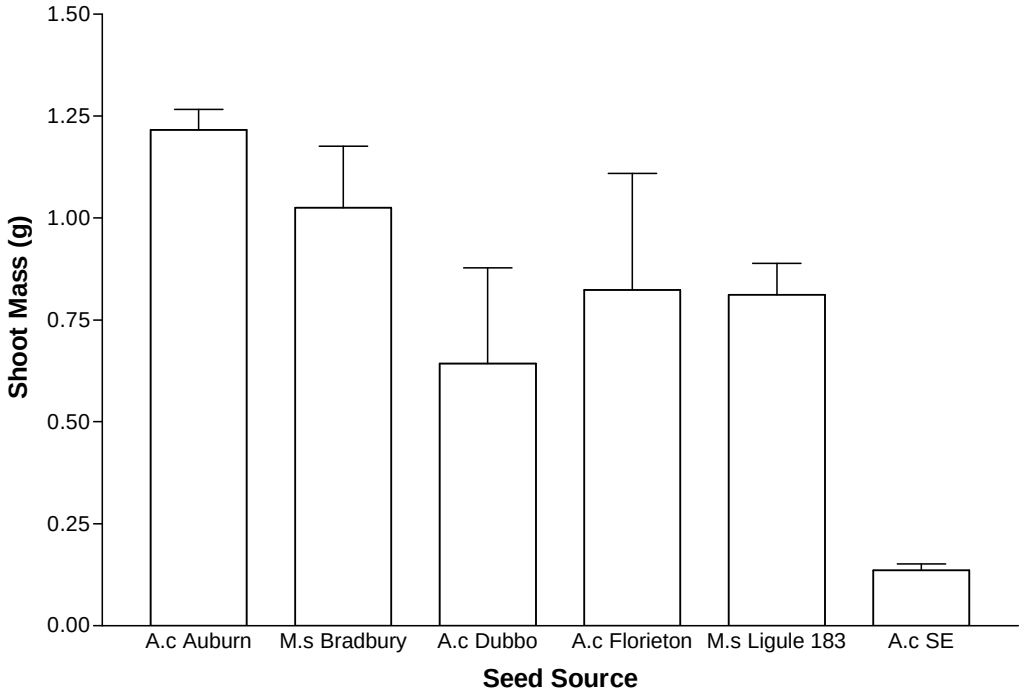

Figure 99 - Shoot mass for populations in colander pilot study.

There was also no difference in root:shoot ratio found between populations (df=5, p=0.55), as shown in Figure 100.

15-317

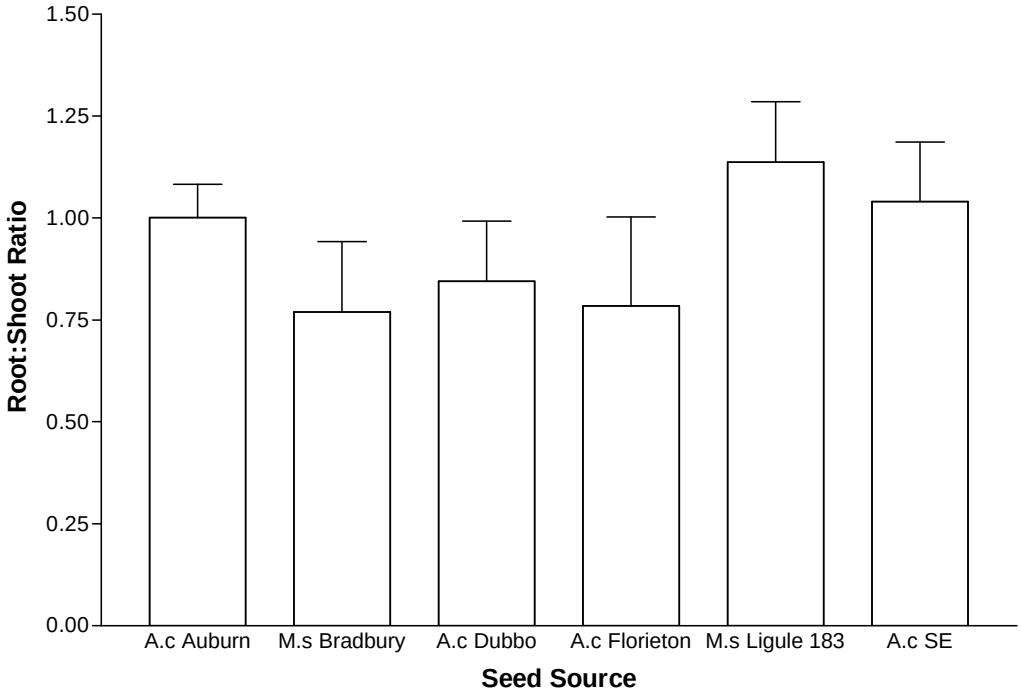

Figure 100 - Root:Shoot ratio for populations in colander pilot study.

Population did have a significant effect in total biomass (df=5, p=0.03), as shown in Figure 101, with a Tukey HSD test showing that population *A.caespitosa* South-East had significantly lower total biomass than other populations. This population appeared to do poorly under the grown conditions provided, with only two plants remaining at the end of the experiment, both showing significantly low growth. When this population was excluded from the analysis and the ANOVAs repeated, there were no significant differences for any measured variable. Many variables, particularly those of deep and shallow root counts, were highly variable, and root counts were considered inaccurate due to difficulties in removing the colanders without breaking roots.

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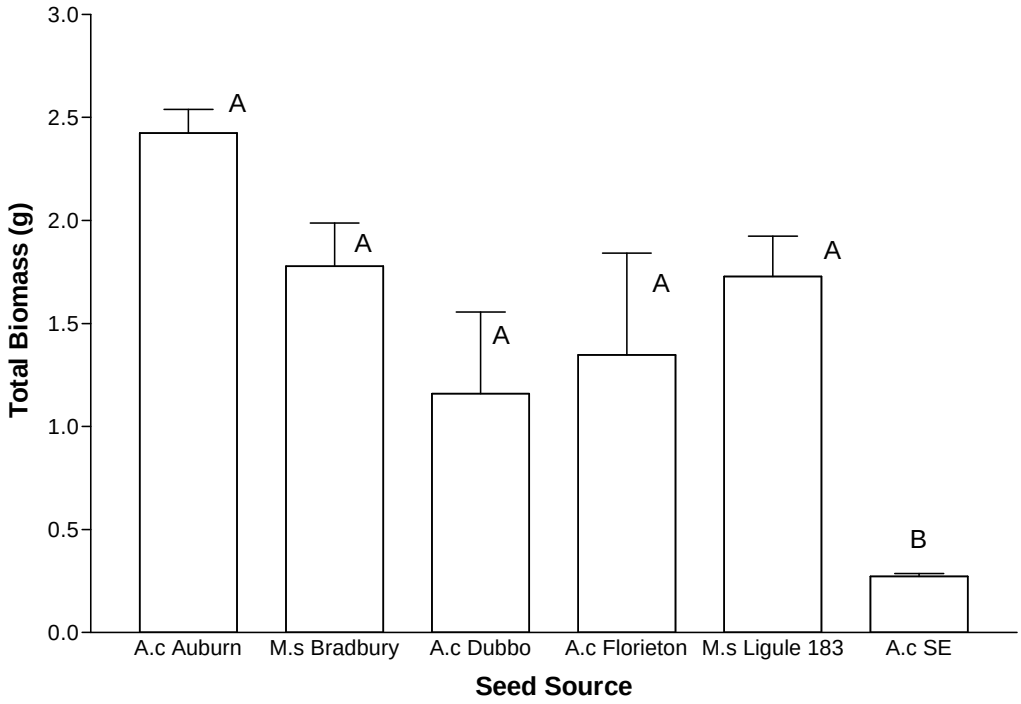

Figure 101 - Total biomass for populations in the colander pilot study.

Discussion

Although there was high variability between populations and species in measured plant growth variables, high variance within populations means no significant differences were found between populations, apart from the significantly low growth in the *A.caespitosa* population from south-east South Australia. The experiment, conducted in a glasshouse under moderate watering, may have provided inappropriate growing conditions for an ecotype sourced from a cool, relatively high rainfall environment.

Overall, this experiment highlighted potential problems with the application of the "colander" technique. It was difficult to remove the colander from the soil without disturbing the soil within the colander, in turn shifting roots and making it unclear

15-319

whether roots extended above or below the 45-degree point. A large number of roots were lost during excavation and root counting, and it was difficult to accurately count fine roots through the colander. Using a rigid, plastic colander with small holes may have provided better results than the wire mesh used in this experiment, but the use of a solid colander may have resulted in soil water redistribution, localized moist areas, and redirection of roots around the solid surface of the colander. The wire mesh technique may be better applied to species with thicker, less breakable roots. A more suitable solution for examining root distribution in this species may be to rely on root depth distribution in the soil profile, rather than root growth angle.

Conclusion

The "colander method", as implemented here, did not provide a sufficiently accurate and reliable method of determining root growth angle distribution, due to high root and soil loss during colander extraction. Measuring root length of mass as it varies with depth may provide more accurate assessment of root distribution than measuring root angle in fine-rooted grasses.

15.2. Ecophysiology pilot study

Introduction

Austrodanthonia caespitosa has been found to show a significant degree of inter-population variability (Robinson and Archer 1988), which may reflect selection and differentiation in response to local environmental influences (Wilson 1996). Plants sourced from regions with small, frequent rainfall events, or constant rainfall

15-320

throughout the year, may show a reduced ability to survive long periods of low soil water availability, and a limited capacity to recover from drought. Similarly, plants may show the greatest growth response under watering regimes that reflect their local climatic conditions, with plants sourced from a region experiencing large, infrequent rainfall events showing reduced growth under small, frequent pulses. A pilot study was conducted to conduct a preliminary examination into differences that may exist between populations in leaf growth, habit, ability to survive and recover from drought, and in photosynthetic efficiencies over time.

Methods

An experiment was set up to measure growth rates and photosynthesis parameters for different populations of *Austrodanthonia caespitosa*. Seeds of five populations (SA020, SA023, VIC003, NSW004 and NSW005) were germinated on damp filter paper in petri dishes. Ten 20cm plastic pots were filled with Mt. Compass Loam (Jeffries, Wingfield). Six seedlings of each population were planted in the pots on March 26, 2004, with each population being planted in two pots. The two sets of pots represented a "pulsed" and a "constant" watering regime. Plants in the "constant" treatment were watered well several times a week by an automatic watering system, while "pulsed" pots were watered once a fortnight manually. From April 8 until May 17, number of leaves and length of longest leaf were measured weekly.

The watering system failed to water the constant treatment from May 25 until June 7. On June 7 and June 8, constant treatments were supplied with additional 20mm

15-321

equivalent watering events. On June 8, a Pulsed Amplitude Modulation (PAM) chlorophyll fluorescence system was used to measure photosynthetic activity of one leaf on all plants in both the "constant" treatment, which had been watered frequently, and the "pulsed" treatment that had not been watered for two weeks. The "pulsed" treatment was re-watered and PAM measurements were taken again on all plants the following day. PAM measurements continued weekly for the following month. Light intensity was variable during PAM measurements, but an artificial light source was used in an attempt to maintain PPFD between 800 and 900 $\mu mol\ m^{-2}\ s^{-1}$.

Results

Measurements of leaf length over time for pulsed plants is presented in Figure 102, and for constant watering plants in Figure 103. Plants in the pulsed treatment, particularly those from populations SA020, SA023, and VIC003 appear to grow longer leaves than those in the constant watering treatment. The effect of pulsed water supply is also clear from the graphs, with plants in the pulsed treatment, particularly those from the SA020, SA023 and VIC003 populations, showing bursts of growth followed by flatter periods, while the growth curve for the constant watering treatment is smoother.

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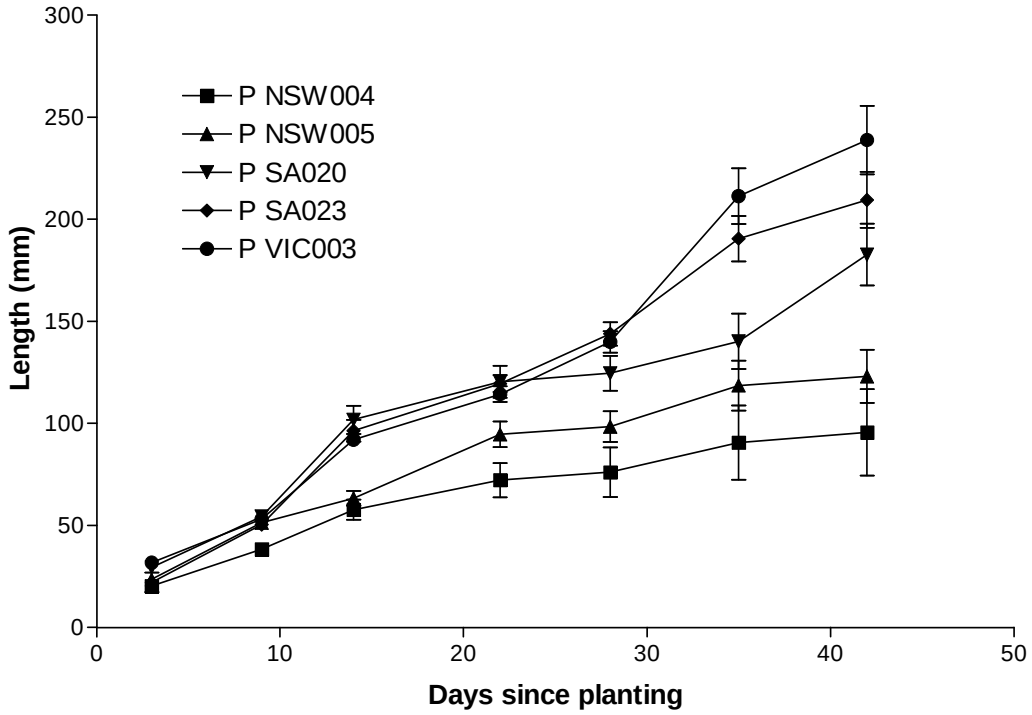

