Investigations into the ecology and management of the invasive plant *Galenia pubescens* within the native temperate grasslands of Victoria, Australia

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Abstract

Plant invasions of natural ecosystems are one of the major threats to the conservation of biological diversity across nearly all biogeographical regions in the world. This thesis focuses on *Galenia pubescens* (Carpet weed) as a case study of possible approaches to the potential management of environmental weed species. *G. pubescens* is a woody prostrate perennial plant that is becoming a serious threat to Australian temperate grasslands, surrounding agricultural areas and conservation reserves. It is indigenous to South Africa and was first recorded in Australia in the early 1900s, and it is an aggressive competitor against native species. It is difficult to control, and little information exists about its effects on natural ecosystems, and aspects of its biology and ecology.

This study has investigated some important ecological characteristics of *G. pubescens'* seeds and has experimented with different management strategies in combination with chemical control. It has also considered the potential for the expansion of the distribution of this species across suitable habitat in Australia under predicted elevated CO_2 and drought conditions.

Seeds of *G. pubescens* are shown to be able to germinate over a broad range of temperatures, but short bursts (5 minutes) of high temperatures (80ºC to 120ºC replicating possible exposures to a fire) reduced seed germination. Seed germination was positively favored by light and declined rapidly in darkness, decreasing by > 80% at a depth of only 0.5 cm in soil. This suggests that fire regimes might be useful in removing mature plants and controlling seed numbers on the surface, and that subsequent native seeding of undisturbed areas may assist in the long-term management of this noxious weed.

A trial was conducted to determine the effect of treatment with a plant essential oil (pine oil) to limit the seed germination and seedling emergence of *G. pubescens.* This trial showed that the effects of pine oil application were significant ($P<0.05$). Germination was completely inhibited by application of pine oil at 5% or higher concentrations directly to seeds, and seedling emergence was reduced by between 90 and 100% in pot trials. These outcomes demonstrate the potential viability of pine oil as a long-term control option for this species.

In field-based experiments, control strategies including herbicide control with glyphosate, organic herbicide control with pine oil, the application of mulch, and the addition of native seeds to the available seedbank (and all possible combinations of these techniques), were tested. The study showed that one single treatment of a *G. pubescens* infestation without undertaking a secondary treatment is insufficient to control the *G. pubescens* infestation or to encourage native regeneration, but that a combined strategy, employing all the aforementioned techniques, is more effective. It is however suggested that full regeneration of the area may not be possible unless further restoration programs are instituted after the cycle of *G. pubescens'* treatment has been completed. The thesis also assessed the control effects of chemical control combined with a prescribed spring burning. Assessment of the resulting aboveground vegetation of *G. pubescens* has shown that a combination of chemical control and late-spring burning can reduce the cover of non-native species such as *G. pubescens,* suggesting that this could be a useful tool in their management.

Finally, this study has supported the view that the growth of *G. pubescens* will be significantly enhanced in a future climate with an enriched atmospheric CO_2 concentration. These climatic changes will have important implications for management of this noxious weed in the future.

Statement of Authorship

Except where explicit reference is made in the text of this thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No other person's work has been relied upon or used without due acknowledgement in the main text and bibliography of the thesis.

Signed: _____	Signed: _____
Dated: _____	Dated: _____
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Candidate	Principal Supervisor

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Chapter 1: General introduction

Background

Galenia pubescens – a dominant weed

Galenia pubescens (Eckl. & Zeyh.) is a prostrate perennial herb indigenous to South Africa (Arnold & De Wet, 1993). It is from the Aizoaceae family (Ice plants) and is a C_4 plant that has adapted to grow in the harsh dry deserts of southern Africa (Landrum, 2002). It is commonly known as 'blanket weed', 'carpet weed' or 'green galenia' and is considered a serious environmental weed in many countries including Australia, where it invades dry coastal vegetation, lowland grassland and grassy woodland, dry sclerophyll forest and woodland, and rocky outcrop vegetation (Carr *et al*., 1992).

Galenia pubescens was first reported in Victoria in the early 1900s (Australia's Virtual Herbarium, 2014) and has since spread through most southern states in Australia (Prescott & Venning, 1984), with infestations being particularly severe in the upper Hunter region of New South Wales (Cook, 2013). *G. pubescens* forms a roughly circular mat on the ground, growing out from a deep central stem in a similar habit to *Polygonum aviculare* (wireweed) (Cook, 2013). *G. pubescens* has been found to smother other plants, and infestations have been linked with statistically-significant lower levels of native species richness and diversity (Garćia-de-Lomas *et al*., 2010). Its smothering habit of growing over surrounding vegetation leads to its dominance in many situations.

At the local level, infestations of *G. pubescens* are becoming an increasing issue within the Western Grassland Reserve in Victoria where it dominates areas previously occupied by native grassland species. *G. pubescens* is a drought-tolerant, salt-tolerant herbaceous plant, and, importantly, it has been found to have a higher extractable nitrate reductase activity than for any previously observed higher plant species

(Kleinkopf *et al*., 1976). Williams (1979) showed that these high levels of nitrates and soluble oxalates produced by *G. pubescens* can be toxic to stock. Williams (1979) also showed that when *G. pubescens* grows on infertile soils, these chemicals were not found to be in high levels, but when grown in fertile soils these chemicals can accumulate to toxic levels.

Notwithstanding its toxic potential, *G. pubescens* is eaten by cattle (Simmonds, 2000). Cattle use their very strong tongue to wrap around the plant and pull it from the ground. This action has been observed to result in some level of control. However, other stock do not have this capacity and rabbits will not eat *G. pubescens,* leading to its dominance in areas inhabited by rabbits (Phil Teledo, pers. comm).

Whilst *G. pubescens* was reportedly introduced into the United States of America from South Africa because of its potential use as a fire-retardant species (Kleinkopf *et al*., 1976), in Australia, anecdotal reports suggest that *G. pubescens* infestations that have been strategically burnt do not recover well. *G. pubescens* produces very small oval seeds (1-2 mm, Figure 1.2, Dashorst & Jessop, 1990) that are not adapted for soil burial. It is likely that a large number of seeds may sit in very shallow depressions in the soil, or be situated just on the soil surface.

1.0 The context of this research

1.1 The concept and meaning of the term 'Weed'

The term 'weed' has been defined as 'a plant that is growing where it is not wanted' (Richardson *et al*., 2000; Weber, 2003; Benvenuti, 2007). To this can be added that weeds cause many negative economic, environmental or social impacts (Cook *et al*., 2015). Economic impacts of weed infestation include the costs of control, and decreases in agricultural yield and loss of stock, all of which contribute to a reduction

in farm income (Sinden *et al*., 2004). Environmental impacts of weeds include the degradation of natural ecosystems through reduction in biodiversity, an increase in fire risk, loss of habitat for native fauna, and reduced ecosystem amenities (Eiswerth *et al*., 2002; Jacob *et al*., 2004).

The status of a plant as a weed is dependent on where it is growing, since a species which is a native in one region may be considered to be a major weed in another region (Pickering & Ann, 2010). A weed is also understood to be any plant that requires some form of action to be taken to reduce its effect on the economy, the environment, human health and social amenities (Richardson *et al*., 2000). Because of their tendency to spread and compete with native species, weeds are also known as invasive plants, and this has meant that many plants introduced into Australia in the last 200 years are now classified as weeds (Sinden *et al*., 2004). In this respect, previous studies have documented that most of the woody plants that have so far been naturalised in Australia were introduced deliberately for ornamental or other purposes, while some have been introduced accidentally as contaminants of other seed lots or in ship's ballast (Mulvaney, 2001; Groves *et al*., 2005).

1.2 A brief review of the problem of weeds

Weeds reduce the quantity and quality of agricultural, horticultural and forest products in Australia, which negatively affects both the industry and consumers. They have severe impacts on the efficiency of the agricultural industry and interfere with the balance of the natural environment. In this latter respect, weeds can lead to a reduction in biodiversity, as some invasive weeds can out-compete most native plants to create vast monocultures. In doing so, weeds can degrade the habitat of native wildlife due to competition with, and reduction of, populations of native plants which may be food

species, or provide other resources, for wildlife (Foxcroft & Richardson, 2003; Singh *et al*., 2014).

Weeds that have direct, negative economic impacts, such as requiring control costs and causing yield losses, may be termed 'economic' weeds. These associated costs tend to particularly affect primary production industries and can represent a significant loss for the producers. For instance, weeds were estimated to cost the American agricultural sector US$26.4 billion annually (Pimentel *et al*., 2005). An invasive species in the Idaho rangelands, *Centaurea solsitialis* (yellow star thistle) was assessed to have an economic impact totaling US$12.7 million per year (Julia *et al*., 2007; Oster *et al*., 2015). Weeds are estimated to cause the New Zealand agricultural sector productivity losses of approximately US$340 million per year (Beck *et al*., 1997). The economic cost of weeds in Australia has been estimated to be about AU$3,300 million annually in weed control activities for grain growers (Llewellyn *et al*., 2016). The actual cost of the environmental weeds is difficult to calculate, but it is expected that the cost will be similar to, if not greater than, that estimated for agricultural industries (Sinden *et al*., 2004). While this figure excludes many ecosystem 'goods and services' such as water flow, drainage and biodiversity (which are difficult to quantify in monetary terms), it does include weed control costs in natural areas.

Plant invasion into natural ecosystems is one of the major threats to the conservation of biological diversity across nearly all biogeographical regions globally (Richardson *et al*., 2000; Aronson *et al*., 2014), but the impact of environmental weeds on the natural environment is a topic that has been little studied in Australia (Vilà *et al*., 2011; Fraser *et al*., 2015). The rate of invasion of the Australian environment by introduced plants has increased linearly since European settlement (Groves, 1986), however, it is now thought that it may be increasing exponentially (Carr, 1993). Some weed

invasion prior to European settlement probably also occurred, although at a considerably slower rate than has been found post-settlement. For instance, a documented example of a pre-European invasion is that of *Tamarindus indicus* (tamarind) on the northern Australian coastline which is believed to have been introduced by Macassans on their annual fishing expeditions to Australia from about 1700, and certainly prior to the first visit to the region by the European explorer Matthew Flinders in 1803 (Macknight, 1976).

Key questions that need to be asked before control programs are undertaken for environmental weeds include determining the impact of weeds on native species and ecosystem functions, the threshold densities at which there is little or no impact of the weed on conservation values, and what factors can be manipulated to reduce that impact.

Environmental weeds threaten nearly all biological communities in Australia. Although weeds appear to degrade many natural ecosystems, quantitative measures of their impact on those systems are relatively rare. Information needed to establish priorities for the control of weeds in natural ecosystems include determination of the mechanisms of weed invasion, the ecological impact of the weeds and the threshold points for decline in biodiversity values as weed invasions proceed.

1.3 Current concepts of weed invasion processes

It has been noted (Lockwood *et al*., 2013) that invasion by a species is never a single event, but rather a complex process involving several stages. As a consequence of this perspective, it has been postulated that for an invasive species to achieve a foothold in an alien environment, it is likely to pass through four main stages. These stages have been termed (i) introduction, (ii) colonisation, (iii) naturalisation and (iv)

invasion (Figure 1.1) (Emerton & Howard, 2008). Richardson *et al*., (2000) challenged the un-occupied niche concept that was generally accepted (but never proven) by reporting that invasions can include a special class of plants that have the ability to enter and occupy already fully-inhabited plant communities without further assistance from humans or the environment. Additional refinements to this process are sometimes considered, such as the intrinsic biology traits of the plant species (i.e. reproductive capacity, dispersal capacity, competitive ability, quick growth and early flowering) and extrinsic factors or host environment (i.e. climate, soil type and land use): these are both important factors in determining the success of invasive plant species (Radosevich *et al*., 2003).

The introduction stage commences once a non-native plant has entered a region, which may be a country, ecosystem or habitat which is not its native range. This process could be intentional or unintentional as far as human agency is concerned. The former category refers to the process whereby humans deliberately introduce a species into a new territory for various purposes including forestry, food production, horticulture, agriculture, biological control, plant trade, decoration, agroforestry, research, landscaping and botanical collections (Radosevich *et al*., 2003). Alternatively, the introduction stage could occur through an unintentional process when the species enters the new territory 'accidentally' such as in or on the belongings of travelers, on agricultural machinery, or by natural means such as floods or strong wind events.

The next stage, colonisation, refers to the establishment of a species in a new location. This takes place when individual plants, which have been introduced to a new territory, survive and form a population capable of reproducing in the new environment. The establishment of exotic weed species capable of surviving in areas such as roadsides

can be regarded as an important colonisation stage (Cousens & Mortine, 1995). The rate at which the colonisation phase takes place is often referred to as its intrinsic rate of increase. Thus, colonisation is thought to depend more on biological functions than environmental ones, despite the importance of both during this stage.

Naturalisation then occurs once the species that has been successfully colonised starts to reproduce and spreads naturally with no human assistance. During the naturalisation phase, the species can begin to have negative impacts on the natural fauna and indigenous flora.

The final stage, known as the invasion phase, occurs when a foreign species that has been naturalised spreads. This is usually to the disadvantage of other species (naturalised or native) present in the location. It is during this phase that some disruptions do or may occur in the ecosystem.

In addition to these intrinsic phenomena, extrinsic factors also influence the rate of introduction by affecting the distribution and success of germinating seeds (Radosevich *et al*., 2003). These extrinsic factors (such as soil-type, climate, land use and the condition of the environment) greatly influence the likelihood of the plant's initial success in the introduction phase. The colonisation and explosive growth phases are, however, closely associated with the intrinsic rate of increase for the invasive species. Therefore, the intrinsic biology of the species has more potential in the estimation of colonisation rates and for management options when compared to extrinsic factors. Finally, both factors (intrinsic and extrinsic) might play an important role in defining the success-rate and invasion-potential of the invasive species (Ortega & Pearson, 2005).

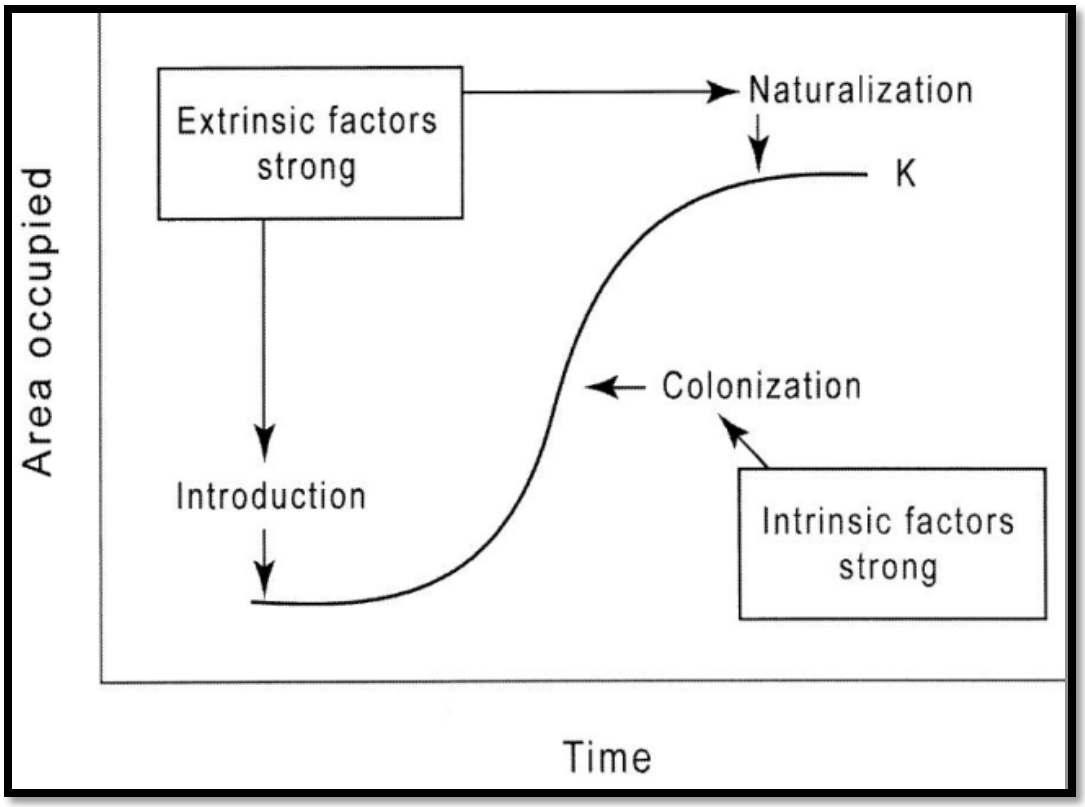

Figure 1.1. The invasion processes: introduction, colonisation, and naturalisation/or invasion of plants, and the relationship of extrinsic and intrinsic factors on these phases of population growth (Emerton & Howard, 2008)

1.4 Characteristics of invasive species

To become established in a new environment, an individual plant or group of plants need certain characteristics to enable them to cope well with their new conditions (Hoffman & Parsons, 1991). When established, such invasive plant species can affect native biodiversity and lead to the extinction of many species because of their aggressive nature and ability to alter soil conditions (Downey, 2006).

According to Hengstum *et al*. (2014), opportunistic and aggressive invasive plant species have certain characteristics that run counter to those of native plant species

and to the possibility of sustainable area management, making native plants both vulnerable to the initial invasion and resistant to control methods. Invasive plant species often have self-compatibility and seed longevity, as well as rapid seedling growth (Hengstum *et al*., 2014). Further, Orians (1986) has suggested that successful invaders also spread quickly because of human and non-human vectors, where tourists, migrants and animals can inadvertently disperse these species, as can abiotic factors such as wind, water and machinery. Many invasive species grow and spread very quickly because they reach sexual maturity rapidly (Shaw *et al*., 2015). They produce many seeds and also have high genotypic and phenotypic plasticity. Many studies demonstrate that the majority of invasive species engage in chemical competition with plants growing nearby by generating allelopathic toxins (Reichard & Hamilton, 1997; Kolar & Lodge, 2001; Jeschke & Streyer, 2008). Even though these invasive species may have common characteristics with native plants, Sutherland (2004) has claimed that invasive plants have a wider range of demographic characteristics and play varying ecological as well as socio-cultural roles. This broad range of adaptations found in weedy species makes it difficult to predict all the characteristics of any one particular invasive plant species (Sutherland, 2004). Aside from the invasive species' characteristics, the features of the invaded area also contribute to the success of an invasion. For example, degraded and fragmented landscapes that do not support much native growth are often those that are the most vulnerable to invasion.

1.5 Exotic plants in temperate Australian grasslands

Grasslands are natural ecological systems dominated by grasses with less than 10% natural tree or shrub cover. They contain many grass species and an even greater diversity of other herbs. Grasslands are among the most species-rich plant

communities in Australia (Faithfull, 2012). Exotic plants are now present almost universally in temperate Australian grasslands, and include a wide diversity of annual, biennial and perennial forbs, and annual and perennial grasses (Trémont, 1994; Kirkpatrick *et al*., 1995; Groves & Whalley, 2002; Faithfull, 2012). Lowland grassland in Victoria was considered by Carr *et al*., (1992) to be one of the weediest of the broad vegetation formations in Victoria, with 344 exotic taxa, of which 87 were considered very serious weeds. Between one quarter and one third of the flora in each of the main grassland regions consists of exotics, and weed invasion is a major problem for the survival of the native flora (Kirkpatrick *et al*., 1995; Groves, 2004).

1.6 A description of the invasive species *Galenia pubescens*

1.6.1 Origin

The genus *Galenia* comprises 27 species, all of which have natural distributions limited to southern Africa (Jacobs & Highet, 1990). There, it is particularly characteristic of the west and south-west, especially along the margins of the Karoo region in South Africa, although some *Galenia* taxa occur along the southern coastal belt. All the taxa are lowland plants and are apparently absent from the main summer rainfall areas. Of particular interest to this investigation is that two of the *Galenia* taxa, *G. secunda* and *G. pubescens*, are now naturalised weeds in lowland areas of Australia (Wallace, 1982).

