CANOPY DIEBACK AND DECLINE IN PINK GUMS, EUCALYPTUS FASCICULOSA, F.MUELL IN THE MT LOFTY RANGES AND ADJOINING PLAINS

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Submitted for the degree of Doctor of Philosophy

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April 2017

CONTENTS

ABSTRACT

In Australia, widespread decline of *Eucalyptus* species has been occurring largely since the 1970s. In southern Australia, pink gums (*Eucalyptus fasciculosa* F. Muell) occur within a restricted area, and have been assigned a conservation rating due to perceived canopy decline. This study aimed to describe the population structure and canopy condition of pink gums in the Mt Lofty Ranges and surrounding plains, and to distinguish between causes of short-term dieback and long-term decline. I hypothesised that decline in pink gum woodlands was most likely to be because of one or more of the following: (1) long-term and continuous mistletoe infection; (2) declining annual rainfall commencing several decades ago, particularly for trees growing in deep sandy soils and (3) reduced groundwater availability that commenced well before 2003. Dieback was most likely to be associated with insect attack. Within the study region, pink gums occupy a wide gradient of rainfall, soil type and topography. A regional survey between 2009 and 2012 recorded that the mean canopy intactness for 2814 pink gums was 70%, but canopy condition varied widely between, and often within, pink gum woodlands. The habitat variables that contributed most to variation in canopy intactness were mistletoe prevalence, followed by insect damage. In almost 30% of populations there were no seedling pink gums.

Between 2009 and 2011, mean canopy intactness of 1751 pink gums declined from 71% to 66%, 21 pink gums died, and no recruitment was recorded. Leaf production in pink gums was greatest during the above-average rainfall in the summer of 2010/11. This was followed by localised high levels of gum leaf skeletoniser (*Uraba lugens*) and lerp (*Cardiaspina densitexta*). Canopy loss from these insects was concluded to be cyclic and short term.

The canopy intactness of 161 pink gums and their mistletoe load, was recorded between 2003 and 2015. The net number of live mistletoes declined by two thirds, with the greatest net decline occurring during three consecutive below-average rainfall years. While the mean canopy intactness of pink gums was the same in 2003 and 2015, pink gums whose canopy comprised at least 20% mistletoe, recorded significant canopy loss during consecutive years of below-average rainfall.

Comparison of aerial photographs between 1949 and 2005 for two pink gum woodlands indicated that canopy decline began in the mid-1970s, coinciding with lowering of groundwater levels and increased mistletoe infection, and the beginning of an ongoing decline in autumn rainfall. These pink gums occurred on deep sands with approximately 500 mm annual rainfall, a habitat that would be more prone to autumn soil drying.

Because of the lack of recent recruitment at many pink gum woodlands, it may be necessary to restore and/or maintain populations with revegetation. A glasshouse experiment involving manipulating water supply to three pink gum provenances showed that there was genetic variation between provenances. This will provide the option in revegetation projects, of matching selection of seed sources to projected climate change. However, in a separate six year study, 12 of 34 pink gum juveniles remained the same height, with insect defoliators and competition from nearby trees considered largely responsible for suppressing growth. The potential slow growth rate of pink gums should be incorporated into revegetation planning.

This study provided evidence that pink gums may be at least partially groundwater dependent. Within the same woodland, where all habitat factors other than depth to groundwater appeared to be equal, pink gums sited over shallower groundwater tables were significantly taller and had greater canopy volumes.

A key finding was that pink gums occurring on deep sands with moderate mean annual rainfall for the study region and with high mistletoe loads, had low canopy intactness. Areas of future research should focus on: which populations of pink gums are at least partially groundwater dependent; the interaction between pink gum water stress and mistletoe abundance over long time frames; and differences between pink gum provenances in drought response and growth rates. Future monitoring of survey sites established in this study would provide information on pink gum canopy and population trends, and mistletoe population trends.

DECLARATION

Name: Sonia Julie Croft **Program:** Doctor of Philosophy

I certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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ACKNOWLEDGEMENTS

Funding for the project was provided by a Frederick Sandholz Postgraduate Scholarship and the Native Vegetation Council (S.A.). Thank you to my supervisors, David Paton and Jose Facelli for their encouragement and advice. I acknowledge the valuable comments both original examiners made towards this re-submitted thesis. Thank you to anonymous reviewers of articles submitted to journals for future publication. Thank you to all landholders who provided permission for me to work on their properties. Special thanks to Roland Chatterton, Tony and Cathy Skewes, Andrew and Margaret Black, and Tim and Tanya Milne, for allowing me to repeatedly visit their pink gum woodlands, their hospitality and ongoing interest. Thank you to my husband, Tim Croft, for assistance with field work. Thank you to Matt Ward for providing the inspiration for this thesis.

Introduction

Tree decline and dieback

Tree decline is a worldwide problem affecting individual trees, woodlands and forests (Allen *et al.* 2010; Horton 2012). In Australia, accelerated decline of *Eucalyptus* species is widespread and is documented as occurring largely from the late 1970s onwards (Close and Davidson 2004; Jurskis 2005; Lowman and Heatwole 1992). In recent years, long-term non-reversible canopy loss and short-term reversible canopy losses have been referred to as eucalypt decline and eucalypt dieback, respectively (Horton 2012; Jurskis, 2005). Trees suffering decline typically have canopies with sparse foliage and a large proportion of dead branches, along with a reduced root system (Reid and Landsberg 1999). Decline most commonly manifests as the death of twigs and branches which generally starts at the tips (DEH 2005). The loss of adult foliage may stimulate epicormic growth on branches and trunks. In long-term decline, eventually epicormic growth also dies, leaving dead twigs and branches. Both dieback and decline can, and often do, occur as the result of interacting biotic and abiotic environmental stresses (Horton 2012).

Causes of eucalypt decline and dieback in Australia

Decline is generally considered to be due to a range of interacting biotic and abiotic factors (Ross and Brack 2015). Causes of *Eucalyptus* decline in Australia include long-term changes in soil, water, atmospheric composition and climate (Cunningham *et al.* 2007). Decline of isolated paddock trees, often referred to as rural tree decline, is most commonly due to trees suffering from: decreased water availability due to soil compaction and competition with improved pasture, increased salinity, loss of soil fungi, altered soil nutrients, and/or changed pest/predator and biodiversity balance (Close and Davidson 2004). These changes often lead to increased insect and/or parasite attacks on the remaining paddock trees.

Factors contributing to rural tree decline have less relevance in woodland, where trees are of near-natural density and retain a native understorey. For woodland *Eucalyptus* trees, the main causes of decline are climate change, regional hydrological changes, pathogens (including *Phytophthora*), altered fire regimes and long-term altered abundances of parasites (most notably, mistletoe) (Jurskis 2005). Long-term hydrological changes have contributed to 70% of river red gum (*Eucalyptus camaldulensis*) forests on the Victorian Murray River floodplain being in some state of dieback (Cunningham *et al.* 2010). Recent research found that in long unburnt *Eucalyptus* woodlands in Western Australia and Tasmania, a high shrub cover developed and eucalypts were exposed to greater water stress (Bushfire CRC 2014). In eastern Australia, eucalypt dieback associated with psyllids and bell miners (*Manorina melanophrys*) is known as bell miner associated dieback (BMAD) and is a serious issue (Horton 2012). Long-term alteration of forest structure, soil nutrients and abundances of bell miners and psyllid insects have been implicated in BMAD. Tree decline due to long-term drought and heat stress is increasingly being observed both globally and in Australia (Booth *et al.* 2015; Brouwers 2013; Dalmaris *et al.* 2015). For example, the south-west of Western Australia has been getting dryer and warmer since the 1980s, which has been linked to forest decline and tree mortality throughout the region. Evans *et al.* (2013) go further and state that most past tree decline events in Australia have drought as the inciting factor. While dieback is commonly associated with insect attack, these attacks are generally a secondary factor that occurs as a result of other underlying causes such as nutrient imbalances or water stress (Ross and Brack 2015). Dieback of *Eucalyptus macrorhyncha* in New South Wales, for example, was attributed to drought followed by secondary attack from stem borers (Pook and Forrester 1984).

Existing approaches to studying causes of eucalypt canopy decline and dieback

Models of eucalypt dieback and decline

Several researches have proposed general models of eucalypt decline and dieback. Landsberg and Wylie (1983), White (1986), Jurskis and Turner (2002) and Jurskis (2005) submitted models broadly stating that in response to water stress, the trees produced nutrient-rich foliage which attracts high numbers of leaf attacking insects. Landsberg and Wylie's (1983) model suggested that the more palatable and nutrient-rich epicormic growth attracted more insects which led to a feedback loop in which there are cycles of defoliation and insect attack. Eventually the decline in carbohydrate reserves is sufficiently great to cause tree death. Jurskis and Turner (2002) stated that changed land management, including the exclusion of low intensity fire, has led to increased understorey vigour leading to increased insect presence on trees with increased susceptibility to pathogens. These models identify variables contributing to dieback and decline, and possible interactions between the variables. As such, they are useful for placing specific studies within a wider framework, providing guides as to possible causes of dieback, and for identifying knowledge gaps in dieback and decline research. Mackay *et al*. (1984), for example, concluded that insects were responsible for New England dieback, based on Landsberg and Wylie's (1983) model.

Determining correlations: multi-factor studies

Where the cause of *Eucalyptus* decline is not known, a common approach is to conduct broadscale surveys of tree canopy intactness and associated independent variables including: leaf damage symptoms, mistletoe presence, site factors (aspect, slope, soil, landform element), land management factors (grazed, fertilizer application) and stand density (Cunningham *et al*. 2007; Mackay *et al.* 1984; Reid *et al.* 2007). Statistical models can then determine if there are significant correlations between independent variables and canopy intactness, and possible interactions between variables. For example, Cunningham *et al.* (2007) found that percentage live basal area, plant area index and crown vigour were most strongly correlated with the condition of river red gum stands and that there was little difference in water potential and chlorophyll fluorescence among stands in good and poor condition. Reid *et al*. (2007) concluded that a suite of environmental parameters, specific to each *Eucalyptus* species, was likely to be attributable to canopy decline. The authors concluded that potential interactions between water stress, insect damage, the old age of trees and cockatoo damage may all be important in the decline of river red gums. For poplar box (*Eucalyptus populnea*), however, the most important factors were likely to be falling shallow water tables during drought, insect damage and herbicide drift (Reid *et al*. 2007). Mackay *et al*. (1984) determined correlations between various tree parameters, site factors, land management factors and stand density, and canopy intactness for *Eucalyptus* species in the New England region of New South Wales.

Determining causes: single factor and multi-factorial experiments

Studies that reveal correlations between environmental factors and canopy intactness enable the formulation of hypotheses aimed at determining causes of canopy decline. Experiments that manipulate one or more independent variables, while controlling other independent variables enable alternative hypotheses to be falsified or supported. For example Mackay *et al.* (1984) concluded that a plausible mechanism of tree death was exhaustion of energy reserves due to repeated insect defoliation. To test this hypothesis Mackay *et al.* (1984) subsequently conducted a single factor experiment, with treatment trees receiving insecticide treatment, and control and treatment trees being matched for species, crown condition and tree height. Their study supported the hypothesis that insect defoliation was a primary cause of tree death in the New England region.

Determining the role of fire in the decline of alpine ash (*Eucalyptus delegatensis*) is a further example of how broadscale studies have determined correlations between canopy intactness and environmental parameters, and which have been followed by experimental studies to test hypotheses. The decline of alpine ash has been consistently correlated with the development of a dense understorey where fire has been excluded for long periods (Close *et al*. 2009). This finding has been the basis for experiments studying the role of fire in tree decline (Bushfire CRC 2014). Horton (2011), for example, established study plots in long unburnt and frequently burnt woodlands to examine the impact of fire frequency on mycorrhizal fungi and forest tree health.

A third example of correlative studies leading to experimental studies concerns lerps (various species of psyllids), sap-sucking insects that can cause extensive defoliation and possibly death of *Eucalyptus* species (Stone and Urquhart 1995). Environmental parameters that have been associated with lerp outbreaks include an abundance of immature succulent foliage, host trees growing as single species dominants, high leaf nutrients, warmer than average seasonal temperatures, high soil moisture; absence of flowering in host trees, and low populations of natural control agents (Collett 2002). In a more specific study, Hall *et al*. (2015) identified that minimum winter temperature correlated highly with psyllid abundance in a population of grey box (*Eucalyptus moluccana*), at western Sydney, New South Wales. Steinbauer *et al*. (2015) developed a model with feedback loops between bell miners, leaf nitrogen content and psyllid abundance in order to promote understanding of bell miner associated dieback. In an experimental study, Brennan and Weinbaum (2001) determined that the presence of a waxy coating on blue gum (*Eucalyptus globulus*) leaves increased resistance to psyllid species. Collectively, these studies contribute to building a more comprehensive understanding of the role of lerps in *Eucalyptus* decline and dieback.

Over-abundant mistletoe is commonly associated with *Eucalyptus* dieback in southern Australia (Watson 2011), but whether mistletoes are primarily a cause or a symptom of *Eucalyptus* decline is still unclear. By conducting a controlled mistletoe removal experiment, however, Reid *et al*. (1994) were able to show that eucalypts hosting mistletoe had significantly reduced trunk diameters and tree survival rates.

The commonly observed association between *Eucalyptus* decline and drought in Australia, has led to the physiological response of different *Eucalyptus* species to water stress being extensively studied in glasshouse experiments (e.g. Bader *et al.* 2011; Jakob 2016, Lin 2012; Merchant 2007; Ngugi 2004). However, the exact physiological mechanisms that cause climate-related tree death are not clear (McDowell *et al.* 2011). Several mechanisms have been proposed including hydraulic failure and depletion of carbon reserves, but further research is required to enable possible selection of drought tolerant species in conservation programs. Poot and Veneklaas (2013) compared the water-use strategies of two *Eucalyptus* species in an area where they co-occurred as well as in areas that represented a more typical habitat for each species. They concluded that water relations in *Eucalyptus* species are not only affected by climate, but also by the interaction of the plants with the soil substrate. These studies have shown that predicting the response of *Eucalyptus* species to drought depend upon numerous interacting factors, which may require carefully designed experiments to determine interactions between variables.

Spatial analysis

Spatial analysis, as a tool for investigating *Eucalyptus* dieback and decline, relies upon finding landscape variables that correlate with different levels of canopy health. For example, Pook *et al.* (1966) noted that different levels of dieback were associated with different soil textures and/or aspects. Wilting tended to occur on the lighter-textured, stonier, or shallower soils, especially on steep and sunny aspects, whereas trees growing on adjacent heavier-textured soils were not visibly affected. This led Pook *et al.* (1966) to conclude that summer rainfall is both effective and essential for *Eucalyptus* species occurring on soils of low water-holding capacity. More recently, specialist spatial software programs have been used in the analysis of spatial patterns of tree health to infer causes of dieback. Martin *et al.* (2001) used spatial analysis to explore the causes of dieback in a reserve on the urban fringe of Victoria. Their results discounted several suggested causes of dieback, including bell miner associated dieback, phytophthora and changed water conditions, but did not provide firm conclusions as to the cause of dieback. Spatial analysis, therefore is a useful tool to determine correlations between *Eucalyptus* decline and environmental parameters. Determining underlying causes however, requires further experimental testing.

Historical aerial photography

In Australia, there have been surprisingly few studies that have used historical aerial photography to document changes in tree health. Wen *et al.* (2009) analysed a series of photos taken over a 40 year period and determined that the long-term decline in crown condition of river red gums corresponded with a reduction in flooding. Herwitz *et al.* (2000) also successfully used aerial imagery to correlate changes in tree health to habitat changes, namely canopy changes in a Californian forestry reserve were related to drought years and years of above average rainfall. As with spatial analysis tools, however, analysing historical aerial photography establishes correlations rather than causal mechanisms.

Adults versus juveniles

Most field studies of *Eucalyptus* decline, including drought-induced decline have focussed upon adult trees (Standish *et al.* 2015). A recent exception is a field study by Standish *et al.* (2015) which recognised the need to understand drought impacts on *Eucalyptus* seedlings to fully predict the impact of drought on woodland trees. In contrast, glasshouse experiments to assess water relations of Australian tree species have exclusively used seedlings or juveniles (e.g. Dalmarris 2012). These glasshouse experiments have variously studied *Eucalyptus* species' responses to water stress by recording physiological parameters such as leaf water potential, water use efficiency and stomatal conductance (Bell and Williams 1997). This information helps determine how a species may cope with water stress.

Short-term versus long-term studies

Most studies on eucalypt dieback and decline have been relatively short term, often less than five years, due largely to limitations imposed by research tenures and/or funding. Lunt (2013) notes, for example, that there have been very few long-term studies of how alternating dry and wet periods affect Australian ecosystems. Hence, most studies are snap shots of *Eucalyptus* health and do not follow tree conditions through time.

Multi-disciplinary

Where the causes of *Eucalyptus* decline are multiple and interacting, a multi-disciplinary approach may be required to determine the role of causal factors. Investigating the causes of decline in tuart (*Eucalyptus gomphocephala*) is an example of a large and integrated research project (Barber and Hardy 2006). Early stages of the research collected data from 46 sites across the environmental range of tuarts and explored relationships between habitat variables and tuart canopy health. The tuart research has subsequently: investigated water and nutrient use under different environmental parameters; developed a model to describe the morphological/ physiological condition of tuart in relation to edaphic factors; and investigated pathogens associated with tree decline and if these are related to predisposing environmental factors (Barber and Hardy 2006). Preliminary findings indicated greatest tuart decline was correlated with higher rainfall, finer and shallower soil, higher groundwater alkalinity and salinity, and greater rates of groundwater salinity increase. Despite the study occurring during one of the driest 18 month periods on record, critical water potentials for loss of xylem function were rarely recorded. Severe canopy decline was also associated with poor root health, a lack of association with mycorrhizal fungi and low levels of nutrients observed in the foliage of tuart. Such research demonstrates the complex range of biotic and abiotic factors that may be involved in *Eucalyptus* decline, and which spans across disciplines such as ecohydrology, soil science, plant ecology and entomology.

Thesis Research: pink gums (*Eucalyptus fasciculosa* F. Muell)

The focus of this thesis was on dieback and decline of pink gums (*Eucalyptus fasciculosa* F. Muell), a species of habitat, conservation and commercial (honey production) value. Pink gums are largely confined to South Australia, occurring in the southern Mt Lofty Ranges, the South East and Kangaroo Island regions, and with just a small incursion of approximately 10 km into western Victoria. Despite this relatively restricted distribution, pink gum is adapted to a range of habitats “equalled by few other eucalypts native to South Australia” (Boomsma and Lewis 1980). The impetus for this thesis was the findings and recommendations of Ward’s (2005) research on mistletoe dispersal in pink gum woodlands, and Paton *et al.*’s (2005) regional study of *Eucalyptus* species in the south-east of South Australia. Both Paton *et al.* (2005) and Ward (2005) found that of the *Eucalyptus* species studied, pink gums had the lowest levels of canopy intactness and that there was an urgent need to understand the causes of pink gum decline in the Mt Lofty Ranges and South East regions of South Australia, respectively.

Pink gums are currently listed as Rare under South Australian legislation (*National Parks and Wildlife Act 1972).* The listing was the result of concern over declining canopy health and gradual loss of trees (Jason van Weenan, pers. comm. 2009). For the South East region of South Australia, it is estimated that only 2% of the original pink gum woodland remains and hence may be regarded as a vulnerable vegetation type (Croft and Carpenter 1996).

Corresponding figures have not been calculated for Kangaroo Island and the Mt Lofty Ranges regions of pink gum's distribution. The most commonly attributed causes or symptoms of dieback and decline in pink gum are mistletoe infection (Ward 2005), psyllid ("lerp") attack (White 1970; White 1986; Paton *et al.* 2005), climatic extremes (White 1969), altered groundwater levels (Ward 2005), nutritional deficiencies implicated with "Mundulla Yellows" (Paton *et al.* 2005) and secondary salinity (Camp 2004).

Existing studies of pink gum

Paton *et al.* (2005) recorded the canopy condition and reproductive output of 11 *Eucalyptus* species located within a 900 km^2 area in the upper South East. The authors found that pink gums had the highest incidence of lerps, box mistletoe (*Amyema miquelii*) and leaf yellowing. Of the 86 pink gums surveyed in vegetation patches (as opposed to paddock trees or roadside trees) approximately 75% had a canopy intactness of ≤ 60%.

Ward's (2005) study of patterns of mistletoe dispersal in pink gums remains the only comprehensive study quantifying pink gum canopy condition in the Mt Lofty Ranges. Ward (2005) surveyed 1200 pink gums from 28 transects within 12 *National Parks and Wildlife Act 1972* Reserves. The mean level of canopy intactness for these pink gums was 52% which Ward (2005) stated was "cause for concern". Ward also found that 38% of surveyed pink gums were infected with live and/or dead mistletoes, which was significantly higher than for any other *Eucalyptus* species in the region. Ward (2005) did not record a significant association though between the level of pink gum canopy intactness and mistletoe infection levels. Ward suggested future research should include investigating the use of groundwater by pink gums, as well as the effects of mistletoe removal on the physiology of pink gums.

White's (1970) study of the pink gum lerp (*Cardiospina densitexta* Taylor) included a discussion of the distribution of pink gums. From observations of uprooted pink gums in the South East of South Australia, White (1970) concluded that they are shallow-rooted and because of this, their distribution is largely determined by their sensitivity to changes in soil water. Based on the premise that pink gums are shallow-rooted, White (1970) reasoned that pink gum colonizes soils that are not prone to waterlogging and yet have the capacity to store sufficient water to enable survival over the summer. White (1970) also stated that there are periodic outbreaks of pink gum lerp in the South East, and less frequently in the Mt Lofty Ranges. White's (1970) study did not involve an assessment of pink gum canopy condition.

Despite the conservation and economic values of pink gums and studies quantifying their poor canopy condition, I have located only three published studies that have researched aspects of pink gum's ecophysiology. Sinclair (1980) recorded water potential and stomatal conductance of pink gums at Belair National Park, in the Mt Lofty Ranges, and compared these parameters with co-occurring species during a summer drought in 1977/78. Sinclair recorded higher (less negative) water potentials in pink gums compared to nearby stringybarks (*Eucalyptus obliqua*). He concluded that pink gums were most likely to be suffering less water stress than stringybarks because pink gums had a deeper, more extensive and/or more effective root system. Whittington and Sinclair (1988) studied the water relations between mistletoe (*Amyema miquelii*) and its pink gum host occurring in a pink gum woodland near McLaren Vale (in the Mt Lofty Ranges), during summer to winter in 1985. Amongst other findings, they concluded that pink gums partially closed stomata at low water potentials and thus coped with summer heat and water deficits without showing physiological stress. In the most recent study of pink gum physiology, Jianmin and Sinclair (1993) found water potentials were high in both burnt and adjoining unburnt pink gums during summer. They concluded that pink gum was a deep-rooted species that could access adequate water in this location, even in summer. The limited number of physiological studies suggest that pink gums are adapted to cope with periods of water stress by being deep rooted and by stomatal closure. These findings however, conflict with White's (1970) observations that pink gums in the South East region of South Australia are shallow rooted, which can perhaps be partially explained by the authors studying pink gums that occurred in different soil textures and soil depths. The conflicting findings indicate the need for further study on pink gum rooting depth and water-stress coping mechanisms.

Aims and methodology of this thesis

The above summary of pink gum studies suggests that there are potentially multiple causes of pink gum canopy decline and/or dieback, including mistletoe infestation, insect attack (including lerps), altered groundwater supplies and possibly nutritional deficiencies. The Mt Lofty Ranges and adjoining plains was chosen as the study region because pink gums occupy wide rainfall, depth to water table, soil and elevation gradients within this region, and hence are likely to experience a range of environmental stresses.

This thesis attempts to answer several questions in relation to pink gum canopy condition in the Mt Lofty Ranges:

1. Are there spatial patterns in pink gum canopy health, and are these related to biotic and/or abiotic factors?
2. What habitat variables correlate with short-term changes in pink gum canopy intactness (dieback) and with long-term changes in canopy intactness (decline)?
3. What is the long-term prognosis for pink gum canopy health?

The studies were driven largely by the following hypotheses.

Hypothesis 1: Spatial patterns in pink gum canopy intactness are associated with biotic or abiotic factors including mistletoe prevalence, lerp infestation, rainfall and soil texture.

Hypothesis 2: Short-term changes in pink gum canopy intactness (dieback) are correlated with increased levels of insect activity.

Hypothesis 3: Pink gums with high mistletoe loads are predisposed to canopy decline during prolonged periods of below-average rainfall.

Hypothesis 4: Long-term changes in pink gum canopy intactness are due to declining water availability and/or prolonged mistletoe infestation.

Hypothesis 5: Experiments manipulating water supply and/or mistletoe loads will help determine underlying causes of pink gum decline.

This thesis included studies that determined correlations between pink gum canopy intactness and habitat variables, and also experimental studies in which the causes of low levels of canopy intactness were investigated. The research spanned several disciplines including investigations of ecohydrology in attempting to determine groundwater use by pink gums; physiological measurements of pink gum leaf water potential; and ecological recording of pink gum tree parameters and habitat variables. Changes in pink gum canopy intactness, leaf production and/or leaf damage were recorded at both the scale of individual leaves and whole of canopy assessments. The multi-pronged approach was aimed at determining what canopy changes in pink gums are short- term from which pink gums can recover, and what are permanent changes.

In order to document the range of canopy intactness levels within and between pink gum populations, and the range of habitat variables in which pink gum naturally occur, Chapter 1 involved a survey of pink gum populations that were geographically spread throughout the study region. Correlations between pink gum canopy intactness and habitat variables were determined, and interactions between variables were examined with general linear regression models.

Chapter 2 aimed to determine what variables contributed to short-term and reversible changes in pink gum canopies (dieback). I recorded pink gum canopy intactness, mistletoe presence and leaf damage at 58 sites in 2009/10 and then again in 2011/12, and at nine study sites these variables were recorded every two to four months, for two years. Pink gum canopy intactness was correlated with environmental parameters. In a separate study, observations of lerp signs and canopy intactness of pink gums during the course of a lerp insect outbreak were used to test the hypothesis that the impact of lerps on pink gums was localised and short term (Chapter 5).

Chapter 3 tested the hypothesis that pink gums with high mistletoe loads were predisposed to canopy decline during periods of below-average rainfall. Baseline monitoring in 2003 and 2005 of over 200 pink gums in several reserves in the Mt Lofty Ranges by Ward (2005) provided a rare opportunity to document changes in pink gum canopies and their mistletoe load over 12 years, which included prolonged periods of below-average rainfall.

Chapter 4 explored the hypothesis that long-term changes in pink gum canopy intactness at two woodlands were due to declining rainfall, increased mistletoe infestation and altered groundwater. This was done by examining historical aerial photography, a literature review and analysis of rainfall records.

Having established correlations between pink gum canopy intactness and environmental factors at different time scales in chapters 1 to 5, chapters 6, 7 and 8 used experiments to determine causes of low canopy intactness. Chapter 6 documents a mistletoe removal experiment in a low rainfall pink gum woodland to test the hypothesis that mistletoe is the primary cause of low canopy intactness and not low and declining rainfall. Chapter 7 hypothesised that the growth rate of juveniles recorded over a six year period would significantly increase following supplementary watering and above-average rainfall, and that there would be differences in growth rates between individuals due to small scale environmental factors. Chapter 8 tested the hypotheses that pink gum juveniles have lower growth parameters when water is limited, and that pink gum provenances differ in biomass parameters and water use strategies, in a glasshouse experiment. To explore the hypothesis that pink gums are at least partially groundwater dependent, Chapter 9 correlates pink gum size and canopy intactness with depth to groundwater.

Although pink gums are largely confined to a small area of South Australia, the aims of the research apply more widely to tree decline elsewhere in Australia, Factors affecting pink gum canopies such as insect damage, long-term declining rainfall, mistletoe infestation, inter-population genetic variance and groundwater dependence are common to *Eucalyptus* species throughout Australia. Distinguishing factors associated with short-term dieback from factors associated with long-term canopy decline will help focus research and conservation activities on underlying causes of canopy decline.

Chapter 1 Pink gum canopy condition and population structure in the Mt Lofty Ranges and adjoining plains

1.1 Abstract

This study documented pink gum (*Eucalyptus fasciculosa*) population structure, canopy condition, and habitat variables associated with low levels of canopy intactness, in the Mt Lofty Ranges and adjoining plains. Between 2009 and 2011, 2814 pink gums were surveyed from 78 survey sites. The mean canopy intactness of these pink gums was 70% (SE ± 0.6) and at 33% of sites the mean canopy intactness was > 80%. Canopy intactness was significantly higher for pink gums > 8 m tall. For each level of canopy intactness, there was a wide geographic range of pink gums, and wide variation in annual rainfall totals, soil types, mistletoe (*Amyema miquelii*) frequencies, tree densities and levels of insect damage. The only variables to record a significant correlation with pink gum canopy intactness were: the percentage of trees with mistletoe at a site, the mean number of mistletoes per pink gum, the percentage of leaves with lerp (*Cardiaspina densitexta*) damage, and the number of pink gums per hectare. A Generalised Linear Model also revealed there was a significant interaction between rainfall and the percentage of trees with mistletoe on canopy intactness. At the 10% level, there was a significant interaction between mean annual rainfall, soil group and mistletoe prevalence. Mistletoes, either live or dead, was present at 65% of sites, including 11% of sites where only dead mistletoes were present. In 2011, lerps were present at six sites, all within an area of 50 km^2 and believed to represent an outbreak uncommon for the region. Almost 90% of the 2814 pink gums had a diameter at breast height (DBH) of < 30 cm with the highest frequency (22%) being within 5–10 cm DBH. At 28% of sites there were no pink gums with a DBH of < 5 cm. These were all sites with a mean annual rainfall of < 650 mm. Mean annual rainfall at the survey sites ranged from 359 mm to 940 mm per annum. At the higher rainfall limits of pink gum occurrence it appears that regeneration is more or less continuous, and occurs mainly from lignotubers or root suckering, while at the lower rainfall locations regeneration is episodic, possibly occurring after release of grazing pressure and/or exceptionally high annual rainfall, and is from seeds.

1.2 Introduction

Decline of *Eucalyptus* species is a major issue in Australia, and particularly species of temperate woodlands (Reid and Landsberg 1999). The major causes of *Eucalyptus* decline include one or more of the following, often interacting, factors: insect population outbreaks (Stone 1996), over-abundance of mistletoe (Reid *et al*. 1994), changes in soil properties (Czerniakowki *et al*. 2006), altered fire regimes (Jurskis 2005, Close *et al.* 2009), climate change (Evans *et al*. 2013) and altered hydrological regimes (Cunningham *et al*. 2010). Absence of fire has also led to an increase in fire-sensitive species such as native pines (*Callitris* species) and drooping sheoak (*Allocasuarina verticillata*) which have been associated with the decline of co-occurring *Eucalyptus* species (Lunt 1988). In South Australia's temperate woodlands altered hydrological regimes, salinization and weather-induced stress are major causes of dieback, and insect attack is a primary feature of trees showing signs of ill-health (Paton *et al*.1999). In particular, Paton *et al.* (1999) highlighted pink gums (*Eucalyptus fasciculosa*) and red gums (*Eucalyptus camaldulensis*) as being species of concern in South Australia. The introduction to this thesis outlines more recent assessments of the health of *Eucalyptus* species in the South East region of South Australia (Paton *et al.* 2005) and in the Mt Lofty Ranges (Ward 2005) in which the poor canopy health of pink gums is again highlighted. Both studies recommended that there was an urgent need to research the causes of pink gum canopy decline. More specifically, Ward (2005) recommended future research include a study of groundwater dependency by pink gums and mistletoe removal experiments.

The natural distribution of pink gums is largely confined to South Australia (Figure 1.1). Due to the wide range of habitats occupied by pink gums in the Mt Lofty Ranges and adjoining plains, and Ward's (2005) findings of low levels of canopy intactness of pink gums, this region was chosen for the present study. Records of pink gum for the study region are shown in Figure 1.2. Within the study region and/or the South East region of South Australia, the most commonly attributed causes or symptoms of dieback and decline in pink gum are mistletoe infection (Ward 2005), psyllid ("lerp") attack (Paton *et al.* 2005; White 1970), climatic extremes (White 1969; White 1970), altered groundwater levels (Ward 2005), nutritional deficiencies implicated with "Mundulla Yellows" (Paton *et al.* 2005) and secondary salinity (Camp 2004). As a genus, *Eucalyptus* species are intolerant of competition and particularly those species which have an open crown (Florence 2000), as have pink gums

(Boomsma and Lewis 1980). However, there have been no studies on the tolerance of pink gum to competition.

This study aimed to document the canopy intactness and population structure of pink gums within the Mt Lofty Ranges and adjoining plains. A second aim was to record environmental parameters including rainfall, soil texture, depth to water table, pathogens and insect presence at pink gum populations, and to determine what parameters were associated with low levels of pink gum canopy intactness. I hypothesised that there would be correlations between pink gum canopy intactness and: lerp presence, mistletoe abundance, soil texture, rainfall, and salinity of the shallowest groundwater layer. On the assumption that the conservation status of pink gums reflected a declining regional population, I hypothesised that the population age structure (as measured by trunk diameter) would be skewed towards larger trees. This information will inform future research into pink gum decline, and also direct management of pink gum to where it is most needed, for example it will guide where to revegetate in areas where pink gum canopy condition is poor and/or there is population decline.

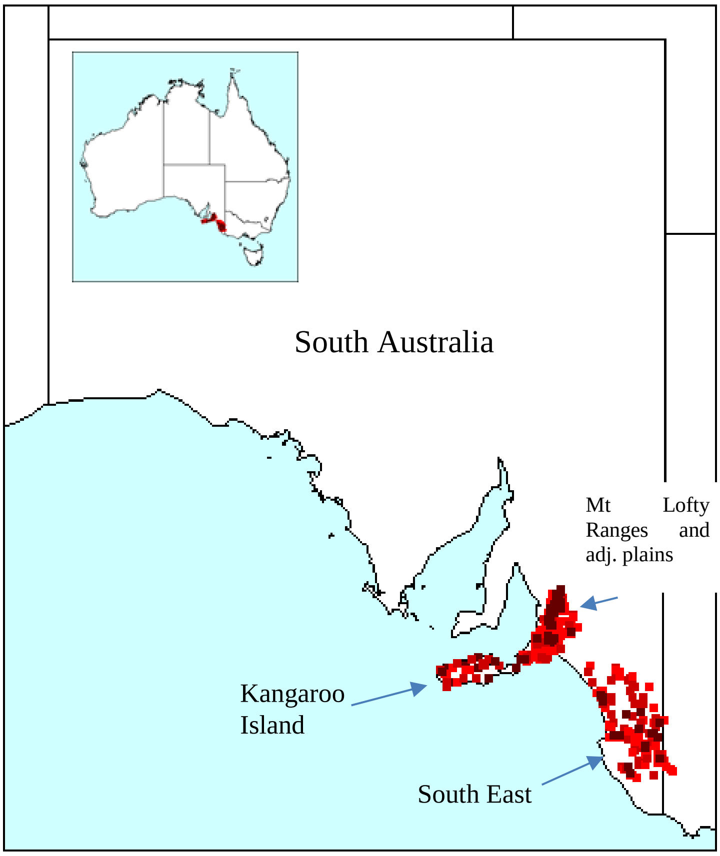

Figure 1.1 Location of specimen records for pink gum (*Eucalyptus* fasciculosa) held by the State Herbarium of South Australia (2015). Locations are on Kangaroo Island, the Mt Lofty Ranges and the South East, with a small incursion into Victoria.

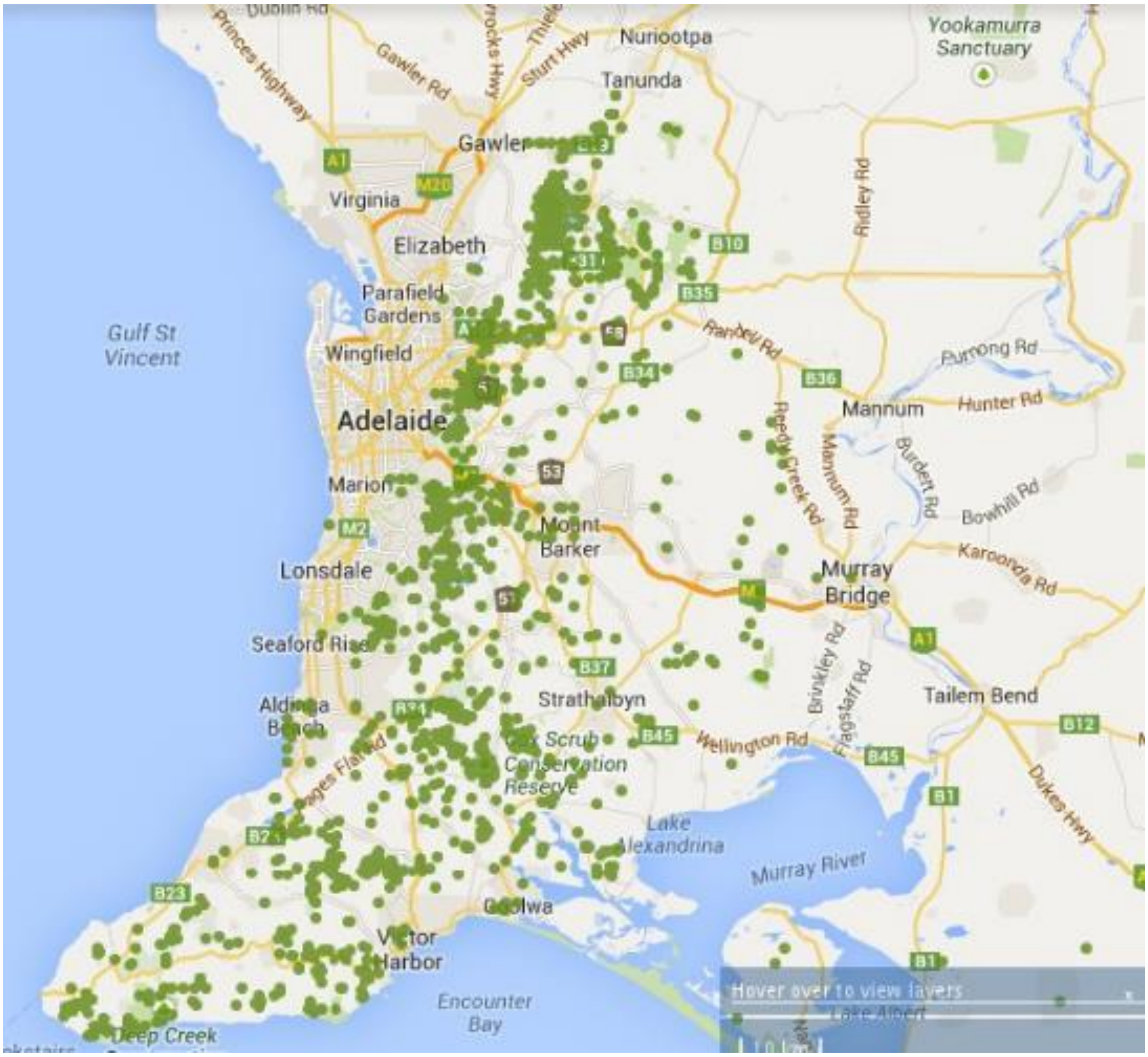

Figure 1.2 Records of pink gum within the Mt Lofty Ranges and adjoining plains, held in various databases collated by the Atlas of Living Australia (2016a)

1.3 Methods

Study region, site selection and trees surveyed

The study region is the Mt Lofty Ranges and adjoining plains (Figure 1.1). The northern boundary is the northern limit of pink gum's natural occurrence. The south-eastern boundary coincides with a natural disjunction in pink gum occurrence between the plains east of the Mt Lofty Ranges and the regional locality known as the upper South-East (White 1970b). Pink gums were surveyed at 78 locations or "survey sites" within 41 woodlands (Appendix A and Figure 1.3). Nine sites were surveyed in spring/summer 2009/10 and 69 sites were surveyed in spring/summer 2011/12. Appendix A includes the survey period for each site. As outlined in the Introduction to this thesis, causes of tree decline in paddock trees are often different to factors affecting tree health in woodlands. This study's aim was to determine factors associated with low levels of canopy intactness in pink gum woodlands. Site selection criteria were:

- Pink gums were an overstorey tree and comprised at least 50% of the total number of all trees present in the overstorey.
- There was no evidence that pink gum density had been reduced by more than 20% due to mechanical clearance and/or long-term domestic stock grazing.
- Sites had not been burnt for at least five years prior to the survey.
- Sites had not been grazed by domestic stock for at least five years prior to survey.
- Within each site, slope and aspect were relatively constant.

The 78 sites were chosen as follows:

- Twenty-one sites were those randomly chosen by Ward (2005) to study mistletoe dispersal and/or attributes of pink gums that attracted mistletoe birds. These sites were all within *National Parks and Wildlife Act 1972* reserves.
- Thirty-four sites were randomly chosen from Biological Survey of South Australia sites (DEWNR 2009) where pink gum had been recorded as an overstorey dominant or co-dominant.
- Twenty-three sites were targeted to gain a greater spread of soil types and annual rainfall totals.

In total, 50 sites were on Crown Land, three sites on land held by non-government organisations and 25 sites were on private property.

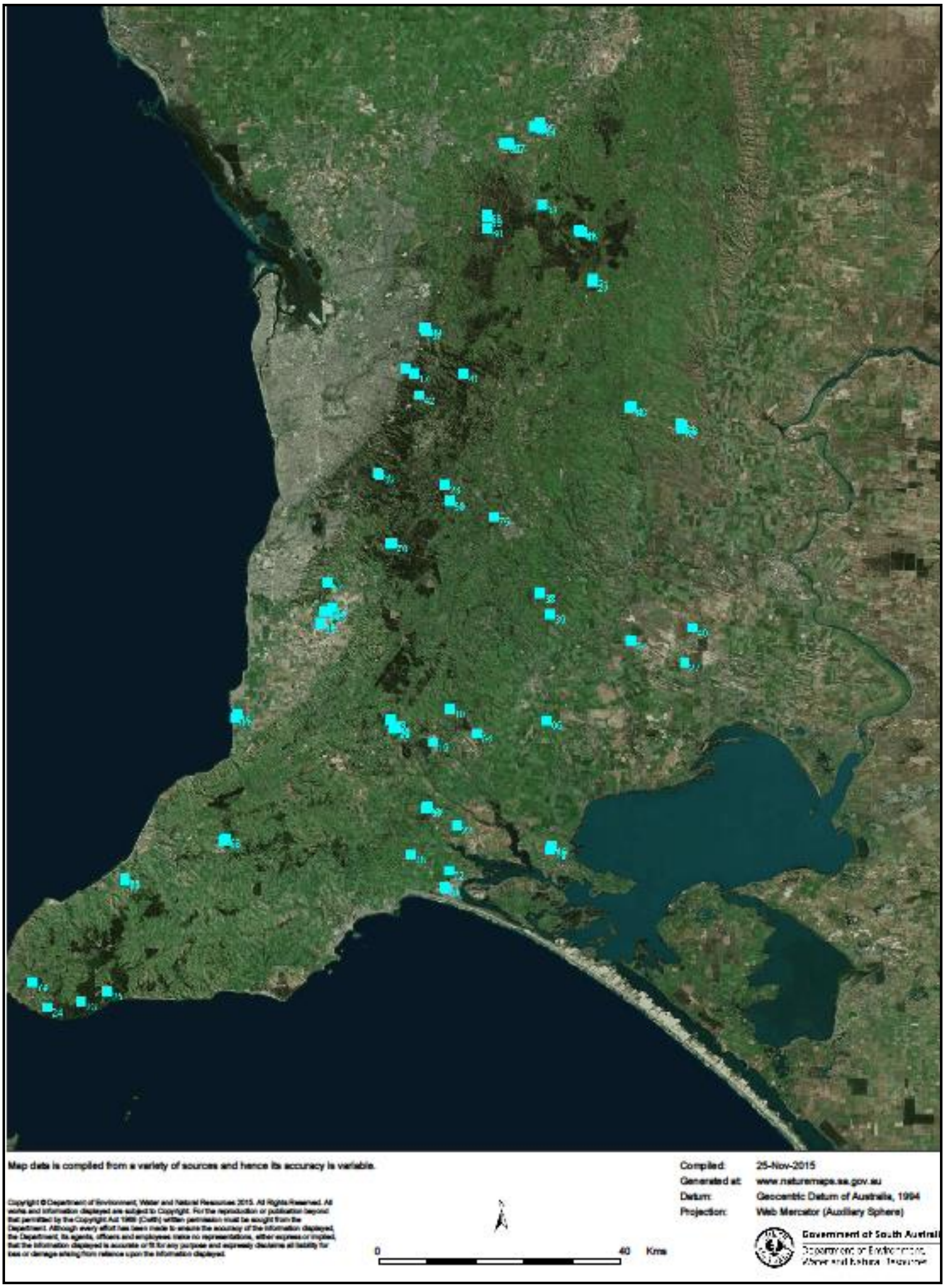

Figure 1.3: Location of regional study sites. Figure compiled using NatureMaps (2016)

Number of trees surveyed and size of survey sites

At each survey site, the first pink gum recorded was that closest to the randomly determined location coordinate. All individuals of tree species that were in the overstorey layer, that were nearest to the first pink gum, were surveyed regardless of their height. All overstorey species were recorded to see if there was an association between total tree density and pink gum canopy condition. The number of trees surveyed at each survey site, and hence the survey site dimension, was determined by the time available with at least 30 trees being recorded at 80% of sites. Sites ranged from 140 m^2 to 6400 m^2. In total, I surveyed 2814 pink gums and 643 non-pink gums from the 78 sites.

Abiotic parameters recorded at study sites

Appendix A summarises abiotic habitat parameters recorded at each survey site. Soil group was obtained from existing soil mapping (DEWNR 2007b). This mapping determined 15 broad soil groups within South Australia (DEWNR 2007b).

The distance to groundwater and salinity (total dissolved solids) of the shallowest aquifer were obtained from Data SA (2015a and Data SA (2015b).These datasets were created by extrapolating from existing drill hole data and hence may not reflect fine-scale variation in depth to groundwater or salinity.

Rainfall and elevation data were obtained from raster layers downloaded from GeoScience Australia (2009).

The number of years since fire for each site was recorded from NatureMaps (2016).

Tree morphology and canopy condition parameters

Within each survey site, the location of each surveyed tree was recorded using a GPS, combined with noting the distance and direction from specified trees. The following parameters were recorded for all surveyed trees, regardless of their height.

Canopy intactness was a visual assessment of the percentage of living foliage compared to the estimated potential amount that would be present in a fully extended canopy (Cunningham *et al.* 2007). This definition, therefore, included short-term foliage loss such as that due to insect attack. It also allowed for dead branches to be present but canopy intactness to be recorded as 100% if regrowth foliage was considered to have compensated for past loss. It was also possible to record 0% canopy intactness and yet the tree to still be alive, for example, where there had been 100% defoliation and/or death of leaves due to insect and/or other biotic agents.

Diameter at breast height (DBH) was recorded for all trunks at 1.3 m above ground, on the upslope side of the tree. Where trees were multi-stemmed, an “effective” tree DBH was calculated as the square root of the sum of all squared stem DBHs (Hoover 2008):

$$\surd \sum_{1}^{n}(stem\ diameter^{2}) \qquad \text{Equation 1-1}$$

Tree height was estimated for the highest point of the tree (including dead branches). I recorded tree height by extrapolation of a known height (a 2 m pole) (NVMU 2015). The precision of repeat measurements decreased with increases in tree height beyond 5 m tall.

Dead trees were recorded and included in the analysis where at least 50% of the canopy branching remained. Epicormic growth was estimated visually as the percentage of live foliage that contained epicormic shoots. Shoots were considered epicormic if they had initiated from buds in the bark of mature stems and were < 3 cm in diameter (Cunningham *et al*. 2007).

Leaf damage was assessed for 1795 live pink gums from 68 survey sites between September 2011 and January 2012. At these randomly chosen sites, at least the first 50% of trees surveyed were scored for leaf damage. I observed the entire canopy for up to one minute, with the aid of binoculars where necessary, and estimated the percentage of the entire canopy leaves that contained leaf damage in the following categories: lerp presence, dead leaves, leaf yellowing (interveinal chlorosis), fungal symptoms (leaf speckle and spotting), skeletoniser damage and chewed leaves. For fungal symptoms, skeletoniser and chewed leaves, leaves were scored if approximately greater than 1% of leaves had more than 10% of the leaf area with symptoms. Where present, the following symptoms of leaf or stem damage were also noted: galls, gum leaf scale (*Eriococcus* spp.), leaf blister and powdery mildew. The percentage of leaves per canopy with signs of salinity damage (dead marginal patches) was recorded. Leaves of salt-affected plants have dead patches, particularly at the margins and tips (DEH 2005).

I recorded the percentage canopy intactness of each live mistletoe, the number of dead mistletoes (*Amyema miquelii*) still present on the host tree and, where obvious, the number of former mistletoe attachments. I also estimated the percentage of the combined pink gum and mistletoe foliage biomass that was comprised of mistletoe.

Pink gum population structure

In the absence of a reliable and/or practical measure for accurately ageing pink gums, the diameter at breast height (DBH) was used as a surrogate measure of relative pink gum age. Based on the largest live or dead DBH recorded per tree (for both single and multi-trunked trees), pink gums were arbitrarily divided into DBH classes, beginning at 0 cm DBH for trees < 1.3 m tall; and for trees ≥ 1.3 m tall, 0.1–2 cm, 2.1–5 cm, then 5 cm increments up to 20 cm DBH, and then 10 cm DBH increments up to 120 cm. The number of pink gums, and the total number of trees per hectare was calculated by measuring the survey site dimensions in the field using a 50 m tape, and then extrapolating the number of pink gums and the number of trees in the known survey area to a hectare. Reineke's Stand Density Index (SDI) adjusts the trees per hectare by incorporating the mean effective DBH for a site, and was calculated following Shaw (2006):

$$\text{SDI per survey site} = \text{number of pink gums per ha x } (\text{mean effective DBH}/25)^{1.605} \quad \text{Equation 1-2}$$

Aldinga woodland case study

Aldinga Scrub Conservation Park (hereafter, Aldinga) is a 266 ha woodland remnant on the coastal plain south of Adelaide, with pink gums the dominant overstorey tree. Within Aldinga, pink gum heights, canopy intactness and mistletoe loads vary widely. To determine if there were spatial patterns in these attributes, in September 2011 I surveyed pink gums in a grid pattern across the park. I surveyed pink gums along east-west aligned grids that were 200 m apart. Along each grid line, at 100 m intervals, I recorded pink gum height, canopy intactness and mistletoe load from the nearest five or six pink gums. A total of 606 pink gums were surveyed.

Statistical analysis

All statistics were performed using SPSS software version 23. Associations between continuous variables and canopy intactness (site means) were determined using Pearson's Correlation (variables were normally distributed and linearly related). Associations between the categorical variables, soil group and time since fire, were determined using a General Linear Model. Combinations of the variables that showed significant correlations ($p < 0.1$) with canopy intactness were used in construction of a general linear model (SPSS software version 23). The setting of a significance level of 10% rather than 5% ensured that key explanatory variables were not overlooked in recording possible interactions. These variables were percent of trees with mistletoe ($N = 78$, $R = -0.38$, $p = 0.005$), mean number of mistletoes per pink gum ($N = 78$, $R = -0.301$, $p = 0.007$), pink gum stand density index ($N = 78$, $R = 0.28$, $p = 0.014$), percent of leaves per tree with lerp ($N = 78$, $R = 0.59$, $p = < 0.001$). Assumptions regarding normal distribution of residuals were validated. In addition, interactions between mean annual rainfall, soil group and depth to groundwater were explored, because of the perceived importance of depth to groundwater in influencing canopy condition. Because of the high degree of collinearity between the mistletoe explanatory variables, these variables were not explored in the same model.

One-way ANOVA was used to determine if there were differences in: size of pink gums in different rainfall zones; canopy intactness between pink gums in each of the eight soil groups; and canopy intactness of pink gums at Aldinga with different mistletoe loads. Independent t- tests (two-tailed) were used to determine if the canopy intactness of pink gums over 8 m tall was different to pink gums ≤ 8 m tall, and to see if there were differences in canopy intactness between pink gums hosting mistletoe and pink gums not hosting mistletoe.

1.4 Results

Habitat

Eight soil groups were represented in the 78 survey sites (Table 1.1). Almost 40% of sites were on shallow acidic soils on rock (group K) and 25% were on sand over clay (group G).

Table 1.1: Major soil groups of this study's 78 survey sites (based on DEWNR 2007b).

Soil Group	Soil Description	Number of survey sites
B	Shallow soils on calcrete or limestone	6
D	Hard red-brown texture contrast soils with alkaline subsoil	2
F	Deep loamy texture contrast soils with brown or dark subsoil	3
G	Sand over clay	21
H	Deep Sand	4
J	Ironstone soils	4
K	Shallow to moderately deep acidic soil on rock	30
L	Shallow soils on rock	8

Study sites ranged from 5 m to 507 m above sea level (ASL). Mean annual rainfall ranged from 359 mm to 940 mm. Within the low (< 470 mm), medium (470-650 mm) and high (> 650 mm) annual rainfall categories, there were 14, 35 and 29 sites, respectively. The sites also spanned a wide range of slopes, ranging from flat land up to slopes of 32^0, although 84% of sites occurred on slopes of 10^0 or less.

Population structure and tree size

Ninety percent of the 2814 pink gums surveyed had a maximum individual stem DBH of < 30 cm, with the highest frequency of pink gums being 5–10 cm DBH (Figure 1.4). The largest DBH recorded was 120 cm. At 19% of sites, there were no pink gums present with a DBH > 20 cm. For low rainfall sites, however, the most frequent DBH class was 20–30 cm DBH (Figure 1.5). Conversely, pink gums with a DBH of < 5 cm were absent from 28% of sites. These were all sites with either low or medium rainfall (< 650 mm mean rainfall per annum). There were significant correlations between the proportion of trees at a site with DBH < 5 cm and both rainfall ($R = 0.53$, $N = 78$, $p < 0.01$) and sites that had been burnt in the previous 35 years ($R = -0.49$, $N = 78$, $p < 0.01$). Namely, sites with the highest proportion of small pink gums tended to be high rainfall sites and/or sites that had been recently burnt. In turn, there was a strong correlation between mean annual rainfall and whether the site had been burnt within the previous 35 years ($R = -0.69$, $N = 78$, $p < 0.01$).

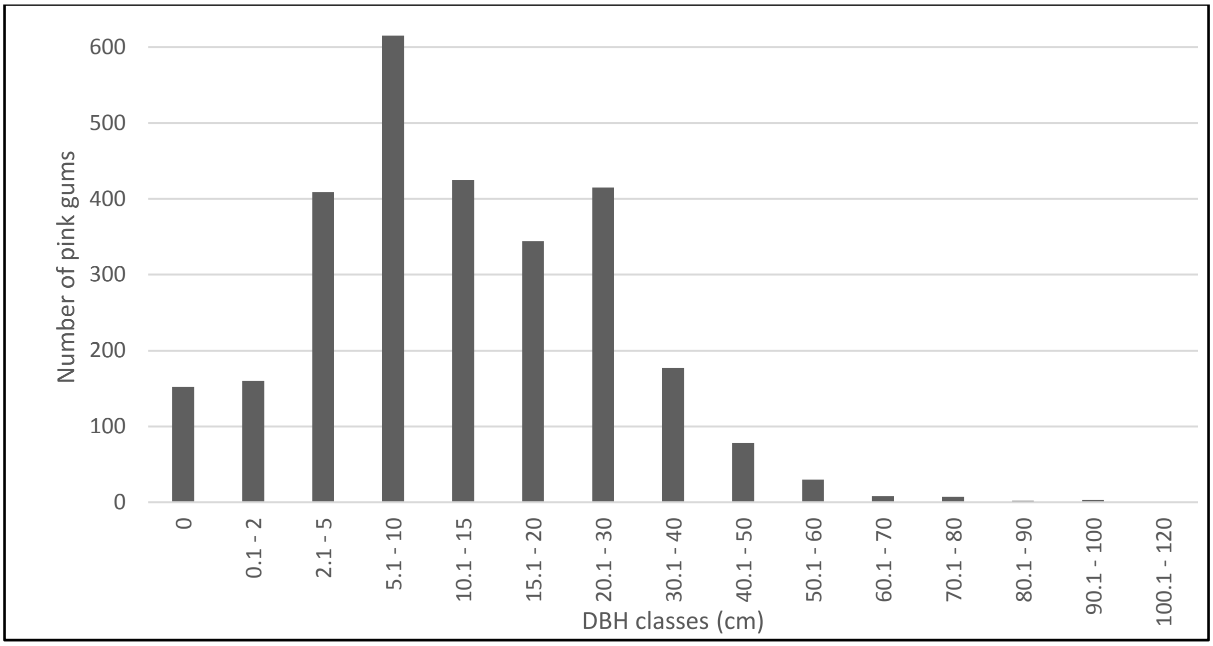

Figure 1.4: Frequency of pink gums within DBH classes. 2814 pink gums were surveyed from 78 regional survey sites

The distribution of DBH classes for sites from each of high, medium and low rainfall locations is shown in Figure 1.5. The mean maximum DBH for high rainfall sites was 9 cm (SE ± 0.2) which was significantly lower than the mean maximum DBH for low and medium rainfall sites of 18 cm (SE ± 0.5) and 18 cm (SE ± 0.4), respectively (ANOVA F = 176.8, $p < 0.001$). At high rainfall sites 71% of pink gums had a maximum stem DBH of < 10 cm, compared with 28% and 34%, respectively for low and medium rainfall sites. In summary, the low rainfall sites were dominated by larger pink gums, with relatively few small trees whereas high rainfall sites were dominated by smaller pink gums with relatively few large trees. There was a highly significant correlation between maximum DBH per tree and pink gum height (N = 2814, R = 0.80, $p < 0.001$). Figure 1.6 shows the distribution of pink gum height classes at survey sites.

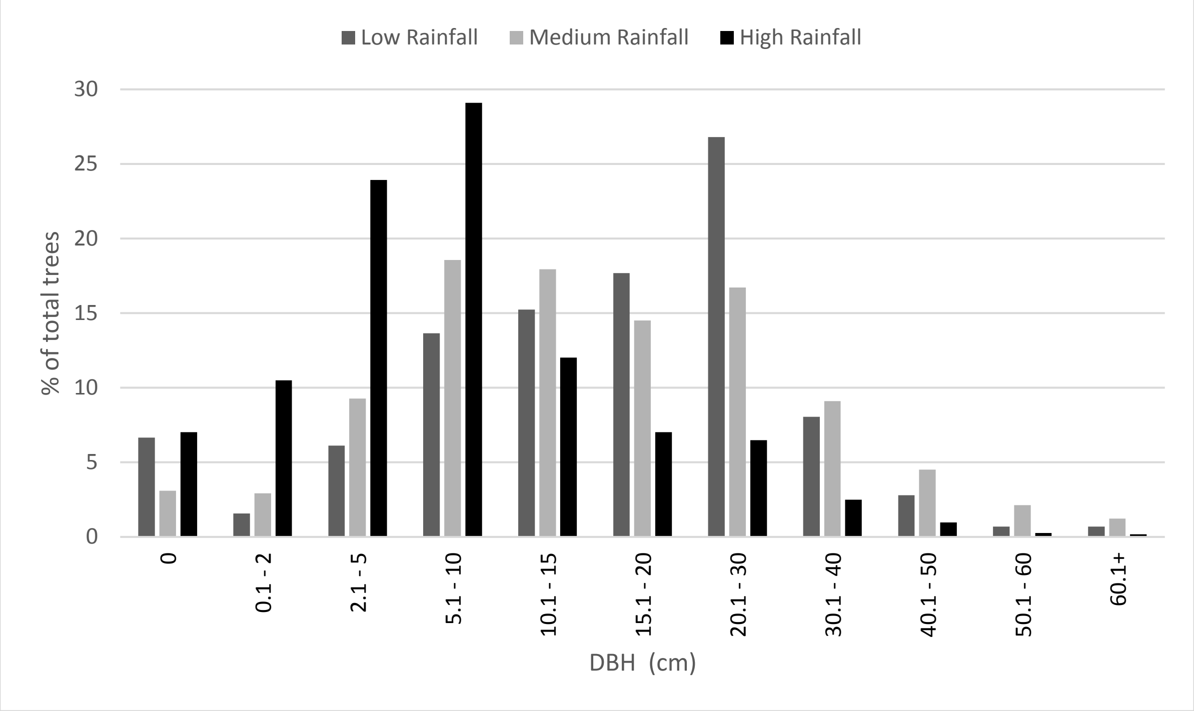

Figure 1.5: Percentage of pink gums within DBH size ranges (cm) for survey sites with high (> 650 mm), medium (470 – 650 mm) and low (< 470 mm) mean annual rainfall. The 0 cm DBH class represents pink gums < 1.3 m tall.