Figure 102 - Length of longest leaf over time for pulsed treatment

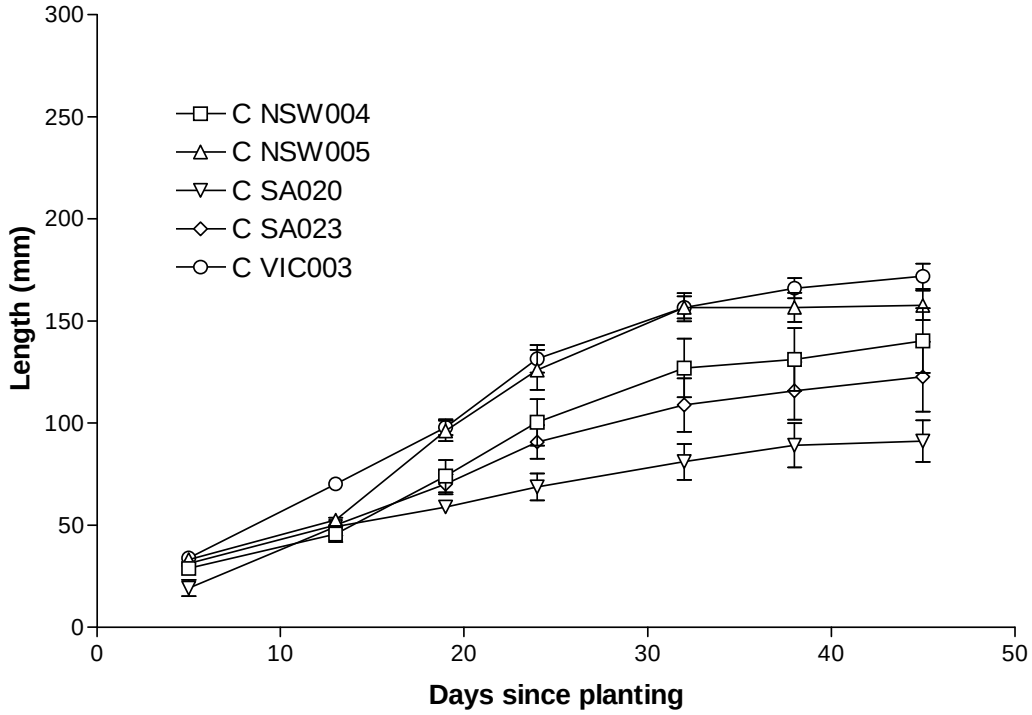

Figure 103 - Length of longest leaf over time for constant watering treatment

15-323

A 2-way ANOVA was performed to examine differences in leaf lengths between populations and watering treatment at the final measurement. The effect of watering treatment was highly significant (df=1, p=0.0006), with the pulsed treatment producing longer leaves than constant watering treatment. Source population was also highly significant (df=4, p<0.0001), with at Tukey HSD test revealing that VIC003 had significantly longer leaf length than NSW004, NSW005 and SA020. The interaction term was also significant (df=4, p<0.0001), with Figure 104 indicating that the New South Wales populations appear to have shorter leaves with the pulsed treatment, while South Australian and Victorian populations have longer leaf length with the pulsed treatment

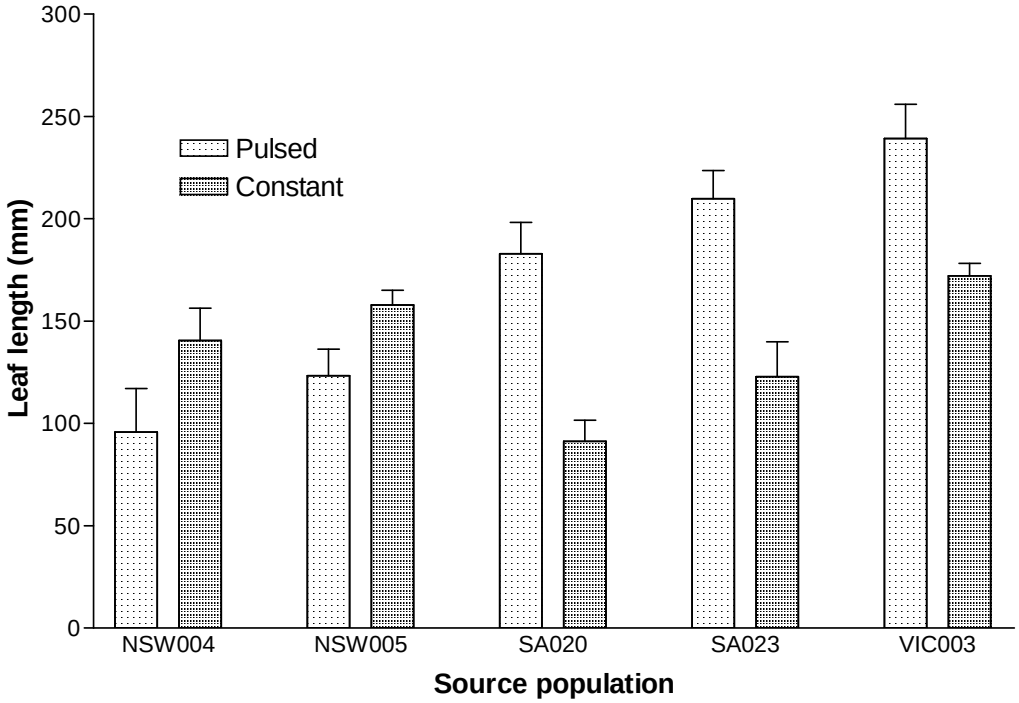

Figure 104 - Final longest leaf length for population and watering treatment

15-324

Leaf counts over time for plants in the pulsed watering treatment is presented in Figure 105, and for the constant watering treatment in Figure 106. Plants from population VIC003 appeared quite morphologically distinct, producing more leaves than the other populations, particularly in the pulsed watering treatment. Under constant watering, South Australian populations appeared to produce significantly fewer leaves than the other populations.

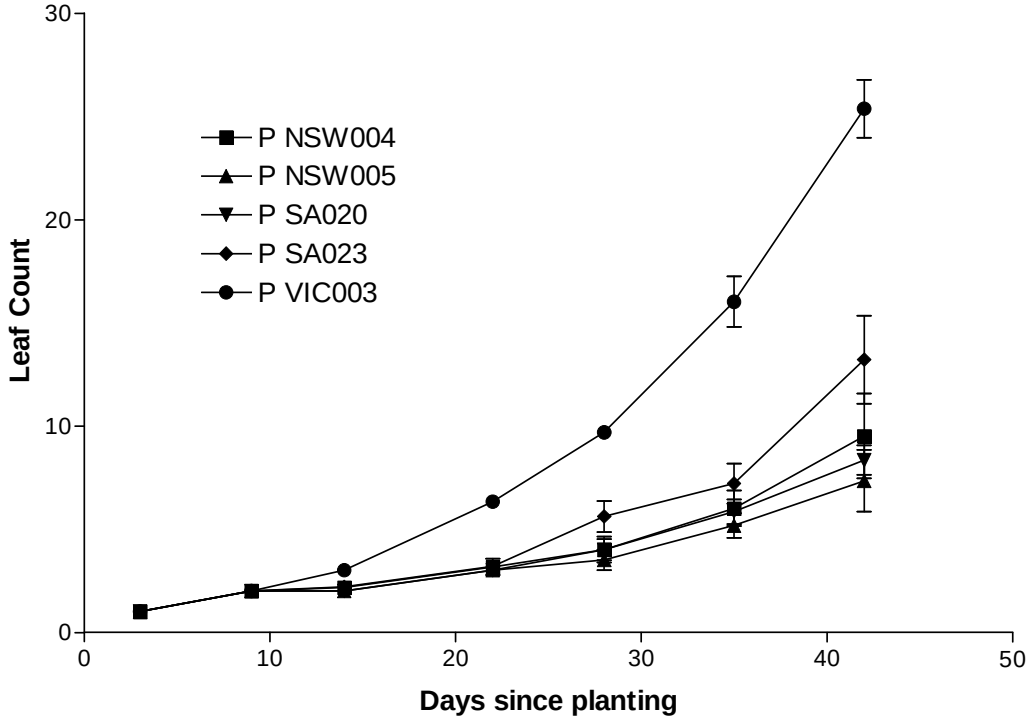

Figure 105 - Leaf count over time for pulsed watering treatment

15-325

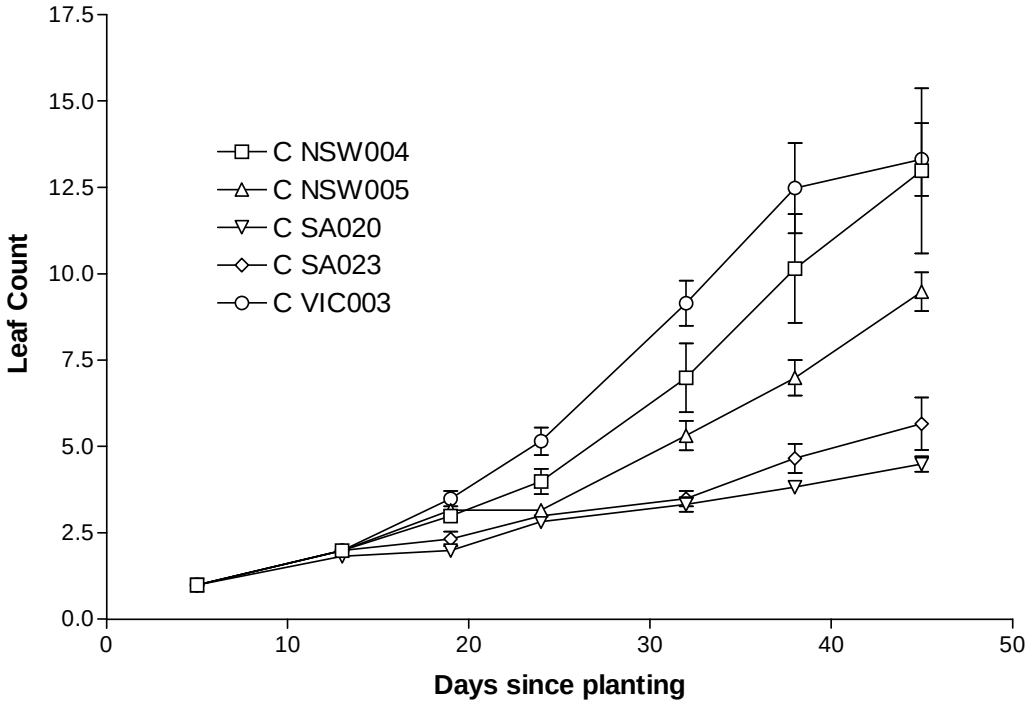

Figure 106 - Leaf count over time for constant watering treatment

A 2-way ANOVA was performed to examine differences in leaf counts at final measurement between watering treatment and population source. Watering treatment had a significant effect (df=1, p=0.0003), with the pulsed treatment producing greater numbers of leaves. Population also had a significant effect (df=4, p<0.0001), with a Tukey HSD showing population VIC003 to have significantly greater leaf numbers than other populations, and population SA020 having significantly fewer leaves than NSW004. The interaction term was also significant (df=4, p<0.0001), with population SA023 and VIC003 having greater leaf numbers in the pulsed treatment than in the constant watering treatment (Figure 107).

15-326

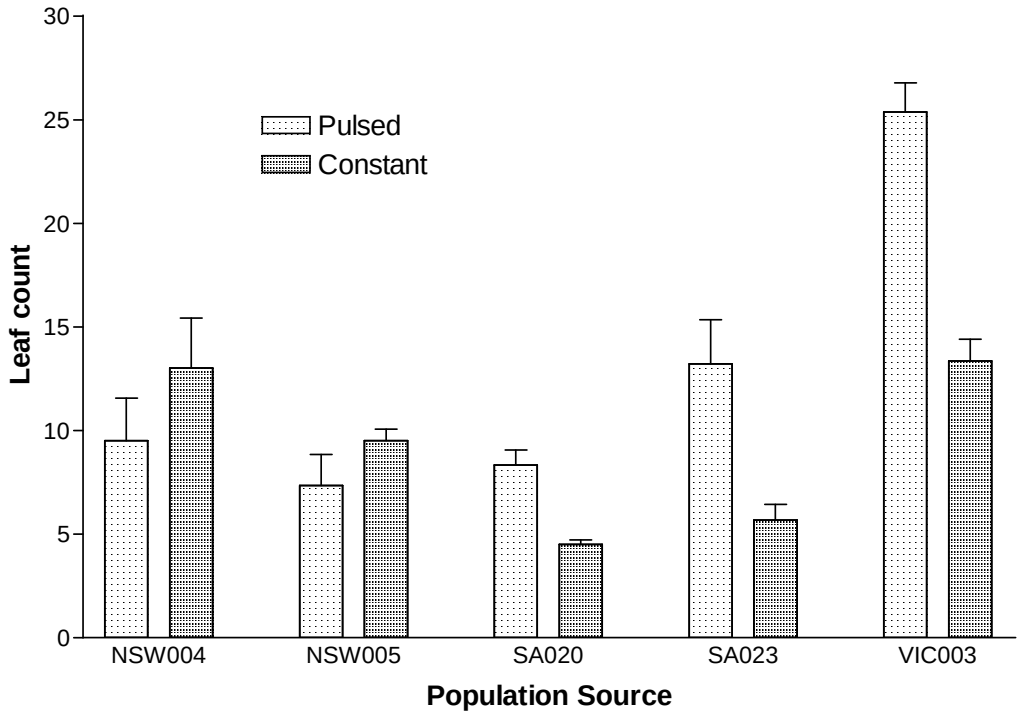

Figure 107- Final leaf count for population and watering treatment

Chlorophyll fluorescence data were highly variable from measurement to measurement, owing to variations in light intensity between measuring days. Although an effort was made to compensate for this with an artificial light source, day 15 was excluded from the following graphs as it was overcast and values were very low.