1.6.2 Morphology

Galenia pubescens is a soft grey-green, often mat-forming shrub. It is a low growing (up to 30 cm high), spreading (in excess of 150 cm), and perennial broad leaf plant. It has a taproot system that can penetrate to 2 m in the ground. The stems are covered in scale-like hairs and are woody at the plant base. The leaves are ovate to spathulate, are covered with hairs and are alternately arranged on the stem. As the plant matures,

it produces many branches with flowers. *G. pubescens* flowers throughout the year, with maximum flower densities occurring in the spring. The flowers are white, with a slight pink tinge, and tend to yellow with age. They are small in size (2-3 mm across), sessile (i.e. without a stalk) with five petals, and are found in the axils (between the leaf and the branch). The fruit is a capsule 2-3 mm long, which is dry and leathery, with five angles which contain shiny, black seeds up to 1 mm in length (Figure 1.2) (Jacobs & Highet, 1990; Cunningham *et al*., 1992; Walsh & Entwisle, 1996).

1.6.3 Propagation

Galenia pubescens is a prolific seed producer, giving it the potential to build up a large seed bank very quickly. It produces from between 95,000 to 100,000 seeds plant^{-1} yr^{-1}, which typically makes up greater than 50% of the total soil seed bank in a particular infested area (García-de-Lomas *et al*., 2010). Up to 400 million *G. pubescens* seeds ha^{-1} can be present in or on the surface soil. Seeds are known to be spread by a wide range of vectors, including vehicles, farm machinery, animals, pasture and crop seed lots, wind, humans and moving water (García-de-Lomas *et al*., 2010).

1.6.4 Climatic conditions

The seeds of *G. pubescens* normally germinate in spring (in temperate Australia: end of September/start of October) and early autumn (March/April); the average maximum/minimum temperatures for these seasons are 20 to 14.3^{o}C and 23.1 to 17.8^{o}C respectively. Maximum germination occurs at available soil moisture between 40% and 60%. The availability of soil moisture in early spring is essential both to germinate the seed and to establish seedlings. The germination of *G. pubescens* seeds depends on sufficient rain to leach germination inhibitors from the seed (García-de-Lomas *et al*., 2010).

1.6.5 Habitat

Galenia pubescens prefers moist or seasonally wet conditions, such as temperate grasslands and road sides. However, it adapts well to saline conditions (Mahmood *et al*., 2016), for example in Macquarie's Pier rocks, and parts of North Stockton in NSW, where it grows along a windbreak fence. During the last 30 years, infestations of *G. pubescens* have adapted to warm conditions (Cook, 2013). It occurs on roadsides, in pasture, waste lands, mine-reclamation sites, native bushland, and lawns. In the study site, this species has also invaded ex-arable land.

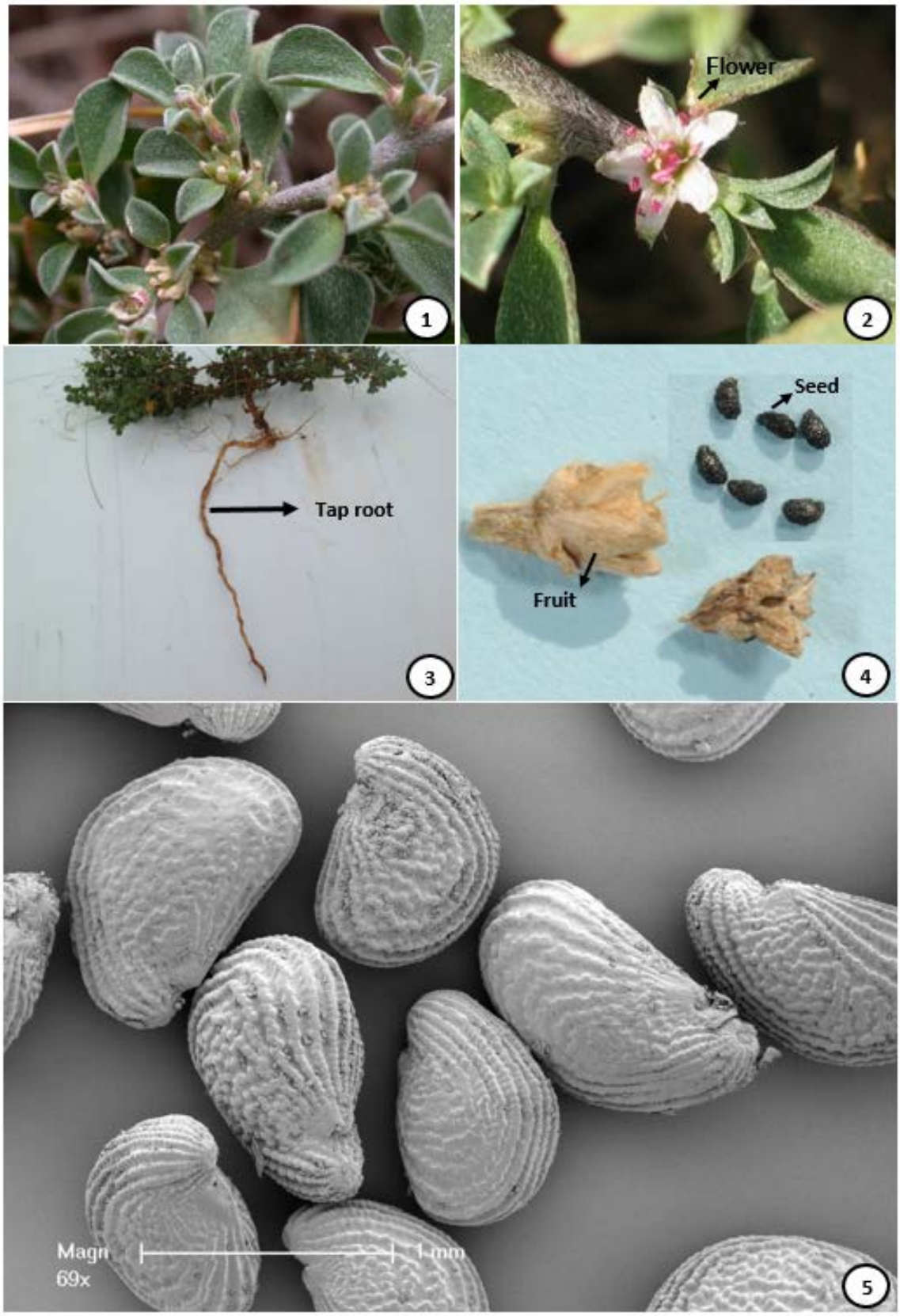

Figure 1.2. *Galenia pubescens* parts: (1) Leaf (2) Flower (3) Root (4) Seed and fruit (5) Electronic microscopic imaging used for seeds.

1.7 Historical and current distribution of *Galenia pubescens* in Australia

Galenia pubescens was introduced to Australia in early 1900 as a stabilizing species for mining areas and to control soil erosion at mining sites and along roadsides (Cook, 2013). Furthermore, this species may also act as an effective fire retardant for fire-sensitive hillsides (Kleinkopf, 1976). Due to its spreading growth habit and invasive characteristics, it is now widely distributed throughout Australia. It has been recorded in parts of South Australia (Kloot, 1986), New South Wales (Mort, 1949), Western Australia (Hussey *et al*., 2007) and Victoria (Walsh, 1996). Due to its widespread distribution throughout New South Wales and Western Australia, *G. pubescens* is currently listed as a declared weed in these two states (The Department of Economic Development, Jobs, Transport and Resources, 2014).

In Australia *G. pubescens* is commonly found in disturbed arid, coastal and saline areas (Hussey *et al*., 2007). It is clear from the modelled distribution of *G. pubescens* in Australia (Figure 1.3a), that there is a very high potential for the species to establish in coastal areas. This potential distribution was confirmed by Hussey *et al*. (2007) who reported that in Western Australia, *G. pubescens* is widespread along the coast, and is commonly found in areas of disturbed vegetation.

The potential distribution for *G. pubescens* is a function of climate, soil and land use in the state of Victoria (Figure 1.3c). Currently in Victoria, this species is noted in both disturbed and relatively undisturbed areas on the western fringe of Melbourne (Figure 1.3b) (Australia's Virtual Herbarium, 2014). In the Geelong and Melbourne coastal areas, *G. pubescens* frequently occurs in the hinterland, on beach sand, and occasionally in arid zone environments. Furthermore, in the Bacchus Marsh-Melbourne district of western Melbourne, it occurs in both woodland and native grassland vegetation communities.

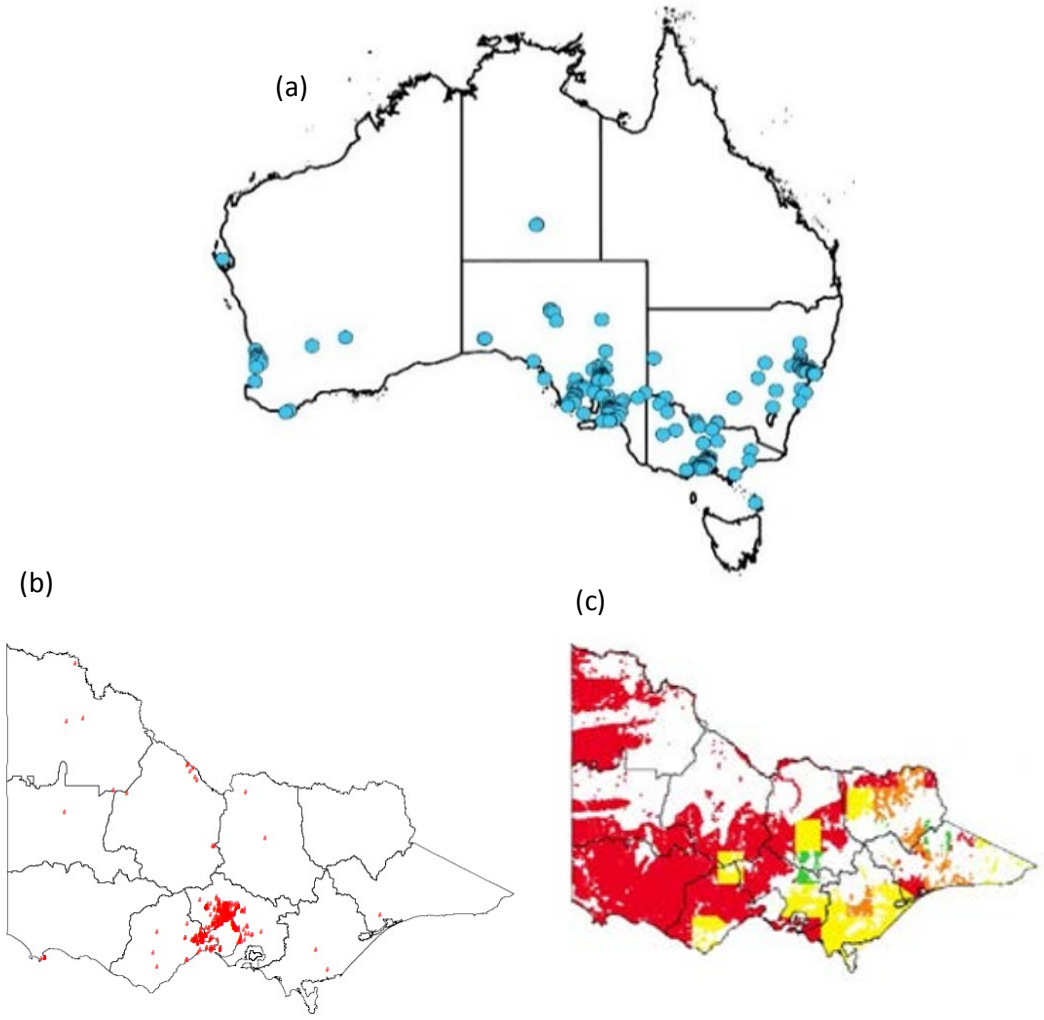

Figure 1.3. The distribution of *Galenia pubescens*: current distribution in Australia (a): blue spots refer to *G. pubescens* distribution. Current distribution in Victoria (b): red spots refer to *G. pubescens* distribution. Potential distribution in Victoria (c): colours indicate probability of *G. pubescens* infesting these areas; Red = very high, Yellow = high, Orange = medium, Green = likely. In the non-coloured areas the plant is unlikely to establish itself (Australia's Virtual Herbarium. 2014).

1.8 The main features that may predict the invasion success of *Galenia pubescens*

The genus *Galenia* was not represented in the native flora of Australia, and the majority of the species of the family Aizoaceae now found in Australia are alien or invasive plants such as *Cryophytum crystallinum* (crystalline), *Litocarpus cordifolius* (heartleaf iceplant) and *Tetragonia implexicoma* (bower spinach) (García-de-Lomas *et al*., 2010; Klak *et al*., 2013). The *Galenia* species have some characteristic features which may give them a competitive advantage when introduced into new environments. The small size of the seeds produced by *G. pubescens* are a feature commonly found in invasive plants (Kolar & Lodge, 2001), and their size may contribute to the plant's rapid spread along transport corridors (Hansen & Clevenger, 2005; Gabbard & Fowler, 2007). Indeed, this phenotypic dissimilarity between native flora and *G. pubescens* may be a key factor that gives it a competitive advantage over native flora, and this property has already been related to invasion success of other exotic plants (Lockwood *et al*., 2001; Ricciardi & Atkinson, 2004), including species such as *Carpobrotus spp*. and *Mesembryanthemum crystallinum*. (Suehs *et al*., 2005).

1.9 The Western Grassland Reserves in Victoria

In 2010, the Victorian Government has set aside 15,000 hectares as a reservation to protect native grasslands in Melbourne's west (Victorian Government Department of Sustainability and Environment, 2011). The natural grasslands are some of the most important areas for grassland preservation in Australia. The grasslands of Victoria's fertile volcanic plain stretch west from Melbourne almost to the South Australian border. They offer a rich agricultural opportunity, and were a historically important inducement for the foundation of Melbourne by graziers in the 1830s (Batman, 1835). As a consequence of agricultural development, at least 95% of the grasslands area

has been cleared. Intact examples are rare, and as a result the survival of many native grassland species is threatened. Lowland grasslands have suffered similar declines around the world, and their conservation is a global challenge (Lunt, 1991; Manel *et al*., 2001; Williams *et al*., 2005).

The establishment of the current reserves is a result of the Victorian Government's decision to expand Melbourne's Urban Growth Boundary (UGB). The expansion will inevitably result in the loss of native vegetation and habitat for native plants and animals, some of which are listed under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). A Strategic Impact Assessment (SIA) on the potential effect of expanding Melbourne's UGB (Faithfull, 2012), conducted by the Victorian Government, and recommended several ways of mitigating the environmental losses, including the establishment of reserves. These reserves are being established outside the UGB, in areas now covered by a Public Acquisition Overlay.

The major mechanism of mitigating losses from within the expanded UGB is termed 'offsetting'. Within this mechanism, these offsets aim to counterbalance biodiversity loss arising from local habitat destruction by enhancing, creating and/or protecting similar but separate habitat (Gordon *et al*., 2011). This policy for native vegetation is occurring under Victoria's existing native vegetation framework (*Victoria's Native Vegetation Management A Framework for Action*, hereafter The *Vegetation Framework*, NRE, 2002). DSE's Native Vegetation Credit Register will administer and track all offset trades utilising the same approach as the established Bush Broker scheme. Other offsets for species habitat will be guided by prescriptions for Matters of National Environmental Significance approved by the Federal Minister for the Environment, Heritage and the Arts as part of the SIA (Faithfull, 2012).

The Western Grassland Reserves accommodate all grassland offsets from clearance within the expanded UGB, and most of the offsets for individual species. Grassland offsets arising elsewhere will be accommodated by alternative means. The grassland reserves (formed due to actual or planned clearing for the expansion of Melbourne) thus form a closed offset system, composed of multiple offset events that are co-located and amalgamated in the reserve areas. The reserves also provide offsets in advance of the clearing. An important purpose of the SIA was to improve outcomes for the environment by planning ahead (Dripps, 2010). Modelling of reservation and offset options supports this approach (Gordon *et al*., 2011).

1.9.1 Relationship between agricultural disturbance and exotic plant invasion

The term 'disturbance' as used in this thesis follows the definition of Van Andel and Van den Bergh (1987), which is: "a change in conditions which interferes with the normal functioning of a given biological system". Due recognition is given to the fact that 'normal' functioning may at times be unknown or poorly described (the ecological history of these grasslands being poorly understood), suggesting that there is a continuing risk of tautology, since a particular change in conditions can only be defined as a disturbance by measuring effects on the system.

Grazing of Australian temperate native grasslands resulted in invasions by exotic plants that were dispersed by livestock and in fodder; these plants were favoured by the types of disturbance associated with hard-hooved ungulates. Numerous studies have demonstrated that exotic plant invasions in Australian temperate grasslands are enhanced by disturbance, including livestock grazing, nutrient enrichment and cultivation (Lunt, 1997). McIntyre and Lavorel (1994a; 1994b), for instance, found that the exotic species richness of the grasslands in the New England Tablelands in NSW was enhanced by anthropogenic water enrichment and soil disturbance. However,

sound generalisations about the relationship between particular disturbances and exotic plant invasions have remained elusive, and there is consensus that at least some weeds can invade without major anthropogenic habitat alteration (D'Antonio *et al*., 1999).

Correlations between weed density on the one hand, and reduction in cover and abundance of a native plant on the other, imply a direct negative interaction. However, the affected species could be reacting in an opposite way to some independent environmental factor or an altered disturbance regime resulting from human activity. It is generally difficult to determine if the invading species or the altered conditions are the cause of such changes (Weiss & Noble, 1984; Huenneke *et al*., 1990; Woods, 1997). If anthropogenic disturbance is the cause, management should be arranged to address the disturbance in the first instance, rather than attempting to directly control the weed.

1.10 The overarching research objective of this study

Gaining more detail about, and insight into, the ecological characteristics of *G. pubescens,* will assist land managers to take suitable control actions for this species at an early rather than late stage in its invasion into new areas. In addition, current management of *G. pubescens* in Australia in infested tracts is aimed at achieving short-term goals; and while this appears to be effective, the gains are only temporary: the long-term success of these approaches is uncertain. Therefore, the overall research objectives of this study are: (i) to better understand the ecology of the species *G. pubescens* and (ii) to explore possible long-term management options via a study of the effects of various control methods in the western Victorian grasslands (iii) and

to test the responses of this species under climate change (especially ones involving an atmospheric CO_2 concentration elevation).

1.11 Research question

To facilitate the development of a novel method to control *G. pubescens* in the Western Grassland Reserves in Victoria and internationally, the following research question has been proposed:

How can we manage or eradicate *Galenia pubescens* in native grasslands?

To help answer this question, the following question groups are proposed:

(i) Ecology

Sub-question group 1. What is the species composition of soil seedbank in areas infested by *G. pubescens* infested areas? Is the species richness and seedbank composition reflected in the above-ground vegetation? Does the seed bank contain similar growth forms to the above-ground vegetation? And in a *G. pubescens* infested area, which species dominate the seedbank? Are these species native or exotic? These questions were investigated in Chapter 2.

Sub-question group 2. What are the influences of various environmental factors on seed germination and seedling emergence of *G. pubescens*? This question is examined in Chapter 3

(ii) Management

Sub-question group 3. What is the most suitable dosage of pine oil to effectively deplete the seedbank of *G. pubescens*? This will be looked at in Chapter 4.

Sub-question group 4. Which control techniques (chemical, pine oil, carbon addition, seeding, or combinations of these) can be observed in the field to be more effective for *G. pubescens* management? This will be the substance of Chapter 5.

Sub-question group 5. Is dose-prescribed fire an effective control technique for reducing the population of *G. pubescens* on degraded ex-grassland reserve within the southeast Werribee-River area, Victoria-Australia? This investigation will be reported in Chapter 6.

(iii) Modelling

(iv) **Sub-question group 6**. How will *G. pubescens* respond to climate change in the future (one involving atmospheric CO_2 concentration elevation and reduced soil moisture availability)? See Chapter 7.

1.12 Significance of this study

The intention of this investigation is to make a contribution to the solution of the significant problem of weed management that is facing those charged with controlling agricultural and environmentally sensitive areas across the world. Although this investigation is focused upon the Western Grasslands of Victoria, Australia, where there is an immediate application of the research outcomes from this study, it is anticipated that they will also inform weed management strategies more broadly in a range of similar environments throughout the world.