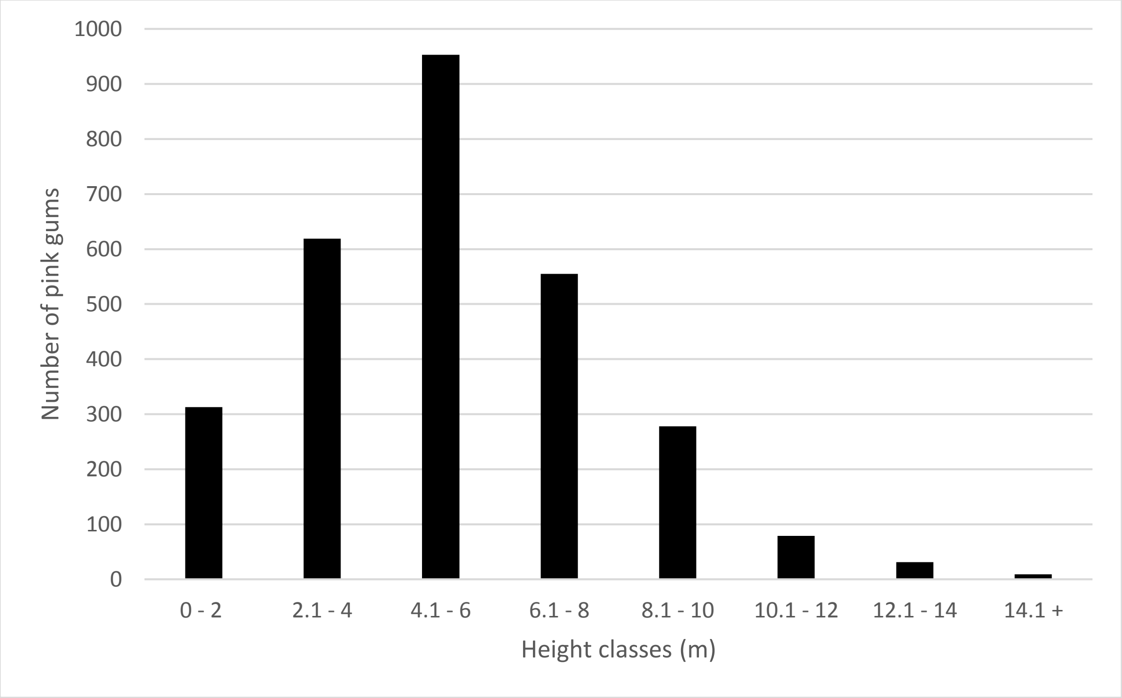

Figure 1.6: Frequency of pink gums within height classes. 2814 pink gums were surveyed from 78 regional survey sites.

Regeneration

No pink gums with juvenile leaves (leaves opposite rather than alternate) and < 10 cm tall were observed at any survey site. Opportunistically, however, I observed three seedlings at Aldinga Scrub in the ash bed in 2012, approximately one year post fire. At only three sites were the juvenile trees (< 1.3 m high) well away from the canopies of adult trees. These seedlings were likely to have originated from seed, while those under the adult canopies were generally within a metre of the trunk and likely to have originated from lignotubers (refer Table 1.2 and Appendix A for site details). Clayton (site 13) was a low rainfall site where pink gum recruitment last occurred in 1992 (Dr Andrew Black landholder, pers. comm. 2009) when the yearly rainfall total was nearly 1.8 times the long-term mean annual rainfall (Bureau of Meteorology 2015). Recruitment at Highland Valley (site 40) occurred in 2005 after the release of heavy grazing, and coincided with an above-average rainfall year (Dr Tim Milne, landholder pers. comm. 2009), while recruitment at Mt Beevor (site 45) followed a wildfire (Peter Atkinson landholder pers. comm. 2009).

Table 1.2 Mode of regeneration at all pink gum survey sites where more than 10% of pink gums were < 1.3 m tall.

Site name	Site #	% pink gums < 1.3 m	Mean annual rainfall (mm)	Year of last known fire(s)*	Mode of regeneration thought most likely**
Belair	11	18	940	1978	Lignotuber
Clayton	13	80	404	No known fire	Seed
Finniss	29	13	768	1983	Lignotuber
Finniss	30	20	768	1983	Lignotuber
Goolwa	32	12	465	1983	Lignotuber
Highland Valley	40	16	537	na	Seed
Mt Beevor	45	33	557	2005	Seed
Mt Crawford	47	12	617	No known fire	Lignotuber
Mt Magnificent	49	24	768	1983	Lignotuber
Totness	77	22	742	1983	Lignotuber

*Fire data obtained from NatureMaps (2016). All fires were summer wildfires

** Seed regeneration was thought most likely where the < 1.3 m tall trees were located well beyond the nearest adult canopy and lignotuber regeneration was thought most likely where the trees were within 1 m of an adult trunk.

Although 90% of the 2814 pink gums had a DBH of < 30 cm, the largest DBH recorded was 120 cm at Belair (site 12) and nine trees had a DBH of at least 80 cm. These nine trees were recorded from six sites (Table 1.3). There was a large variation in rainfall, soil group, slope, elevation, and vegetation community structure between these sites, e.g. the trees with the two largest DBH were from the sites with the highest and lowest mean annual rainfalls (Table 1.3). The tallest pink gums recorded were 18 m, and were from Belair (site 12) and Sandergrove (site 66). These sites had different soil groups and a difference in mean annual rainfall totals of over 430 mm.

Table 1.3: Selected habitat parameters of the sites containing pink gums with the nine largest DBH's recorded from the 78 survey sites. Soil group data obtained from DEWNR (2007b)

Site # and Name	Largest DBH (cm)	Mean annual rainfall (mm)	Elevation (m ASL)	Slope (^{0})	Soil Group	Dominant Overstorey Trees
12 Belair	120 (1 tree) 80 (1 tree)	940	440	1	Shallow soils on rock	Pink gum – Blue Gum*
63 Rockleigh	100	359	229	0	Sand over clay	Pink gum – Native Pine**
64 Sandergrove	95	518	94	6	Sand over clay	Pink Gum
38 Wistow	90	537	285	9	Shallow to moderately deep acidic soil on rock	Pink gum
75 Totness	85	742	408	8	Shallow to moderately deep acidic soil on rock	Pink gum – Stringybark***
43 Mt Beevor	80 (3 trees)	557	420	5	Deep loamy texture contrast soils with brown or dark subsoil	Pink gum – Blue gum

*Blue Gum = *Eucalyptus leucoxylon* ssp. *leucoxylon*
**Native pine = *Callitris gracilis*
***Stringybark = *Eucalyptus obliqua*

Pink gum stand density

There was a significant correlation between mean annual rainfall and the number of pink gums per hectare (R = 0.60, N = 78, $p < 0.001$). The three sites with ≤ 45 pink gums per hectare all received < 400 mm annual rainfall and had no known fire history (sites 34, 27 and 63 in Appendix A). Conversely, the eight sites with the highest number of pink gums per hectare were all high rainfall. Pink gum density at these sites ranged from 1100 trees per hectare at Cromer (site 20, 729 mm rainfall) to 3040 trees per hectare at Deep Creek (site 23, 794 mm rainfall). The 30 sites with the lowest tree densities all had no recorded fire histories. There was a significant correlation between pink gum canopy intactness and trees per hectare (N = 78, R = 0.24, p = 0.03). There was a similar correlation between pink gum intactness and Reinecke's SDI (N = 78, R = 0.27, p = 0.01).

Regional pink gum canopy intactness

The mean canopy intactness for the 2814 pink gums surveyed between 2009 and 2011 was 70% (SE ± 0.6). Mean canopy intactness was at least 80% at one third of sites, and at 80% of sites it was at least 60%. The mean canopy intactness for all pink gums with a maximum individual stem DBH of ≤ 5 cm and with a DBH > 5 cm was 70% for both groups. Pink gums over 8 m tall had a mean canopy intactness of 76% which was significantly greater than the 69% for pink gums less than 8 m tall (independent t-test 2-tailed N = 2814, t = -6.32, $p < 0.001$). Within each category of canopy intactness (including ≥ 90%), there was a wide geographic range (Figure 1.7), and a wide range of annual rainfall totals, soil groups, mistletoe frequencies, tree densities and levels of insect damage (Table 1.4).

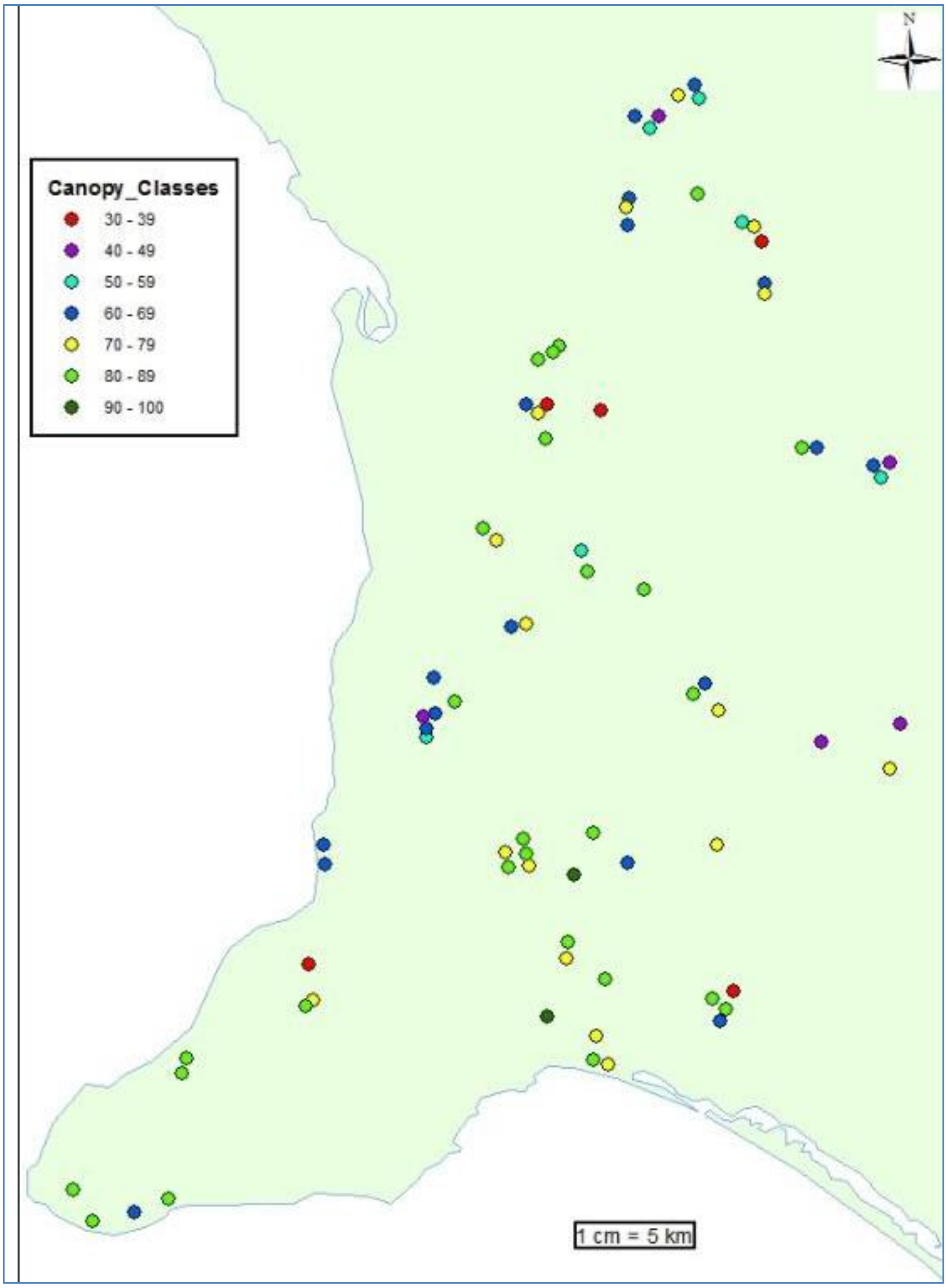

Figure 1.7: Mean pink gum canopy intactness classes for 78 survey sites in the study region, recorded during 2009 - 2011.

Table 1.4: Number of survey sites within each pink gum canopy intactness class and associated range within selected habitat parameters recorded 2009–2011. Heading explanations are as follows:

*Low rainfall = < 470 mm; Medium rainfall = 470–650 mm; High rainfall = > 650 mm

**Soil Codes: H = Deep sand; G = Sand over clay; D = Hard red-brown texture contrast soils with alkaline subsoil; B = Shallow soils on calcrete or limestone; F = Deep loamy texture contrast soils with brown or dark subsoil; J = Ironstone soils; L = shallow soils on rock; K = Shallow to moderately deep acidic soils on rock

[#] Percentage of sites with > 5% of trees containing live or dead mistletoe

[##] Percentage of sites where the mean percentage of canopy with obvious insect chewing and or skeletoniser damage is ≥ 10%

Mean % canopy intact-ness	# survey sites	Mean annual rainfall range (mm)	% sites low medium and high rainfall*	Soil codes** (% sites)	All tree Stand Density Index range	Elevation (m)	% sites with mistletoe[#]	% sites with insect damage[##]	% sites with lerp present
20–29	1	827	H (100	K(100)	1420	500	0	100	100
30 – 39	5	404 – 686	L (20) M (40) H (40)	F(20) G (20) K (60)	456 – 2423	7–507	80	20	60
40 – 49	5	359 – 896	L (40) M (40) H (20)	B(20) G (60) K (20)	64 – 1723	80–415	100	50	20
50 – 59	5	368 – 617	L (40) M (60) H (0)	B(20) G (40) K (40)	13 – 735	76–450	80	25	20
60 – 69	17	404 – 829	L (6) M (53) H (41)	F(6) G(29) H(18) J(12) K(35)	276 – 1079	8–462	71	23	0
70 – 79	19	359 – 940	L (26) M (32) H (42)	B(10) F(6) G(32) J(10) K(32) L(10)	38 – 7947	9–459	53	53	0
80 – 89	22	404 – 788	L (9) M (50) H (41)	B(5) D(9) G(9) H(5) K(50) L(22)	289 – 14719	7–389	18	7	0
90 – 100	4	404 – 694	L (25) M (50) H (25)	B(25) G (50) L (25)	731– 1917	5–438	50	25	0

Of all the variables recorded at survey sites, there was a significant correlation ($p < 0.05$) with pink gum canopy intactness for only the following variables: the percentage of trees with mistletoe at a site (N = 78, R = -0.375, p = 0.005), the mean number of mistletoes per pink gum at a site (N = 78, R = -0.3, p = 0.007); the mean percentage of leaves with lerp damage (N = 78, R = -0.59, $p < 0.001$), and the pink gum stand density index (N = 78, R = - 0.29, p = 0.01). There was no significant interaction between soil group and mean annual rainfall, (GLM, F = 0.456, p = -0.77), nor with depth to groundwater and soil group or rainfall. There was a significant interaction at the 10% level, however, between mean annual rainfall, soil group and mistletoe (GLM, F = 2.03 p = -0.10).

Running a GLM with only the variables: percent trees with mistletoe at a site (F = 10.7, p = 0.002) and mean percent leaves with lerps at a site (F = 29.7, $p < 0.001$), the equation was: Site canopy intactness (%) = (-0.167 x % trees with mistletoe) – (0.57 x % leaves with lerps).

Mean canopy intactness for each soil group ranged from 67% (SE ± 3) for sand over clay soils to 84% (SE ± 2) for shallow acidic soils on rock, but there were no significant differences in mean canopy intactness between soil groups (ANOVA F = 0.996, p = 0.442).

Mistletoe and pink gum canopy intactness

The minimum pink gum height from which I recorded the presence of a mistletoe was 1.6 m. Of the 2606 pink gums surveyed that were least 1.6 m tall, box mistletoe (*Amyema miquelii*), either live or dead, was present on 27% of trees and at 50 (64%) of the 78 sites. Live mistletoe was present on 17% of the 2606 trees and dead mistletoe (or remains of mistletoe) was present on 20% of trees. Seven sites had only dead mistletoes present. Of pink gums bearing live or dead mistletoes, the mean number of mistletoes (live and dead combined) was 4.4 (SD ± 4.4, N = 709).

The mistletoe variable with the strongest association to pink gum canopy intactness at a site was the percentage of pink gums ($\geq$ 1.6 m tall) containing mistletoe at a survey site (N = 78, R = -0.38, $p < 0.001$). There was a weaker correlation between the total number of live and/or dead mistletoes per canopy and canopy intactness for individual pink gums $\geq$ 1.6 m tall (R = - 0.21, N = 2606, $p < 0.001$). The association was stronger between the number of dead mistletoes (including former attachments) present and canopy intactness (R = -0.29, N = 2606, p < 0.001). There was almost no association, however, between the number of live mistletoes and canopy intactness (R = -0.06, N = 2606, p =0.001). The significance of the negative correlation between the number of live and dead mistletoes per pink gum canopy and canopy intactness increased as pink gum host height increased, reaching a maximum correlation of R = -0.49 for trees at least 11 m tall (Table 1.5).

Table 1.5: Persons correlation values (r) for number of live and or dead mistletoe per pink gum and pink gum canopy intactness for 2606 pink gums in different height categories (from 78 survey sites). For all values, p was < 0.001)

Pink gum height range (m)	# of pink gums	% pink gums with mistletoe	r value
1.6–3.0	413	6	-0.25
3.1–5	802	24	-0.24
5.1–7	742	36	-0.27
7.1–9	440	36	-0.38
9.1–11	124	27	-0.37
> 11	85	27	-0.49

There was a significant difference between the mean canopy intactness of all pink gums that contained either live or dead mistletoe (namely 56%, SE ± 1.2) and pink gums that contained no mistletoe (74%, SE ± 0.6) (independent t test, t = -13.3, N = 2606, p < 0.001). A Generalised Linear Model also revealed there was a significant interaction between mean annual rainfall and percentage of trees with mistletoe (F = 4.923, p = 0.03), mistletoe being more common at the lower rainfall sites (Figure 1.8).

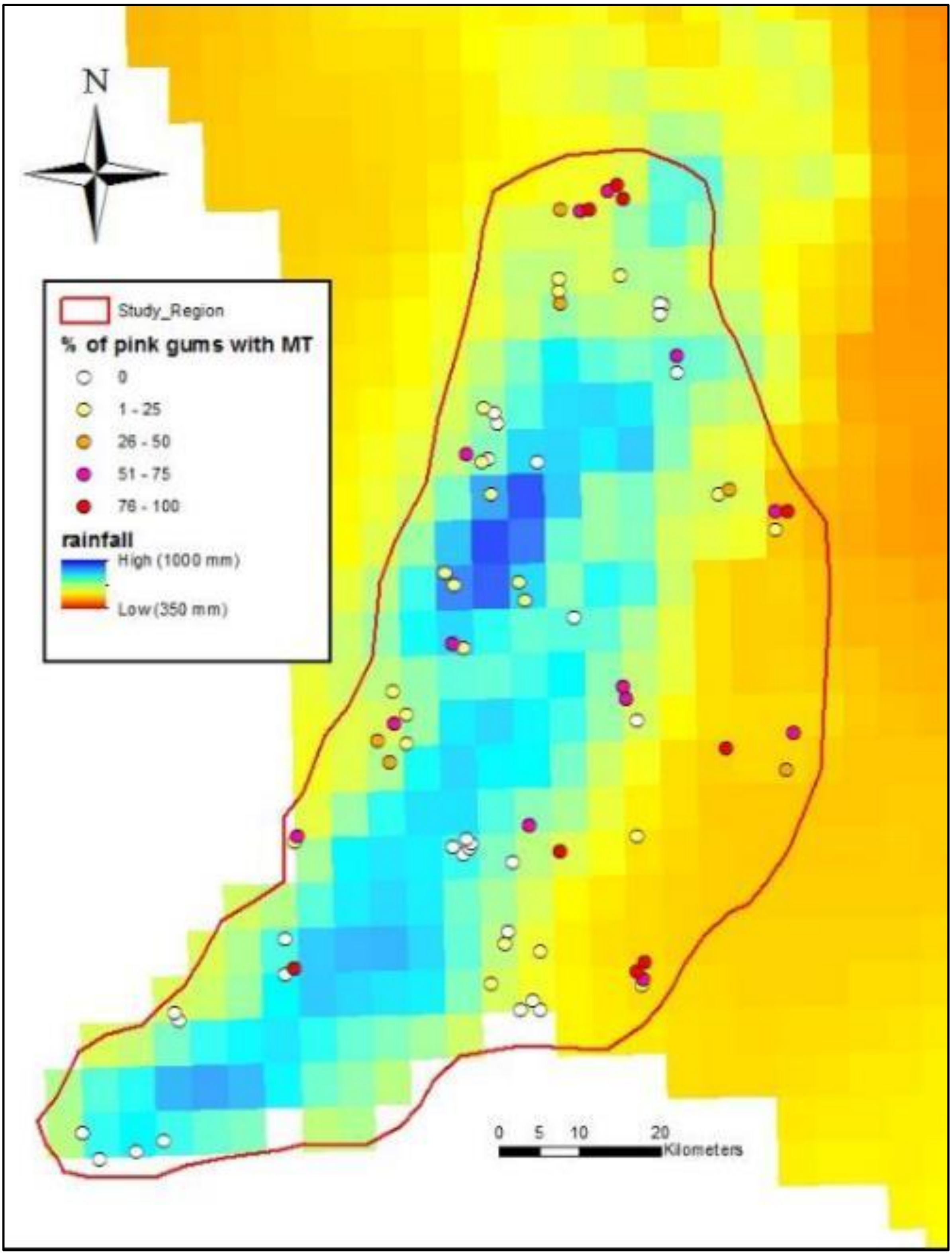

Figure 1.8: Percentage of pink gums carrying live or dead mistletoe per survey site superimposed upon rainfall. Highest and lowest rainfall pixels are approximately 1000 mm and 350 mm, respectively.

Leaf and bark damage

Lerps were present at six of the 78 survey sites, with the mean percentage of canopy leaves affected by lerps ranging from 7–100%. Because this variable showed the highest correlation with pink gum canopy intactness of any of the recorded variables (N = 78, R = -0.58, $p < 0.001$), the impact and extent of lerps is further explored in Chapter 5.

Signs of chlorosis (yellowing) were detected at two sites only, namely at a coastal cliff at Deep Creek and on deep sandy soil north of Middleton. At Deep Creek and Middleton, approximately 30% and 5% of pink gums, respectively had < 5% of leaves affected by chlorosis. Galls and gum tree scale (*Eriococcus* spp.) were recorded on less than 1% of trees, and on these trees, were confined to < 5% of branches. Leaf blisters (resulting from leaf blister sawflies (*Phylacteophagus* spp.) were recorded on trees at only seven sites, and on < 5% of leaves at each site. Powdery mildew was recorded on only five trees at two sites. Insect chewing damage and skeletoniser damage were scored on 50% and 20% of trees surveyed, being scored when more than 1% of leaves on the tree had greater than an estimated 10% of leaf area affected.

Because pink gums have smooth bark, if borers were present, then borer holes would be conspicuous but borer holes were observed on only five dead trunks.

Salinity

Leaves with signs of being salt-affected (dead margins) were recorded at only one site, Aldinga and at only five of the surveyed pink gums at this site. This site was 200 m from the coast, and dead patches on the leaves were most likely due to salt spray rather than soil salinity, as the affected leaves were on the coastal side of the canopy. The extrapolated salinity of the groundwater layer (which is approximately 6 m deep) is 5500 TDS (Data SA 2015b), or about 6 dS/m which is in the moderate range (FAO 1986). There was no correlation between the salinity of the shallowest groundwater layer and canopy intactness (R = -0.03, N = 78).

Aldinga case study

The correlation between mistletoe presence and canopy intactness for the 606 surveyed trees at Aldinga mirrored that of the regional pink gum population, there being a moderately significant correlation between the total number of live and dead mistletoes present and the pink gum host's canopy intactness ($R = -0.41$, $p < 0.01$). The mean canopy intactness of the 606 Aldinga pink gums was 58%. However, the mean canopy intactness of pink gums with no mistletoes (live or dead) was 73%, significantly higher than the mean of 49% for pink gums with mistletoe (Independent t test, $N = 606$, $p < 0.001$. Lower canopy intactness was associated with the presence of any mistletoe load, but mean canopy intactness was not significantly further diminished above the presence of six or more live and/or dead mistletoes (Table 1.6).

Table 1.6: Mean canopy intactness of 606 pink gums at Aldinga for different mistletoe classes. Superscripts of the same letter represent no significant differences in means using one-way ANOVA.

# of live and/or dead mistletoes per host canopy	mean pink gum % canopy intactness ± SE	number of pink gums
0	73 ± 1.5^a	231
1 - 2	60 ± 2.3^b	151
3 - 5	55 ± 3.5^b	88
6 – 10	33 ± 3.4^c	77
11 –20	33 ± 4.0^c	47
> 20	33 ± 7.5^c	12

1.5 Discussion

Pink gum habitat

Accounts of pink gum's natural distribution note the wide range of habitats occupied by this species. Boomsma and Lewis (1980) stated that pink gums occupied a range of habitats "equalled by few other eucalypts native to South Australia". Specht and Perry (1948) documented pink gum's presence on a wide variety of soil types ranging from skeletal acidic soils in the higher rainfall parts of the Mt Lofty Ranges to "relatively rich red-brown earths in a rainfall as low as 15 inches [380 mm] per annum". In a study of the ecology of eucalypt species in the central Mt Lofty Ranges, Green (1994) recorded pink gum across a range of altitudes, rainfalls and soil types, and reported that pink gum was found growing with most other eucalypt species in his study region. Hyde (1999) also noted the wide habitat range occupied by pink gums on the eastern flanks of the Mt Lofty Ranges. My survey sites covered an equally wide and comparable range of habitat attributes as described by these studies. The dominance of pink gum survey sites on soils of low fertility – either rocky acidic soils or deep sandy soils reflects both the bias of historical vegetation clearance, and perhaps a natural preference of pink gums for well-drained soils. Despite approximately 87% of native vegetation in the study region having been cleared since European settlement (DEH 2009), the extant distribution of pink gums still occurs over a range of habitat parameters likely to be comparable to that of its pre-European distribution. This means that should there be major shifts in climate, there will still be suitable habitats in which pink gums are currently surviving.

Pink gum size and correlation with water availability

The present population of the study region is dominated by trees with a DBH of < 30 cm, and especially so at higher rainfall sites. The dominance of smaller pink gums at high rainfall sites is likely to be due at least in part to pink gums at many high rainfall sites being regrowth from fire, and because the higher rainfall sites were associated with skeletal infertile soils. Interestingly it was not mean annual rainfall *per se* that was the main correlate of potential pink gum size, but localised environmental factors. The two largest diameter trees recorded during the survey were from the sites with the highest and lowest mean annual rainfalls. The six largest diameter trees were from six different woodlands, comprising a wide range of soils, slopes, rainfall, and spanning a wide geographic range. The amount of soil water available to pink gums is influenced by not only rainfall, but also soil texture, depth, rock content and seasonality of rainfall. Some of the largest pink gums and/or pink gums with the most intact canopies were on sandy soils and in low rainfall environments. This suggests that at some sites pink gums were using water from deeper in the soil profile, and possibly even groundwater, for at least several months of the year. No direct measurements of pink gum rooting depth have been done but during the current study, I opportunistically observed pink gum roots exposed by soil excavation. On these plants, the roots were over 20 cm thick at 1 m below ground level. Chapter 9 explores the influence of depth to groundwater on pink gum size attributes. However, further work on pink gum rooting depths and sources of water throughout the soil profile, including groundwater would increase our understanding of pink gum's ability to withstand drought and climate change.

Regeneration

The size range of pink gum trunks (DBH) indicates not only habitat differences, but also large differences in recruitment, and recruitment periodicity between sites. The relative lack of pink gums with small DBH's at sites with lower rainfall and/or no fire history, suggests that rainfall and/or fire influence regeneration. At higher rainfall sites, pink gum regeneration occurs regularly, while at the lower rainfall sites, regeneration is highly episodic. Twelve of the 14 sites that contained no pink gums with DBH's of < 5 cm received < 600 mm mean annual rainfall per year. Further, none of these sites had a known fire history, nor were they grazed. During my study, I opportunistically observed localised but abundant pink gum juveniles (< 1.3 m tall) outside the formal survey sites, at Talisker and at Hindmarsh Tiers, both high rainfall locations (means of 770 mm and 815 mm rainfall per annum, respectively) on the Fleurieu Peninsula. These juveniles seemed to have regenerated from seed, as they were well beyond the canopy of the nearest mature pink gums. However, the lack of recruitment at low to moderate rainfall sites, is not due to an inability to produce viable seeds (S. Croft unpublished data). A possible explanation that requires further study is that low rainfall sites have inadequate rainfall to secure seedling establishment and survival.

Anecdotal evidence I gained during the regional study suggested that pink gum seedlings may be highly sensitive to competition, such as from introduced grasses and herbs. For example, the pink gums at the Wistow sites were subject to low to moderate but continuous levels of rabbit and kangaroo grazing, and I recorded no pink gum regeneration, all trees being even-aged mature trees. However, in the adjoining paddocks, where there was high but intermittent grazing by horses, numerous pink gums of a range of age and height classes were present. Possibly, the heavy horse grazing reduced pasture biomass and so upon removal of horse grazing, pink gum seedlings that germinated may have been able to establish rather than be eliminated by competition from groundcover vegetation. Should conditions occur that favour prolific germination at lower rainfall sites in future (such as exceptionally high rainfall years), increased grazing pressure and/or invasion by introduced species may prevent successful recruitment of pink gums. Active management of pink gum habitats may be needed to encourage seedling survival. Conversely, if well above-average rainfall events become even more infrequent, pink gum populations may decline over the long-term at low rainfall sites. The sensitivity of pink gums to competition (at least at the seedling stage) and the impact of removal of grazing upon regeneration are possible areas of future research into determining how habitat may be manipulated to encourage pink gum recruitment.

My observations during the regional study suggest that pink gum regeneration was generally from lignotuberous growth of existing root stock, rather than from seeds. Furthermore, this lignotuberous regeneration was largely confined to higher rainfall sites. At most sites where pink gums < 1.3 m tall were present, they were within a few metres of a mature tree trunk. Without substantial soil excavation, however, it was not possible to determine correctly if they were from the same root system as the nearby mature tree, but it is thought this was likely in most cases. This assertion was supported by my observations of pink gum juveniles at Morialta (site 43) at the end of a dry spring and summer in February 2013. Despite being less than a metre tall, the juveniles had recently put on new leaf growth and appeared to be under no water stress. If these were seedlings with relatively shallow roots, I would have expected the new leaves at least to be wilting and/or leaf loss. The suspected dominance of lignotuberous regeneration in pink gums is supported by White's (1970) observation that in the South East of South Australia, most pink gums are multi-stemmed and twisted, "having originated as suckers from roots, stumps and lignotubers". At higher rainfall sites, therefore, the relatively high occurrence of smaller pink gums suggests that recruitment (largely from lignotubers) is sufficient to sustain the populations in the medium to longer term.

Canopy intactness and spatial patterns

The majority of pink gum woodlands in the Mt Lofty Ranges and adjoining plains contain pink gums with moderate to high levels of canopy intactness. Ward's (2005) regional study recorded a mean canopy intactness of 48% for pink gums, substantially lower than the 70% that I recorded. These findings, however, cannot be directly compared. Nearly 80% of Ward's transects were from only seven pink gum woodlands, all of which had high mistletoe levels. My results showed that low pink gum canopy intactness is localised, with larger areas of the study region containing pink gums with moderate to relatively high pink gum canopy intactness.

Further, within a pink gum woodland, there were often significant differences in pink gum canopy intactness (and often in pink gum size). At Cromer, Deep Creek, Rockleigh, Finniss, Scott Creek, Aldinga Scrub and Clayton, for example, pink gums varied greatly in size and/or canopy intactness between sampling sites within the same woodland. I hypothesised that differences in mistletoe loads, soil texture and depth, depth to water table, tree density, fire, and in the short term, insect attack are associated with differences in canopy intactness and pink gum size – and forms the basis of remaining studies in this thesis.

Litchfield (1956) and White (1970) concluded that in the South East region of South Australia (refer Figure 1.1), the distribution and abundance of pink gum seems to be largely determined by available soil moisture. In particular, White (1970) suggested that pink gums are very sensitive to seasonal extremes in soil moisture. More generally, Specht and Perry (1948) stated that water availability, as controlled by slope, aspect, soil texture and annual rainfall was an important factor controlling the distribution pattern of eucalypts (including pink gum) in the central region of the Mount Lofty Ranges. The current study recorded significant correlations between the presence of lerps and mistletoes and the number of trees per hectare, but there was no correlation between pink gum canopy intactness and either slope, aspect, soil texture or annual rainfall. However, there was a significant interaction between mistletoe and rainfall, and at the 10% level, between mistletoe, rainfall and soil type. This latter interaction supported the observation that survey sites on sandy soils with < 550 mm rainfall often contained high mistletoe loads with low canopy intactness levels. These sites were at Aldinga, Altona Sandy Creek, Onkaparinga and McLaren Vale. In contrast, pink gums occurring on deep sands where rainfall was over 600 mm and mistletoe was absent (at Cox Scrub) contained highly intact canopies. On rocky acidic soils in high rainfall zones, the condition of pink gums also varied. At Montacute, the condition of pink gums in 2009 was very poor but lerps were present and this site had been burnt in the previous 10 years. In contrast, on similar soils, pink gums were in very good condition in 2009 at Anstey Hill, but lerps were absent.

In the study region, pink gum leaves did not show any signs of yellowing - a key symptom of pink gums in poor condition in the South East of South Australia (Paton *et al.* 2005), nor were scale, leaf galls, or leaf blister present on more than 1% of trees. Insect chewing damage and skeletoniser damage were scored on 50% and 20% of trees surveyed, respectively. Borers were not specifically observed, and borer holes in dead trunks were recorded from only five trees. Pink gums in the study region are unlikely to be particularly susceptible to wood boring insects. Paton *et al*. (2005) recorded the presence of borers in six *Eucalyptus* species in the South East of South Australia, where it was *Eucalyptus leucoxylon* that recorded twice the incidence of borers as four other species, including pink gums. Because insect damage was correlated with pink gum canopy intactness, Chapter 2 and Chapter 5 explore further the hypothesis that insect damage is strongly associated with canopy dieback, rather than longer term decline.

Mistletoe and host canopy intactness

The percentage of pink gums (at least 1.6 m tall) containing mistletoe, and the mean number of mistletoes per pink gum at a survey site were the variables with the strongest correlation with the mean canopy intactness of pink gums, for a survey site. There was not, however, a significant association between the number of live mistletoes and canopy intactness. This finding agrees with Ward (2007) who recorded “no strong relationship between the number of live mistletoes and canopy dieback ($r^2 = 0.01$)” for 1200 pink gums surveyed within pink gum dominated woodlands. Possibly, this is because considering only live mistletoes does not account for the impact of past live mistletoes (which are now dead) on the pink gum canopy. Chapter 3 further explores the relationship between pink gum canopy intactness and mistletoe loads, and in particular the hypothesis that high mistletoe loads predispose pink gums to canopy decline during prolonged periods of below-average rainfall.

Chapter 2 Seasonal and two-yearly changes in pink gum canopy intactness: 2009 – 2012

2.1 Abstract

Distinguishing between short-term fluctuations in canopy intactness is important to maximise the effectiveness of vegetation management for conservation gain. To this end, pink gum (*Eucalyptus fasciculosa*) canopy attributes were recorded seasonally at nine survey sites within five woodlands, from winter 2010 to summer 2011/12; and at 58 survey sites in both 2009/10 and 2011/12. All sites occurred in the Mt Lofty Ranges region, South Australia, where the poor canopy condition of pink gums has been noted as being of concern. Short-term reversible canopy changes were hypothesised to be associated with large deviations from seasonal rainfall totals and increased abundance of leaf-damaging insects. At the seasonally-monitored sites, the mean canopy intactness of the 162 pink gums surveyed was 61% in both winter 2010 and summer 2011/12. The greatest change in mean canopy intactness at a survey site was a decline of 12% for a near coastal site. The greatest seasonal increases in mean canopy intactness, although still < 10%, occurred during summer 2010/11, when up to 2.5 times the long-term mean rainfall was received. For all seasonally-monitored sites, at least 90% of total leaf production between August 2010 and February 2012 occurred in spring/summer 2010/11. During the study period there were net increases of 52, 17 and 2 live mistletoes at the three sites where mistletoes were present. The pre-dawn leaf water potentials of pink gums ranged from site means of -1.3 MPa to -1.7 MPa in summer 2011 and from -1.3 to -2.3 MPa in summer 2012, well below the -3.5 MPa considered to be the critical water potential required to cause leaf damage in pink gums.

For the 1751 pink gums surveyed from 58 sites in both 2009/10 and 2011/12, there was a statistically significant decline in mean canopy intactness from 71% to 66%. Canopy intactness changed by at least ± 25% in 14% of these trees. No new pink gums were recorded at any of the 58 sites and 21 trees died. The net number of live mistletoes increased by 21% for all the 1751 trees combined. Declines in pink gum canopy intactness were most commonly associated with leaf damage from insects, particularly lerps (*Cardiospina densitexta*), cup moth larvae (*Doratifera quadriguttata*) and/or gum leaf skeletoniser (*Uraba lugens*). The greatest increases in canopy intactness were correlated with trees with the lowest levels of canopy intactness in 2009/10. When the five sites with lerp present were excluded

from the analysis, there was no significant change in mean canopy intactness between 2009/10 (70% intactness) and 2011/12 (69% intactness).

2.2 Introduction

Decline of woodland and forest trees is a worldwide phenomenon (Ciesla and Donabauer 1994). In Australia chronic decline of *Eucalyptus* trees has increased since the late 1970s because of factors such as extended drought and altered fire regimes (Jurskis and Walmsley 2012). The various factors contributing to *Eucalyptus* decline can be classified as: (i) predisposing factors which put a permanent stress on the tree e.g., change in land use, climate change (ii) inciting factors which are usually of short duration e.g. insect attack, short-term weather events and (iii) contributing factors such as parasites which invade the tissues of trees and reduce the chance of recovery (after Manion 1981 cited in Old 2000). The tree may completely recover from inciting factors unless it has been predisposed by other factors. In determining conservation priorities, therefore, it is important to distinguish between short-term fluctuations from which the vegetation can naturally recover, and long-term declines, from which the vegetation is unlikely to recover without active intervention (Pullin *et al.* 2013; Rumpf *et al*.2009).

To determine the cause (s) of tree decline, a range of parameters can be examined. These can be classified into physiological parameters, and parameters that describe outward appearance such as canopy intactness. Physiological responses to stress, such as changes in leaf water potential, photosynthesis, leaf temperature and stomatal opening, are usually measured at scales from minutes to weeks, whereas changes in outward appearance are usually measured at longer time scales as they are the result of cumulative physiological effects (Ben-Gal *et al.* 2010). Changes in trees over longer time frames include leaf production and leaf death, changes in plant size, phenological changes and branch death. Generally, physiological based monitoring on its own does not quantify longer-term cumulative effects. The regional study (Chapter 1) showed that mistletoe infection and insect attack were most strongly associated with low levels of pink gum canopy intactness

This chapter monitored both physiological changes (in the form of water potential) and changes in outward appearance of pink gum canopies, including insect presence, mistletoe infection and canopy intactness, at intervals ranging from 3-24 months. I hypothesised that changes in water potential would reflect short-term changes in weather and not be indicative of long-term declining canopy intactness. Changes in insect presence, leaf production and leaf loss, were hypothesised to be short term and associated with seasonal rainfall fluctuations. Changes in mistletoe abundance over the study period were hypothesised to reflect long-term trends.

2.3 Methods

2.3.1 Seasonally-monitored sites

To document seasonal morphological and physiological changes in pink gums, I surveyed pink gums from nine sites each season between August 2010 (winter) and February 2012 (summer). The sites were in five pink gum woodlands which included the northern, southern and western occurrences of pink gum in the study region (Figure 2.1). The sites also represented a range of pink gum sizes, canopy intactness levels, mistletoe loads, and habitats (Table 2.1). Rainfall, soil, elevation and landform details are summarised in Table 2.2 (refer 1.3 for methods of recording these attributes).

Figure 2.1: Location of study sites monitored seasonally 2010 – 2012, all located within the Mt Lofty Ranges and surrounding plains, South Australia (imagery from Google Earth). Coordinates (UTM GDA94 and Zone 54) are as follows: Aldinga Easting 307800 Northing 6171410, Highland Valley Easting 310650 Northing 6105290, Aldinga Easting 268680 Northing 6091030, Clayton Easting 311260 Northing 6073800 and Goolwa Easting 297460 Northing 6070700.

Table 2.1: Pink gum attributes for the nine study sites recorded in winter 2010. All pink gums were > 2.3 m tall.

Attribute	**Clayton**			**High-land Valley**	**Aldinga**			**Altona**	**Goolwa**
	A	**B**	**C**		**B**	**C**	**D**		
# of pink gums surveyed	12	10	11	31	16	12	18	24	28
Mean % canopy intactness (±SD)	71 (± 28)	94 ± 7	46 (± 22)	79 (± 18)	66 (± 25)	39 (± 25)	40 (± 28)	47 (± 32)	64 (± 25)
Mean tree height (m)	6.4	7.3	4.6	7.1	4.1	6.1	5.1	6.0	6.1
Tree height range (m)	4–8	5–10	3–6	4–11	2–6	3–9	3–10	3–8	3–12
Mean DBH*(cm) (±SD)	29 (±16)	28 (±11)	13 (± 13)	34 (± 13)	8 (± 3)	20 (± 7)	16 (± 5)	15 (± 8)	26 (± 9)
% pink gums with live and/or dead mistletoe	42	82	100	0	6	75	100	96	0

* For multi-trunked trees, the trunk with the largest DBH was recorded

Table 2.2: Rainfall, landform element and upper soil texture of nine study sites within five pink gum woodlands

Woodland name and size (ha)	**Mean annual rainfall (mm)**	**Study Site**	**Elevation (m)**	**Landform element**	**Soil Type**
Clayton **75 ha**	400	A	9	Dune crest	Deep sand
		B	7	Flat	Sand over clay
		C	7	Swale	Sand over clay
Highland Valley **2 ha**	535	A	245	Upper slope	Sandy-loam
Aldinga **266 ha**	507	B	19	Dune slope	Sand to 1–2 m over sandy clay
		C	14	Flat	
		D	16	Flat	
Altona **150 ha**	500	A	225	Flat	Sand (to 0.5 m) over sandy clay
Goolwa **1.5 ha**	465	A	13	Plain	Sand to sandy-loam

Within the Clayton and Aldinga woodlands, multiple survey sites were chosen to represent a range of pink gum canopy conditions. The first tree within each site was arbitrarily chosen. The other pink gums surveyed were the nearest live trees to the first tree, and that were at least 3 m from any other surveyed tree. At the Goolwa, Highland Valley and Altona woodlands, all trees were within a one hectare area, and were chosen to represent a range of tree canopy intactness levels. At each survey site, the following parameters were measured at two to four monthly intervals, from winter 2010 to summer 2012: Survey dates are shown in Table 2.3.

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Table 2.3: Survey dates for seasonally-monitored parameters at five pink gum woodlands

Pink gum woodland	**Survey date**						
Highland Valley	26/7/10	24/11/10	1/2/11	30/5/11	11/8/11	3/11/11	31/1/12
Clayton	7/8/10	29/11/10	26/3/11	7/5/11	23/8/11	17/11/11	24/2/12
Aldinga	4/8/10	17/11/10	22/2/11	27/5/11	3/8/11	31/10/11	6/2/12
Altona	22/8/10	3/11/10	16/3/11	24/5/11	15/8/11	7/11/11	3/2/12
Goolwa	11/8/10	5/11/10	7/3/11	17/5/11	5/9/11	11/11/11	26/1/12

For the seasonally-monitored sites, the following parameters were recorded:

Pink gum canopy intactness

This was a visual estimate of the amount of foliage present expressed as a percentage of the possible maximum canopy for the tree. I considered that changes in canopy intactness of < 10% were within the range of observational accuracy of my scoring rather than actual changes.

Leaf production and longevity

During winter 2010, I numbered leaves on at least five trees per survey site and on at least three branchlets per tree. To obtain actively growing branches, branchlets were chosen from the outside of the canopy and as high in the canopy as within reach from a step ladder. This was up to 4 m off the ground. Table 2.6 provides details of the numbers of branchlets tagged and leaves numbered. I marked the location on a branch from which to begin leaf numbering with both a paint pen and metal tags. At the first recording, I consecutively numbered all leaves beyond the marked location with a paint pen. Thereafter, each survey date I recorded which numbered leaves remained, and continued to number new leaves sequentially (after Lowman and Heatwole 1992).

Mistletoe

For each mistletoe (*Amyema miquelii*), I estimated the percentage of canopy that was intact, ranging from zero (dead and/or no live foliage present) to 100%. I recorded the proportion of the combined pink gum and mistletoe foliage that was comprised of mistletoe foliage.

Leaf damage

For the Altona, Aldinga, Clayton and Goolwa study sites, I estimated the percentage of all canopy leaves that had greater than 10% of the leaf area damaged by insect chewing, skeletoniser activity, psyllid (“lerp”) activity or fungal spots, as well as the percentage of canopy leaves that were dead. These parameters were also recorded for the leaves that were numbered to determine leaf production (refer above).

Trunk diameter

I recorded the diameter of all trunks at 1.3 m above ground (referred to as diameter at breast height, DBH)

Stem and leaf water potential

Midday stem water potential (Ψ), midday leaf Ψ and pre-dawn leaf Ψ were recorded on one occasion during each of summer 2010/11, winter 2011 and summer 2011/12 at the Altona, Clayton and Aldinga survey sites. Water potential dates, weather details and number of trees sampled for each site are provided in the Results section. For each tree, leaf and stem Ψs were recorded from the same stem. Where I recorded a second leaf Ψ and stem Ψ from the same tree, this was from a separate part of the canopy. Leaf Ψ was recorded on fully exposed and fully expanded leaves selected from the outer canopy. Stem Ψ was recorded by placing black plastic bags covered with foil over clumps of leaves, approximately one hour before recording water potential. The bag was left on long enough to allow the water tension in the leaf to come to equilibrium with the water tension in the stem (Cleary *et al.* 2009). The trees recorded were those nearest the centre of the study site, with the number of trees recorded depending upon the time available at midday or before dawn. Midday leaf Ψ and pre-dawn Ψ are assumed to be the minimum and maximum diurnal water potential values, respectively (Damesin and Rambal 1995). Hence, the absolute difference between midday and predawn Ψ (ΔΨ) was calculated, as this is an indication of water stress. With increasing water stress, ΔΨ will generally become smaller (Damesin and Rambal 1995), because predawn Ψ will be a greater value (more negative) and become closer to midday Ψ.

Rainfall

Figure 2.2 shows seasonal rainfall received during the survey period compared with mean seasonal rainfall for the study woodlands. Rainfall data for each site was obtained from the nearest long-term weather station with a similar elevation. There were no nearby weather stations with long-term data for Aldinga (hence seasonal means are not known). Clayton rainfall was based on the Milang weather station 024576*;* Altona on the Rosedale weather station 023343*;* Goolwa based on the Goolwa weather station 023718, and Highland Valley based on a 110% correction factor (interpolated from discussions with landholder) applied to the nearest weather station at Strathalbyn 023747 (Bureau of Meteorology 2015). Between spring 2010 and autumn 2011, rainfall was above average, and between winter 2011 and summer 2011/12 was below or near-average.

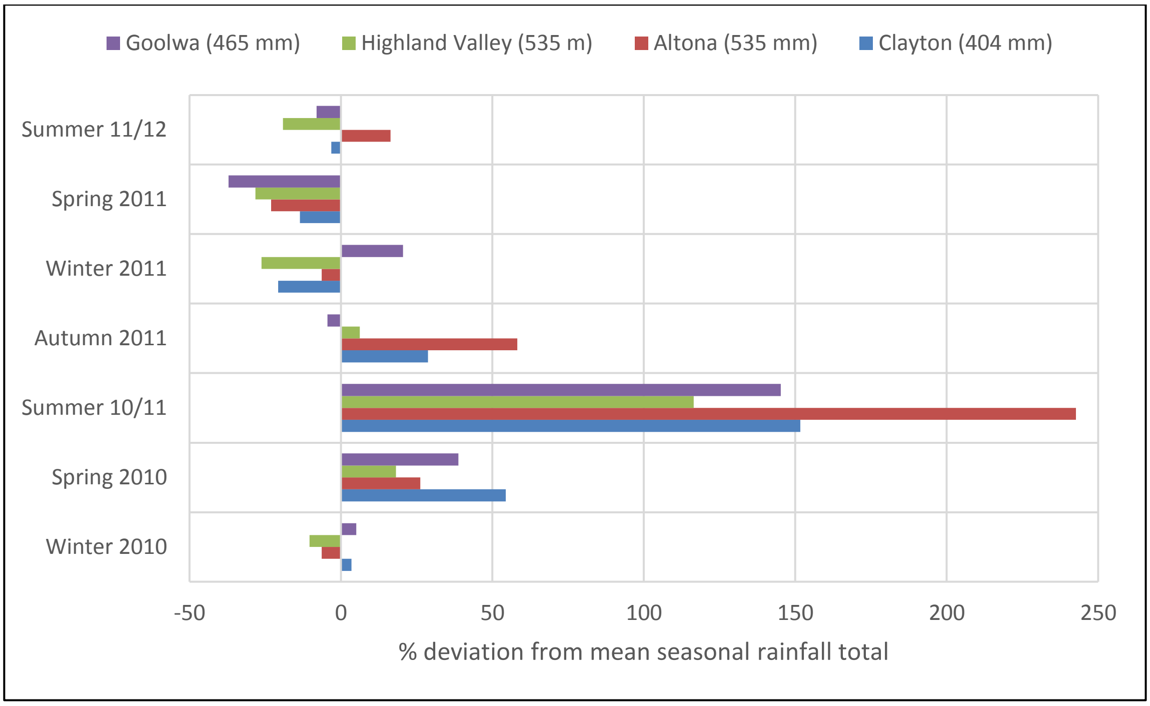

Figure 2.2: Percent deviation from mean seasonal rainfall totals for Clayton, Altona, Goolwa and Highland Valley sites from winter 2010 to summer 2012. Summer 10/11 = December 2010 to February 2011; summer 11/12 = December 2011 to February 2012. Data obtained from Bureau of Meteorology (2015).

Statistical analysis: seasonal sites

Seasonal changes in canopy intactness were compared using a repeated measures ANOVA (with a Greenhouse-Geisser correction when the assumption of sphericity was not met (Lund

Research Ltd 2013). For each survey site, differences in the levels of leaf damage categories between survey periods were assessed using one-way ANOVA and the Welch test as the assumption of homogeneity of variance was not met. Comparison of mean canopy percentages for a survey site between the start and end of the recording period were tested using paired samples t-tests (after testing for normality of variance). Pearson's correlation was used to determine an association between the canopy intactness of a tree in August 2010, and the magnitude of change in canopy intactness (after removing two outliers). Comparison of water potential recordings and canopy intactness changes between survey sites for a single reporting period were analysed using one-way ANOVA and the Welch test when the assumption of homogeneity of variance was not met. Pearson's correlations were calculated for associations between canopy intactness and leaf production, insect damage to leaves and water potential. All analyses were done using SPSS version 23.

2.3.2 **Two-yearly monitored sites**

I surveyed pink gum canopy intactness, tree height and mistletoe percent canopy intactness for 1751 individual pink gums from 58 of the regional sites (refer Chapter 1) in both spring/summer 2009/2010 and spring/summer 2011/12, hereafter referred to as visit 1 and visit 2, respectively. The sites surveyed for both visits are shown in Appendix A. Individual pink gum trees were re-located using a combination of GPS points and field notes made during visit 1 (namely, noting the distance and bearing of one tree from another and/or by using tree dimensions). Pink gum canopy intactness, mistletoe canopy intactness and tree height were recorded using the methods for the seasonally-monitored trees.

I surveyed leaf damage attributes from 1189 pink gums from 51 survey sites in both spring/summer 2009/2010 and spring/summer 2011/12. I visually scanned the entire canopy, using binoculars where necessary, for up to 30 seconds to determine the percentage of all canopy leaves that had more than 10% of the leaf area affected by chewing insects, skeletonisers, psyllids ("lerps") and/or fungal symptoms. The percentage of dead leaves per canopy was also estimated on these 1189 trees.

Statistical analysis: two-yearly monitored sites

Comparison of mean canopy intactness for all trees between 2009 and 2012 was made using paired samples t-test, after testing for normality of distribution. Pearson's correlations were made between canopy intactness and mean annual rainfall.

2.4 Results

2.4.1 Seasonally-monitored sites

Canopy intactness

The nine survey sites contained a total of 162 pink gums, whose mean canopy intactness was 61.6% (SE ± 2.3) in winter 2010 and 61.1% (SE ± 2.2) in summer 2011/12. There was a < 10% change in canopy intactness for 67% of trees. Of those trees that recorded at least a 10% change, 16% recorded an increase and 17% recorded a decrease. There was a canopy change of at least 25% in 7% of trees. Between August 2010 and February 2012 mean canopy change for an individual survey site was at least ± 10% only for Clayton site B (-10%) and Aldinga site B (-12%) (Table 2.4). The differences in mean canopy intactness between 2010 and 2012 were significant for these sites (paired sample t test, $t = 2.46$, $p = 0.03$ for Aldinga B; and $t = 2.61$, $p = 0.03$ for Clayton B). A repeated measures ANOVA with a Greenhouse-Geisser correction determined that mean canopy intactness for Aldinga site B and Clayton site B did not differ significantly between seasons other than between the start and end recording period ($F = 4.06$, $p = 0.03$).

Table 2.4: Mean change in canopy intactness of 162 pink gums at nine survey sites between August 2010 and January 2012; percentage of trees showing > 10% change in canopy; and maximum individual tree canopy change for each site

Study site	Mean % canopy intactness at start (± SD)	Mean change in canopy intactness ± SD	% of trees with > 10% canopy change	Greatest % canopy change within a single tree
Highland Valley	79 ± 18	+1 ± 6	3	+20
Clayton A	71 ± 29	-1 ± 8	8	-20
Clayton B	94 ± 7	-10 ± 12	36	-30
Clayton C	46 ± 25	+4 ± 15	46	+25 and -25
Aldinga B	66 ± 14	-12 ± 20	50	-60
Aldinga C	39 ± 25	+9 ± 11	25	+30
Aldinga D	40 ± 28	-2 ± 12	28	+28
Altona	47 ± 30	0 ± 8	8	-25
Goolwa	64 ± 25	+3 ± 10	30	+35

There was a weak but significant correlation between the canopy intactness of a tree in August 2010, and the magnitude of change in canopy intactness (R = -0.36, df = 159, $p < 0.001$) (Figure 2.3). The mean canopy intactness in 2009/10 of trees that recorded at least a 10% increase, was 39% (SE ± 3.6), which was significantly lower than for trees which recorded "no change" (65% SE ± 2.9) and for trees that recorded a decrease in canopy intactness (71% SE ± 4.3) (ANOVA Welch test F = 12.67 $p < 0.001$).

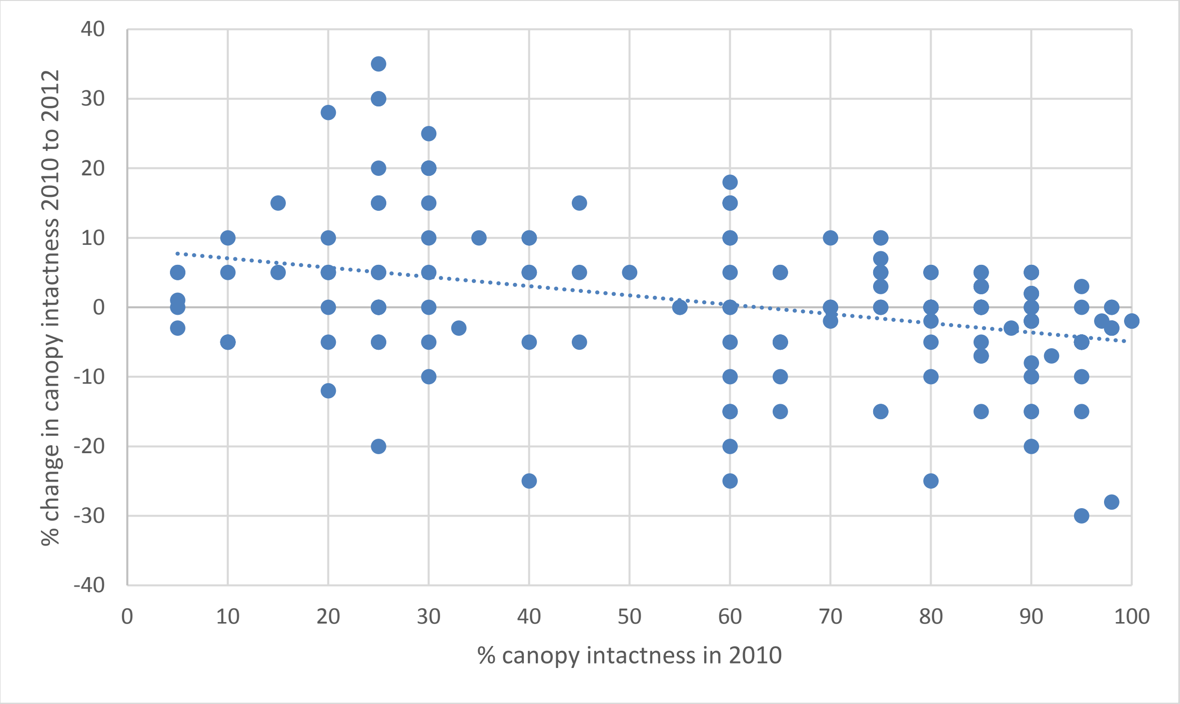

Figure 2.3: Percentage change in canopy intactness between 2010–2012 compared with initial canopy intactness for 160 pink gums (two outliers were removed). Some data points represent multiple trees where the initial canopy intactness and change in canopy intactness was the same for two or more trees.

For the nine survey sites, the intra-seasonal change in mean canopy intactness ranged from 1% at Altona to 9% at Aldinga site C (Table 2.5). The greatest seasonal increases in mean canopy intactness, although still < 10%, most commonly occurred during summer December 2010 to February 2011, when rainfall received was up to 250% the long-term summer average. At Highland Valley, for example, during summer 2010/11 none of the pink gums declined in canopy intactness and one third of trees increased between 10% and 20% in canopy intactness. The greatest negative seasonal changes most commonly occurred during summer 2011/12 (Table 2.5).

Table 2.5: Mean intra-seasonal changes in canopy intactness per survey site. Greatest positive changes are in green and greatest negative changes are in yellow.

Survey site	**Spring 2010**	**Summer 10/11**	**Autumn 2011**	**Winter 2011**	**Spring 2011**	**Summer 11/12**	**Net change: Winter 2010 to Summer 2012**
Highland Valley	-3	**6**	-2	1	1	**-2**	**+1**
Clayton A	0	1	0	0	**-3**	0	**-1**
Clayton B	0	-3	0	-2	-1	**-3**	**-10**
Clayton C	2	1	**3**	1	**-3**	0.0	**+4**
Aldinga B	0	-3	0	-2	-2	**-6**	**-12**
Aldinga C	0	**9**	9	0	0	0	**+9**
Aldinga D	0	**3**	-1	0	-1	**-3**	**-2**
Altona	0	**1**	-1.0	0	0.1	**-1**	**0**
Goolwa	-0.3	**4**	0	-1	0	0	**+3**

Leaf production and loss

For the nine survey sites, at least 90% of all leaves produced between August 2010 and February 2012 occurred between September 2010 and early March 2012 (spring and summer). In contrast, only about 5% of all leaves were produced during spring/summer 2011/12. As well as most leaf production occurring in summer, most leaf loss also occurred in summer and to a lesser extent spring, with greatly reduced leaf loss in autumn and winter (Table 2.6). For all sites combined the percentage of total leaves lost during the survey period was: spring 2010 (12%), summer 2010/11 (31%), autumn 2011 (9%), winter 2011 (5%), spring 2011 (16%) and summer 2011/12 (27%). For the Aldinga, Highland Valley, Altona and Clayton sites individually and collectively, there were no significant correlations between the number of new leaves produced on the tagged branchlets and the tree's canopy intactness (with R values ranging from -0.09 at Aldinga (df = 15, p = 0.74) to -0.3 at Highland Valley (df = 4 and 4, p = 0.63).

Table 2.6: Leaf production and loss for nine survey sites in five woodlands, August 2010 to February 2012

Parameter	**Aldinga B, C, D**	**Clayton A, B, C**	**Highland Valley**	**Altona**	**Goolwa**
# of branches tagged at start	51	38	23	62	41
# of leaves numbered at start	1539	1787	766	1676	1199
# of new leaves recorded	903	669	199	1485	446
# of leaves numbered at start but not present in February 2012, or dead	1347	1095	691	896	611
% net leaf loss/gain *	-28	-24	-64	35	-14
spring/summer 2010/11 leaf production as % of total number of new leaves for whole survey period	90	95	94	80	93
spring/summer 2011/12 leaf production as % of total number of new leaves for whole survey period	6	4	3	16	5
Season in which most new leaves recorded	Summer 2010/11 (84%)	Summer 2010/11 (68%)	Summer 2010/11 (71%)	Spring 2010 (54%)	Spring 2010 (48%)
Season of most leaf loss	Summer 2010/11	Summer 2010/11	Summer 2010/11	Summer 2011/12	Summer 2010/11
Seasons with net leaf gain**	1,2	1,2	nil	1,2,3	1,2,3
Seasons with net leaf loss**	3,4,5,6	4,5,6,7	1,2,3,4,5,6	4,5,6	4,5,6

*% net leaf loss or gain = (total number of new leaves – total number of missing and dead leaves)/number of leaves initially numbered in 2010

** 1 = spring 2010; 2 = summer 2010/11; 3 = autumn 2011; 4 =winter 2011; 5 = spring 2011; 6 = summer 2011/12

Leaf retention

The percentage of leaves numbered in August 2010 that were still present in January/February 2012 (approximately 18 months later) varied from 24% and 25% at Aldinga and Goolwa, respectively to 51% at Altona, with Clayton and Highland Valley retaining 44% and 40%, respectively of the original leaves.

Leaf damage

All of the five pink gum woodlands recorded the greatest leaf damage from skeletoniser, chewing and fungal symptoms between spring 2011 and summer 2011/12, which was significantly higher than the previous spring-summer 2010/11 period (Table 2.7). No signs of psyllid presence were recorded on any leaves at any of the nine survey sites.