PSII quantum efficiency (Fq'/Fm') over time since rewatering is shown from Figure 108 to Figure 112. The high degree of variability from day to day is evident, as are the generally low values of Fq'/Fm', which should be close to 0.8 for a photosystem operating at maximum efficiency. In general, values for the pulsed treatment appear higher than those for the constant watering treatment, and there does not appear to be a significant and consistent change from day 0 to day 1, after the addition of a watering

15-327

event, and from day 1 to the end of measurements as the soil dried out. Visually, many plants in both treatments were quite wilted by day 27, but it is on this day that the most consistent differences between pulsed and constant watering treatment can be seen.

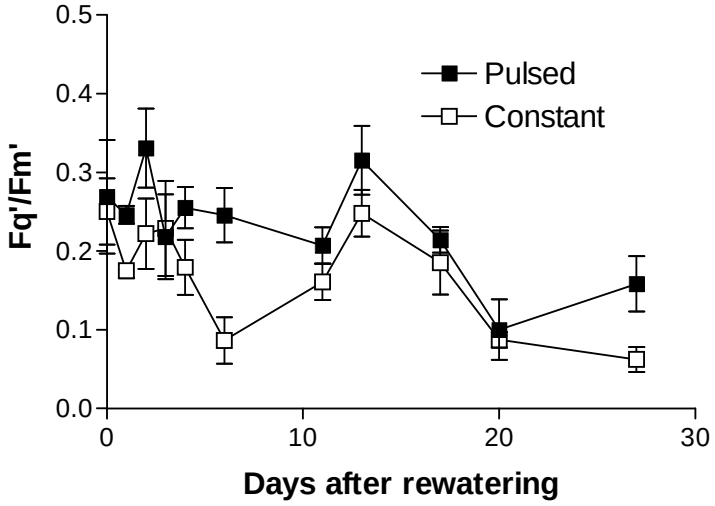

Figure 108 - PSII quantum efficiency (Fq'/Fm') for population NSW004

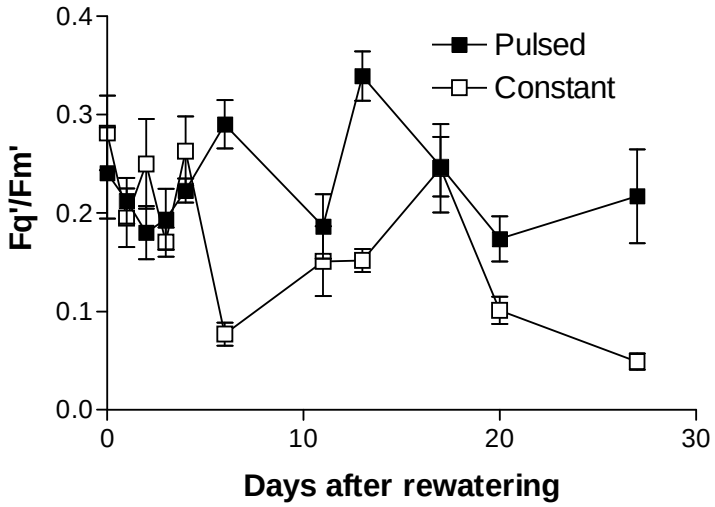

Figure 109 - PSII quantum efficiency (Fq'/Fm') for population NSW005

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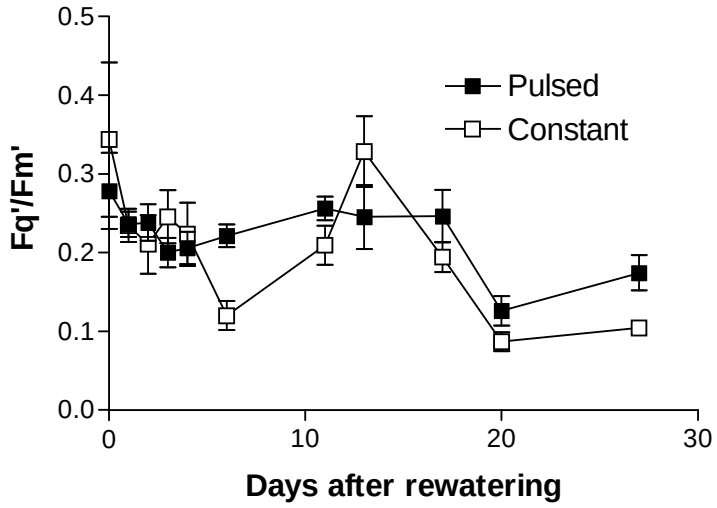

Figure 110 - PSII quantum efficiency (Fq'/Fm') for population SA020

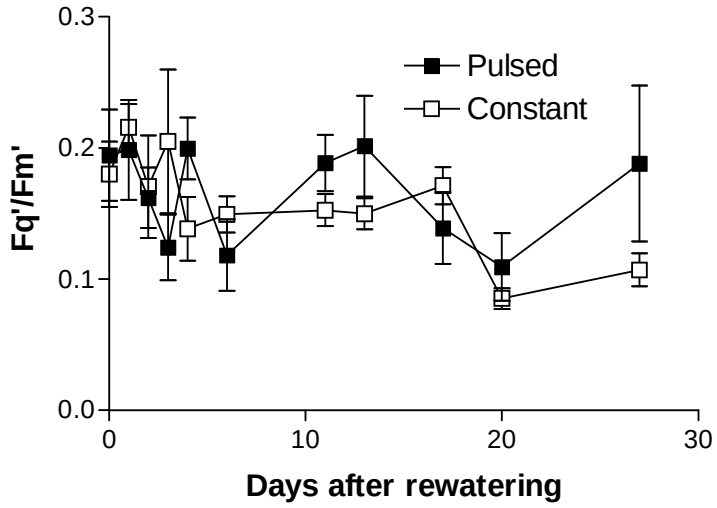

Figure 111 - PSII quantum efficiency (Fq'/Fm') for population SA023

15-329

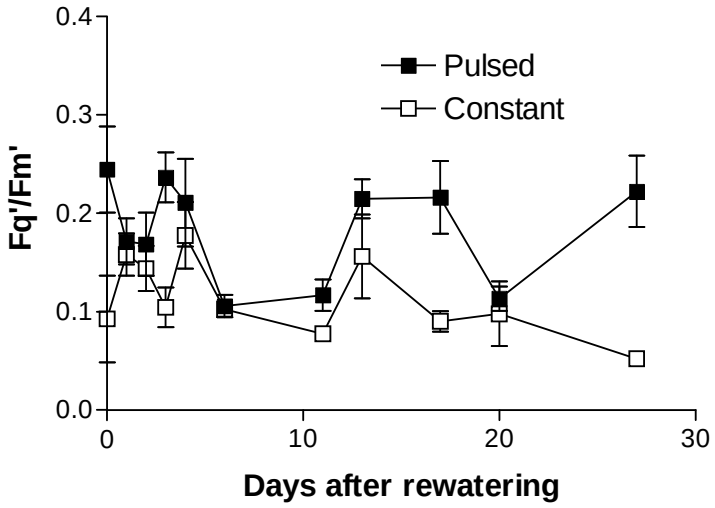

Figure 112 - PSII quantum efficiency (Fq'/Fm') for population VIC003

A 2-way ANOVA was performed on PSII quantum efficiency measurements (F_q'/F_m') taken on day 27 to examine differences in photosynthetic activity between watering treatments and populations. Watering treatment had a highly significant effect (df=1, $p<0.0001$) with the pulsed treatment having significantly higher F_q'/F_m' than the constant watered plants. There were no significant differences between populations (df=4, p=0.847) and the interaction term was not significant (df=4, p=0.244). Results are graphed in Figure 113.

15-330

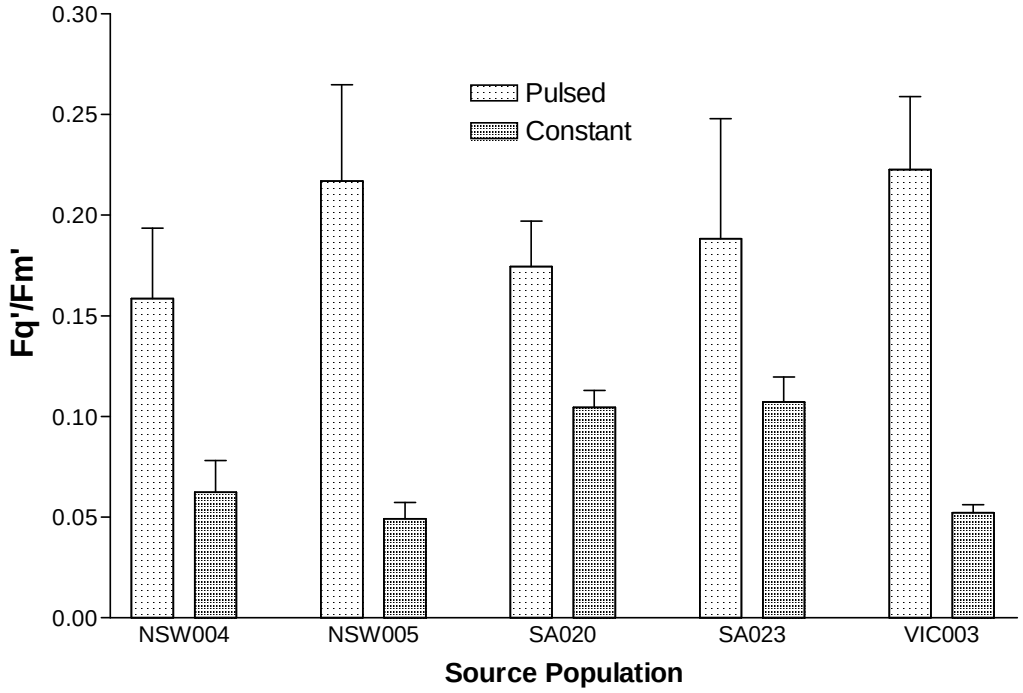

Figure 113 - Fq'/Fm' at day 27 for populations and watering treatments

Discussion

A number of differences in leaf variables were identified between populations, with South Australian and Victorian populations showing the opposite response to pulse regime than New South Wales populations. For example, a strongly pulsed watering regime resulted in longer leaf lengths and more leaves in the SA and Vic populations, while the pulsed treatment resulted in shorter leaves and fewer leaves in the NSW populations. The large amount of water delivered in pulse events had a clear effect on the growth response of some South Australian and Victorian populations, with SA023 and VIC003 in particular showing rapid growth in the measurement periods following water application, while growth in New South Wales populations was more constant. This may indicate a greater capacity of western populations to utilize large watering

15-331

events compared to eastern populations, although this is unexpected, as the climate in the west of the sampling range is biased towards small events, and constant rainfall over winter, while New South Wales populations experience infrequent large events that may occur at any time during the year. A rapid growth response to high rainfall input would be expected in the later case. Leaf length and number are considered useful surrogates for plant biomass, as water limitation is expected to affect leaf extension rates. Population VIC003 showed noticeably higher leaf production throughout the experiment, compared to all other populations.

Despite attempts to standardize light input during PAM chlorophyll fluorescence measurements, daily light conditions had a greater impact on measurements than watering treatments, and the data collected was extremely noisy. This suggests that the technique may be unsuitable for application under natural light conditions, where acclimatization of plants to the daily conditions has a large impact on the photosynthetic measurements. Decay in photosynthetic efficiency over time was not clear from the graphs, but in all populations it appears that plants grown under a strongly pulsed rainfall regime prior to drought maintained higher photosynthetic efficiencies, especially towards the end of the drought period. This indicates some plasticity and acclimatisation by the plants, with a pulsed water regime potentially resulting in the development of plant morphology and physiology better able to cope with extended drought than plant grown initially under constant water availability. No root distribution measurements were taken in this study, but root distribution may be an important plastic response that may assist survival in drought. Large rainfall events

15-332

penetrate to deep soil layers, which may promote the development of deep roots, while deep roots are considered useful in drought survival (Bolger, Rivelli *et al.* 2005; Hurd 1974).

Conclusion

There were clear differences between populations of *Austrodanthonia caespitosa*, primarily in the morphological leaf measurements, although plastic shifts in these characters with water regime were in the opposite direction to those expected for ecotypes sourced from the locations chosen. PAM chlorophyll fluorescence measurements were extremely noisy due to daily variation in natural light input, highlighting the need to conduct PAM measurements under controlled light conditions. However, there were differences between watering regimes that suggested plants subjected to a pulsed watering regime may be better able to cope with extended drought than those grown under a constant water supply.

15.3. Water Use Efficiency Determination

Introduction

The evolutionary algorithm model requires as input parameters for water use efficiency, reflecting water use per gram of assimilated biomass, and photosynthesis rate, in grams of biomass assimilated per unit of light input, under optimum conditions. Instantaneous water use efficiency, as measured with gas exchange systems, may not give an accurate measure of water use over the life of the plant, so instead plant growth was correlated with water use under water limited conditions, with corrections made for

15-333

evaporative and deep drainage water loss. Previous uses of dry matter accumulation to estimate water use efficiency have faced problems from not counting roots, and not taking into account soil evaporation (Fischer and Turner 1978), but both problems are overcome in this experiment.

Methods

In order to obtain growth parameters for the model, an experiment was set up to measure integrated water use efficiency over the growth season of two populations of *Austrodanthonia caespitosa*, by regressing total biomass against total water applied. Thin washcloth squares were placed in the bottom of 18 20cm diameter plastic pots to stop soil loss, and the pots were filled with washed propagating sand (Jeffries, Wingfield). Soil was left to dry in pots in a glasshouse for five days.