The local context of this investigation

The Victorian Government is in the process of making compulsory acquisitions of 15,000 ha of grasslands between Werribee and the You Yangs in the State of Victoria to offset green wedge land sold to developers across Melbourne. Approximately 1,200 ha have been acquired at this stage. The aim is to develop, maintain and preserve indigenous grasslands that are currently one of Australia's most threatened ecosystems (Williams *et al.,* 2005; Gibson-Roy & McDonald, 2014).

Galenia pubescens, being one of the exotic perennial broad leaf species invading this region, is posing a significant threat to the preservation of indigenous grassland, and

acts as a barrier to grassland restoration efforts through its domination of vast stretches of land within this proposed Western Grasslands area. A large financial investment is being made on the future acquisition, maintenance and rehabilitation of these grasslands for the next 40 years. In addition, providing core information about the life cycle and control methods for *G. pubescens* will be important for developing and screening several possible new techniques for the restoration of these grasslands after the removal of *G. pubescens.*

The wider national and international context of this investigation

In Australia, at least 3000 plant species have become 'naturalised' with approximately 450 of these now classified as exotic species (Groves *et al.,* 2005). It is anticipated that the results of this local investigation will contribute to the development of management strategies to control *G. pubescens* in areas of Victoria and Australia where similar concerns about its spread have been raised. In addition, this species is established in other countries outside its natural distribution range, and so the outcomes of this local study may be relevant internationally. It is also anticipated that the results of the management section of this study may assist in the development of control strategies for other weed species with similar genetic and ecological characteristics. For example, *G. pubescens* has a deep tap root that enables it to access ground water under times of moisture stress (Kleinkopf *et al.,* 1976). Other deep-rooted weedy summer-growing perennial species such as *Solanum elaeagnifolium* (silverleaf nightshade) and *Physalis viscosa* (starhair groundcherry) have caused serious pasture and crop damage by diminishing the soil water availability for desirable native species (Arcioni, 2004; Faulkner *et al.,* 2006; Stanton *et al.,* 2011).

The significance of the Galenia pubescens study for weed management

Galenia pubescens is only one of a host of exotic weeds that have become naturalised in the Western Grasslands region. It is particularly aggressive, and previous attempts to remove the weed, and to restore the affected areas with indigenous species, have been unsuccessful. It is the possibility of combining these two approaches of weed removal and native restoration ('bottom up' and 'top down' approaches) that is a key aspect of this investigation. If this dual research objective can be achieved, it will provide significant information to enable the eradication of other exotic species and their replacement with native grassland species. One significant problem which has been identified in this regard is the large and persistent seedbank of perennial exotic weeds which are a major factor restricting the ability to rehabilitate native grasslands, and currently no techniques are available to adequately solve this problem (Meyer *et al*., 1990).

1.13 Thesis structure

This thesis is presented in eight chapters (Figure 1.4). The first (current) chapter has introduced the background, significance and objectives of this study. It has also reviewed the relevant literature on the invasive weed ecology, including concepts and definitions of invasive plant species, characteristics of successful invaders, and global and Australian perspectives of the invasive plant problem. This chapter also reviewed the ecology, population biology and distribution of *G. pubescens*, with the aim of establishing the level of current knowledge available and identifying gaps which might be investigated. Following this introductory chapter, the six subsequent experimental chapters (Chapters 2–7) are presented in order to meet the objectives of the thesis by investigating discrete aspects of the problem. In Chapter 2 the thesis reports on the characterisation of the seedbank and the nature of the standing vegetation found on

degraded ex-grassland reserves from the southeast Werribee-River, Victoria, Australia. Chapter 3 describes a series of individual studies to investigate the effects of various environmental factors on germination and emergence of *G. pubescens* seeds. Chapter 4 presents laboratory and glasshouse trials, carried out to determine the most efficient and cost-effective dosage of pine oil needed to deplete the seedbank of *G. pubescens*. Chapter 5 presents a field experiment in which an area of native grassland might be restored that has been severely disturbed by the removal of native grasses and by external manipulations in the interests of increasing agricultural of fertility, and which has also been heavily invaded by *G. pubescens*. In this study, a number of different control techniques to reduce the population of the mature plants above ground and for destroying the seedbank were investigated. Chapter 6 reports on the effects of prescribed fire and a season of burning on reducing the population of *G. pubescens* on former grassland which is currently part of a reserve. Chapter 7 reported the effects of elevated carbon dioxide and drought on the growth and physiology of *G. pubescens*. Chapter 8, summary and conclusions, provides recommendations for management practices and future research.

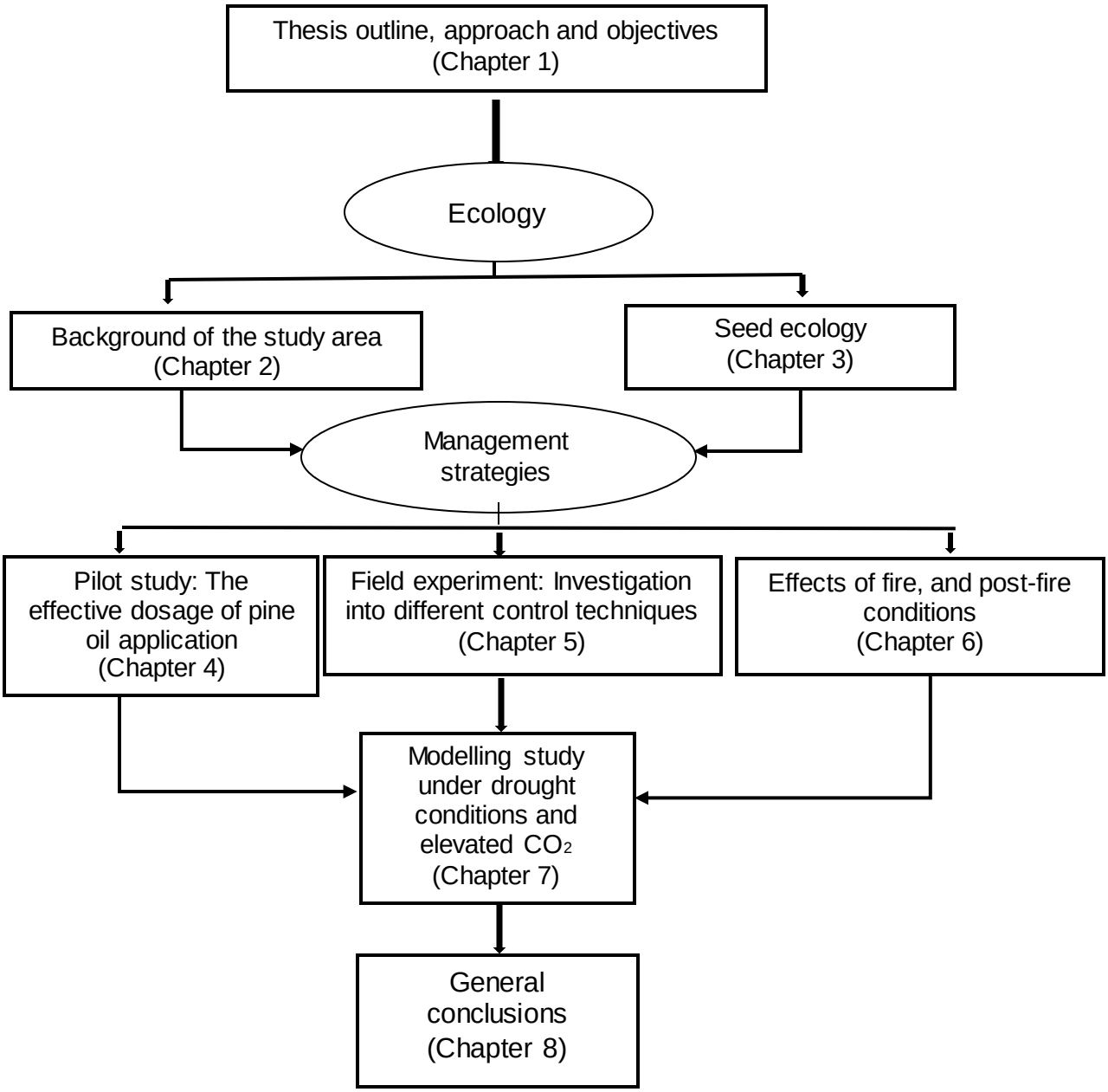

Figure 1.4. Conceptual framework of thesis structure.

Part One

The ecology of *Galenia pubescens*

This part of the thesis consists of two chapters (2 and 3). Chapter 2 reveals the degree of degradation of the site area and describes the composition of the current vegetation (in terms of both percentage cover and contribution to the soil seedbank for all species detected). Chapter 3 describes an investigation of the influence of various environmental factors on the seed germination of *G. pubescens,* which has been the basis of a peer-reviewed publication:

> Mahmood, A. H., Florentine, S., Chauhan, B. S., McLaren, D. A., Palmer, G. C., & Wright, W. (2016). Influence of Various Environmental Factors on Seed Germination and Seedling Emergence of a Noxious Environmental Weed: Green Galenia (*Galenia pubescens***).** *Weed Science* (doi:http://dx.doi.org/10.1614/WS-D-15-00184.1).

A copy of this paper is provided in the Appendix **A** of this thesis.

Chapter 2: Characterization of the Seedbank and Standing Vegetation on a Degraded Ex-grassland Reserve Southeast of the Werribee River, Victoria, Australia

2.1 Summary

Grasslands provide multiple benefits to humans. They are one of the main feed resources for livestock and they are also important for watershed functions, biodiversity, and recreation. The area of grassland that is the focus of this study lies to the south east of the Werribee River in Victoria, Australia. In 2010, this area was acquired by the Victorian Government as a planned reserve of about 15,000 hectares in order to protect the existing native vegetation and, ideally, to return the grasslands to a high quality ecosystem capable of supporting flora and fauna for future generations. It is necessary to ascertain the current ecological condition of these grasslands in order to establish as accurately as possible what obstacles there might be to the achievement of these restoration aims.

A detailed study was therefore made of the seedbank properties and of the aboveground vegetation of a representative section of this grassland. An estimate of the soil seedbank composition was made using the seedling emergence method. The constituent types of aboveground vegetation, and the relative percentage of cover of each constituent type was also measured, and the relationship of these results to the findings of the seedbank study was explored.

A total of 7246 germinants, representing 26 families, 28 genera and 32 species, emerged from 272 soil samples collected from the study site. Aboveground vegetation estimates revealed that the long-lived perennial *G. pubescens* was dominant across all plots. This species was also dominant in the seedbank composition. *G. pubescens*

alone comprised 67.3% of the seedbank composition, while all other weed species comprised 25.7%. Native herbs occupied just 7% of the total seedbank composition. The numbers of seeds germinated from the soil samples in the seedbank and the numbers of plants recorded as standing vegetation were significantly positively correlated for *G. pubescens* but not for other plant groups.

The study thus established that *G. pubescens* was the most dominant species in the selected plots, and that *G. pubescens* is therefore to be considered a major obstacle to the process of restoration. On these grounds, this species was selected as a case study for further investigation into its ecological features and possible management strategies for reducing weed infestation in grasslands. These investigations will be presented in the following chapters.

2.2 Introduction

Grasslands provide multiple benefits to communities and the environment. They support food and fibre production, assist in water collection and reticulation, are a home to a wide range of flora and fauna, and they provide space and facilities for a range of different recreation activities. They are considered particularly important for their environmental significance and thus must be maintained in the context of broader ecosystem management (Galvin *et al*., 2006; Yahdjian *et al*., 2015).

Australian native grasslands have been considerably affected by European farming practices on an increasing scale since European arrival in 1788. For example, the conversion of original grasslands for grazing and cropping purposes has led to many serious environmental changes. Cropping reduces the seedbank composition of native species and often requires the use of specialised fertilisers which advantages the growth of exotic plants. Grazing damages the soil structure, largely through topsoil compaction and over-utilisation, together with the addition of soil nutrients which

disadvantage native species (Kirkham *et al*., 2014). In short, as far as the biodiversity of native grasslands are concerned, so-called 'benign' farming activities have both reduced the natural biodiversity, and have prepared the way for non-native species. In passing, we note that the negative effects of introduced farming techniques were not restricted to Australian grasslands, and these effects will also have had some repercussions on other types of ecosystems across the globe.

Notwithstanding the larger agricultural picture, the disturbance of grassland has long been recognised as a central component shaping ecological systems worldwide (Turner *et al*., 2003). Understanding how the seedbank and vegetation changes in response to disturbance regimes provides an insight into how resilient a community is to perturbations, which mechanisms drive regeneration, and what steps should be taken to restore community composition and structure (Hopfensperger, 2007). One of the most pressing threats to grassland biodiversity is posed by biological invasions by non-native plant species. These invasions have occurred in almost every type of native ecosystem worldwide, and have caused hundreds of native biological extinctions. (Baillie *et al*., 2004; Dobson, 2005).

A major reason for the success of such invasive plant species is their capacity to produce vast numbers of seeds annually, and the fact that these seeds often remain viable for long periods of time. Indeed, grassland seedbanks and their relationships to vegetation have been the subject of much recent attention (Vieira *et al*., 2015). The understanding of the potential of a seedbank to alter grassland composition, its potential for restoring species-rich pastures (McDonald *et al*., 1996) and maintaining floristic diversity (Bakker *et al*., 1991; Willems *et al*., 1993; Amiaud & Touzard, 2004) are some factors that have motivated researchers to compare the composition of the aboveground vegetation with seed reserves hidden in the soil. The presence of seeds

in disturbed habitats is determined by the relationships between the original plant assemblages, propagule production and the seed reserves in the soil (Harvolk *et al*., 2015).

Any management study therefore, should begin with the seedbank composition of the target species, and an adequate knowledge of the plant cover (Grubb, 1977; Wisheu & Keddy, 1991). As a first step in this investigation, a monitoring study was carried out to assess the aboveground vegetative cover and the composition of the soil seedbank in a degraded former agricultural grassland area southeast of the Werribee River in Victoria, Australia**.** The primary aim of this study was to discover which species, if any, dominated the area, and to assess its seedbank composition. The second aim was to determine the relationship, if any, between the dominant aboveground vegetation and its seedbank.

This information will assist in the choice of the most effective control program. The development of such a program will help land managers to act effectively against exotic species, to plan for community responses to disturbances, and restore diversity.

2.3 Materials and methods

2.3.1 Study site

The study was conducted in former agricultural grassland at the Cobbledick's Ford site. Cobbledick's Ford is located on the Werribee River to the south of Mount Cottrell and northwest of Werribee, in southern central Victoria, Australia (37º 49' 5.63" S 144º 34' 58.77"E) at an altitude of 66 m. The study area is 37.8 km southwest of Melbourne's Central Business District (Figure 2.1).

Figure 2.1. Map of Victoria, showing the location of the study area. The red circle (●) marks the site.

2.3.2 Geology

The surface geology of much of the region is dominated by the remnants of Cainozoic volcanism, and there are numerous eruption points indicating the past presence of broad, moderately thick lava flows in the northern, central and western sectors. The most widespread geological material outcropping in the area is the basalt of the Newer Volcanics, ranging from 2.5 million to 5 million years ago. Individual flow thickness varies from 2 metres to over 10 metres, and the entire sequence is, in places, almost 100 metres thick (Condon, 1950*;* Hare & Cas, 2005*).*

2.3.3 Soil and climate conditions

Soils are predominantly yellow-brown clay-loams with shallow rock outcrops. Its composition is pH 6.2, P 9.0 mg/kg, K 521.0 mg/kg with an organic carbon percentage of 2.98. The area has a Mediterranean-type climate with warm to hot and dry summers and cooler winters when most precipitation occurs. The long-term (30 years) annual

average rainfall is 539 mm at the nearest recording station (RAAF (Royal Australian Air Force) Laverton station, 37.86 °S, 144.76 °E, 20 m a.s.l situated 20 km from the study site). The average daily maximum and minimum temperatures are 20.7°C and 10.3°C respectively (Bureau of Meteorology, 2014).

2.3.4 Soil seedbank sampling

To assess the seedbank composition, soil samples were collected at the end of spring, in November 2013, when germination had ended and before any new seeds were dispersed. These soil samples were taken from 64 plots, each being 6 m x 6 m, chosen for their heavy degree of infestation. Within each plot, five soil samples (which included leaf and litter) were randomly collected using a soil corer (10 cm diameter x 5 cm depth), resulting in a total of 64 × 5 = 320 soil samples over a total area of one hectare. Soil samples were placed in individually labelled zip-lock bags, then transferred to a glasshouse situated at Federation University Australia, Mt Helen Campus, in Ballarat, Victoria, for seedbank determination. Prior to the seedbank germination trial, litter and small stones were removed. All of the sieved soil was used for the germination trial, and any leaves found were thoroughly shaken to free all possible seeds (Leckie *et al*., 2000).

The seedling emergence method, being the most frequently used method in soil seedbank studies (Baskin & Baskin, 1998), was used to determine the number of viable seeds in the soil. In the present study, it was assumed that the number of seedlings detected by the seedling emergence method is the number of buried viable seeds, which would indicate the number of readily germinable seeds in the soil. The soil samples were spread out on 2.5 cm thick layers of sterilised sand in plastic seed trays (17 cm x 11 cm x 7 cm), the soil forming an approximate 3.5 cm thick layer in each tray. Four seed trays with sterilised sand only were placed among the sample

trays to test for contamination by local or airborne seeds. No seedlings were found in these control trays during the course of germination trial. The seed sample trays were labelled and placed into larger white butchers' trays (44 cm x 36 cm x 7 cm) to be used as reservoirs to facilitate watering from below, and to ensure minimal disturbance of the samples. The seed trays were kept continuously moist by watering based on need; the moisture content was assessed on a twice-weekly basis. The glasshouse was not equipped with a temperature control mechanism; its ventilation was such that the interior and exterior temperatures were approximately equal.

To avoid differences in light exposure, the position of the trays was changed every 2 weeks. All seed trays were checked each week for seedlings emergence. Newly emerged seedlings were identified, counted and removed. Unidentifiable seedlings were transplanted into pots containing commercially available potting mix and watered regularly; these were grown until species identification was possible (Diaz-Villa *et al*., 2003). Soil samples were maintained and checked for emerging seedlings for approximately six months, as a shorter period of study may have resulted in an underestimation of the persistent seedbank (Baskin & Baskin, 1998). During the experiment period, soil samples were stirred five times to bring any ungerminated seeds to the surface to increase the possibility of seeds being exposed to light. The total number of seedlings that emerged was used as the measure of viable seeds in the seedbank.

2.3.5 Aboveground vegetation sampling

Species composition of vascular plants in the aboveground vegetation was sampled in October, 2013. The same plots were used from which seedbank soil samples were extracted. Plant basal cover and species composition were estimated using the transect line method (Ellenberg & Mueller-Dombois, 1974; Rodriguez & Jacobo,

2010). Within each 6 m x 6 m plot, four 5 m transects were arranged at 1 m intervals across each plot (Figure 2.2). To avoid possible interference from 'edge effects' the first transect line was set out 1 m from the corner posts. Using the point intercept technique, measurements were taken every 20 cm for each transect. This resulted in 25 intercept points per transect and 100 intercept points for each plot. At each intercept point, a record was made regarding whether the ground at that point was: bare ground or rock, covered with litter, or covered by vegetation. Where covered by vegetation, the plant species was recorded.

The point intercept method was also used to characterise percentage cover by species, that is, at each of 100 intercept points within the 64 6 x 6 m plots, a record was made of the species present. This enabled a mean percentage score over all found species across all plots.

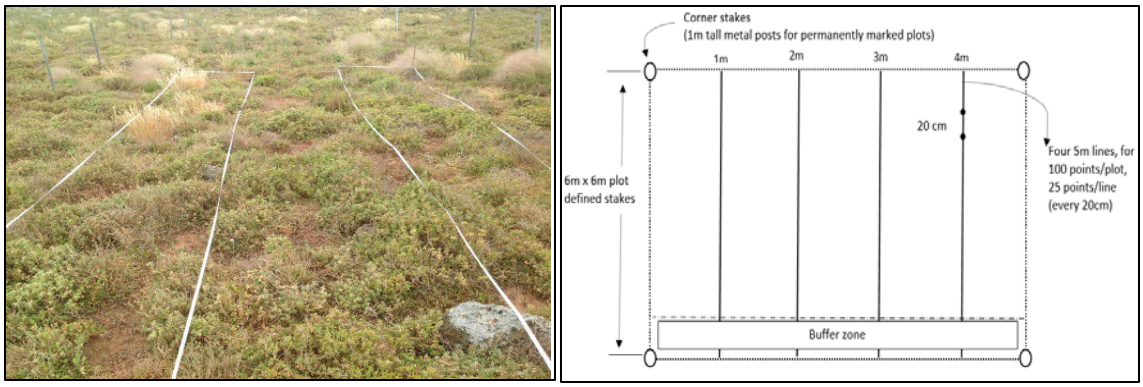

Figure 2.2. 6 m x 6 m plot showing the transect line method for vegetation sampling.