Table 2.7: Mean percentage of leaves (rounded to the nearest percent) on tagged branchlets that were dead or had at least 10% of the leaf area with chewing, skeletoniser, and/or fungal symptoms for the seasonally-monitored survey site trees – Altona, Goolwa, Aldinga and Clayton. Superscripts shows homogeneous subgroups using ANOVA Welch test and Games-Howel post-hoc test.

Survey season	**% Chewed**	**Skeletonised**	**Fungal**	**Dead**
winter 2010	2 ± 2^{A}	8 ± 3^{AB}	2 ± 2^{A}	1 ± 0.2
spring 2010	8 ± 3^{A}	8 ± 2^{ABC}	7 ± 3^{A}	1 ± 0.2
summer 10/11	10 ± 2^{AB}	3 ± 1^{A}	5 ± 1^{AB}	5 ± 1
autumn 2011	19 ± 3^{BC}	4 ± 1^{A}	14 ± 3^{BC}	2 ± 1
winter 2011	23 ± 3^{CD}	11 ± 2^{ABC}	20 ± 3^{CD}	1 ± 0.2
spring 2011	32 ± 3^{DE}	17 ± 2^{C}	26 ± 3^{D}	2 ± 1
summer 11/12	35 ± 2^{E}	17 ± 2^{BC}	29 ± 3^{D}	4 ± 1
F	24.37	10.24	17.37	3.09
p	< 0.001	< 0.001	< 0.001	0.05

Leaf water potential

The summer pre-dawn mean leaf water potentials (Ψ) for Clayton and Aldinga in March 2011 were relatively high (less negative), namely -1.5 MPa and -1.3 MPa, respectively (Table 2.8). In February and March 2012, mean predawn leaf Ψs were still greater than -2 MPa for all sites, but were more negative than the 2011 recordings, reflecting the hotter and drier weather conditions preceding and during the recording period in 2012 (Table 2.8). For both summer 2011 and summer 2012, mean midday leaf Ψ ranged from -2.4 MPa at Clayton site B to -3.3 MPa at Altona. Mean midday summer leaf Ψ for all three Aldinga sites was -2.8 MPa in 2011 and -2.5 MPa in 2012. For each recording period the mean of individual tree differences between pre-dawn and mid-day leaf water potential (ΔΨ) was always less negative than -2 MPa, and summer and winter diurnal ranges were of similar magnitude. For all sites, the differences between stem and leaf water potentials were also relatively low, ranging from -0.1 to -1.1 MPa. For example, for the three Clayton sites combined, there was a maximum difference of only 0.3 MPa between seasonal mean midday leaf Ψ readings, and surprisingly no difference between the winter 2011 and summer 2012 means. Similarly, for the Clayton sites, there was only a maximum difference of 0.4 MPa in the mean stem water potential (SWP) between any recording period.

Table 2.8: Mean (± SD) predawn leaf water potential and mean leaf and stem water potentials recorded in MPa during summer 2010/11, winter 2011 and summer 2011/12 for Highland Valley, Clayton, Aldinga and Altona sites. Values were the mean of all trees. One to two samples per tree were recorded.

Recording date		**Clayton (N = 18–30)**		**Aldinga (N = 32)**		**Altona (N =14)**	
		WP (MPa)	**Temp Cloud Rain***	**WP (MPa)**	**Temp. Cloud Rain***	**WP (MPa)**	**Temp Cloud Rain***
Altona = 17/2/11 Aldinga = 1/3/11 Clayton = 6/3/11 (Late summer/ early autumn	Predawn leaf Ψ	-1.5 ± 0.2	19^{0}, 70% 15 mm	-1.3 ± 0.2	21^{0}, 70 % 0 mm	not recorded	30^{0}, 5% 13 mm
	Midday leaf Ψ	-2.8 ± 0.5		-2.8 ± 0.3		-3.3 ± 0.4	
	Midday stem Ψ	-2.0 ± 0.3		-2.1 ± 0.2		-2.3 ± 0.3	
	Mean of individual trees' diurnal range (ΔΨ)	**1.0**		**1.4**		not recorded	
	Midday leaf Ψ minus midday stem Ψ	-0.8		-0.7		-1.0	
Altona = 14/8/11 Aldinga = 2/8/11 Clayton = 3/8/11 (late winter)	Predawn leaf Ψ	-1.3 ± 0.2	20^{0}, 0% 11 mm	nr	17^{0}, nr 32 mm	-1.0 ± 0.2	20^{0}, 0% 10 mm
	Midday leaf Ψ	-2.7 ± 0.4		-1.9 ± 0.4		-2.0 ± 0.4	
	Midday stem Ψ	-2.1 ± 0.3		-1.4 ± 0.3		-1.4 ± 0.4	
	Mean of individual trees' diurnal range (ΔΨ)	**2.0**				**1.1**	
	Midday leaf Ψ minus midday stem Ψ	-0.6		-0.5		-0.6	
Altona = 2/2/12 Aldinga = 4/3/12 Clayton = 3/2/12 (Late summer)	Predawn leaf Ψ	-1.9 ± 0.3	39^{0}, 5% 0.6 mm	-1.6 ± 0.4	34^{0}, 80% 6 mm	-1.5 ± 0.1	29^{0}, 0% 2 mm
	Midday leaf Ψ	-2.7 ± 0.4		-2.5 ± 0.6		-3.1 ± 0.4	
	Midday stem Ψ	-2.4 ± 0.4		-2.4 ± 0.3		-2.3 ± 0.2	
	Mean of individual trees' diurnal range (ΔΨ)	**1.5**		**1.0**		**1.7**	
	Midday leaf Ψ minus midday stem Ψ	-0.3		-0.1		-1.0	

*Temp = Maximum temperature recorded on the survey date, at nearest weather station

Cloud = % cloud cover which was a visual estimate of mean cloud cover during the water potential recording period

Rain = cumulative rainfall in the seven days prior to recording.

Correlation between water potential and canopy intactness

For all trees at Clayton, Altona and Aldinga, for all recording periods there was no correlation between ΔΨ and mean canopy intactness (R = -0.09, N = 90, p = 0.40). For all sites, on all recording dates, there were no significant correlations between tree canopy intactness and midday leaf Ψ, midday stem Ψ or predawn leaf Ψ. The Pearson correlation and significance values for all Aldinga and Altona survey trees assessed over three recording dates (summer and winter) are shown in Table 2.9.

Table 2.9: Correlation between water potential parameters and pink gum canopy intactness for all water potential recordings at Aldinga (all sites combined) and Altona sites between winter 2010 and summer 2011/12

Water potential (Ψ) parameter	**Aldinga**			**Altona**		
	N*	r	p	N	r	p
Midday pink gum leaf Ψ	191	0.06	0.41	46	-0.10	0.50
Midday pink gum stem Ψ	172	0.02	0.76	45	-0.11	0.46
Pre-dawn pink gum leaf Ψ	49	-0.14	0.34	28	-0.08	0.70

* Total number of water potentials measured

Mistletoe

During the survey period, the net number of live mistletoes increased at each of the three woodlands where mistletoe was present, namely Clayton, Aldinga and Altona. The increase in the number of mistletoes varied widely though, both between sites within a woodland, and between pink gum woodlands. At the lowest rainfall site, Clayton, the number of live mistletoes increased from 105 to 157. Despite a relatively high baseline mistletoe population and moderate mean annual rainfall, the number of live mistletoes at Altona increased by only two, from 37 to 39, and there was an 8% decline in the percentage of trees containing live mistletoes. There was an increase of only one live mistletoe at each of Aldinga sites B and C, despite site C having a higher mean canopy intactness level, canopy volume and baseline number of live mistletoes.

2.4.2 **Two year monitored sites**

Canopy intactness

There was a significant net decline in canopy intactness of the 1751 trees surveyed in both 2009 and 2011, from 71% to 66% (paired samples t-test, $t = 10.25$ $N = 1751$, $p < 0.01$). Canopy intactness changed by > 10% in 22% of trees (declining in 15% and increasing in 7% of trees), and by at least 25% in 14% of trees (declining in 11% and increasing in 3%). During visit 1, 96 trees were dead (hence there were 1656 live trees). An additional 21 trees died by 2011, equating to 0.6% loss per year. Of these, 13 had a canopy intactness of 10% or less during visit 1 and 10 trees were < 2.3 m tall. The additional 21 dead trees were from 13 pink gum woodlands representing a range of soil types, rainfall and geographic locations. No new pink gums were recorded at any of the sites.

At nearly 80% of the survey sites (45 of 58 sites), there was a < 10% change in mean canopy intactness. There was a ≥ 10% decline in mean canopy intactness at 11 of the 58 sites compared with a ≥ 10% increase at two sites. The greatest mean declines were at the five sites which had an outbreak of lerps (sites 42, 78, 43, 17 and 8, refer Appendix A), where the mean declines ranged from 20% to 50%. There was a weakly significant negative correlation between a tree's canopy intactness in 2010 and the magnitude of change in canopy intactness ($N = 1654$, $R = -0.26$, $p < 0.001$). That is, trees with lower levels of canopy intactness generally showed the greatest increases in canopy intactness.

Skeletoniser and leaf chewing damage

The percentage of trees recorded with skeletoniser damage (> 1% of leaves having at least 10% of the leaf surface area skeletonised) increased from 10% in visit 1 to 28% in visit 2 and the mean percentage of canopy with skeletoniser damage per host tree increased from 11% to 20%. Skeletoniser damage was recorded from 34 sites during both visit 1 and 2. The percentage of trees with chewing damage (> 1% of leaves having at least 10% of the leaf surface area chewed) increased from 21% to 64% and the mean percentage of leaves per host tree with chewing damage increased from 14% to 24%.

Mistletoe

Between 2009 and 2011, for the 1751 pink gums recorded on both occasions, the number of live mistletoes present increased by 202, from 764 in 2009 to 966 in 2011. The number of live mistletoes increased in 39% of pink gums, remained the same in 55% and declined in 6% of pink gums. Of the 34 re-surveyed sites where mistletoes were present either during visit 1 or visit 2, the number of live mistletoes declined at seven sites, stayed the same at eight sites and increased at 19 sites. Mt Beevor (refer Appendix A, site 43) was the only site where mistletoes were recorded during visit 2 but not visit 1.

2.5 Discussion

Changes in canopy intactness

This study aimed to document changes in pink gum canopies between 2009/10 and 2011/12 in the Mt Lofty Ranges and surrounding plains. Rainfall totals for summer 2009/10 were up to 2.5 times mean long-term totals, hence detectable changes in canopy parameters was considered likely. However, at only two of nine seasonally-monitored sites was there a greater than 10% change in mean canopy intactness between August 2010 and January 2012. The lack of a detectable change in canopy intactness for the remaining seven sites, which included all sites where mean canopy intactness was < 50%, suggests that the current canopy condition of most of the surveyed pink gums is due to factors operating over time frames much longer than two years. Similarly, for the 58 regional sites that were surveyed once in 2009/10 and once in 2011/12, there was a > 10% change in mean canopy intactness at 20% of sites and only a 5% change in the mean canopy intactness of the 1751 trees. When the five sites with lerp present were excluded from the comparison, there was no significant change in mean canopy intactness between visit 1 (70% intactness) and visit 2 (69% intactness). For approximately 80% of the 1751 pink gums, changes in canopy intactness were less than the precision of recording this parameter, namely < 10%. Despite the relatively stable mean canopy intactness of the 58 regional sites and the nine sites that were monitored seasonally, canopy intactness did change by at least 25% (both increases and decreases) in 14% of trees, with the greatest changes in individual trees at each survey site ranging from 20% to 60%. Longer term monitoring, therefore, is required to determine if the apparent population stability in canopy intactness over two years was temporary.

Over the two year survey period, no new pink gums were recorded within the 58 survey sites and 21 of the pink gums died, equating to a net loss of about 0.6% per year. The majority of trees that died had either < 10% canopy intactness at the start of the survey and/or were less than 2.2 m tall. The loss of trees was spread across 13 pink gum woodlands representing a range of habitats and there was no obvious single contributing factor causing their death. Long-term monitoring of pink gums at the survey sites is required to determine population trends of pink gums in the study region and whether the net loss of pink gums during the study period reflects a long-term population decline. The need for long-term monitoring applies more generally to *Eucalyptus* species in Australia. Lunt (2013) noted that there is no long-term monitoring system to document how forests across south-east Australia change over time, and so no sense of long-term trends.

For the nine seasonally-monitored sites, the greatest increases in canopy intactness occurred during the high rainfall spring/summer 2010/11 period. At least 90% of leaves produced between August 2010 to February 2012 occurred in spring/summer 2010/11. In contrast, only about 5% of all leaves were produced during spring/summer 2011/12. The most obvious explanation for the yearly differences was that rainfall was up to 250% above average during spring/summer 2010/11 compared with average rainfall received during spring/summer 2011/12. For seven of the nine sites, most leaf production occurred during summer 2010/11. The dominance of summer leaf production suggests that summer rains are just as important as spring and winter rains for pink gum growth. Pook *et al*. (1966) also concluded that summer rainfall was especially important for *Eucalyptus* species occurring on soils with low water-holding capacity. This has implications for the long-term canopy condition of pink gums should there be shifts in the seasonality of rainfall. Declines in summer rainfall may be just as detrimental to leaf production as declines in winter and spring rainfall.

The much reduced leaf production during 2011/12 may have been due to both much-reduced rainfall, along with the increased abundance of leaf-eating insects during spring 2011 removing a high proportion of growth buds. The greatest negative seasonal changes in canopy intactness for sites occurred during spring 2011 and summer 2011/12 when there were generally low levels of new growth and high levels of insect damage to leaves.

Both the seasonal and two year monitoring showed that leaves damaged by skeletoniser insects, leaf chewing insects and lerps were more prevalent in 2011/2012 than 2009/10. In

2011/12, there was an almost three-fold increase (10% to 28%) in the number of trees with skeletoniser damage. Although the current study did not quantify the abundance of leaf-eating insects, most of the leaf damage appears to have been caused by the gum leaf skeletoniser, *Uraba lugens* which I observed on leaves at the majority of both the two year monitored and seasonal sites. I also observed the four-spotted cup moth caterpillar, *Doratifera quadriguttata*, a leaf eater, at sites in 2011 but not in 2009.

Although there was no significant correlation between mean annual rainfall and the mean levels of insect damage at the 58 survey sites, the widespread increase in leaf damaging insects (psyllids, gum leaf skeletoniser and cup moth larvae) may have been due to the above-average rainfall in 2010/11 following three years of below-average rainfall: Outbreaks of gum leaf skeletoniser in eastern Australia have been associated with drought and flood cycles (Farr *et al.* 2004). Gum leaf skeletoniser is a native Australian defoliator of many *Eucalyptus* species and related species and outbreaks have caused significant damage to natural and commercially managed forests in Australia (Berndt and Allen 2010). Following the above-average rainfall in 2011 and subsequent dry years of 2012 and 2013, there were anecdotal accounts of outbreaks of cup moth caterpillars also being widespread in south-eastern Australia and leading to defoliation of large tracts of eucalypts (Lunt 2013). The mean percentage of leaves per canopy affected by skeletoniser and chewing damage in 2011/12 was 20% and 24% respectively. Even though this was an increase on 2010 levels, it is still within “non-outbreak” levels. After a thorough examination of the literature, Stone (1991) estimated that in non-outbreak situations on mature *Eucalyptus* trees in forests, the average level of insect defoliation ranges from approximately 3 to 20% leaf area loss annually. These figures are supported by Lowman and Heatwole (1992). During an outbreak trees can be substantially or completely defoliated. However, healthy mature eucalypts have a high capacity to recover from periodic attacks by insect herbivores, especially if the attack occurs in spring at the beginning of the growing period (Stone 1991). Because the maximum mean level of insect damage during the current study was approximately 20%, the impact of leaf-eating insects (primarily by cup moth larvae and gum leaf skeletoniser) on pink gums was likely to be temporary. Both cup moth larvae and gum leaf skeletoniser, however have a preference for juvenile *Eucalyptus* trees and their capacity to recover from damage is not as great as mature trees (Stone 1991). Chapter 7 explores damage to juvenile pink gums during the study period.

For both the seasonal and two year monitored sites, pink gums with relatively low levels of canopy intactness in 2009/10 showed the greatest increases in canopy intactness. Trees with initially lower levels of canopy intactness had the greatest scope to respond to the above-average rainfall during the survey by large increases in leaf production. Conversely, trees with near intact canopies generally declined in canopy extent. This was partly because trees with intact or near intact canopies had little scope for increasing canopy intactness. Also the short-term influence of increased insect activity on pink gum leaves possibly outweighed increases in leaf production in pink gums with more intact canopies. Irrespective of this, the results showed that individual pink gums are capable of relatively rapid increases in canopy intactness, indicating that pink gums have the potential to recover from canopy dieback. As noted above, however, the study also recorded that the majority of deaths occurred for pink gums whose canopy intactness was < 10% in 2009/10. Further study, is required to determine if there are thresholds in canopy intactness below which canopy recovery is not possible from defoliation, for various levels of water availability.

Water potential

For all the seasonally-monitored sites there were no significant correlations between tree canopy intactness and water potential parameters, including pre-dawn, midday water potential and diurnal range in water potential. In the current study, the mean ΔΨ remained low in both summer 2011 and 2012. The low diurnal range in leaf water potential for these periods reflects the relatively low pre-dawn leaf water potentials, suggesting that the trees were not water stressed. This study recorded both leaf and stem water potentials. Leaf water potential measures the water potential in individual transpiring leaves whereas stem water potential measures the water potential of the whole stem (Bogart 2006). The difference between stem water potential and leaf water potential can provide an indirect measurement of mean leaf transpiration, which varies with soil moisture conditions and vapour pressure deficit in the atmosphere. For all my survey sites, the differences between stem and leaf water potentials were relatively low, ranging from 0.1 to 1.1 MPa indicating that the trees were not transpiring more (and hence not under significantly more water stress) in summer than in winter. Over both summer 2011 and summer 2012, midday summer leaf Ψ at all sites ranged from -2.4 MPa at Clayton site B to -3.3 MPa at Altona. These values are similar to Whittington and Sinclair's (1988) recordings for pink gum mean midday leaf Ψ of -3.0 MPa and -3.2 MPa in March 1985, following a dry summer. Whittington and Sinclair (1988) suggested that -3.5 MPa is a critical water potential at which partial stomatal closure occurs in pink gums. Clayton-Greene (1983) also reported -3.5 MPa was the critical value for causing stomatal closure in *Eucalyptus melliodora* and *E. microcarpa*. Pook and Forrester (1984, cited in Snowden 2000) observed that leaf water potential values between -3 MPa and -5 MPa were required to cause leaf damage in *Eucalyptus macrorhyncha* and *E. rossii.* The present study recorded values that were less negative than -3.5 MPa, further indicating that the pink gums were not under severe water stress during summer 2011 or summer 2012. Water potential measurements reflect short-term responses to short-term changes in water availability, whereas midday water potential readings reflect changes over minutes or hours, and pre-dawn readings reflect changes in water availability over several days to several weeks (Cleary *et al.* 2009). This may explain why Camp (2004) found that physiological indicators of *Eucalyptus* species health in the South East of South Australia did not correlate well with visible signs of tree damage. Similarly, Cunningham *et al.* (2007) recorded little difference in water potential and chlorophyll fluorescence amongst river red gum (*Eucalyptus camaldulensis*) that were in good and poor condition.

Mistletoe

Between 2009 and 2011, there was a 25% net increase in the number of live mistletoes hosted by the 1751 pink gums, which included an above-average rainfall period. The number of live mistletoes also increased at the seasonally-monitored sites. The relative increase in mistletoes, however, varied widely both between trees within a woodland, and between woodlands. Of the 34 re-surveyed sites where mistletoes were present in either 2009/10 or 2011/12, the number of live mistletoes declined at seven sites and increased at 19 sites. Because the regional study (Chapter 1) recorded mistletoe as having the greatest correlation with pink gum canopy intactness, the long-term trend of mistletoe numbers is critical in predicting the long-term trend in canopy intactness of pink gums. The two year study was not long enough to determine longer term trends in the regional mistletoe population. Longer term trends in mistletoe prevalence and their association with varying yearly rainfall totals are further explored in Chapter 3.

2.6 Conclusion

Between 2009 and 2012, most declines in pink gum canopy intactness were due to insect attack on already healthy trees and most increases in canopy intactness were associated with greater leaf production following above-average rainfall. Over time frames of three months to two years, attributes relevant to measuring changes in pink gum canopies included leaf damage and leaf production. However, because the regional pink gum population was stable during the study period, the relatively low levels of canopy intactness appear to be due to causes that have been operating at time scales of much greater than two years. Water potential recordings indicated that the pink gums were not under stress at the time of measurement. Long-term monitoring of canopy intactness, and water potential measurements over periods that cover a wide spectrum of waters stress levels in pink gums, will help to determine the trend in pink gum canopy intactness. Future repeat monitoring of the regional survey sites is required to determine if the death of 21 pink gums combined with no recruitment over the two years is typical and so assess if pink gum populations are declining.

Chapter 3 Canopy condition of mistletoes (*Amyema miquelii*) and their pink gum (*Eucalyptus fasciculosa*) hosts 2003–2015

3.1 Abstract

Mistletoes are hemi-parasitic plants relying upon their host for water. Mistletoe proliferation has been implicated in tree decline around the world, including south-eastern Australia. This study compared the canopy condition of box mistletoe (*Amyema miquelii*) and their pink gum (*Eucalyptus fasciculosa*) hosts between 2003 and 2015 within three woodlands in the Mt Lofty Ranges and adjoining plains, South Australia. The study included two periods of below-average rainfall, 2006–2009 and 2011-2015, and an absence of fire. I hypothesised that pink gums with high mistletoe loads would have greater canopy loss during periods of below-average rainfall than pink gums with low or absent mistletoe infection. While the mean canopy intactness of pink gums in 2003 and 2015 was the same, there was a net decline of 60% in the number of live mistletoes. Between 2003 and 2015, fluctuations in mean pink gum canopy intactness and in the net number of live mistletoes corresponded with periods of above- and below-average rainfall. There was no evidence of brush-tailed possums, a primary mistletoe forager, at any survey woodland, nor of a decline in avian mistletoe pollinators and dispersers. Pink gums whose canopies comprised at least 20% mistletoe recorded a decrease in canopy intactness between 2010 and 2015, while there was no change in pink gums whose canopies comprised less than 20% mistletoe. At each recording period, the mean canopy intactness of pink gums which hosted a net increase in live mistletoes was significantly greater than that of pink gums which recorded a net decrease in live mistletoes. For all survey dates, the mean canopy intactness of pink gums hosting live and/or dead mistletoes was significantly lower than that of pink gums with no live and/or dead mistletoes. This high mistletoe load predisposed pink gums to canopy decline during prolonged periods of below-average rainfall, whereas pink gums with low-level mistletoe infestation were more resilient to canopy decline.

3.2 Introduction

Mistletoes are hemi-parasitic plants occurring in much of the worlds' temperate, subtropical and tropical regions (Watson and Rawsthorne 2013). In many parts of the world their natural abundance has increased due to altered environments (Mathiasen *et al*. 2008). In south-east

Australia, localised increases in box mistletoe (*Amyema miquelii*) have been implicated in the decline of *Eucalyptus* species (Norton and Reid 1997). Despite this, there are few long-term studies on the comparative canopy condition of mistletoe and its host (Turner and Smith 2016), particularly under changing environmental conditions (Spurrier and Smith 2007). Because mistletoes have only a limited ability to regulate water loss, mistletoes are expected to display greater sensitivity to water stress than their hosts (Watson 2015). Yet, most (northern hemisphere) studies have shown that mistletoe increases the risk of drought-induced mortality of its host (Dobbertin and Rigling 2006; Mutlu *et al.* 2016; Rigling *et al.* 2010; Spurrier and Smith 2007). In a two year study, Spurrier and Smith (2007) recorded high mortality of mistletoe-bearing trees during a drought, but a 97% survival rate of mistletoe-free trees. Conversely, Reid and Lange (1988) recorded increased mistletoe death and no host death during a severe drought in central Australia. Similarly, Cirocco *et al*. (2016) recorded that the impact of a hemiparasite (*Cassytha pubescens*) on its host was least when the host experienced low water status.

Factors influencing mistletoe prevalence and intensity include the presence of natural predators, and in particular, brush-tailed possums (*Trichosurus vulpecula*), the population of mistletoe pollinators and mistletoe seed dispersers, fire history, storm events, light, and host water and nutrient relations (Norton and Reid 1997; Watson 2015). Of these factors, Watson (2009) proposed that "host quality", incorporating such elements as the host's access to water and nutrients, was a key variable in accounting for the non-random occurrence of mistletoes in many systems. Norton and Stafford Smith (1999) also concluded that mistletoes are more likely to establish on hosts with greater access to water and nutrients. Similarly, Miller *et al.* (2003) recorded higher rates of mistletoe establishment on hosts with high predawn water potentials, while Reid and Stafford Smith (2000) recorded higher germination rates of mistletoe on their *Acacia* hosts during above-average rainfall years. Watson (2009) further questioned whether mistletoe infection subsequently diminishes host quality, thus reducing the likelihood of further infections.

Because mistletoe relies upon its host for water, changing climatic patterns may influence the relationship between host and mistletoe. In the Mt Lofty Ranges (MLR) and adjoining plains, South Australia, there has been a downward trend in annual rainfall totals, since the mid-1970s, largely due to declining autumn rainfall, thus extending the summer drought (Goyder Institute 2015). In the MLR, box mistletoe (*Amyema miquelii*), hereafter referred to as mistletoe, has a greater frequency on pink gums (*Eucalyptus fasciculosa*) than on any other host species (Ward 2005). Pink gum woodlands form a major component of the MLR woodlands and provide key habitat for a number of declining bird species (Szabo *et al.* 2011). Within the MLR, concern has been expressed for the low level of pink gum canopy health (Ward 2005). Because of the high prevalence of mistletoe in pink gums and its negative impact on hosts when it becomes overabundant, the long-term trends of pink gum canopy intactness and mistletoe abundance will help determine if mistletoe infestation in pink gums is a cause for concern in a drying climate. The MLR represents an island of higher rainfall native woodland that is separated by hundreds of kilometres from similar habitat in south-eastern Australia, and hence has been described as a 'canary landscape' for temperate woodlands elsewhere in Australia (Szabo *et al.* 2011). The long-term trends in comparative mistletoe and pink gum health, therefore, may serve as an indicator of host/mistletoe health in other temperate *Eucalyptus* species in south-east Australia.

Without long-term monitoring of mistletoe and host tree population dynamics factors correlated with the abundance of mistletoes at a tree and stand level, and their subsequent impact on the host population will remain unclear. Determining such correlations will enable hypotheses on underlying causes of misletoe and/or pink gum canopy decline to be tested experimentally. This study compared the canopy condition of pink gums and their mistletoe population at several points in the period 2003-2015 in the Mt Lofty Ranges during which, yearly rainfall totals varied widely. The aims were to determine at three pink gum woodlands whether: mistletoe presence was correlated with host canopy intactness; pink gum canopy intactness was increasing, decreasing or stable; mistletoe population fluctuations coincided with periods of above or below-average rainfall; mistletoe predisposed the host to canopy loss during periods of below-average rainfall; and mistletoe loss or recruitment correlated with the canopy intactness of the host.

3.3 Methods

In 2003 Ward (2005) tagged 198 pink gums within three woodlands in the Mt Lofty Ranges: Aldinga (109 trees), Sandy Creek (40) and Onkaparinga (49) (Figure 3.1). Ward (2005) selected 75 of the Aldinga trees by choosing the nearest three pink gums at randomly selected grid coordinates, for a mistletoe population study, referred to hereafter as the Aldinga population study trees. The remaining trees at Aldinga and all trees at Sandy Creek and Onkaparinga were chosen by Ward to determine traits of pink gums that influence mistletoe establishment. Ward (2005) chose one mistletoe-free pink gum and one infected pink gum within quadrats along belt transects. Hence only the Aldinga population study trees can be used as an indication of mistletoe prevalence (percentage of pink gums infected with mistletoe). In 2003, for all 198 trees, Ward scored the number of mistletoes in each pink gum, the canopy intactness of the mistletoes and the canopy intactness of each pink gum. In 2005, Ward again scored the number of live mistletoes present in each tree (Ward 2005). Of the 198 trees originally tagged by Ward, 27 were subsequently burnt in control burns in 2011 (17 at Aldinga and 10 at Onkaparinga), and I could not locate a further 10 trees. Of Ward's 75 population study trees, I relocated 52 unburnt trees, between 2009 and 2015. Hence I revisited 161 trees between November 2009 and March 2010, and again in April 2011, November 2011 and November 2015. These 161 trees had no known fire history.

Figure 3.1. Location of pink gum woodlands, monitored 2003–2015 to record changes in pink gum canopy intactness and mistletoe prevalence, and location of Kent Town weather station. Image compiled using Google Earth. 8/10/16.

Site descriptions

Survey sites were chosen by Ward as they represented areas of high mistletoe infestation. All sites are crown land conserved under the *National Parks and* Wildlife *Act* 1972. Aldinga is a 267 ha coastal plain woodland, surrounded by housing, agricultural land and a constructed wetland (NatureMaps 2016). Onkaparinga is a 154 ha woodland, which is surrounded by agricultural and peri-urban land. Sandy Creek is a 158 ha woodland and surrounded by woodland, agricultural land and quarries (NatureMaps 2016). At Aldinga and Onkaparinga, pink gum was the dominant overstorey tree and at Sandy Creek pink gum varied from being the sole dominant to co-dominant with native pine (*Callitris gracilis*) or peppermint box (*Eucalyptus odorata*). The projective foliage cover of the pink gums ranged from 10-30% at the survey sites (Croft pers obs. 2011), using the methodology outlined in Department of Sustainability and Environment (2004). All survey sites supported a native shrub and sedge understorey with an estimated combined projective foliage cover of 10–30% (Croft per obs. 2011). Mean annual rainfall at Sandy Creek, Aldinga and Onkaparinga is approximately 535 mm, 510 mm and 540 mm, respectively (GeoScience Australia 2009). The predominant soil group at Aldinga is deep sand, and at Sandy Creek and Onkaparinga, sand over clay (Data SA 2016). The slope at all sites was less than 1%.

Parameters recorded

In 2009, I recorded mean tree height of the 161 survey pink gums as 6.2 m (SE ± 0.3). Tree heights ranged from 3 to 9 m at Aldinga, ($\bar{x}$ = 6 m SE ± 0.2), 3 to 10 m at Onkaparinga, ($\bar{x}$ = 5.8 m SE ± 0.3) and 4 to 10 m at Sandy Creek, ($\bar{x}$ = 7.2 m SE ± 0.1). The Sandy Creek trees were significantly taller (ANOVA, $F = 12.47$, $p < 0.001$). Mean canopy intactness for all trees was 54% SE ± 2.3) and was not significantly different between sites (with means for Aldinga, Onkaparinga and Sandy Creek being 51% SE ± 3.4, 54% SE ± 5.0 and 60% SE ± 0.3, respectively (ANOVA $F = 1.32$, $p = 0.27$). In 2010, as part of the regional study (Chapter 1), I surveyed the nearest pink gums from between two to four locations within each of the current study's woodlands. For each woodland the percentage of pink gums with live or dead mistletoes (and the total number of pink gums surveyed) was 56% (45 trees) at Onkaparinga, 57% (69 trees) at Sandy Creek and 63% (69 trees) at Aldinga. For the three woodlands combined, live or dead mistletoe was present on 59% of pink gums.

In 2009, I was initially accompanied by Ward to ensure consistency in scoring of attributes recorded in common with Ward (2005). For pink gums, I recorded tree height, trunk diameter at breast height (DBH), canopy volume, and canopy intactness. Canopy intactness was a visual assessment of the percentage of the potential crown that contained live foliage. The potential crown was determined by the extent of the existing branching structure (Cunningham *et al.* 2007). No survey trees had a fire history (NatureMaps 2016). Multi-trunked trees that included dead trunks could still have 100% canopy intactness if growth elsewhere in the canopy was considered to have compensated for lost growth. To determine the precision of my estimates, I measured the same 30 trees at each of two survey sites at Aldinga, twice within 14 days. Based on these repeated measures of the same trees my visual estimates of canopy intactness were considered to be accurate within +/- 10%. As per Ward (2005), an approximate canopy volume was calculated from canopy depth and width using the formula for the volume of an ellipsoid, namely volume = $4/3\Pi abr$, where a and b are the north-south and east-west diameters divided by 2, respectively and r was the difference between the height of the maximum and minimum canopy foliage. I recorded the trunk diameters by either measuring with a tape measure (for lengths < 2 m) or pacing out the distance from the trunk to the edge of the canopy at the four cardinal compass points. The heights of the maximum and minimum canopy foliage were measured using a 2 m pole, or if greater than 2 m high, by extrapolation using the 2 m pole (NVMU 2015). Because tree heights were < 10 m, this method was considered accurate to within 0.5 m for heights up to 6 m and within 1 m for heights over 6 m. Trunk diameters were measured at 1.3 m above ground using a tree diameter tape. Maximum DBH was the greatest individual stem diameter for each tree.

I recorded the number of live mistletoes that were visible from ground level in each survey pink gum. The smallest mistletoe recorded was 15 cm vertical diameter. I did not actively search for smaller mistletoes to be consistent with Ward’s (2005) data - of the 297 mistletoes Ward recorded at Aldinga, 295 were at least 15 cm in diameter. Because of the very open canopy of pink gums (Boomsma and Lewis 1980) and because all trees were less than 10 m tall, the likely detection of mistletoes of > 15 cm diameter was high. As the haustorium of box mistletoe forms only at the primary point of infection (Reid and Yan 2000), each woody haustorium was recorded as one mistletoe. For each live mistletoe, I recorded the percentage of the canopy that was intact. As with host canopy, this was an assessment of the percentage of the potential canopy present. It was possible, therefore, to record a mistletoe as having 0%

canopy intact, but still be alive, and to subsequently regain foliage. I recorded the number of dead mistletoes still present on the host tree and, where obvious, the number of former mistletoe attachments. I visually estimated the percentage of the combined host and mistletoe foliage that was mistletoe foliage. Because individual mistletoes were tagged only at Aldinga (and only in 2003), and the majority of the tags had fallen off by 2009, the fate of individual mistletoes could not be tracked. Hence I followed the net changes in numbers of live and dead mistletoes on marked trees over time rather than mistletoe survival and deaths. At each survey date, I searched for the presence of pink gum lerps (*Cardiospina densitexta*) on all pink gums, whether there were signs of leaf-eating insects or leaf discolouration in the canopies, and noted if brush-tailed possum scats (a key mistletoe herbivore) were present.

Rainfall

Rainfall records were obtained from the Bureau of Meteorology's official weather station for the region (Kent Town station number 023090, Bureau of Meteorology 2015). This station is situated on the plains adjoining the Mt Lofty Ranges (Figure 3.1). Long-term average rainfall for the Kent Town station is 543 mm, which is within 10 mm of the yearly rainfall averages for Sandy Creek and Onkaparinga and within 35 mm of Aldinga's annual rainfall average (data obtained from NatureMaps 2016). I calculated the percent deviation of rainfall received from mean annual rainfall for each year from 2002–2015 inclusive (Figure 3.2). Above-average rainfalls in 2003 followed a severe (bottom 5 percentile) rainfall deficiency in 2002 (as defined by Bureau of Meteorology 2015). Rainfall for 2006 was the lowest yearly rainfall on record for the Kent Town weather station and this was followed by a further three years of below-average rainfall. Rainfall was near- or above-average in 2010 and 2011, followed by near- or below-average rainfall during 2012-2015.

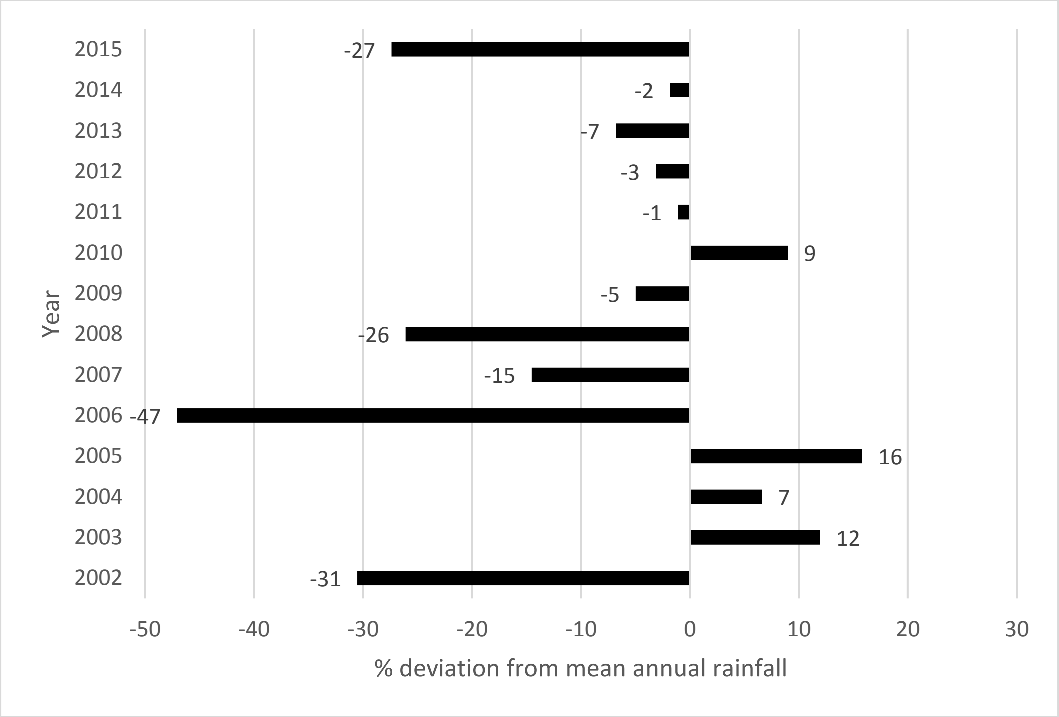

Figure 3.2: Percentage deviation from mean annual rainfall for the Kent Town weather station (023090) located in the study region, and which is representative of the three pink gum study sites (Bureau of Meteorology 2015)

Statistical analysis

All analyses were done using SPSS Version 23 software. Pearson's Product-Moment correlation was used to determine if there were significant associations between mistletoe abundance (numbers of live and/or dead mistletoes) and pink gum canopy intactness (one-tail testing). An independent t-test was used to compare the means of canopy intactness for pink gums with mistletoes and for those without mistletoes at each recording period, to further assess if there were significant correlations between mistletoes and canopy intactness.

A one-way ANOVA with repeat measures was used to test if there were differences in mean pink gum canopy intactness between survey periods. A Greenhouse-Geisser correction was used as the assumption of sphericity was not meet, and a Bonferroni post-hoc test was used to determine which means were different. These analyses were undertaken to show if canopy intactness was stable, declining or increasing over the medium to longer term. Trees which died were scored as having zero percent canopy intactness. One-way ANOVA was used to determine if the number of live mistletoes per host changed between reporting periods. A significant lowering of the mean number of mistletoes per host would suggest mistletoes were either under greater water stress, not establishing and/or suffering greater predation.

To test the hypothesis that mistletoe predisposes pink gums to canopy loss during periods of below-average rainfall, pink gums were divided into two groups: heavily infested pink gums whose canopy comprised at least 30% mistletoe and lightly infested pink gums whose canopy comprised less than 30% mistletoe, this percentage being recognised as a lower critical value for causing significant reduction in host canopy vigour (Reid and Yan 2000). A repeated measures ANOVA, with Greenhouse-Geisser correction, was used to determine if there were significant differences within each group between survey periods. This analysis was repeated by dividing mistletoes into two groups based on whether the canopy comprised at least 20% mistletoe, or not. A significant decline in host canopy intactness in the high mistletoe load group and a lack of decline in the low mistletoe load group during below-average rainfall would not falsify the hypothesis that mistletoe predisposed pink gums to canopy loss during periods of below-average rainfall.

Pearson’s correlations were undertaken to assess associations between net changes in mistletoe numbers for individual trees and host tree canopy intactness (two-tail testing), and to assess associations between mistletoe loss or recruitment and host canopy intactness.

3.4 Results

Between November 2003 and November 2015, there was a 60% decline in the net number of live mistletoes (525 to 208 mistletoes) for the 161 trees (Table 3.1). By 2015, the number of live mistletoes as a percentage of 2003 totals was 48% at Aldinga, 32% at Onkaparinga and 8% at Sandy Creek. The number of trees hosting live mistletoes declined from 102 to 69. The number of live mistletoes per host was significantly higher in 2003 and 2005 than for the other recording periods (Table 3.1). In contrast, the mean canopy intactness of the 161 pink gums was not significantly different in 2003 and 2015. The greatest declines in the number of live mistletoes occurred during prolonged periods of below-average rainfall, and the greatest increases in mean pink gum canopy intactness occurred during above-average rainfall years (Figure 3.3).

Table 3.1: Changes in the number of live mistletoes present, and the number of pink gums hosting live mistletoes between 2003 and 2015 (161 pink gums surveyed). The Aldinga mistletoe population study subset of trees was additionally monitored in 2005 by Ward (2007). Means for the number of live mistletoes per host were compared using One-way ANOVA. Means for pink gum canopy intactness were compared using repeat measures ANOVA (Bonferroni correction to determine which means were different). Superscripts represent homogenous groups.

Attribute	**November 2003**	**2005**	**Nov 2009**	**April 2011**	**Nov 2011**	**Nov 2015**
# of live mistletoes	525		238 (↓ 55%)	251 (↑ 5%)	255 (↑ 2%)	208 (↓ 18%)
# of live mistletoes Aldinga population study subset	257	301 (↑ 17%)	167 (↓ 80%)	180 (↑ 7%)	181 (↑ 0.5%)	139 (↓ 23%)
% (and number) of pink gums hosting live mistletoe (161 trees)	63% (102)		44% (71)	45% (72)	44% (71)	43% (69)
% (and number) of pink gums hosting live mistletoe Aldinga population study subset	77% (40)	87% (45)	71% (37)	75% (39)	71% (37)	69% (36)
# of live mistletoes per host tree (± SE) (F = 5.12, $p < 0.001$)	5.1 ±0.5^{A}	6.7 ± 1.1^{A}	3.4 ±0.4^{B}	3.5 ± 0.5^{B}	3.6 ± 0.6^{B}	3.0 ± 0.4^{B}
Mean pink gum canopy intactness (F = 8.09, $p < 0.001$)	53 ± 2.5^{A}	not recorded	54 ± 2.3^{A}	60 ± 2.5^{B}	62 ± 2.3^{B}	57 ± 2.4^{AB}
Proportion of canopy comprised of mistletoe	not recorded	not recorded	8.6 ± 1.5	8.4 ± 1.5	9.2 ± 1.7	10.6 ± 1.7

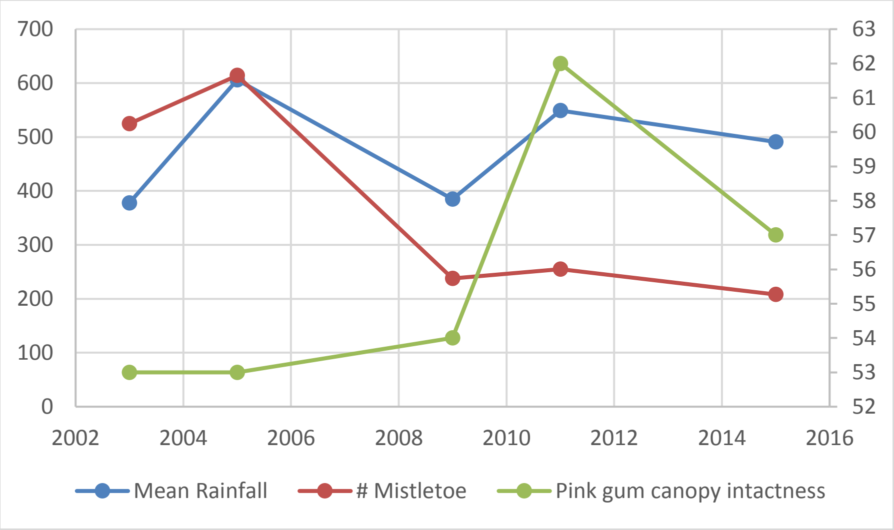

Figure 3.3: Comparison of mean yearly rainfall received and number of live mistletoes (left axis) and mean pink gum canopy intactness (right axis) recorded in 161 pink gums occurring in Sandy Creek, Aldinga and Onkaparinga woodlands in the Mt Lofty Ranges between 2003 and 2015. 2005 was recorded only for a subset of 52 pink gums from Aldinga woodland. Mean rainfall represents the mean of annual rainfall received between recording years.

The greatest inter-survey decline in the net number of live mistletoes occurred between 2003 and 2009 when there was a net decline of 55%. For the Aldinga population study trees, this decline was recorded between 2005 and 2009, which followed an increase between 2003 and 2005. There was both a decline in the number of live mistletoes per host tree and the number of trees hosting live mistletoes. Based on the results for the Aldinga subset, it was assumed that the net decline in live mistletoes between 2003 and 2009 for all the study trees occurred between 2006 and 2009/2010.

Between November 2009 and November 2011, the net number of live mistletoes present increased from 238 to 255, a 7% increase. The mean pink gum canopy intactness also increased from 54% to 62% during this period, when yearly rainfall totals were above average. Between November 2011 and November 2015, the net number of live mistletoes declined from 255 to 208, an 18% decline, and mean pink gum canopy intactness declined by 5%. This coincided with four consecutive years of below-average rainfall in the study region.

Although the mean pink gum canopy intactness in December 2015 was not significantly different from November 2003, there were approximately even numbers of pink gums that increased (56 trees, 35%), decreased (61 trees, 38%) or showed no change (44 trees, 27%) in canopy intactness. Sixteen of the 161 trees (10%) declined by at least 40% and 29 trees (18%) increased in canopy intactness by at least 40%. Between 2003 and 2015, 12 of the 161 survey pink gums died. The five trees that died between 2003 and 2009 all had less than 50% canopy intactness in 2003 and contained from 1-11 live mistletoes. Seven trees died between 2011 and 2015, of which five scored < 8% canopy intactness in 2011 and contained from 2-11 mistletoes. The remaining two trees were seemingly healthy in 2011, with 85% and 95% canopy intactness and recording zero and one live mistletoe respectively.

For those pink gums whose canopies comprised < 30% mistletoe in 2009, mean canopy intactness increased between 2009 and 2011 but then remained statistically the same until 2015 (Table 3.2). However, for pink gums whose canopies comprised at least 30% mistletoe in 2009, there was no change between 2009 and 2011, and a significant decline between 2011 and 2015 (from 21% to 8%). Similar results were obtained for means of pink gum canopies which comprised < 20% mistletoe and at least 20% mistletoe.

Table 3.2: Comparison of pink gum canopy intactness means between pink gums whose canopies comprised at least 20% or 30% mistletoe (24 and 18 trees, respectively) and pink gums whose canopies comprised less than 20% or 30% mistletoe (137 an 143 trees respectively) using ANOVA repeated measures. Superscripts represent homogeneous subgroups.

Survey period	**Mean pink gum canopy intactness**			
	Pink gums whose canopy comprised < 30% mistletoe in 2009	Pink gums whose canopy comprised ≥ 30% mistletoe in 2009	Pink gums whose canopy comprised < 20% mistletoe in 2009	Pink gums whose canopy comprised ≥ 20% mistletoe in 2009
	54 ± 2.3^{A}	25 ± 5.2^{A}	59 ± 2.3^{A}	26 ± 4.8^{A}
April 2011	60 ± 2.3^{B}	24 ± 5.2^{A}	65 ± 2.2^{B}	27 ± 4.9^{A}
November 2011	62 ± 2.3^{B}	21 ± 4.8^{AB}	68 ± 2.2^{B}	24 ± 4.8^{A}
November 2015	57 ± 2.4^{AB}	9 ± 2.7^{C}	64 ± 2.2^{A}	16 ± 4.9^{B}
	$F = 13.7$ $p < 0.001$	$F = 12.7$ $p < 0.001$	$F = 6.04$ $p = 0.012$	$F = 5.35$ $p = 0.007$

Correlation between pink gum canopy intactness and mistletoe infestation levels

For all survey dates, the mean canopy intactness of pink gums hosting live and/or dead mistletoes was significantly less than the mean canopy intactness of pink gums with no live and/or dead mistletoes (Table 3.3). For both groups, there was no difference in mean canopy intactness between the survey periods. For all survey years there were significant negative correlations between the number of live and dead mistletoes and pink gum canopy intactness and between live mistletoes only and host canopy intactness (Table 3.4).

Table 3.3: Comparison of mean canopy intactness of mistletoe-free pink gums and mistletoe-hosting pink gums at each recording period in 2009, 2011 and 2015 (using independent t-test, one-tailed). The number of trees with mistletoe was 112 and without mistletoe was 49. Comparison of differences in mean canopy intactness between recording periods for pink gums that were not hosting mistletoes, as well as those that were bearing mistletoes used one-way ANOVA.

Survey recording month/year	Pink gums with no live or dead mistletoe	Pink gums with live or dead or former mistletoe attachments	T value	Significance p value (one tail)
mean canopy intactness ± SE				
Nov 2009	64.9 ± 3.3	49.2 ± 2.9	-3.56	0.001
April 2011	72.2 ± 3.3	54.1 ± 2.8	-3.79	< 0.001
Nov 2011	75.4 ± 3.1	55.6 ± 2.9	-4.09	< 0.001
Nov 2015	73.5 ± 3.0	49.6 ± 3.0	-4.8	< 0.001
F value	2.134	1.233		
p	0.097	0.297		

Table 3.4: Correlation values (r) between the number of live, or live and dead mistletoes present in a pink gum canopy and pink gum canopy intactness for 161 pink gums in 2003, 2009, 2011 and 2015 from three pink gum woodlands (two-tailed test)

Survey month/year	Live mistletoes only		Live plus dead plus former attachments of mistletoes	
	r	p	r	p
Nov 2003	-0.268	0.001	-0.264	0.001
Nov 2009	-0.278	0.001	-0.168	< 0.003
April 2011	-0.359	< 0.001	-0.182	0.021
Nov 2011	-0.380	< 0.001	-0.233	0.003
Nov 2015	-0.413	< 0.001	-0.178	0.0024

Mistletoe establishment and host canopy intactness

There was a significant correlation between pink gum initial canopy intactness in 2003 and the net change in mistletoe numbers for that tree (R = 0.356, p < 0.001), namely, the lower the initial canopy intactness, the greater the loss of live mistletoes (Figure 3.4).

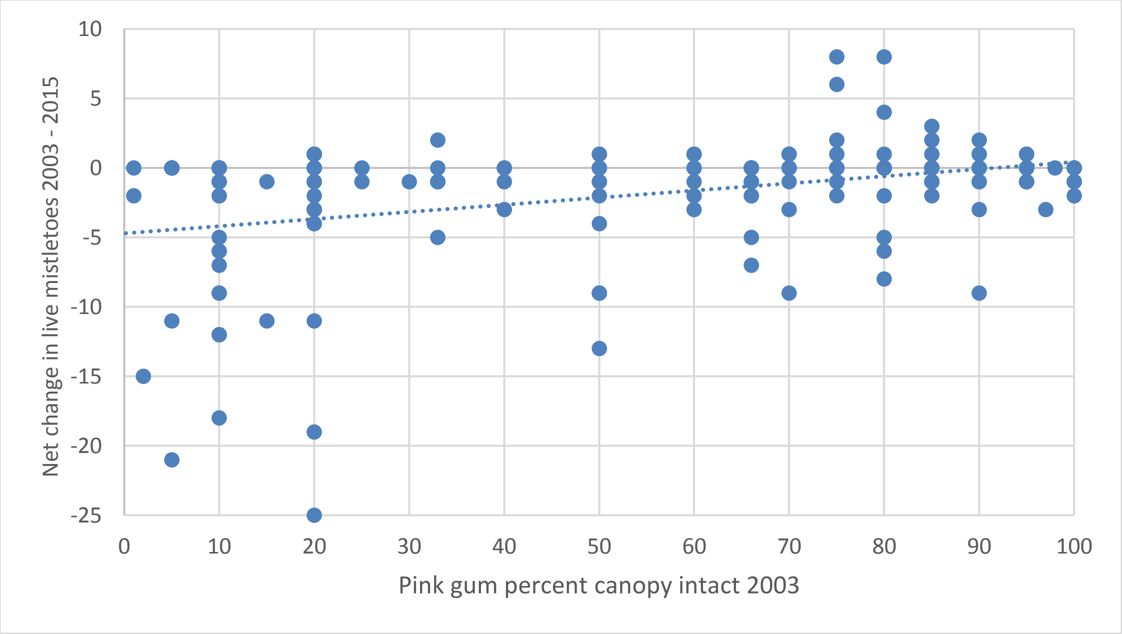

Figure 3.4. Correlation between the canopy intactness of pink gums in 2003 and the net change in the number of live mistletoes between 2003 and 2015

At each recording period, the canopy intactness of pink gums with a net increase in the number of live mistletoes was significantly greater than pink gums which recorded a net decrease in live mistletoes (Table 3.5). The largest increases in live mistletoes were within existing host trees, rather than into previously non-infected trees. Of the 59 uninfected pink gums in 2003, almost 20% (10 trees) had become infected with mistletoes by 2015, with a combined total of 11 new mistletoes. Of the trees with existing mistletoe loads in 2003, 15 trees recorded a net increase of live mistletoes, and a combined net increase of 42 live mistletoes. In 2009 there was no significant correlation between the number of live and dead mistletoes and pink gum host canopy volume (N = 161, R = -0.0458, p = 0.553).

Table 3.5: Comparison of mean canopy intactness for pink gums with: a net decline, net increase, and no change in the number of live mistletoes. For each time period, the mean canopy intactness of pink gums is for the beginning of the period. The five pink gums that died were excluded after 2009. Figures in brackets in the table represent the number of pink gums.

2003-2009		2009-2011		2011-2015	
Mistletoe change group	Mean pink gum canopy intactness ± SE	Mistletoe change group	Mean pink gum canopy intactness ± SE	Mistletoe change group	Mean pink gum canopy intactness ± SE
Net decline (75)	49 ± 3.7	Net decline (25)	41 ± 6.0	Net decline (27)	38 ± 5.7
No change (66)	54 ± 4.1	No change (105)	58 ± 2.7	No change (101)	64 ± 2.6
Net increase (20)	68 ± 5.3	Net increase (26)	62 ± 5.2	Net increase (28)	74 ± 3.1
F value	3.154		4.235		15.318
p value	0.045		0016		< 0.001

3.5 Discussion

This study recorded a 60% reduction in the net number of live mistletoes at three pink gum woodlands between 2003 and 2015 and a 40% reduction in the number of trees hosting live mistletoes, while the mean canopy intactness of pink gums was the same in 2003 and 2015. The woodlands were of similar size, soil, rainfall and vegetation type and all had a high mistletoe prevalence and intensity in 2003.

For the study trees, there was a significant correlation between pink gum canopy intactness and the number of live mistletoes per host for all survey dates. This correlation was even stronger for the total of live and dead mistletoes per host and pink gum canopy intactness. This finding contrasts with Ward (2005) who recorded no significant correlation between pink gum canopy intactness and the number of live mistletoes. Ward’s data was based on 1200 pink gums in 12 woodlands of which the current study trees were a subset. Of Ward’s study trees, 32% were infected with mistletoe with a mean of 2.8 live mistletoe per host tree.

The current study’s trees however recorded 63% of trees hosting mistletoe and a mean of 5.1 live mistletoes per host in 2003. This higher prevalence and intensity of mistletoes per host tree may explain the contrasting findings between the two studies. The weaker correlation in the current study between canopy intactness and the number of live mistletoes (not including dead mistletoes) was most likely due to the large loss of live mistletoes after 2003 and the canopy condition of pink gum being a reflection of *past* live mistletoe infection. The presence of high mistletoe loads is likely to be correlated with the relatively poor canopy condition of pink gums at the study sites, with all sites collectively having a mean canopy intactness of about 57%.

A second aim was to determine long-term trends in pink gum population canopy intactness at three woodlands where the canopy intactness of pink gums was low, ranging from 51% to 60%. Between 2003 and 2015, despite mean canopy intactness not changing, 73% of pink gums showed at least a 10% change in canopy intactness, with comparable numbers of pink gums increasing and decreasing in canopy intactness. The increase in canopy intactness of some pink gums was sufficient to offset declines in other trees. There were, however, 12 deaths of the 161 survey trees. The study design did not specifically monitor pink gum recruitment, but no new pink gum seedlings were noted along the study transects. Longer-term monitoring pink gum population size as well as canopy intactness would help determine population trends.

This study also aimed to determine whether pink gums with high mistletoe loads suffered greater canopy loss during below-average rainfall periods than pink gums with low or absent mistletoe. Mistletoe plants rely upon their host for water and solutes, hence their ability to survive depends to a large extent upon the water relations of their host (Watson 2011). To maintain the movement of water from their host, mistletoes have higher rates of transpiration and lower water potentials than their host (Whittington and Sinclair 1988). The stomata of mistletoes remain open at night, even in hot or dry conditions, whereas the host will conserve water by closing its stomata (Watson 2011). Reid and Yan (2000) noted that while heavily infected hosts often die with the onset of seasonally dry conditions, water stress may disadvantage mistletoes more than lightly infected hosts. Watson (2015) also noted that mistletoes display greater sensitivity to drought than their hosts, and periodic dry spells may reduce mistletoe numbers, even in the absence of host deaths. The current study recorded mean canopy intactness of all pink gums being the same in 2003 and 2015, but there was a difference between pink gums whose canopies had high mistletoe loads (canopies comprising at least 20% or 30% mistletoe) and those with low or no mistletoe loads (canopies comprising < 20% or < 30% mistletoe). Following four years of below-average rainfall (2006–2009), pink gums with low mistletoe loads recovered canopy intactness between 2009/10 and 2011 when rainfall was average or above average, and mean canopy intactness remained the same between 2011 and 2015 when rainfall was below average for four consecutive years. In contrast, pink gums with high mistletoe-bearing canopies did not recover canopy intactness between 2009 and 2011 and then recorded further significant declines between 2011 and 2015. These findings suggest that the mistletoes are predisposing pink gums to drought stress. Similar findings were recorded by Spurrier and Smith (2007) and Rigling *et al.* (2010). Spurrier and Smith (2007) concluded that during periodic severe drought conditions in the Mojave, California, many host trees that supported mistletoe, died during periodic severe drought, whereas almost all trees without mistletoe infestations survived. Rigling *et al.* (2010) suggested that pine mistletoe increases the risk of drought induced mortality of its host making trees more susceptible to drought stress when growing in a xeric environment due to the enhanced needle loss. During a severe drought in South Australia, in 1982, Reid and Lange (1988) recorded no deaths amongst 48 surveyed host plants, *Acacia papyrocarpa* but a combined mortality of 21% of the two species of mistletoes infecting them (*Amyema quandang* and *Lysiana exocarpi*).

A fourth aim was to determine whether the mistletoe population was increasing, decreasing or stable in the pink gum woodlands. The study recorded a 60% decline in the net number of live mistletoes between 2003 and 2015, but within this period there were fluctuations in the numbers of live mistletoes. Net numbers of live mistletoes increased between 2003 and 2005, declined between 2005 and 2009 before recovering slightly by 2011, and then again declining between 2011 and 2015. The presence of mistletoe is determined by multiple factors including climatic conditions, abundances of mistletoe predators and dispersers, and characteristics of the host tree and landscape structure, all of which operate at different spatial scales (Roura-Pascual *et al.* 2012*).* All the current study's host trees were the same species (*Eucalyptus fasciculosa*) and with similar mean, minimum and maximum heights at each woodland. The spacing of pink gums and the native shrub understorey at each woodland was also similar. All woodlands were between 150 and 280 ha and surrounded by a mix of agricultural land and peri-urban land use. The trees were also within the core of each woodland area and unlikely to be affected by severe wind events. During each survey period, no fallen branches were recorded on the ground. There were also no signs of brush-tailed possums, a key herbivore of mistletoes, (Watson 2011) at any survey date. Turner and Smith (2016) recorded a three-fold increase in mistletoe numbers between 1990 and 2004 in a *Eucalyptus* forest in south-east Australia. The authors suggested that the mistletoe population increase was part of a natural cycle of boom and bust controlled largely by occasional high-intensity wildfires. However, the study did not examine the health of the host trees nor possible fluctuations in mistletoe numbers within the study period. No trees in the current study of pink gums had a known fire history, hence alternative explanations are required for fluctuations in mistletoe numbers.

Changes in the population of mistletoe dispersers are a possible explanation for changes in mistletoe abundance. Mistletoes are dispersed primarily by the mistletoebird (*Dicaeum hirundinaceum*) but also by the spiny-cheeked honeyeater (*Acanthagenys rufogularis*) in drier habitats outside the Mt Lofty Ranges (Reid and Yan 2000). The population of mistletoebirds in the study region is considered stable and is unlikely to have declined in the study sites (David Paton pers. comm. August 2016). Caterpillars are also known herbivores of mistletoes (Watson 2015) but caterpillars were not recorded on the host trees or the mistletoes during the survey periods. *Amyema* mistletoe species are, however, short-lived relative to their hosts (Watson 2009). Reid and Yan (2000) cite a 15 year study of *Amyema* which concluded average lifespans of 10 years. It is likely, therefore, that a number of mistletoe deaths in the current study were due to mistletoes reaching their life expectancy, and if this were the case, the decline in net mistletoe numbers would have been largely due to a decline in successful establishment. This in turn is likely to have been affected by the host "quality" (Watson 2009). This study recorded greater mistletoe establishment on pink gums with more intact canopies. At each recording period, the canopy intactness of pink gums with a net increase in live mistletoes was significantly greater than pink gums which recorded a net decrease in live mistletoes. There was no significant correlation between the number of live and dead mistletoes and pink gum host canopy volume, indicating that canopy intactness rather than canopy volume was more important in influencing mistletoe recruitment levels.