Seeds of two populations of *Austrodanthonia caespitosa* from extreme ends of the survey range (SA023, NSW005) were germinated on moist filter paper in petri dishes. Pots were randomly assigned to nine levels of watering (150, 200, 250, 300, 400, 500, 600, 800, 1000 mm a^{-1}) and two populations. On April 26, 2005, volumetric soil water content was measured using a Theta probe, and nine seedlings were planted in each pot.

Plants were watered twice a week, with an amount of water designated to equate to the annual rainfall total, with rainfall 70% concentrated in winter. During watering, pots were placed over trays to catch drainage 15 minutes after watering, by which time drainage from pots had stopped, drained water was weighed.

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On October 2, 2005, photosynthesis of plants in the 400 and 1000mL treatments of populations were measured using a Cirrus 2 portable photosynthesis system, under 1500μm PAR and ambient (350ppm) CO_2 concentration.

Plants in each pot were harvested on October 10, 2005. Soil was washed from the root systems over a 2mm sieve, and roots and shoots in each pot were separated and dried in an oven at 80°C for four days. Roots and shoots were then weighed, and a linear regression was performed, regressing total biomass against total water applied minus drainage, with the x-intercept indicating evaporative loss, and the slope indicating water use efficiency.

Results

Drainage from each pot was subtracted from the total water added to provide a measure of the total water available after loss to drainage. A linear regression was performed for total plant dry weight versus available water for NSW005 and SA023 populations (Figure 114).

15-335

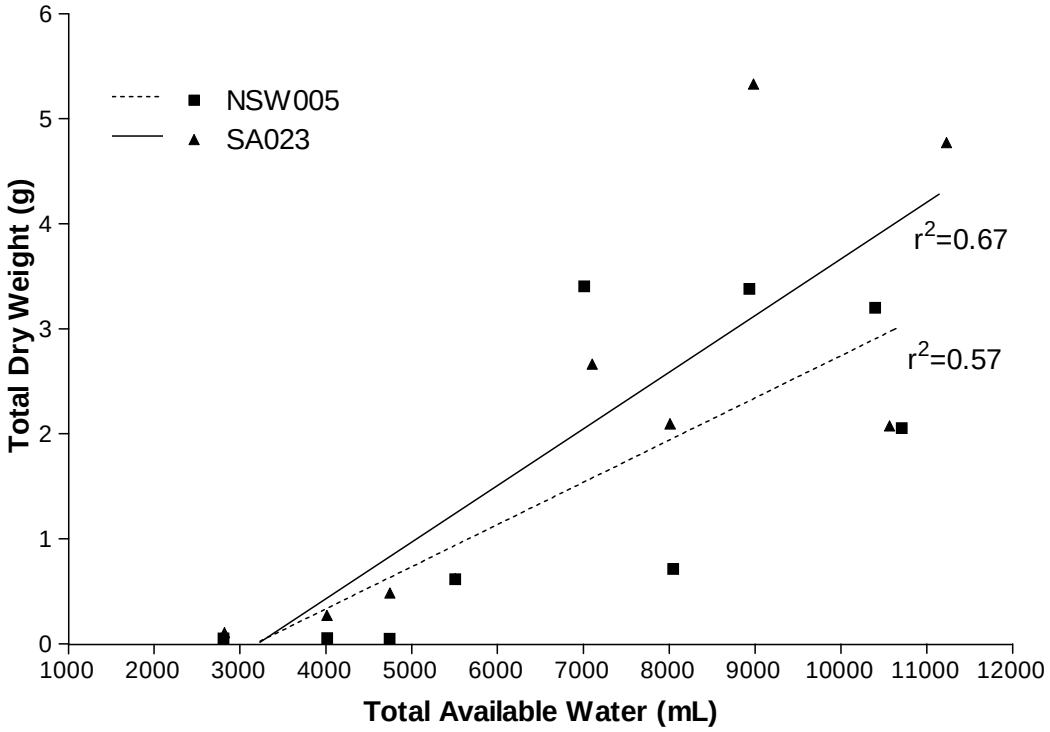

Figure 114 - Linear regression of dry weight per mL of water available for *Austrodanthonia caespitosa*

The r^2 value for the SA023 plants was 0.671. The slope, 0.000539 g mL^{-1}, was significantly different from zero (p=0.0069, n=9). The x-axis intercept of the regression line for the SA023 population was 3188mL. The r^2 value for the NSW005 plants was 0.57. The slope, 0.000402 g mL^{-1}, was significantly different from zero (p=0.019, n=9). The x-axis intercept of the regression line for the NSW005 population was 3150mL.

The peak value of photosynthesis rate obtained for *Austrodanthonia caespitosa* by gas-exchange measurement was 13.8 μmol m^2 sec^{-1} CO_2.

15-336

Discussion

Regressing total plant growth against total water addition gives a measurement not of instantaneous water use efficiency, which is a function of transpiration rate and photosynthetic rate, but of integrated rainfall use efficiency (Le Houérou, Bingham *et al.* 1988)over a growing season. The slopes and x-axis intercepts calculated by this method provide biologically important information. The slope indicates the amount of biomass produced per unit of water added, indicating water use efficiency. The SA023 population appeared to have a higher WUE than the NSW005 population, and these two extremes of WUE values are utilized in the genetic algorithm model to provide a range of trait values. The x-axis intercept indicates the amount of water not used for growth, representing evaporation from the soil surface, and, as expected, this value was similar between populations, indicating soil evaporation did not differ between the populations due to, for instance, different leaf area index or shading. The peak photosynthesis rate value obtained with the portable photosynthesis system was used as a fixed plant parameter in the genetic algorithm model.

Conclusion

Water use efficiency and photosynthesis rates for use in the model were determined. The plant growth method for determining water use efficiency appeared to accurately separate the effects of transpiration and evaporation, as evidenced by the coinciding x-axis intercepts for the watering versus growth regressions for the two populations. The two populations had different water use efficiencies, with the South Australian ecotype having higher water use efficiency than the New South Wales ecotype.

15-337

16. APPENDIX C – INCOMPLETE AND FAILED EXPERIMENTS

16.1. Pilot seminal root growth angles

Introduction

Much early research into root gravitropism has considered it a binary system, with roots either affected by gravity, and hence growing vertically, or unaffected by gravity and growing horizontally (Bennet-Clark, Younis *et al.* 1959), subject to additional influences such as hydrotropism (Takahashi 1994). However, roots may in fact possess a gravitropic set-point angle, a genetically determined angle from the vertical at which roots grow, which may change over time to produce a characteristic root architecture (Digby and Firn 1995). Seminal seedling roots may display a set angle of growth which reflects the over all distribution of the root system, with large angles from the vertical indicating a shallow root distribution, and small angles indicating a deep root distribution. Growing germinated seeds in beakers of agar gel (Oyanagi 1994; Oyanagi, Nakamoto *et al.* 1993a) and measuring the angle of root growth after a period of time is one simple method of determining seminal root growth angles of seedlings. This technique was applied to two ecotypes of *Austrodanthonia caespitosa*, to determine the applicability of the technique, and to explore genotypically controlled variations in root angle within this species.

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Methods

A pilot study was performed to trial a method of examining seminal root growth angles in germinated seeds. The technique involved growing seeds in transparent jars of agar gel (Oyanagi 1994; Oyanagi, Nakamoto *et al*. 1993a). Agar gel was made up in batches of 800ml. RO water was heated to a 75°C, and 1.6g of food-grade agar powder was added to the water and stirred vigorously with a magnetic stirrer. The heat was removed but magnetic stirring continued until the temperature fell to 70°C. Air was then pumped through the solution in order to oxygenate the gel, until the temperature had reached 40°C. The solution was then poured into 100mL beakers, covered with plastic film, and left to cool at 20 degrees C.

Seeds of two commercially available *Austrodanthonia caespitosa* populations, "Auburn" and "South East" (Blackwood Seeds) were germinated on moist filter paper. After 3 days, when seminal roots were 5mm long, 10 seeds of each population were placed in the agar gel jars, 5mm below the surface with the seminal root oriented horizontally. Seedlings were stored in the dark at 20 degrees C in a controlled climate room. Root growth was observed daily and attempts were made to measure the angle of the root tip from the horizontal after 4 days.

Results

No results were obtained from this experiment because of difficulties in measuring seminal root growth angles through round jars. In addition, cotyledons of many of the seedlings were observed to be close to the horizontal, indicating that the seedlings had

16-339

fallen over due to the agar solution being too low in concentration.

Conclusion

Application of this technique relies upon a suitable concentration of agar to produce a gel that prevents seedlings toppling and changing angle due to the weight of shoots or leaves on growing plants. Additionally, measurements of root growth angles may be easier to obtain if seedlings are grown in straight-sided, rather than curved beakers. Another technique, which may be useful in determining seminal root growth angle, is described by (Bonser, Lynch *et al.* 1996), where roots are grown along vertically oriented filter paper inside plastic envelopes.

16.2. Paddock experiment

Introduction

Austrodanthonia caespitosa is highly variable across its range, and grows under a wide range of rainfall regime, including seasonal rainfall distributions ranging from a strong winter bias, in South Australia, to equal probability of rainfall occurrence in any season in New South Wales. Plants growing under a particular seasonal rainfall distribution have faced selective pressure due to seasonal rainfall shortage, and may have evolved morphology and phenology best suited to make use of the local rainfall regime. For instance, South Australian populations, which face low and unpredictable rainfall during summer, may be expected to show early dormancy and low response to summer rainfall addition, while New South Wales populations that receive rainfall over summer

16-340

and may show a greater growth response to experimental water addition over summer.

This experiment aimed to investigate growth differences between ecotypes sourced from across a gradient in seasonal rainfall distribution, under two watering treatments, one imposing summer drought, under natural South Australian rainfall, while the other watering treatment involved additional watering over summer. The experiment also aimed to measure soil water content, to compare the capacity of different populations to reduce soil water content over summer, and hence the capacity to reduce deep drainage.

Methods

A paddock at the Roseworthy Campus of the University of Adelaide (34.5259 S, 138.6882 E) was prepared for an experiment examining differences in growth and seasonal water usage between different populations of *Austrodanthonia caespitosa*. 96 $1m^2$ plots were marked out in rows in the paddock. In June 2004, the area was sprayed with glyphosate (Roundup, Monsanto) to kill existing weeds.

On June 11, 2004, seeds from seven populations of *A.caespitosa* (SA023, SA021, SA020, VIC006, NSW-004, NSW-005) were germinated in petri dishes on moist filter paper. After four days, seedlings were planted in shallow seedling trays filled with sandy loam soil (Jeffries, Wingfield), with 12 seedlings per tray. Trays were left in the open in Adelaide under natural weather conditions.

Between August 11 and 22, seedlings were transplanted into plots in the paddock.

16-341

There was a population treatment (six populations plus an empty control), by a watering treatment (summer watering versus summer drought), with six replicates of each treatment, and random placement of experimental units in a grid in the paddock. The centre $0.7m^2$ of each plot was raked to remove any remaining weed biomass, and twenty plants of *A.caespitosa* were planted in each plot. 2L of water was applied to each plot after planting to aid establishment, followed by another 2L the following week.

By early October 2004, it was noted that a second crop of weeds had seriously encroached upon all plots, shading out *A.caespitosa* seedlings, and the experiment was abandoned.

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17. MODEL SOURCE CODE

The evolutionary algorithm model was developed in Python 2.3, an open-source interpreted language available for *nix and Microsoft Windows based operating systems. The application relies on a number of third-party libraries, including Psyco, a speed optimisation module, and PythonCardPrototype and wxPython, for the graphical interface. The source code presented here will not run as a stand-alone application, and requires the PythonCard definition files on the included CD-ROM for the graphical user interface.

```
#!/usr/bin/python

# Austrodanthonia caespitosa Evolutionary Algorithm Model
# Grant Williamson
# University of Adelaide

"""
__version__ = "$Revision: 1.6 $"
__date__ = "$Date: 2002/07/29 17:44:55 $"
"""

# Load required modules
from PythonCardPrototype import model
from math import *
from random import random, randint
from wxPython import wx
import psyco

# Initalize Psyco optimization module
psyco.full()

# Define main program class, as controlled by the user interface
class Genes(model.Background):

    def on_menuFileExit_select(self, event):
        self.Close()

    def on_Start_mouseClick(self,event):
        global lock
        self.keepDrawing = 1
        # Display header text for output data in results window.
        self.components.Display.AppendText(
"%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t\n" % ("day", "rd0" ,"rd1","rs","max_w",
"wue", "seed_w", "seed_t","smavg","rm0avg","rm1avg","rm2avg","ddavg"))
        # Run the model for the number of replicates defined in the program window.
        for repeat in range(int(self.components.replicates.text)):
            # Each model generation runs for two years.
            self.components.done.max=int(365*2)
            # Call main program loop
```


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```
            self.main()

        self.keepDrawing = 0

    def on_Clear_mouseClick(self,event):
        self.components.Display.Clear()

    def on_Stop_mouseClick(self,event):
        self.keepDrawing = 0



    # Initialize global environmental variables, and variables controlling model execution
    def initenv(self):
        global dout
        global SoilK, FC, WP, SoilSA
        global MON_EV, MON_DAYS
        global RF, Temp, MON_Temp
        global c_count
        global EV
        global Depth
        global efunc
        global fastbreed
        global srl
        global resp
        global Env_Lat
        global fit
        global lock
        global reportall
        global vpdvar
        global RH

        #Report all = set 1 to report entire best plants array
        reportall = 0

        #Set lock to 1 to Lock WUE and PS genes, otherwise allow evolution of these genes
        lock = 0
        lockstring = "Lock:" + str(lock)
        self.components.Display.AppendText(lockstring)

        #Select Fitness function
        #1 = Highest Productivity
        #2 = Lowest WUE
        fit = 1

        #VPD function - set to 1 to use the VPD function to 1, or set to 0 to ignore it
        vpdvar = 1