2.4 Statistical analysis

Soil seedbank composition and aboveground vegetation cover was analysed using a one-way analysis of variance (ANOVA) and Duncan's multiple-range test with $p = 0.05$. All analyses were conducted with MINITAB Release 17. Seedbank and aboveground vegetation were assessed for similarity using the Sørensen similarity coefficient to assess similarity of species' composition. The Sorensen similarity coefficient was

calculated as S=2a/(2a+b+c), where **a** is the number of species shared in common between two samples, **b** is the total number of species in the first sample, and **c** is the total number of species found in the second sample (Arroyo *et al.*, 1999).

2.5 Results

2.5.1 Seedbank composition and diversity

A total of 7246 seedlings belonging to 32 plant species, representing 28 genera and 26 families were recorded in seedbank soil samples (Table 2.1). The germinants were categorised into four major plant functional groups: annual grasses, perennial grasses, annual forbs, and perennial forbs. The seedling emergence period varied considerably among these different plant groups. Most of the dicotyledonous plants emerged between the first and the fourth week, followed by grasses whose emergence continued for four months. Stirring of the soil samples in the later stages of the experiment did not result in the germination of more seedlings.

Table 2.1. Seedbank composition and diversity of germinated species from soil samples. Fourteen species (marked *) were found aboveground as well as among the germinated species. The symbols C_3 and C_4 represent the plants' photosynthetic pathways.

Species	Common name	Family		C_3/ C_4	Status	Dicot /Monocot
Annual grasses						
Lolium rigidum	Ray grass	Poaceae	*	C_3	weed	Monocot
Vulpia myuros	Fescue	Poaceae	*	C_3	weed	Monocot
Perennial grasses						
Pennisetum alopecuroides	Foxtail grass	Poaceae		C_4	noxious weed	Monocot
Austrostipa bigeniculata	Stipa	Poaceae		C_4	native	Monocot
Nassella trichotoma	Serrated tussock	Poaceae	*	C_3	noxious weed	Monocot
Cyperus rotundus	Nutsedge	Cyperaceae	*	C_4	weed	Monocot
Annual forbs						
Polycarpon tetraphyllum	four-leaved	Caryophyllaceae	*	C_4	weed	Dicot
Helichrysum luteoalbum	Cudweed	Asteraceae		C_4	weed	Dicot
Sonchus asper	Sow thistle	Asteraceae	*	C_4	weed	Dicot
Sonchus oleraceus	Sow thistle	Asteraceae		C_4	weed	Dicot
Cirsium vulgare	Spear thistle	Asteraceae		C_4	weed	Dicot
Chenopodium pumilio	Goosefoot	Amaranthaceae	*	C_4	native	Dicot
Chenopodium nutans	Saltbush	Amaranthaceae	*	C_4	native	Dicot
Galium aparine	Bedstraw	Rubiaceae		C_4	weed	Dicot
Veronica arvensis	Speedwell	Plantaginaceae		C_4	weed	Dicot
Lythrum hyssopifolia	Loosetrife	Lythraceae		C_4	weed	Dicot
Polygonum aviculare	Knotweed	Polygonaceae		C_4	weed	Dicot
Centaurium erythraea	Centaury	Gentianaceae		C_4	weed	Dicot
Calandrinia eremaea	Centaury	Portulacaceae		C_4	native	Dicot
Medicago polymorpha	Burclover	Fabaceae	*	C_4	weed	Dicot
Crassula decumbens	Rufous crassula	Crassulaceae		C_4	native	Dicot
Crassula sieberiana	Australian stonecrop	Crassulaceae	*	C_4	native	Dicot
Heliotropium europaeum	Heliotrope	Boraginaceae		C_3	weed	Dicot
Oxalis corniculata	wood sorrel	Oxalidaceae	*	C_3	weed	Dicot
Asphodelus fistulosus	Onion weed	Xanthorrhoeaceae	*	C_4	noxious weed	Monocot
Juncus bufonius	Toad rush	Juncaceae		C_4	weed	Monocot
Perennial forbs						
Galenia pubescens	Carpet weed	Aizoaceae	*	C_4	noxious weed	Dicot
Arctotheca calendula	Capeweed	Asteraceae		C_4	weed	Dicot
Trifolium repens	White clover	Fabaceae	*	C_4	weed	Dicot
Verbascum thapsus	Great mullein	Scrophulariaceae		C_3	noxious weed	Dicot
Solanum nigrum	Black nighthade	Solanaceae		C_4	toxic weed	Dicot
Epilobium billardierianum	Willow-herb	Onagraceae		C_4	weed	Dicot
Total number of species						32
Total number of germinants						7246

Of the total 7246 seedlings, annual forbs comprised the largest group (measured by the number of species within each plant category) and contained 20 species, but only 34.4% of the total seedling emergence. Perennial forbs were the next most abundant category among the emergents, making up only six species but comprising 69.5% of the total seedling emergence. Annual grasses and perennial grasses comprised only 4.0% and 2.0% respectively of the total richness of seedling emergents (Table 2.2).

Table 2.2. Numbers of seedlings within each category, and composition percentage of seedlings germinating from the soil seedbank. Percentage composition is shown with mean number ± standard error.

Category	Total number of seedlings in each category	Percentage composition of total number of seedlings
Annual grasses	267	4.1
Perennial grasses	135	2.04
Annual forbs	2269	34.4
Perennial forbs	4585	63.6

The average seed density across all plots in the study site was 159.7 seedlings per square metre, with the dominant families being Aizoaceae, Caryophyllaceae and Asteraceae. The most abundant species in the total seedbank was *Galenia pubescens* (carpet weed) at 273 seedlings per m^2 or 68.2%, followed by *Polycarpon tetraphyllum* (four-leaved) at 175 seedlings per m^2 or 43.75%; *Oxalis corniculata* (wood sorrel) at 85 seedlings per m^2. These three species made up 87.4% of all seeds recorded in the samples (Table 2.3).

Table 2.3. Total number of germinants for each species from soil samples. Percentage composition is shown with mean number ± standard error.

Species	Total number of seedlings in each species	Percentage composition of total number of seedlings
Annual grasses (totals)		
Lolium rigidum	194	2.9 ± 19.7
Vulpia myuros	73	1.1 ± 5.3
Perennial (grasses +sedges) (totals)		
Pennisetum alopecuroides	13	0.1 ± 0.8
Austrostipa bigeniculata	12	0.2 ± 1.9
Nassella trichotoma	76	1.6 ± 5.6
Cyperus rotundus	34	0.5 ± 2.1
Annual forbs (totals)		
Polycarpon tetraphyllum	716	10.8 ± 52.9
Helichrysum luteoalbum	31	0.5 ± 2.1
Sonchus asper	28	0.4 ± 3.8
Sonchus oleraceus	89	1.4 ± 5.5
Cirsium Vulgare	7	0.1 ± 0.9
Chenopodium pumilio	168	2.5 ± 7.3
Chenopodium nutans	17	0.6 ± 1.8
Galium aparine	3	0.1 ± 0.7
Veronica arvensis	2	0.03 ± 0.5
Lythrum hyssopifolia	2	0.03 ± 0.5
polygonum aviculare	6	0.09 ± 0.8
Centaurium erythraea	25	0.4 ± 2.2
Calandrinia eremaea	15	0.2 ± 3.1
Medicago polymorpha	9	0.1 ± 1.03
Crassula decumbens	74	1.1 ± 4.3
Crassula sieberiana	191	2.9 ± 12.7
Heliotropium europaeum	78	1.7 ± 11.4
Oxalis corniculata	610	9.6 ± 18.5
Asphodelus fistulosus	127	1.9 ± 15.1
Juncus bufonius	71	1.07 ± 10.2
Perennial forbs (totals)		
Galenia pubescens	4434	67.3 ± 118.7
Arctotheca calendula	9	0.2 ± 1.6
Trifolium repens	12	0.2 ± 0.8
Verbascum Thapsus	126	1.9 ± 6.2
Solanum nigrum	3	0.04 ± 0.5
Epilobium billardierianum	1	0.01 ± 0.3
Total seedlings germinated	7246	100
No. of species	32	

After separating all observed plant species into 'Australian native' or 'weed species' categories, weeds were found to be the most dominant plant type in the soil seedbank of the study site, comprising 26 species or 93% , while Australian native species (grasses and herbs) comprised a total of six, or 7% (Figure 2.3).

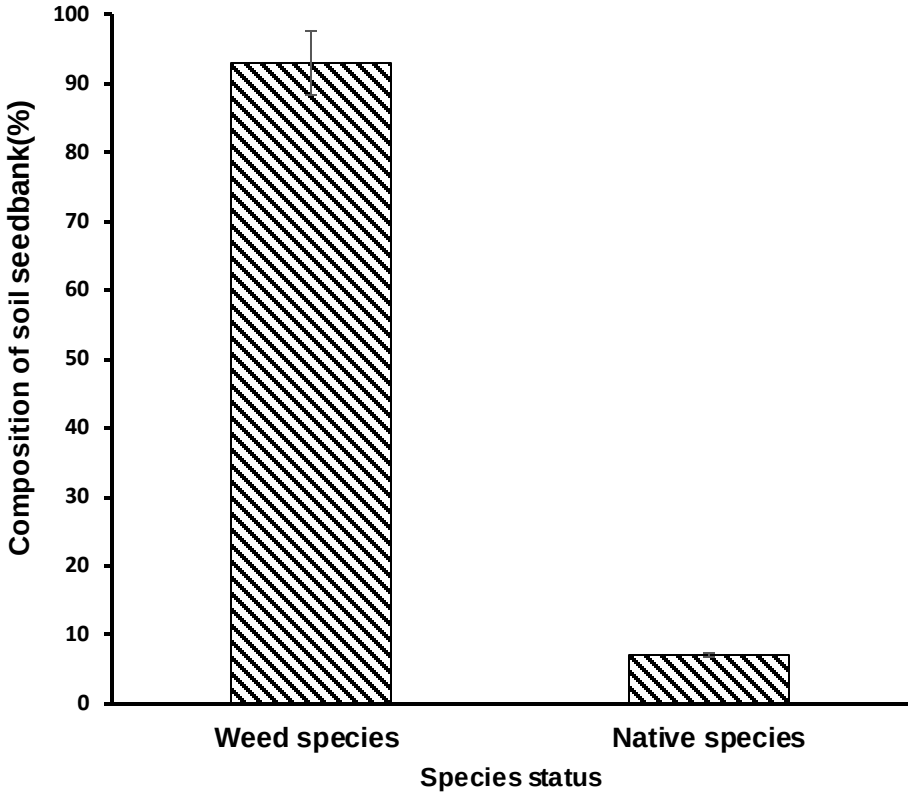

Figure 2.3. Proportion in soil seedbank composition of weed species to native species germinated from all plots. Vertical bars represent ± standard error of the mean.

2.5.2 Aboveground vegetation

Overall percentage cover of vegetation was estimated at 93.2% across the area sampled. A total of 14 species was recorded in the aboveground plant community; of these, three species belonged to the family Poaceae, and the remaining 11 species came from 11 other families (Table 2.4).

Classification of extant aboveground vegetation resulted in the following: annual forbs (seven species; 50% of all discovered species); perennial forbs (3 species; 21.4%); annual grass (two species; 14.3%); perennial grass (two species; 14.3%). Native species represented only 4.5% of the total recorded species, with the remainder being introduced, and these were predominantly classifiable as broadleaf weeds (three species). The most dominant species of weed was *G. pubescens*, which represented 66.4% of the total cover; *Polycarpon tetraphyllum*, gave 9.35% cover and *Vulpia myuros* 5.4% (Table 2.5).

Table 2.4. Identification of aboveground species detected via transect line survey across all selected plots. The symbols C_3 and C_4 represent the plants' photosynthetic pathways.

Species	Common name	Family	C_3/C_4	Status	Dicot / monocot
Annual grasses					
Lolium rigidum	Ray grass	Poaceae	C_3	weed	Monocot
Vulpia myuros	Fescue	Poaceae	C_3	weed	Monocot
Perennial grasses					
Nassella trichotoma	Serrated tussock	Poaceae	C_3	noxious weed	Monocot
Cyperus rotundus	Nutsedge	Cyperaceae	C_4	weed	Monocot
Annual herbs					
Polycarpon tetraphyllum	Four-leaved	Caryophyllaceae	C_4	weed	Dicot
Sonchus asper	Sow thistle	Asteraceae	C_4	weed	Dicot
Chenopodium pumilio	Goosefoot	Amaranthaceae	C_4	native	Dicot
Medicago polymorpha	Burclover	Fabaceae	C_4	weed	Dicot
Crassula sieberiana	Australia stonecrop	Crassulaceae	C_4	native	Dicot
Oxalis corniculata	Wood sorrel	Oxalidaceae	C_3	weed	Dicot
Asphodelus fistulosus	Onion weed	Xanthorrhoeaceae	C_4	noxious weed	Monocot
Perennial forbs					
Galenia pubescens	Carpet weed	Aizoaceae	C_4	noxious weed	Dicot
Trifolium repens	White clover	Fabaceae	C_4	weed	Dicot
Solanum nigrum	Black nighthade	Solanaceae	C_4	toxic weed	Dicot
Total number of species					14

Table 2.5. Mean percentage cover of aboveground vegetation across all selected plots. Percentage cover is shown with mean number ± standard error.

Vegetation cover	Total number of intercept points where each species was detected	Mean percentage cover ± standard error
Galenia pubescens	4221	66.42 ± 1.31
Oxalis corniculata	112	1.75 ± 0.25
Sonchus oleraceus	97	1.51 ± 0.22
Crassula sieberiana	198	3.09 ± 0.47
Vulpia myuros	350	5.46 ± 0.72
Chenopodium pumilio	91	1.42 ± 0.33
Polycarpon tetraphyllum	599	9.35 ± 0.80
Cyperus spp.	31	0.45 ± 0.18
Lolium rigidum	93	1.45 ± 0.41
Nassella trichotoma	73	1.14 ± 0.22
Trifolium repens	25	0.39 ± 0.10
Asphodelus fistulosus	24	0.37 ± 0.10
Verbascum thapsus	26	0.4 ± 0.16
Solanium nigram	3	0.04 ± 0.03
Total number of point intercepts with vegetation cover	**5943**	**93.24 ± 0.38**
Vegetation absent		
Bare soil	226	3.53 ± 0.36
Rock	201	3.14 ± 0.45
Total number of point intercepts without vegetation cover	**727**	**6.67 ± 0.40**

2.5.3 Relationship of soil seedbank composition to aboveground vegetation

The relationship between the composition of the seedbank and the aboveground vegetation was calculated using the Sørensen's similarity coefficient. A total of 32 species germinated from the seedbank, but only 14 species were recorded aboveground (Table 2.1). The only pattern revealed by Sørensen's coefficient values was that seedbanks from all selected plots were generally more similar to aboveground vegetation (66.7%) with regard to non-native species (including *G. pubescens)* while the similarity value for native species was very low.

2.6. Discussion

The above results reveal that in all 64 plots of the study area, weeds were dominant. This was established in three ways: from the seedbank weed composition (93.4% of the seeds that germinated were weed species); from the analysis of species type (a total of 26 weed species were found, but only six native species were present); and from the vegetation cover assessment, which showed that weeds were present at 88.7% of the intercept points. The composition of the germinable seedbank and the aboveground vegetation were moderately similar in terms of their presence at the overall site according to Sørensen's similarity index. All the 14 recorded aboveground species were also represented in the germinated seedlings from the seedbank in the soil samples.

It is clear from these results that the degradation of grasslands at the study area has resulted in, or partly been caused by, weed infestation. Regardless of the cause however, this infestation would appear to be one the key factors to be considered in any future restoration program. In addition, amongst all the weed species, *G. pubescens* was found to be the most dominant; comprising 66% of the seedbank. This species (and no other) was found in all soil samples. It is therefore this species that should be the main target of remedial action.

The rapid adaptation, colonisation and strong competitive ability of *G. pubescens* in highly disturbed (degraded) areas may explain its proliferation at the site. Previous research has suggested that the species diversity of grassland plant communities is significantly reduced by the presence of *G. pubescens* (García-de-Lomas *et al*., 2010), and the present study has demonstrated a correlation between high *G. pubescens* presence and low species diversity across the community. This is in concert with the observation that the successful establishment of native grass species in grasslands is

thought to be limited by the presence of invasive plants (Nyamai *et al.*, 2011). Previous work has found that the overall relative density of native species becomes lowest at high exotic weed infestation levels (Baillie *et al*., 2004; Dobson, 2005). This present study also shows that greater *G. pubescens* presence can negatively affect native species numbers. As stated, the 2013 analysis of the study site confirmed the dominance of *G. pubescens,* and it is expected that the *G. pubescens* management approaches will have significant impacts upon this grassland, with a positive change in vegetation composition (both in the above- and below-ground community) towards the more extensive presence of native species. In conclusion, the data show that the study area is currently most negatively affected by *G. pubescens* colonisation. This plant plays a major role in the suppression of native species, and poses a strong threat to biodiversity. Having established this link, work now needs to be done upon this plant's biology and ecology in order to design more effective and efficient control measures. These matters will be addressed in the following chapters.

Chapter 3: Influence of Various Environmental Factors on Seed Germination and Seedling Emergence of Carpet Weed (*Galenia pubescens*)

3.1 Summary

Galenia pubescens was first recorded in Victoria and New South Wales in the early 1900s and has since become a serious weed threatening temperate Australian indigenous grasslands. Until recently, little was known about its seed ecology, including its germination, seed longevity and seedling emergence under various environmental conditions. Therefore, we investigated the effects of various factors that could affect the *G. pubescens* seedbank in both laboratory and field experiments in order to inform the development of long term management strategies. This study shows that seeds of *G. pubescens* were able to germinate over a broad range of temperatures, but that short bursts (5 minutes) of high temperatures (80 ˚C to 120 ˚C simulating possible exposure to a fire) significantly reduced seed germination. Seed germination was positively favoured by light, indicating that buried seed will remain in a dormant state until disturbed. Field seed burial studies showed that seed viability declined significantly after 30 d. This suggests that, if *G. pubescens* propagules can be properly managed, the viable seedbank is likely to decline. This may contribute to long-term management of *G. pubescens*. Similarly, the emergence of *G. pubescens* seedlings decreased with increases in planting depth. Seedling emergence was greatest (53% of seeds present) for seeds placed on the soil surface and decreased considerably as planting depth increased from 0.5 to 1 cm. No seedlings emerged from seeds placed at depths of 2 to 5 cm. Water stress greatly reduced germination of *G. pubescens* seeds (45% of seeds present in experiments with osmotic potentials below -0.2 MPa). Germination was completely inhibited at water potentials of -0.4 to

-1.0 MPa. Seeds of *G. pubescens* are moderately tolerant to salinity, with over 50% of seeds germinating at low levels of salinity (60 mM NaCl), and moderate germination (48.67%) occurring at 120 mM NaCl. *G. pubescens* can germinate in both alkaline (pH 10 – 83%) and acidic (pH 4 – 80%) conditions suggesting that the seeds are capable of germination and survival in a wide range of environmental conditions. The results of this study may assist in developing tools and strategies for the long term management of this noxious weed in Victoria and other parts of Australia.

3.2 Introduction

Galenia pubescens' control programs have proved to be unsuccessful in the past, primarily due to the extensive distribution of the species, and the abundant production of seeds, which persist in the soil for long periods of time (García-de-Lomas *et al*., 2010). The increasing abundance of this species in temperate native grasslands in Australia is likely to be related, in part, to aggressive seedling emergence and the difficulty associated with reducing the extensive and persistent soil seedbank. This observation implies that this weed will persist in the landscape for many years unless seed reserves can be significantly diminished. It is imperative, therefore, that more detailed investigations of the factors that lead to the reduction of *G. pubescens* seed viability are carried out using localised control and eradication programs in order to assist more confident and effective control implementation.