By a process of elimination of alternatives, the most likely explanation for the fluctuations in mistletoe numbers during the survey period is prolonged periods of below-average rainfall. This study has also shown there was a decline in both the number of trees hosting live mistletoe and the average number of mistletoes per host during periods of below-average rainfall, with a slight increase in mistletoe abundance during higher rainfall periods. The driest period was 2006 to 2009, during which time mistletoe numbers declined by approximately 50%. The most dramatic decline in mistletoes was at Sandy Creek where the number of live mistletoes declined from 92 in 2003 to 16 in 2009 and to just seven in 2015 while at Aldinga and Onkaparinga the net number of live mistletoes reduced by 52% and 68%, respectively between 2003 and 2015.

3.6 Conclusion

Pink gums with moderate to high mistletoe loads continued to decline in canopy intactness following and during below-average rainfall periods, while pink gums with low to absent mistletoe loads were able to recover canopy intactness following prolonged periods of below-average rainfall. Future research is required to determine if the resilience of pink gums can be sustained during even longer and/or more severe periods of below-average rainfall than that experienced during the study period. If climate change predictions of longer and more severe droughts eventuate, then decreased rainfall may serve as a natural control on over-abundant mistletoes elsewhere in south-eastern Australia.

Chapter 4 Long-term changes in pink gums

4.1 Abstract

The mean canopy intactness of pink gums (*Eucalyptus fasciculosa* F. Muell) was 56% and 61% in two woodlands, Altona and Aldinga, in the Mt Lofty Ranges region in 2012. Over 50% of all trees surveyed containing mistletoe (*Amyema miquelii*). Comparison of aerial photos dated between 1949 and 2005 indicated that pink gum canopy intactness and total canopy area at the two woodlands peaked in the 1970s before commencing a declining trend. The start of canopy decline coincided with increased mistletoe infection, a long-term decline in autumn rainfall, and possibly, declining groundwater levels. The decline in autumn rainfall effectively extends the summer dry season, and this is exacerbated on sandy soils such as occurs at Altona and Aldinga. In addition, at the Altona woodland, there has been an estimated doubling of the area occupied by native pines (*Callitris gracilis*) in the pink gum study area. These may have competed with pink gums for resources. Between at least 2003 and 2015, the pink gum population at Altona and Aldinga may have temporarily stabilised after a period of high canopy loss in the preceding two to three decades.

4.2 Introduction

Tree decline is a worldwide phenomenon, and linking causal agents to decline events is often difficult, particularly when there are multiple and often interacting causes (Garkaklis and Behn 2005). Throughout the world, however, climate change has been attributed as a major cause of tree decline and/or changes in species distribution (Allen *et al*. 2010; Calder and Kirkpatrick 2008; van Mantgem *et al.* 2009). In Australia, climate change, and particularly declining rainfall, has also been implicated in tree decline, especially in the south-west and south-east of the continent (Dalmaris 2012). For example, the decline of wandoo (*Eucalyptus wandoo*) in south-west Western Australia, began in the mid-1970s and coincided with significant declines in rainfall, and especially declines in rainfall occurring at the start of the wet season of May to July, thus prolonging summer drought. Climate change, however, is often acting in conjunction with, or exacerbating other stressors, including increased habitat fragmentation, altered pathogen levels and/or changing land management (Calder and Kirkpatrick 2008).

In the Mt Lofty Ranges, pink gums (*Eucalyptus fasciculosa*) occupy a wide rainfall gradient, range of soil types and topographies. Chapter 1 documented a wide range in canopy intactness of pink gums throughout the Mt Lofty Ranges, including 11 of 78 survey sites having a mean canopy intactness of < 50%. Chapter 2 recorded a 5% decline in canopy intactness of pink gums between 2009/10 and 2011/12 at 58 survey sites. However, three other pink gum woodlands had the same mean canopy intactness in 2003 and 2015, despite significant changes in mean canopy intactness within this twelve year period. Hence, it seems likely that the current regional population condition of pink gums reflects processes that operate at longer time scales, possibly greater than 10 years. Measurements of leaf water potential are a key physiological indicator of water stress. However, measurements of pink gum leaf water potential during 2010 to 2012 did not provide insights into long-term canopy decline because no pink gums measured during the study period had high levels of water stress (Chapter 2).

Documenting changes in pink gums over time spans much longer than two years is needed to determine underlying causes of canopy decline and long-term trends in pink gum canopy intactness. This chapter uses historical aerial photography as a tool to help determine when pink gum canopy decline began at two woodlands. Such studies are rare in Australia, but include Calder and Kirkpatrick (2008) who used a time series of aerial photographs to show that severe droughts over the last several decades had coincided with the most rapid decline of cider gum (*Eucalyptus gunnii*) in Tasmania. Lehman *et al.* (2008) also used historical aerial photography to analyse multi-decadal patterns in tree cover in relation to the frequency and season of fire, feral grazing and rainfall patterns.

I hypothesised that for two pink gum woodlands where the current canopy intactness of pink gums is low, this was most likely to be because of one or more of the following: (1) long-term and continuous mistletoe infection; (2) reduced groundwater availability that commenced well before 2003; and (3) declining annual rainfall commencing several decades ago, particularly for pink gums growing in deep sandy soils. The aims of this chapter were to use historical aerial photography, long-term rainfall records, a literature review and historical data on groundwater levels to document the time frame and possible explanations for long-term changes in pink gum canopy intactness. Historical aerial photography records for the study region were chosen over using satellite imagery because aerial photography records extend back to 1949 and have a higher resolution.

4.3 Methods

4.3.1 **Literature review**

A review of literature pertaining to pink gum ecology and distribution was done to determine if there were any references to pink gum health.

4.3.2 **Long-term rainfall trends**

Rainfall trends since 1975 were analysed by calculating the cumulative deviation from annual mean rainfall for various weather stations in the Mt Lofty Ranges. Cumulative deviation from mean annual rainfall is calculated by taking the mean rainfall for each year from the actual rainfall for each year. This difference is then cumulatively added to produce a trend line (Department for Water 2010). An upward slope indicates a period in which the rainfall is greater than the average, while a downward slope indicates a period in which the rainfall is below the average (Department for Water 2010). Weather stations selected for analysis were those close to the two pink gum woodlands in this chapter's study, and which contained long-term and relatively complete data. For occasional months when data were not collected, the rainfall totals from the nearest weather station were substituted for the missing values.

4.3.3 **Historical aerial photos**

I compared a series of large scale aerial photos, dated between 1949 and 2005, to quantify projected canopy areas of pink gums (after Herwitz *et al*. 2000). The pink gums were analysed from two locations: Aldinga, a pink gum woodland on the coastal plain 45 km south of Adelaide, and from Altona, a pink gum woodland 45 km north-east of Adelaide and at the northern limits of pink gum's natural occurrence. At both sites, the soils comprise a sand layer of 1–2 m over a deep clay layer (pers obs. S Croft 2010). For each location, the area analysed was approximately 1.5 ha. The historical aerial photos were obtained from Mapland (2011). The aerial photos were scanned into digital raster form and then orthorectified (corrected for geometric distortions). This scanning and orthorectification was done by David Gibson (GIS officer, Department for Environment, Water and Natural Resources) in 2012. Photo scales varied from 1:14000 to 1:40000. To enable orthorectification, I obtained 44 spatial reference points ("Ground Control Points") in and adjacent to Aldinga and 30 spatial reference points for the Altona remnant, in both cases using a differential GPS.

I viewed the scanned photographs using ArcGIS 10.3 software and compared canopy areas between different photos. To analyse canopy changes of individual pink gums or pink gum clusters at Aldinga, I digitised all pink gum canopy outlines within a defined area using images dated 1949, 1969, 1979, 2002 and 2005. For Altona, I digitised pink gums within a defined area, in and near my field survey site (site number 5, Appendix A), using images dated 1949, 1979 and 2001. The defined areas chosen at Aldinga and Altona contained relatively large trees or clusters of trees whose canopies could be distinguished from other trees/clusters. Because the resolution of the photos analysed was similar, I considered this method to be accurate in recording relative changes in canopy size between different photo years.

4.3.4 **Changes in groundwater**

I reviewed the literature to provide a basis for understanding likely changes in water tables at the two sites. Aldinga and Altona study sites were in the McLaren Vale Prescribed Wells Area (PWA) and Barossa Prescribed Water Resources Area (PWRA) regions, respectively. All study sites in these prescribed areas were on sand over clay soils, receiving about 500 mm rainfall per year, and mistletoe loads were high. A PWA and a PWRA are prescribed under legislation if it is determined that regulation is required to ensure the proper management of that resource (AMLR NRM 2015). Of the 78 regional study sites (refer Chapter 1), 11 occurred within the McLaren Vale PWA and the Barossa PWRA (Figure 4.1). Of the 11 regional study sites that were in these prescribed areas, 10 sites had a mean canopy intactness of less than 70%. Of the remaining regional study sites only two other sites (sites 23 of 67, Appendix A) had a mean canopy intactness of less than 70%.

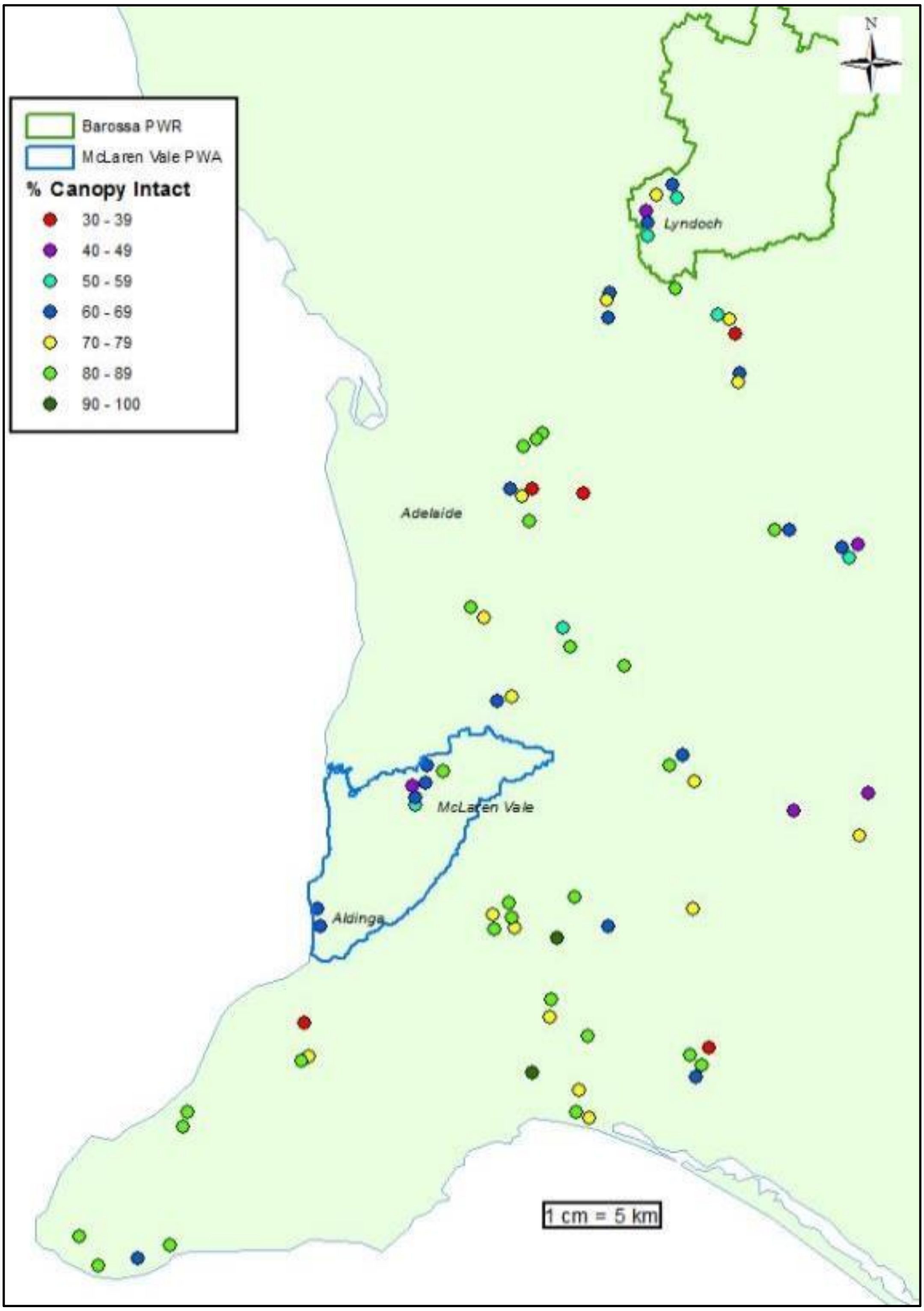

Figure 4.1: Mean percent pink gum canopy intactness at regional sites 2009 – 2011 showing location of McLaren Vale Prescribed Wells Area and Barossa Prescribed Water Resources Area. Of the 11 regional study sites that were in these prescribed areas, 10 sites had a mean canopy intactness of less than 70%.

4.4 Results

4.4.1 Historical accounts of pink gums

The earliest accounts of the ecology of eucalypts in the Mt Lofty Ranges focussed on describing relationships between habitat attributes and species distributions, along with floristic composition of communities (e.g. Adamson and Osborn 1924; Martin and Specht 1961; Specht and Perry 1948). While acknowledging this focus, no mention is made of the health of any eucalypt species, including that of pink gums, or of the prevalence or otherwise of mistletoe, implying that these were not considered issues at the time. However, various observers have noted that pink gum has a naturally sparse and open canopy. Boomsma and Lewis (1980) noted that in open scrub formation pink gum often has "a skimpy crown which appears scarcely adequate for survival". Boomsma (1981) stated that pink gum has "a thin crown which yields little shade", while Nicolle (1997) stated that pink gum has a "fairly open crown". Most of the larger pink gums with a fuller canopy are likely to have occurred on more fertile soils with higher soil water availability, and hence have been preferentially cleared for agriculture since European settlement.

In response to perceived threats to the conservation values of Aldinga, a report on the area's history and natural values was compiled (Wollaston 1989). This report considered that the greatest threats to the vegetation were weed invasion and proliferation of walking tracks. Earlier, Kraehenbuehl (1983) had noted the dieback of shrubs in Aldinga (but not of the *Eucalyptus* trees), which he attributed to the above-average summer temperatures in 1982 and 1983, low rainfall in 1982 and draining of nearby swamps to the east and south of the park with consequent lowering of the water table. In a survey of the northern part of Aldinga, Fatchen (1986 cited in Kraehenbuehl 1989) recorded dieback "of large pink gums near the remnant swamp margins". There is no mention of mistletoe in Kraehenbuehl (1989), nor in the current park vegetation management plan (Kraehenbuehl and Holton, 2001). Presumably, therefore, mistletoe was not as prevalent in the 1980s and/or not seen as a long-term threat to pink gums; and perhaps widespread net loss of pink gum canopy was not evident before the 1980s. Apart from the above literature, I have not located any other pre-1980 published references to pink gum condition in the Mt Lofty Ranges.

At the Altona study site woodland, the landholder recalls that pink gums were at their peak condition in the late 1970's, since which time they have been slowly declining (Roland

Chatterton, landholder pers. comm. 2011). The landholder also noted that the soil in the Altona woodland is now generally drier than in the late 1970s, observing that drainage lines which were once seasonally wet are now permanently dry.

4.4.2 **Long-term rainfall trends**

The nearest rainfall stations for the Aldinga and Altona survey sites are Willunga and Lyndoch respectively (station number 23753 and 23309 Bureau of Meteorology 2015). Since 1975, at these rainfall stations, there has been a declining trend in the amount of rainfall received annually, and for all seasons (Figure 4.2 to Figure 4.7). More generally, in southern Australia, the declining annual rainfall trend has largely been due to a decline in autumn rainfall with the start of the wet season (May to August) occurring later and bringing less rainfall (Bureau of Meteorology and CSIRO 2014).

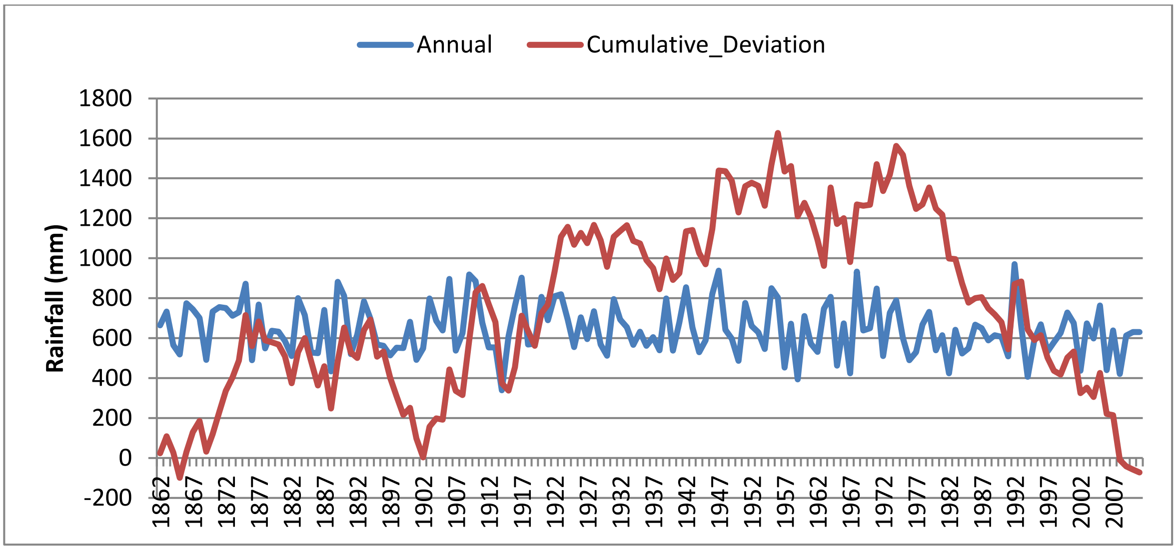

Figure 4.2: Annual rainfall and cumulative deviation from mean annual rainfall since 1862 at Willunga weather station. Figures 4.1 to 4.6 were compiled from data for Willunga station number 23753 and Lyndoch station 23309 (Bureau of Meteorology 2015). Cumulative deviation from mean annual rainfall is calculated by taking the mean rainfall for each year from the actual rainfall for each year. This difference is then cumulatively added to produce a trend line.

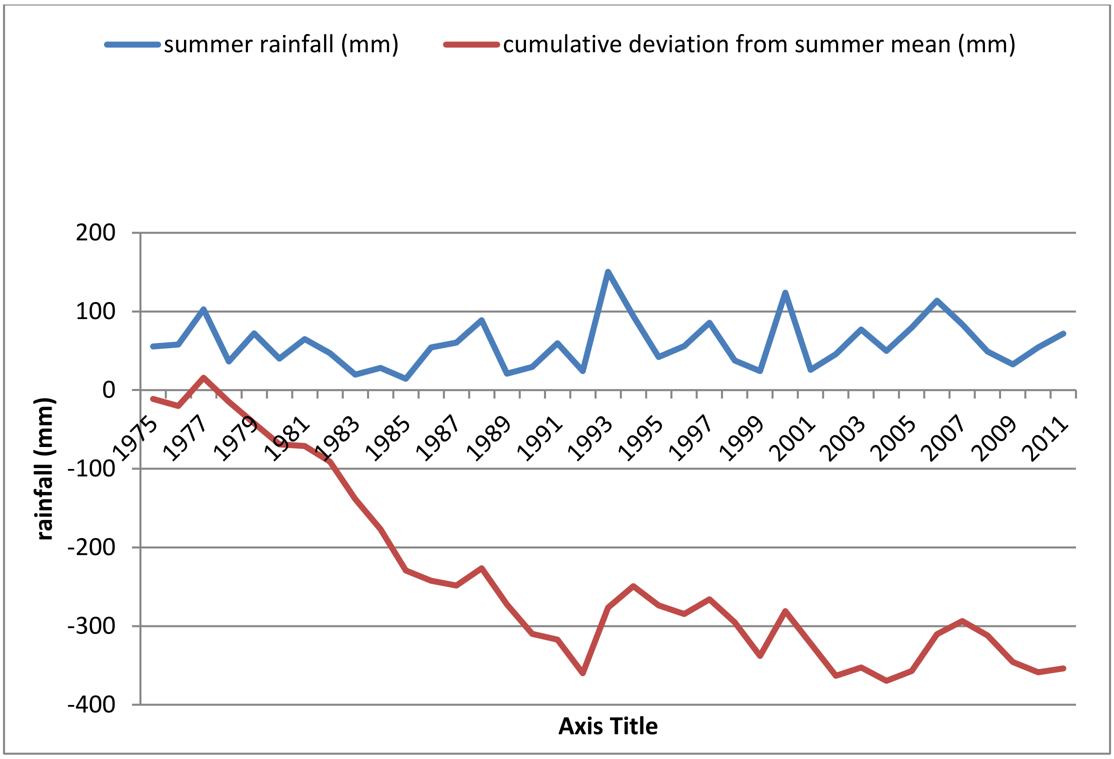

Figure 4.3: Willunga summer rainfall and cumulative deviation from mean summer rainfall 1975 to 2011

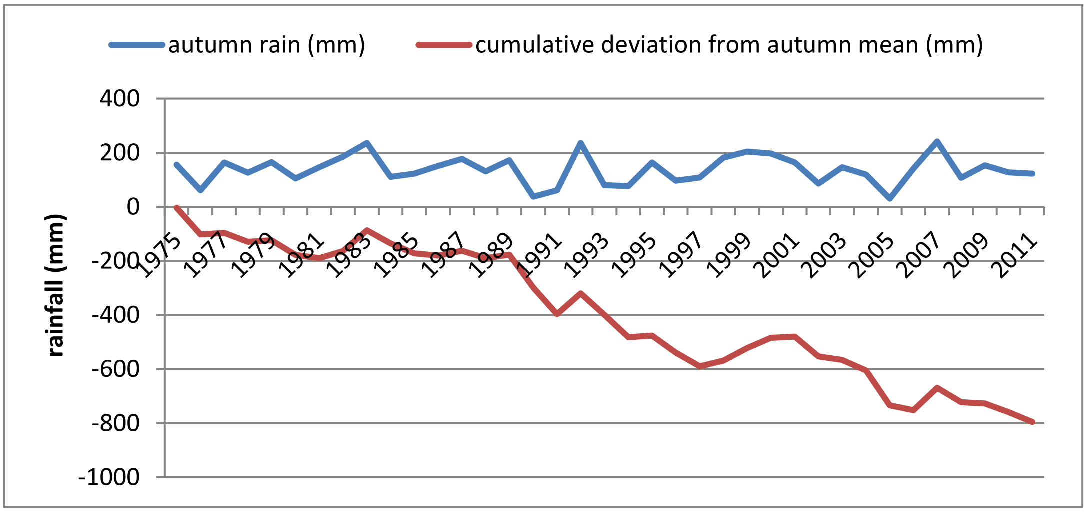

Figure 4.4**:** Willunga autumn rainfall and cumulative deviation from mean autumn rainfall 1975 to 2011

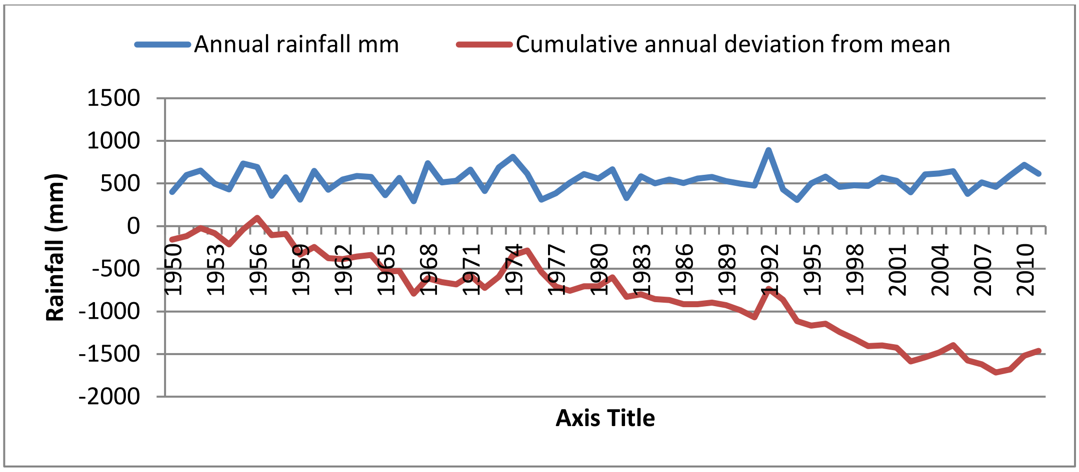

Figure 4.5**:** Annual rainfall and cumulative deviation from mean annual rainfall 1950 – 2011 at Lyndoch weather station

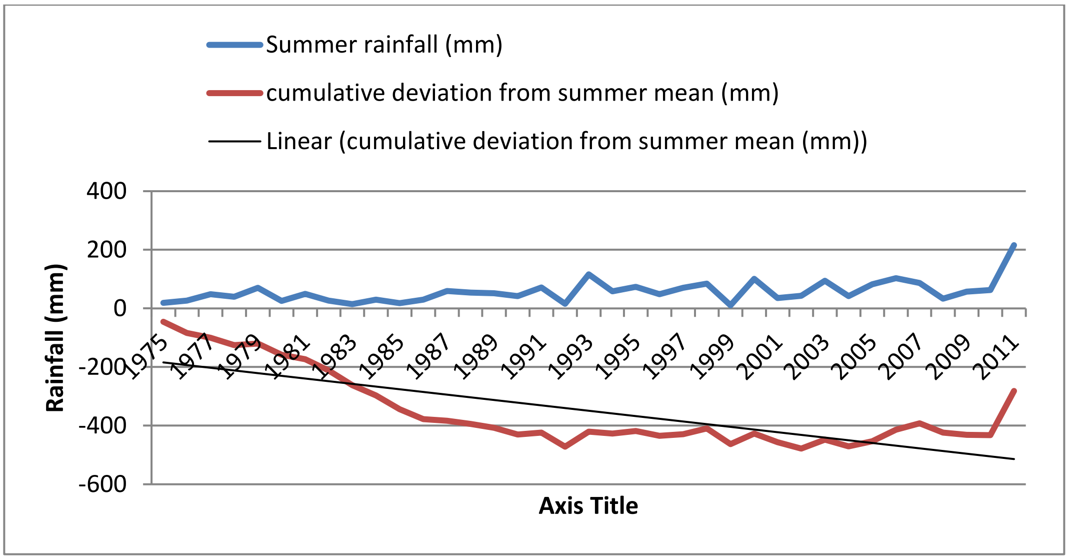

Figure 4.6: Lyndoch summer rainfall and cumulative deviation from mean summer rainfall 1975 to 2011

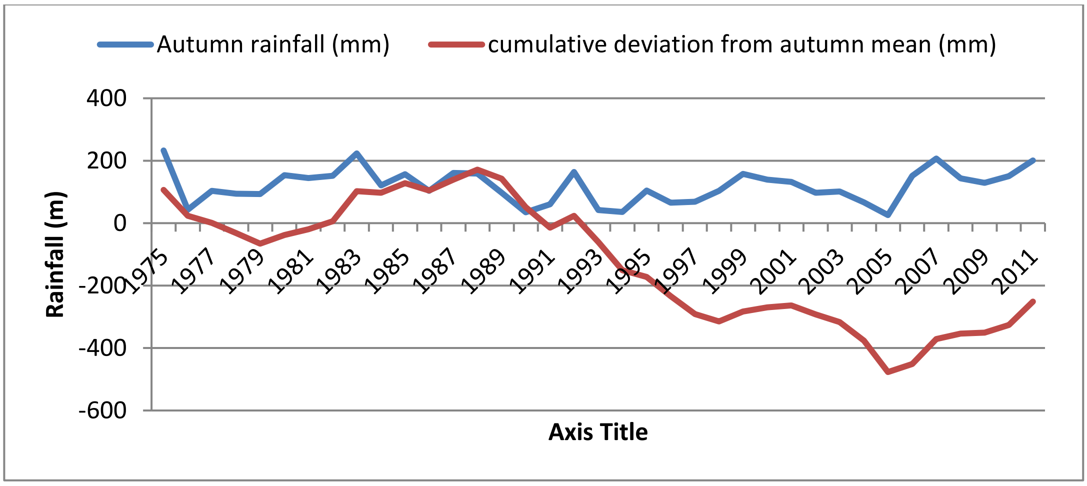

Figure 4.7: Lyndoch autumn rainfall and cumulative deviation from mean autumn rainfall 1975 – 2011

4.4.3 **Historical aerial photos: changes in canopy area**

When surveyed in 2012 as part of the current study, mean canopy intactness of pink gums at survey sites at Altona and Aldinga were 56% and 61%, respectively. The percentage of trees containing live and/or dead mistletoe was 80% at Altona and 55% at Aldinga.

Aldinga

Between 1949 and 2005, the area occupied by pink gum canopies was greatest for the 1969 imagery, after which it steadily declined until 2005 (Figure 4.8). Between 1949 and 1969 the total canopy area of the measured trees increased by almost 20%, which was accompanied by recruitment of pink gums. Because the 1949 individual tree canopies appeared to be complete, the canopy area increase appears to have been largely due to an increase in canopy size rather than an increase in canopy intactness, and to a lesser extent, due to additional trees being present. During this period the cumulative deviation from mean annual rainfall at the nearest long-term weather station (Willunga) showed an upward trend. From the 1970s however, there has been a decline in canopy intactness, accompanied by declining annual, spring and summer rainfall. Between 1969 and 2005, the canopy area of the measured trees declined by over 50% with several trees no longer discernible on the aerial image (Figure 4.9 to Figure 4.12).

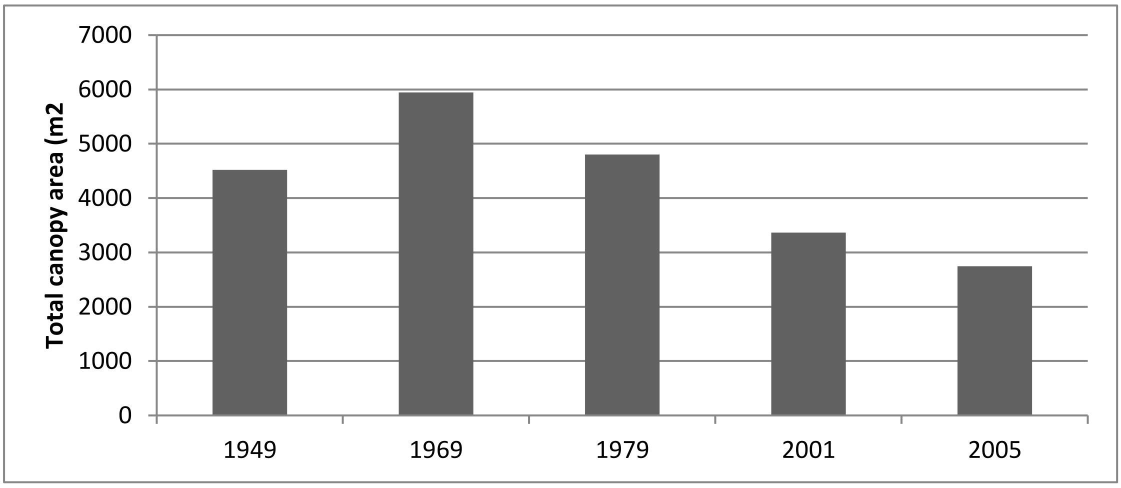

Figure 4.8: Total canopy area of trees (m^2) within a predefined area at Aldinga digitised from aerial photos dated 1949, 1969, 1979, 2001 and 2005. Trees measured are shown in Figures 4.8 to 4.11.

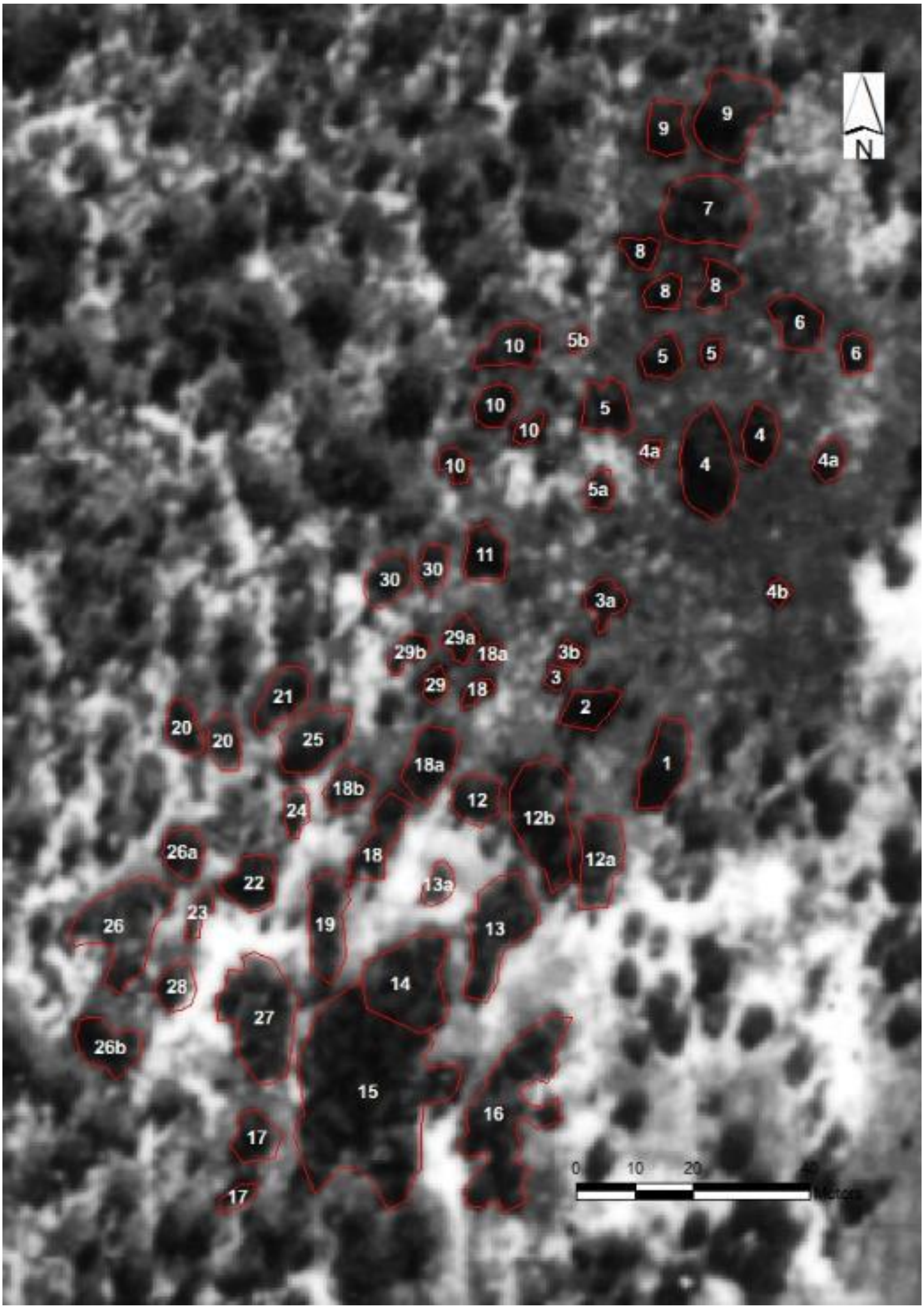

Figure 4.9: Outline of tree canopies within pre-defined area at Aldinga, digitised onto a 1949 scanned aerial photo

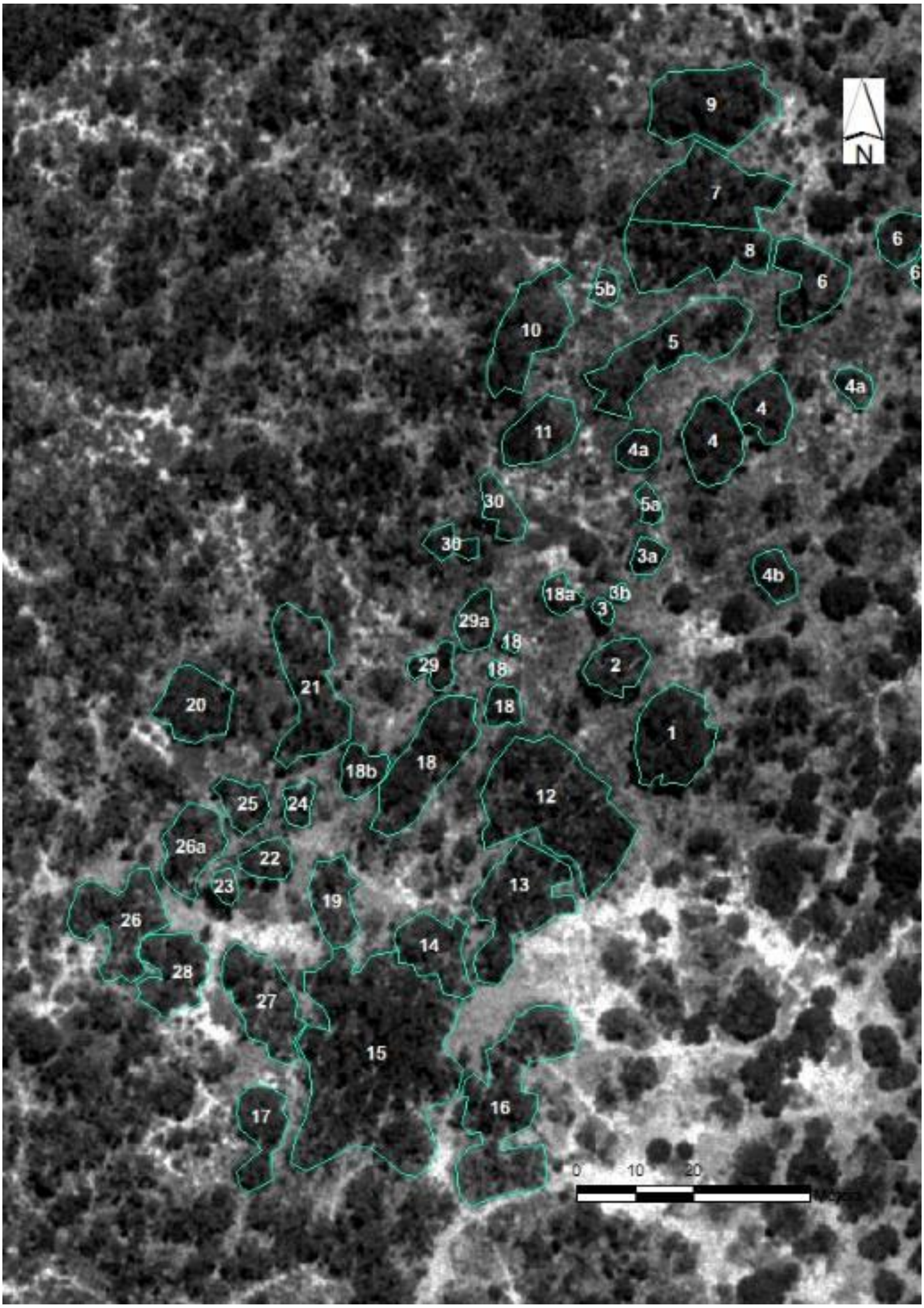

Figure 4.10: Outline of tree canopies within pre-defined area at Aldinga, digitised onto a 1969 scanned aerial photo

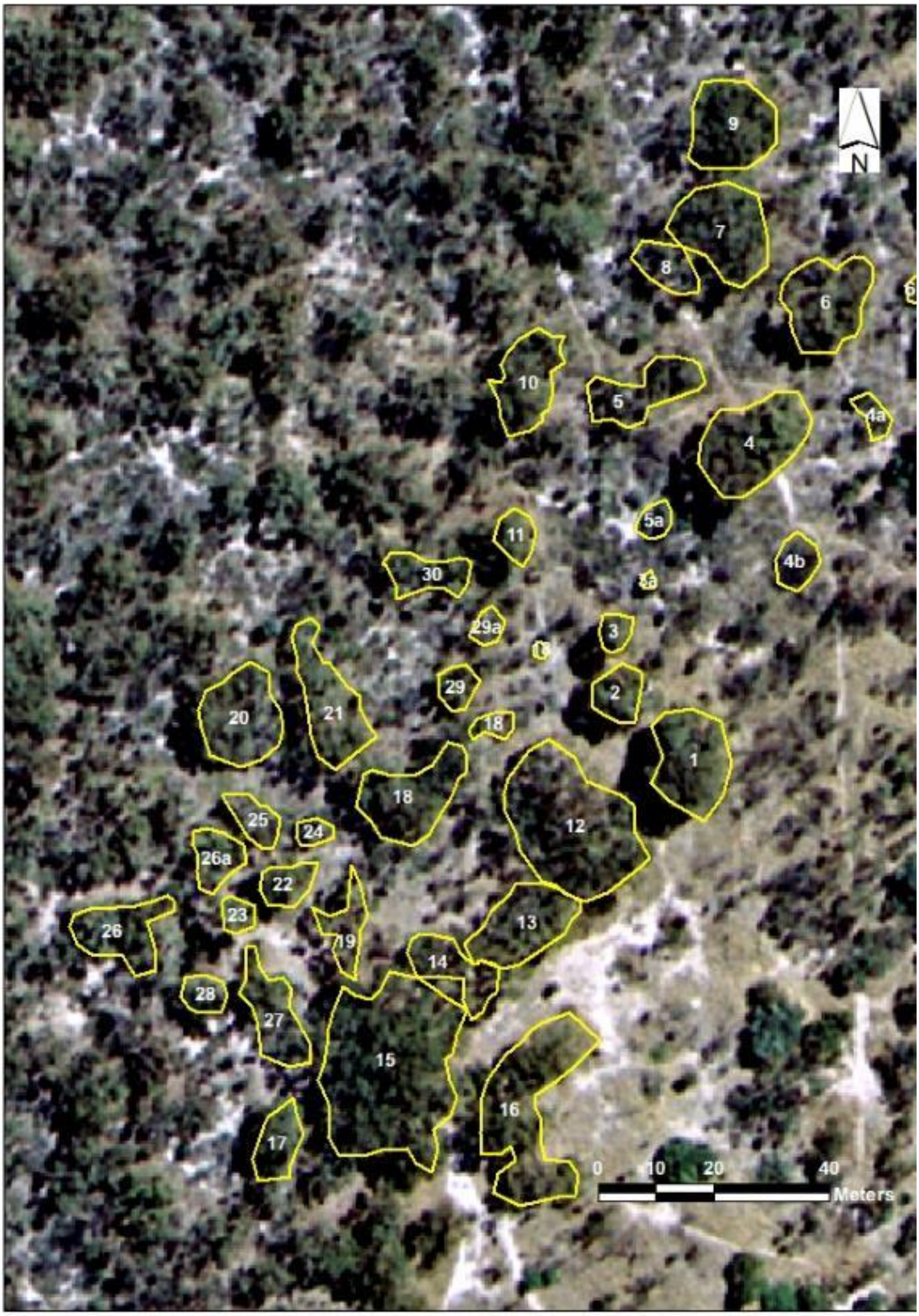

Figure 4.11: Outline of tree canopies within pre-defined area at Aldinga, digitised onto a 1979 scanned aerial photo

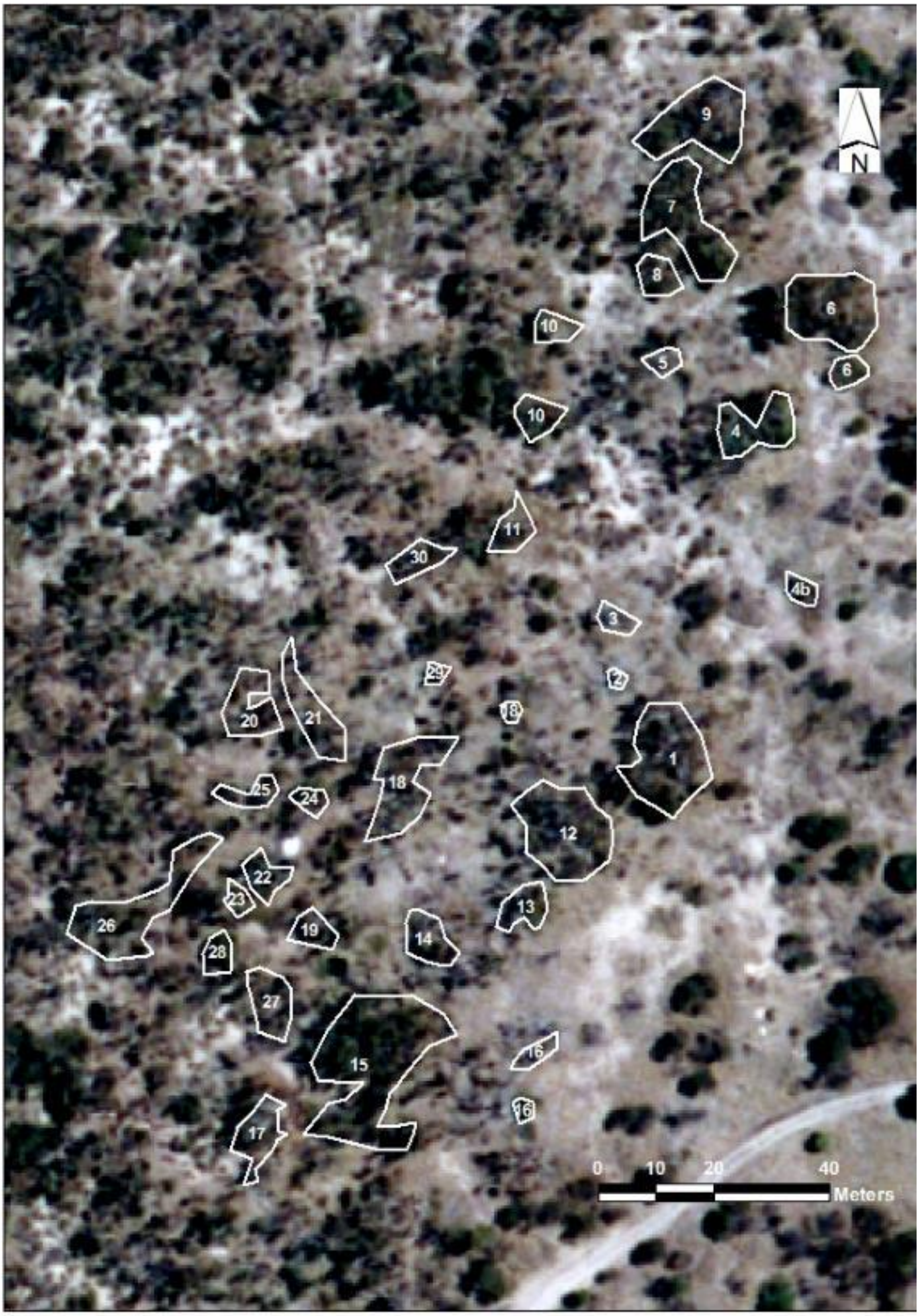

Figure 4.12: Outline of tree canopies within pre-defined area at Aldinga, digitised on a 2005 scanned aerial photo

Altona

Between 1949 and 1979, there was a 25% increase in canopy area of the pink gums within the study area. Between 1979 and 2001, however, there was a 65% decline in the canopy area of pink gums within the same area. The total canopy areas in 1949, 1979 and 2001 were approximately 16 800 m^2, 21 000 m^2 and 7 400 m^2, respectively. The magnitude of canopy decline and the years of decline corresponded with that of the Aldinga trees. In the 1949 and 1979 aerial photos for Altona of the area encompassing the 50 study trees, there were no mistletoes and few native pines (*Callitris gracilis*) were obvious. By 2001, however, the canopy of the 50 pink gums had declined and/or become less distinct, mistletoe was present in many of the larger pink gum canopies, and native pines appeared to be as numerous as the pink gums (Figure 4.13–Figure 4.14). Mistletoe has a distinct light green colour in the imagery, and the native pines were clearly discernible because of their compact dark green canopies. Imagery taken in 2014 shows the area occupied by native pines had increased still further (Figure 4.16).

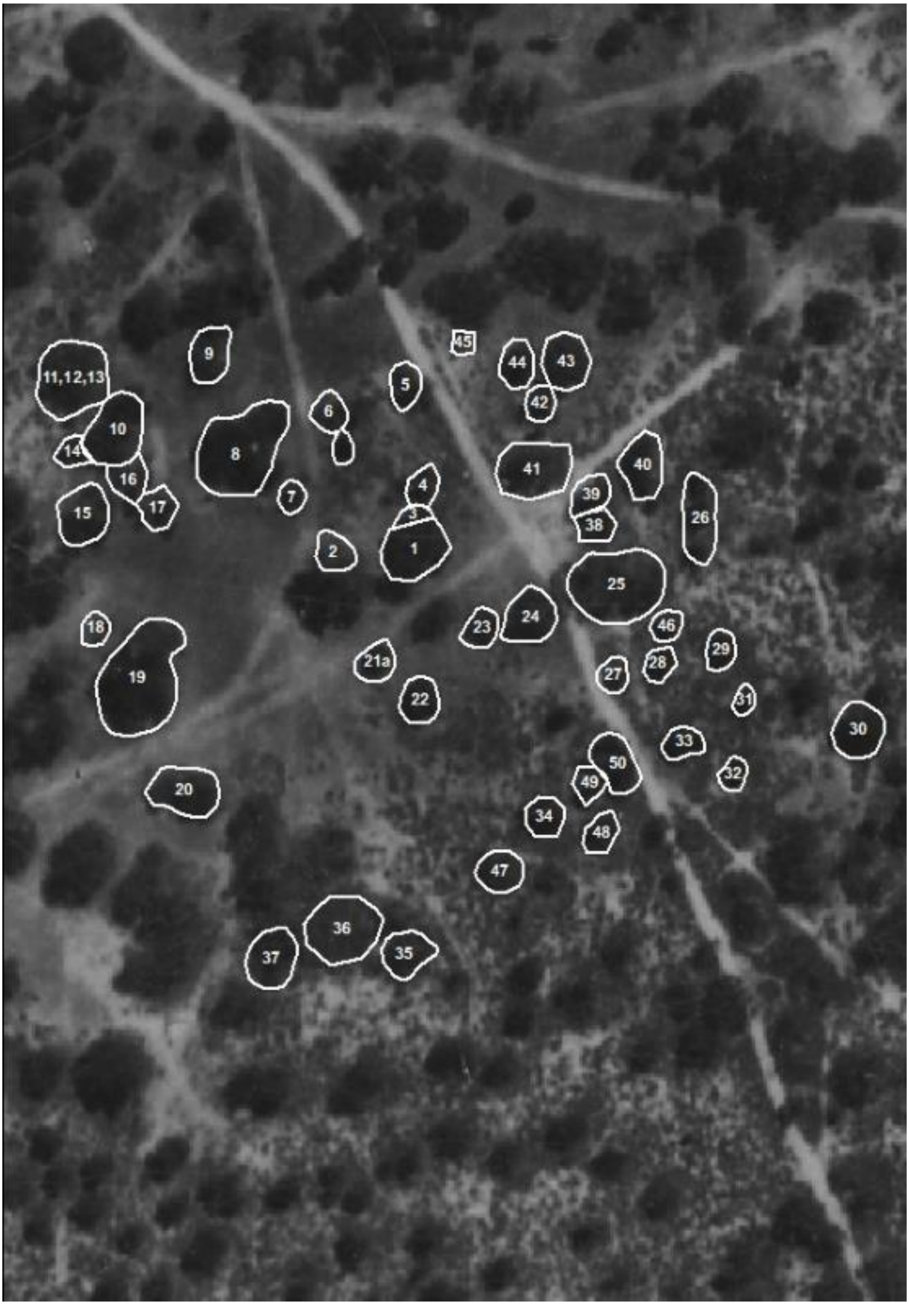

Figure 4.13: Digitised pink gum canopy outlines within the pre-defined area at Altona, 1949 aerial photo. The area is dominated by pink gums, with an absence of native pine and mistletoe.

Figure 4.14: Digitised pink gum canopy outlines within the pre-defined area at Altona, 1979 aerial photo

Figure 4.15: Digitised pink gum canopy outlines within the pre-defined area at Altona, 2001 aerial photo

Figure 4.16. 2014 imagery showing that native pines (*Callitris gracilis*) are now at least co-dominant in the survey area (darker and denser canopies than pink gums)

4.4.4 **Changes in groundwater regimes**

In the Barossa PWRA, groundwater in the upper aquifer is recharged by contemporary rainfall (Department for Water 2010). Monitoring of wells, which began in the 1970s, showed that groundwater level trends have a broad relationship with rainfall, and annual fluctuations in this aquifer are relatively large. Between 1972 and 1987, water levels generally declined by up to 0.25 m/year (AMLR NRM 2009). By 1987 water levels had begun to stabilise before rising again in 1992 following a wet spring. From 1994 to 2002 water levels again declined generally by around 0.25 m/year.

The extrapolated depth to groundwater at the Altona study site is 8-11 m (Data SA 2015). There are four groundwater wells within a 2.5 km radius of the study site (Well numbers: BRS025, BRS006, BRS012 and BRS015). Monitoring of these wells has occurred between 1987 and 2015, and at all wells groundwater has fluctuated from 2-6 m, with increases or declines reflecting annual rainfall. Figure 4.17 shows depth to groundwater fluctuating between about 9 and 11 m between 1988 and 2014 for observation well BRS015. It is likely that groundwater at the Altona study site has also fluctuated according to rainfall. In summary, depth to groundwater in the Barossa PWRA fluctuates according to rainfall, and has shown a declining trend since about 1975. The historical aerial photography also shows a decline in canopy intactness of pink gums at the Altona study site sometime after 1979.

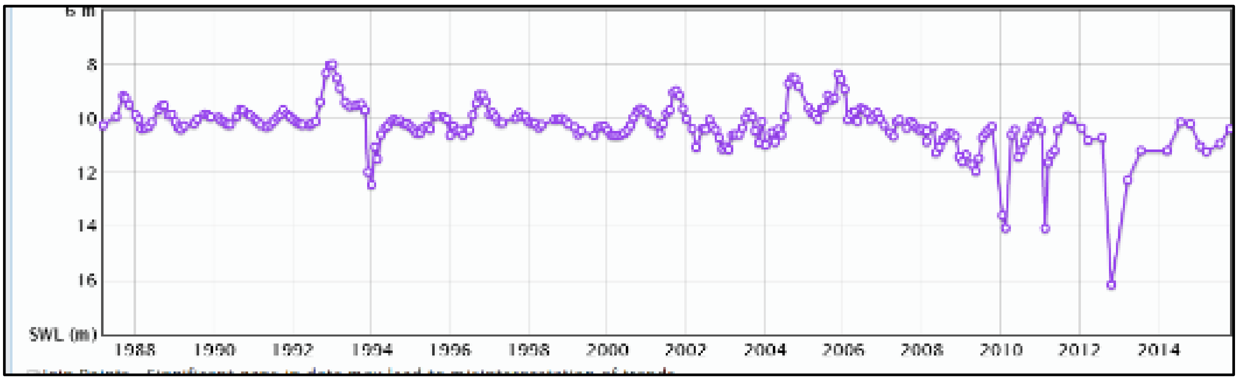

Figure 4.17: Hydrograph of depth to groundwater (Standing Water Level (SWL is shown in the Y axis) for observation well BRS015, between 1988 and 2014. Increases in 1992 reflect above-average rainfall years and decreases after 2008 reflect below-average rainfall years. Graph reproduced from Data SA (2015)

McLaren Vale PWA

The groundwater aquifers underlying Aldinga are the Quaternary Aquifer and the Willunga Formation (Department for Water 2012). The Quaternary Aquifer is relatively shallow and comprises sands and interbedded clays and also, unique to the Aldinga area, is a perched aquifer (Clarke 2002). Recharge of the Quaternary and perched aquifer is predominantly derived from local rainfall. The typical soil profile for Aldinga is a freely draining sand layer overlying a low permeability clay up to 20 m thick upon which a perched water table develops during the wetter months of the year (Ecological Associates Pty Ltd 2003). The depth of the sand layer varies from zero (in the north east) where there is seasonal water, up to about 3 m (based on piezometers in and near the park boundary (Data SA 2015). Because piezometers are all located near the park boundaries, much of the subsurface hydrology of the perched water table can only be inferred (Ecological Associates Pty Ltd 2003). The perched water table aquifer receives direct rainfall recharge and also subsurface flow originating north of Aldinga. The sloping upper surface of the clay subsurface layer means that groundwater that accumulates on the clay layer tends to flow west toward the coastline and southeast. Most water is discharged from the perched system by evapotranspiration or downward leakage into the clay layer (Ecological Associates Pty Ltd 2003).

Groundwater monitoring at Aldinga only began in 1988 and since that time groundwater levels have remained relatively stable (Ecological Associates Pty Ltd 2003). However, groundwater levels are likely to have been lowered before the 1980s. Commencing in the late 1940’s storm water diversion channels resulted in the gradual drying out of formerly large areas of swampy ground in Aldinga (Parks and Leisure Australia 2009) by both removing surface water flows and lowering the groundwater. The lowered water table is believed to have caused poorer pink gum health (Ecological Associates Pty Ltd 2003).

For most of the pink gum-dominated area at Aldinga, the extrapolated depth to groundwater is at least 10 m deep (Data SA 2015). As at the Altona study site, pink gums may have historically accessed groundwater at Aldinga, especially if groundwater levels were historically shallower, and if this were so, would have been affected by lowering of groundwater levels. Ecological Associates (2003) concluded that much of the Aldinga vegetation was historically dependent on flooding associated with the underlying perched water table. The extent and duration of flooding appears to have declined because of the diversion of storm water north of Aldinga Beach Road, the drainage of flooded land to the east of the Scrub and the interception of the perched water table by drains. Ecological Associates (2003) identified a need for replenishment of soil moisture in the scrub and proposed a wetland along the northern boundary of the scrub with provision for infiltration. Consequently the Hart Road wetland was constructed in 2009 and a water reuse scheme completed in 2013 (City of Onkaparinga 2013). The potential monitoring of the impact of this wetland on the Aldinga scrub was outside the time frame of the current study.

4.5 Discussion

The absence of published data on the condition of pink gums in the Mt Lofty Ranges before the 1970s suggests that poor canopy condition was not an issue. Comparison of aerial photographs of pink gum woodlands at Aldinga and Altona show that the decline in pink gum canopies at these sites began in the late 1970s. Between circa the mid-1970s and 2005 there was a 50% to 65% decline in the total canopy area of pink gums within parts of the pink gun woodlands at Aldinga and Altona. The most likely explanations for the decline in these pink gums since the late 1970s are: a long-term decline in autumn rainfall since the 1970s, coinciding with an increase in mistletoe abundance and decline in groundwater levels, and at Altona, competition with native pines.

Groundwater has only been monitored since about 1975 in the study areas that incorporate Aldinga and Altona. Prior to these areas being proclaimed as a Prescribed Water Resource Area and Prescribed Well Area in the 1980s (and water extraction controlled under legislation) it is likely that there was higher groundwater extraction. In addition, at Aldinga there was diversion of surface and subsurface water away from the park which began in the 1950s (Parks and Leisure Australia 2009). The series of historical photos also show that there was an increase in mistletoe abundance in the 1970s. This increase is most likely due to a combination of increased fragmentation of vegetation remnants, fire suppression and fewer leaf-eating marsupials (Reid and Yan 2000; Watson 2015).

Throughout the Mt Lofty Ranges study region the influence of long-term declining autumn rainfall and increasing summer temperatures is likely to be greatest at sites where pink gums occur on deep sandy soils (such as the Barossa and McLaren Vale areas) and where pink gums are not accessing groundwater in summer and autumn. This is because sandy soils have less water holding capacity. Pook *et al*. (1966) found that the *Eucalyptus* communities most affected by a severe drought in 1965 in eastern Australia were those growing in lighter textured and stonier soils whereas trees growing on heavier textured soils were not visibly affected. They concluded that for soils with lower moisture storing capacity, the winter-spring carry-over is insufficient to meet summer-autumn demand of woodland communities in south-eastern Australia, unless supplemented by summer-early autumn rainfall. For pink gums growing on light-textured soils, therefore, a decline in autumn rainfall may be having an impact on their long-term canopy intactness if they do not have access to groundwater.

A much larger long-term study is required to quantify the sources of water (soil and/or groundwater) and the depths at which soil water is obtained by pink gums in different parts of the study region. This would help determine the influence of past and future climate trends in areas where pink gums could be partially groundwater dependent. The rooting depth of pink gums is not known, but Jianmin and Sinclair (1993) concluded that pink gums have "deep roots" This would be consistent with most *Eucalyptus* species, which on a global scale, have consistently deep roots (Jackson *et al*. 1996). Canadell *et al*. (1996) reported that sclerophyllous trees from Mediterranean regions (like the study area) had consistently deep roots, with a mean maximum rooting depth of 12.6 ± 3.4 m (N = 11). Robinson *et al.* (2006) found that within seven years of planting, mallee *Eucalyptus* species were using soil water to at least 10 m deep. At both Aldinga and Altona the groundwater is likely to be at about this depth. It is possible therefore that pink gums may have historically used groundwater, but lowering of groundwater in the last several decades may have meant that pink gum roots can no longer access this resource. Evidence that pink gums are using groundwater in some of the regional survey sites is presented in greater depth in Chapter 9.