        #Latitude of site, used for calculating solar integral
        Env_Lat = 35

        #Output frequency - set to 1 to output each generation's genes, or 0 to output at end
of replicate
        dout = 0

        #Respiration 0 = none, 1 = decay 2 = even 3 = monthly lag
        #Currently defined in user interface
        resp = int(self.components.respiration.text)

        # Soil conductivity between layers, in proportion of water content difference
        SoilK = 0.1

        # Define Water Field Capacities FC (% by volume)
        # Remove # marks from required soil type

        #Sand
        #FC = [0.15,0.15,0.15]
        #WP = [0.07,0.07,0.07]

        #Loam
        FC = [0.3,0.3,0.3]
```


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```
        WP = [0.14,0.14,0.14]

        #Clay
        #FC = [0.4,0.4,0.4]
        #WP = [0.21,0.21,0.21]

        #Standard
        #FC = [.377,.377,.377]
        #WP = [.04,.04,.04]

        # Define depth of each layer (cm)
        Depth = [10.0,10.0,20.0

        # Define soil surface area (cm2)
        SoilSA = 100.0

        # Define Specific Root Length (cm per g)
        srl = 10000

        #Climate Tables for a range of locations
        TEMP_Den = [32.5,32.0,28.7,23.4,18.6,15.1,14.4,16.4,19.6,23.6,27.5,30.5]
        HUM_Den = [30,33,37,44,55,63,62,55,48,40,34,30]
        EV_Den = [9.7,8.8,6.5,3.9,2.0,1.3,1.3,2.0,3.3,5.3,7.5,9.1]

        TEMP_Ber = [ 31.9 ,  31.5,   28.1,   22.9,   18.0,   14.0,   13.4,   15.4 ,  19.0 ,
22.9,   27.1,   30.4]
        HUM_Ber = [30 ,    40 ,   37   ,  44  ,   56   ,  60   ,  64 ,   57  ,   49  ,   43   ,
40   ,  26 ]

        TEMP_Wya = [32.4 ,  31.8 ,  28.7 ,  23.5 ,  18.5 ,  14.8 ,  14.0 ,  15.8 ,  19.2 ,
23.0 ,  26.8  , 30.5]
        HUM_Wya = [33  ,   36  ,   35    , 41   ,  53  ,   59   ,  57   ,  51  ,   47  ,   43
,   34  ,   32]

        TEMP_Pri = [28.3  , 26.9  , 25.8  ,22.3  , 19.4 ,  16.4  , 15.9  , 17.0  , 19.6   ,22.5
,  24.5  , 26.5 ]
        HUM_Pri = [49   ,  53   ,  52   ,  54   ,  65  ,   68  ,   66  ,   59    ,55   ,  54 ,
49   ,  54 ]
        EV_Pri = [ 8.4  ,  7.8  ,  6.1 ,   4.0  ,  2.5   , 1.7   , 1.9  ,  2.5 ,   3.5  ,  5.2
,7.0  ,  7.9]

        TEMP_Kad = [ 30.5 ,  30.5,   27.6  , 23.5 ,  19.2   ,16.3 ,  15.4 ,  16.7   ,19.5  ,
22.8   ,26.0,   28.1]
        HUM_Kad = [34  ,   35  ,   38   ,  46 ,    57   ,  63     ,63   ,  57 ,    51  ,   42
,   36  ,   36]

        TEMP_Bun = [30.2 ,  29.8  , 26.7 ,  22.7  , 17.4  , 13.3  , 13.7 ,  15.3  , 16.7 ,
21.4 ,  25.2  , 28.3 ]
        HUM_Bun = [31   ,  34  ,   37  ,   45   ,  56  ,  60,   69   ,  64  ,   52  ,   33   ,
37  ,   33]
        EV_Bun = [8.4  ,  7.6  ,  5.7   , 3.4   , 1.8  ,  1.4 ,   1.5  ,  1.9  ,  2.7  ,  4.0
, 6.0  ,  7.9]

        TEMP_Cla = [ 29.5 ,  29.0  , 26.9 ,  21.6 ,  17.2  , 14.0  , 13.2 ,  14.5   ,17.5 ,
21.0   ,24.6 ,  27.4]
        HUM_Cla = [30   ,  33 ,    35  ,   45 ,    56 ,    63   ,  63    , 58  ,   50   ,  43
,   35 ,    32 ]

        TEMP_Kei = [29.7  , 29.9  , 26.8  , 22.4 , 18.3  , 15.5 ,  15.0  , 16.1  , 18.5 ,  21.4
,  24.6 ,  27.5]
        HUM_Kei = [ 33   ,  33 ,    38 ,    46  ,   59    , 66  ,   65   ,  59   ,  55    , 47
,   40   ,  36 ]

        EV_Pad = [  8.3  ,  7.8  ,  5.7 ,   3.5 ,  2.0  ,  1.4  ,  1.5  ,  2.0 ,   2.8  ,  4.3
,   6.0,   7.4 ]

        TEMP_Mur = [28.7  , 29.2  , 26.4,   23.3   ,19.5  , 16.6  , 16.2  , 17.2 ,  19.6 ,
22.4  , 25.3,   27.3]
        HUM_Mur = [37  ,   37  ,   42  ,   45   ,  53   ,  60    , 58   ,  53   ,  48   ,  43
,   38  ,   38]
```


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```
        TEMP_Str = [ 27.4  , 27.4  , 25.4  , 21.8  , 18.3  , 15.6 ,  14.8 ,  15.9 ,  18.3 ,
21.0 ,  23.9 ,  26.1]
        HUM_Str = [42  ,   43   ,  45 ,    52   ,  61  ,   66 ,    64   ,  60     ,56  ,   50
,   45    , 43 ]

        TEMP_Vic = [  24.5  , 24.5,   23.4  , 21.0 ,  18.5 ,  15.9  , 15.4 ,  16.2  , 18.2 ,
20.1 ,  21.8 ,  23.5]
        HUM_Vic = [69  ,   71  ,   71    , 71  ,   76  ,   81   ,  79 ,    74  ,   70   ,  65 ,
66 ,    65]

        EV_Ade = [  8.2  ,  7.7  ,  5.6  ,  4.0,    2.5   , 1.9  ,  1.9  ,  2.5  ,  3.7  ,  5.2
,   6.6   , 7.8]

        TEMP_Ser = [  29.3 ,  28.6  , 25.9  , 21.2 ,  17.0  , 14.1  , 13.5 , 15.0  , 17.5
,20.6 ,  24.1   ,27.0]
        HUM_Ser = [  32    , 37  ,   39  ,   50  ,   64   ,  66 ,    67   ,  61   ,  54   ,  51
,  45   ,  37]

        TEMP_Sta = [ 27.4 ,  28.0  , 24.5  ,20.1  , 15.9  , 12.8 ,  12.2 ,  13.6  , 15.5 ,
19.1  , 22.2  , 25.5 ]
        HUM_Sta = [37 ,    36  ,   42   ,  50  ,   62   ,  70  ,   69   ,  64  ,  58  ,   51 ,
45  ,   39]
        EV_Sta = [7.9 ,   7.2  ,  5.3  ,  3.1   , 2.0  ,  1.5 ,   1.6  ,  2.0  ,  2.7  ,  4.1
,  5.1  ,  7.1]

        TEMP_Don = [29.6  , 29.8 ,  26.3 ,  21.7   ,17.2  , 14.0,   13.3 ,  14.8 ,  17.1 ,
20.7  , 24.3,   27.3]
        HUM_Don = [29   ,  30   ,  36  ,   42    , 56   ,  63   ,  62  ,   56  ,   52 ,   43  ,
35   ,  30]

        TEMP_Wyc = [ 30.5  , 30.8,  27.2  , 22.4  , 17.6  , 13.8   ,13.5  , 15.2   ,18.0 ,
21.9  , 26.4  , 29.3]

        TEMP_Boo = [31.4  , 31.0  , 27.6 ,  22.5  , 17.9 ,  14.4 ,  13.9 ,  15.7  , 18.6 ,
22.2 ,  26.2 ,  29.5]
        HUM_Boo = [ 28   ,  32  ,   37  ,   44  ,   57 ,   63  ,   62  ,   56  ,   49  ,   43
, 32 ,   31 ]

        TEMP_Num = [ 30.7  , 30.8  , 27.9  ,22.5 ,  17.7 ,  13.2 ,  13.0 ,  15.2   ,18.0  ,
22.3 ,  26.9  , 30.2 ]
        EV_Num = [ 8.7  ,  7.9  ,  5.7   , 3.3  ,  1.8  ,  1.1  ,  1.2 ,   1.9   , 2.9  ,  4.4
,  6.5   , 8.5]

        NSW001 = [TEMP_Den, HUM_Den, EV_Den]
        NSW002 = [TEMP_Ber, HUM_Ber, EV_Den]
        NSW003 = [TEMP_Ber, HUM_Ber, EV_Den]
        NSW004 = [TEMP_Ber, HUM_Ber, EV_Den]
        NSW005 = [TEMP_Wya, HUM_Wya, EV_Den]
        NSW006 = [TEMP_Wya, HUM_Wya, EV_Den]
        NSW007 = [TEMP_Wya, HUM_Wya, EV_Den]

        SA001 = [TEMP_Pri, HUM_Pri, EV_Pri]
        SA002 = [TEMP_Kad, HUM_Kad, EV_Pri]
        SA003 = [TEMP_Kad, HUM_Kad, EV_Pri]
        SA004 = [TEMP_Bun, HUM_Bun, EV_Bun]
        SA006 = [TEMP_Bun, HUM_Bun, EV_Bun]
        SA007 = [TEMP_Cla, HUM_Cla, EV_Bun]
        SA008 = [TEMP_Cla, HUM_Cla, EV_Bun]
        SA009 = [TEMP_Cla, HUM_Cla, EV_Bun]
        SA010 = [TEMP_Kei, HUM_Kei, EV_Pad]
        SA012 = [TEMP_Ser, HUM_Ser, EV_Pad]
        SA013 = [TEMP_Ser, HUM_Ser, EV_Pad]

        VIC001 = [TEMP_Sta, HUM_Sta, EV_Sta]
        VIC003 = [TEMP_Don, HUM_Don, EV_Sta]
        VIC004 = [TEMP_Don, HUM_Don, EV_Sta]
        VIC005 = [TEMP_Wyc, HUM_Don, EV_Sta]
        VIC006 = [TEMP_Boo, HUM_Boo, EV_Sta]
        VIC007 = [TEMP_Num, HUM_Boo, EV_Sta]

        SA020 = [TEMP_Mur, HUM_Mur, EV_Ade]
```


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```
        SA021 = [TEMP_Str, HUM_Str, EV_Ade]
        SA022 = [TEMP_Vic, HUM_Vic, EV_Ade]
        SA023 = [TEMP_Vic, HUM_Vic, EV_Ade]


        # Define which location to use for climate data
        LOCA = VIC007

        # Select between using standard "Adelaide" evaporation data
        # Or data from the sample location defined above
        MON_EV=[7.1,6.7,4.9,3.0,1.9,1.4,1.5,2.1,3.0,4.3,5.7,6.5]
        #MON_EV = LOCA[2]

        # Days in Each Month used for averaging in MON_DAYS
        MON_DAYS=[31,28,31,30,31,30,31,31,30,31,30,31]

        # Define and Set daily rainfall to zero
        RF = 0.0

        # Define temperature variable
        Temp = 0.0

        # Select between using standard "Adelaide" monthly temperatures
        # Or data from sample location defined above
        MON_Temp=[28.9, 29.3, 26.0, 22.4, 18.9, 16.0, 15.3, 16.5, 18.8, 21.6, 24.6, 26.7]
        #MON_Temp = LOCA[0]

        # Select between using standard "Adelaide" 3pm relative humidity data
        # Or data from sample location defined above
        RH=[37,37,42,47,56,62,61,56,52,46,41,40]
        #RH = LOCA[1]

        # Define Counter for rainfall frequency
        c_count=0
        # Define Daily evaporation
        EV=0

        # Define evaporation method, 0 = soil water content, 1 = soil potential
        efunc=0

        # Define swapping of subgenes (0) or whole genes(1)
        # Swapping subgenes (bits) results in slower evolution, but more complete exploration
of the trait space
        fastbreed=0


    # Function to convert from binary chromosome to actual gene values, for a given plant
    def fillgenes(self,plantid):

        global lock

        plantid['g1']=self.bin2dec(plantid['cs1'][0:8])
        plantid['g2']=self.bin2dec(plantid['cs1'][8:16])
        plantid['g3']=self.bin2dec(plantid['cs1'][16:24])
        plantid['g4']=self.bin2dec(plantid['cs1'][24:32])
        plantid['g5']=self.bin2dec(plantid['cs1'][32:40])
        plantid['g6']=self.bin2dec(plantid['cs1'][40:48])
        plantid['g7']=self.bin2dec(plantid['cs1'][48:56])
        plantid['g8']=self.bin2dec(plantid['cs1'][56:64])

        # Defines whether or not to lock WUE and PS genes
        if lock==0:
            plantid['g9']=self.bin2dec(plantid['cs1'][64:72])
        else:
            plantid['g9']=255

        if lock==0:
            plantid['g10']=self.bin2dec(plantid['cs1'][72:80])
        else:
            plantid['g10']=255
```


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```
        plantid['g11']=self.bin2dec(plantid['cs1'][80:88])
        plantid['g12']=self.bin2dec(plantid['cs1'][88:96])
        plantid['g13']=self.bin2dec(plantid['cs1'][96:104])
        plantid['g14']=self.bin2dec(plantid['cs1'][104:112])
        plantid['g15']=self.bin2dec(plantid['cs1'][112:120])
        plantid['g16']=self.bin2dec(plantid['cs1'][120:128])
        return()