A variety of environmental factors generally influence seed germination in weeds, including temperature, light, moisture, fire (temperature and smoke), salinity, soil acidity, and depth of seed burial. These factors can, separately or jointly, affect seed germination (Florentine *et al*., 2006; Chauhan *et al*., 2006a; Collin *et al*., 2013; Koutsovoulou *et al*., 2014). Of these, it has been shown that temperature and light are the most important environmental signals regulating seed germination and

subsequent survival and distribution of weed species (Florentine *et al*., 2006, Javaid & Tanveer, 2014). In particular, it has been shown that, when other factors such as moisture, salinity and acidity are not limiting (Martinkova *et al*., 2006), temperature plays a major role in determining the periodicity of seed germination and the distribution of species (Baskin & Baskin, 1998). Temperature can also affect the percentage and rate of germination through its effects on seed deterioration, loss of dormancy and by affecting the direct germination process itself (Kebreab & Murdoch, 1999).

Light is also an important requirement for germination for some environmental weeds, although this varies between species. Some are insensitive to changes in light levels whilst others are inhibited by any exposure to light during germination (Carta *et al*., 2014). It has also been shown that the response of seeds to light can control the timing of germination, and this can be a crucial factor in the survival of the resulting seedlings and their growth and fitness in subsequent life stages (Pons, 2000). These differences to exposure to light for many species from different habitats can be understood, because research has indicated that responses to light are mediated by phytochrome perception of the light environment (Lindig-Cisneros & Zedler, 2001), and these levels of perception vary widely for exotic species.

In Australia, surface fire has been used for many years as one of the options to control invasive species. In particular, prescribed fire events have been widely used to manage weed infestation generally across Australian grasslands (Morgan, 1999; 2001; Russell-Smith *et al*., 2000; Goergen *et al*., 2006) and so it was thought that fire might be an important management tool for containing *G. pubescens* and thus maintaining healthy grasslands on the Victorian Volcanic Plains (Morgan, 1999). However, because fire increases the surface temperature of the soil matrix and

produces significant levels of smoke, it is also thought that these two components, either separately or together, may actually serve as germination cues and/or significantly affect the activity of weed seedbanks (Tieu *et al*., 2001; Gashaw & Michelsen, 2002; Clarke & French, 2005; Wright & Clarke, 2009). In this respect, smoke has been widely acknowledged as a key agent in the release of seed dormancy and may positively interact with heat to promote this release (Smith *et al*., 1999). De Lange and Boucher (1990) investigated plant-derived smoke as a germination cue and found that smoke products can stimulate germination in many South African species. In addition, seed morphology may facilitate germination after fire because seeds are more likely to survive when buried in the soil. Therefore, it is essential to understand the effect of heat and smoke on *G. pubescens* before applying fire control techniques.

Depth and duration of burial also have an impact on the germination, dormancy and the viability of seeds. It has been shown that germination requirements generally become less specific after a period of seed dry storage (El-Keblawy, 2014; Bebawi *et al*., 2015) which may occur when seeds are buried in dry soil. In addition, the nutrients for seed germination at various soil depths have significant influence on germination (Grundy *et al*., 1996). Understanding the factors that relate to seed burial and survival are essential, because normally only the seeds close to the soil surface are able to germinate and emerge (Grundy *et al*., 2003).

Moisture stress and salinity also affect seed germination. Water is a basic requirement for germination, because it is essential for enzyme activation, breakdown, translocation, and use of reserve storage material (Beyranvand *et al*., 2013). Water limitation may delay, reduce or prevent germination and later have an effect on the growth of the plant (Javaid & Tanveer, 2014). Conditions of moisture stress may

enable some weeds to take advantage of limitations in the germination or growth of other species. Related to this, it has been shown that salinity stress affects seed germination either through dehydrating osmotic effects, by biologically preventing or delaying germination, or through ion toxicity, which can render the seeds unviable (Huang & Redmann, 1995; Guan *et al*., 2009).

Seed germination can also be affected by soil pH levels. Whilst the response of plants to different pH values varies among species, many plants can only tolerate a pH range in their environment between 5 and 10. Beyond this range, high concentrations of ions can be directly toxic to plants. Recent work has shown, however, that there are some weeds that may germinate over a broad range of pH values. For example, seeds of *Senna obtusifolia* (sicklepod) germinated at pH 3 to 10 (Norsworthy & Oliveira, 2006), whilst seeds of *Eclipta prostrate* (eclipta) and *Emex spinosa* (spiny emex) can germinate over a range of pH 4 to 10 (Chauhan & Johnson, 2008; Javaid &Tanveer, 2014).

Although it is known that *G. pubescens* is spread mainly by seeds, there has, until now, been little understanding of the environmental factors that affect the seed germination of this species in Australia. A greater understanding of the effect of environmental factors on seed germination in *G. pubescens* is needed to help to develop suitable and cost effective weed control options (Collin *et al*., 2013; Koutsovoulou *et al*., 2014). Further, information about how these factors affect germination of this species may help to prevent its invasion of new areas. Results may also assist in the development of control strategies for other weed species with similar ecological characteristics. Therefore, the objectives of this study were: (i) to investigate the effects of temperature, light, heat, smoke, burial depth, water stress, salinity and

pH on germination and seedling emergence, and (ii) to examine the seed viability of *G. pubescens* under field conditions.

3.3 Materials and methods

3.3.1 Seed collection and processing

Mature seeds of *G. pubescens* were collected from over 300 mature plants in the Werribee region (37º 49' 5.63" S 144º 34' 58.77" E), at an altitude of 66 m, near Ballan Road, southeast of the Werribee River, Victoria, Australia during October 2013. Seeds were considered matured when the capsule turned brown. Uncleaned seeds were placed in a labelled bag and taken to the Mt Helen Campus of Federation University Australia, and stored at room temperature (16 to 27 °C) and at relative humidity from 30 to 50% until use.

Seeds of *G. pubescens* were surface-sterilised by rinsing in 1% w/v sodium hypochlorite for 1 min, and then washed clean with double-distilled water before the start of each germination trial. Germination tests were conducted by placing 25 seeds evenly in a 9 cm diameter Petri dish lined with Whatman® No.10 filter papers, and moistened with 5 ml sterile distilled water or a treatment solution to ensure adequate moisture for the seeds. The Petri dishes containing the seeds were then placed in seed germinating cabinets (Thermoline Scientific and Humidity Cabinet, TRISLH-495-1-SD, Vol. 240, Australia), which were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux of 40 µmol $m^{-2}s^{-1}$. Daily observations were taken for seed germination for a period of 30 d from the date of sowing. Seeds were considered germinated when the radicle was approximately 2 mm long and cotyledons had emerged from the seed coat (Ferrari & Parera, 2015). At the conclusion of the germination test, non-germinated seeds were checked for viability using the 2,3,5-

triphenyltetrazolium chloride (TTC) test (Waes & Debergh, 1986; Saatkamp *et al*., 2011).

3.3.2 Effects of temperature regime and photoperiod

The effects of temperature on seed germination were investigated by exposing the seeds to three alternating temperature regimes (17/7, 20/10, and 30/20 °C) under two photoperiods of 12/12 h light/dark and 24 h of continuous darkness. Four replicates of 25 seeds of each treatment were used. These temperature regimes were selected to reflect temperature variations during the period of spring to winter in Victoria. The dark treatment was achieved by covering Petri dishes with aluminium foil.

3.3.3 Effects of heat and smoke water

In order to examine the effects of fire on seed germination, a laboratory experiment was established with four levels of heat and two concentrations (0 and 10%) of smoke water. For the heat treatments, seeds were exposed for: (i) 17/7°C [control treatment (no heat); this temperature regime was found optimum in the previous trial on the temperature regime and photoperiods for germination of *G. pubescens* seeds], (ii) 40 °C (simulating a hot day in summer), (iii) 80 °C (simulating a cool burn), and (iv) 120 °C (simulating heat from an hot burn). To apply heat shock, seeds were treated at the desired temperature for 5 min in a preheated convection oven and then removed to room temperature for cooling. Seeds in the unheated control were not placed into the oven.

Smoke treatments were conducted using commercially available aqueous smoke water, which is produced by Grayson Australia, a smoke-flavoring company, by burning eucalyptus wood and concentrating the smoke in aqueous solution (Read *et al*., 2000). A 10% solution of this smoke water was used (after Tieu *et al*., 2001). Subsequent to the heat treatment, one half of the heated seeds were soaked in 25 ml

of distilled water, and the other half was soaked in 25 ml of 10% smoke water solution for 20 h at room temperature. Then four replicates of 25 seeds of each treatment were incubated at 17/7 °C temperature under 12:12 h light and dark photoperiods. Seeds were checked daily for physical changes and the matrix moistened when necessary.

3.3.4 Effects of seed age and field burial on seed germination

To examine the seed viability of *G. pubescens* under field conditions, a 1-year seed burial experiment was established at the ex-grassland reserve near southeast Werribee-River (37^{O} 82', 144^{O} 57'), Victoria, Australia during December 2013. This site was heavily infested with *G. pubescens.* A total of 24 stainless steel mesh envelopes (135 by 95 mm) were made to carry out this experiment. These permeable metal mesh envelopes were used to create conditions close to natural soil conditions (water and air diffusion and microorganisms). Each envelope contained 25 firm cleaned seeds of *G. pubescens,* and was buried in the field and covered with 4 cm of surface soil. The site of each buried envelope was marked with a metal picket and recorded with GPS for easy relocation. Four seed envelopes were exhumed every three months (from 4th January 2014 to 4th January 2015) for viability testing. Control seeds were kept at the room temperature and four replicates of 25 seeds of six age groups (0, 1, 4, 7, 10, and 13 months) were tested for both germinability and viability. Seeds of one to 13 months were kept in dry storage (17 °C) following collection; then, every three months, seeds were exposed to the same germination and viability tests as described in the previous experiment.

3.3.5 Effects of depth on seedling emergence

The impact of seed burial depth on seedling emergence was examined. Soil used for this trial was collected from the study site and sterilised to kill any viable seeds. For

soil moisture movement and aeration, sterilised sandy soil was placed to a depth of 1 cm at the base of each plastic pot (10 cm x 6 cm x 6 cm) which was lined with paper towel and then filled with the sterilised field collected soil. Fifty seeds of *G. pubescens* were placed at 0, 0.5, 1, 2, 3, 4, and 5 cm depths of the sterilised field collected soil, using three replicates for each depth in separate punnets. Punnets were individually labelled with the sample number and placed into large butcher trays (28 cm long x 44 cm wide x 5.5 cm high) to facilitate watering from below to ensure minimal disturbance of seed samples. The positions of punnets within the glasshouse were changed every second week to reduce environmental bias. Trays were regularly placed in water so that soil was moist but not flooded. All the trays were placed in a growth chamber set at 17/7 °C temperature under 12/12 light and dark photoperiods. A seed or seedling was considered to have germinated or emerged when the protruding radicle achieved the length of 2 mm beyond the seed coat (Amri, 2010). Daily observations and counts were made of seedling emergence, with the final counts being made two months after the last emergence of new seedlings.

3.3.6 Effect of salinity stress on seed germination

Sodium chloride (NaCl) treatments were carried out, in which 10 ml aliquots of the treatment solution were applied directly to 25 seeds on each Petri dish. NaCl solutions were applied at 0%, 0.01%, 0.05%, 0.25%, 1.25% 2.5%, and 6.0% concentrations. All 10 ml aliquots were dripped directly onto the seeds. Petri dishes were incubated at 17/7 °C (17 °C during day light and 7 °C during night time) temperatures under a 12/12 h light/dark (cool white Philips 18 W/840 at 60 µmol m^{-2}) lighting regime. Treatments were replicated three times and maintained in a constant temperature cabinet, with Petri dishes being rotated within the cabinet every second day. This range of NaCl treatments was selected to reflect the level of salinity occurring in typical Australian

disturbed soils (Chauhan *et al*., 2006a). Germination of *G. pubescens* seeds were determined for a period of 30 days.

3.3.7 Effect of osmotic stress on seed germination

To investigate the effect of osmotic stress on seed germination, *G. pubescens* seeds were tested for germination in aqueous solutions of polyethylene glycol (PEG) with an average molecular weight of 8000, prepared to obtain osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa by dissolving 0, 91.6, 129.5, 183.1, 224.2, 258.9, and 289.8 g, respectively, of polyethylene glycol 8000 (Sigma-Aldrich Co., 3050 Spruce St., Louis, MO 63103) in 1 L of distilled water (Michel, 1983). Three replicates of 25 seeds of *G. pubescens*, at each level of PEG, were incubated at 17/7 °C (day/ night) under 12/12 h light and dark. Germination of *G. pubescens* seeds was determined for a period of 30 days.

3.3.8 Effect of pH on seed germination

The effect of pH on seed germination was examined by using buffer solutions with pH values 4, 5, and 6 (to simulate an acidic medium), 7 (neutral), and 8, 9, and 10 (to simulate an alkaline medium) with distilled water (pH 6.3) as a control. The solutions were prepared according to the method as described by Chauhan *et al*., (2006a, b) using potassium hydrogen phthalate, and were adjusted to pH 4 with 1 N hydrogen chloride (HCl). A 2 mM solution of MES [2-(N-morpholino) ethanesulfonic acid] was adjusted to pH 5 and 6 with 1 N sodium hydroxide (NaOH). A 2- mM solution of HEPES [N- (2-hydroxymethyl) piperazine- N- (2-ethanesulfonic acid)] was adjusted to pH 7 and 8 with 1 N NaOH. The pH 9 and 10 buffers were prepared with 2-mM tricine [N-Tris (hydroxmethyl) methylglycine] and adjusted with 1 N NaOH. Petri dishes were incubated at 17/7 °C (day/ night) temperature cycles under 12/12 h light and dark photoperiods as described earlier. pH treatments were applied, using approximately 5

ml aliquots of the treatment solution, directly to 25 seeds on each Petri dish. All aliquots were dripped directly onto the seeds. Petri dishes were incubated at 17/7 °C alternating temperature and 12/12 h light/dark (cool white Philips 18 W/840 at 60 µmol m^{-2}) lighting. Treatments were replicated three times and maintained in a constant temperature cabinet (T), with Petri dishes being rotated within the cabinet every second day. Germination of *G. pubescens* seeds were determined for a period of 30 days.

3.4 Statistical analyses

At the end of these trials, the final germination percentage (FG%), viability adjusted germination (VAG%), mean germination time (MGT), time to 50% germination (T_{50}), time to start germination (TSG), and germination index (GI), were calculated using the formulas below:

Final germination percentage (*FG %):*

$$FG\% = \frac{SG}{TS} \times 100 \qquad [1]$$

where SG is the total number of seeds germinated and TS is the total number of seeds planted (Wang *et al*., 2009).

The viability adjusted germination:

$$VAG = \frac{germination\%}{viability\%} \times 100 \qquad [2]$$

where *germination %* is the final germination percentage and *viability %* is the percentage of alive seeds with (TTC) (Jefferson *et al*., 2008)

Mean germination time (MGT):

Mean germination time was calculated according to Salehzade *et al*. (2009):

$$MGT = \frac{\sum Dn}{\sum n} \quad [3]$$

where n is the number of seeds that were germinated on day D and D is the number of days counted from the beginning of germination.

Time to 50% germination (T_{50}):

The time to 50% germination (T_{50}) was calculated according to Coolbear *et al*., (1984), as modified by Farooq *et al*., (2005):

$$T_{50} = ti + \frac{\left(\frac{N}{2} - ni\right)(tj - ti)}{nj - ni} \quad [4]$$

where N is the final number of seeds that have germinated and n_i, and n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Time to start germination (TSG):

Number of days from seed sowing to first germination (Bu *et al*., 2007).

Germination index (GI):

Germination index (GI) was calculated as described by Bu *et al*., (2007); Wu and Du (2007) using the following equation.

$$GI = (\sum_i (60 - i)x\ Gi)x100\ /(60\ xGN) \quad [5]$$

where *i* = number of days since the day of sowing, Gi = number of seeds germinated on day *i*, and *GN* = total number of germinated seeds.

Germination (%) values at different concentrations of NaCl and osmotic potential were fitted to a three-parameter logistic model using SigmaPlot 2013 (version 13). The model fitted was

$$G(\%) = G_{max}/[1 + (x/x_{50})g] \quad [6]$$

where G is the total germination (%) at concentration x, G_{max} is the maximum germination (%), x_{50} is the NaCl concentrations or osmotic potential for 50% inhibition of the maximum germination and g indicates the slope.

A three-parameter logistic model:

$$E(\%) = E_{max}/[1 + (x/x_{50})e] \quad [7]$$

was fitted to the seedling emergence (%) obtained at different burial depths, where E represents the seedling emergence (%) at burial depth x, E_{max} is the maximum seedling emergence, and e indicates the slope.

All the experiments were repeated (except for the seed age and field burial experiment) and the data were combined from the two repeated experiments. One-way analysis of Variance (ANOVA) was conducted using the SuperANOVA software program (*Abacus Concepts Inc* in Berkeley, California). Residual plots of final germination percentage data were transformed to arcsin as appropriate and re-analysed. The data presented here are uniform means. Means were compared using Tukey's HSD comparison (Day & Quinn, 1989).

3.5 Results

3.5.1 Effects of temperature regime and photoperiod

The effects of three alternating temperature regimes (17/7, 25/10, and 30/20 °C) under two photoperiods of 12/12 hours light/dark and 24 hours of continuous darkness on germination of *G. pubescens* seeds are presented in Figure 3.1. A two-way ANOVA showed that only the light regime had a significant effect on the VAG ($F=76.580$, $P<0.01$), while no significant effect was detected for temperature ($F=1.900$, $P=0.350$)

or any interaction between light and temperature regimes (F=2.720, P=0.091). The data were therefore pooled to perform a one-way ANOVA. A significant difference (F=65.690, P=0.000) was detected between 12/12 hours light/dark and 24 h dark conditions. This factor was found to explain a substantial part of the overall difference in germination (R^2=74.91). Seeds of *G. pubescens* germinated at all temperatures tested with 86, 78 and 81% germination at 17/7, 20/10, and 30/20 °C, respectively. Germination of 34, 51 and 30% of seeds was observed at temperature regimes of 17/7, 20/10 and 30/20 °C, respectively under the 24 hours dark cycle (Figure 3.1).

Greater than 90% of seeds of *G. pubescens* were viable for all temperature regimes when the photoperiod was 12/12 h light/dark (Table 3.1). Time to achieve 50% germination of *G. pubescens* seeds decreased with increasing temperature when seeds were exposed to continuous darkness. More specifically, T_{50} values were 9.5 and 10.6 days respectively, at a temperature regime of 17/7 C with 12/12 hours light/dark and 24-hours dark cycles. The lowest T_{50} time to germination (2.8 days) was recorded at 24 hours dark, in 30/20 °C (Table 3.1).