At Altona, native pines (*Callitris gracilis*) have increased from being occasional trees in the mid-1970s to being co-dominant or even dominant trees. The increase in native pines is likely to be largely due to the long-term absence of fire, but also to the drier soil conditions thought to have occurred at Altona since the mid-1970s. This is because native pines are fire-sensitive (Cameron 2003) and are adapted to lower rainfall than pink gums. Native pines occur on well-drained soils in south-eastern Australia in areas of 250–500 mm rainfall (Cameron 2003), which is slightly more arid than the habitat of pink gums, whose distribution occurs within 350 – 900 mm rainfall range. Cohn *et al.* (2012) found that in a study examining the impacts of encroaching *Callitris* on *Eucalyptus* trees in 360 to 600 mm rainfall study areas, *Eucalyptus* trees were more likely to be drought stressed at lower rainfalls, where canopy trees were at higher densities and soil moisture likely to be a limiting resource. As a genus, *Eucalyptus* species are intolerant to very intolerant of competition (Florence 2000). In general competition–intolerant species have naturally thin foliage and open crowns and canopies. The naturally sparse canopy of pink gums, may indicate that pink gums are at the lower end of the scale for *Eucalyptus* species tolerating competition. My observations, therefore, indicate that pink gum may be repressed by competition from native pines as a result of the long-term absence of fire.

4.6 Conclusion

Using a combination of historical aerial photography, rainfall records and groundwater data, the decline of pink gums at two sites in the Mt Lofty Ranges appears to have coincided with long-term declining rainfall, increased mistletoe infestation and historical lowering of groundwater levels which began in the 1970s. Increased density of native pines may also be a factor at one woodland. Monitoring of pink gums between 2003 and 2015, however, at one of these populations suggests that the canopy intactness may have stabilised (Chapter 3). Whether this period of stable canopy intactness is long term will most likely be determined by future rainfall trends. The main factors, therefore, considered likely to have reduced pink gum canopy intactness over the last several decades are long-term shifts in soil moisture availability and/or mistletoe infection.

Chapter 5 Pink gum (*Eucalyptus fasciculosa*) recovery from isolated outbreak of lerp (*Cardiaspina densitexta*) in the Mt Lofty Ranges, South Australia

5.1 Abstract

Pink gums (*Eucalyptus fasciculosa*) are listed under the South Australian *National Parks and Wildlife Act*, 1972 as having a Rare conservation status. The pink gum lerp (*Cardiaspina densitexta* Taylor 1962) is a sap-sucking insect and has been implicated in the poor canopy condition of pink gums. This study looked at the impact of lerps on pink gums in the Mt Lofty Ranges and adjoining plains, South Australia. In 2009/10 lerps were recorded at only two of 66 survey sites spread across the distribution of the species in the study region. In early 2011 an isolated outbreak of lerps was recorded on the western slopes of the Mt Lofty Ranges, which lasted approximately two years. Towards the end of 2011, all pink gums at the study's four survey sites within the lerp outbreak region were at least 80% defoliated, and fruit, buds and flowers were largely absent from lerp-affected pink gums. There was no significant change in mistletoe (*Amyema miquelii* Lehm. ex Miq) canopy intactness on lerp-affected trees. In July 2015 lerps were not recorded on any pink gums at the four study sites within the lerp outbreak region. At three of these sites, pink gums had recovered their pre-lerp canopy intactness of between 70% and 90%. At the fourth site, where pink gums were already affected by lerps prior to the outbreak, the pink gum canopy intactness remained significantly less than the 40% canopy level prior to the lerp outbreak. In July 2015, at all four sites, both buds and flowers were present on approximately the same percentage of trees as occurred pre-lerp infestation.

The 2011 outbreak coincided with an exceptionally wet summer, following five years of below or near-average rainfall. Lerps are not considered to be a cause of long-term canopy decline in pink gums in the Mt Lofty Ranges and surrounding plains.

5.2 Introduction

Psyllids are sap-sucking insects with *Cardiaspina* and *Glycaspis* the most common genera in Australia (Hall *et al.* 2015). The nymphs of psyllids exude a sugary protective cover, which is also referred to as lerp (Stone and Urquhart 1995). At low population levels lerps cause little damage. However, outbreaks have been recorded regularly in *Eucalyptus* forests of southern and eastern Australia causing extensive damage (Hall *et al.* 2015). The sap sucking feeding of both the adult and nymph lerp insect causes death of leaf cells and the leaves to change colour from green to purple/red to brown, giving the tree a burnt appearance (Stone and Urquhart 1995). This is followed by premature leaf fall and in some cases all old leaves can be lost from the canopy. Outbreaks generally last two to three years. During this time there are several psyllid generations and cycles of canopy defoliation and regrowth. Whole stands are commonly affected (Collet 2001). Most eucalypt trees can cope with high psyllid numbers for a couple of seasons, and recover after the lerp population declines. Prolonged and repeated defoliation, however, can result in dieback, and even tree death (Phillips 1992). In eastern Australia, extensive dieback of eucalypt forests is associated with over-abundant populations of psyllids (*Glycaspis* spp.) and over-abundant bell miners (Wardell-Johnson *et al.* 2005), referred to as bell miner associated dieback. There are no records of outbreaks of *Glycaspis* species on eucalypts in South Australia but outbreaks of *Cardiaspina albitextura* are common on red gums (*Eucalyptus camaldulensis* Dehnh.) and *Cardiaspina densitexta* on pink gums (*Eucalyptus fasciculosa* F.Muell) (Phillips 1992). The *Cardiaspina* genus is the most damaging psyllid to eucalypt species (Collett 2001).

Pink gum is largely confined to South Australia, occurring in the southern Mt Lofty Ranges, the South East and Kangaroo Island regions, and with just a small incursion of approximately 10 km into western Victoria. In South Australia, concerns over the perceived poor health and lack of recruitment in pink gums led to pink gums being listed in 2008 as a Rare species in South Australia under the *National Parks and Wildlife Act* 1972 (Jason van Weenan, pers. comm. 2009). In a study assessing tree health in the south-east of South Australia, Paton *et al*. (2005) recorded the pink gum lerp on 40% of pink gums, which was the most common symptom associated with loss of pink gum canopy condition.

White's (1966) study remains the most comprehensive study of pink gum lerp ecology in South Australia. White (1970b) reported that the pink gum lerp, normally breeds only on pink gum and is most abundant in the South East region of South Australia, appearing to be "much less numerous" throughout the Mount Lofty Ranges and absent from Kangaroo Island. However, White (1970b) noted that from time to time there may be sudden enormous increases in the numbers of *Cardiaspina densitexta*, where its abundance may increase in regions outside of the South East of South Australia. White (1969) associated lerp outbreaks with climate stress. Paton *et al.* (1999) noted that outbreaks of lerp have increased in frequency since the 1950s. In early 2011, one such outbreak occurred in the Mt Lofty Ranges region of South Australia. This provided the opportunity to assess the impact of lerps on both healthy pink gums and on pink gums under pre-existing stress. I hypothesised that a favourable combination of climatic and environmental factors led to the outbreak and that the lerp outbreak would be geographically confined to a small area of the study region. I also hypothesised that pink gums with high levels of canopy intactness prior to the lerp outbreak would recover their pre-outbreak canopy intactness. Pink gum populations with low levels of canopy intactness prior to the lerp outbreak would not recover their canopy intactness. The study aimed to document: the extent of pink gum lerp in the study region, the impact of a single lerp outbreak on pink gum canopy intactness and phenology, the recovery or otherwise of pink gum canopies post the outbreak, and where present, the impact of lerps on mistletoe canopy intactness.

5.3 Methods

The regional study of pink gums (Chapter 1) surveyed 78 sites between 2009/10 and 2011/12. Of these, 66 sites were surveyed between October 2009 and March 2010. Survey site selection, pink gum tree selection and methods to record pink gum canopy attributes are detailed in Chapter 1. The survey included scanning the canopy for signs of obvious insect damage. To determine if pink gum lerps (hereafter referred to as lerps) were present, I visually scanned the entire canopy, using binoculars where necessary, for up to 30 seconds. For each tree a rapid estimate was made of the percentage of leaves that contained lerps or signs of lerp activity, collectively referred to as “lerp signs”. The lerp populations were most obviously observed by the presence of the waxy lerp case – the outer covering on leaves within which the nymphal stage of the psyllid insect develops - and/or necrotic patches where lerps had fed upon the leaves.

In spring/summer 2011/12, all pink gums from 58 of the 66 sites surveyed in 2009/10 were re-surveyed which again included searching for lerp presence. Additionally, pink gums were surveyed at 12 new survey sites in 2011/12, which also increased the geographic area surveyed for lerps. For the 58 repeat survey sites, the same pink gums were surveyed each visit. Individual pink gum trees were re-located using a combination of GPS points and field notes made during the first visit, namely, noting the distance and bearing of one tree from another and/or by using tree dimensions.

Survey to record impact of lerps on pink gum canopy

To investigate the impact of lerps on pink gum hosts during a lerp outbreak, four sites where lerps were recorded during the 2011/12 survey, were additionally surveyed in December 2011/January 2012 and in July 2015. These sites and survey dates are shown in Table 5.1.

Table 5.1: Survey month and year for four sites where lerps were recorded in 2011, and number of pink gums surveyed

Survey site	No. of pink gums	Survey month/year			
Wadmore Park	29	Dec 2009	April 2011 & Oct 2011	Jan 2012	July 2015
Montacute	37	Dec 2009	Oct 2011	Jan 2012	July 2015
Anstey Hill	29	Oct 2009	Sep 2011	Dec 2011	July 2015
Morialta	25	Dec 2009	Oct 2011	Jan 2012 & Feb 2013	July 2015

To determine the impact of lerps on the pink gum host at each of the above four sites, the following attributes were recorded at each survey date (unless stated otherwise).

Pink gum canopy intactness was a visual assessment of the percentage of living foliage compared to the estimated potential amount that would be present in a fully extended canopy (Cunningham *et al.* 2007). The number of live mistletoes and the estimated percentage of mistletoe canopy that was intact was recorded. The percentage of old leaves and dead leaves that contained lerp signs was estimated by visually scanning the entire canopy. The mean percentage of old and dead leaves with lerp signs was calculated from the individual tree estimates. The percentage of the pink gum canopy volume that comprised new, old and dead pink gum leaves was recorded for survey dates in 2011 and 2012. New leaves were leaves of the current season's growth - soft and pale green leaves, flexible and free of blemishes; old leaves were leaves more than one year old – thick, dark green, and rigid; and dead leaves were recorded where all of the leaf was discoloured (after White 1966). The abundance of flowers, buds and fruit for the whole tree was quantified using an adaptation of Paton's (2008) 'hand span' method. The mean number of fruit, buds and flowers over the whole canopy, per 'hand span' was estimated, with the area outlined by a hand span equal to about 0.04 m^2. Hence data collected was numerical rather than categorical.

Seasonal rainfall totals, prior to, during and after the outbreak (2005 to 2014), were subtracted from long-term seasonal rainfall means. Rainfall data was obtained from the Bureau of Meteorology's official weather station at Kent Town (weather station Kent Town Bureau of

Meteorology 2015), approximately 10 km from the lerp outbreak area. This was the weather station nearest to the lerp outbreak that held long term and complete records.

Statistical analysis

All statistical analyses were undertaken using SPSS software version 23. For each survey site, a one-way ANOVA with repeat measures was used to test if there were differences in mean pink gum canopy intactness between survey periods. A Greenhouse-Geisser correction was used as the assumption of sphericity was not meet, and a Bonferroni post-hoc test was used to determine which means were different. For each survey site, I tested for differences in the percentages of surveyed trees with lerps between survey periods by arcsine transforming the data and then using one-way ANOVA. Mean mistletoe canopy intactness was calculated on all live mistletoes and for mistletoes that had died since the previous recording date. For the Wadmore and Morialta survey sites, differences in mean mistletoe canopy intactness between survey periods were analysed with one-way ANOVA. For each survey site, I tested for differences in the abundance of buds, fruits and flowers between survey periods by one-way ANOVA using the Welch test (as there was no homogeneity of variance between means, nor was the distribution normal). Where significant differences were found for canopy intactness or the abundance of buds, fruits and flowers, post hoc comparisons were undertaken using Tukey HSD.

5.4 Results

Regional occurrence of lerps

Of the 66 sites surveyed in 2009/10 (“visit 1”), lerp signs were recorded on pink gums at only two sites – Montacute on the western boundary of the study region and Mt Crawford near the north-eastern boundary. At the Montacute survey site, lerp signs were recorded on 75% of the surveyed pink gums, and on a mean of 54% of old and dead leaves for affected trees. At Mt Crawford, 80% of the surveyed pink gums contained lerp signs, but with a mean of only 2% of old and dead leaves on those trees with lerp signs.

In 2011/12 (“visit 2”), in addition to the Montacute and Mt Crawford sites, lerps were recorded at a further six sites. These additional sites had also been surveyed in 2009/10 and were at Wadmore Park, Anstey Hill (two sites), Black Hill (two sites) and Morialta (one site). No lerp signs were recorded in the 14 survey sites surveyed only in 2011. Figure 5.1 shows the location of survey sites, and where lerp activity was recorded during visit 2. During visit

2, lerps were recorded within an area of approximately 50 km^2, excluding the low-level lerp site (Mt Crawford). Within this area, all sites received over 600 mm mean annual rainfall, had been burnt within the last 30 years, and pink gum densities ranged from 320 to 620 trees per hectare (Table 5.2). The pink gums were relatively small trees with mean tree heights ranging from 3.2 to 4.2 m. All survey sites were within public land reserves and contained woodlands with a native understorey. The occurrence of lerps at the Mt Crawford survey site did not appear to have increased in extent in 2011/12, being confined to only one of three survey sites within this woodland, and for each individual tree, no more than 10% of leaves contained signs of lerps.

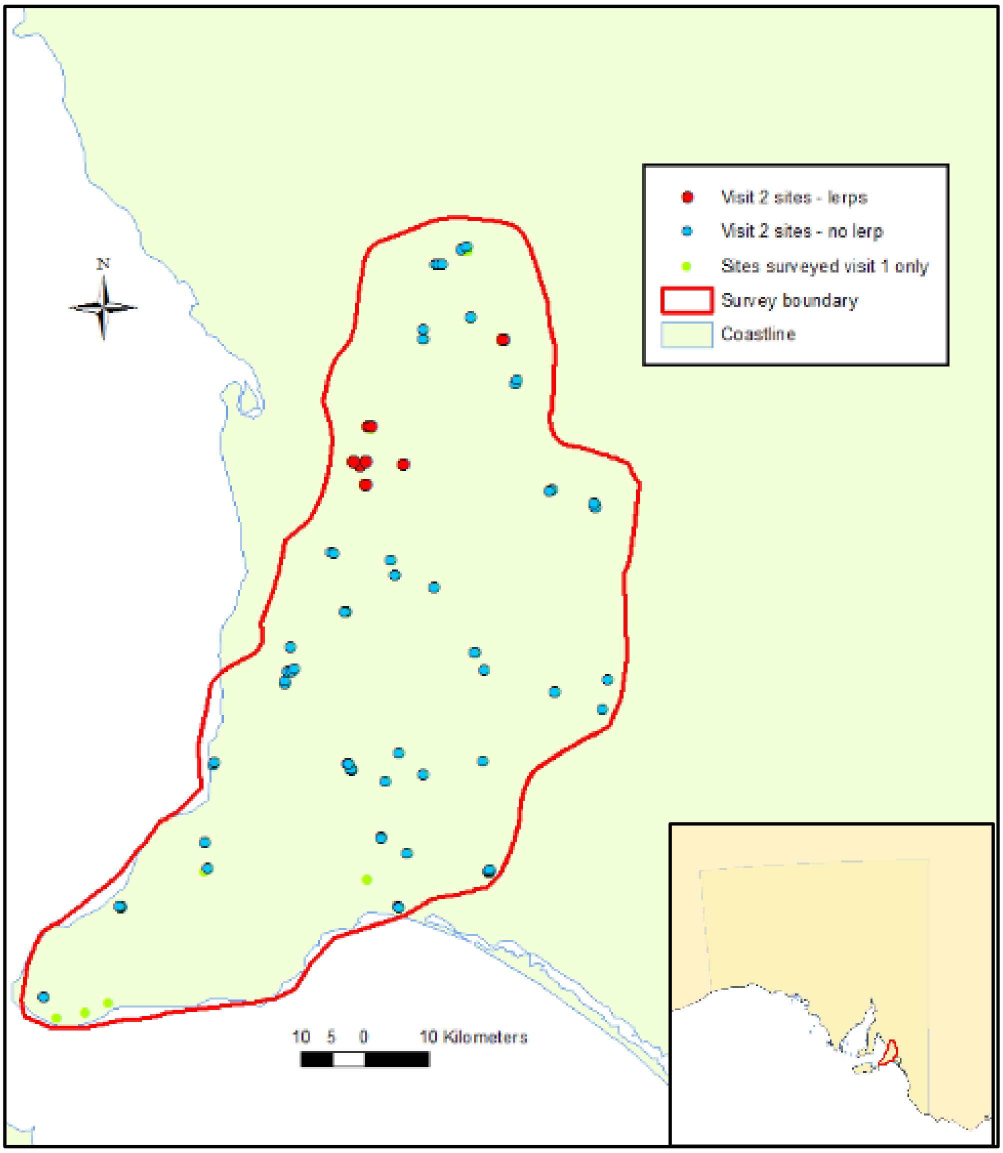

Figure 5.1. Location of pink gum survey sites surveyed during visit 2 (blue dots), visit 1 only (green dots) and survey sites where lerps were recorded during visit 2 (red dots).

Table 5.2: Pink gum height, stand density, fire history and mean annual rainfall at survey sites where lerps were recorded 2011/2012

Survey site (and # pink gums)	Number of pink gums surveyed	Mean tree height (m) ± SE	Last fire(s)*	Mean annual rainfall (mm)**	Elevation (m)	Number of pink gum trees ha^{-1} ***
Anstey Hill 1	29	4.2 ± 1.5	16/2/83	638	304	480
Anstey Hill 2	47	3.2 ± 1.0	16/2/83; 21/3/95	638	381	620
Black Hill 1	29	3.2 ± 1.1	14/1/1985	686	323	600
Black Hill 2	37	3.6 ± 1.9	14/1/85; 14/10/08	686	457	320
Montacute	37	4.1 ± 1.6	16/3/83; 30/3/10	827	500	540
Morialta	25	4.4 ± 2.3	31/12/83; 11/10/04	896	415	580
Wadmore Park	29	4.3 ± 1.9	14/1/85?	686	149	390
Mt Crawford	16	5.3 ± 1.8	> 35 years	617	450	320

*NatureMaps (2016)
** GeoScience Australia (2009).
***the number of pink gums within the survey site was extrapolated to a hectare

Climate

Visit 1 (October 2009 to March 2010) occurred during the fourth consecutive year of below-average rainfall. Between autumn 2006 and spring 2010 inclusive, seasonal rainfall totals were below average for 16 of the 19 seasons. Visit 2 (October 2011 to March 2012) followed an exceptionally wet summer (Figure 5.2). For many weather stations in the study region, the December 2010 rainfall totals were the highest on record (Bureau of Meteorology 2010).

The above-average rainfall between December 2010 and February 2011 coincided with below-average maximum daily temperatures. The mild summer maximum temperatures were followed by milder winter and spring temperatures with all months recording above-average daily minimum temperatures. In contrast, mean monthly minimum temperatures for winter 2012 (June, July and August) were all below long-term averages, but then were again above average for winter 2013. Towards the end of the lerp outbreak, monthly mean maximum temperatures were above average for 14 consecutive months between September 2012 and October 2013.

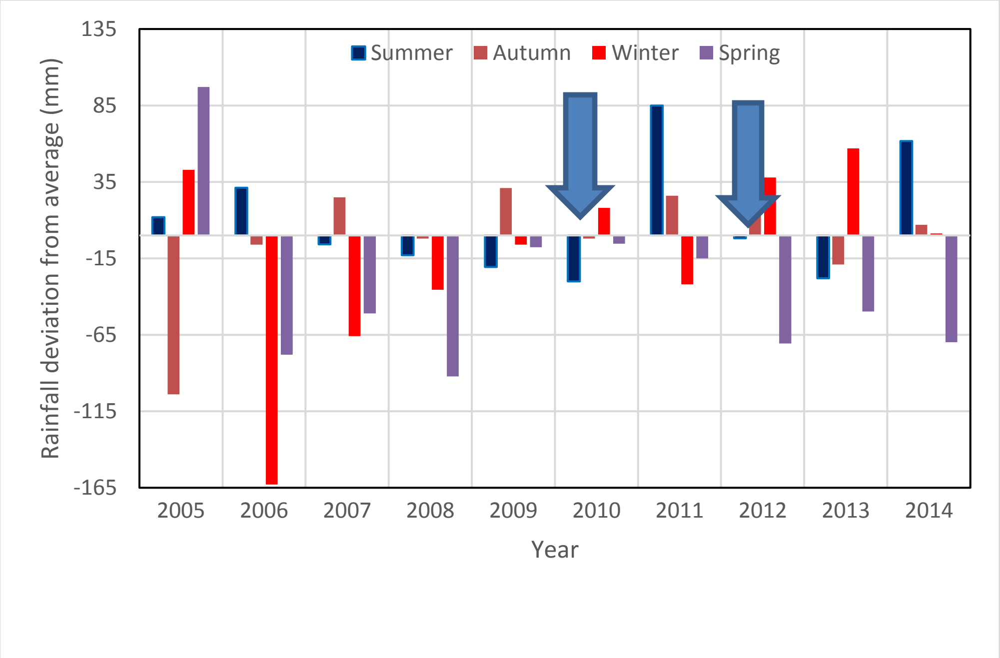

Figure 5.2: Percent deviation of seasonal rainfall received from mean seasonal rainfall for the years 2005 – 2014, at the nearest long-term weather station with complete data (Bureau of Meteorology 2015, Kent Town weather station number 023090). Blue arrows indicate visit 1 and visit 2 survey periods.

2011 lerp outbreak and impact on pink gum canopies

In April 2011, all surveyed pink gums at Wadmore Park had lerp, and on 50% of the surveyed pink gums all leaves were affected. In spring 2011, lerps were recorded on 90–100% of all surveyed pink gums at Anstey Hill, Blackhill, Montacute and Morialta. Mean canopy intactness had significantly reduced from spring 2009 levels at Wadmore, Blackhill and Morialta sites (Figure 5.3). In spring 2011, the decline in canopy intactness was greatest at Wadmore, where mean canopy intactness declined from 75% pre-outbreak to 10% in spring 2011. On 50% of trees, the only live leaves present were new leaves. At Anstey Hill and Montacute, the significant canopy decline from 2009 levels did not occur until summer 2011/12.

At all sites, defoliation was followed by new leaf growth. At Wadmore and Morialta, new leaf growth comprised 70% of the canopy in summer 2011/12 and canopy intactness had increased from the previous spring levels (Figure 5.3). The Wadmore and Morialta sites were additionally surveyed in summer 2012/13. At this time, the pink gums were continuing to recover their pre-lerp canopy intactness levels despite further re-infection(s) of lerps during 2012.

By winter 2015, no lerp signs were visible at any of the survey sites. At Morialta, Anstey Hill and Wadmore Park, there was no significant difference in canopy intactness with the 2009 (pre-lerp infestation) canopy levels (Figure 5.3). The recovery of pink gum canopies at Morialta, Anstey Hill and Wadmore where mean canopy intactness was greater than 70%, contrasts with the Montacute site. At Montacute mean canopy intactness was only 40% in 2009, and 75% of surveyed pink gums were affected by an isolated lerp population. Further, all the surveyed trees at Montacute were burnt by a prescribed burn in March 2010. In spring 2011, no new leaf growth was present on 50% of the Montacute pink gums and there was continued canopy decline in summer 2012, including the death of two trees. By 2015, the 23% mean canopy intactness was still significantly lower than the 40% in 2009. Table 5.3 presents a more detailed analysis of the progression of the lerp infection at Wadmore.

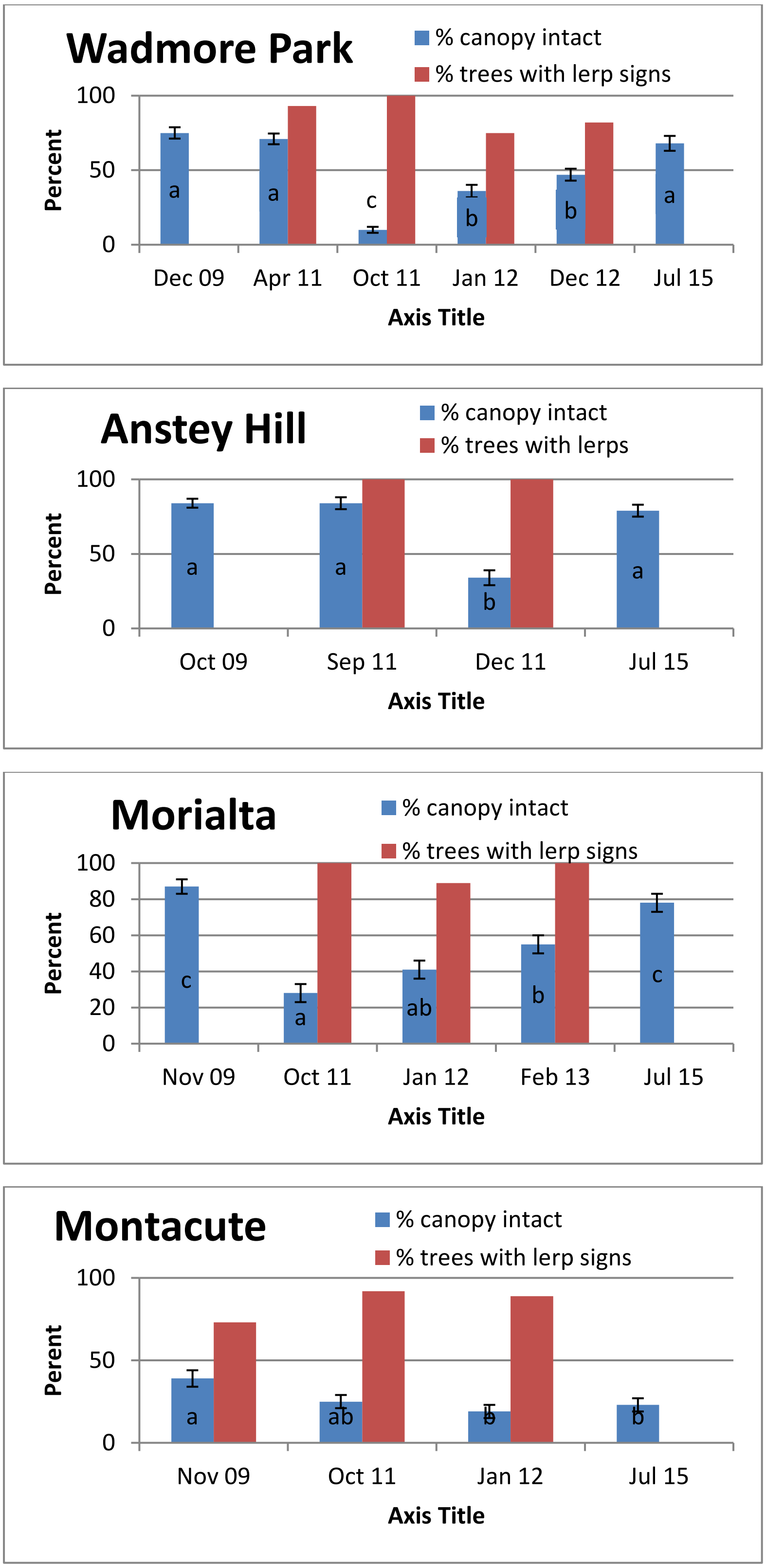

Figure 5.3: Mean canopy intactness and percentage of surveyed trees with lerp signs at Anstey Hill, Montacute, Morialta and Wadmore survey sites between spring 2009 and winter 2015. Error bars show SE. Data labels show significantly different canopy means. F values (one-way ANOVA) were 31, 33, 42 and 4, respectively, with p values being less than 0.05 for Anstey Hill, Morialta, Wadmore and Montacute. Lerp signs were leaves with lerp case and/or necrotic patches on leaves. The Blackhill site was surveyed only in spring 2009 and spring 2011 and is not shown in the figure.

Table 5.3: Percentage of leaves (other than new leaves) with lerp signs, mean pink gum canopy intactness and fruiting, flowering and bud abundance (with standard error) between December 2009 and July 2015 for 29 live pink gums at Wadmore Park survey site. Fruiting, flowering and bud abundance obtained by the handspan method (Paton 2008).

Attribute	Dec 2009	April 2011	Oct 2011	Jan 2012	Dec 2012	July 2015
Mean % canopy intact	75 ± 4	71 ± 4	10 ± 2	36 ± 4	47 ± 4	68 ± 5
Mean % of old and/or dead leaves per canopy with lerp**	0	83 ± 6	96 ± 3	15 ± 4	64 ± 7	0
Mean % canopy comprised of new leaf growth**	nr	nr	71 ± 8	70 ± 2	20 ± 2	nr
% trees with lerp*	0	97	100	75	83	0
% of trees with fruit	27	66	69	69	59	62
Mean fruiting abundance***	4 ± 2	29 ± 7	16 ± 4	12 ± 3	6 ± 2	12 ± 4
% of trees with buds and/or flowers	62	69	0	0	0	59
Mean flowering and/or bud abundance***	45 ± 12	24 ± 7	0	0	0	9 ± 2

* lerp here refers to lerp casing or evidence of lerp presence – brown/necrotic leaves due to past nymph feeding

** refer to methods for definition of new and old leaves

*** number of reproductive units present per handspan averaged for the entire canopy, present at the time of survey

Phenology changes

During October 2009 to December 2009, flowers or buds were present on at least two survey trees at each site at Wadmore, Anstey Hill and Morialta, but absent from Blackhill and Montacute (the latter was where trees were already affected by an isolated lerp population). However, between October 2011 and January 2012, no buds or flowers were recorded on any pink gums at all four survey sites. To place this in a regional context, between September 2009 and February 2010, flowers or buds were present at 31 of 44 survey sites spread across pink gum's distribution in the Mt Lofty Ranges (Croft unpublished data). Between September 2011 and February 2012, buds or flowers were present at 30 of the same 44 survey sites (and the trees surveyed were the same as those in 2009/10).

In addition to being surveyed in spring 2011, Anstey Hill, Wadmore, Morialta and Montacute were surveyed in summer 2011/12. At Anstey Hill, Morialta and Montacute buds or fruit were present in spring 2011 but not a few months later in summer 2011/12. For example, at

Anstey Hill, in September 2011, when lerps were first recorded on the trees, 60% of trees contained buds or flowers. Yet, under three months later, no flowers or buds were recorded on any trees. At Wadmore, buds or fruit were present in 69% of trees in April 2011 but were absent just six months later in October 2011 and were still absent in January 2012 and December 2012 (Table 5.3). At Wadmore, fruiting abundance significantly declined between April 2011 and December 2012, before increasing in July 2015. Between October 2011 and December 2012, fruit remaining on the survey trees were shrivelled and appeared to be those that were present in April 2011. In July 2015, flowers and buds were present at all four sites.

Mistletoe

Mistletoes (*Amyema miquelii*) were present on pink gums at the Wadmore and Morialta survey sites. Despite the lerp infestation resulting in the loss of at least 75% of old leaves on all the surveyed pink gums at Wadmore Park and Morialta in 2011/12, the total number of live mistletoes remained the same or increased during the lerp infestation period (Table 5.4) There was no significant difference in the mean canopy intactness of mistletoes for any survey period (ANOVA, F = 0.865, p = 0.507). Mistletoe was absent from Anstey Hill and Montacute.

Table 5.4: Number of live mistletoes (MT) (*Amyema miquelii*), canopy intactness and percentage of total canopy foliage in 2009 prior to the lerp outbreak,) during the outbreak (autumn 2011 to summer 2012/13) and post outbreak (July 2015) at Wadmore and Morialta survey sites.

	# live MTs	# pink gums with live MTs	# pink gums with live or dead MTs	Mean MT canopy intact (± SD)	MT % of total tree foliage (± SD)	# new MTs	# MTs that died
WADMORE PARK							
Dec 09	17	7	17	53 ± 42	8 ± 6	na	na
April 11	19	7	17	66 ± 40	9 ± 5	2	0
Oct 11	19	7	17	73 ± 36	84 ± 26	1	1
Jan 12	19	6	17	83 ± 38	61 ± 37	1	1
Dec 12	22	8	17	84 ± 36	46 ± 40	3	0
Jul 15	19	8	17	86 ± 45	13 ± 14	2	5
MORIALTA							
Dec 09	10	4	10	68 ± 40	10 ± 7	na	na
Oct 11	13	5	11	70 ± 38	51 ± 33	3	0
Jan 12	13	5	11	73 ± 36	59 ± 32	0	0
Feb 13	12	5	11	73 ± 40	63 ± 24	0	1
Jul 15	10	4	11	61 ± 44	26 ± 15	0	1

5.5 Discussion

This study has shown that the pink gum lerp (*Cardiaspina densitexta*) is usually of highly restricted occurrence in the Mt Lofty Ranges and surrounding plains, but that there can be localised outbreaks. Historical outbreaks of pink gum lerp in the study region have not been documented in the published literature, but anecdotally the outbreak in 2011 was considered the worst "in a long time" (Peddie 2011). The lerp infested sites were recorded opportunistically as part of a wider study of pink gums in the Mt Lofty Ranges and surrounding plains. During 2011, lerps were first recorded in April 2011, at Wadmore Park. Because the majority of existing leaves on the pink gums were still alive in autumn 2011, it was assumed that the lerp outbreak occurred earlier in 2011, or possibly late in 2010. The outbreak coincided with the exceptionally wet summer of 2010/11, following five years of below or near-average rainfall. Lerp outbreaks have also been recorded in eastern Australia after unusually dry and/or wet conditions (Stone and Urquhart 1995). In response to the threat posed by lerp outbreaks to eucalypts, an increasing number of studies have focussed on factors influencing lerp outbreaks (Hall *et al.* 2015; Horton 2012). In recent years it has been proposed that lerps respond positively to intermittently stressed plants (Stone *et al.* 2010). This is a modification of White's (1970) hypothesis that lerp outbreaks followed a period of prolonged water stress, without necessarily the ensuing wet conditions. The 2011 outbreak in the study region conforms with Stone *et al.*'s (2010) view.

Although not a primary aim of this study, an appraisal of the environmental conditions existing at the start of the lerp outbreak may help predict the frequency of future pink gum lerp outbreaks in the Mt Lofty Ranges region. In addition to unusually dry and/or wet conditions, Collett (2001) suggested several interacting environmental factors combine to provide conditions that are favourable for lerps to rapidly increase in numbers. These factors include an abundance of leaves of appropriate age on tree hosts, host trees growing as single species dominants, high leaf nutrients, warmer than average seasonal temperatures, enough soil moisture for the host foliage to be fully turgid as this assists the sucking action of the nymphs and adults, absence of flowering in host trees, and low populations of natural controlling agents. Within the Mt Lofty Ranges, several of these factors were present during the pink gum lerp outbreak: an exceptionally high rainfall summer following drought, milder than average summer temperatures; the host trees were the sole overstorey dominant; and, warmer than average seasonal minimum temperatures.

Hall *et al.* (2015) found that minimum winter temperature was a key driver of the rate of population growth for a *Cardiospina* species in an outbreak on *Eucalyptus moluccana*, New South Wales. Although Hall *et al.* (2015) could not identify the psyllid definitively to species level, the *Cardiospina* had similarities with other species of this genus, in particular with *Cardiaspina densitexta* and *Cardiaspina tenuitela*. During the present study, mean daily minimum temperatures for the months leading up to or at the start of the outbreak were above long-term averages for the October 2010 to February 2011 period. The mean minimum temperature for the winter months of July, August and September in 2011 were also warmer than long-term averages. Further, the study sites all received above-average yearly rainfall totals. The sites also contained relatively high densities of trees. Hall *et al.* (2015) noted that tree density may contribute to the build-up of lerp populations at the beginning of an outbreak. Therefore, in 2011, there was a favourable combination of climatic and environmental factors leading to the outbreak. In turn, the above-average summer temperatures in early 2013 in turn may have contributed to the decline of the lerps.

Climate change predictions for the Mt Lofty Ranges suggest a decrease in rainfall in all seasons and increasing heatwaves (Goyder Institute 2015). If exceptionally wet conditions are a requirement (and possibly following a drought) for lerp outbreaks in the study region, then based on current climate predictions, pink gum lerp outbreaks will continue to be highly sporadic and occasional. Further, if outbreaks do occur, they are not likely to be prolonged beyond two to three years due to the prediction of increased temperature extremes.

The second aim of this study was to document the impact of lerps on pink gum canopies, flowering and mistletoes hosted by pink gums, during and following a lerp outbreak. Of the 10 main psyllid genera within Australia, *Cardiaspina* are the most damaging to *Eucalyptus* species. When feeding, *Cardiaspina* species secrete substances that cause localised death of the leaf cells that possibly lead to defoliation of canopies (Stone and Urquhart 1995). At all four study sites within the present study, pink gum canopy intactness significantly declined due to defoliation. At the Wadmore site there was complete loss of old leaves on 50% of trees. At three of the four study sites, defoliation was followed by large increases in leaf production in spring and summer, even during ongoing lerp infestations. At these sites pink gum canopy intactness was high prior to the lerp outbreak, and the pink gums had recovered their pre-canopy intactness levels approximately 18 months post the outbreak. However, at the Montacute site where pink gums had not recovered their pre-2011 canopy intactness, the pink gums were already in poor health and affected by lerps prior to the outbreak. A fire in

2010 may have caused further stress on the trees. During this two year lerp outbreak, therefore, the ability and/or speed of pink gum recovery may depend on their initial canopy intactness.

At Wadmore, no buds or flowers were present when surveyed in October 2011, January 2012 and December 2012. The mean abundance of fruit that were on the trees also declined from April 2011 to December 2012. Pink gums do not flower every year (David Paton 2015 pers. comm.) and the timing of flowering also varies greatly (Croft unpublished data), in common with other *Eucalyptus* species (Wilson 2002). Hence the lack of flowering at the lerp infestation sites cannot be attributed directly to the lerp infestation. However, during the lerp outbreak, buds were present in spring 2011 at Wadmore and Anstey Hill but were not present three months later. Bud production had resumed though, approximately 18 months after the outbreak ended. Elsewhere in Australia, there is evidence that loss of flowering is associated with over-abundant psyllids. For example, in eastern Australia an over-abundance of psyllids (*Glycaspis* sp.) (associated with over-abundance of bell miners) reduces eucalypt flowering (Horton 2012). This can eventually eliminate the seed production necessary for forest regeneration (Bell Miner Associated Dieback Working Group 2004). Apart from impairing future regeneration of the lerp-affected tree, reduced flowering will also impact fauna relying on nectar. In South Australia, pink gums are a key floral resource for the bee-keeping industry in South Australia (Paton 2008). Reduced pink gum flowering will directly impact upon this resource. In the Mt Lofty Ranges, the 2011 outbreak was restricted to a highly localised area where bee-keeping is not conducted. In the South-East region of South Australia, however, lerp outbreaks on pink gums are more frequent, and the production of honey from pink gums has declined (David Paton 2015 pers. comm.). Lerp outbreaks in commercial bee-keeping areas, particularly if of an extended duration, could be detrimental to the commercial bee-keeping industry. The lack of flowering in pink gums during a lerp outbreak will also impact upon fauna that rely on this resource.

The health of mistletoes did not appear to be affected by the lerp infestation. During the outbreak, the canopy intactness of mistletoes increased. This suggests that during the outbreak the pink gums were not under water stress and/or that defoliation did not impact upon the mistletoes’ ability to extract water from its host.

In July 2015, lerps were not recorded at any of the study sites within the 2011 lerp outbreak area. However, pink gum lerps were opportunistically recorded on pink gums near Mt Torrens in the north-east of the study region (pers obs. S. Croft 2015). From brief observations, I noted that less than 10% of canopy leaves contained lerp signs and there had been no premature leaf loss. It appears therefore, that at any one time, there are small and highly localised lerp populations present in the study region, which are not causing long-term damage to pink gums.

5.6 Conclusion

In the Mt Lofty Ranges region, the presence of lerps is not a major factor contributing to long-term canopy decline in pink gums. Although there seems to be localised lerp populations always present in the region, in most years these remain of highly restricted occurrence and/or remain at low levels. Localised outbreaks of lerps are considered to be sporadic and cyclical, and not likely to diminish the long-term canopy condition of pink gums. It is unlikely that environmental conditions favouring outbreaks are likely to increase in frequency, but should this not be the case, the ability of pink gums to recover from such outbreaks may depend upon their pre-existing canopy intactness.

Chapter 6 Impact of box mistletoe (*Amyema miquelii*) on host pink gums (*Eucalyptus fasciculosa*) in a low rainfall woodland

6.1 Abstract

Long-term declining rainfall and increased mistletoe infection have been implicated in *Eucalyptus* dieback in temperate Australia. Yet these factors have largely been studied in isolation. This study examined the impacts of mistletoe (*Amyema miquelii* Lehm. ex Miq.) on its host, pink gum (*Eucalyptus fasciculosa* F. Muell) in a low rainfall woodland, and included a mistletoe removal experiment. The study site was at Hartley, on the plains east of the Mt Lofty Ranges, South Australia. The mean annual rainfall of 380 mm is at the lower rainfall limit of pink gums' natural occurrence, and where rainfall has also been declining since 1975. The mean canopy intactness of all live and dead pink gums at the study woodland was 48%, and 90% of the pink gums contained mistletoe, or evidence of former mistletoe attachment. There was a significant association between the canopy intactness of live pink gums and the number of dead and/or old mistletoe attachments, but not with live mistletoes. Over 15 months, dimeter increases of pink gum branches without mistletoe were twice that of branches hosting mistletoe. There was a significant correlation between the increase in host canopy intactness and the number of mistletoes removed from the treatment trees. Evidence that long-term declining rainfall in an already low rainfall habitat, did not contribute to pink gum stress included: highly intact canopies were represented in all age classes of pink gum; almost all non-mistletoe bearing branches were over 90% intact; and there was a strong correlation between host epicormic growth and the percentage of canopy that comprised mistletoe. Thus, mistletoe is the primary cause of low pink gum canopy intactness in this low and declining rainfall environment, rather than being superimposed on existing long-term stress.

6.2 Introduction

Tree decline in Australia and world-wide has been linked to multiple, often interacting biotic and abiotic factors (Sanguesa-Bareda *et al.* 2012). In southern Australia, long-term declining rainfall and localised abundant mistletoe infections have been implicated in the decline of eucalypts, although these factors have usually been studied in isolation (Jurskis 2005; Reid *et al.* 2007). Mistletoe is the common name for over 1400 species of plants in four families, found on most continents (Barlow 2012). Most attach to and penetrate the branches of a tree or shrub by a haustorium, through which they absorb water and nutrients from the host plant (Barlow 2012). In undisturbed systems, mistletoes are generally rare (Watson 2015). Some species, though, have become locally over-abundant, for example, dwarf mistletoe (*Arceuthobium* spp.) in North America and *Viscum album* subspecies in Europe (Matiasen *et al.* 2008). Box mistletoe (*Amyema miquelii* Lehm. ex Miq.), family Loranthaceae, is the most common and widespread of Australia's approximately 90 mistletoe species (Atlas of Living Australia 2016b; Fagg 2014). Its numbers appear to have increased as a result of fragmentation of the natural landscape, removal of natural enemies and reduced fires (Watson 2011).

Because mistletoe obtains water and nutrients from its host, it is likely to create further stress on their host trees if they are already suffering water stress (Rigling *et al*. 2010). Yet there has been little research in Australia on whether drought and/or low rainfall intensifies the impact of mistletoe. Northern hemisphere studies have concluded that pine mistletoe (*Viscum album* ssp. *austriacum* L.), family Santalaceae, increases the susceptibility of its host to drought stress when growing in a xeric environment (Sanguesa-Bareda *et al*. 2012). Conversely, mistletoes from the Loranthaceae family in Australia have been observed to suffer more than their host during drought conditions, reflecting mistletoes' limited ability to regulate water loss (Watson 2011). A further view is that long-term mistletoe effects on tree growth and canopy intactness in low rainfall areas may be cumulative, acting when mistletoe has become a major part of the canopy and after successive drought years or declining rainfall (Sanguesa-Bareda *et al*. 2012).

To what extent mistletoe is the primary underlying cause of canopy decline or merely an added stress on trees already under long-term stress (and particularly, water stress) remains unclear (MacRaild *et al*. 2009). Mistletoe removal experiments allow the effect of mistletoes on the host's canopy to be quantified by comparing trees with mistletoes with "matching" trees in which all mistletoes have been removed (Watson 2009). Despite widespread concern about the impact of mistletoes on host trees, particularly *Eucalyptus* trees, in Australia only a few mistletoe removal studies have been undertaken (Reid *et al*. 1994; Reid and Stafford Smith 2000; Jaunay and Miles 2008). Reid *et al*. (1994) recorded smaller trunk diameters in trees with mistletoe, and significantly higher survival rates, trunk diameter growth increments, and greater foliage, in trees with mistletoe removed than trees retaining mistletoe.

Reid and Stafford Smith (2000) found that once mistletoes had been removed, larger trees were disproportionately infected by reinvading mistletoes and trees that had been infected previously were more likely to be reinfected. In a one year study, Juanay and Miles (2008) recorded increased canopy condition in both pink gums where mistletoe had been retained and in pink gums where mistletoe had been removed. More recently, mistletoe removal studies have documented the impact of mistletoe as a keystone resource for the broader ecological community, with Watson and Herring (2012) finding that woodlands from which all mistletoe had been removed lost approximately 20% of their bird species richness.

In the Mt Lofty Ranges and adjoining plains, pink gum (*Eucalyptus fasciculosa* F. Muell.), is the most common host of box mistletoe (*Amyema miquelii*) (Ward 2007), hereafter referred to as mistletoe. Mistletoe presence was the variable that had the strongest association with low levels of pink gum canopy intactness (Chapter 1). This thesis also recorded mistletoe as being most common at lower rainfall sites in the study region, but there was no significant correlation between rainfall *per se* and pink gum canopy intactness (Chapter 1). In a low rainfall pink gum woodland east of the Mt Lofty Ranges, South Australia, pink gums were recorded with high levels of mistletoe and low levels of canopy intactness (refer Appendix A, site number 34). Canopy intactness was defined as the percentage of the potential crown that contained foliage and is highly correlated with overall stand condition (Cunningham *et al*. 2007). I hypothesised that pink gums with mistletoe removed would have greater canopy intactness, leaf production and branch diameter increases than trees with mistletoe retained. A second hypothesis was that mistletoe infection and not declining rainfall was the primary cause of low levels of canopy intactness in the pink gum woodland. The aims of this study were to use a mistletoe removal experiment to investigate:

- the impact of mistletoe infection on host canopy intactness and growth
- the capacity of pink gum canopies to recover after mistletoe removal
- whether mistletoe is the primary cause of low levels of pink gum canopy intactness or whether mistletoe is a stress that is superimposed on other stresses acting on pink gums, particularly low and declining annual rainfall.

6.3 Methods

Study site description

The study site was a 10 ha pink gum open woodland at Hartley, 50 km south-east of Adelaide, at $35^0 21^1$S, $139^0 03^1$E. The woodland retained native shrub and sedge understorey, with exotic species inconspicuous, and had not been grazed by domestic stock since approximately 2003. The soil was a red sandy-loam, generally about 30 cm deep, over a calcrete layer, and also with outcropping calcrete. The woodland was surrounded by cleared land, but with five similar woodland patches within a 2 km radius. Mean annual rainfall is 380 mm (Hartley weather station number 23822 Bureau of Meteorology 2015); hence the pink gums at this site are near the lower rainfall limit of their natural distribution (Atlas of Living Australia 2016a). Figure 6.1 shows the percentage deviation from mean annual rainfall for the study site between 1992 and 2011, with eight years receiving at least 10% less than the annual mean and five years receiving at least 10% more than the annual mean. The Bureau of Meteorology (2016) defines below average rainfall as being yearly rainfall totals in the lowest 30% of historical totals. Based on this definition, the years 2006, 2007 and 2008 were all below average. Since 1975 there has been a downward trend in total rainfall received (Figure 6.2).

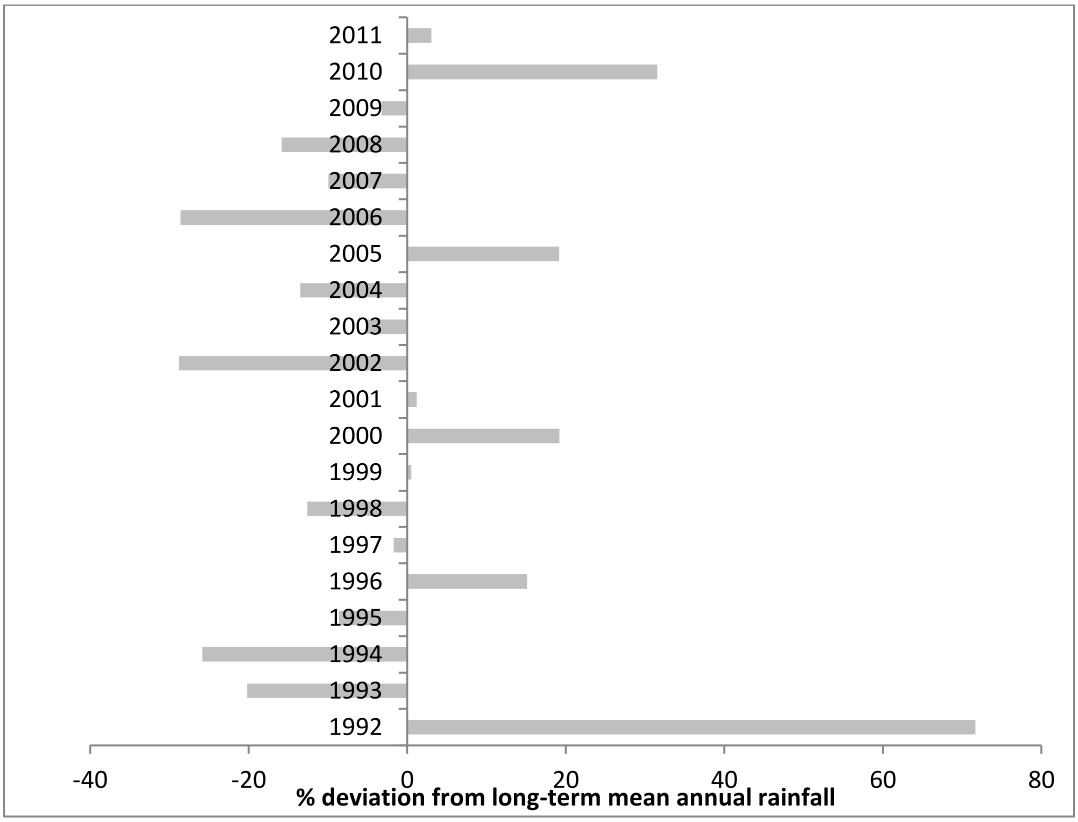

Figure 6.1: Percent deviation of yearly rainfall, 1992 – 2011, for the Hartley weather station (# 23822), from long-term mean annual rainfall (Bureau of Meteorology 2015)

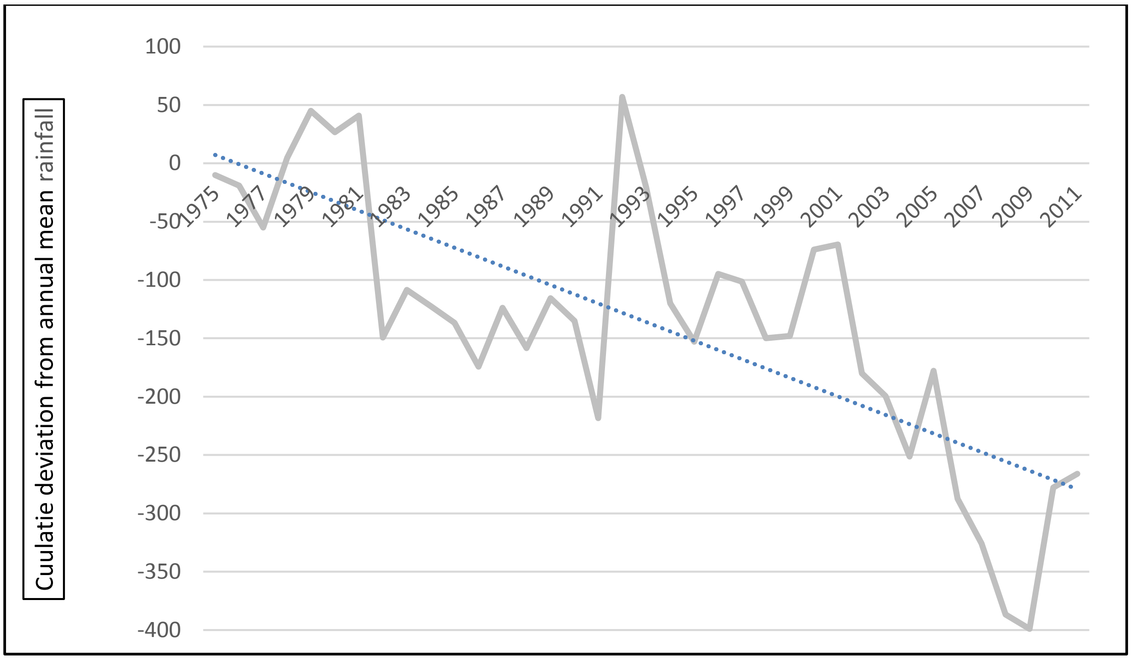

Figure 6.2: Cumulative deviation from annual mean rainfall for the Hartley study site, 1975 to 2011. Compiled from annual rainfall totals for the Hartley weather station (# 23822), (Bureau of Meteorology 2015). Cumulative deviation from mean annual rainfall is calculated by taking the mean rainfall for each year from the actual rainfall for each year. This difference is then cumulatively added to produce a trend line.

Pink gum tree parameters

Between September 2010 and January 2011, I surveyed all pink gum trees within the 10 ha woodland, recording canopy intactness, tree height and the number of live and dead mistletoes. Tree height was estimated for the highest point of the tree (including dead branches). I recorded tree height by extrapolation of a known height (a 2 m pole) (NVMU 2015). For live pink gums, tree height was used as a surrogate measure for tree age (trunk diameters were not recorded for all trees). Trees were placed into five height classes: 2.1- 4 m, 4.1-5 m, 6.1-7 m, 7.1-8 m, and 8.1-9 m.

Control and treatment trees: parameters monitored

On 2 September 2010, I selected 20 pink gums divided into 10 pairs, each member of a pair being matched for similar canopy intactness and numbers of live mistletoes present (after Reid *et al*. 1994). The following parameters were recorded on 2 September 2010, and after mistletoes had been removed (in October 2010) at two to three monthly intervals until either December 2011 or February 2012, as detailed below.

Pink gum canopy intactness was recorded in September 2010, January, March, June, September and November 2011, and February 2012. Canopy intactness was a visual assessment of the percentage of the potential crown that contained foliage (Cunningham *et al*. 2007). This definition allowed for dead branches to be present but canopy intactness to be recorded as 100% if regrowth foliage was considered to have compensated for past loss. Epicormic growth was estimated visually as the percentage of live foliage that comprised epicormic shoots. Shoots were considered epicormic if they had initiated from buds in the bark of mature stems and were < 3 cm in diameter (Cunningham *et al*. 2007). The percentage of total pink gum foliage comprised of epicormic leaves was recorded in September 2010, January, March and June 2011, and February 2012. I recorded the number of live mistletoes that were visible from ground level and from searching within the canopy while standing on a 2 m ladder. The horizontal and vertical diameters and percentage canopy intactness of each mistletoe plant was recorded on the same dates as host canopy intactness. The smallest mistletoe recorded was 5 cm in vertical diameter and with four leaves. Because of the very open canopy of the pink gums (Boomsma and Lewis 1980), including trees with low canopy intactness, and because all trees were less than 6 m tall, the likely detection of mistletoes of at least 5 cm diameter was high. As the haustorium of box mistletoe forms only at the primary point of infection (Reid and Yan 2000), each woody haustorium was recorded as one mistletoe. I also estimated the percentage of the combined pink gum and mistletoe canopy that was comprised of mistletoe foliage. I calculated mistletoe volume using the formula for a cone:

$V = 1/3\pi r^2 h$, where h = vertical diameter and r^2 = maximum horizontal radius.

Mistletoe dimensions were estimated from ground level.

Immediately before mistletoe removal on 4 October 2010, branch diameters were measured with callipers, to the nearest 0.1 mm. For the control trees (mistletoe retained), I measured the diameter of the branch containing mistletoe and where present, the diameter of the corresponding location on the adjoining forked branch that contained no mistletoe. For the treatment trees, the diameters of branches where mistletoes had been removed were measured and, where present, the diameters of the adjoining forked branch where there were no mistletoes. The number of branches measured for mistletoe removal, mistletoe retained and no mistletoe present was 29, 31 and 32, respectively. The measured location on the branch was proximal and where within reach, 10 cm from the mistletoe attachment or former mistletoe attachment, but where beyond my reach, up to 100 cm from the mistletoe attachment. All branch diameters were subsequently re-measured in January, June, September and December 2011. I marked the location of the diameter to be measured by inserting map pins to a depth of approximately 3 mm, and painting the branch circumference. For each branch, I recorded the orientation of the callipers in relation to the pin and the stem, to ensure the callipers were placed in the same location on each branch for subsequent measurements. Based on recordings of three repeat measurements of each branch diameter each survey date, the branch diameter measurements were accurate to within 0.2 mm. This technique for measuring branch diameters relied upon seasonal visits to ensure pins and circumference lines could be re-located before pins were dislodged and/or bark shed.

Following mistletoe removal, I recorded the number of epicormic shoots and leaves that had resprouted from the branch from which mistletoe had been cut, on the 9th January, 13th June, 2nd September, 29th December 2011 and on 17th February 2012. Leaf damage due to insect chewing, skeletoniser damage, senescence (all of the leaf area being a red colour) and lerp activity was recorded in September 2010, January, March, June, September and November 2011. These parameters were scored by estimating the percentage of leaves estimated to have greater than 10% of the surface area damaged, and in the case of lerps, any presence. For six treatment trees and five control trees, two to four branchlets were tagged per tree, and all leaves were numbered from a marked location to the terminal end of the branchlet. A total of 36 branchlets were monitored. All branchlets chosen were within 4 m of the ground, this being the maximum height that could be safely accessed via a ladder. Trees not monitored contained only branchlets > 4 m above ground.

In addition to the above regularly monitored parameters, in September 2011 I recorded: canopy intactness of non-mistletoe infected branches, and signs of mistletoe on larger dead branches during September 2011, for the 20 survey trees being used in the mistletoe removal experiment. To help determine if the impact of mistletoe on host canopy intactness was confined to its host branch, the percentage canopy intactness of the forked branch adjoining that bearing mistletoe (or from which mistletoe had been removed) was scored. To help determine the contribution of mistletoe to pink gum canopy loss in the study area, all the dead trunks and limbs of the 20 study trees were examined for evidence of past mistletoe infection. I defined trunks as the central parts of the tree from which limbs grew. Limbs were the larger branches leading directly off the trunk that defined the shape of the canopy and had relatively little foliage leading directly off the limb. Four of the 20 trees had fallen limbs and one tree had a fallen trunk on the ground. These were not recorded for presence of mistletoe because they appeared to have been long dead, and it was not possible to tell if the branch had been alive or dead at the time of breaking off from the tree. Box mistletoe have a high water content making them relatively heavy compared to the host foliage, hence infected branches are more susceptible to breakage (Watson 2015).

Mistletoe removal

All mistletoes were removed from the 10 treatment trees on 4 October 2010. The host branch was cut as close to, and proximal to the haustorium, and done so that no pink gum foliage was removed. In all cases, the pink gum branch beyond the mistletoe had died prior to the mistletoe being removed.

Statistical analysis

All statistical analyses were conducted using SPSS version 23. For both the mistletoe removal study trees, and for all pink gums in the study woodland, the impact of mistletoe infection was assessed by looking at associations between mistletoe abundance and epicormic growth, using Pearson’s correlation, after checking for normality of distribution and lack of outliers. I grouped all the 94 live pink gums present in the 10 ha woodland into five “percent canopy intactness” classes: ≤ 20%; 2– 40%; 4– 60%; 61–80% and 81–100%. Differences in the proportion of live and dead mistletoes between host canopy intactness classes were determined using a Pearson’s goodness of fit test. To determine differences in canopy intactness between different pink gum height classes, I used one-way ANOVA. Two-way ANOVA was used to determine if there was any interaction between tree height and mistletoe abundance on canopy extent.

For the mistletoe removal treatment and control trees, differences in mean stem diameters between control and treatment groups were tested using one-way ANOVA (the variances of the data were homogeneous). Paired t-tests were used to compare changes in mean canopy intactness between treatment and their control trees, and differences in leaf production between treatment and control trees. The capacity of pink gums to recover from mistletoe removal was assessed by absolute counts of pink gum epicormic leaves on the branch from which mistletoe had been removed, and by recording canopy intactness following removal in both treatment and control trees.

6.4 Results

6.4.1 **Pink gum population parameters**

The 10 ha woodland at Hartley had 94 live pink gums and 26 dead pink gums (78% and 22% respectively). The mean percent canopy intactness of the live pink gums was 62% (SD ± 26) and for live and dead pink gums combined was 48% (SD ± 34). Despite the relatively low mean pink gum canopy intactness for the study site, 20% of the live pink gums had a canopy intactness of at least 80% and the highest frequency class was 71– 80% canopy intactness (Figure 6.3). There was no significant difference in canopy intactness between the different height classes (Figure 6.4) (one-way ANOVA, F = 1.1, p = 0.367). There was also no interaction between height and mistletoe abundance in determining canopy intactness (Two way ANOVA F = 0.981 p = -0.436).