    # Calculates the altitude of the sun above the horizon for a given time and day of the year
    # Sun calculations from : http://www.squ1.com/index.php?http://www.squ1.com/solar/solar-
position.html
    def sunaltitude(self,latitude,dayofyear,time):
        pi = 3.1415927
        decT = 2.0*pi*((dayofyear-1.0)/365.0)
        declination = 0.322003-22.971*cos(decT)-0.357898*cos(2*decT)-
0.14398*cos(3*decT)+3.94638*sin(decT)+0.019334*sin(2*decT)+0.05928*sin(3*decT)
        hourangle = radians(15.0*(time-12.0))
        altT = sin(radians(declination))*sin(radians(latitude))
        altU = cos(radians(declination))*cos(radians(latitude))*cos(hourangle)
        altcalc = asin(altT + altU)
        altitude = degrees(altcalc)
        return altitude

    # Calculates the daily solar integral for a given day of the year, based on hourly sun
altitudes
    def dayintegral(self,dayofyear,latitude):
        solarint = 0.0
        for hour in range(24):
            altitude = self.sunaltitude(latitude,dayofyear,hour)
            if altitude > 0.0:
                fromzenith = 90-altitude
                period = 1500.0 * cos(radians(fromzenith)) * 0.36
                solarint = solarint + period
        return solarint

    # Initializes array of plants
    def initplants(self,tss):
            #Initial Size of plant array
            global ss, lock, SoilSA
            global pdict

            # Defines the "dictionary" that descibes each plant's variables
            # cs1 = Binary Chromosome
            # g1...16 = Gene values
            # age = age of plant in days
            # phen = phenological stage, seed or growing
            # sm = above-ground dry weight, shoot mass (g)
            # rm0...2 = dry weigh of each root layer (g)
            # sa = specific leaf area, cm per g
            # WCE = water use efficiency, in mL of water per G of growth - no longer used
            # CC = allowed seed density - no longer used
            # DD = record of total deep drainage from this plant
            # tm = record of total dry weight for this plant
            pdict= {'cs1':'',
             'g1':0.0, 'g2':0.0, 'g3':0.0, 'g4':0.0,
             'g5':0.0, 'g6':0.0, 'g7':0.0, 'g8':0.0,
             'g9':0.0, 'g10':0.0, 'g11':0.0, 'g12':0.0,
             'g13':0.0, 'g14':0.0, 'g15':0.0, 'g16':0.0,
              'age':0.0, 'phen':'seed', 'sm':0.1, 'rm0':0.001, 'rm1':0.0, 'rm2': 0.0, 'sa':
220.0, 'WCE': 258.93, 'CC':50.0, 'DD':0.0, 'tm':0.0 }

            # Define plant array
            global plant
            plant=[]

            # Define soil array
            global soil
            soil=[]

            # Define deep drainage array
            global DD
```


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```
            DD=[]

            global ps
            global genep
            global deadp
            global maxseed
            global maxage

            global Env_Lat


            counterx = 0

            # Fill plant array with inital random plants
            global plant
            print "Generating initial random genotypes"
            while counterx < tss:
                counterx = counterx + 1
                plant.append(pdict.copy())

                # Define water content, in mL per cm2 surface area
                l0 = 1.0
                l1 = 1.0
                l2 = 2.0


                soil.append([l0 * SoilSA,l1 * SoilSA,l2 * SoilSA])
                DD.append(0.0)
                bitstore=''
                countery = 0

                # Generate random genome for plant
                while countery < 128:
                    countery=countery + 1
                    if random() > 0.5:
                        bit='1'
                    else:
                        bit='0'
                    bitstore = bitstore + bit
                plant[len(plant)-1]['cs1']=bitstore
                plant[len(plant)-1]['age']=1
                self.fillgenes(plant[len(plant)-1])

    # Convert binary chromosome data to integer gene value
    def bin2dec(self,bin):
        return
int(bin[7])+int(bin[6])*2+int(bin[5])*4+int(bin[4])*8+int(bin[3])*16+int(bin[2])*32+int(bin[1])
*64+int(bin[0])*128

    #Generate new seed with a specific genotype
    def newplant(self, bitstore):
         global plant, DD,soil,pdict
         plant.append(pdict.copy())
         soil.append([100.0,100.0,200.0])
         DD.append(0.0)
         plant[len(plant)-1]['phen']='seed'
         plant[len(plant)-1]['cs1']=bitstore
         self.fillgenes(plant[len(plant)-1])

    # Generate a new array of plants by recombining genes from the array of fit plants
    def mixitup(self, bestplant):
        global plant
        global soil
        plant = []
        soil = []
        nobest = len(bestplant)
        generate = 0
        while generate <= 300:
            generate = generate + 1

            planta=bestplant[int(randint(0,nobest-1))]
```


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```
            plantb=bestplant[int(randint(0,nobest-1))]
            bitstore = ''
            gene = 0

            # Randomly select genes from the two parent plants to produce the child plant
            while gene < len(planta['cs1']):

                bita=planta['cs1'][gene:gene+8]
                bitb=plantb['cs1'][gene:gene+8]

                gene=gene+8
                if random() < 0.5:
                    newbit=bita
                else:
                    newbit=bitb
                bitstore = bitstore + newbit

            # Apply a random mutations to the genome
            for muts in range(3):
                if random()<0.99 :
                    #print "Mutation is going to occur"
                    if random()<0.5:
                        mutbit='0'
                    else:
                        mutbit='1'
                    place=int(randint(0,127))
                    #Need to fix this
                    if place==127:
                        newbitstore=bitstore[:127]+mutbit
                    else:
                        newbitstore=bitstore[:place]+mutbit+bitstore[place+1:]
                    #print bitstore,newbitstore
                    bitstore=str(newbitstore)

            # Create a new plant with the given genome
            self.newplant(bitstore)


    # Calculate Vapour Pressure Deficit's impact on WUE, based on temperature and relative
humidity
    def vpd(self, wue, Temp, RelH):
        global RH
        global vpdvar
        if vpdvar ==0:
                return wue
        es = 0.6108*exp((17.27*Temp)/(Temp+237.3))
        ee = (RelH*es)/100
        vpdef = es - ee
        wue = wue * vpdef
        return wue

    #Function to generate a gaussian probability
    def gausprob(self,x,o,u):
        gp=(1.0/sqrt(2.0*pi*o*o))*exp(-(pow(x-u,2.0))/(2.0*o*o))
        return(gp)

    # Function to manage seed germination
    # Elements of this function are no-longer used, due to discrete generations
    # However, it does introduce some variation in germination time
    def seedgerminate(self,idx,seed_storage, germinate_comp, germ_curve, livecount, cc,
seed_water, seed_temp, flower_stop):
        global soil, plant, Temp
        plantwilldie = 0
        #If plant is seed
        if plant[idx]['phen'] == 'seed':
           #If seed is too old, flag it as going to die
           if (float(plant[idx]['age']) >= (50.0+(6.25*seed_storage)) or
float(plant[idx]['age']) > (300.0+(1.5625*flower_stop))):
              plantwilldie=1

           else:
```


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```
            #Give seed chance at germination dependent on live plant density
            prop=(float(livecount)/((float(germinate_comp)/16.0)+1.0))/cc
            #prop=0.1
            if random() >= prop:
                 #print "Random density test passed:"
                 seedage=plant[idx]['age']
                 #Generate Gaussian probability distribution for seed germination.
                 gdist=self.gausprob(seedage, 200.0, (60.0+germ_curve*6.25))
                 #Then give seed a chance of germinating based on it's age and peak
germination time
                 if random() < (gdist+0.2):
                     #Age distribution test passed
                     if soil[idx][0]>(50.0+((seed_water/256.0)*100)) and Temp <
(18.0+(float(seed_temp)/64.0)):
                          #Water and temp test passed, set to grow.
                          plant[idx]['phen']='grow'
                          plant[idx]['age'] = 1.0

        return(plantwilldie)

    #Function to perform photosynthesis and allocate biomass.
    def plantps(self, idx, ps_gene, rs_ratio,root_depth_1, root_depth_2, solar, max_water,
wue_gene, Temp, RelH):
        global daylength, plant
        if plant[idx]['phen'] == 'grow' or plant[idx]['phen']=='flower':
           # WUE is calculated by normalizing the WUE gene value between the maximum and
minimum
           #experimentally determined WUE values for Austrodanthonia caespitosa
           #Solar = total daily photons (umol cm-2) = calculated
           #PEff = assimilated mol carbon per mol photons = 0.0092
           #MolC = weight of 1 umol of carbon in g

           wue = 1/(0.0004017 + (wue_gene * 0.000000539607))
           newwue = self.vpd(wue, Temp, RelH)
           PEff = 0.0092
           MolC = 0.00001201

       #Calculate the potential maximum assimilated carbon
           DWL = plant[idx]['sm'] * plant[idx]['sa'] * solar * PEff * MolC

           # Remove water from soil, and return the water deficit
           W_def = self.suck_soil(DWL, idx, max_water, newwue)

           # Allocate carbon to shoots and roots, moderated by the water deficit
           self.grow_leaves(DWL, W_def, idx, rs_ratio, newwue)
           self.grow_roots(DWL, W_def, idx, rs_ratio, root_depth_1, root_depth_2, newwue)

           return

    # Allocate biomass to leaves, based on RS Gene, assimilation, and water deficit
    def grow_leaves(self,totalG, W_def, idx, rs_ratio, wue):
        global plant, soil, dayA

    #Total potential biomass to be allocated to leaves, based on RS_Ratio gene
        G = totalG*((rs_ratio/512.0)+0.25)

    #Calculate percentage of this to be added to leaves, based on water deficit.
        needed = wue * totalG
        if W_def > 0:
            percent = (needed - W_def) / needed
        else:
            percent = 1

        APA=percent * G

        sG = G * percent

    #Add biomass to leaves.
        plant[idx]["sm"] = plant[idx]["sm"] + sG
        dayA = dayA + sG
        return
```


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```
    # Allocate biomass to root layers, based on RS, RD0 and RD1 genes, assimilation, and water
deficit
    def grow_roots(self, totalG, W_def, idx, rs_ratio, root_depth_1, root_depth_2, wue):
        global plant, dayA

    #Total potential biomass to be allocated to roots, based on RS_Ratio gene
        G = totalG * (1-((rs_ratio/512.0)+0.25))

    #Calculate two allocation proportions from RD_0 and RD_1 genes
        prop1 = root_depth_1/256.0
        prop2 = root_depth_2/256.0


    #Use the RD_0 gene to divide allocation between the top layer, and the bottom two
    #Use the RD_1 gene to divide allocation between the bottom two layers
        rl0 = prop1
        leftover = 1.0-prop1
        rl1 = leftover * prop2
        rl2 = leftover * (1.0-prop2)



        #Calculate percentage of actual carbon we can allocate to the roots based on water
availability.
        needed = 0.0
        if W_def > 0.0:
            needed = wue * totalG
            percent = (needed - W_def) / needed
            if percent < 0.0:
                percent = 0.0

        else:
            percent = 1.0

        dG = G * percent

        #Allocate biomass to the three root layers.
        plant[idx]["rm0"] = plant[idx]["rm0"] + (dG * rl0)
        plant[idx]["rm1"] = plant[idx]["rm1"] + (dG * rl1)
        plant[idx]["rm2"] = plant[idx]["rm2"] + (dG * rl2)
        dayA = dayA + (dG * rl0) + (dG * rl1) + (dG * rl2)
        return

    # Determine if plant should be die due to permanent wilting.
    # This is an optional function
    def plantdess(self, idx, rd0, rd1):
        global plant, soil, WP, Depth, SoilSA

        if soil[idx][0] < (WP[0]*Depth[0]*SoilSA) and soil[idx][1] < (WP[1]*Depth[1]*SoilSA)
and soil[idx][2] < (WP[2]*Depth[2]*SoilSA):
            dead = 1
        else:
            dead = 0

        return dead

    #Extract water from soil
    def suck_soil(self, DWL, idx, max_water, wue):
        global plant, soil, Depth, WC, FC, WP, SoilSA, srl
    #Keep a record of total root mass
        totalroots = plant[idx]["rm0"] + plant[idx]["rm1"] + plant[idx]["rm2"]
        #Water defecit - how much water we DON'T have in the soil
        W_def = 0.0
        #Calculate proportion of roots in each layer
        prop_0 = plant[idx]["rm0"] / totalroots
        prop_1 = plant[idx]["rm1"] / totalroots
        prop_2 = plant[idx]["rm2"] / totalroots