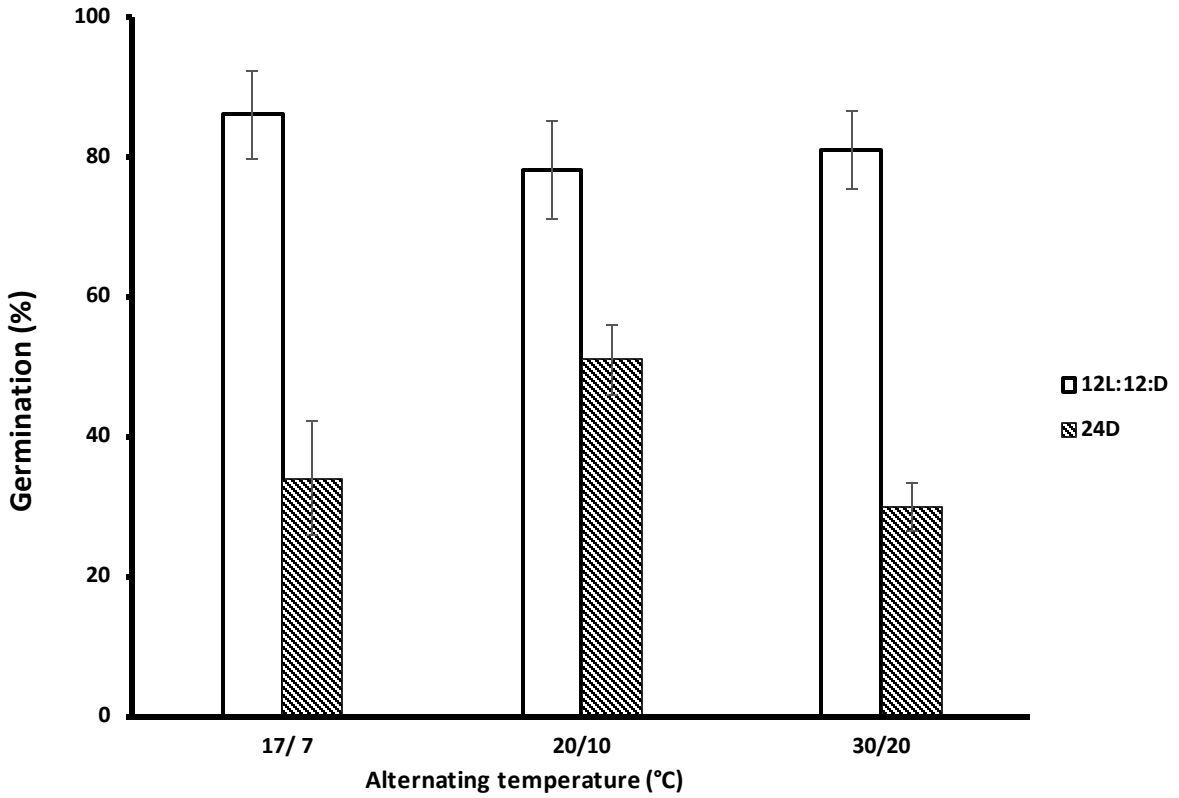

Figure 3.1. The effects of alternating temperature and photoperiod regimes on the germination of *Galenia pubescens* seeds. Vertical bars represent ± standard error of the mean.

The mean period of time (MGT) to reach the final germination percentage of the seeds decreased with increasing temperatures, whereas the photoperiod did not have a significant effect (P>0.05). In particular, the longest period of time for a final germination seed value occurred at a temperature regime of 17/7 °C (9.5 days for 12:12 light: dark cycle and 10.9 days for a 24 hour dark cycle), while the shortest mean germination time occurred at 30/20 °C in both photoperiod regimes; these values were 3.8 d in light/dark and 3.6 days in darkness (Table 3.1). Time to start (TSG) *G. pubescens* seed germination was lowest at 30/20 °C (1.0 days), when the photoperiod was 12/12 hours light/dark, whereas the maximum GI was recorded at 17/7 °C under 24-hours continuous darkness.

Table 3.1. Effects of temperature and light treatments on germination of *Galenia pubescens* seeds. Comparisons between temperature treatments are shown within columns. VAG %, viability adjusted germination; MGT, mean germination time; T_{50}, time to 50% germination; TSG, time to start germination; GI, germination index. L/D = 12 hr of Light and 12: hr of Dark, D = 24 hr Dark.

Treatments		***VAG %***	***MGT (d)***	***T_{50} (d)***	***TSG (d)***	***GI***
Alternating temperature (°C)	**Light**					
17/7	**12:12 L/D**	91.3	9.5	9.5	5.7	5.7
	24 D	38.7	10.9	10.6	8.3	8.3
20/10	**12:12 L/D**	94.1	5.7	5.2	4.0	4.0
	24 D	61.1	6.4	5.1	4.0	4.0
30/20	**12:12 L/D**	92.3	3.9	2.9	1.0	2.0
	24 D	44.4	3.6	2.8	2.0	3.0

3.5.2 Effects of heat and smoke water

The final percentage of germination for the control seeds not exposed to elevated temperature and heat plus smoke, was 86% ± 4.81SE (Figure 3.2a). In both treatments, (i) elevated temperature and heat plus smoke with increasing temperature and (ii) increasing temperature and applying smoke water, reduced the final germination percentage. In particular, when the seeds were exposed to both increasing temperature and smoke water, the final germination percentage decreased dramatically. The interaction between heat and smoke water significantly (P<0.05) reduced germination and viability except at 40 °C (Figure 3.2a, b).

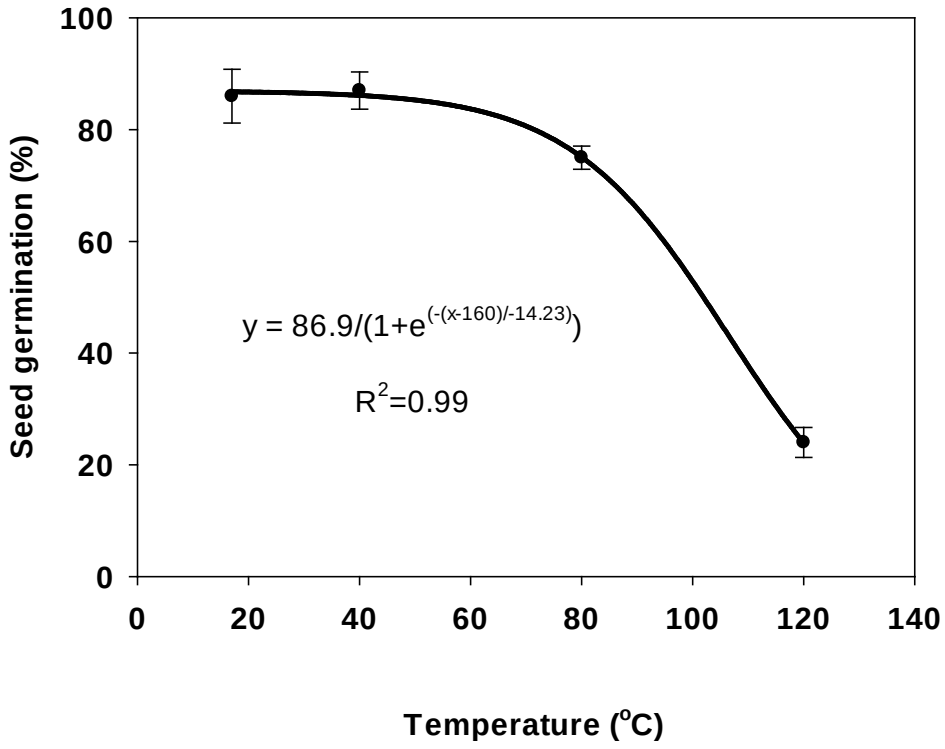

Figure 3.2a. Effects of temperature on seedling emergence of *Galenia pubescens*. The bold line represents Sigmoid, three-parameter fitted to the data. Vertical bars represent ± standard error of the mean.

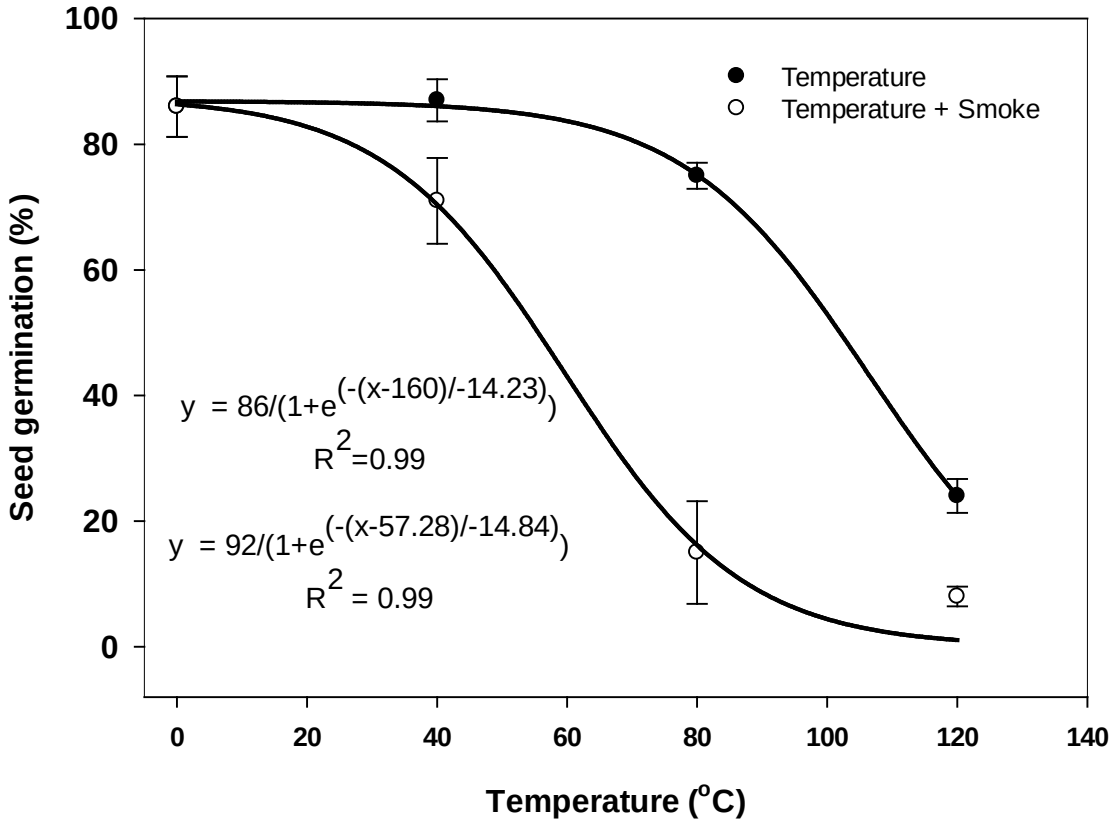

Figure 3.2b. Effects of temperature and smoke on seedling emergence of *Galenia pubescens*. The bold line represents Sigmoid, three-parameter fitted to the data. Vertical bars represent ± standard error of the mean.

A three-parameter sigmoid model {G (%) = 86.87/[1+$e^{(-(x106.32)/-14.17)}$] R^2 = 0.99} was fitted to examine the final germination (%) when seeds were exposed to different temperature regimes and subsequently placed in the 17/7 °C germination incubator (Figure 3.2a, b). *G. pubescens* seed germination was reduced from 86% to 24% when the temperature was increased to 120 °C. It is important to notice that just over 24% seeds were viable after exposed to a five minute burst of heat at 120 °C.

A three-parameter sigmoid model, {G (%) = 87.51/[1+$e^{(-(x59.51)/-13.79)}$] R^2 = 0.99} was fitted to the germination values (%) observed when the seeds were exposed to similar

heat and 10% v/v smoke water (Figure 3.2b). The seed germination (%) was reduced from 87 % to 8 % when exposed to both 120 °C heat and smoke water. The decline in seed germination % due to the smoke water was very significant (F=18.76; P=0.001) (Figure 3.2b).

3.5.3 Effects of seed age and field burial on seed germination

The overall germination percentage of the seeds declined with duration of burial compared with (control) seeds that were not buried (Figure 3.3). The germination of fresh seeds before burial was over 90%. This was reduced to less than 50% in the first 30 days of burial (4 cm) and to 39% after one year. In contrast, for seeds stored in dry conditions (not buried), the germination was 87% in the first 30 d and remained at this percentage for up to one year. The outcomes of this study indicate that, after one year of burial, the median proportion of buried viable, non-germinated seeds ranged from 35% to 55% for *G. pubescens*.

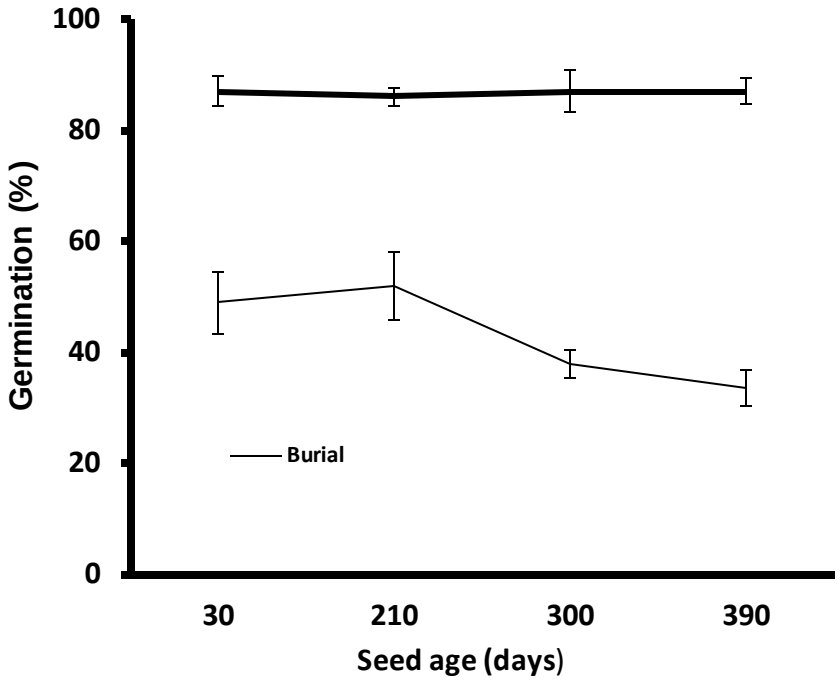

Figure 3.3. Effects of dry storage and field burial on germination of *Galenia pubescens* seeds. Vertical bars represent ± standard error of the mean.

3.5.4 Effects of depth on seedling emergence

Seedling emergence of *G. pubescens* was influenced by planting depth (Figure 3. 4), and our experiments showed that seedling emergence of *G. pubescens* decreased with increased planting depth. Seedling emergence was greatest (53%) for seeds placed on the soil surface and considerably decreased as planting depth increased from 0.5 to 1 cm, with no seedlings emerging from seeds placed at depths of 2 cm or greater.

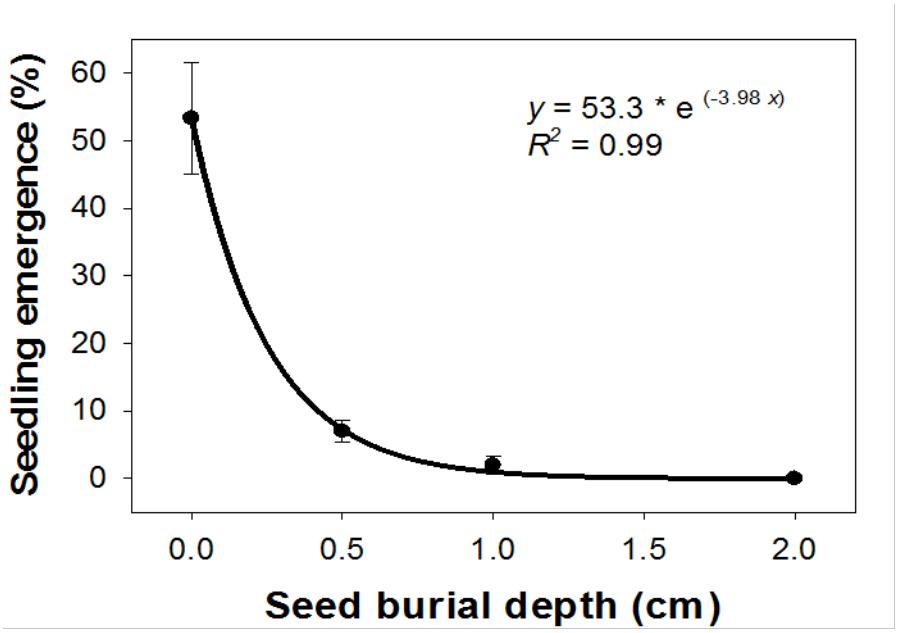

Figure 3.4. Effects of burial depth on seedling emergence of *Galenia pubescens*. The bold line represents an exponential decay, single, two-parameter fitted to the data. Vertical bars represent ± standard error of the mean.

3.5.5 Effect of salinity stress on seed germination

A three-parameter logistic model {G (%) = 71.08/ [1 + ($e^{-(x-133)/-13.6}$)], R^2 = 0.91} was fitted to the germination values at different concentrations of NaCl for *G. pubescens* (Figure 3. 5). Data show that the highest germination (82.7%) occurred in the control treatment (no stress) and germination reduced to 71.3% and 54.7% with increasing concentrations of NaCl to 10 mM and 60 mM, respectively. Germination was less than

50% at 120 mM NaCl and it was completely inhibited at 180 mM or higher concentrations of NaCl. The concentration of NaCl required for 50% inhibition of the maximum germination, estimated from the fitted model, was 115.2 mM.

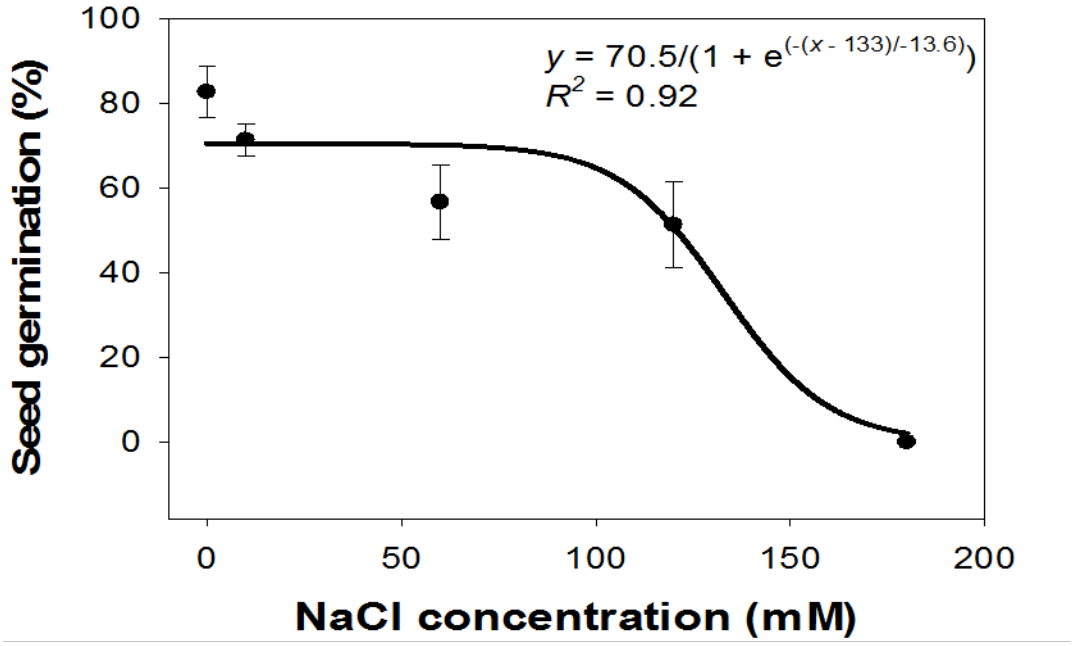

Figure 3.5. Effect of sodium chloride (NaCl) concentrations on seed germination of *Galenia pubescens*. The bold line represents a three-parameter logistic model fitted to the data. Vertical bars represent ± standard error of the mean.

3.5.6 Effects of osmotic stress on seed germination

The percentage of seed germination at different osmotic potentials is presented in Figure 3.6. A three-parameter logistic model {G (%) = 82.1/ ($e^{(x/-21)/0.03}$], R^2 = 0.99} was fitted to germination percentages. Germination of *G. pubescens* decreased from 82% to 45% as osmotic potential decreased from 0 to -0.2 MPa. Germination was completely inhibited at an osmotic potential of -0.4 MPa or lower. However, more than 45% of seeds germinated at an osmotic potential of -0.2 MPa, indicating that some seeds of *G. pubescens* can germinate under marginal water stress conditions.