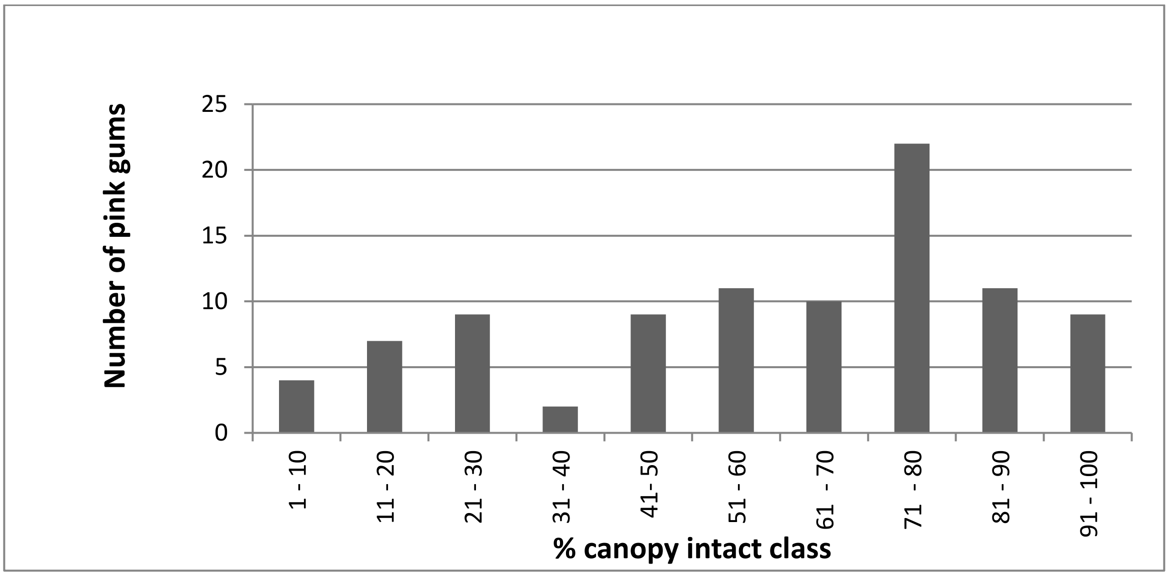

Figure 6.3: Number of live pink gums within each "percent canopy intactness" class for the 10 ha pink gum woodland at Hartley. Trees were surveyed between September 2010 and January 2011.

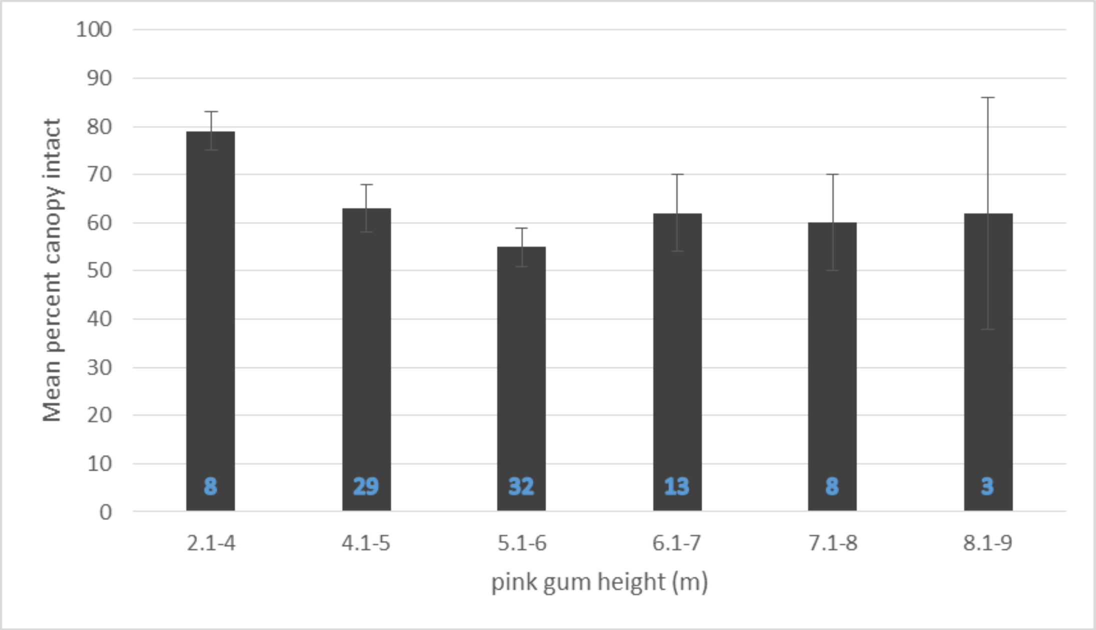

Figure 6.4: Mean canopy intactness in September 2010 for pink gums in different height classes within the Hartley population (one pink gum was 0.5 m and was not included). Error bars show SE. Data labels represent number of pink gums within each height class

Of the live pink gums, 70% contained live mistletoe and 89% contained live, dead and/or old mistletoe attachments. There was a strong negative correlation between the canopy intactness of live pink gums and the percentage of canopy comprised of mistletoe (N = 94, R = - 0.61, $p < 0.01$). There was a weak but significant negative association between the canopy intactness of live pink gums and the number of dead and/or old mistletoe attachments, after removal of one outlier (N = 93, R = - 0.28, p = 0.007) (Figure 6.5). However, there was no significant association between pink gum canopy intactness and the number of live mistletoes (N = 94, R = 0.04, p = 0.69), or the total number of live and dead mistletoes (N = 93, R = - 0.16, p = 0.12).

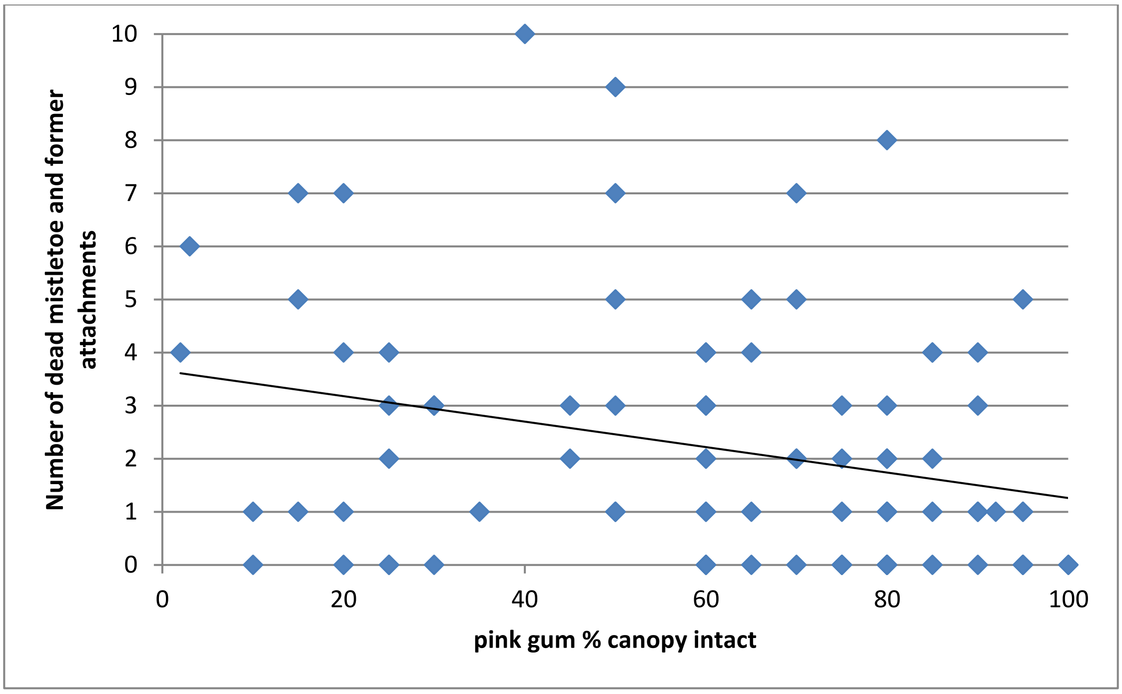

Figure 6.5: Correlation between pink gum canopy intactness for all live pink gums at the Hartley study site, and number of dead and former mistletoe attachments per tree.

The ratio of live to dead mistletoes within a canopy increased as pink gum canopy intactness increased. The 2:1 ratio of live to dead mistletoes occurring in the 80–100% class was significantly higher than the approximately 1:1 ratio of all other canopy condition classes ($\chi 2 = 16.9$, df = 4, $p < 0.05$) (Table 6.1). As might be expected, there was a significant negative correlation between pink gum canopy intactness and the percentage of total canopy foliage that comprised mistletoe ($R = -0.55$, $N = 94$, $p < 0.001$). The percentage of canopy comprised of mistletoe was significantly greater for canopy intact classes 1–20% and 21-40% than for the higher percentage canopy intact classes (Table 6.1).

The mean percentage of pink gum foliage that comprised epicormic growth was 20% (SD ± 32). There was a significant correlation between the percentage of pink gum foliage that comprised epicormic growth and the percentage of canopy foliage that comprised mistletoe ($R = 0.39$, $p = 0.004$) and there was a significant negative correlation between the percentage of pink gum foliage that comprised epicormic growth and pink gum percent canopy intactness ($R = –0.57$ $p < 0.001$).

Table 6.1: The proportion of the mean number of live to dead mistletoes and the mean percentage of total canopy comprised of mistletoe for each pink gum canopy intactness class for the 94 live mistletoes in the Hartley study site. Homogeneous subgroups have the same superscript.

Pink gum canopy intact (%) range	No. of live pink gums	Proportion of mean number of live to dead mistletoes	Mean % of combined host and mistletoe canopy comprised of mistletoe (± SE)
1–20	11	0.8^{A}	25 ± 7.5^{A}
21–40	11	0.8^{A}	19 ± 4.5^{A}
41–60	20	0.7^{A}	4 ± 1.2^{B}
61–80	17	1.0^{A}	4.8 ± 1.0^{B}
81–100	35	2.0^{B}	4.5 ± 1.2^{B}

6.4.2 **Treatment and control trees**

Baseline parameters

Baseline values for tree sizes, canopy intactness and mistletoe loads for the 10 treatment and 10 control trees recorded on 2 September 2010 immediately before mistletoe removal are shown in Table 6.2. There was no difference between treatment and control trees in tree height, trunk DBH, canopy intactness, number of live mistletoes or percentage of canopy comprised of epicormic growth. Before removal, there were 82 live mistletoes, 27 dead mistletoes and 40 former attachments evident on the 20 study trees. The mean canopy intactness of the live mistletoes was 60%, and one third of the live mistletoes had < 20% canopy intactness. There was a highly significant negative correlation between the percentage pink gum canopy intactness and the number of dead mistletoes and former mistletoe attachments ($N = 20$, $R = -0.70$, $p < 0.01$) (Figure 6.6). However there was no significant correlation between the number of live mistletoes and the canopy intactness of the host trees. There was a strong positive correlation between the percentage of pink gum foliage that comprised epicormic growth and the percentage of total canopy foliage that comprised mistletoe ($R = 0.76$, $N = 20$, $p < 0.001$).

Table 6.2: Range in baseline values of parameters for pink gum control (N = 10) and treatment (N = 10) trees, and for all 20 trees. The t values and significance for the difference between control and treatment trees are also shown (independent t test).

Parameter	**Mean ± SE**					**Range in values**
	All trees	Control	Treatment	t value	p value	
Pink gum percent canopy intactness	58 ± 7	57 ± 10	59 ± 9	-0.16	0.88	2–95
# live mistletoes per tree	4.1 ± 0.7	3.9 ± 0.9	4.3 ± 1.1	-0.29	0.77	1–12
Percentage of pink gum foliage comprised of epicormic growth	11 ± 5	19 ± 10	3 ±2	1.57	0.13	0–100
Maximum pink gum trunk diameter at breast height (cm)	23 ± 1	2 ± 1	23 ± 1	-0.41	0.68	17–29
Pink gum height (m)	4.7 ± 0.2	4.9 ± 0.3	4.5 ± 0.2	1.32	0.20	3.2–6

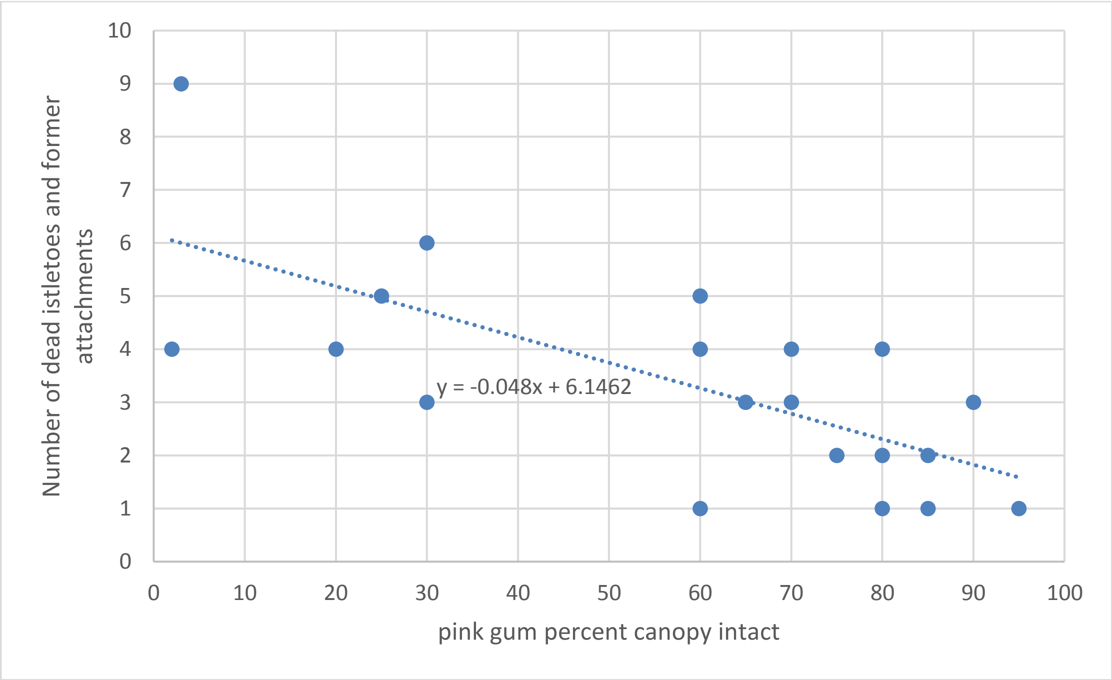

Figure 6.6: Correlation between pink gum canopy intactness for 20 study trees, and number of dead and former mistletoe attachments per tree.

Dead Trunks and Limbs

Of the 24 dead trunks in the 20 study trees, 22 had either dead mistletoe present or evidence of past mistletoe attachments. Of the 45 dead limbs (refer Methods for definition), 29 had either dead mistletoe present or evidence of past mistletoe attachments. For most dead limbs, former mistletoe attachments remained only, suggesting that the mistletoe and the branch were long dead. Where there were dead limbs with no evidence of dead mistletoe or past attachments, these branches appeared to have been long dead and may or may not have hosted mistletoe in the past.

Canopy intactness of mistletoe and non-mistletoe infected live branches

Live branches with mistletoe had no pink gum foliage beyond the point of attachment. For the 20 study trees, forked branches that adjoined mistletoe-bearing branches (including those prior to mistletoe removal) were highly intact, with 88% having at least 90% of the branch canopy intact. The remaining live canopy limbs and branches on each tree without mistletoe were also observed to be at least 90% intact.

Mistletoe recruitment

Based on pink gum branch diameter as a surrogate measure of mistletoe age (as per Reid *et al*. 1994) mistletoe recruitment appears to have been continuous in the study trees, there being a normal distribution in the spread of stem diameters bearing live mistletoes (Figure 6.7). The length of time a dead mistletoe remains on a host branch is highly variable and during which time the dead branch can suffer shrinkage (personal observation S. Croft). Hence, the diameters of branches bearing dead mistletoes were not recorded in the present study.

Between October 2010 and February 2012, there were seven new mistletoes spread amongst six of the treatment trees, and no deaths of mistletoes. For the 10 treatment trees, this was a mean of 0.4 new mistletoes per tree per year. With the exception of one tree, recruitment occurred on trees with 80% or more of the canopy intact. Three mistletoes established less than two months following mistletoe removal (on other branches), with establishment of the remaining mistletoes being evenly spread over the remainder of the survey period. In contrast, there were no new mistletoes recorded in any of the control trees, and five of the existing mistletoes died. Hence for the 20 trees, there was a net increase of two mistletoes.

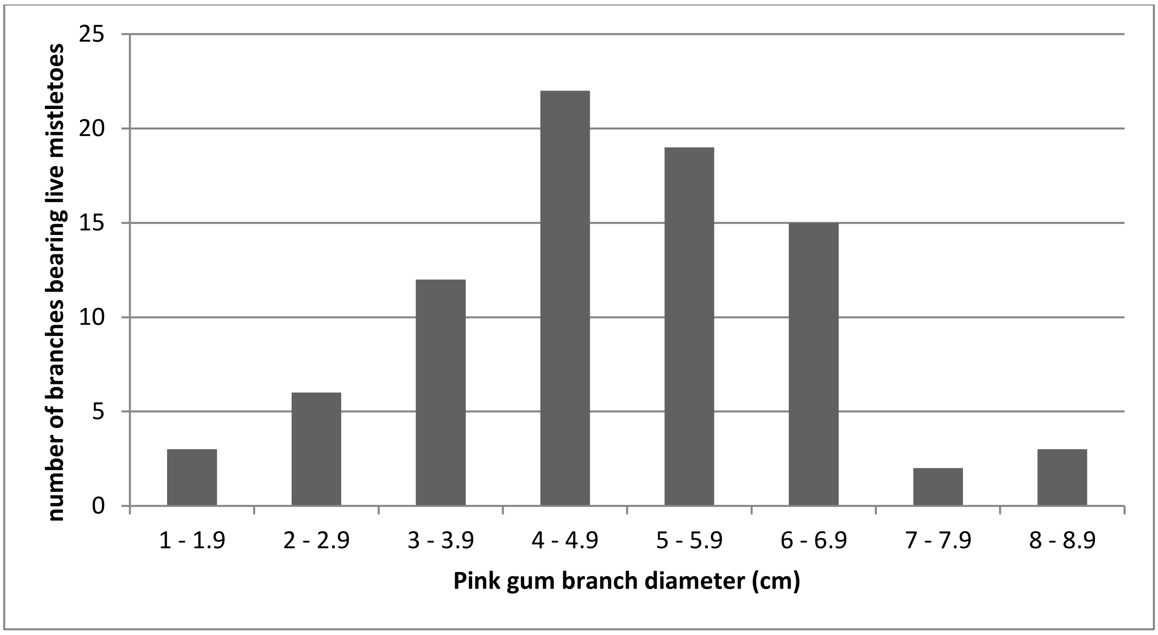

Figure 6.7: Distribution of pink gum branch diameters bearing live mistletoes, amongst the 10 treatment and 10 control trees (diameter measured proximal to, and where possible, 10 cm from the mistletoe attachment, but where beyond reach, up to 100 cm from the attachment)

Diameter increases of mistletoe- and non-mistletoe-bearing branches

At the start of the survey on 4 October 2010, the mean diameter of branches hosting mistletoe (including those branches where mistletoes were subsequently removed) was 43.0 mm (SD ± 16) compared with a mean diameter of 47.0 mm (SD ± 15) for branches not hosting mistletoe, but this difference was not significant (independent sample t-test, df = 90, t = -1.14, p = 0.58). Between 4 October 2010 and 29 December 2011, control branches (no mistletoe) had a mean increase in stem diameter of over twice that of both branches retaining mistletoe and branches where mistletoe was removed (Table 6.3) but these differences were not significant (ANOVA F = 0.85, p = 0.431).

Table 6.3: Mean increase in pink gum branch diameters of control branches (no mistletoe), mistletoe-bearing branches and branches from which mistletoe was removed, between 4 October 2010 and 29 December 2011. Control branches adjoined mistletoe branches on the same forked branch system. Mistletoe was removed on 4 October 2010.

Branch status	Number of branches	Mean increase in branch diameter (mm)± SD
Control	32	1.6 ± 2.3
Mistletoe-bearing	30	0.8 ± 4.0
Mistletoe removed[§]	24	0.5 ± 3.4

[§] seven stems that died after mistletoe was removed were not included in the analysis.

The mean net change in diameters of all treatment and control branches between 4 October 2010 and 29 December 2011 was 1.0 mm (SD ± 3.4 mm), with a range of -4 mm to 11 mm. Branches with no mistletoe showed a < 1 mm mean increase in branch diameter at all survey periods, the mean increases off-setting decreases due to bark shedding during the summer monitoring periods (Figure 6.8). For branches with mistletoe retained, there was a mean net diameter decline of 0.7 mm in January 2011 due to bark shedding, before increasing in June 2011 and thereafter remaining relatively steady (Figure 6.9).

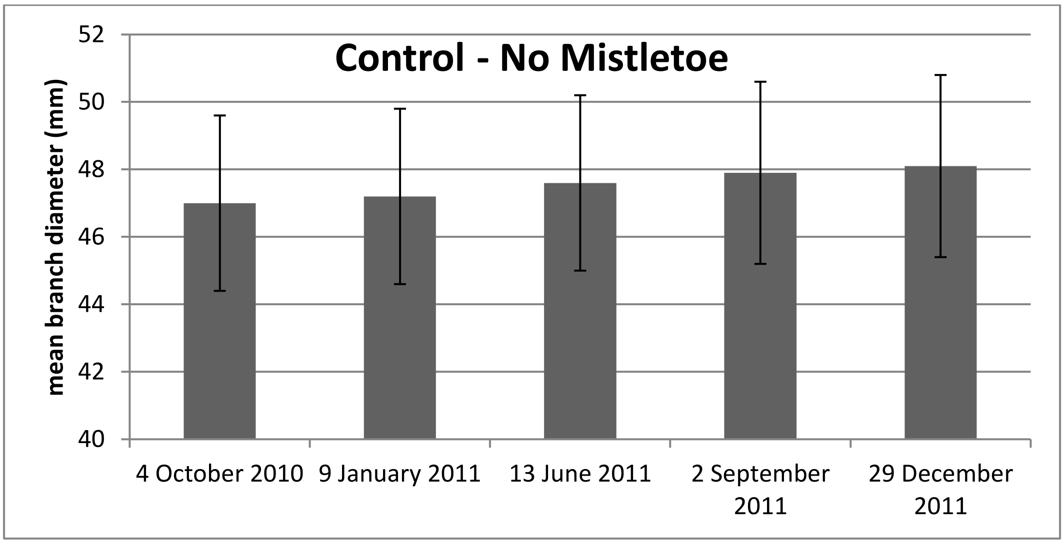

Figure 6.8: Seasonal changes in mean diameter (mm) of the 32 branches with no mistletoe present. Error bars show SE.

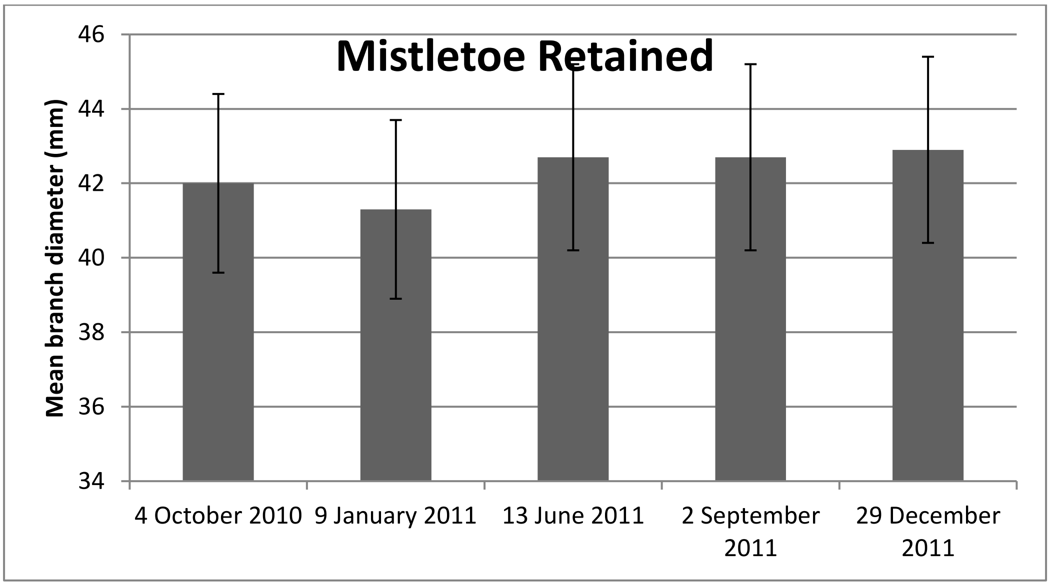

Figure 6.9: Seasonal changes in mean diameter (mm) of the 31 branches with mistletoe retained. Error bars show SE.

Whole tree canopy changes

Trees that had mistletoe removed increased in mean canopy intactness by 4.3% (SD ± 9) between October 2010 and February 2012. This compared with a mean decrease in canopy intactness of 3% (SD ± 9) for control trees (mistletoe retained). The difference between treatment and control groups was significant at the 10% level (paired t-test, df = 9, t = 2.05, p = 0.071). I considered I was able to estimate canopy intactness within an error margin of 10% (refer Methods, Chapter 3). On this basis, two control trees declined in canopy intactness (by -10% and -25%), and three treatment trees increased in canopy intactness (by 10%, 15% and 20%). Changes in mean canopy intactness between recording periods (every two to three months) were < 5% and not significantly different for control trees (ANOVA F = 0.019, p = 0.99) or treatment trees (ANOVA, F = 0.06, p = 0.99). The mean canopy intactness for all 20 study trees was 58% in both September 2010 and February 2012.

There was a positive correlation between the increase in host canopy intactness and the number of mistletoes removed from the treatment trees (N = 10, R = 0.59, p = 0.035). On trees where mistletoe were retained, there was almost no association between the change in pink gum canopy intactness and the number of mistletoes (N = 10, R = -0.12, p = 0.75).

Epicormic response to mistletoe removal

Of the 29 branches where mistletoes were removed, there was an epicormic response on 22 (75%) branches. Five of the 29 branches died after the mistletoe was removed, four of which were on trees with < 20% canopy intactness. Seventy-six percent of epicormic leaves that were recorded within the 15 month survey period were present within three months of mistletoe removal. After this initial rapid response, there was a continued increase in the number of new epicormic shoots and leaves, even 15 months after mistletoe removal, albeit at a much reduced rate of increase.

There was a significant association between the number of new epicormic shoots on trees where mistletoe was removed, and the canopy intactness of the host pink gums (N = 29, R = 0.39, p < 0.05) and also between the number of new epicormic shoots on trees where mistletoe was removed and the increase in pink gum canopy intactness during the survey period (N = 29, R = 0.46, p < 0.05). All mistletoes were removed from the treatment trees. No new epicormic shoots were recorded on control trees (namely, trees where mistletoes were retained).

New leaves

The mean maximum number of new pink gum leaves per tagged stem for treatment and control trees was 42 (SE ± 18) and 31 (SE ± 17) respectively, but this difference was not significant (Independent samples t-test, df = 9, t = 0.44, p = 0.67). There was no significant correlation between a tree's canopy intactness and the mean number of new leaves per stem for that tree (N = 11, R = 0.05, p = 0.88). No new leaves were recorded on 9 of the 25 tagged stems.

Leaf symptoms

Lerp activity was not recorded on any pink gum trees for the duration of the study. Between September 2010 and June 2011, no more than 1% of leaves were scored with skeletoniser damage, and for all but two trees in September 2010, no more than 5% of leaves were scored as either chewed or senescent or dead. In November 2011, leaf damage levels were still very low, with the percent of leaves chewed ranging from 5–20%, senescent leaves 0-10% and no more than 1% of leaves were skeletonised or dead on any tree.

6.5 Discussion

Mistletoe: primary cause of pink gum canopy decline?

This study sought to disentangle the impacts of mistletoe from other possible causes of low canopy intactness by conducting a controlled mistletoe removal experiment. Box mistletoe (*Amyema miquelii*) is the most widespread mistletoe species in Australia (Atlas of Living Australia 2016a), occasionally occurring in densities exceeding 500 plants ha^{-1}, although 7– 10 plants ha^{-1} is more typical of undisturbed forest (Turner 1991; Watson 2009). The 28 live mistletoes ha^{-1} recorded at the Hartley study site represents a relatively high density. The mean canopy intactness of the Hartley study site pink gums was 48% which compares with a mean of 70% for live and dead pink gums surveyed during a study of pink gums in the Mt Lofty Ranges and adjoining plains (Chapter 1).

The most obvious possible explanations for the higher percentage of dead trees and the relatively low levels of canopy intactness for the Hartley study site are the high frequency of box mistletoes, the declining trend in mean annual rainfall (which is already at the lower limit of pink gums' natural distribution), or long-term water stress due to mistletoe infection superimposed upon a declining and low mean annual rainfall. The latter explanation would conform with a general model for long-term tree dieback, namely that predisposing factors expose the trees to long-term stress which make the trees more susceptible to short-term stresses (Galiano *et al*. 2010). The predisposing factors of a declining and low mean annual rainfall could make pink gum more susceptible to seasonal drought and/or mistletoe. In the 20 years prior to the study there were 10 years with > 5% below average annual rainfall, and only five with > 5% above average annual rainfall, resulting in a downward trend in annual rainfall totals.

At the Hartley study site, however, there was no evidence to support low mean annual rainfall contributing to canopy decline. Despite the low mean level of canopy intactness, 29% of trees had 80% or more of their canopy intact. These trees were widely, and seemingly randomly, spaced within the woodland and interspersed with dead trees. Assuming tree height is a surrogate measure for pink gum age, there was no significant correlation between canopy intactness and tree age: both relatively old and young trees were represented by trees with highly intact canopies. During the 2010 to 2012 survey period, the 20 trees involved in the mistletoe removal experiment did not appear to be under stress with no signs of bark cracking, branch dieback, high leaf loss in summer, or a high percentage of dead leaves (after Pook *et al.* 1966). Further there were no signs of unnaturally high levels of insect attack, and a complete absence of lerp infection. The canopy intactness of the 20 treatment and control trees was first recorded in September 2010, which followed three years of below-average rainfall. The canopy intactness was also recorded in February 2012, following an above-average rainfall year (Bureau of Meteorology 2015). If low mean annual rainfall was contributing to low levels of canopy intactness, then it might be expected that the pink gums would show an increase in canopy intactness during 2011. However, mean canopy intactness remained the same. The high variability in pink gum canopy intactness levels combined with the lack of response to above-average rainfall suggests that low mean annual rainfall *per se* was not the primary cause of low levels of canopy intactness.

During the survey period, new epicormic growth was recorded only on branches from which mistletoe had been removed suggesting that the trees were not under stress from other factors, including drought stress. Further, the canopy intactness of branches without mistletoe, including those on adjoining branches was in almost all cases, over 90%.

The most likely explanation for low levels of pink gum canopy intactness at the Hartley study site is high levels of mistletoe infection. For the 20 pink gums used in the mistletoe removal experiment (treatment and control), there was a highly significant correlation between canopy intactness and the number of dead mistletoes and former mistletoe attachments (N = 20, R = -0.71, $p < 0.01$). Although short-lived relative to their eucalypt host, individual box mistletoe plants can live for several decades (Watson 2009). The significant correlation between pink gum canopy intactness and the number of dead mistletoes, but not with the number of live mistletoes, indicates that the present pink gum canopies are largely a legacy of past mistletoe infection. For the mistletoe removal study trees, most of the dead branches appeared to have been long dead. Of the 20 trees included in the mistletoe removal study, almost 75% of the dead trunks and limbs had evidence of past mistletoe infection. This was further evidence that the current canopy condition reflects ongoing and past mistletoe infection levels. Thus studies of mistletoe impact on pink gum canopy intactness ideally require studies over several decades.

My study also found that the pink gums with the most intact canopies supported the highest ratio of live to dead mistletoes which may reflect that the healthiest trees may be hosting more recent infections. Highest numbers of live mistletoes were associated with canopies that were 80% or more intact. This was well above the 59% mean canopy intactness for all the 20 study trees and may indicate that mistletoe have higher infection and/or establishment rates on healthier trees and/or the seed vectors of mistletoe prefer more intact canopies. The latter explanation is considered less likely as Ward (2007) found that mistletoebirds favoured dead branches of pink gums for alighting, inferring that they prefer more open and hence less intact canopies.

Within the study area, soils were sandy-textured and only 30 cm deep, before a calcrete layer was encountered. To survive in such a low rainfall environment with hot dry summers, the pink gum roots would have to be penetrating deeper into the soil profile through cracks in the calcrete layer. This seems quite possible because roots of mallee-form eucalypts (at this site pink gums were often multi-stemmed) have been found at a depth of 28 m (Nulsen *et al.* 1986). Differential access to water, if a factor, may also be a reason to explain variability in canopy intactness between the pink gums in this woodland. Determining the source(s) of water for these pink gums would reveal if the pink gums in this low rainfall environment have access to reliable soil water at depth or to a perched water table below the calcrete layer.

Mistletoe impacts on host growth and canopy

This study indicated that the morphological impacts of each mistletoe on the host tree were confined to the host branch. Branches that adjoined mistletoe-bearing branches were healthy, with 88% having 90% or more of the branch canopy intact. Hence adjoining branches of the same forked branch system were seemingly unaffected by mistletoe on adjoining branches.

The scope for measuring the impact of mistletoe on its host is reduced because the distal portion of the host branch dies after box mistletoe infection (Whittington and Sinclair 1988; Watson 2011). For example, it is not possible to record leaf area index of foliage on mistletoe-bearing versus non mistletoe-bearing branches. Attributes recorded to measure the impacts of mistletoe removal in Australia have focussed on changes in host trunk diameter, host canopy intactness, and percentage of total host canopy that comprises epicormic growth (Jaunay and Miles 2008; Reid *et al*. 1994). Reid *et al*. (1994), for example, observed smaller trunk diameters in trees with mistletoe. Because mistletoe plants obtain water from their host, it is likely that pink gum branches hosting mistletoe would have a reduced water and nutrient supply, and hence have decreased branch diameters compared with adjoining non-mistletoe bearing branches. I measured the diameters of the relatively small mistletoe-bearing branches, and where present, adjoining forked branches, rather than the main trunk. This was because the impacts of mistletoe on canopy intactness appeared to be confined to the host branch. I am not aware of any other similar Australian studies. In northern hemisphere studies, however, Rigling *et al*. (2010) found that dwarf mistletoe infection reduced branch diameters and branch length in Scots pine and Barbu (2012) found that White Mistletoe (*Viscum album*) causes a significant reduction in needle length of Silver Fir (*Abies alba*). Although, these studies were on mistletoes in the Santalaceae family and on conifer hosts, the mistletoes still receive water and nutrients from their host through a haustorium, as is the case for *Amyema miquelii* (family Loranthaceae). The mean diameter of pink gum branches hosting mistletoe was 4 mm less than branches without mistletoe but this difference was not significant. Changes in diameters over the recording period for 75% of branches were less than 2 mm, reflecting the very low growth rates of pink gum in this low rainfall environment. Although branches with no mistletoe recorded an increase in diameter, twice that of branches with mistletoe, the difference was also not significant. A longer-term study is required to determine the impact of mistletoe on host branch diameters. A similar study is recommended to determine if growth rate is lower on younger pink gums and/or pink gums in higher rainfall environments hosting mistletoe, in which the growth rate of the pink gums would be expected to be naturally higher than the present study's trees.

Although this study recorded a strong and significant correlation between the percentage of pink gum canopy comprised of epicormic growth and the percentage of canopy comprised of mistletoe, the epicormic response may be due to factors other than and/or in addition to stress caused by mistletoe parasitism. This study, therefore, quantified epicormic growth (number of shoots and leaves) on the branch from which mistletoe was removed. The magnitude of the epicormic growth response was more a reflection of whole tree canopy intactness rather than the number, size and/or canopy intactness of the mistletoe removed. Conversely, the five branches that died following mistletoe removal were from four trees which were in the bottom seven trees for canopy intactness levels. The results showed that pink gums with more intact canopies have greater capacity to recover canopy following mistletoe removal.

There was no correlation between the number of new leaves produced on selected stems, and with either pink gum canopy intactness or mistletoe abundance. Because the host pink gum branch dies beyond the mistletoe attachment and is normally devoid of host leaves upstream of the attachment, non-host stems had to be selected for leaf monitoring. However, this study showed that even adjoining branches seem to be unaffected by nearby mistletoe infection, hence numbering leaves on adjoining stems will not necessarily reflect the impact of mistletoe on the host stem.

This study did not last long enough to investigate the impact of mistletoe removal on mortality rates of host trees. This would require at least 10 years of measuring mortality of non-mistletoe-infected trees with mistletoe-infected trees within the same woodland, and with all other environmental variables being equal. Given the speed with which mistletoes re-established on trees, on-going removal would also be required.

6.6 Conclusion

Although the study site pink gums were occurring at the lower rainfall limits of their natural distribution, the major contributing factor to their decline appears to be high levels of mistletoe infection, rather than declining and low rainfall. The impact of mistletoes on host canopy intactness seemed to be largely confined to the host branch with non-mistletoe bearing branches being intact. The negative correlation between the number of dead and former mistletoes and canopy intactness suggested that the present canopy intactness results from mistletoe infections over several decades or more. The capacity of pink gums to recover canopy after mistletoe removal was more a function of pre-existing canopy intactness, rather than related to the number or volume of mistletoes removed. Because of the very low growth rates of pink gums in this low rainfall environment, longer term studies are required to determine if mistletoe infection reduces the branch diameter growth rate of its host, and whether mean canopy intactness levels will remain stable with ongoing rainfall decline and/or high mistletoe infection levels. Further studies are required to determine if the pink gums are buffered against low and declining rainfall by having access to deep soil water or a perched water table.

Chapter 7 Growth of juvenile pink gums (*Eucalyptus fasciculosa*)

7.1 Abstract

Conservation and management of threatened tree species requires an understanding of the factors influencing the early growth of juveniles, including their response to changing rainfall patterns. In the Mt Lofty Ranges South Australia, concern has been expressed over the poor canopy condition of pink gums (*Eucalyptus fasciculosa*) (F. Muell). If pink gum canopies have been declining due to declining rainfall, then I hypothesised that above-average rainfall and supplementary watering would increase growth rates. The influence of additional water on individual trees, however, may be variable. Growth parameters of 34 juvenile pink gums in a natural setting were recorded at varying intervals between 2010 and 2016. During December 2010 to February 2011, the juveniles received twice the mean summer rainfall and 50% of the trees received up to four times the average seasonal rainfall with supplementary watering. During this period, there was no significant difference in growth parameters between watered and unwatered trees. All trees, however, collectively recorded a four times greater mean height increase, 15 times greater shoot extension and twice the leaf production compared with the average rainfall and no supplementary watering of spring/summer 2011/12. Between September 2010 and August 2016 mean stem diameters of the 34 juveniles, almost doubled, and mean height increased 40%. However, between September 2010 and August 2016, 35% of trees recorded a < 5 cm height increase and almost 75% of trees recorded a < 1 cm increase in stem diameter. Over the study period, 30 of the 34 juveniles recorded an increased lignotuber diameter.

The above-average summer rainfall in 2010/11 rainfall was followed by an outbreak of gum leaf skeletoniser (*Uraba lugens*) and a 24% decrease in mean canopy intactness during spring 2011. Six of the study trees lost tagged branches due to animal damage. There was a highly significant correlation between stem diameter increase between September 2010 and August 2016, and the amount of foliage cover within a 20 m radius of each juvenile. For some juveniles, it appeared that proximity to adult trees, leaf damage by the gum leaf skeletoniser and possibly, inherent variation between individuals, were more important in determining growth than water supply. The findings have implications for revegetation programs, which need to consider potential very slow growth rates of pink gums.

7.2 Introduction

In southern Australia, temperate eucalypt woodlands have been extensively cleared and now occur as remnants of varying size and isolation within predominantly agricultural landscapes (Yates and Hobbs 1997). Increasingly, efforts are focussed on reversing the processes of degradation and decline. Revegetation programs are now commonly undertaken from property to landscape scales and include enlarging existing remnants and linking remnants with revegetated corridors (Yates and Hobbs 1997). Successful establishment of planted species, however requires high resource input. In addition to planting techniques, weed and grazing control (Greening Australia 2012), a knowledge of factors affecting juvenile growth rates is critical for planning and implementing revegetation programs. Surprisingly, Clarke's (2002) study is one of the few field studies on growth and survival rates of juvenile *Eucalyptus* species. Clarke (2002) concluded that the major causes of juvenile tree death in the New England region of New South Wales were the combined effects of insect defoliation, cold temperatures, and low soil moisture.

Although a lack of adequate moisture is often reported as a major factor that reduces the survival of *Eucalyptus* seedlings and juveniles (Garau *et al*. 2008), experiments manipulating the water supply of *Eucalyptus* juveniles have most commonly been done in controlled glasshouse environments rather than in natural settings (Duan 2014; Li and Wang 2003; Ngugi *et al*. 2003). In one of the few studies involving supplementary watering of *Eucalyptus* trees in their natural environment, Barton and Montagu (2006) found that red gums (*Eucalyptus camaldulensis*) grew faster both in height and diameter when grown at wide spacing and when irrigated.

In South Australia, pink gums (*Eucalyptus fasciculosa*) are largely confined to the south-eastern part of South Australia, but occur in areas of annual rainfall varying from 350–900 mm (Atlas of Living Australia 2016a). The decline of pink gum woodlands has been recognised (Paton *et al*. 2005) and has led to the provision of management guidelines (Tucker 2005), but these largely focus on generic principles of weed and grazing control. In moderate to lower rainfall areas of pink gum's natural distribution, recruitment of pink gums is rare (Chapter 1), hence active planting of pink gums may be necessary to replace dead trees and/or former areas of pink gum woodlands. Understanding factors that affect the growth rates of juvenile pink gums, therefore, will assist in determining revegetation needs. The aims of this study were to document the growth of juvenile pink gums when exposed to different environmental factors, including variable seasonal rainfall, herbivory and intraspecific competition. To do this I recorded the growth of pink gum juveniles in a woodland, between September 2010 and August 2016. This included providing supplementary water to juveniles during spring/summer 2010/2011. I hypothesised that pink gum juveniles would significantly increase growth rates in response to above-average water received. I also hypothesised that there would be differences in growth rates between individual pink gums due to intraspecific competition and herbivory.

7.3 Methods

Site details

The study site was a two hectare pink gum woodland at Highland Valley in the Mt Lofty Ranges, 240 m above sea level and with a mean annual rainfall of approximately 540 mm. The woodland contained mature pink gums with a herb and grass dominated understorey and unusually, 40 pink gum juveniles that had germinated in 2005 after the removal of domestic stock grazing (Dr Tim Milne 2009, pers. comm. landholder). The woodland was on a hill crest with a slope of 3^0 and a westerly aspect. Surface soil was generally a sandy loam texture. The nearest borehole was 20 m from one of the juvenile trees, and located on the same contour. The groundwater depth at this hole was 80 m when last recorded in 1991 (Government of South Australia 2015).

Study design

Within the pink gum woodland, 34 juveniles were chosen representing a range of heights and geographic spread. The location of the 34 trees is shown in Figure 7.1. Individual juveniles were between two and five metres from the nearest adult or juvenile tree. At the start of the study in September 2010 the juveniles were five years old and between 0.3 m and 0.95 m high, with a mean height of 0.55 m (SD ± 0.2). The juvenile canopies ranged from 80–100% intact with a mean 95% canopy intactness (SD ± 7). There were no obvious signs of insect damage on more than 5% of the leaves within each individual canopy.

Figure 7.1: Location of study site and location of study juveniles (black and white circles) within a 2 ha pink gum woodland, Highland Valley South Australia (scale1:2000). Black circles represent juveniles that were provided with supplementary water during October 2010 to March 2011.

Juvenile tree parameters

Juvenile parameters were recorded at several periods between September 2010 and August 2016 (Table 7.1). Canopy intactness was an estimate of the amount of foliage present compared to the potential maximum foliage present for the tree taking into account branching structure. The total number of canopy leaves was recorded in increments of 10 leaves up to 50 leaves, and thereafter in increments of 50 leaves namely: 10, 20, 30, 40 and 50 leaves, 51-100, 101–150 and so on. For canopies with > 300 leaves, leaves were counted on an estimated 10% of the canopy and then multiplied by 10 to determine total canopy leaves. Leaf production and loss was recorded by using a paint pen to number all leaves on two tagged branches. In September 2010, I initially numbered 1922 leaves on 68 branches. I visually estimated that between 30% and 95% of the leaves on each of the juveniles were numbered. Individually numbered leaves were subsequently monitored at approximately two-monthly intervals until February 2012, to record if the numbered leaves were still present. New leaves on the tagged branch were sequentially numbered. Dead leaves on the tagged stems were also recorded and identified as such. In August 2011, six tagged stems were broken off (presumably by kangaroos) from six trees. New stems were marked as substitutes to record leaf production and shoot extension. For the two tagged branches on each tree, I recorded shoot length by measuring from a marked location at the proximal stem end to the distal growing tip. For the whole tree canopy I recorded the percentage of live and dead leaves with an estimated > 10% of the leaf surface area skeletonised or chewed. For each tree, using vernier callipers, I measured the diameter of one stem, to the nearest 0.1 mm. All stems were < 20 cm from ground level. To increase the accuracy of repeat measures I painted a line around the stem circumference and ensured the same orientation of callipers in relation to the stem when diameters were re-measured. Using a tape measure, I recorded tree height from the ground to the highest live growing tip. The diameter of that portion of the lignotuber visible at ground level was recorded.

Table 7.1: Juvenile parameters and months recorded

Recording month/ year	Canopy Intactness	Total no. of canopy leaves	Height	Stem diameter Shoot extension	Leaf loss/ production	Lignotuber diameter	Leaf Damage
Sep 10	√	√	√	√	√	√	√
Dec 10	√	√	√	√	√		√
Feb 11	√	√	√	√	√		√
May 11	√	√	√	√	√		√
Aug 11	√						
Sep 11	√	√	√	√	√		√
Nov 11			√	√	√		√
Dec 11	√	√	√	√	√		√
Feb 12	√	√	√	√	√		√
Aug 15	√		√				
Aug 16	√	√	√			√	√

Supplementary watering

Within the pink gum woodland, 17 pairs of juvenile trees were chosen, with trees in each pair matched as closely as possible for height and canopy volume, and proximity to each other. All juveniles were at least 5 m apart. To determine if above-average water in spring and summer increased growth rates, 17 of the 34 juveniles were randomly chosen and provided with additional water. I provided five trees with a single pulse of water during the first week of October, November and December 2010; seven trees received a single pulse of water during the first week of January, February and March 2011; and five trees with a single pulse of water during the first week of each month from October, 2010 to March 2011. For all trees each watering consisted of 100 L of water. I applied water using 9 L buckets with a single

5 mm diameter hole in the base, to reduce flow rates. Water was applied at the rate of 3.3 L per minute (100 L every 30 minutes). I assumed that the lateral root spread of the watered trees was about twice that of the canopy projection of the tree (O'Grady *et al.* 2002), hence water was applied to an area within a 0.7 m radius of the tree stem, namely over 1.5 m^2. At each tree there was no litter layer, but approximately 90 to 100% of the ground area was covered with wild oats (*Avena barbata*) and/or spear-grasses (*Austrostipa* spp.). It was recognised that the water applied to each juvenile was also likely to be used by roots of nearby mature trees. However the supplementary watering was still considered valid to determine the impact of substantial amounts of additional water on the juvenile population as a whole, rather than on specific individuals. Ideally, this part of the experiment could have been improved by applying water uniformly to the whole woodland area encompassed by the juveniles or physically partitioning roots of individual trees.

Monthly rainfall

Long-term mean annual rainfall for the nearest weather station, Strathalbyn, 12 km to the south east of the study site is 490 mm (Bureau of Meteorology 2015). From rainfall isohyets, mean annual rainfall for the study site is 537 mm per annum (GeoScience Australia. 2009). Therefore, long-term monthly rainfall averages and actual monthly rainfall totals for the study site during the survey period were assumed to be 1.1 times greater than those for Strathalbyn (Table 7.2).

Rainfall received compared with supplementary watering

During the supplementary watering period from October 2010 to March 2011, the study site received approximately the mean spring rainfall but double the long-term mean summer rainfall (Table 7.2). In contrast, the following spring and summer rainfall totals in 2011/12 were 25% and 10% below long-term mean seasonal totals. The 100 L of supplementary water was applied over 1.5 m^2, hence was the equivalent of 67 mm over this area (1 mm of rainfall is equivalent to one litre over 1 m^2). Because the juvenile canopies occupied < 1 m^2, this water was likely to have penetrated within the majority of the juvenile root zone, except for the October and March waterings where there was approximately 50% overland flow.

Table 7.2: Mean and actual monthly spring and summer rainfall totals during the watering period - October 2010 to March 2011 - and amount of supplementary watering**.**

Month/Year	Long-term mean rainfall (mm)	Rainfall received (mm)	Supplementary watering - rainfall equivalent (mm)
Summer 2009/10	73	40	not applicable
Autumn 2010	127	131	
Winter 2010	203	182	
Spring 2010	137	149	200
Summer2010/11	73	153	200
Spring 2011	137	104	not applicable
Summer 2011/12	73	64	
Summer 2012/13		45	
Summer 2013/14		100	
Summer 2014/15		56	
2011 total	547	460	
2012 total		627	
2013 total		546	
2014 total		428	
2015 total		449	

*Rainfall figures for the study site were based on a correction factor (i.e. 110%) applied to the nearest weather station at Strathalbyn (Bureau of Meteorology website, weather station 023747, opened 1887).
**100 litres applied over 1.5 m^2 to 17 juveniles (1 litre = 1 mm over 1 m^2)

Water potential

I recorded midday stem and leaf water potential (Ψ) for juvenile study trees using a Scholander pressure bomb. Recording dates and midday daily temperatures were: 16 February 2011 (30 ^{0}C), which was towards the end of the supplementary watering; and 12 February 2012 (22 ^{0}C). On both occasions, measurements were taken from 18 juveniles. From each tree, I obtained two samples for leaf water potential measurements, and one sample for stem water potential. For stem water potentials, I enclosed several leaves in black plastic and covered this with foil, one to two hours before measurement. For all measurements, cut leaves were immediately placed in a zip-lock plastic bag and the water potential was measured within two minutes of excision. Stem Ψ is considered more representative of the water stress of the entire plant and less variable than leaf Ψ (Chone *et al.*2001).

Soil texture and soil moisture

I used a hand auger to obtain soil profiles from four locations, all within 2 m of study trees, and to a depth of 0.7–1.2 m. The depth of soil profiles was limited by rocks and/or lack of cohesiveness. Profiles were obtained on 30 July 2010, 7 October 2010, 15 March 2011, 31 May 2011 and 18 January 2012. At each survey date, profiles were obtained within 1 m of the previous profile location. From each profile, I collected a soil sample where there was a distinct change in soil texture or colour. Soil samples were placed in glass jars and sealed using electric tape. In the laboratory, for each sample I calculated: soil texture by fractionation (Colorada University 2003), and gravimetric water content (the mass of water per unit mass of dry soil), calculated as (wet soil weight – dry soil weight)/wet soil weight) x 100). Soil was dried at 105 ^{0}C for 24 hours. Gravimetric water content was multiplied by an approximate bulk density value for sandy loam soils (namely 1.5) to covert to volumetric water content. Within the soil profile depth sampled, soil textures varied from loamy sand, sandy loams, and sandy clay loam.

Surrounding canopy cover

For each juvenile, the area (m^2) of tree canopy within a 20 m radius was digitised using ArcGIS version 10.2. The aerial imagery was dated 2012, with a pixel size of 30 cm. The canopy cover was digitised at a scale of 1:200.

Statistical analysis

All statistical analyses were done using SPSS Version 23. Mean growth parameters were compared between trees provided with supplementary watering and non-watered trees, using paired t-tests. Pearson’s correlation was used to determine associations between change in juvenile stem diameter and height, and the combined projective foliage cover within a 20 m radius of the juvenile. Growth parameters for all juvenile trees combined were compared between different recording periods using a repeated measures General Linear Model (the growth parameters were the dependent variable and were normally distributed). The Greenhouse-Geisser correction factor was used when the assumption of sphericity had been violated. For stem diameter and height parameters, outlying values were also removed.

7.4 Results

Comparative growth of watered and unwatered trees

During the watering period and ensuing six weeks, 28 September 2010 to 19 May 2011, there was no significant difference in growth parameters (stem diameter, height, and leaf production) between watered and unwatered trees. Hereafter, the results refer to all 34 juveniles collectively.

Growth rates compared over different years

Stem diameters and height increased significantly between September 2010 and February 2011, but not between September 2011 and February 2012 (Table 7.3). There was also no significant height increase between February 2011 and August 2015. There was a 50% increase in mean stem diameter (9.4 cm to 14.3 cm) in the first 12 months of recording but between September 2011 and August 2016, increases in stem diameters averaged only 13% each year (increasing from means of 14.3 cm to 23.8 cm, a 66% increase over six years). For 25 trees there was a < 1 cm increase in stem diameter between 2010 and 2016 (Figure 7.2). Twelve of the 34 trees recorded a < 5 cm height increase between September 2010 and August 2016 (Figure 7.2).

Table 7.3: Mean stem diameters (cm) for all 34 juvenile pink gums at different times between September 2010 and August 2016. Repeat measures ANOVA with Greenhouse Geisser correction. Superscripts show significantly different homogeneous means**.** For stem diameters, three outliers were removed and one outlier was removed for heights.

Recording date	Mean stem diameter ± SE	Mean height (cm) ± SE
28/9/10	$8.5 \pm 0.7^{\mathbf{A}}$	$50 \pm 2.5^{\mathbf{A}}$
7/2/11	$9.7 \pm 0.7^{\mathbf{B}}$	$60 \pm 3.5^{\mathbf{B}}$
19/5/11	$11.0 \pm 0.8^{\mathbf{C}}$	$62 \pm 3.8^{\mathbf{B}}$
26/9/11	$12.0 \pm 0.9^{\mathbf{C}}$	$62 \pm 4.2^{\mathbf{B}}$
21/2/12	$11.8 \pm 1.0^{\mathbf{C}}$	$63 \pm 4.6^{\mathbf{B}}$
14/8/15	not recorded	$67 \pm 5.3^{\mathbf{BC}}$
13/8/16	$16.1 \pm 1.5^{\mathbf{D}}$	$70 \pm 6.4^{\mathbf{C}}$
F	37.53	9.13
p	< 0.001	0.002

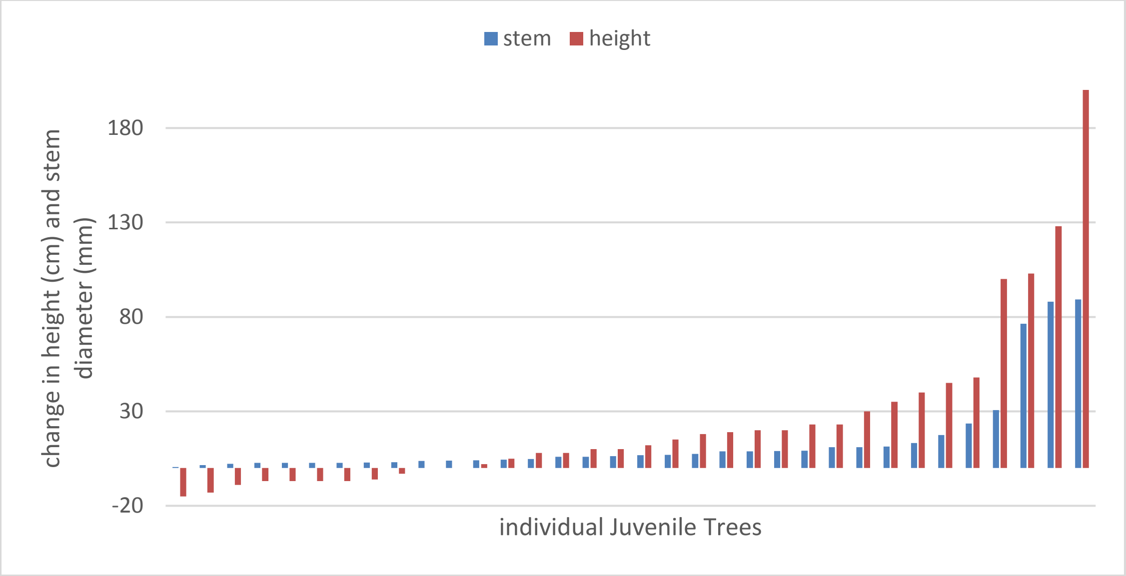

Figure 7.2: Change in stem diameter (mm) and height (cm) between September 2010 and August 2016, for the 34 juvenile pink gums.

Total number of canopy leaves

A paired t-test showed that there was no significant difference in the mean number of canopy leaves at the start and end of the experiment (t = -1.562, p = 0.128) with the mean number of total leaves per canopy at the start being 170 (SE ± 19) and at the end being 295 (SE ± 83). Thirteen trees had fewer leaves in August 2016 than in September 2010, there was no change in in nine trees, and there was an increase in leaf number in 16 trees (Figure 7.3).

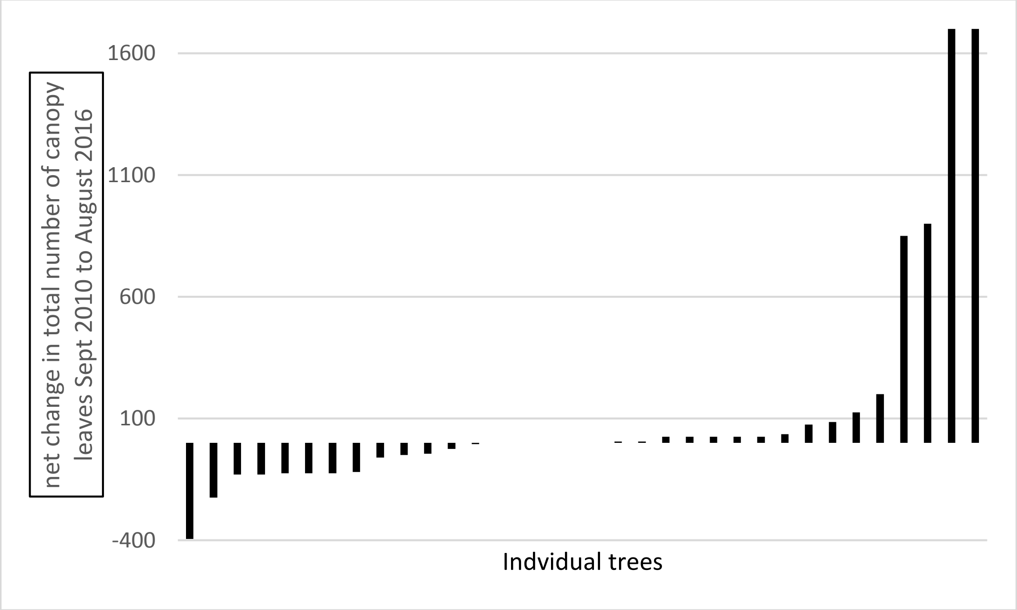

Figure 7.3: Change in total number of canopy leaves for 34 juvenile pink gums, between September 2010 and August 2016

Leaf production

Mean leaf production between September 2010 and February 2011 was 77 (SE ± 16) leaves per two marked branches for each tree – over twice that of the mean leaf production between September 2011 and February 2012 (37 ± 14) and significantly higher (paired t-test, df = 33, t = 4.0, $p < 0.001$). There was a strong positive association between the increase in height between September 2010 and May 2011 for an individual tree and the increase in height between May 2011 and August 2016 (Pearson's correlation, R = 0.77, df = 33, $p < 0.001$). Namely, trees that grew most in height during the above-average rainfall/watering period tended to grow most in height during the following five years.

Factors affecting growth other than water supply

Between September 2010 and February 2011, although the mean height increase for the 34 juveniles was 10.8 cm (SE ± 2.8 cm) and significantly greater than for any other recording period, 50% of the trees recorded a ≤ 5 cm height increase, including five trees with no height increase. This was despite summer rainfall being over twice the long-term average. Twelve of the 34 trees (35%) recorded a < 5 cm height increase between September 2010 and August 2016 and 50% recorded a < 5 cm increase between September 2011 and August 2016. Between September 2011 and August 2016, 25 trees recorded a < 1 cm increase in stem diameter.

Insect damage

Between September 2010 and August 2016, the mean canopy intactness for the 34 juveniles declined 29% from 95% (SE ± 1.2) to 66% (SE ± 5.7). The largest seasonal mean change in canopy intactness for all trees was a 24% decline in spring 2011, after which mean canopy intactness gradually increased until February 2012 (Figure 7.4). Between September 2010 and February 2012, almost 50% of all leaves were lost during spring 2011 (Figure 7.5). This loss coincided with an outbreak of gum leaf skeletoniser (*Uraba lugens*) in spring 2011 when 30 of the 34 juveniles contained leaves with > 10% of their leaf area skeletonised. In September and November 2011, *Uraba lugens* individuals were seen on canopies and a mean of 50% (SE ± 7) and 40% (SE ± 6), respectively of all numbered leaves had skeletoniser damage. In November 2011, 20% of the 68 tagged stems had no live leaves left. Following extensive defoliation, there was a net leaf gain during summer 2011/12. The juveniles with a < 5% change in canopy intactness between September 2010 and 2016 did not have obvious skeletoniser damage during the recording periods. For all recording periods between

September 2010 and 2016, there was a significant negative correlation between canopy intactness and the percentage of all adult leaves per canopy with skeletonised leaves (N = 321, R = -0.39, p < 0.39). There were no significant correlations between the percentage of leaves with chewing symptoms and canopy intactness, nor with the percentage of leaves with fungal symptoms and canopy intactness (N =321, R = 0.003, p = 0.96 and R = -0.02 p = 0.725, respectively).