        #Maximum water to be extracted from each layer based on root proportions
        ext_0 = prop_0 * (DWL * wue)
```


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```
        ext_1 = prop_1 * (DWL * wue)
        ext_2 = prop_2 * (DWL * wue)

        #Calculate maxex = maximum water extractable per day - mL per cm of root length
        maxex = 0.02 + (max_water / 256.0)*0.02
        max_0 = plant[idx]["rm0"] * srl * maxex
        max_1 = plant[idx]["rm1"] * srl * maxex
        max_2 = plant[idx]["rm2"] * srl * maxex

    #For each layer determine what is less - the maximum based on proportion
    #Or the maxiumum based on extraction rate, and adjust wanted water accordingly

        if max_0 < ext_0:
            W_def = W_def + (ext_0 - max_0)
            ext_0 = max_0
        if max_1 < ext_1:
            W_def = W_def + (ext_1 - max_1)
            ext_1 = max_1
        if max_2 < ext_2:
            W_def = W_def + (ext_2 - max_2)
            ext_2 = max_2


        #Extract water from each layer

        if soil[idx][0] > (WP[0]*Depth[0]*SoilSA):   #If water in layer 0 greater than wilting
point, proceed
            if ext_0 < soil[idx][0]-(WP[0]*Depth[0]*SoilSA):  #If the "wanted" water is less
than the available water
                soil[idx][0] = soil[idx][0] - ext_0  #Extract the "wanted" water - no deficit
from this layer
            else:  #Other wise, if wanted water is greater than available water
                W_def = W_def + (ext_0 - (soil[idx][0]-(WP[0]*Depth[0]*SoilSA))) #Add any water
not available to the deficit
                soil[idx][0] = WP[0]*Depth[0]*SoilSA
        else:
            W_def = W_def + ext_0  #If water in layer 0 less than wilting point, then add the
"wanted" water to the deficit

    #Layer 1
        if soil[idx][1] > (WP[1]*Depth[1]*SoilSA):
            if ext_1 < soil[idx][1]-(WP[1]*Depth[1]*SoilSA):
                soil[idx][1] = soil[idx][1] - ext_1

            else:
                W_def = W_def + (ext_1 - (soil[idx][1]-(WP[1]*Depth[1]*SoilSA)))
                soil[idx][1] = WP[1]*Depth[1]*SoilSA
        else:
                W_def = W_def + ext_1

    #Layer 2
        if soil[idx][2] > (WP[2]*Depth[2]*SoilSA):
            if ext_2 < soil[idx][2]-(WP[2]*Depth[2]*SoilSA):
                soil[idx][2] = soil[idx][2] - ext_2
            else:
                W_def = W_def + (ext_2 - (soil[idx][2]-(WP[2]*Depth[2]*SoilSA)))
                soil[idx][2] = WP[2]*Depth[2]*SoilSA
        else:
            W_def = W_def + ext_2

    #Return the water deficit based on how much water was available, and how much was extracted
        return W_def

    #Function to switch plant to flowering
    #No-longer used with discrete generations
    def flowering(self,idx, flower_matur):
        global plant, Temp
        if (plant[idx]['age'] >= 200.0+(0.3125*flower_matur) and plant[idx]['phen']=='grow' and
Temp > 23.0):
           plant[idx]['phen'] = 'flower'
        if (plant[idx]['phen'] == 'flower') and Temp > 28.0:
```


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```
            plant[idx]['phen'] = 'grow'

    #Function to manage all growth on each plant, and gene values
    def growplant(self, idx, livecount, solar, Temp, RelH):
        global genep, maxseed, maxage, deadp,plant, lock

        plant[idx]['age']=plant[idx]['age']+1.0

        #Put genes into appropriate variables.
        #Gene controlling ability to germinate given competitor density
        #As expected, maximised, therefore look for trade-off
        #will fix at 14.95 - can vary this to define how competitive plants are
        #germinate_comp=plant[idx]['g1']
        germinate_comp = 14.95 * 16

        #Seed Lifespan - How long will the seed last in the seedbank before it is no longer
viable
        #Appeared fixed  but unrelated to rainfall - will fix at 11.6
        #seed_storage=plant[idx]['g2']
        seed_storage = 11.6 * 16

        #Germination curve - probability of germinating (normal distribution) after production
        germ_curve=plant[idx]['g3']
        #germ_curve=7.5 * 16


        #Soil Water - amount of water required in top layer of soil before germination can
occur
        seed_water=plant[idx]['g4']

        #Temperature - Temperature range within which seed is able to germinate
        seed_temp=plant[idx]['g5']

        #RS Ratio - gene controlling allocation to roots / shoots
        rs_ratio=plant[idx]['g6']

        #Root Depth Distribution - genes controlling critial mass after which roots proceed to
the next layer
        root_depth_1=plant[idx]['g7']
        root_depth_2=plant[idx]['g8']

        #Max Water Extraction - maximum rate at which roots can extract water per root mass
    #Locked if required
        if lock == 0:
            max_water=plant[idx]['g9']
        else:
            max_water=255.0


        #WUE - Water use efficiency, or water required per g of carbon assimilated
    #locked if required
        if lock == 0:
            wue_gene=plant[idx]['g10']
        else:
            wue_gene=255.0


        #Photosynthetic rate - carbon assimilation per light input
    #Currently fixed
        #ps_gene=plant[idx]['g11']
        ps_gene = 4 * 16

        #Dessication - time of low water availability before plant dies
        #Currently, plants don't die with low water availability - will need to include this
        #dessicate=plant[idx]['g12']
        dessicate = 7.5 * 16

        #Flowering maturity - age of plant before flowering can occur
        flower_matur=plant[idx]['g13']
```


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```
        #Flowering temp - temperature integral from start of winter before flowering can occur
        #Currently, temperature regime is always the same, so it looks like this isn't variable
        #Therefore, fix at 7.5 for the moment
        #flower_temp=plant[idx]['g14']
        flower_temp=7.5 * 16

        #Flowering time - point (temperature? water balance?) determine when flowering will
stop
        #Unresponsive to rainfall, and appeared to be maximised, therefore look for a trade-off
        #Will fix at 14.75
        #flower_stop=plant[idx]['g15']
        flower_stop = 14.75 * 16

        #Flowering allocation - proportion of biomass allocation to reproduction / seed output
during flowering
        #Fixed and unresponsive = fix at 7.5
        #flower_ratio=plant[idx]['g16']
        flower_ratio=7.5 * 16


        #genec[repro]=genec[repro]+1.0

        genep[0]=genep[0]+root_depth_1
        genep[1]=genep[1]+root_depth_2
        genep[2]=genep[2]+rs_ratio
        genep[3]=genep[3]+ps_gene
        genep[4]=genep[4]+seed_storage
        genep[5]=genep[5]+germ_curve
        genep[6]=genep[6]+seed_water
        genep[7]=genep[7]+seed_temp
        genep[8]=genep[8]+max_water
        genep[9]=genep[9]+wue_gene
        genep[10]=genep[10]+dessicate
        genep[11]=genep[11]+flower_matur
        genep[12]=genep[12]+flower_temp
        genep[13]=genep[13]+flower_stop
        genep[14]=genep[14]+flower_ratio
        genep[15]=genep[15]+germinate_comp

        #Manage soil seed bank
        plantwilldie=self.seedgerminate(idx,seed_storage, germinate_comp, germ_curve,
livecount, plant[idx]['CC'], seed_water, seed_temp, flower_stop)

        if plant[idx]['phen'] == 'grow' or plant[idx]['phen'] == 'flower':
            diedess = self.plantdess(idx, root_depth_1, root_depth_2)
        else:
            diedess = 0

        diedess = 0

        #Call Photosynthesis and resource allocation
        if plant[idx]['phen'] <> 'seed':
            self.plantps(idx, ps_gene, rs_ratio,root_depth_1, root_depth_2, solar, max_water,
wue_gene, Temp, RelH)

        #Determine if plant is to start flowering
        self.flowering(idx, flower_matur)

        #Kill plant if (a) past a certain age or (b) seed in soil becomes unviable

        if plantwilldie==1 or diedess == 1:

           deadp=deadp+1
           return 1
        else:
           return 0

    #-----------------------------------------------------------------
    # Soil Water Flux Function
    # Calculates movements between layers based on
    # A simple model of hydraulic conductivity
```


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```
def flux(self, idx):
    global SoilK, soil

#Record starting water content of each layer
    T0=soil[idx][0]
    T1=soil[idx][1]
    T2=soil[idx][2]

#Calculate moisture differences between layers
#And proportion of water to be redistributed
    DIF01=T0-T1
    CHANGE01=DIF01*SoilK
    DIF12=T1-T2
    CHANGE12=DIF12*SoilK

#Redistribute water
    NT0=T0-CHANGE01
    NT1=T1+CHANGE01-CHANGE12
    NT2=T2+CHANGE12

#Update soil water layers
    soil[idx][0]=NT0
    soil[idx][1]=NT1
    soil[idx][2]=NT2


# Calculate Soil Water Content by Volume
# Returns water content (%) by volume for a layer
def WCV_function(self,idx,WCV_Layer):
    global Depth, soil, SoilSA
    tvol = Depth[WCV_Layer]*SoilSA # Volume of layer
    return soil[idx][WCV_Layer]/tvol

#Convert the day of the year to a month, for use in climate averages
def Day_To_Month(self, Day):
    global MON_DAYS
    tot=0
    amonth=0
    tmonth=0
    for month in MON_DAYS:
        tot=tot+month
        amonth=amonth+1
        if Day > tot:
            tmonth=amonth
    return tmonth

# Calculate Soil Percentage Saturation SP for a layer  (%)
def SP_function(self, idx, SP_Layer):
    global WP, FC, Depth, SoilSA, soil
    FCml = FC[SP_Layer] * Depth[SP_Layer]*SoilSA  # Field capacity in mL
    SP = soil[idx][SP_Layer]/FCml
    return SP

# Soil Water Potential Function
# Returns water potential O (kPa) for a layer
# Based on filter-paper measurements
# No longer used.
def SO_function(self, SO_Layer):
    global Depth
    WCVolume=self.WCV_function(SO_Layer) + 0.001
    return 0.0085 * pow(WCVolume, -4.4879)

#RF_Function - returns daily rainfall amount, depending on the rainfall model being used
#Fixed, seasonal or real.
def RF_function(self, RF_Month):
    global rainfile
    global c_count
    if (self.components.RainfallData.selected=="Artificial"):
        if c_count == 0:
            The_RF=self.components.rainmm.text
```


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```
        else:
            The_RF=0.0
        c_count=c_count+1
        if c_count == int(self.components.rainfreq.text):
            c_count=0
        return The_RF
    elif (self.components.RainfallData.selected=="Seasonal"):
        if (RF_Month <=2) or (RF_Month >=9):
            thefreq = self.components.rainfreqsum.text
            themm = self.components.rainmmsum.text
        else:
            thefreq = self.components.rainfreqwin.text
            themm = self.components.rainmmwin.text
        if c_count==0:
                The_RF = themm
        else:
                The_RF = 0.0

        c_count=c_count+1
        if c_count >= int(thefreq):
            c_count=0



        return The_RF
    else:
        todayval=rainfile.readline()
        if not todayval:
            rainfile.seek(0)
            todayval=rainfile.readline()

        return todayval

#Convert mm of rainfall to mL, based on soil surface area
def mm_to_mL(self,mm):
    global SoilSA
    return mm * (SoilSA / 10.0)

#Function to infiltrate rainfall into the three soil layers
#As a cascade
def infiltrate(self, idx, infil_RF):
    global Depth, plant, soil, SoilSA
    global FC
    infil_TOP=infil_RF
    infil_mm = self.mm_to_mL(infil_TOP)

#Layer 0
#If soil water content is less than field capacity then continue
    if soil[idx][0] < (FC[0]*Depth[0]*SoilSA):
    #Calculate how much water would be needed to saturate this layer
        tofill = (FC[0]*Depth[0]*SoilSA)-soil[idx][0]
    #Calculate whether the rainfall input exceeds this amount
        if infil_mm <= tofill:
       #If yes, add all the rainfall
            soil[idx][0] = soil[idx][0] + infil_mm
            infil_mm = 0
        if infil_mm > tofill:
       #If no, fill the layer, leave the rest for the next layer.
            infil_mm = infil_mm - tofill
            soil[idx][0] = soil[idx][0] + tofill

    #Layer 1
    if soil[idx][1] < (FC[1]*Depth[1]*SoilSA):
        tofill = (FC[1]*Depth[1]*SoilSA)-soil[idx][1]
        if infil_mm <= tofill:
            soil[idx][1] = soil[idx][1] + infil_mm
            infil_mm = 0
        if infil_mm > tofill:
            infil_mm = infil_mm - tofill
            soil[idx][1] = soil[idx][1] + tofill
```


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```
    #Layer 2
    if soil[idx][2] < (FC[2]*Depth[2]*SoilSA):
        tofill = (FC[2]*Depth[2]*SoilSA)-soil[idx][2]
        if infil_mm <= tofill:
            soil[idx][2] = soil[idx][2] + infil_mm
            infil_mm = 0

        if infil_mm > tofill:
            infil_mm = infil_mm - tofill
            soil[idx][2] = soil[idx][2] + tofill

#Returns any unallocated water as deep drainage
    return(infil_mm)

#Determine daily evaporation from climate records
def EV_function(self, Day):
    global PRF,cloudy, MON_EV, MON_DAYS
    EV=MON_EV[self.Day_To_Month(Day)]
    return EV

#Respiration function
def respire(self, idx,Temp,Temp1, Temp2, rflag):
    global dayR


    p2 = 0.000625
    p1 = 0.0693

    #Depth variable Respiration
    if rflag==1:
        Temp0 = Temp
        Temp1 = Temp-5.0
        Temp2 = Temp-10.0

        resp = p2 * exp(Temp * p1)
        resp0 = p2 * exp(Temp0 * p1)
        resp1 = p2 * exp(Temp1 * p1)
        resp2 = p2 * exp(Temp2 * p1)

        plant[idx]['sm'] = plant[idx]['sm'] * (1-resp)
        plant[idx]['rm0'] = plant[idx]['rm0'] * (1-resp0)
        plant[idx]['rm1'] = plant[idx]['rm1'] * (1-resp1)
        plant[idx]['rm2'] = plant[idx]['rm2'] * (1-resp2)

    #Even soil respiration
    if rflag==2:

        resp = p2 * exp(Temp * p1)
        resp0 = p2 * exp(Temp * p1)
        resp1 = p2 * exp(Temp * p1)
        resp2 = p2 * exp(Temp * p1)

        plant[idx]['sm'] = plant[idx]['sm'] * (1-resp)
        plant[idx]['rm0'] = plant[idx]['rm0'] * (1-resp0)
        plant[idx]['rm1'] = plant[idx]['rm1'] * (1-resp1)
        plant[idx]['rm2'] = plant[idx]['rm2'] * (1-resp2)

    #Monthly lags with deeper soil
    if rflag==3:
        resp = p2 * exp(Temp * p1)
        resp0 = p2 * exp(Temp * p1)
        resp1 = p2 * exp(Temp1 * p1)
        resp2 = p2 * exp(Temp2 * p1)

        plant[idx]['sm'] = plant[idx]['sm'] * (1-resp)
        plant[idx]['rm0'] = plant[idx]['rm0'] * (1-resp0)
        plant[idx]['rm1'] = plant[idx]['rm1'] * (1-resp1)
        plant[idx]['rm2'] = plant[idx]['rm2'] * (1-resp2)