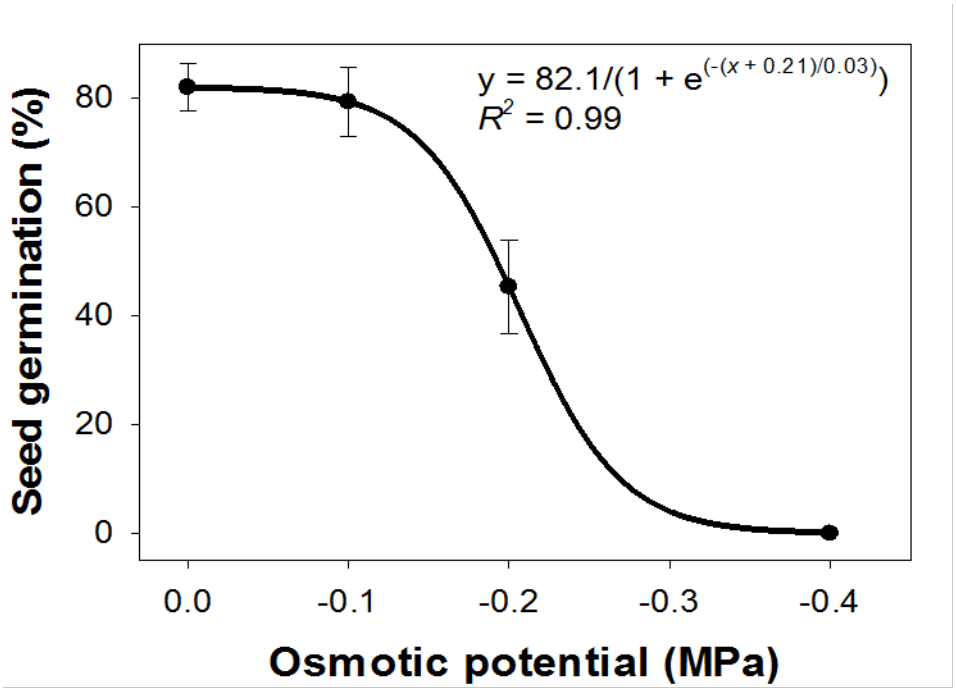

Figure 3.6. Effect of osmotic potential on seed germination of *Galenia pubescens*. The bold line represents a three parameter logistic model fitted to the data. Vertical bars represent ± standard error of the mean.

3.5.7 Effects of pH on seed germination

Galenia pubescens seeds had greater than 70% germination over a pH range of 4 to 10 (Figure 3.7). The highest percentages of final germination was 83% at pH 10 (alkaline medium) and 80% at pH 4 (acidic medium). This indicates that pH is not likely to be a limiting factor for germination of *G. pubescens* in most soil types in agricultural or non-agricultural areas of Australia.

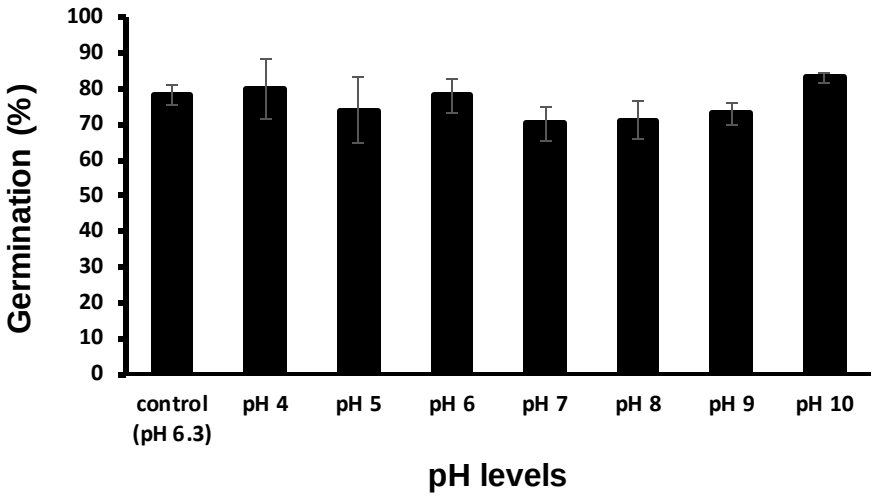

Figure 3.7. Effect of pH solutions on seed germination of *Galenia pubescens* seeds. Vertical bars represent ± standard error of the mean.

3.6 Discussion

This study clearly shows that light is a key factor for germination of seeds of *G. pubescens*. It is essential to point out here that during the 24 h dark treatment used in this study, seeds were briefly exposed to light whenever we examined for seed germination. It is possible that if seeds were kept completely in darkness, lower rates of germination may have been observed. Germination under alternating light and dark cycles was significantly higher than in continuous darkness. A similar effect has been reported for other invasive species (Çirak *et al*., 2004; Florentine *et al*., 2006; Amri, 2010). This type of light-controlled germination is associated with phytochrome. The sensitivity of seeds to the spectral quality of the light mediated by phytochrome is frequently observed within species that grow in open areas. The results of this study thus imply that the seeds of *G. pubescens* are light dependent for germination. Higher germination of *G. pubescens* seeds in light suggests that its germination and subsequent emergence in the field will be favored by the presence of seeds at, or near, the soil surface. Our results are similar to those of Chauhan *et al*. (2006a), who

found that annual *Sonchus oleraceus* (sow thistle) had greater seedling emergence under a no-till system as compared with the system that buried seeds deeper in the soil.

Galenia pubescens forms large circular prostrate mats under which seeds tend to accumulate. Once the branches die off, seeds which have accumulated on the surface of the soil are exposed to light, and when conditions are suitable, all the viable seeds germinate. This characteristic might be a key factor underlying the high capability of this species to rapidly colonise new areas. The ability of *G. pubescens* seeds to germinate at various temperature regimes ranging from 17/7 °C to 30/20 °C may explain why this species has so successfully germinated and subsequently been able to establish in a wide range of environmental conditions in Australia, and in other parts of the world.

The effect of fire, even after these experiments, is still contested. Historically, fire has been used as a control tool for managing weeds in Australia (Russell-Smith *et al*., 2000; Read *et al*., 2000; Tieu *et al*., 2001). Our field observations also show that a large number of *G. pubescens* seeds are confined to the surface layers of the soil, but these seeds are generally covered with thick foliage cover of the mature *G. pubescens* plants. Removal of the foliage cover with methods other than fire is likely to expose seeds to conditions which will encourage germination. However, the use of fire, and specifically the effects of the smoke and high temperatures associated with fire, may permit removal of mature plants and reduce the viability of seeds stored in the surface soil. Other studies have shown that heat shock from fire negatively affects germination of weed seeds, either by desiccating the seed coat (Jeffery *et al*., 1988; Brits *et al*., 1993), or by directly damaging the embryo (Van de Venter & Esterhuizen, 1988). In addition, smoke has been shown to physiologically inhibit seed germination, by

sensitising seeds to many of the known plant hormones involved in seed germination (Strydom *et al*., 1996; Gardner *et al*., 2001). On the other hand, studies have shown that the enhanced effect of smoke on the germination of various seeds is independent of other aspects of fire, such as heat and the production of ash (Willis *et al*., 2003).

The findings of this present study show that although seeds located on the soil surface may be destroyed by high temperatures and combinations of high temperatures and smoke, any seeds located in deeper soil layers may escape the heat from the fire, particularly in the case of a less severe fire (Todorović *et al*., 2010). Such seeds may thus remain viable until brought to the soil surface by various factors such as by soil erosion. This implies that if fire is to be employed as a tool to control *G. pubescens*, it would be essential to implement restoration efforts after a burn. Establishment of rapidly growing native species should occur either by broadcasting seeds or planting seedlings, and if a closed canopy of native species can be quickly developed, this is likely to prevent the subsequent germination and reestablishment of *G. pubescens*.

Our results are similar to those of Bouwmeester and Karssen (1992) and Wijayratne and Pyke (2012) who demonstrated that buried seeds of *Polygonum persicaria* (ladysthumb) and *Artemisia tridentata* (sagebrush) lost viability with increasing duration of burial. The percentage of seed longevity decreased with the length of the time of burial, which suggests that in undisturbed conditions, the problem of *G. pubescens* may be reduced in the long-term, if new seeds are not added to the seedbank. However, the relatively slow decrease in seed viability from 30 days to one year suggests that it may have a very long lived seedbank. This invasive weed is likely to remain problematic under field conditions due to the very large number of seeds it produces.

Higher seedling emergence for seeds positioned on the soil surface is consistent with the positive effects of light on germination and lower seedling emergence due to increasing depth of burial, and this has been reported for several weeds (Chauhan & Johnson, 2009; Tang *et al*., 2015). Light penetration is generally limited to the first few millimeters of the soil (Woolley & Stoller, 1978) and thus seeds positioned deeper in the soil do not receive light. Because of *G. pubescens'* requirement for light, limited light penetration is considered to be the most likely reason for the observed low emergence of buried seeds. However, some seeds (4%) buried at 1 cm depth did emerge, suggesting that additional factors might be responsible for the lack of emergence from deeply buried seeds.

One possible explanation of this low germination rate is based on gas diffusion, which is inversely correlated with burial depth, and also to the presence of CO_2 deriving from soil biological activity that might also be implicated (Benvenuti *et al*., 2001).

Other invasive species such as *Mimosa invisa* (giant sensitive plant) had only a 50% inhibition of the maximum germination at high salt level 255 ± 3.5 mM NaCl (Chauhan & Johnson, 2008). This outcome suggests that even at high levels of soil salinity, the invasive *G. pubescens* seeds may germinate, and those types of soil are common in some parts of Australia (Chauhan *et al*., 2006a). Similarly, Kleinkopf (1976), reported in the USA that *G. pubescens* has a high capability to germinate under salt stress.

A previous study reported that at an osmotic pressure of -0.7 MPa, 50% of the available seedbank of *Eragrostis tenella* (lovegrass) was inhibited from germination (Chauhan, 2013). On the other hand, the small-flowered *Malva parviflora* (mallow) showed no germination at an osmotic potential of - 0.6 MPa (Chauhan *et al*., 2006b). Because drought is an important factor limiting seed germination, and this study

indicates that *G. pubescens* is favoured by a moist environment, these results explain the association between low water availability and reduced germination of this species. Findings from this study indicated that germination of the *G. pubescens* species declined with increasing osmotic stress. The ability to germinate over a wide-range of pH values supports the view that *G. pubescens* is adapted to a wide range of soil conditions. This characteristic is common for many weed species such as *Synedrella nodiflora* (synedrella) and *Emex spinosa* (spiny emex) (Chauhan & Johnson, 2009; Javaid & Tanveer, 2014). Germination under a variety of soil conditions aids the ability of a weed to invade diverse habitats.

In summary, the results of this study indicated that seeds of *G. pubescens* can germinate under a wide range of environmental conditions, including those which are too harsh for native species. Germinating seeds of *G. pubescens* are tolerant to salinity but are sensitive to water stress. However, seeds germinated over a broad pH range, which indicates that soil pH is not a limiting factor for germination of this species. Seed germination in *G. pubescens* was found to be stimulated by light, indicating that buried seeds are likely to remain in a dormant state until disturbed. Whilst seeds of *G. pubescens* were able to germinate over a broad range of temperatures, it was found that high temperatures, and high temperatures combined with smoke, reduced germination. This suggests that fire may be a useful tool in decreasing the germination of this species, particularly for seeds stored in surface soil layers. This study has identified a weakness in the life cycle of *G. pubescens* that land managers can exploit in order to effectively manage such exotic weeds. Commitment to follow-up control for the first few years following control of original infestations should be sufficient to run down the seedbank to a negligible level. However, the elimination of localised infestations does not necessarily mean that the threat of *G.*

pubescens is removed. There is a risk of reestablishment even if the local seed reserves are depleted and all mature plants are killed. A wide range of environmental conditions provide suitable environments for this species, which can quickly re-establish new populations over large areas and which in turn produce very large numbers of seeds.

Part Two

Management strategies for Galenia pubescens

Part Two consists of three chapters. Chapter Four presents a pilot trial carried out to determine the most efficient dosage of pine oil to deplete the seedbank of *G. pubescens*. Chapter Five describes a field experiment carried out on an area of native grassland that had previously been severely disturbed by the removal of native grasses and external manipulations to aid fertility. At the time of the study it was infested by *G. pubescens.* In this study, the effectiveness of a number of different control techniques intended to reduce the population of aboveground mature plants and to destroy or radically deplete the seedbank was investigated. Chapter Six reports on the effects of prescribed fire and a season of burning upon the population of *G. pubescens* in different plots on the same, heavily infested, native grassland area.

Chapter 4: Effect of Pine Oil on the *Galenia pubescens* Soil Seedbank: Implications for Weed Management

4.1 Summary

In this study, experiments were carried out to determine the most effective dosage rate of a pine essential oil to limit the seed germination and seedling emergence of *G. pubescens.* A laboratory trial consisted in the application of pine oil at different concentrations of 0%, 0.5%, 1.0%, 2.5%, 5.0%, 10.0% and 15.0%) both directly and indirectly (fumigant) to determine the best dose-response application. The effect of pine oil application was both environmentally safe and significant in all concentrations (P<0.05) when compared to the untreated seeds: 1.0% or greater concentrations of pine oil reduced germination by more than 90% when applied directly to the seeds. Germination was completely inhibited by applications of pine oil at 5.0% or higher concentrations. In a pot trail, results also showed significant pine oil dose-response effects on reducing seedling emergence by between 90 and 100%. This demonstrates that pine oil could be used as a tool to destroy seedbanks of unpalatable plants without the need to initiate the germination process in all seeds. Such knowledge can be applied to help management agencies and programs in the control of dominant introduced weed species and in the conservation and restoration of biodiversity.

4.2 Introduction

Galenia pubescens control programs have proven to be unsuccessful in the past, primarily due to the extensive distribution of the species, and its capacity to produce large amounts of seeds annually. Seeds of *G. pubescens* are positively favoured by light for germination, indicating that buried seed will remain in a dormant state until disturbed (García-de-Lomas *et al*., 2010; Mahmood *et al.,* 2016). The increasing abundance of this species in temperate native grasslands in Australia is likely to be related to prolific seedling emergence and the difficulty associated with reducing the extensive and persistent soil seedbank (Cook, 2013). Chapter 3 identified a weakness in the seeds of *G. pubescens* that land managers can exploit in order to achieve effective management. Commitment to follow-up control for the first few years following the original control of infestations should be sufficient to deplete the seedbank to a negligible level. This observation implies that this weed will persist in the landscape for many years unless seed reserves can be significantly diminished.

It has been noted that effective control of *G. pubescens* has not been achieved despite the use of various chemical methods (Cook, 2013). Chemical methods involving the use of synthetic herbicides have had limited success among many weed species (Paudel & Gupta, 2008). This is because such treatments effect only aboveground plants, and have not demonstrated any success in reducing the seedbank composition. Moreover, chemical methods are environmentally unfriendly and hazardous. On the other hand, the use of bio-herbicides such as essential oils, which do effect the seedbank (McLaren, & Butler, 2015), are not just eco-friendly, but are also a cost effective method of weed control (Duke *et al*., 2002).

Pine oil (BioWeed™ and BioSeed™) is a distilled essential oil of *Pinus radiata* (radiata pine), produced by Certified Organics Pty Ltd which is a joint Australian/New Zealand

company. BioWeed™ and BioSeed™ herbicides are not systemic herbicides. They work by stripping the outer coating of plant and seed material that comes into contact with the preparation, causing cell collapse and desiccation.

Previous studies have shown that essential oils have a strong inhibitory effect on the seedling growth and seed germination. For instance, McLaren, and Butler (2015), stated the significant effects of essential oil products both directly and fumigant on seed germination of serrated tussock (*Nassella trichotoma*). In another study seed germination percentage was declined of *Amaranthus retroflexus* (redroot pigweed), *Chenopodium album* (lambsquarters) and *Rumex crispus* (curly dock) declined after treatment with essential oil application (Salamci *et al*., 2007). The essential oil (pine oil) of radiata pine has been shown to control *Orobanche ramosa* (branched broomrape) and *Nassella trichotoma* (serrated tussock) seedbanks as a part of South Australian and Victorian weed eradication programs (Mathews *et al*., 2006; McLaren *et al*., 2012).

Although some studies have been carried out on the effects of pine oil on the other species, little is known of the effect of pine oil on *G. pubescens*. The overall aim for this trial was to understand the suitable dosage rate of essential oil (pine oil) to deplete soil-stored seed of *G. pubescens.* In order to determine whether essential oils can be used as a tool to eradicate or minimize elevated soil-seedbank density of this invasive species in agricultural and environmental areas, two further experiments were conducted: (i) a laboratory trial was carried out to investigate the effect of different concentrations of pine oil on seed germination percentage, and (ii) a pot trial was conducted to investigate the effect of different concentrations of pine oil on seedling emergence.

4.3 Materials and methods

4.3.1 Experiment 1: Laboratory tests to determine the direct and fumigation effects of different concentrations of pine oil on germination of *Galenia pubescens* seeds.

4.3.1.1 Seed harvesting and processing

Galenia pubescens seeds were collected from several populations of mature plants in the Werribee region (37º 49' 5.63" S 144º 34' 58.77" E), at an altitude of 66 m, near Ballan Road, southeast of the Werribee River, Victoria, Australia during 2013. Uncleaned seeds collected in the field were placed in a labelled bag and transported to the Seed Ecology Laboratory at Federation University Australia, Mt Helen Campus in Ballarat, Victoria. Seeds were placed in an air-tight glass bottle and stored at (21°C) and at relative humidity of between 30 and 50% until use.

4.3.1.2 Pine oil application

The experiment was set up to assess whether the essential oil (pine oil) could reduce *G. pubescens* seed viability. Due to the high volatility of the essential oils, a trial was also initiated to determine whether pine oil had a fumigant impact on seed germination. To test this, Petri dishes were separated into halves by the use of plastic dividers. Filter papers were also halved. This enabled a comparison of directly treated seeds with those that only received the volatile fumes.

Prior to the start of the germination trial in the laboratory, seeds were surface-sterilised by rinsing in 1% w/v sodium hypochlorite for 1 minute, and then washed clean with sterilised double-distilled water. In each treatment, 25 mature and healthy *G. pubescens* seeds were selected from the seed containers and placed onto Whatman® No. 10 filter paper on each side of a 9 cm diameter Petri dish, so that each dish contained a total of 50 seeds.

One side of the Petri dishes was treated directly with pine oil (1.5 ml) at seven separate concentrations: 0.0 (control), 0.5, 1.0, 2.5, 5.0, 10.0, and 15.0% with four replications. The other side received only water. The two halves of the Petri dishes were labelled as treated and untreated. Petri dishes were then air sealed with paraffin plastic seal and adhesive tape to reduce evaporation and to investigate volatile vapour effects of essential oils. All Petri dishes were then placed in an incubator (Thermoline Scientific & Humidity Cabinet, TRISLH-4951-SD, Vol. 240, Australia) at 17/7°C (day/night) under 12/12 light and dark photoperiod, which was equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux of 40 µmol $m^{-2}s^{-1}$.

Daily observations were taken for seed germination for a period of 30 days from the date of sowing. Seeds were considered germinated when the radicle was approximately 2 mm long and cotyledons had emerged from the seed coat (Ferrari & Parera, 2015). At the conclusion of the germination test, non-germinated seeds were checked for viability using the 2,3,5-triphenyltetrazolium chloride (TTC) test (Waes & Debergh, 1986; Saatkamp *et al*., 2011).

4.1.1.3 Scanning electron microscope imaging

The seeds treated with pine oil were examined and photographed with a stereomicroscope. In addition, scanning electron microscope imaging was used to inspect the morphological changes caused by pine oil on the testa (outer coat) of the *G. pubescens* seeds. The seeds treated with pine oil were also examined and photographed with a stereomicroscope. Seed samples for scanning electron microscopy were prepared as follows. Seeds were fixed in 2.5% glutaraldehyde at room temperature for 24 hours. The seeds were rinsed in distilled water and dehydrated in increasing concentrations of ethanol consisting of 10, 30, 50, 70, 90% ethanol in water followed by two changes of 100% ethanol for 30 minutes at each step.

The samples were dried in a Bal-Tec CPD 030 critical point dryer (Balzers, Liechtenstein, Germany) and mounted onto 12 mm aluminium stubs with double-sided carbon tabs. The samples were then coated with gold using a Xenosput sputter coater (Dynavac, Wantirna South, Australia) and imaged with the Philips XL30 field-emission scanning electron microscope (Philips, Eindhoven, Netherlands) at a voltage of 2.0 kV at the Electron Microscopy Laboratory of the Botany Department at University of Melbourne.

4.3.2 Experiment 2: Pot trial to determine the effects of different dosage rates of pine oil application on seedling emergence of *Galenia pubescens*.