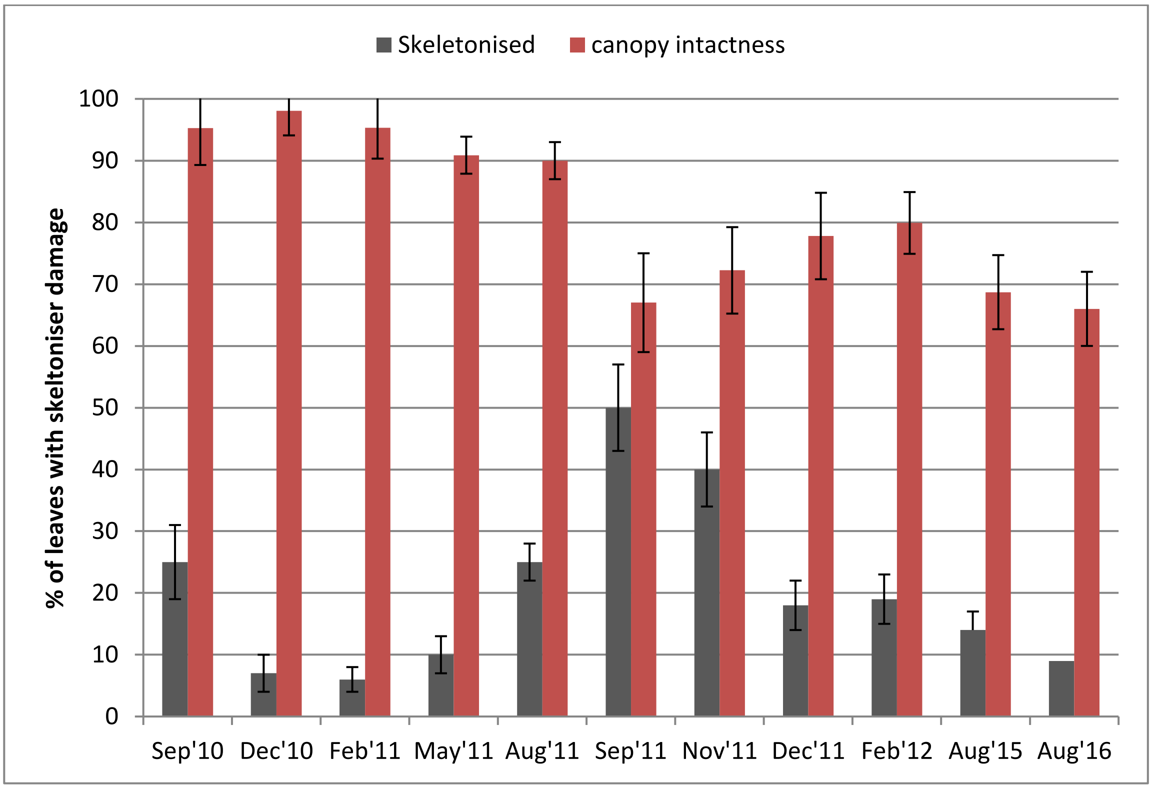

Figure 7.4: Mean percentage of leaves recorded with obvious (> 10% of leaf area) skeletoniser damage for 34 juvenile pink gums at Highland Valley from September 2010 to February 2012. Error bars show SE.

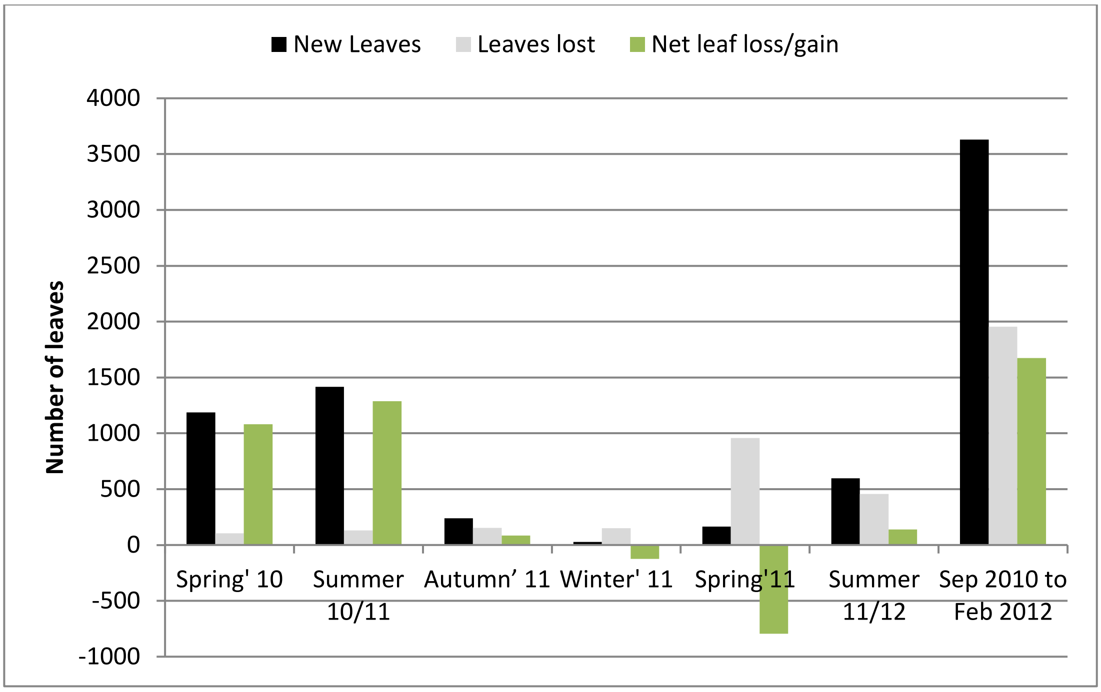

Figure 7.5: Net leaf gain/loss each season for 34 juvenile pink gums. Leaf production and loss was recorded on two tagged stems from each juvenile. Initially, 1244 leaves were numbered on 68 tagged stems (two per tree) with a mean of 18 ± 8 (SE) leaves per tagged stem.

Lerps

Lerps were recorded only in August 2015 when lerp cases were recorded on nine of the 34 juveniles, and on between 5% and 50% of the canopy leaves.

Kangaroo damage

Between September 2010 and February 2012, six of the tagged branches were damaged or broken off, presumably by kangaroos.

Competition

Between September 2010 and August 2016, there was a highly significant negative correlation between change in juvenile stem diameter and the combined projective foliage cover within a 20 m radius of the juvenile (Pearson’s correlation, $R = -0.64$, $p < 0.001$, (Figure 7.6). A similar association existed between the change in juvenile height between September 2010 and August 2016 and combined projective foliage cover within a 20 m radius of the juvenile ($R = - 0.70$, $p < 0.001$). Between September 2010 and August 2016, only four of the 34 juveniles recorded a height increase of > 40 cm, and these juveniles had the least amount of canopy cover from surrounding pink gums within a 20 m radius.

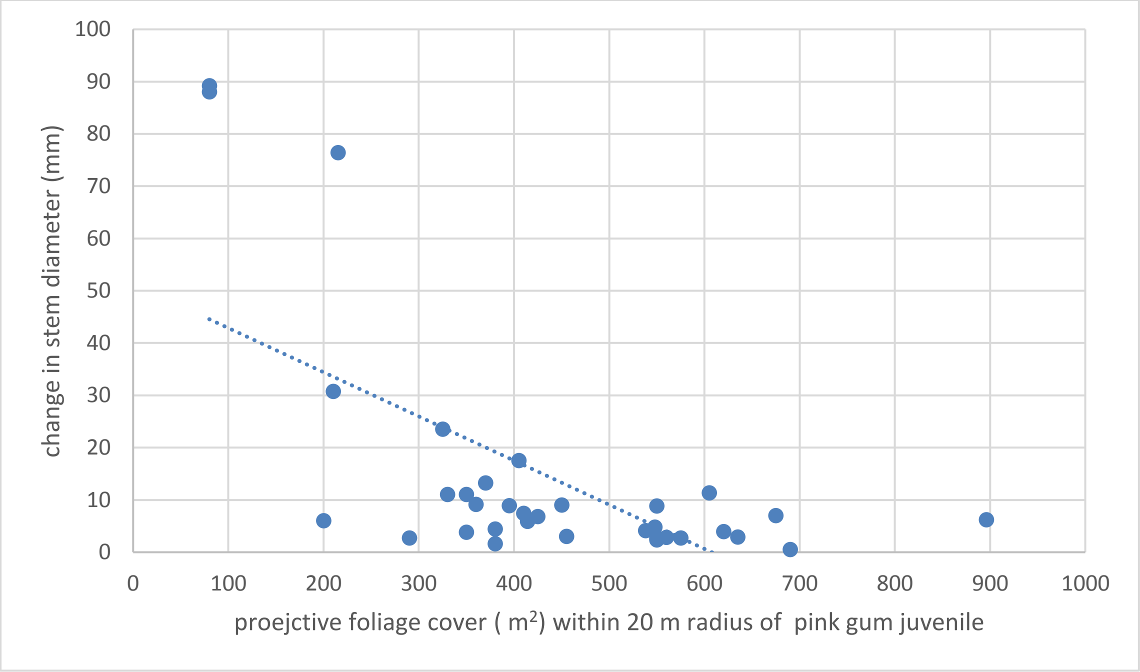

Figure 7.6: Scatter diagram showing the negative and significant correlation between change in juvenile stem diameter (between September 2010 and August 2016) and the projective foliage cover (m^2) within a 20 m radius of the juvenile**.**

Midday leaf and stem water potentials

On 16 February 2011, near the end of summer and the supplementary watering, the mean midday stem water potential was -2.3 MPa (SE ± 0.1) for both watered and non-watered juveniles. This was not significantly different to the mean stem water potential of -2.5 MPa (SE ± 0.11) on 12 February 2012. In contrast, the mean leaf water potential for the juveniles in February 2011 was - 2.62 ± 0.05 MPa which was significantly higher than the mean leaf water potential for February 2012 of -2.95 ± 0.12 MPa (repeat measures, GLM F = 22.5, $p < 0.001$).

Soil moisture and soil water potential

At depths of 25 and 50 cm, and 2 m from juvenile trees, soil volumetric water ranged from 16%–28% in March 2011 (Table 7.4). In January 2012, for the same profiles and depths, soil volumetric water recordings ranged from 5–17%, reflecting the much drier season.

Table 7.4: Soil volumetric water (%) for four soil profiles recorded in October 2010, March 2011 and January 2012. One gravimetric water calculation was done for each depth within each profile

Recording date	Soil depth (cm)	Soil volumetric water (%) for different treatments			
		Profile 1	Profile 2	Profile 3	Profile 4
7/10/10	20–25	16	11	nr	6
	45–50	33	27	nr	16
	60–70	33	27	nr	16
15/3/11	20–25	18	23	28	16
	45–50	27	27	23	16
	60–70	27	27	23	16
18/1/12	20–25	not recorded	5	11	8
	45–50	not recorded	17	5	10
	60–70	not recorded	17	5	10

Lignotuber diameter

In September 2010, the diameters of the lignotubers of the 34 pink gums ranged from 2– 9 cm, with a mean diameter of 4.3 cm (SE ± 0.3 cm) In August 2016 the mean lignotuber diameter was 6.1 cm (SE ± 0.4 cm). The 1.8 cm increase was significant (paired t-test, t = - 6.114, df 33, $p < 0.001$).

7.5 Discussion

Five-year-old pink gums responded to the above-average 2010/11 summer rainfall with significant increases in height, leaf production, stem diameter and stem length. During this period, all these growth parameters were significantly greater than during the corresponding period the following year. The lower rainfall totals during summer 2011/12 was reflected in lower soil moisture levels and more negative leaf water potential values than compared with summer 2010/11. Hence reduced water may have been one factor in the lower mean growth rates of the juvenile pink gums in 2011/12.

The increased growth in summer 2010/11, however, did not apply to all pink gum juveniles. During this time, 50% of the study trees recorded a < 5 cm increase in height. Furthermore, there was negligible growth of the plants for the next five years, despite some of those years having average to above average rainfall. Between September 2010 and August 2016, 35% of the study trees recorded a < 5 cm increase in height and 70% (25 trees) recorded a < 1 cm increase in stem diameter. Further, trees that grew most in height during the above-average rainfall/watering period tended to grow most in height during the following five years. These findings suggest that there were factors in addition to water supply controlling the juvenile growth. Possible potential factors include insect herbivory, competition from other plants and inherent variation between individual trees.

In spring 2011, following the high rainfall summer of December 2010 to February 2011, there was an outbreak of gumleaf skeletoniser insect (*Uraba lugens*), which was associated with extensive defoliation of the juveniles. There was a significant negative correlation between the percentage of leaves damaged by skeletoniser insects, and the increase in tree height. Insect defoliation has been found to be a major factor responsible for death of *Eucalyptus* seedlings in south-eastern Australia, and particularly in the New England region of New South Wales (Clarke 2002; Lowman and Heatwole 1992). Although Wills *et al.* (2004) recorded no mortality of jarrah (*Eucalyptus marginata*) juveniles in Western Australia that were subject to up to 15 consecutive years of complete insect defoliation, the chronic defoliation did lead to a complete cessation of stem growth. The suppressed growth of pink gums and absence of mortality during the study period may reflect a similar defoliation resistance in pink gums, combined with intra-specific competition. The interaction between insect outbreaks and changing rainfall patterns and their combined impact on trees is only just beginning to be explored, but is critical in predicting patterns of tree mortality (Anderegg *et al.* 2015).

The juveniles were growing in a mature pink gum woodland and it is likely that the surrounding mature trees quickly used the available water resource. For example, Zeppel *et al*. (2008) found that semi-arid woodland trees of New South Wales quickly increased their water use when pulses of rainfall occurred. For the current study, there was a highly significant correlation between change in pink gum height and stem diameter, and the amount of projective foliage cover within a 20 m radius. Whether the apparent sensitivity of pink gums to competition was due to light, soil nutrients and/or water was not determined by this study. It is possible that some of the variation in growth response between pink gum juveniles was due to inherent differences in the growth potential of the juveniles themselves, namely some trees were predisposed to greater growth rates. For example, Ashton (1975) noted the great variation in shoot growth between *Eucalyptus regnans* seedlings.

The finding that surplus water in summer 2010/11 did not result in significant growth of approximately 50% of individuals during this period, may have implications for pink gum revegetation programs. Where plantings are very dense and/or soil fertility is limited, and/or insect herbivory is high, the provision of additional water may have limited benefit. It may also take an extended period to re-establish pink gums in some habitats as indicated by the almost static height of 35% of the juveniles over six years, despite receiving an exceptional amount of water in spring/summer 2010/11. Soil moisture readings during the high rainfall summer indicated that the soil was approaching saturation point. Similarly, midday stem water potential recordings indicated that the trees were not under water stress. In a separate study over two years (Chapter 2) I recorded the height of juvenile pink gums in pink gum woodland, near Clayton, approximately 30 km south east of the present study site, and where mean annual rainfall is 400 mm (regional study site number 13, refer Appendix A). At this site in 2009, the mean height of 23 pink gums that were 17 years-old, was 0.7 m (SD ± 0.4 m) but their height had increased by 25% to 0.9 m (SD ± 0.5 m) in 2011.

The study recorded increases in lignotuber growth in all but four of the juveniles, but suppressed increases in height in 50% of the juveniles. The slow growth rate of up to 50% of the pink gum juveniles at the Highland Valley study site and at the Clayton woodland in all but exceptional rainfall years, is comparable with the growth rate and development of juvenile jarrah (*Eucalyptus marginata*), a Western Australian eucalypt. Jarrah grows in a Mediterranean climate and hence experiences hot dry summers. Szota (2009) recorded a slow growth rate of jarrah, stating that the shoots can remain < 1.5 m for 15–20 years until the lignotuber is about 10 cm in diameter. Clarke (2002) also noted the connection between the possession of a lignotuber and suppressed growth rates in five-year-old *Eucalyptus* species. As a generalisation, *Eucalyptus* species possess lignotubers in areas with low rainfall and slow growth rates (Florence 2000). In September 2010, the diameters of the lignotubers of the pink gum juveniles in this study ranged from 2–9 cm. By 2016, all but four of the juveniles recorded increased lignotuber diameters. If the growth rate of pink gums is comparable to jarrah (based on lignotuber size) then up to half of the study trees may still have been in the phase of very slow above-ground growth, while roots and lignotubers were being developed.

The season of rainfall may also be significant in determining the magnitude of growth in pink gums. Between September 2010 and May 2011, which included summer rainfall being twice the long-term mean, height and stem diameter growth rates were greater than that of the following five years between September 2011 and August 2016. During this latter period, summer rainfall was generally below average in each year, while yearly totals were close to long-term means. Above average rainfall in summer therefore may be critical for significantly increasing pink gum growth. Since 1970 there has been a 15% decline in late autumn and early winter rainfall in the southeast of Australia (which includes the study site) (Bureau of Meteorology and CSIRO 2014). Delays and declines in autumn rainfall may exacerbate the slower growth rates, unless summer rains are above average. When habitat variables required for pink gum growth are non-limiting, high summer rainfall may be more important in increasing pink gum growth than high annual rainfall totals *per se*. Indirect effects of high rainfall on pink gums such as increased insect herbivory and the interaction between herbivory and climate stress, also needs further long-term study. Population genetic studies may also help determine if there are inherent differences in growth rates between individuals. Broader implications of the study are that the response of a species to climate change may vary according to local small scale habitat factors.

Chapter 8 Growth response of pink gum (*Eucalyptus fasciculosa*) provenances to various water regimes

8.1 Abstract

Widespread dieback of *Eucalyptus* species in southern Australia is increasingly being attributed to long-term declines in rainfall. Pink gums (*Eucalyptus fasciculosa* F. Muell) are largely confined to southern South Australia, but within parts of this range concern has been expressed for their high levels of dieback, possibly due to long-term declines in water availability. A glasshouse experiment was conducted to determine if pink gum juveniles had lower growth parameters when water was limited, if pink gum provenances differ in biomass parameters and water use strategies; and if there were differences in the response to water stress between three pink gum provenances within the Mt Lofty Ranges. Pink gum juveniles from three provenances within the Mt Lofty Ranges, were subjected to five levels of water stress in a glasshouse, over a period of four months. Control plants were watered to field capacity every two to three days, while treatment plants received either: 100% (T100), 75% (T75), 50% (T50) or 25% (T25) of the mean water loss of control plants. For all provenances, significantly reduced leaf production, more negative water potentials, and increased root to shoot ratios resulted only when water supply was reduced to 25% of the control plants' water loss. At the end of the experiment, control plants had almost 50% greater biomass and double the number of leaves of the T100 plants. While the performance of all provenances to variable water stress was not significantly different, there were differences in biomass parameters between the provenances. The high rainfall Myponga provenance juveniles had a significantly higher mean total biomass, stem diameter and number of leaves than the lower rainfall Currency Creek and Blewitt Spring juveniles. The Myponga juveniles also used significantly more water than the Currency Creek and Blewitt Spring provenances. Despite the lack of a significant interaction between provenance and water stress, the Myponga juveniles recorded the lowest biomass parameters when subject to the greatest water stress. The study's findings support the trend to match tree provenance traits, with revegetation requirements, including water usage, desired potential biomass and growth rates. The lack of an interaction between water stress and provenance however, suggests that all populations in the Mt Lofty Ranges may be equally susceptible to ongoing decline of autumn rainfall and hotter summers. Further research on the responses of different pink gum provenances to water stress is recommended.

8.2 Introduction

Tree decline due to long-term drought and heat stress is increasingly being observed both globally and in Australia (Dalmaris *et al.* 2015). For example, the drying and warming trend in the south-west of Western Australia since the 1980s has been linked to forest decline throughout the region (Evans *et al*. 2013). The long-term survival of a tree species will depend *inter alia* on its ability to tolerate changing climate regimes. Water stress is the most important abiotic factor limiting survival of eucalypt juveniles (Garau *et al.* 2008). In southern Australia's Mediterranean climate, successful tree establishment requires the ability to survive summer drought and heat in the first year of growth (Holloway-Phillips *et al.* 2015). Several studies have investigated the responses of juvenile *Eucalyptus* species to drought stress, most of which have found differences in responses between provenances (Tuomela *et al.* 2000; Li and Wang 2003; Ngugi *et al.* 2003; Garau *et al.* 2008). *Eucalyptus* species that occur over a wide habitat range often have the ability to exhibit a range of phenotypes depending on environment, but additionally are often genetically differentiated across environmental gradients (McLean *et al.* 2014*)*. Increasingly, in revegetation projects, selection of seed sources to match projected climate changes is being considered (Lowe 2016; McLean *et al.* 2014; Prober *et al.* 2015).

Pink gums (*Eucalyptus fasciculosa*) are largely confined to the south-eastern temperate zone of South Australia, but within this area, they occupy a wide habitat range (Boomsma and Lewis 1980). For example, within the Mt Lofty Ranges and adjoining plains, pink gums occur at sites receiving from 340 mm to 900 mm rainfall per year; on deep sandy soils to shallow duplex soils, acid to alkaline soils, and from coastal plains to over 500 m above sea level (Chapter 1). The outward appearance of pink gums is similarly diverse, ranging from single-trunked woodland trees up to 18 m tall to gnarled multi-trunked trees less than 4 m tall (Chapter 1). Within parts of its natural range, concern has been expressed for the low level of pink gum canopy intactness (Paton *et al.*2005; Ward 2007). Litchfield (1956) and White (1970) noted that pink gums are confined to soils not prone to waterlogging, but which have the capacity to store sufficient water to meet the needs of pink gums over summer. The monitoring of pink gums in the Mt Lofty Ranges between 2003 and 2015 recorded significant fluctuations in canopy intactness coinciding with periods of above- or below-average rainfall (Chapter 3). Historical aerial photographs of two pink gum woodlands in the Mt Lofty Ranges also suggest that the decline of pink gums at these sites commenced in the 1970s, coinciding with a long-term decline in rainfall and providing further evidence that water stress is a cause of declining canopy condition (Chapter 4).

Although the distribution of pink gums covers a large rainfall gradient, local water availability depends upon not only rainfall, but factors such as tree density, soil texture, depth to groundwater and topography (after Dalmaris 2012). Even so, it may be expected that pink gum populations at the lower rainfall margins of pink gum's natural distribution will be better adapted to low water availabilities. With continuing declining rainfall predicted in southern Australia (Bureau of Meteorology and CSIRO 2014), determining if populations have different adaptations to water stress is important for revegetation and restoration planning (after Dalmaris 2012).

The aims of this study were to examine the morphological and water potential responses of pink gum juveniles to varying levels of water stress in order to determine if: pink gum juveniles have lower growth parameters when water is limited; pink gum provenances differ in biomass parameters and water use strategies; and if there were differences in the response to water stress between three pink gum provenances within the Mt Lofty Ranges. I hypothesised that the high rainfall provenance would have the greatest increase in biomass when water was non-limiting, but would have the lowest biomass increase under extreme drought conditions.

8.3 Methods

For the water stress experiment, 150 six-month old pink gum juveniles were purchased from State Flora, Murray Bridge. These juveniles had been grown in potting mix from seed sourced at three provenances within the Mt Lofty Ranges: Currency Creek, Myponga and Blewitt Springs (Figure 8.1). Fifty juveniles were from each of the three provenances. The mean annual rainfall and soil type for these locations are shown in Table 8.1. Soil data was provided by State Flora, Murray Bridge. Details of the number of trees from which seeds were sourced to propagate the seedlings were not available. On the 30 March 2010, prior to the start of the experiment there was no significant difference in the height of the juveniles between the three provenances, with the Blewitt Springs, Currency Creek and Myponga provenances having mean heights (± SE) of 44.5 ± 0.7 cm, 43.0 ± 0.9 cm and 42.9 ± 1.0 cm, respectively (ANOVA, F = 1.0, p = 0.39). Two weeks after purchase, I transferred the seedlings to larger pots, 175 mm deep x 170 mm wide, with a 3.3 L capacity, containing a peat-sand mix, and with a pH of 6 to 6.5. The mix also contained approximately 1% lime and 1% artificial fertiliser. All juveniles were watered to field capacity (the amount of water held in the soil before being drawn away by gravity), every 48 to 72 hours, for six weeks after purchase until the experimental watering regime commenced.

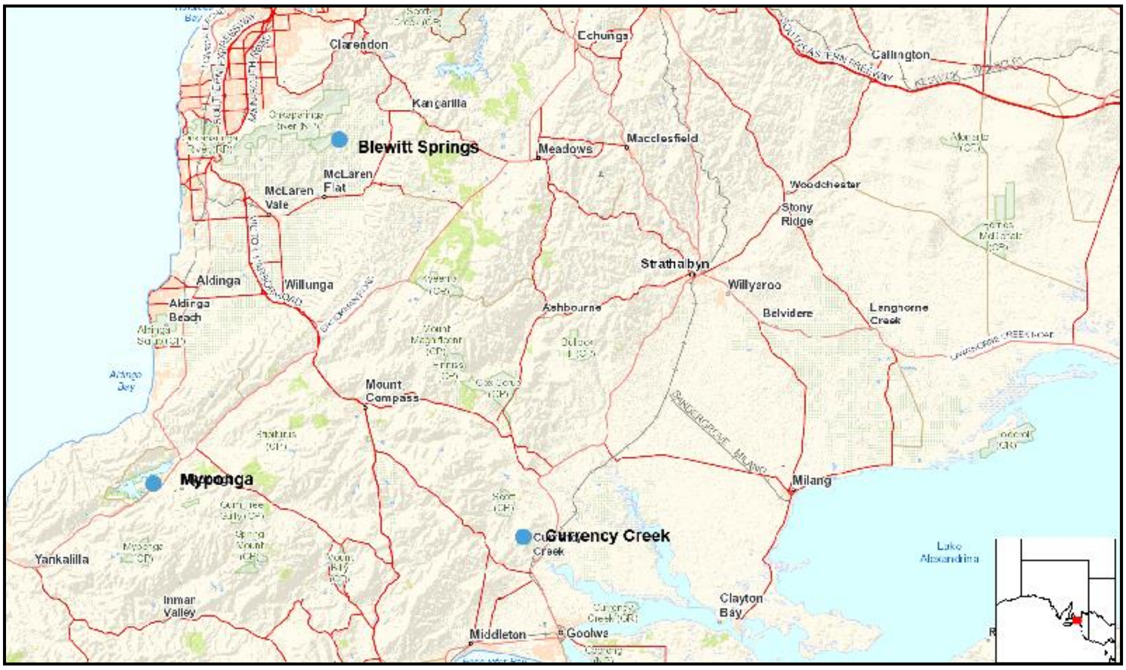

Figure 8.1: Location of pink gum provenances used in glasshouse experiment (Blewitt Springs, Myponga and Currency Creek)

Table 8.1: Pink gum provenances used in watering experiment, long-term average annual rainfall and surface soil type. Rainfall represents mean annual rainfall 1976–2005 (NatureMaps 2016)

Provenance locality	Latitude	Longitude	Mean annual rainfall (mm)	Surface soil texture
Blewitt Springs	$35^{0}10'04''$ S	$138^{0}35'50''$ E	643	clay loam
Currency Creek	$35^{0}27'14''$S	$138^{0}45'40''$ E	480	sandy
Myponga	$35^{0}23'50''$ S	$138^{0}25'10''$ E	765	clay

To test whether pink gum provenances responded variably to water stress, 10 plants from each of the three provenances were randomly chosen to receive one of five watering regimes. Control plants were watered every 48 to 72 hours, to saturation (namely, all pore spaces were filled with water). Once throughflow had ceased and field capacity was reached, I weighed all the control pots. The control pots were then re-weighed 48 to 72 hours later, just prior to watering, and the difference in pot weight was calculated. T100 plants within each provenance received the mean weight loss of control plants for each respective provenance in grams of water. T75, T50 and T25 plants received 75%, 50% and 25%, respectively of the mean weight loss in grams of water. The experimental watering regime continued for 16 weeks, commencing on 19 April 2010 when the pink gums were 7½ months old and finishing four months later on 9 August 2010 (autumn to winter). Temperatures in the greenhouse were not regulated, hence were similar to outside temperatures. Outside maximum daily temperatures ranged from 12 ^{0}C to 30 ^{0}C during the experiment (Bureau of Meteorology 2015).

Immediately before commencing the watering regimes, and every eight weeks thereafter, I added 4 grams of soluble fertiliser (Thrive®) to each pot. A wetting agent was applied two weeks after the first experimental watering, to ensure even distribution of water throughout the soil. All plants were randomly rotated in the glasshouse every two to three weeks, to minimise possible environmental variation within the glasshouse. At the start of the experiment I added a surface layer of 150 grams of propagation gravel to each pot to minimise evaporative water loss from the soil. A pesticide, Pyrethrum®, was applied on four occasions during the experiment to control sap-sucking insects.

I recorded midday and pre-dawn leaf water potential on three occasions between day 33 and 97 of the experiment, using a Scholander pressure bomb. Not all plants could be measured each recording event, due to time restrictions, and hence plants chosen for water potential measurements were chosen at random. Within the two hours available for water potential measurements, I recorded water potential for either 45 or 60 juveniles, these being either three or four juveniles (out of a possible 10 juveniles) selected from each of the five watering regimes for each of the three provenances. Pre-dawn measurements used the same trees as the previous day's midday measures.

All plants were harvested upon completion of the experiment. I removed all leaves from all plants while still fresh, and sorted them into three categories: emergent leaves, young leaves and fully-expanded leaves. Emergent leaves were defined as linear leaves < 0.5 cm long; young leaves as 0.5–3 cm long; and fully-expanded leaves as all leaves > 3 cm long. Fresh leaves were scanned to determine leaf area.

After recording leaf area, all components of the plants (leaves, stems and roots) were dried at 80 ^{0}C for 48 hours. I measured the following attributes on dried material: height, stem diameter at 1 cm above the lignotuber, lignotuber diameter, specific leaf area, biomass, and root to shoot ratio. Height was measured as the distance between the stem base (immediately above lignotuber) and the shoot tip. Specific leaf area (SLA) is the one-sided area of a fresh leaf divided by its oven-dry mass, expressed in m^2 kg^{-1} or (correspondingly) in mm^2 g-^{1} and is normally calculated on fully expanded leaves (Cornelissen *et al.* 2003). Leaf biomass was recorded separately for emergent, young and fully-expanded leaf categories. The root to shoot ratio was calculated as the ratio of the dry mass of roots to dry mass of leaves and shoots combined.

Statistical analysis

All analyses were done using SPSS software package version 23. After testing for homogeneity of variance and normal distribution of the dependent variables, I used Two Way ANOVA to compare the effect of the different provenances and the different watering regimes on biomass, number of leaves and height, and to determine possible interactions between these two independent variables. To determine which provenances and/or watering regimes had significantly different means, I used two-way ANOVA post-hoc Tukey's honestly significant different test. To determine which treatments had significantly different means within a single provenance I used one-way ANOVA Welch tests (as variances were not homogeneous). To determine if there were differences in biomass parameters between the provenances, I used one way ANOVA and Tukey post hoc tests. During and at the end of the experiment differences between provenances in mean water use and water potential were determined with One Way ANOVA.

8.4 Results

Effect of watering regime

Watering regime had a significant effect on all recorded variables. At the end of the experiment, the control trees had a significantly higher biomass for all biomass components (total biomass, stems and leaves, and root weight), and significantly greater lignotuber diameter than any of the treatments and the T25 plants had a significantly lower biomass than any of the other treatments (Table 8.2). For all treatments combined, the control plants' mean total biomass was 40% greater than the T100 (100% water replacement) plants and 85%

greater than T50 plants. In turn, T100 plants had a significantly higher biomass than T50 plants, which had a significantly higher biomass than the T25 plants.

Table 8.2: Mean biomass ± SD (gram dry weight) parameters for control and treatment pink gums at the end of the experiment. Values within a column followed by the same superscript letter are not significantly different (One-way ANOVA, Tukey post hoc test)

Treatment	Total biomass (g)	Root biomass (g)	Stem + leaves biomass (g)	Stem biomass (g)
Control	14.6 ± 6.8^{A}	2.7 ± 1.3^{A}	11.9 ± 5.9^{A}	6.2 ± 2.0^{A}
T100	10.5 ± 3.8^{B}	2.1 ± 1.1^{B}	8.4 ± 3.3^{B}	3.8 ± 1.6^{B}
T75	8.1 ± 2.6^{BC}	1.7 ± 0.6^{B}	6.7 ± 2.1^{BC}	3.1 ± 1.0^{BC}
T50	7.8 ± 2.5^{CD}	1.8 ± 0.7^{B}	6.0 ± 2.0^{CD}	2.9 ± 1.0^{BC}
T25	5.5 ± 1.5^{E}	1.4 ± 0.4^{B}	4.1 ± 1.3^{D}	2.1 ± 0.7^{C}
F	21.1	8.7	25.0	28.7
p	< 0.01	< 0.001	< 0.001	< 0.001

As with total biomass, the amount of water received by plants had a highly significant effect on leaf production and/or retention, stem diameter, root to shoot ratio, lignotuber diameter and SLA (Table 8.3). At the end of the experiment, the mean number of fully-expanded leaves (> 3 cm long) for control plants was over 30% greater than that of T100 plants. Control and T25 plants had the highest and lowest mean stem diameters, respectively. The mean root to shoot ratio was highest for T25 plants, but there was no significant difference between Control, T100, T75 and T50 plants. The mean lignotuber diameter of control plants was significantly larger than T75 plants, and T100 plants were significantly larger than the T25 plants.

At the end of the experiment, control and T100 plants had a greater number of emergent and young leaves (< 3 cm long) than T75 and T50 plants, with control plants having double the number of leaves of T75 and T50 plants. In turn, T75 and T50 plants had three times the number of young leaves as did T25 plants, with new leaf production having almost ceased in T25 plants at the end of 16 weeks of treatment. The SLA of T25 plants was less than control plants. Although there was an increase in SLA with increasing water received, the differences between adjoining treatment levels were not significant (Table 8.3). Control plants had a significantly higher (less negative) predawn water potential than T50 and T25 plants at all recording periods (Table 8.4). For each treatment level, the midday water potential showed no clear trends between recording dates (Table 8.5). For the recordings on days 48, 71 and 94, T25 plants had a more negative water potential than the T75, T100 and control plants.

Table 8.3: Comparison of the mean number of leaves, stem diameter, root to shoot ratio, lignotuber diameter and Specific Leaf Area (± SD) for each watering regime at the end of the 16 week watering experiment. Values within a column followed by the same superscript letter are not significantly different (one-way ANOVA, Tukey post-host test)

Treatment	Parameter mean ± SD					
	# of leaves > 3 cm	# of leaves 0.5–3 cm	stem diameter (mm)	root to shoot ratio	Lignotuber diameter (mm)	SLA
Control	35 ± 20.0 ^{A}	56 ± 34^{A}	3.4 ± 1.0^{A}	0.25 ± .03^{A}	10.8 ± 2.1^{A}	4.4 ± 0.9 ^{A}
T100	25 ± 13.8^{B}	43 ± 26^{AB}	2.7 ± 0.7^{B}	0.27 ± .03^{A}	9.4 ± 2.6^{AB}	4.1 ± 0.6 ^{AB}
T75	20 ± 7.5^{BC}	28 ± 19^{B}	2.6 ± 0.6^{B}	0.26 ± .03^{A}	8.3 ± 2.4^{BC}	4.1 ± 0.7 ^{AB}
T50	19 ± 7.1^{BC}	28 ± 18^{B}	2.4 ± 0.6^{B}	0.31 ± .04^{AB}	8.9 ± 2.1^{BC}	3.8 ± 0.7^{BC}
T25	13 ± 5.8^{C}	9 ± 13^{C}	2.3 ± 0.5^{C}	0.36 ± .04^{B}	7.9 ± 1.8^{C}	3.4 ± 0.5^{C}
F	13.7	26.7	14.2	5.4	9.4	8.9
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 8.4: Mean values (± SD) for pre-dawn water potential measurements (MPa) for control and treatment plants during and at the end of the experiment. Values within a column followed by the same superscript letter are not significantly different (one-way ANOVA, Tukey post-host test)

Treatment	Water potential (MPa) value for different dates		
	Day 49 23 May 2010	Day 58 17 June 2010	Day 95 24 July 2010
Control	-0.7 ± 0.1^{A}	-0.6 ± 0.2^{A}	-0.8 ± 0.2^{A}
T100	-0.8 ± 0.1^{AB}	-0.8 ± 0.2^{AB}	-1.0 ± 0.2 ^{AB}
T75	-0.8 ± 0.2^{AB}	-0.9 ± 0.2^{AB}	-1.0 ± 0.2^{AB}
T50	-1.1 ± 0.4^{B}	-1.2 ± 0.3^{BC}	-1.2 ± 0.2^{B}
T25	-1.6 ± 0.6^{C}	-1.5 ± 0.8^{C}	-1.2 ± 0.1^{B}
F value	10.7	8.0	5.6
P value	< 0.001	< 0.001	0.001

Table 8.5: Mean values (± SD) for midday WP measurements (MPa) for control and treatment plants during and at the end of the experiment. Values within a column followed by the same superscript letter are not significantly different (one-way ANOVA, Tukey post-host test)

Treatment	Mean water potential (MPa) value for different dates		
	Day 48 7 June 2010	Day 57 16 June 2010	Day 94 23 July 2010
Control	-0.9 ± 0.3^{A}	-0.5 ± 0.1^{A}	-0.9 ± 0.3^{A}
T100	-1.0 ± 0.3^{A}	-0.6 ± 0.2^{A}	-1.1 ± 0.2^{AB}
T75	-1.9 ± 0.9^{B}	-0.9 ± 0.3^{A}	$-1.3 \pm 0.\ 2^{BC}$
T50	-1.9 ± 0.8^{B}	-1.4 ± 1.0^{AB}	-1.4 ± 0.2^{CD}
T25	-3.8 ± 0.9^{C}	-2.0 ± 1.3^{B}	-1.6 ± 0.3^{D}
F value	23.0	7.2	18.9
P value	< 0.001	< 0.001	< 0.001

Effect of provenance

The study also aimed to determine if pink gum provenances differ in biomass parameters and water use strategies, namely do the populations display genetic variation? The high rainfall Myponga plants had a significantly higher mean total biomass, stem plus leaf biomass, and stem biomass than the lower rainfall Blewitt Springs provenance (Table 8.6). The Myponga plants' mean stem diameter was greater than both the Currency Creek and Blewitt Spring means (Table 8.7). The Currency Creek plants had a significantly lower mean root biomass (Table 8.6), mean root to shoot ratio (due to the lower root biomass) and lignotuber diameter than the Myponga and Blewitt Spring plants (Table 8.7)). The lower mean root to shoot ratio also applied to Currency Creek plants at all treatment levels. Although the Myponga provenance plants collectively had the highest mean total biomass, highest number of leaves and biggest stem diameters, this applied to control plants, T100, T75 and T50 plants, but not to T25 plants. For the T25 plants, the Myponga provenance had the lowest means for all these parameters (Table 8.8). There was no difference in the mean SLA for each provenance, with mean values ranging from 3.9 to 4.0 (Table 8.8). The lack of a significant difference in height between the three provenances remained throughout the experiment, with mean heights (± SD) of dried plants being 52 ± 14, 53 ± 14 and 53 ± 14 cm for Blewitt Springs, Currency Creek and Myponga provenances, respectively. There were no significant differences between the provenance means for any of the pre-dawn or midday recordings of water potentials**.** This was true for both the mean of all treatment plants within a provenance and for specific treatments within each provenance.

Table 8.6: Comparison of biomass means for the three provenances (including all treatment and control trees) showing homogeneous subsets. Values within a column followed by the same superscript letter are not significantly different (Two way ANOVA, Tukey post hoc test)

Provenance (and mean annual rainfall (mm))	Biomass component dry weight (g)			
	Total biomass (± SD)	root	Stem + leaves	stem
Blewitt Springs (643)	8.4 ± 3.8^{A}	2.0^{B}	6.6^{A}	3.1^{A}
Currency Creek (480)	8.9 ± 4.2^{AB}	1.5^{A}	7.2^{AB}	3.6^{AB}
Myponga (765)	10.6 ± 5.9^{B}	$2.^{B}$	8.5^{B}	4.2^{B}
F value	4.8	8.71	4.95	6.9
P value	0.01	< 0.001	0.009	0.001

Table 8.7: Comparison of the mean number of leaves, stem diameter, root to shoot ratio, lignotuber diameter and specific leaf area (SLA) (± SD) for each watering regime (including all treatment and control trees) at the end of the experiment. Values within a column followed by the same superscript letter are not significantly different (Two way ANOVA, Tukey post hoc test)

Provenance	Parameter mean ± SD					
	Total number of leaves > 3 cm	Total number of leaves 0.5–3 cm	stem diameter (mm)	Root to shoot	Lignotuber diameter (mm)	SLA
Blewitt Springs	20 ± 11^{A}	30 ± 29	2.4 ± 0.7^{A}	0.32 ± 0.1^{A}	9.9 ± 1.9^{A}	4.0 ± 0.7
Currency Creek	22 ± 13^{B}	34 ± 29	2.6 ± 0.6^{A}	0.24 ± 0.1^{B}	7.8 ± 2.5^{B}	3.9 ± 0.7
Myponga	25 ± 1^{BC}	35 ± 26	3.0 ± 0.8^{B}	0.31 ± 0.1^{A}	9.5 ± 2.3^{A}	4.0 ± 0.8
F value	2.96	0.35	12.9	8.5	15.25	0.695
P value	0.05	0.94	< 0.001	< 0.001	< 0.001	0.50

Table 8.8: Comparison of mean total biomass (gram dry weight) (± SD) between provenances for all plants combined, control plants; and treatment plants, at the end of the treatment. For each provenance, values within a column followed by the same superscript letter are not significantly different (ANOVA welch test), i.e. superscripts show significant differences across treatments within each provenance.

Treatment	Mean total biomass (gram dry weight) (± SD)		
	Blewitt Springs	Currency Creek	Myponga
All plants	8.4 ± 3.8	8.9 ± 4.2	10.6 ± 5.9
Control	13.8 ± 7.3^{A}	13.7 ± 3.8^{A}	17.8 ± 7.7^{A}
T100	8.6 ± 2.9^{B}	11.1 ± 4.2^{A}	11.9 ± 3.8^{B}
T75	8.3 ± 1.7^{B}	7.3 ± 2.2^{B}	8.9 ± 2.1^{CD}
T50	7.1 ± 1.6^{B}	6.7 ± 2.6^{B}	9.6 ± 3.1^{CD}
T25	5.8 ± 1.7^{B}	5.8 ± 1.4^{B}	4.8 ± 1.4s ^{D}
F value	5.34	11.00	11.5
p value	0.002	< 0.001	< 0.001

Water use

In line with the greater mean total biomass and number of leaves of Myponga plants, the mean water use of the Myponga control plants at each recording date was always greater than either the Blewitt Springs or Currency Creek plants. For all 38 measurement dates, the mean water use for Myponga plants was 77 mL (SD ± 25), compared with the mean use of 61 mL (SD ± 20) for both Currency Creek and Blewitt Springs plants (one-way ANOVA F = 6.9, $p < 0.05$). For each treatment level the mean water use of Myponga plants for all recordings was higher than the water use for plants coming from Blewitt Springs and Currency Creek (Table 8.9).

Provenance interaction with water supply?

The third aim of this study was to determine whether there were differences in the response to water stress between the three pink gum provenances. For all biomass and growth variables measured (including biomass, number of leaves, stem and lignotuber diameter), two way ANOVA revealed there was no significant interaction between provenance and treatment. Namely, the response of each provenance to water stress was not significantly different. However, as outline above, there were significant main effects for both the independent variables, watering regime and provenance, on biomass.

Table 8.9: Mean water use (± SD) every 2-3 days (mL) for each treatment within each provenance over the 38 recording periods between 24 April 2010 and 9 August 2010. Values within a column followed by the same superscript letter are not significantly different (one-way ANOVA, Tukey post-host test)

Provenance	Mean water use (mL)			
	T100	T75	T50	T25
Blewitt Springs	98 ± 30^{A}	73 ± 22^{A}	49 ± 15^{A}	24 ± 7^{A}
Currency Creek	97 ± 32^{A}	73 ± 24^{A}	49 ± 16^{A}	24 ± 8^{A}
Myponga	124 ± 41^{B}	93 ± 31^{B}	62 ± 21^{B}	31 ± 10^{B}
F value	6.91	6.91	6.93	6.92
p value	0.002	0.002	0.001	0.002

8.5 Discussion

The experimental manipulation of water to juvenile pink gums showed that the amount of water supply had a significant impact on all biomass parameters measured, and on water potential. The second major finding was that for all pink gum juveniles combined, the plants needed to be severely water deprived (25% replacement water only) for the growth response to be significantly different from plants receiving 100% replacement water, over the 16 week experiment. At the end of the experiment, the T25 plants had approximately half the mean number of fully-expanded leaves of control plants. The reduced number of leaves was due to both fewer leaves being produced and increased loss of fully-expanded leaves. By the end of the experiment, leaves were still being produced in control and T100 plants, but leaf production had almost ceased in T25 plants. A key mechanism for the pink gum juveniles coping with reduced water, therefore, was reducing the number of leaves present. Accelerated leaf fall during drought appears to be an adaptation of *Eucalyptus* species, in general, to a dry climate (Florence 1996). For the pink gum juveniles, coupled with this mechanism, there was an increase in the root to shoot ratio, which only became significantly different in the highly water stressed T25 plants. For the duration of the experiment, the 25% replacement water amount was also the critical water supply that significantly impacted water potential. In the controlled glasshouse conditions, therefore, the pink gum juveniles only began making a significant diversion in growth allocation between roots and shoots when under extreme water stress. Because severe water stress manifested itself in leaf loss and reduced growth, it may be a potential driver of pink gum canopy decline in the field.

Conversely, the provision of excess water to pink gums resulted in significant increases in stem and leaf biomass over plants provided even with 100% replacement water. After 16 weeks of treatment regimes, the control plants had 40% greater biomass than the 100% replacement water plants and 85% greater biomass of T50 plants. In a similar study, Li and Wang (2003) found that at the end of a five month glasshouse experiment, the mean total biomass of 100% replacement water treatments in three provenances of *Eucalyptus microtheca* was 20% to 40% greater than 50% water-replaced treatments, comparable to the current study's results. Hence, it appears that pink gums have the capacity to allocate water in excess of that required for maintenance, to increasing biomass.

In addition to pink gums recording different biomass parameters in response to different water levels, the study also showed that there was provenance-level variation in morphological traits and water use strategies. Throughout the experiment, the high rainfall Myponga provenance plants had the highest mean total biomass, root biomass, and stem-plus-leaf biomass for the control plants and for all treatment plants except the T25 plants. The Myponga T25 plants had the lowest biomass and number of leaves. The Myponga juveniles also consistently used more water than the Blewitt Springs and Currency Creek plants. Bleby (2003) noted that when compared on a sapwood area basis, maximum daily rates of water use in *Eucalyptus* species decrease with decreasing rainfall. Bleby found that *Eucalyptus* species from more mesic sites (e.g. *Eucalyptus regnans)* tend to have a larger capacity for water use than species from more xeric sites (e.g. *Eucalyptus salmonophloia*). Similarly, Li and Wang (2003) found that *Eucalyptus microtheca* populations from low rainfall areas have conservative water use strategies and slow growth rates, whereas populations from areas with mild short-term droughts consume available water rapidly until almost all water from the soil is exhausted, contributing to fast growth rates. Similarly, in the current study, the Myponga plants receiving only 25% water replacement may have exhausted the soil water prior to that of the other provenances. The finding that the high rainfall Myponga plants consistently used significantly more water than the Currency Creek or Blewitt Springs plants has potential implications for the long-term conservation of pink gums. If pink gums from high rainfall areas were used in revegetation programs, they may require more water than pink gums from lower rainfall areas and/or suffer higher mortality rates if not provided with supplementary water.

The Currency Creek plants had a significantly lower root biomass, and a correspondingly lower root to shoot ratio than the Blewitt Springs or Myponga plants. This result was surprising because the Currency Creek plants were from the lowest rainfall region, where it may be expected that plants would have a higher root to shoot ratio. This is because plants of drier habitats tend to allocate proportionately more carbon to roots than plants of wetter habitats (Monk, 1966). A higher root to shoot ratio in drier habitats increases the chances of water uptake and reduces water loss through transpiration. Coupled with the lower root biomass, the Currency Creek plants also had a significantly smaller lignotuber diameter. Lignotubers are basal woody swellings that contain dormant vegetative buds from which new branches form if the above ground parts of the plant are damaged (Walters *et al.* 2005). There were seven plants that did not contain an apparent lignotuber, all of which were from Currency Creek, and six of which were from T75, T100 or control plants. The development of lignotubers is thought to be influenced by environmental conditions, especially the availability of water and nutrients (Walters *et al.* 2005). Contrary to this prevalent view, though, Walters *et al*. (2005) found that in three *Eucalyptus* species, lignotuber development appeared to be strongly influenced by genotype rather than environment. The most likely explanation for the absence of and/or relatively small lignotubers in the Currency Creek plants, therefore, is that lignotuber development is both genetically and environmentally determined. The lower root mass and lignotuber size for the low rainfall Currency Creek plants possibly reflects inherently lower water stress levels in the Currency Creek plants, and hence this provenance may be better adapted to tolerate lower levels of water availability. Alternatively, the Currency Creek genotype may be less prone to producing large lignotubers and/or lignotuber development may be relatively delayed. The results showed therefore, that within the study region there are differences between provenances in water use strategies and possibly also in mechanisms for coping with water stress. This has implications for choosing between provenances in planning pink gum revegetation programs and also differences between provenances in coping with an increasingly dry climate.

Specific leaf area (SLA), defined as the ratio between leaf blade area and its dry weight, reflects the physiological cost of producing leaf area (Feng *et al*. 2008). Lower values of SLA tend to correspond with long leaf life spans and resource-poor (including water) environments (Cornelissen *et al.* 2003). Species that grow in dry environments often reduce water loss by producing smaller, thicker leaves resulting in a decrease in SLA (Schulze *et al.* 2006). In the current study, SLA ranged from a mean of 3.7 for T25 fully-expanded leaves to

4.3 for control plant fully-expanded leaves. Both values are in the mid-range of SLA values reported for *Eucalyptus* species (Turner *et al.* 2006). The SLA was significantly lower in T25 plants than in plants that were subjected to other watering regimes. There were no significant differences in SLA between provenances. The significant difference in SLA between watering regimes suggests that SLA in pink gum juveniles is influenced mainly by environmental factors.

Although the study showed that pink gum growth was determined by both water supply and genotype (provenance), there were no interactions between water supply levels and genotype. That is, the response to drought stress was not significantly different. This finding indicates that all populations of pink gums may be similarly affected should climate trends of decreasing rainfall continue. The finding that the high rainfall Myponga plants had the highest biomass for control plants, T100, T75 and T50 plants but the lowest biomass for T25 plants, is at variance with the lack of an interaction between provenance and water supply. It would appear that the higher water use strategy of Myponga plants enables a greater biomass, given sufficient water supply, but when water supply is severely restricted, there is less capacity for growth than the lower rainfall populations. Further work is required to determine if there are physiological differences between pink gum populations.

Future research

The seedlings were purchased from a commercial nursery and information regarding how many source trees were represented in each provenance was not available. It is not known therefore, how representative the seedlings were of the entire provenance. The results, however, suggest significant differences between the populations and future research involving known mother plants taken over a wider geographic area is warranted. Further work on the physiological mechanisms of water use and water conservation between different populations would assist in determining further if different populations have different water stress adaptations.

8.6 Conclusion

Pink gum growth is determined by both water supply and genotype. Within pink gums in the Mt Lofty Ranges region, there is some genetic specialisation for local conditions, as well as capacity for some plasticity in response (after McLean *et al*. 2014). This enables restoration practitioners to choose the most appropriate seed source strategy, for example by matching specific provenances with predicted rainfall levels at a revegetation site (Prober *et al.* 2015). For restoration programs where supplementary water may be available, choosing seed from a low rainfall population may be most suitable. Alternatively, if supplementary water was available, and high growth rates were desired, then seed sourced from high rainfall populations may be optimal.

The significantly greater biomass of control plants compared with T100 plants showed that pink gums have the capacity for greatly increased growth if they receive more water than that required for maintenance activities, given that light, nutrients and other resources are non-limiting. Conversely, most of the biomass parameters measured were only significantly impacted when pink gums were receiving only 25% replacement water levels. This indicates that pink gum juveniles could have a high capacity to survive low soil water potentials in their natural environment for at least several months. The experiment also showed that there were significant differences between provenances in biomass components and water use. Hence, should there be shifts in long-term rainfall patterns, provenances may differ in their ability to adapt to possible reduced rainfall regimes. Future restoration projects therefore should consider seed sourcing biased toward the direction of predicted climate change, but not exclusive to it (Prober *et al.* 2015).

Chapter 9 Influence of depth to groundwater on pink gum (*Eucalyptus fasciculosa*) size attributes

9.1 Abstract

This study aimed to determine if differences in size attributes of pink gums (*Eucalyptus fasciculosa* F. Muell) within the same woodland were related to depth to groundwater. Two to three sites were selected within each of three pink gum woodlands on the plains adjoining the Mt Lofty Ranges. Sites within each woodland received the same rainfall, had the same soil surface texture, elevation, slope and aspect, and had no known clearance or fire history. At the Clayton woodland, pink gums sited on a 2 m deep water table were significantly taller and had larger canopy volumes and trunk diameters than pink gums sited on a 4 m deep water table. At the Aldinga woodland, pink gums adjoining an ephemeral wetland were significantly larger than trees at the other Aldinga sites, where groundwater was 10 to 14 m deep. The shortest pink gums at Aldinga occurred where surface sand was deepest. At the end of a high rainfall summer, for all Clayton and Aldinga sites, soil water potentials were higher than pre-dawn pink gum leaf water potentials, indicating that all parts of the sampled soil profiles (which were up to 11 m deep) were moist enough to potentially supply water to the pink gums. At Clayton, however, even during a high rainfall summer, soil water potentials throughout the profile were consistently more negative for the deeper water table site than the shallower water table site. At the lowest rainfall woodland, Rockleigh, pink gums at two sites with sandy soil, equidistant to an ephemeral creek, but with elevation that differed by up to 5 m, also differed significantly in size. Although soil profiles were not obtained at Rockleigh, it was likely that the site containing the larger pink gums had access to a shallower water table. Despite occurring at the lower rainfall limit of pink gum's natural distribution, pink gums at one of the Rockleigh sites had amongst the largest trunk diameters recorded for pink gums in the wider Mt Lofty Ranges region. At some locations on the plains adjoining the Mt Lofty Ranges, pink gums are likely to be accessing groundwater. Historical lowering of groundwater may explain the decline in canopy intactness of some pink gums in the region. Further studies are required to determine from where in the soil profile pink gums are accessing water at other sites in the study region where groundwater is potentially within pink gum rooting depth. Pink gums at such sites may be threatened by possible lowering of water tables due to a drying climate and/or non-sustainable extraction of groundwater.

9.2 Introduction

Declining groundwater levels are a global issue, threatening the supply of drinking and irrigation water and the existence of ecosystems that depend on groundwater, referred to as phreatophytic ecosystems (Eamus 2015). In response to falling groundwater levels in Australia, there has been increased research on the impact of declining groundwater on ecosystems (Harrington and Cook 2014; Sommer and Froend 2011; Tomlinson, 2011). Most of this research on vegetation use of groundwater has largely concerned riparian systems (Holland *et al.* 2006) and semi-arid or arid systems (Swaffer 2014), where it has been well documented that access to groundwater can help plants survive drought (Zolfagher *et al*. 2014). Zolfagher *et al*'s (2014) study, however, showed that even in a mesic environment of approximately 1000 mm rainfall per year, *Eucalyptus* woodlands with groundwater < 5.5 m deep had greater above-ground biomass than those where groundwater was > 9.8 m deep. Determining whether ecosystems are at least partially groundwater dependent will help predict if they are likely to have been, or are likely to be, affected by past and/or ongoing groundwater extraction, and/or periods of drought.

Ward (2007) postulated that a credible hypothesis for poor pink gum condition in the Mount Lofty Ranges (MLR) is that pink gum woodlands are seasonal or episodically dependent upon groundwater, and their poor condition has resulted from the significant changes in hydrology that have occurred over much of the MLR. In a study of the distribution of *Eucalyptus* species over part of Coonalpyn Downs, Litchfield (1956) noted that pink gum is most abundant and frequent on sand plain soils over travertine (a form of limestone), with free to moderate drainage. Litchfield (1956) concluded that it was likely that exploratory roots of pink gums reached the relatively shallow (generally < 8 m) watertables under the travertine plain.

As part of the regional survey (Chapter 1), the mean height, trunk diameter and canopy volume of pink gums were often recorded as being significantly different between survey sites within the same woodland, even when elevation and surface soil texture were the same. Because rainfall, fire and clearance history were the same within woodlands, I hypothesised that the differences in pink gum size were due to different soil water availabilities, which in turn were due to different depths to water table.

The aims of this study were to:

- indirectly determine if pink gums were likely to be at least partially groundwater dependent in some locations by comparing differences in above ground biomass between sites with different depths to groundwater
- determine from where in the soil profile pink gums were obtaining their water by comparing soil water potentials with pre-dawn pink gum leaf water potentials.

9.3 Methods

The study was conducted at nine survey sites located within three pink gum woodlands, situated on the plains adjoining the Mt Lofty Ranges. These woodlands were near Clayton, Aldinga and Rockleigh (Figure 9.1). Within each of these woodlands, there were differences in pink gum sizes, despite sampling sites within the same woodland having the same rainfall and seemingly similar surface soil textures, elevation, and occupying the same landform element (Table 9.1). All woodlands had no known fire or clearance history, were ungrazed by domestic stock, and managed primarily for conservation. At Rockleigh and Clayton, pink gums occur towards the lower rainfall limit of their natural distribution, with mean annual rainfalls of 360 mm and 395 mm per year respectively. The location of the study sites within each remnant are shown in Figures 9.2, 9.3 and 9.4.

Figure 9.1: Location of study woodlands, Clayton, Aldinga and Rockleigh, all on the plains adjoining the Mt Lofty Ranges. Map produced from Google Earth.

Table 9.1: Habitat parameters for three pink gum woodlands containing two or more survey sites

Habitat Parameter	Woodland		
	Clayton	Aldinga	Rockleigh
Woodland size (ha)	60	260	200
Survey sites	A,B	A,B,C,D,E	A,B
Mean annual rainfall (mm)*	395	500	360
Soil Group**	Shallow sand over clay on calcrete	Bleached sand over sandy clay loam	Sand over clay
Elevation (m) ASL**	A and B = 9 m	19-21 m	A = 230-235 m B = 225-230 m
Landform Element	Swale	A, ,C,D = Swale B = bottom of shallow dune E = Fringing wetland	Flood plain
Depth to Groundwater***	A = 4 m B= 2 m	10 – 15 m	5–10 m
Distance between survey sites (m)	330	320 to 500	330

* Bureau of Meteorology (2015)

**Soil Group and Elevation obtained from NatureMaps (2016)

*** Depth to groundwater obtained from soil profiles for the current study (Clayton); piezometers in Aldinga woodland (Aldinga) and Data SA (2015) depth to groundwater dataset (Rockleigh)

Figure 9.2: Location of study sites at Aldinga. Soil profiles were obtained from sites A, B and C.

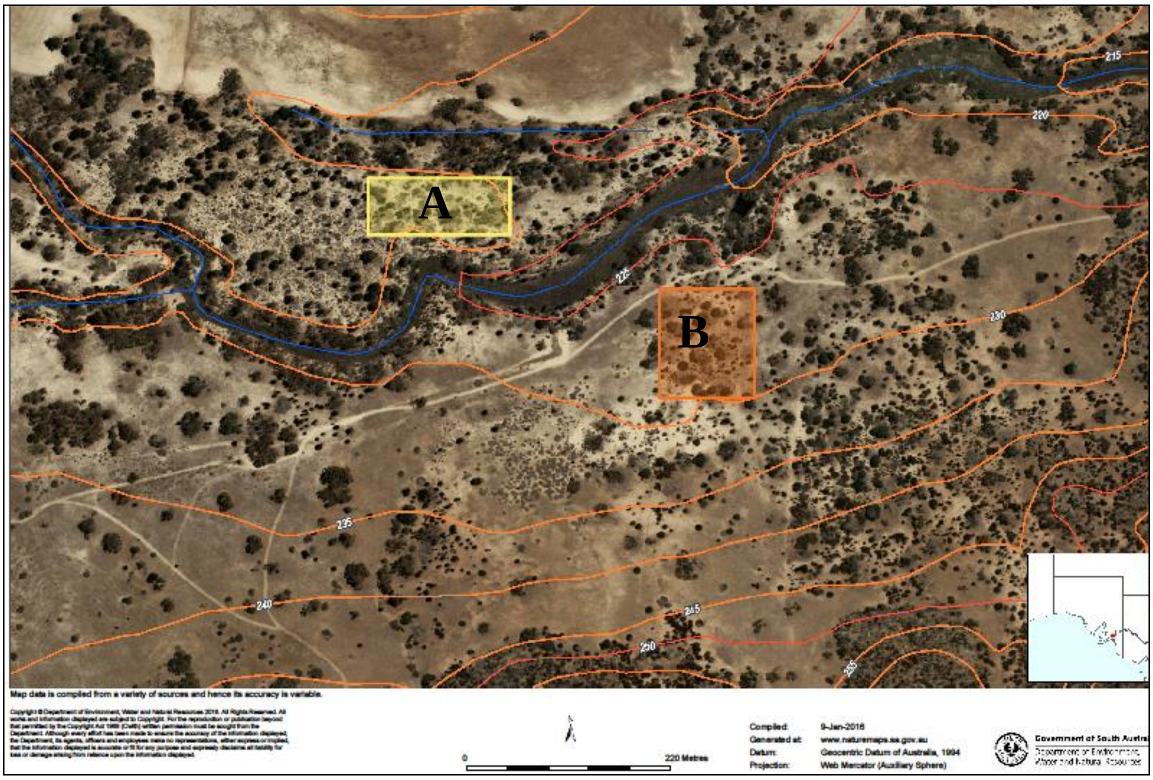

Figure 9.3: Location of study sites A and B at Rockleigh.