#Record respiration total
```


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```
        dayR = dayR + (plant[idx]['sm'] * (resp)) + (plant[idx]['rm0'] * (resp0)) +
(plant[idx]['rm1'] * (resp1)) + (plant[idx]['rm2'] * (resp2))

    #Do daily evaporation - needs modification
    def evaporate(self, idx, Day):
            global efunc, soil
            olddayevap = self.EV_function(Day)
            dayevap=self.mm_to_mL(olddayevap)

            #Evaporation Moderated by soil moisture - ie. Thornthwaite
            if efunc==0:

                pp=(0.7 * dayevap*self.SP_function(idx,0))

                soil[idx][0]=soil[idx][0]- (0.7 * dayevap*self.SP_function(idx,0))

                if soil[idx][0]<0.01:
                    soil[idx][0]=0.0
                soil[idx][1]=soil[idx][1] - (0.05 * dayevap*self.SP_function(idx,1))
                if soil[idx][1]<0.01:
                    soil[idx][1]=0.0
                soil[idx][2]=soil[idx][2]- (0.005 * dayevap*self.SP_function(idx,2))
                if soil[idx][2]<0.01:
                    soil[idx][2]=0.0
            #Evaporation moderated by soil water potential
        # No longer used
            if efunc==1:
                soil[idx][0]=soil[idx][0]- (0.7 * dayevap/(self.SO_function(idx,0)/2))
                if soil[idx][0]<0.01:
                    soil[idx][0]=0.0
                soil[idx][1]=soil[idx][1] - (0.2 * dayevap/(self.SO_function(idx,1)/2))
                if soil[idx][1]<0.01:
                    soil[idx][1]=0.0
                soil[idx][2]=soil[idx][2]- (0.1 * dayevap/(self.SO_function(idx,2)/2))
                if soil[idx][2]<0.01:
                    soil[idx][2]=0.0
            return
        #Experimental function to only evaporate from surface layer.
            if efunc==2:
                pp=(0.7 * dayevap*self.SP_function(idx,0))

                soil[idx][0]=soil[idx][0]- (0.7 * dayevap * self.SP_function(idx,0))

                if soil[idx][0]<0.01:
                    soil[idx][0]=0.0


    #Main Execution Loop
    def main(self):
    #Define global variables
        global dout
        global rainfile
        global ps
        global genep
        global deadp
        global maxseed
        global maxage
        global population
        global plant, soil, DD
        global pdict
        global daylength
        global RF, Temp, EV
        global Env_Lat
        global resp
        global dayR, dayA
        global fit
        global reportall

    #If using real rainfall data, open file
        if (self.components.RainfallData.selected=="Real"):
            rainfilename = self.components.Filename.text
```


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```
        rainfile = open(rainfilename)

#Initialize environmental variables and plant array
    self.initenv()
    self.initplants(300)

#Array to keep track of gene averages
    genep=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]

#Define number of generations to run the model for
    notries = int(self.components.Days.text)
    totalDD = 0.0
    totalG = 0.0
    tries = 0

#Loop for each model replicate
    while tries < notries:
        #print "Try / notries:", tries,notries
        tries = tries + 1
        day=0
        if (self.components.RainfallData.selected=="Real"):
            #If using real daily rainfall data, pick a random start year
       startl = int(randint(1,45))
            skipl = startl*365
            for skipline in range(skipl):
                rainfile.readline()

    #Loop for each generation
        while day < (365*2):

         #Reset record variables
             day=day+1
             counter=0.0
             scounter=0.0
             maxseed=0.0
             deadp=0
             maxage=0.0
             avgshoot = 0.0
             livecount=0.0
             asm0=0.0
             asm1=0.0
             asm2=0.0
             seeds=0.0
             death=0.0
             dayR = 0.0
             dayA = 0.0
             genec=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]
             genep=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]

             #Do environmental functions
             daylength = self.DayL_function(day)
             RF = float(self.RF_function(self.Day_To_Month(day % 365)))
             Temp = float(MON_Temp[self.Day_To_Month(day % 365)])
             RelH = float(RH[self.Day_To_Month(day % 365)])
             Temp1 = float(MON_Temp[self.Day_To_Month((day + 335) % 365)])
             Temp2 = float(MON_Temp[self.Day_To_Month((day + 305) % 365)])
             solar = self.dayintegral((day % 365), (0-Env_Lat))

             #Introduce daily rainfall into soil
             for idx in range(len(soil) -1):

                 DDd = self.infiltrate(idx,RF)
                 plant[idx]['DD'] = plant[idx]['DD'] + DDd


             #Flux between layers
             for idx in range(len(soil) -1):
                 self.flux(idx)
```


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```
                #Run counters of how many plants / live plants we have
                  for plantid in plant:
                    counter=counter+1.0
                    if plantid['phen'] <> 'seed':
                          livecount=livecount+1.0
                          avgshoot = avgshoot + plantid['sm'] + plantid['rm0'] + plantid['rm1']
+ plantid['rm2']

                    else:
                          seeds=seeds+1.0

                  #Keep track of average soil moisture
                  for soilb in soil:
                      scounter=scounter+1.0
                      asm0=asm0+soilb[0]
                      asm1=asm1+soilb[1]
                      asm2=asm2+soilb[2]


                  #Call respiration function

                  if resp>0:
                    for idx in range(len(plant) -1, 0, -1):
                        if plant[idx]['phen'] <> 'seed':
                            self.respire(idx, Temp, Temp1, Temp2, resp)



                  #Call main plant growth loop
                  for idx in range(len(plant) -1, 0, -1):

                      isdead = self.growplant(idx, livecount, solar, Temp, RelH)
                      if isdead == 1:
                        #print "killed plant", idx
                        del plant[idx]
                        del soil[idx]
                        del DD[idx]

                  #Evaporate from soil
                  for idx in range(len(soil) -1):
                      self.evaporate(idx ,day % 365)



                  #self.components.Display.append( "test" )
                  wx.wxSafeYield(self)
                  avgshoot=0.0

               #Update graphical display
                    self.components.done.value=int(day)
                    #Report daily data here

              #This point here is where we sort, and create a new set of 300 plants
              #"plant" is a list of dictionaries
              #ie. plant[3]['age']
              #therefore, can be treated like sorting a list.

              #Calculate total biomass for each plant
              for plantid in plant:
                  plantid['tm'] = plantid['sm'] + plantid['rm0']+ plantid['rm1']+ plantid['rm2']

          #Fitness function - sort plants by total biomass
              def stry(x,y):
                  global fit

                  #Shoot mass
                  #Sort by shoot mass - remember to REVERSE
                  #return cmp(x['sm'],y['sm'])
                  #Sort by total mass - remember to REVERSE
                  if fit == 1:
                      return cmp(x['tm'],y['tm'])
```


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```
            #Sort by Deep Drainage
            if fit == 2:
                return cmp(x['DD'],y['DD'])

        plant.sort(stry)

    #Take a subset of the best 10% of plants
        bestplant = []

        dbflag = 0
        if len(plant) < 10:
            #print "Less than 10 plants!"
            dbflag = 1
        if dbflag ==1:
            #print "Breaking out of loop"
            break
        numbertograb = len(plant) * 0.1
        if numbertograb <= 10:
            numbertograb = 10


        #Reverse list so the "highest" plants are first.
        if fit == 1:
            plant.reverse()

    #Report average biomass, if required
        testavg = 0
        for countplant in range(int(len(plant))):
            testavg = testavg + plant[countplant]['tm']
        #print "Average:", testavg/len(plant)


    #Store best plants in a new array
        for countplant in range(int(numbertograb)):
            bestplant.append(plant[countplant].copy())

    #Create a new array of plants by recombining the genes of the best plants, with the
mixitup function

        statplant = plant[:]
        self.mixitup(bestplant)

    #Report data if set to report after each generation
        if dout==1:
            genec=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]
            genep=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]
            smavg = 0.0
            ddavg = 0.0
            rm0avg = 0.0
            rm1avg = 0.0
            rm2avg = 0.0
            counter = len(bestplant)

            for place in range(len(bestplant)):
                germinate_comp = 14.95 * 16
                seed_storage = 11.6 * 16
                germ_curve=bestplant[place]['g3']
                seed_water=bestplant[place]['g4']
                seed_temp=bestplant[place]['g5']
                rs_ratio=bestplant[place]['g6']
                root_depth_1=bestplant[place]['g7']
                root_depth_2=bestplant[place]['g8']
                max_water = bestplant[place]['g9']
                wue_gene=bestplant[place]['g10']
                ps_gene=bestplant[place]['g11']
                dessicate = 7.5 * 16
                flower_matur=bestplant[place]['g13']
                flower_temp=7.5 * 16
                flower_stop = 14.75 * 16
                flower_ratio=7.5 * 16
```


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```
                    genep[0]=genep[0]+root_depth_1
                    genep[1]=genep[1]+root_depth_2
                    genep[2]=genep[2]+rs_ratio
                    genep[3]=genep[3]+ps_gene
                    genep[4]=genep[4]+seed_storage
                    genep[5]=genep[5]+germ_curve
                    genep[6]=genep[6]+seed_water
                    genep[7]=genep[7]+seed_temp
                    genep[8]=genep[8]+max_water
                    genep[9]=genep[9]+wue_gene
                    genep[10]=genep[10]+dessicate
                    genep[11]=genep[11]+flower_matur
                    genep[12]=genep[12]+flower_temp
                    genep[13]=genep[13]+flower_stop
                    genep[14]=genep[14]+flower_ratio
                    genep[15]=genep[15]+germinate_comp
                    smavg = smavg + bestplant[place]['sm']
                    rm0avg = rm0avg + bestplant[place]['rm0']
                    rm1avg = rm1avg + bestplant[place]['rm1']
                    rm2avg = rm2avg + bestplant[place]['rm2']
                    ddavg = ddavg + bestplant[place]['DD']
                self.components.Display.AppendText(
"%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\n" % (tries, (genep[0]/counter),
(genep[1]/counter)
,(genep[2]/counter),(genep[8]/counter),(genep[9]/counter),(genep[6]/counter),(genep[7]/counter)
, (smavg/counter),(rm0avg/counter) ,(rm1avg/counter),(rm2avg/counter),(ddavg/counter)))


        #self.keepDrawing = 0
        #Report averages for each model replicate - ie. optimal at end of each model run.

        genec=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]
        genep=[0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0,0.0]
        smavg = 0.0
        ddavg = 0.0
        rm0avg = 0.0
        rm1avg = 0.0
        rm2avg = 0.0
        counter = len(bestplant)
        print "Counter:",counter
        for place in range(len(bestplant)):
            germinate_comp = 14.95 * 16
            seed_storage = 11.6 * 16
            germ_curve=bestplant[place]['g3']
            seed_water=bestplant[place]['g4']
            seed_temp=bestplant[place]['g5']
            rs_ratio=bestplant[place]['g6']
            root_depth_1=bestplant[place]['g7']
            root_depth_2=bestplant[place]['g8']
            max_water = bestplant[place]['g9']
            wue_gene=bestplant[place]['g10']
            ps_gene=bestplant[place]['g11']
            dessicate = 7.5 * 16
            flower_matur=bestplant[place]['g13']
            flower_temp=7.5 * 16
            flower_stop = 14.75 * 16
            flower_ratio=7.5 * 16
            if reportall==1:
                if dout==0:
                    self.components.Display.AppendText(
"%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\n" % (tries, root_depth_1, root_depth_2
,rs_ratio, max_water, wue_gene, seed_water, seed_temp,
bestplant[place]['sm'],bestplant[place]['rm0']
,bestplant[place]['rm1'],bestplant[place]['rm2'],bestplant[place]['DD']))

            genep[0]=genep[0]+root_depth_1 #Good
            genep[1]=genep[1]+root_depth_2 #Good
            genep[2]=genep[2]+rs_ratio #Good
            genep[3]=genep[3]+ps_gene
            genep[4]=genep[4]+seed_storage
            genep[5]=genep[5]+germ_curve
```


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```
            genep[6]=genep[6]+seed_water #Good
            genep[7]=genep[7]+seed_temp #Good
            genep[8]=genep[8]+max_water #Good
            genep[9]=genep[9]+wue_gene #Good
            genep[10]=genep[10]+dessicate
            genep[11]=genep[11]+flower_matur
            genep[12]=genep[12]+flower_temp
            genep[13]=genep[13]+flower_stop
            genep[14]=genep[14]+flower_ratio
            genep[15]=genep[15]+germinate_comp
            smavg = smavg + bestplant[place]['sm'] #Good
            rm0avg = rm0avg + bestplant[place]['rm0'] #Good
            rm1avg = rm1avg + bestplant[place]['rm1'] #Good
            rm2avg = rm2avg + bestplant[place]['rm2'] #Good
            ddavg = ddavg + bestplant[place]['DD'] #Good
            totalDD = totalDD + ddavg
            totalG = totalG + bestplant[place]['sm'] + bestplant[place]['rm0'] +
bestplant[place]['rm1'] + bestplant[place]['rm2']
        if dout==0:
            totalDD = totalDD / len(bestplant)
            totalDD = totalDD / int(self.components.Days.text)
            totalG = totalG / len(bestplant)
            totalG = totalG / int(self.components.Days.text)
            self.components.Display.AppendText(
"%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\t%s\n" % (tries, (genep[0]/counter),
(genep[1]/counter)
,(genep[2]/counter),(genep[8]/counter),(genep[9]/counter),(genep[6]/counter),(genep[7]/counter)
, (smavg/counter),(rm0avg/counter)
,(rm1avg/counter),(rm2avg/counter),(ddavg/counter),(totalDD),(totalG)))
            print "Returned Plants"
        #return


if __name__ == '__main__':
    app = model.PythonCardApp(Genes)

    app.MainLoop()
```


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