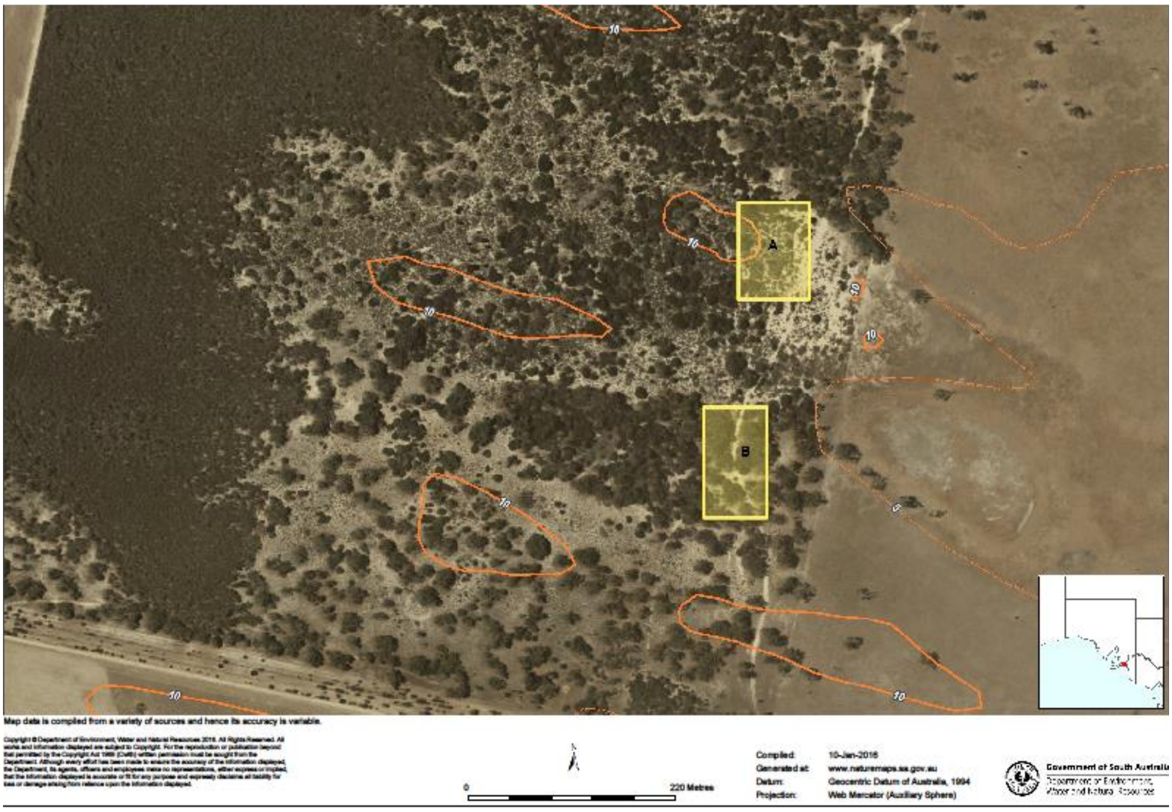

Figure 9.4: Location of soil profile study sites, A and B at Clayton

Pink gum attributes

Between February and March 2011, at each site between 10 and 18 pink gums were surveyed, these being the pink gums closest to the randomly determined first pink gum surveyed (Chapter 1). All pink gums were > 3 m tall. Pink gum attributes recorded were tree height, canopy intactness, canopy volume, maximum and effective diameter at breast height (DBH). Canopy intactness was a visual, subjective assessment of the percentage of living foliage compared to the estimated potential amount that would be present in a fully extended canopy (Cunningham *et al.* 2007). Canopy volume was calculated from canopy depth and width using the formula for the volume of an ellipsoid, where volume = $4/3\Pi abr$, and a and b are the north–south and east-west diameters divided by 2 respectively and r was the difference between the height of the maximum and minimum canopy foliage. The heights of the maximum and minimum canopy foliage were measured using a 2 m pole, or if > 2 m high, by extrapolation using the 2 m pole. Trunk diameters were measured at 1.3 m above ground. Maximum DBH was the greatest individual stem diameter for each tree.

Where trees were multi-stemmed, an “effective” tree DBH was calculated as the square root of the sum of all squared stem DBHs (Hoover 2008):

$$\sqrt{\sum_{1}^{n}(\boldsymbol{stem\ diameter^{2}})} \qquad \text{Equation 9-1}$$

For the Clayton survey sites, Reineke’s Stand Density Index (SDI) was calculated (for the Aldinga sites, insufficient data was recorded to calculate an extrapolated number of pink gums per hectare). This SDI adjusts the trees per hectare by incorporating the mean effective DBH for a site, as follows (Shaw 2006):

$$\text{SDI per survey site} = \text{number of pink gums per ha x (mean effective DBH/25)}^{1.605} \qquad \text{Equation 9-2}$$

Soil profiles

Soil profiles were obtained at Aldinga sites A, B and C on 28 February 2011 and at each of the two Clayton sites on 25 March 2011. Soil profile cores were obtained by a professional driller, using a mechanical drill. At Clayton sites A and B, soil cores reached the water table, and were obtained to depths of about 4 m and 6 m, respectively, while at Aldinga soil cores were taken to depths ranging from 8 to 11 m, the latter all being above the water table. As each core was being drilled, I obtained soil samples every 20 to 50 cm from the drilled core, coinciding with changes in texture, colour and perceived moisture levels. Within five minutes of extraction I stored the soil samples in 500 ml glass jars, sealed with electrical tape. In the laboratory, I determined soil texture, soil pH, soil gravimetric moisture, soil salinity and soil matric potential. Soil texture was determined by the sedimentation method (Colorado University 2015). Namely, I added water to the soil sample and a few drops of detergent. The sample was shaken vigorously for one minute and then the particles were allowed to settle. The percentage of sand particles (settled after 30 seconds), silt (settled after 30 minutes) and clay particles (settled after 24 hours) was used to determine soil texture. I calculated soil gravimetric water by weighing the fresh and dry weight of soil. Soil gravimetric moisture is the mass of water per mass of dry soil. It is calculated as the (wet weight – dry weight)/dry weight. Soils with a coarse texture (sandy soils) have fewer inter-particle spaces and a lower water holding capacity. Conversely clay, with a matrix of very fine pores has a high water holding capacity (Kolb 2011). However, in fine textured soils, water is held more tightly and plants have greater difficulty extracting it. Generally, loams hold large quantities of water that is available for plant use.

Determining which parts of the soil profile were available to pink gums as a source of water

On the morning following the collection of soil profiles, I recorded pre-dawn pink gum leaf water potential (Ψ) values from up to nine pink gums nearest to each of the soil profiles (with two samples from each tree), and compared these with total soil Ψ values to determine which parts of the profile were available to the tree as a source of water (after Holland 2006). Soil Ψ, or the energy of free water, is the sum of the soil matric potential (dryness) and the osmotic potential (saltiness) (Holland 2006). Gravitational potential is considered negligible and not considered in calculating total soil water potential. Water potential is commonly expressed in units of megapascals (MPa). A water potential of 0 MPa is equivalent to free water and more negative values are drier and/or more saline (Holland 2006). For plants to be able to extract water from the soil, a plant's leaf water potential must be less (namely more negative) than the soil Ψ. Soil water potential values that are less negative than pre-dawn leaf water potentials (when stem and leaf water potentials are at a maximum) indicate regions in the soil from which the roots are able to extract water (Holland 2006). Generally the soil Ψ at which plants can no longer extract water (permanent wilting point) is considered to be -1.5 MPa.

I determined soil matric potential (ψ_m) using the 'filter paper' technique (Greacen *et al*. 1989). I calculated soil osmotic potential by converting the soil salinity to osmotic potential. I measured soil salinity using an electrical conductivity (EC) meter using a 1:5 soil to water suspension ($EC_{1:5}$) to give values in dS/m (after adjusting for soil texture) (Cooperative

Research Centre for Viticulture 2006). These readings were then converted to the electrical conductivity of a saturated soil (ECe) using standard conversion factors (Future Farm Industries Cooperative Research Centre 2011). ECe is more ecologically meaningful than $EC_{1:5}$ because it corresponds to the salinity that plants growing in the soil actually experience in saturated soil (Future Farm Industries Cooperative Research Centre 2011). As the soil dries however, the plants experience increasing salinity in the soil solution, until at wilting point, the salinity of the soil solution will be approximately four times the measured ECe. The osmotic potential of a saturated extract can be calculated from the ECe as follows (Ronen 2006):

$$\text{osmotic potential (MPa)} = \text{ECe} \times 0.036. \qquad \text{Equation 9-3}$$

Pre-dawn leaf Ψ is considered to be a good indicator of soil water availability as it is assumed that the plant is in equilibrium with the soil's water potential (Sinclair 1980). For each soil profile at Clayton and Aldinga, I measured pre-dawn leaf Ψ on the day after soil cores were extracted. At each site, I recorded pre-dawn leaf Ψ from between six and nine trees, the actual number being determined by time constraints. From each tree I recorded Ψ on two replicate twigs per tree taken 1–2 m from the ground using a Scholander pressure bomb. Stable isotope analysis was not used as a method to determine if pink gums were accessing groundwater because groundwater was not reached at Aldinga. Stable isotope analysis compares the isotopic signature of twig water to the range of potential water sources in the landscape (Swaffer 2014), namely soil, groundwater and rainfall samples.

Rainfall

In the three months before the soil profile and pre-dawn water potential recording at Clayton (namely, January, February and March 2011), the nearest weather station recorded twice the long-term mean rainfall aggregate for these months (103 mm compared with 55 mm) and 200 mm was received in the four months prior to the survey compared with the long-term mean of 76 mm (Milang weather station, Bureau of Meteorology 2015). No rainfall was received in the 10 days prior to recording pre-dawn leaf water potential. The Aldinga study site also received twice the long-term mean rainfall in the three months prior to the soil and water potential recording dates, namely 125 mm compared with a mean of 62 mm (Sellicks Beach weather station 23871, Bureau of Meteorology 2015). On 19 February 2011, 11 days prior to the pre-dawn water potential recording, 55 mm was received, three times the mean monthly rainfall total.

Statistical analysis

One-way ANOVA and t-tests were used to test if the means of maximum tree height, stem size and canopy volume were different between sites within the same woodland. ANOVA was followed by Tukey's *post hoc* test to determine where any differences lay. Analyses were performed using SPSS software version 23.

9.4 Results

9.4.1 Clayton

Pink gum attributes

Pink gums at Clayton Site B had larger stem diameters, were taller, and had greater canopy volumes and canopy intactness than Clayton Site A trees (Table 9.2).

Table 9.2: Comparison of pink gum parameters between site A and B, Clayton. Different superscripts represent significantly different means.

Parameter (Means)	Site A	Site B	t-test p value
	Mean ± SE	Mean ± SE	
Canopy Intactness (%)	50 ± 6.2^{A}	90 ± 2.9^{B}	< 0.01
Canopy Volume (m^3)	13 ± 5.0^{A}	176 ± 28.3^{B}	< 0.01
Effective DBH (cm)	17 ± 1.5^{A}	40 ± 3.9^{B}	< 0.01
Max DBH (cm)	13 ± 0.8^{A}	28 ± 3.5^{B}	< 0.01
Tree height (m)	4.7 ± 0.3^{A}	7.3 ± 0.5^{B}	< 0.01
Extrapolated # pink gums per hectare	244	257	
Pink gum stand density index	104	371	

Clayton soil profiles

The water table at Site A extended from 4.2 to 6.2 metres below the ground surface and at Site B occurred between 2.2 m and 4.2 m. Site A soil profile comprised sand to 0.7 m deep over sandy-clay to 1.7 m, below which was clay until at least the lower level of the water table. Soil pH was slightly acidic to 0.7 m deep, then alkaline below this depth. Site B comprised loamy-sand to 0.3 m deep over a sandy-clay layer to 1.2 m and then clay until the lower level of the water table. The soil pH was similar to site A being acidic in the upper sandy layer to 0.3 m (pH = 5.6 at 0.1 m and pH = 6.7 at 0.3 m) and alkaline in the lower soil with pH ranging from 8.0 to 8.6. The water table was also slightly alkaline (pH = 7.5 at 4.2 m). Both soils were non-saline throughout the recorded profile.

Pre-dawn pink gum leaf water potentials and soil moisture

At both Site A and B soil water potentials (SWPs) throughout the profile were well above the wilting point of -1.5 MPa, and were at least 1 MPa higher than pink gum pre-dawn water potentials (Table 9.3). However, between 0.5 m and 2.5 m, SWPs were consistently more negative for site A than for corresponding depths at Site B (

Figure 9.5). Gravimetric soil moisture for site A was similar to site B, and ranged from 8–20% which compares with gravimetric soil moisture levels of 20–40% for saturated soils (the level being dependent upon soil texture).

Table 9.3: Comparison of pre-dawn leaf water potentials (MPa), soil water potentials (MPa) and gravimetric soil moisture for Clayton sites A and B, from samples collected on 25 March 2011

Site	Maximum pre-dawn leaf Ψ	Minimum pre-dawn leaf Ψ	Mean pre-dawn leaf Ψ ± SE	Minimum soil Ψ	Maximum soilΨ	Minimum soil gravimetric water	Maximum soil gravimetric water
A	-1.0	-2.1	-1.5 ± 0.1	-0.5	-0.2	8	20
B	-1.5	-1.9	-1.7 ± 0.2	-0.4	-0.2	10	21

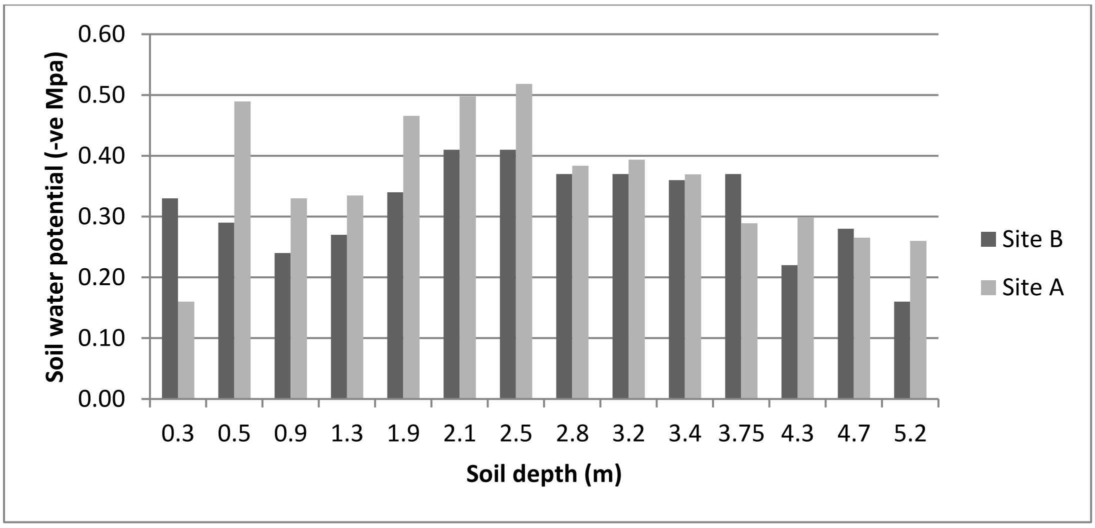

Figure 9.5: Comparison of total soil water potential at Clayton sites A and B at different soil depths from samples collected 25 March 2011

9.4.2 **Aldinga**

Pink gum attributes

Site D trees (adjoining the ephemeral wetland) had significantly larger mean maximum stem DBH, effective DBH, heights and canopy volumes than all other sites. Site B trees (western-most site) were significantly shorter than all other sites, and had significantly smaller diameters than site D trees (Table 9.4).

Depth to groundwater

The soil profile drilling was conducted with the same effort and for the same duration at each site. Soil profile depths were 11 m at site C, 9 m at site A and 7 m at site B, and groundwater was not reached at any site. Based on piezometers installed on the woodland edges, depth to groundwater varies from about 15 m in the north of the woodland to 10 m in the centre (Ecological Associates 2003). In general, the Aldinga woodland comprises a freely draining sandy soil overlying low permeability clay. A seasonal perched water table develops in these sands. This perched water table is highest in the north of the park near the wetlands (adjoining site D) and flows westwards and southwards (Ecological Associates 2003). This perched water table is believed to have been depleted due to diversion of water flowing into the north of the park in the 1950’s (Ecological Associates 2003).

Table 9.4: Comparison of mean canopy dimensions, tree height and stem diameters (± SE) between Aldinga sites A B and C, recorded in February 2011. Different superscripts represent significantly different means.

Site	Number of pink gums	% Canopy Intact	Maximum stem DbH	Effective Diameter (cm)	Height(m)	Canopy Volume (m^3)
A	18	40 ± 6.2^{A}	16 ± 1.2^{AB}	23 ± 2.2^{B}	6.3 ± 0.3^{B}	12 ± 3.7^{A}
B	16	64 ± 3^{BC}	8 ± 0.7^{A}	10 ± 1.2^{A}	3.9 ± 0.2^{A}	6 ± 3.7^{A}
C	12	48 ± 6.9^{AB}	19 ± 2.2^{B}	24 ± 3.9^{B}	6.1 ± 0.5^{B}	43 ± 18^{A}
D	10	78 ± 4.6^{C}	39 ± 5.2^{B}	43 ± 5.4^{C}	8.3 ± 0.5^{C}	190 ± 40^{B}
E	11	70 ± 4.1^{B}	14 ± 1.2^{AB}	14 ± 1.2^{AB}	6.1 ± 0.3^{B}	6 ± 1.2^{A}
F value		6.7	24.6	17.5	17.5	21.5
p		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Soil profiles

Soil profiles at sites A, B and C were similar, being sand in the upper layers and merging into a clay-sand to sandy-clay before becoming clay at about 6 m. The depth of the sand layer was 3.4 m at site B, compared with 2 m for sites A and C. At site C, between 6 and 9 m deep, the texture was heavy clay which could possibly have been an old water table (pers. comm., L. Sandland, drilling contractor 2011). Soils were non-saline (< 4 dS/m) to a depth of 2 m for all three sites. Between 2 and 6 m deep, soils were slightly saline (4.5–9 dS.) for sites A and C but non-saline for the near-coastal site B. Beyond 6 m deep, all soils were slightly saline. Salinity categories are those of FAO (1986).

Pre-dawn pink gum water potentials and soil moisture

Soil water potentials (SWPs), namely matric potential plus osmotic potential, were high at all sites throughout the recorded soil profile (Figure 9.6). Highest SWPs at each site ranged from -0.03 to -0.06 MPa and were between 0.4 to 2 m depth; and the lowest SWPs ranged from -0.37 to -0.47 MPa, which were between 5 to 11 m depth. Between 2 to 5 m, SWPs were between -0.2 and -0.3 MPa. Gravimetric soil moisture increased steadily with depth, ranging from approximately 1–2% at 0.5 m for all profiles, up to 12.9% at 4.8 m (site A), 16% at 8.2 m (site B) and 27% at 8.5 m (site C). Even though more water was present in the soil at greater depth, the matric potential was more negative due to the influence of the clay texture. Soil moisture up to depths of 2.2 m was lowest at site B, being only 1% compared with 4% at site C and 9% at site A.

In line with the high SWPs, pre-dawn pink gum leaf water potentials (LWPs) were also high at all sites (Figure 9.7). Pink gum mean pre-dawn LWPs varied by less than 0.4 MPa between sites, with both sites B and C having the lowest (most negative) LWPs of -1.7 MPa and means of -1.3 MPa. At site A, the lowest and mean pre-dawn LWPs were slightly higher being -1.5 MPa and -1.2 MPa respectively. Maximum pre-dawn LWPs ranged from -0.9 to -1.1 MPa.

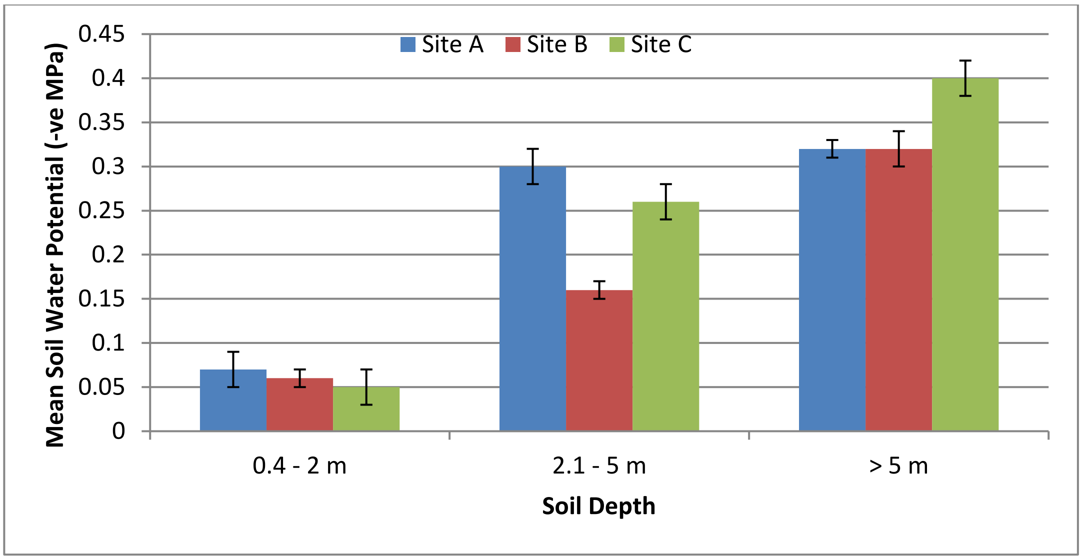

Figure 9.6: Mean soil water potential (MPa) (with SE bars) at different soil depths for sites A, B and C at Aldinga Scrub, 28 February 2011

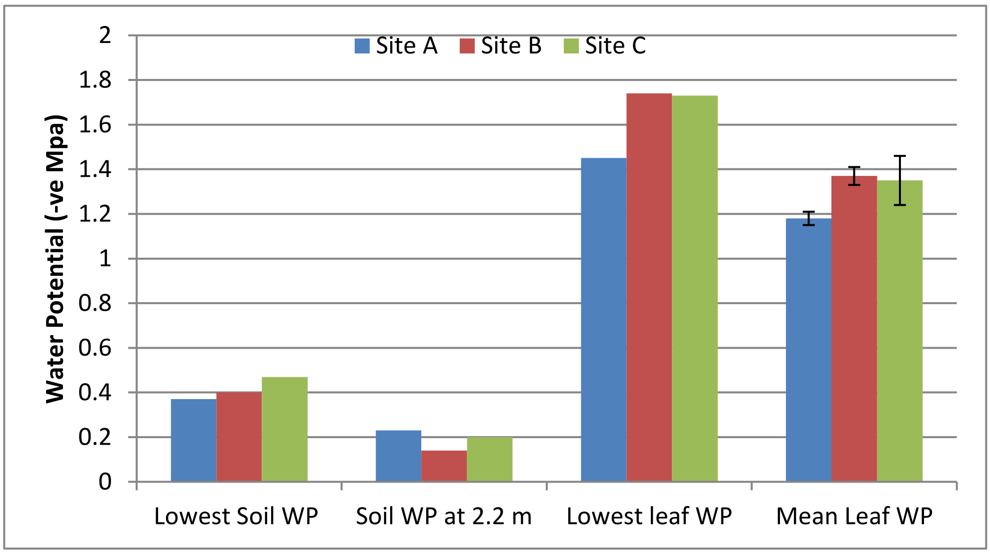

Figure 9.7: Lowest (most negative) soil water potential (SWP) throughout soil profile, SWP at 2.2 m deep; lowest and mean (showing SE bars) pre-dawn pink gum leaf water potential at Aldinga sites A, B and C. Data recorded 28 February 2011

Rockleigh

Site B trees had a significantly greater mean maximum stem DBH, effective DBH, and height than site A trees, but a lower canopy intactness (Table 9.5). Despite occurring at the lower rainfall limit of pink gum's natural distribution, a pink gum with the equal largest DBH of 2814 pink gums recorded in the wider region was recorded at Rockleigh (refer Chapter 1).

Table 9.5: Mean pink gum size attributes and canopy intactness at Rockleigh pink gum survey sites. Recorded March 2011

Size parameter	Site A means ± SE	Site B means ± SE	t value	p value
Effective DBH (cm)	19 ± 3	45 ± 6	-3.8	< 0.001
Maximum DBH (cm)	16 ± 3	36 ± 5	-3.8	< 0.001
% Canopy Intactness	72 ± 6	44 ± 8	2.8	< 0.001
Tree Height (m)	4.6 ± 0.4	8.0 ± 0.4	-5.6	< 0.001

Soil profiles were not obtained at Rockleigh: howeve, both sites had sandy surface textures and were located equidistant from the ephemeral creek, but elevation at site A was up to 5 m higher than site B.

9.5 Discussion

This study provided evidence that where pink gums were accessing groundwater or perched water tables, they were significantly taller than pink gums on nearby sites with a greater depth to groundwater and that was putatively beyond the rooting depth of the pink gums. Based on recent reviews of Australian studies of *Eucalyptus* rooting depth and groundwater use, Zolfager *et al.* (2014) suggested that for *Eucalyptus* species, there is a threshold depth to groundwater in the range 7–12 m for influencing growth. This reflects the maximum rooting depth of most *Eucalyptus* trees. Zolfager *et al*'s (2014) own study showed that groundwater depths of up to 9.8 m deep significantly influenced *Eucalyptus* biomass, despite occurring in a relatively high rainfall region of approximately 1000 mm per year, in New South Wales.

The depth of pink gum roots has not been directly measured in any previous studies. To date, the source of water used by pink gums and/or rooting depth has been only speculated upon through indirect observations (Jianmin and Sinclair 1993; Litchfield 1956; Sinclair 1980; White 1970). From these observations, it would appear that depending upon the soil, underlying rock, depth to groundwater, topography and rainfall, the sources of water used by pink gum will vary. Jianmin and Sinclair (1993) inferred pink gums to be “deep-rooted” based on the relatively high leaf water potential values recorded in pink gums during summer. Jianmin and Sinclair (1993) did not speculate on an actual rooting depth, but noted that the fractured rock strata underlying the shallow soil profile of their study site would have been an important source for any plant with a deep root system. Referring to the distribution of pink gums in the Upper South East region of South Australia (between Keith and the SA/Victorian border), Litchfield (1956) stated that it is likely that exploratory pink gum roots reach the relatively shallow water-tables under the travertine plain (which is generally less than 8 m). During the current study, I opportunistically noted pink gum roots growing within 15 km of the Aldinga study sites in sand over clay soils, similar to Aldinga study sites, the soil having been excavated for a car park. The visible roots were approximately 1.5 m deep, at which depth they were still at least 10 cm in diameter (Figure 9.8). It is likely therefore that the fine roots extended considerably deeper than 1.5 m and into the clay layer. Similar root dimensions were observed in August 2016, on pink gum roots partially exposed at the Highland Valley study site (refer Appendix A), following a storm (pers. obs. S Croft).

Within the Mt Lofty Ranges and adjoining plains, of the 78 pink gum woodland survey sites (Chapter 1), there were 19 sites where the depth to groundwater has been extrapolated as being ≤ 10 m (based on Data SA 2015). This depth is within the postulated rooting depth of *Eucalyptus* species (Zolfagher *et al*. 2014).

At Clayton, trees at Site B overlying a shallow water table (2–4 m below ground) were significantly taller and had larger and had more intact canopies than site A trees where the water table was 4– 6 m deep. The sites were 300 m apart and at the same elevation. At both sites, soils were uniformly non-saline, hence differences in pink gums between the sites appears to be due to different groundwater depths. The deeper water table at site A could be at least 6 m deep during drought, and the shallower water table at site B, up to 4 m deep during drought. The tree roots at the shallower water table are likely to have access to a reliable source of water regardless of seasonal drought conditions and are likely to be the reason for the larger trees, and larger and more intact canopies. The results suggest that the root depth for pink gums at Clayton site A are at least 2 m but possibly less than 6 m.

At Rockleigh, there was also a significant difference in mean tree height and DBH between the two study sites, both having sandy surface soil, were the same distance from an ephemeral creek on the adjoining flood out area, and with no known fire or clearance history. Pink gums at site B averaged 8 m tall with mean maximum DBHs of 36 cm, approximately twice those for trees at site A trees. The most obvious difference between the sites was the slightly lower elevation of site B trees - between 225 and 230 m ASL - compared with between 230 and 235 m ASL at site A. At the survey sites, groundwater levels have been extrapolated to be 5 to 10 m below ground (Data SA 2015). The slightly lower position in the landscape may mean that the depth to water is less at site B (possibly at the minimum end of the extrapolated 5 – 10 m range) and the tree roots may be accessing the groundwater. Groundwater at site A could be closer to the upper end of the extrapolated 5–10 m range which may be beyond the rooting depth of pink gums.

Current groundwater levels at Aldinga are between 10–15 m below ground, but it is likely that the depth to groundwater has been historically lowered, prior to groundwater monitoring commencing in 1988 (Ecological Associates 2003). Diversion of surface water flows and installation of drains in the 1940s and 1950s are likely to have lowered the groundwater. The current depth to groundwater may be beyond the maximum rooting depth of pink gums, but if historical levels were 6–9 m as possibly indicated by the results of the soil profile at site C, then, historically, tree roots may have also had access to groundwater. The relatively low canopy intactness of trees at Aldinga site C may partially reflect this loss of groundwater contact. The significantly taller trees at the ephemeral wetland site D, however, is thought to be due to the presence of the seasonal perched water table which is highest near the wetland tree sites and former surface water ponding in the area (Ecological Associates 2003). Historical anecdotal evidence indicates that the wetland area contained surface water for up to 6 months of the year (Ecological Associates 2003) prior to drainage of the surrounding area.

Source of water within the profile

The Clayton soil profiles and leaf water potentials were recorded in late February and March, when the soil would normally be at its yearly driest and the pink gums under most water stress. However, in the four months immediately preceding the Clayton sampling (December 2010 to March 2011) the locality received 200 mm of rainfall compared with the long-term average of 76 mm (Clayton rainfall station, Bureau of Meteorology 2015), with all four months receiving above-average rainfall. This was reflected in high gravimetric soil moisture levels and soil water potentials throughout the profile that were considerably higher than wilting point. In this unusually wet summer therefore, all the sampled soil profiles would be available to the trees for water extraction at both sites.

Gravimetric soil moisture percentages were also high (approaching soil saturation at some depths), but even at these levels soil moisture potential was higher at site B (shallow water table) than at corresponding depths for site A. Because soil moisture potential was still high at site A, this was not affecting plant water availability. However, in a dry season, if this relative difference in soil water potential was maintained between the two sites in soil profiles then water could become unavailable to trees sooner at site A than at site B. This may partially explain the low canopy intactness of trees at site A.

The water use of a stand of trees is principally a function of the combined basal area of the tree trunks (O'Grady *et al.* 2002). The stand density index of site B trees was almost four times that of site A trees, hence the total water use of site B would be higher than that of site A. Due to the high soil moisture and water potentials throughout the profile, at the time of survey, it was not possible to determine the degree to which site B trees were using groundwater. It would be expected though, that to support the relatively high stand water use of site B trees and maintain the intact canopies, the trees would be relying upon groundwater as a primary source of water in most years during the drier months. The full extent of this groundwater dependency would require long-term sampling throughout all seasons and over several years, including a series of below-average rainfall years. The sampling would include comparison of soil water and leaf water potential measurements, along with isotope analysis of twig, soil and groundwater.

Similarly, at Aldinga, when monitored at the end of summer, water from all parts of the soil profiles would have been available for pink gum roots to uptake water. Because soil water potentials were highest at 0–2 m deep, it is most likely that the pink gums were accessing water from a depth of < 2 m at all sites during the recording period. This depth corresponded to the sandy textured soils, in contrast to the deeper clay soils. As well as water being more tightly held by the clay soil particles, at all sites the heavy clay layer would have also created a lot of resistance to pink gum root growth. The depth of soil profiles at sites A, B and C were 11, 7.2 and 9.4 m but groundwater was not reached. Groundwater depths have been interpreted to be between 10 and 14 m at these sites. As referred to above this is at the deeper end, or even just beyond the range of groundwater depth which *Eucalyptus* species may possibly access. Soil moisture to a depth of 2.2 m was lowest at site B – being only 1% compared with 4% at site C and 9% at site A. Site B was at the base of a shallow dune, compared with sandy flats at sites A and C, and site B had the deepest sand layer. The smaller trees at Site B are likely to have shallower roots and hence may be more prone to water stress in summer. The significant size differences and levels of canopy intactness in pink gums at Aldinga suggests a complex ecohydrology and further studies are recommended to determine from where in the soil profile, pink gums are obtaining water at different times of the year and over a number of years.

Figure 9.8: Exposed pink gum roots at Onkaparinga National Park. The visible roots were approximately 1.5 m deep

9.6 Conclusion

At the Aldinga, Rockleigh and Clayton pink gum woodlands, there were significant differences between sites in mean pink gum height, canopy volumes and canopy intactness levels. Because rainfall was the same across sites within each of the woodlands, differences in pink gum size were most likely due to differences in soil water availability. At Clayton and Rockleigh, this was thought to reflect the different depths of groundwater. At Aldinga, pink gums at a seasonal wetland were significantly taller than all other sites, which in turn were taller than pink gums on a site with the deepest surface sand layer. The pink gum trees at the wetland site were most likely benefitting from the higher perched water table. Because pink gums commonly occur where the depth to groundwater is less than 10 m, this is conceivably within their rooting depth. It is possible that in some locations, therefore, the poor health of pink gum may be due to its partial reliance on groundwater and historical lowering of groundwater tables that has occurred within the region.

During the current study, because the water table was not reached at any site and all the soil profile was relatively moist and potentially available as a source of water, no conclusions were drawn relating to where in the soil profile pink gums were accessing water. Further studies involving soil moisture monitoring at different depths and over several years, are required to predict the resilience of pink gums to fluctuating groundwater levels in different locations.

Thesis Summary and Conclusions

Prior to this thesis, the only regional assessment of pink gums in the Mt Lofty Ranges concluded that a study to determine the causes of pink gum canopy decline was urgently needed. For a species of high conservation, habitat and commercial value, remarkably few studies have been done on pink gum's autecology. This thesis began, therefore, by documenting pink gum canopy condition and population structure in the Mt Lofty Ranges and adjoining plains. This was followed by studies correlating changes in canopy intactness over time periods varying from three months to several decades, with environmental parameters. Subsequent glasshouse and field experiments sought to isolate the impact of mistletoe and various water regimes on pink gum canopy intactness.

Regional pink gum canopy intactness, population structure and recruitment

The regional survey (Chapter 1) recorded 2814 pink gums from 78 survey sites. Canopy intactness was highly variable between sites, with mean canopy intactness being greater than 80% at 26 of the 78 survey sites, but less than 50% at 11 sites. The mean canopy intactness of individual trees was 70%, and for trees at least 8 m tall, was a relatively high 79%. Mistletoe was present at 50 (64%) of the 78 regional survey sites.

The largest trunk diameter at breast height (DBH) was 120 cm, and the tallest pink gum recorded was up to 18 m, suggesting that in optimum habitat, pink gums are capable of being relatively large trees. Optimum habitat was considered to include high water availability, either by rainfall and/or access to groundwater. However, less than 2% of surveyed trees had a DBH of > 50 cm. In general, low rainfall sites (defined in this study as < 470 mm mean annual rainfall) were dominated by pink gums of 20–30 cm DBH, and with relatively few trees of < 10 cm DBH, whereas high rainfall sites were dominated by pink gums of 5–10 cm DBH. The larger percentage of smaller trees at high rainfall sites is partly explained by the higher frequency of fires associated with high rainfall sites.

At 28% of sites there were no pink gums present with a DBH of < 5 cm. These were all sites with a mean annual rainfall of < 650 mm, indicating that recruitment of pink gums at lower rainfall sites is infrequent and episodic. Between 2009 and 2012, 1% of 1751 pink gums died and no recruitment was recorded. Whether this represents a long-term downward trend in the regional population size requires further monitoring.

Based on a literature review and results of the regional pink gum survey, five major hypotheses guided the subsequent studies in this thesis.

Hypothesis 1: Spatial variation in pink gum canopy intactness coincides with environmental parameters.

The regional study recorded that the environmental variables with a significant (negative) correlation with pink gum canopy intactness were mistletoe infestation and lerp infestation. Although there was no significant difference in pink gum mean canopy intactness between different soil groups, pink gums on deep sand-over-clay soils had the lowest mean canopy intactness (67%) and pink gums on shallow acidic soils on rock had the highest mean canopy intactness (84%). Further, a General Linear Model revealed that there was significant interaction at the 10% level between soil texture, mean annual rainfall and mistletoe abundance, on pink gum canopy intactness. For example, the mean canopy intactness of pink gums on sand-over-clay was over 90% at sites receiving approximately 700 mm rainfall and with mistletoe absent (e.g. at Cox Scrub and Ashbourne), but was < 60% at sites with sand-over-clay soils, receiving approximately 500 mm annual rainfall, and with mistletoe present (Altona, Sandy Creek, Noarlunga, McLaren Vale and Aldinga).

Hypothesis 2: Short-term changes in pink gum canopy intactness (dieback) are correlated with increased levels of insect activity.

Chapter 2 and Chapter 7 documented the increased presence on pink gum leaves of gum leaf skeletoniser (*Uraba lugens*) and cup moth caterpillar (*Doratifera quadriguttata)* in spring 2011, following the exceptionally high rainfall summer of early 2011. These insects have been documented as causing widespread damage of *Eucalyptus* trees elsewhere in south-eastern Australia, but this was the first time they have been documented on pink gums. The impact of these insects was most damaging on juvenile pink gums, whereas their abundance on adult pink gums was considered to be within “non-outbreak” levels, from which pink gums would readily recover.

The psyllid insect, pink gum lerp (*Cardiospina densitexta*) is largely confined to pink gums, and is frequently recorded in the south eastern portion of pink gum’s natural distribution in South Australia (White 1968; Paton *et al*. 2005). Although pink gum lerp outbreaks in the Mt Lofty Ranges have occasionally been recorded, the impact and extent of lerps on pink gums in the study region has not been documented. Chapter 5 concluded that for most years pink gum lerps have a highly restricted and inconspicuous occurrence in the study region. In 2011, however, a localised outbreak coincided with an exceptionally wet summer, following five years of below-average rainfall. The outbreak lasted less than two years. Pink gums with a relatively intact canopy recovered from the lerp outbreak, whereas pink gums with low canopy intactness prior to the outbreak did not fully recover their pre-lerp canopy intactness levels.

The study's findings contributes to the body of knowledge on timing of insect outbreaks in response to unusual climatic conditions, and the ability of *Eucalyptus* species to recover, or otherwise, from relatively short-term insect outbreaks.

*Hypothesis 3: Pink gums with high mistletoe (*Amyema miquelii*) loads are predisposed to canopy decline during prolonged periods of below-average rainfall.*

This thesis consistently showed that the canopy intactness of pink gums with mistletoe was significantly lower than pink gums without mistletoe. Chapter 3 compared pink gum and mistletoe canopy intactness between 2003 and 2015 at three pink gum woodlands with deep sandy soils. Pink gums whose canopies comprised at least 20% mistletoe recorded a decrease in canopy intactness between 2011 and 2015, a period of below-average rainfall. Conversely, the mean canopy intactness of pink gums whose canopies comprised less than 20% mistletoe remained the same. It was concluded that for pink gums on deep sandy soils, with moderate rainfall, high mistletoe loads predispose pink gums to canopy decline during prolonged periods of below-average rainfall, whereas pink gums with low-level mistletoe infestation are more resilient to canopy decline. While the mean canopy intactness of pink gums in 2003 and 2015 was the same, there was a net decline of 60% in the number of live mistletoes. Between 2003 and 2015, fluctuations in mean pink gum canopy intactness and the net number of live mistletoes corresponded with periods of above- and below-average rainfall. Mistletoe deaths were generally greatest on trees with lower canopy intactness levels and recruitment of mistletoes was associated with more intact host canopies.

Hypothesis 4: Long-term changes in pink gum canopy intactness are due to declining water availability and/or prolonged mistletoe infestation.

Examination of historical photos at two pink gum woodlands with deep sandy soils, indicated that canopy decline began in the 1970's (Chapter 4). This period also coincided with declining annual rainfall, including declining autumn rainfall. Sandy soils, due to their lower water holding capacity, would be most vulnerable to decreasing autumn rainfall. Conversely, pink gums at five woodlands responded to above-average summer rainfall in early 2011 by significant increases in leaf production. At least 90% of leaves produced between August 2010 and February 2012 occurred in spring/summer 2010/11, with most of this occurring during the summer months. The dominance of summer leaf production suggests that summer rains are just as important as spring and winter rains for pink gum growth. Long-term declines in summer rainfall may be just as detrimental to leaf production as declines in winter and spring rainfall.

The mean canopy intactness of 161 pink gums at three sandy soil woodlands was a relatively low 55% in both 2003 and 2015 (Chapter 3). The fact that the mean pink gum population canopy intactness was the same in 2003 and 2015 supports the premise that the decline in canopy intactness of some pink gum populations in the study region began several decades ago. Several of the studies in the thesis found that there were significant correlations between the number of live and dead mistletoes, and pink gum canopy intactness but not when only live mistletoes were considered. This indicates that pink gum canopy decline is likely to have begun one or more generations of mistletoe ago (namely, several decades ago). This conclusion was further supported by aerial photographs dating from 1949 at the Altona study site in which there was an association between increasing mistletoe levels commencing in the 1970s and declining pink gum canopy intactness.

Chapter 4 also noted that historical lowering of groundwater may be associated with the canopy decline of pink gums. Hence, Chapter 9 compared biomass parameters of pink gums in areas where the depth to groundwater differed, and provided evidence that pink gums in some locations may be accessing groundwater. Within the same woodland, pink gums overlying a shallow groundwater table were significantly taller and with larger and more intact canopies than pink gums overlying a deeper groundwater table. This indirect evidence along with a review of the literature suggests that in some situations pink gums are likely to be deep-rooted, with roots possibly up to 10 m deep. This is within the depth to groundwater for some populations of pink gums, and particularly those in areas of deep sandy soils in moderate rainfall areas. For the Mt Lofty Ranges and adjoining plains, decreases in rainfall are projected for all seasons (Goyder Institute 2015). This may result in lowering of those groundwater tables whose depth is largely rainfall-dependent. Should this occur where pink gums are using groundwater, then a decline of pink gums at these sites is likely.

Hypothesis 5: Experiments manipulating water supply and/or mistletoe loads will help determine underlying causes of pink gum decline.

Most mistletoe in the study region was recorded at low to moderate rainfall locations. To isolate the impact of mistletoes from the impact of low rainfall on pink gums, I conducted a mistletoe removal experiment in a woodland at the low rainfall limit of pink gum's natural distribution (Chapter 6). I concluded that the low canopy intactness was due primarily to mistletoe infection, despite the low mean annual rainfall of 380 mm, which had also been declining since 1975. Evidence that the pink gums were not suffering from a lack of water availability included: there being almost 30% of pink gums with over 80% canopy intactness, which were spread evenly across all age classes; non-mistletoe bearing branches were over 90% intact; no signs of bark cracking or a high percentage of dead leaves; and the increase in canopy intactness following mistletoe removal was correlated with the number of mistletoes removed. This finding varies with the conclusion reached in Chapter 3, which was that high mistletoe loads predisposed their pink gum host to canopy loss during periods of below-average rainfall. An explanation for this may be that pink gums at the low rainfall woodland may be accessing groundwater. Further study is required to determine from where in the soil profile pink gums in this low rainfall environment were sourcing their water. If pink gums were obtaining water from groundwater, then they would be to some extent buffered against low rainfall.

The glasshouse experiment (Chapter 8), showed that there were differences in water use and biomass between three pink gum provenances. The high rainfall Myponga provenance plants had a high water use strategy which enabled them to have significantly greater growth when water supply was sufficient, but had significantly lower biomass, leaf production and/or leaf retention when water was severely limiting. The results have implications in provenance selection for revegetation programmes.

Chapter 7 examined the growth rates and factors affecting growth of pink gum juveniles in the natural environment over six years. Such extended studies of juvenile *Eucalyptus* species are rare. The study found that 35% of the 34 juveniles remained the same height over six years, with likely explanations including competition for resources from nearby mature pink gums, and an outbreak of leaf-eating insects following above-average rainfall. Hence, the growth of pink gum juveniles can be negligible for several years, depending upon habitat factors other than water supply. This finding also has implications for management of revegetation sites.

Future research

A re-survey of the 78 regional study sites established between 2009 and 2011 would provide valuable data on longer-term trends in pink gum canopy intactness levels and population size. Similarly, further surveys of the pink gums and their mistletoe populations that were surveyed between 2003 and 2015, would indicate long-term trends of pink gum canopy condition with high levels of mistletoe infestation. This research would provide a wider understanding of *Eucalyptus* dieback and decline in Australia. Distinguishing short-term causes of canopy dieback, from longer-term causes of decline, will enable management efforts to be directed at treating underlying long-term causes.

Based on available evidence, I consider that for all pink gum populations in the study region, long-term soil moisture availability is a key determinant of pink gum size, canopy intactness and/or mistletoe abundance. To fully understand this relationship, however, would require a comprehensive study of groundwater depth and/or soil profiles within pink gum populations, along with long-term studies of soil, mistletoe and pink gum water potentials over at least 10 years. This would help determine pink gum rooting depth and from where in the soil profile pink gums obtain their water during different seasons and in different habitats. It would also quantify to what extent pink gums can recover from a loss of canopy intactness, when favourable soil water availability resumes. This knowledge would add to the increased focus of research on groundwater dependent ecosystems in Australia, which are threatened by groundwater resources being increasingly used at a non-sustainable rate (Eamus 2015).

Research on germination and establishment requirements of pink gums would help predict future population trends of pink gums in low rainfall environments. Data gathered during this thesis suggested that recruitment events at the lower rainfall limits of pink gum’s natural distribution are rare, and likely to require exceptionally high rainfall years. Similarly, qualitative and/or anecdotal evidence suggested that pink gums may be highly sensitive to competition from co-occurring pink gum and other trees, which may reduce pink gum canopy intactness and possibly also suppress growth and establishment of juveniles.

This study recorded genetic variation between pink gum populations which was reflected in different growth rates and water use strategies. Further study is required to determine the extent of this variation and if populations respond differently to drought. This information will contribute to the emerging body of knowledge on the value of revegetating with seed sourced from habitats that correspond to predicted water availability of the revegetation area.

Prognosis for pink gums in the Mt Lofty ranges and adjoining plains

Despite extensive clearance of native vegetation, pink gums are still widespread and abundant throughout the study region. They occur at all but the very highest elevations, occupy a wide range of soil types and landforms, and have been recorded with all other *Eucalyptus* species present in the region. Although the extant distribution of pink gums is largely confined to soils of low fertility and/or skeletal soils, there are pink gums present on more fertile soils, suggesting that the pre-European distribution of pink gums was even more widespread than at present. For the majority of survey sites where mistletoe was absent, pink gums have relatively high levels of canopy intactness. However, in woodlands where mistletoe is abundant, pink gum canopy intactness is relatively low and this has perhaps distorted the perception of pink gum health in the region.

This thesis found evidence to support the following hypotheses:
Short-term canopy loss (dieback) of pink gums is most likely due to fluctuations in insect activity, from which pink gums can recover if canopies are intact prior to localised outbreak. Long-term decline of pink gums is most likely due to a combination of prolonged mistletoe infestation and declining water availability. Pink gums on deep sands in moderate rainfall areas are most likely to experience water stress in late summer or autumn, if rainfall continues to decline and/or past access to groundwater is not recovered. Pink gums with access to groundwater have larger and more intact canopies than pink gums in habitats matched in all respects other than depth to groundwater. The study also found a correlation between increasing native pine (*Callitris gracilis*) density and declining pink gum canopy intactness.

Hence, there are four factors likely to diminish pink gum canopy intactness in the future: overabundance of mistletoes, particularly in areas of low to moderate rainfall and/or low soil water availability; lowering of groundwater levels in regions where water is currently being extracted and the groundwater table is < 10 m deep; continued reductions in autumn rainfall (climate change) particularly in areas with deep sandy soils; and exclusion of fire in woodlands where native pine (*Callitris gracilis*) is present.

Conversely, the sites where pink gums are considered to be buffered against continued declines in rainfall are those woodlands where pink gum is the dominant overstorey tree and mistletoe is absent or sparse. These areas include much of the southern Fleurieu Peninsula, rocky skeletal soils at high rainfall sites within the central spine and western scarp of the Mt Lofty Ranges, and areas of more fertile soils such as loam or silty loam soils on the eastern flanks of the Mt Lofty Ranges.

Overall, pink gums are secure in the Mt Lofty Ranges and the canopy condition across the region is unlikely to change drastically in the foreseeable future (next 10-20 years). This is because pink gums are widespread and locally abundant in the study region, and occur over a wide range of soils and rainfall levels. Most populations have reasonably intact canopies, or at least have individuals present with high canopy intactness and a capacity to recover from short-term perturbations such as insect outbreaks that are infrequent and localised. Pink gums are likely to be deep-rooted, enhancing their ability to take advantage of any possible increases in water availability at depth, including groundwater. Pink gums also have sufficient genetic diversity from which seeds can be sourced to allow pink gum populations to be successfully re-established in areas where they have been removed historically.

However, while the regional population is likely to remain stable for the medium term, there are localised populations which may continue to decline. These are populations where mistletoe is abundant and/or recent recruitment is lacking. For these populations, planting of pink gums is recommended to replace dead trees. Any re-establishment of populations will increase the distribution and abundance and conservation of the species. Revegetation programs should consider matching growth rates and water usage of the provenance from which seed is sourced, with revegetation requirements and local water supply.

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Appendix A: Regional survey sites - habitat and pink gum parameters

Heading explanations

Site numbers with coloured infill were surveyed in 2009/10 and 2011/12

Source: T = targeted sites; W = Ward (2007) survey sites; BS = biological survey sites (DEWNR 2009) (refer Section 1.3)

Soil group sourced from NatureMaps (2015)

Pink gum dominance calculated as the ratio of the sum of effective DBH for all pink gums at survey site to the ratio of the sum of effective DBG for all non-pink gum trees recorded at a site

Pink gum stand density index = number of pink gums per ha x (mean effective DBH/25)$^{1.605}$

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
01	Aldinga	268132	6090403	T	507	11	0	0	1	Deep Sand	69 ± 7	25	24	0	50	33
02		268475	6090993	W	507	25	0	0	> 35	Deep Sand	61 ± 5	56	6	3	100	186
03	Altona	306964	6170908	BS	518	200	340	1	> 35	Sand over clay	76 ± 6	68	8	65	79	94
04		307961	6170707	BS	478	223	115	2	> 35	Sand over clay	56 ± 7	85	0	5	100	215
05		307812	6171398	BS	518	223	0	0	> 35	Sand over clay	61 ± 7	82	9	12	100	58
06	Angas Plains	310472	6091045	BS	405	51	180	3	> 35	Sand over clay	70 ± 3	7	2	31	100	243

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
07	Anstey Hill	292987	6143014	W	638	336	150	35	13	Shallow to moderately deep acidic soil on rock	83 ± 2	0	44	2	90	156
08		292501	6143498	W	638	304	165	22	28	Shallow to moderately deep acidic soil on rock	34 ± 5	21	17	3	78	147
09		292940	6143487	W	638	381	310	7	16	Shallow to moderately deep acidic soil on rock	88 ± 3	0	53	0	100	107
10	Ashbourne	297245	6092359	BS	681	150	205	5	> 35	Deep Sand	88 ± 1	62	14	22	100	137
11	Belair	286734	6123724	BS	940	389	5	10	33	Shallow soils on rock	83 ± 3	6	64	3	59	252
12		287005	6123520	T	940	440	5	1	33	Shallow soils on rock	77 ± 3	3	16	22	100	436
13	Clayton	311329	6073729	T	404	5	180	1	> 35	Shallow soils on calcrete or limestone	95 ± 2	10	93	5	89	254
14		311286	6073834	BS	404	8	0	0	> 35	Deep Sand	67 ± 5	67	7	34	100	208
15		311315	6073966	T	404	7	0	0	> 35	Sand over clay	85 ± 3	82	0	67	100	371
16		311427	6074255	T	404	7	90	1	> 35	Sand over clay	38 ± 6	93	4	0	100	104
17	Blackhill/Morialta	291396	6137305	BS	686	323	240	22	24	Shallow to moderately deep acidic soil on rock	32 ± 6	18	28	3	58	95
18	Burgar Road, Middleton	292398	6072713	T	543	108	180	2	> 35	Sand over clay	90 ± 3	2	8	35	99	367
19	Cox Scrub	295151	6087826	BS	694	146	70	5	28	Sand over clay	91 ± 2	0	61	0	82	100
20	Cromer	315487	6149995	W	729	459	280	2	> 35	Ironstone soils	72 ± 4	0	51	4	84	191

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
21		315562	6150533	W	729	462	250	1	> 35	Ironstone soils	68 ± 4	55	7	30	100	273
22	Currency Creek	298593	6076762	BS	485	29	0	0	> 35	Hard red-brown texture contrast soils with alkaline subsoil	82 ± 4	14	23	37	82	109
23	Deep Creek	248388	6051961	BS	794	223	161	16	> 35	Shallow to moderately deep acidic soils on rock	69 ± 4	0	63	0	100	237
24		243845	6051077	BS	769	243	270	2	> 35	Shallow soils on rock	83 ± 2	0	63	0	72	81
25		251926	6053399	T	762	239	90	4	> 35	Shallow to moderately deep acidic soils on rock	85 ± 5	0	37	0	68	395
26	Engelbrook	295948	6122437	BS	829	362	70	10	22	Shallow to moderately deep acidic soils on rock	60 ± 7	5	58	0	34	53
27	Ferries McDonald	328978	6099186	T	388	42	0	0	> 35	Shallow soils on calcrete or limestone	75 ± 6	26	5	37	92	24
28	Finniss	289886	6089536	W	768	253	225	20	28	Shallow to moderately deep acidic soil on rock	70 ± 4	0	43	15	100	346
29		289794	6089540	W	768	253	150	10	28	Shallow to moderately deep acidic soil on rock	83 ± 2	0	24	35	100	444
30		289846	6089731	BS	768	281	60	8	28	Shallow to moderately deep acidic soil on rock	80 ± 4	0	32	19	100	258

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
31	Goolwa	297094	6068500	BS	465	9	0	0	28	Shallow soils on calcrete or limestone	77 ± 4	0	20	16	100	157
32		297268	6068249	BS	465	10	0	0	> 35	Shallow soils on calcrete or limestone	85 ± 3	0	3	53	100	140
33	Hale	308390	6160344	BS	628	361	20	10	> 35	Shallow to moderately deep acidic soil on rock	81 ± 3	5	27	0	42	56
34	Hartley	321640	6101996	BS	382	76	0	0	> 35	Shallow soils on calcrete or limestone	51 ± 3	86	5	65	74	14
35	Manning Scrub	279436	6103248	BS	543	117	90	5	> 35	Sand over clay	61 ± 7	46	4	37	100	266
36		279415	6103507	W	543	140	180	2	> 35	Sand over clay	69 ± 4	33	40	2	100	155
37	Wistow	309188	6108092	T	537	290	300	7	> 35	Shallow to moderately deep acidic soil on rock	66 ± 4	76	0	96	100	1207
38		309148	6108037	T	537	285	180	9	> 35	Shallow to moderately deep acidic soil on rock	81 ± 2	69	2	76	100	516
39	Highland Valley	310614	6105314	T	537	239	315	5	> 35	Shallow to moderately deep acidic soils on rock	73 ± 4	0	16	51	100	267
40	Monarto South	329949	6103902	BS	393	80	180	2	> 35	Shallow soils on calcrete or limestone	45 ± 5	74	17	10	99	92
41	Montacute	298143	6137368	BS	827	500	270	20	28	Shallow to moderately deep acidic soils on rock	20 ± 3	0	41	22	90	142

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
42	Blackhill/Morialta	292179	6134325	BS	896	415	110	19	5	Shallow to moderately deep acidic soils on rock	44 ± 4	26	40	7	92	160
43	Mt Beevor	321149	6133420	BS	557	420	310	5	> 35	Deep loamy texture contrast soils with dark subsoil	70 ± 4	30	7	87	83	283
44		320751	6133279	T	557	438	30	9	5	Shallow soils on rock	93 ± 2	7	47	35	98	315
45	Mt Crawford	313852	6156807	BS	617	507	270	13	> 35	Shallow to moderately deep acidic soils on rock	38 ± 4	0	9	23	64	197
46		313443	6157049	BS	617	450	240	4	> 35	Shallow to moderately deep acidic soil on rock	52 ± 6	0	18	12	91	219
47		313699	6156867	T	617	450	240	3	> 35	Shallow to moderately deep acidic soil on rock	71 ± 4	0	0	30	100	264
48	Mt Magnificent	289225	6090583	BS	768	287	350	13	28	Shallow to moderately deep acidic soil on rock	74 ± 5	0	52	12	43	211
49		289356	6090758	T	768	314	270	18	28	Shallow to moderately deep acidic soil on rock	83 ± 4	0	9	22	100	527
50	Mylor	296607	6120206	W	829	317	90	8	31	Shallow to moderately deep acidic soil on rock	83 ± 2	0	52	0	84	177
51		296632	6120212	W	829	317	90	8	31	Shallow to moderately deep acidic soil on rock	67 ± 4	4	67	0	96	196

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
52	Myponga	267019	6073967	BS	788	285	230	12	> 35	Shallow soils on rock	88 ± 3	0	41	0	39	147
53		267308	6074331	W	753	191	320	10	> 35	Shallow to moderately deep acidic soils on rock	72 ± 6	81	18	32	50	131
54	Onkaparinga	279896	6104992	W	543	161	240	5	> 35	Sand over clay	57 ± 8	14	6	0	34	60
55		280466	6105037	W	543	161	120	3	> 35	Sand over clay	61 ± 6	56	13	4	85	106
56		280992	6105520	W	543	153	260	6	5	Sand over clay	85 ± 3	5	20	11	100	379
57		280248	6108930	BS	599	269	120	4	> 35	Shallow to moderately deep acidic soils on rock	62 ± 5	3	37	10	63	90
58	Para Wirra	300822	6158982	W	661	339	355	1	> 35	Ironstone soils	73 ± 6	6	6	17	79	80
59		300852	6158512	W	661	339	260	5	> 35	Ironstone soils	62 ± 6	23	0	62	60	180
60		300983	6156991	W	661	339	355	1	> 35	Ironstone soils	65 ± 6	50	42	12	72	192
61	Rockleigh	327760	6131188	BS	359	229	0	0	> 35	Sand over clay	72 ± 6	69	10	14	61	64
62		327894	6130621	T	368	269	260	8	> 35	Shallow to moderately deep acidic soil on rock	59 ± 9	22	19	27	89	136
63		328060	6131020	T	359	229	0	0	> 35	Sand over clay	44 ± 8	91	0	74	100	92
64	Sandergrove	300997	6089087	BS	518	94	120	6	28	Sand over clay	70 ± 4	88	0	71	100	239
65	Sandy Creek	303030	6168497	W	534	217	330	2	> 35	Sand over clay	67 ± 6	22	0	32	41	130
66		303459	6168500	W	534	217	330	2	> 35	Sand over clay	48 ± 6	63	0	26	100	124
67		303816	6168497	W	534	217	330	2	> 35	Sand over clay	49 ± 8	79	0	47	100	79

Site #	Remnant	Easting	Northing	Source	Mean Annual Rainfall (mm)	Elevation (m)	Aspect (degrees)	Slope (%)	Years since last fire at survey date	Soil group	Mean pink gum % canopy intact ± SE	% of pink gums ≥ 1.6m tall with mistletoe	% pink gums with max DBH of < 5cm	% of pink gums with max DBH > 20 cm	Pink gum dominance	Pink gum stand density index
68	Scott	294567	6079238	T	597	90	0	0	> 35	Hard red-brown texture contrast soils with alkaline subsoil	87 ± 2	0	0	43	100	350
69		294447	6078997	W	597	98	295	5	> 35	Sand over clay	78 ± 5	19	57	0	64	119
70	Scott Creek	289005	6114367	T	829	285	240	7	> 35	Shallow to moderately deep acidic soils on rock	72 ± 4	15	65	0	82	108
71		288770	6114341	T	829	249	270	1	> 35	Deep loamy texture contrast soils with brown or dark subsoil	68 ± 5	56	20	7	100	225
72	Skewes Rd, Goolwa	297653	6070696	T	465	13	0	0	> 35	Sand over clay	76 ± 6	0	0	64	72	122
73	Talisker	241802	6054335	BS	607	284	190	10	28	Shallow to moderately deep acidic soils on rock	83 ± 3	0	3	45	56	437
74		241740	6054340	BS	607	276	240	18	28	Shallow to moderately deep acidic soils on rock	80 ± 4	0	7	32	91	477
75	Totness	302706	6118210	T	742	408	290	8	28	Shallow to moderately deep acidic soils on rock	71 ± 4	0	44	7	82	142
76	Blackhill/Morialta	290191	6137816	W	686	149	250	3	26	Deep loamy texture contrast soils with dark subsoil	34 ± 4	64	28	22	95	168
77	Normanville	253850	6068272	T	538	145	180	16	> 35	Shallow soils on rock	82 ± 2	0	0	84	96	503
78		253793	6068458	T	538	169	270	7	> 35	Shallow soils on rock	81 ± 2	0	0	96	100	333