A pot trial was designed to test whether pine oil could also damage or kill *G. pubescens* seeds in the soil seedbank. This experiment was conducted in order to gain an understanding of the suitable dosage rate of essential oil (pine oil) to deplete the *G. pubescens* soil seedbank.

4.3.2.1 Experimental process

Soil used for this trial was collected from the study site (37^{O} 49' 5.63" S 144^{O} 34' 58.77" E). After that all vegetative materials such as leaves, rhizomes and litter were separated from soil samples using a sieve (4 mm mesh). Approximately 25 kg of cleaned soil was then placed in three biohazard bags and sterilised in the Autoclave (Horizontal, bench-top Autoclave Systec DX – 90, Germany) using a wet cycle run for a period 2.5 hours at 250 °C to kill any viable seeds or propagules.

At the glasshouse, the trial was prepared as follows: for soil moisture movement and aeration, sterilised industrial, seed-free sand soil was placed to a depth of 1 cm in black plastic punnets (10 cm x 6 cm x 6 cm) lined with paper towel. A mean weight of 250 g of well-mixed dry sterilised soil was placed into each punnet. One hundred *G. pubescens* seeds were counted and spread evenly on the top of each punnet. Punnets

were labelled and placed into large white trays (44 cm x 36 cm x 7 cm) to facilitate watering from below, which ensured minimal disturbance of seeds and later, germinants. Trays were watered for 24 hours before treatment application. Pine oil at seven concentrations 0 (control), 0.5, 1.0, 2.5, 5, 10, and 15% was applied using a watering can at a rate of 2 L m^{-2}, equivalent to the rate applied in the South Australian branched broomrape program (Mathews *et al*., 2006). Daily observations and counts were made of seedling emergence, with the final counts being made two months after the last emergence of new seedlings. Emerging seedlings were defined as those with the appearance of the two cotyledons.

4.4 Statistical analyses

In both experiments, at the end of the trial the final germination percentage (FG%) and seedling emergence percentage were calculated using the formula:

$$FG\% = \frac{SG}{TS} \times 100 \ , \qquad \text{(i)}$$

where SG is total number of seeds germinated and TS is total number of seeds planted (Wang *et al*., 1999).

The model fitted to germination at different concentrations of pine oil (direct and vapour) was the single two-parameter exponential decay model:

$$[G\ (\%) = a*e^{-b*x})], \qquad \text{(ii)}$$

where G is the total germination (%) at concentrations of pine oil x, and a and b describe the slopes of the regression curves.

The model fitted to seedling emergence at different concentrations of pine oil was the single two-parameter exponential decay model:

$$[E\ (\%) = a*e^{-b*x})], \qquad \text{(iii)}$$

where E is the estimated emergence (%) as a function of concentration of pine oil x, and a and b describe the slopes of the regression curves.

To ensure reliability, the experiment was repeated and the data were combined from the two repeated experiments. The data presented here are constant means.

4.5 Results

4.5.1 Experiment 1

The effects of seven concentrations of pine oil application for *G. pubescens* seeds treated both directly and by fumigation are presented in Figures 4.1 and 4.2. The data shows that the highest germination (80%) occurred in the control (no treatment). In contrast all concentrations of pine oil (0.5, 1.0, 2.5, 5.0, 10, and 15%; direct and fumigated) significantly ($P<0.05$) affected seed germination. Data showed that at 0.5% concentration, seed germination was 19% and germination was completely inhibited at 5% concentration or over (Figure 4.1).

With regard to the fumigation effects of the volatile pine oil, results of the Petri dish trial clearly show that pine oil has a fumigant effect as well. A concentration of 1.0% caused a decline in seed germination of more than 50% compared to the control (Figure 4. 2). However, as expected, direct application of pine oil to the *G. pubescens* seeds caused a faster reduction in seed germination than the treatments involving only the volatile fumes.

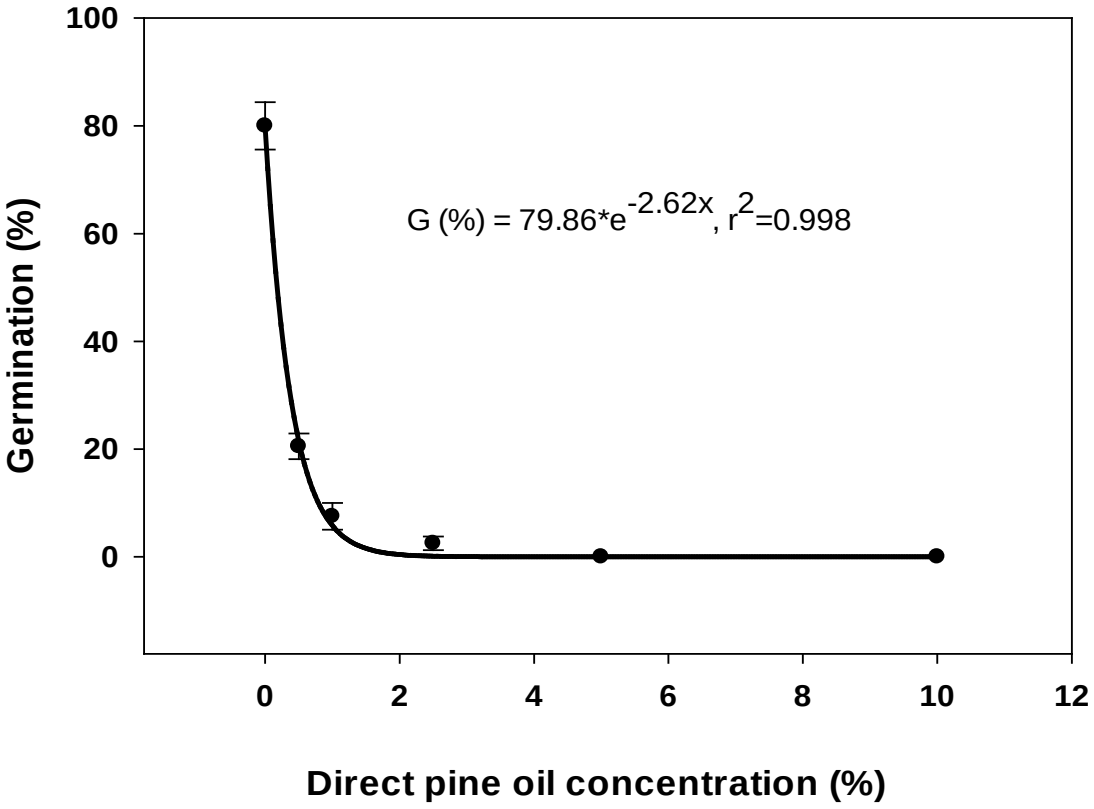

Figure 4.1. Effect of pine oil (direct) on germination of *Galenia pubescens* seeds incubated at 17/7°C day/night temperature in a 12-h photoperiod for 30 d. Line represents the function of single two-parameter exponential decay model [G (%) = a*e-b*x)]

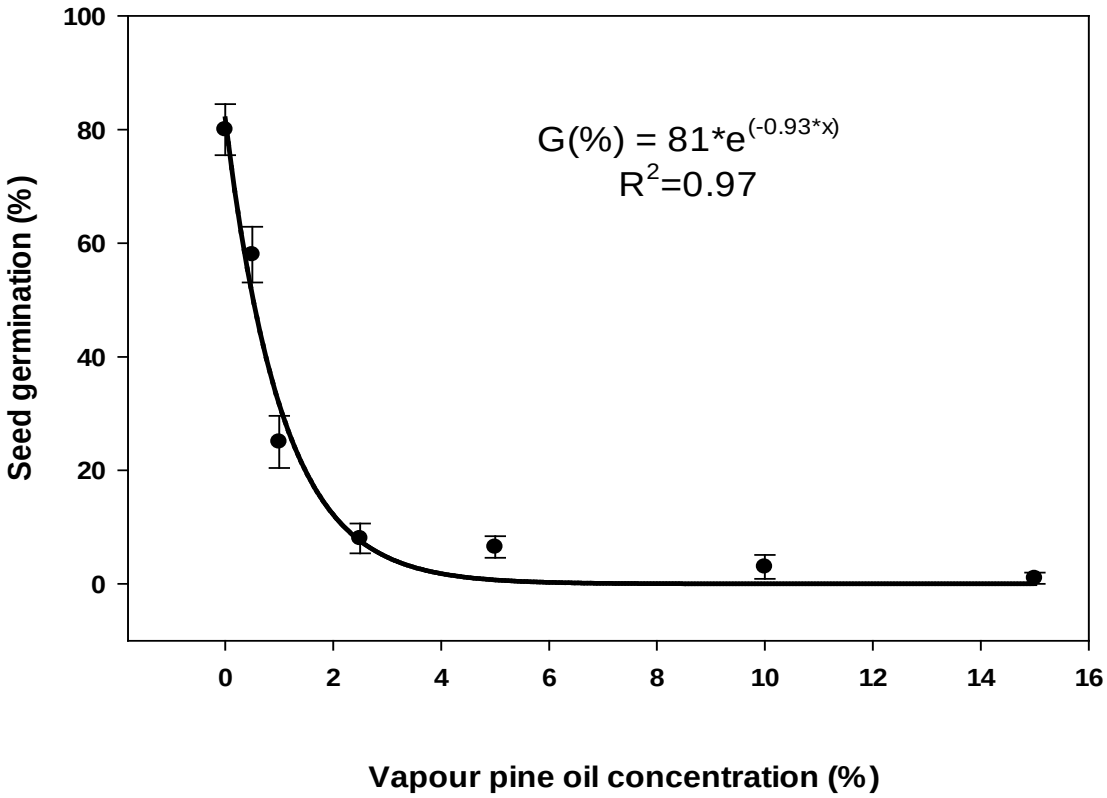

Figure 4.2. Effect of pine oil (vapour) on germination of *Galenia pubescens* seeds incubated at 17/7°C day/night temperature in a 12-h photoperiod for 30 d. Line represents the function of single two-parameter exponential decay model [G (%) = a*e-b*x)]

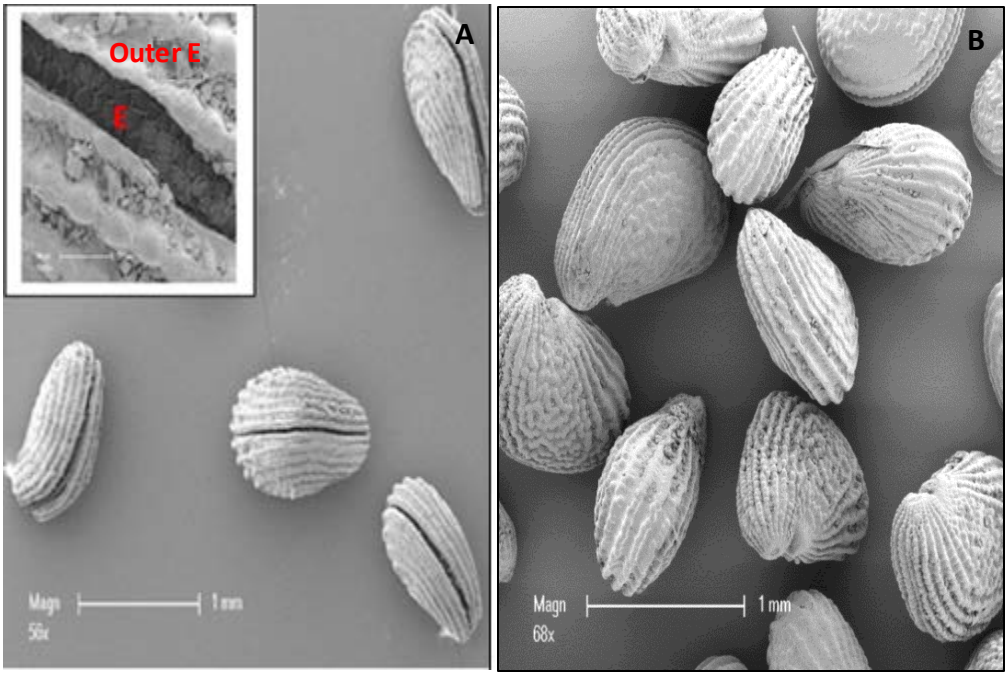

Figure 4.3. Scanning Electron Microscope images showing (A) the effects of pine oil on the outer coat of Galenia pubescens seeds; and (B) untreated (control) seeds. Outer E = outer epidermis (outer coat); E = Endosperm.

4.5.2 Experiment 2

To estimate the effect of pine oil, the percentage of seeds which germinated after treatment with various concentrations was calculated. Seedling emergence of *G. pubescens* in the pot trial was affected significantly by pine oil application. Seedling emergence was greatest (70%) for untreated seeds (control) and considerably decreased as concentrations of pine oil increased from 0.5 to 5%. No seedlings emerged from seeds treated with pine oil at concentrations of 10% and 15% (Figure 4.4).

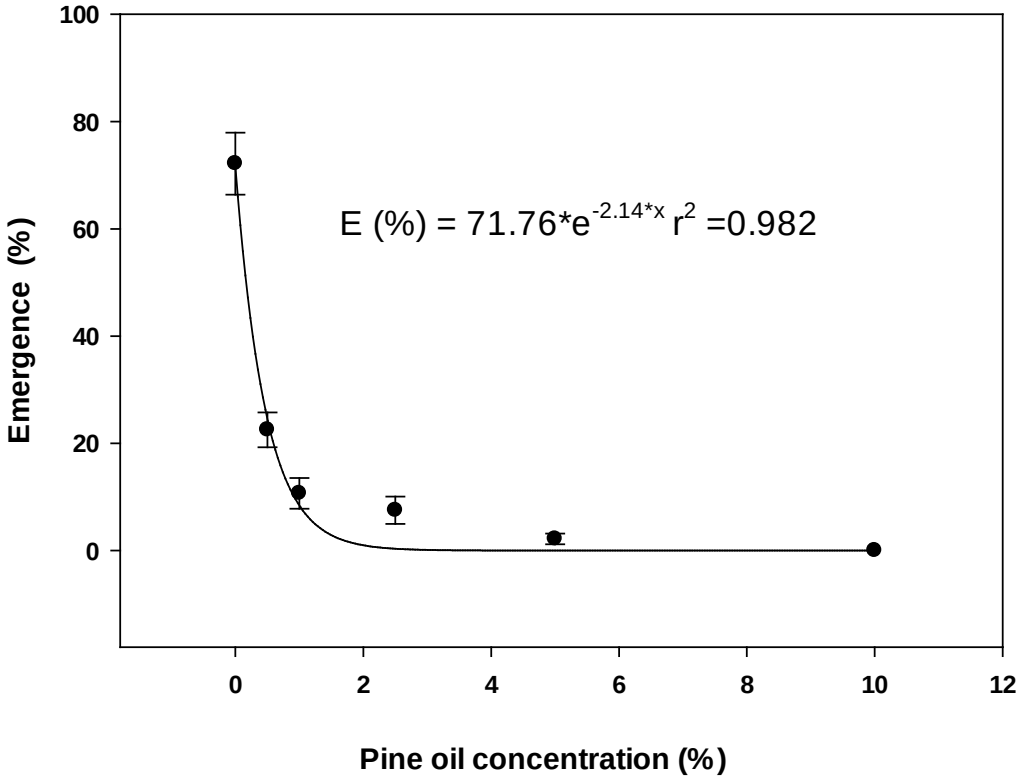

Figure 4.4. Seedling emergence of *Galenia pubescens* in response to different concentrations of pine oil in glasshouse. Line represent the function of single two-parameter exponential decay model [E (%) = a*e-b*x)]. Vertical bars represent standard errors.

4.6 Discussion

The results showed a significant dose-response effect of pine oil working to reduce seed germination and seedling emergence of *G. pubescens*. These results may be interpreted as the physiological or mechanical effect of pine oil inhibiting seed germination. With regard to the physiological impact, there are different kinds of terpenoids in plant essential oils. It is reported that these terpenoids, particularly sesquiterpene, are a group of compounds that exhibit a specific structure activity relationship (Paudel & Gupta, 2008). In addition, pine oil fumes may have penetrated the seed coat and affected the cells within the seed embryo. Being typically lipophilic, cytotoxicity is usually caused by the essential oil molecules passing through the cell wall and the cytoplasmic membrane, disrupting the structure of the different layers of polysaccharides, fatty acids, and phospholipids thus making them permeable (Bakkali *et al.*, 2008).

In a previous study, Lorber and Muller (1976) investigated the mechanisms of the action of terpene compounds by means of microscopic analysis of *Cucumis sativus* (garden cucumber) tips treated with volatile substances extracted from *Salvia leucophylla* (gray sage). These authors observed severe cell damage affecting organelle membranes, in particular the mitochondrial membranes. In another study conducted by (Angelini *et al*., 2003), the results showed that essential oils of *Rosmarinus officinalis* (rosemary), *Thymus praecox* (thyme), and *Ionactis linariifolia* (savoryleaf) are endowed with significant inhibiting activity on in-vitro seed germination. Results from the same study stated that the release of terpene compounds into the soil most probably represents an attempt by the plant to create for itself an environment that is unfavourable to the development of other species. This would ensure more advantageous conditions for that plant in the struggle for survival.

In addition, previous studies have shown that essential oils exhibit phytotoxity against weeds, and that these have a great potential for weed management (Kohli *et al*., 1998; Batish *et al*., 2008; McLaren *et al*., 2012). For example, Kohli *et al*., (1998) reported that essential oil from *Eucalptus tereticomis* and *E. citriodora,* when applied in vapour form, significantly decreases the germination of the noxious weed *Parthenium hysterophorus* (ragweed). Further, fumigation of mature plants with eucalyptus oil vapours reduced growth and the chlorophyll and water content, and also decreased cellular respiration. (Kohli *et al*., (1998) stated that the inhibitory activity of oil was time-dependent, and that a gradual decline in weed growth was observed following increasing periods of oil exposure. The study concluded that applications of essential oil extracted from *Eucalyptus* species is a promising strategy for weed management, provided that the economics of its extraction and application are thoroughly investigated and found to be cost effective. However, the study did not include an evaluation of the toxicity of the oil upon other associated plants and non-target organisms. Future investigations could be made to establish the chemical characterisation of the essential oils in question (pine and eucalypt) in order to extend the findings of the present investigation.

Another observation of relevance relates to the mechanical impact of pine oil (Figure 4.3). This shows that the outer coat split longitudinally in all treated seeds, causing cell collapse and desiccation. The outcomes of this study are similar to previous studies (Singh *et al*., 2005; Paudel & Gupta, 2009), in which the effects of plant essential oils upon seed germination and seedling growth on *Parthenium hysterophorus* are reported. In another study, pine oil has been used successfully to control the seedbank of *Orobanche ramosa* in South Australia's weed eradication program. Field experiments established that 5% concentrations of pine oil could reduce emergent

seed by greater than 95% (Matthews *et al*., 2006). The changes in seedbank composition demonstrates that pine oil application may have important implications for controlling weed seedbanks for biodiversity conservation. The results indicate that regular applications of pine oil produces a significant shift in seedbank composition, with concomitant reduced seedbank richness.

Given that applications of pine oil have no systemic action, its use offers a very high level of crop safety. It can be used in a wide variety of settings when targeting unwanted plants, it can be used in close proximity to nearby favoured plants, and can also be applied throughout the full crop cycle. In a study conducted by Matthews *et al*., (2004), pine oil was used to treat the exotic weed *O. ramosa* infesting large 'broad acre' tracts with the application of the pine oil being made by helicopter. Boom-spray and orchard row applications are also practicable. In terms of its cost effectiveness, pine oil application is relatively expensive. However, this initial, more expensive outlay is more than compensated for by the fact that (i) pine oil applications need not be made annually, and (ii) the weed seedbank composition is radically depleted, leading to vastly reduced re-growth. This will allow significant changes to be instituted for management practices, where spray regimes may be reduced by up to 50%. In consequence, valuable time and cost savings will be achieved.

In another study, pine oil application was found to be relatively safe for use upon *O. ramosa* among native species, where only seven of 58 native species were found to have decreased in species density when measured 12 months after the initial application. This would suggest that pine oil applications should be considered as a suitable treatment option in areas of native scrub and along roadsides to eliminate weeds, since it will do so with only limited negative effects upon native biodiversity (Matthews *et al*., 2004).

Due to its volatility, aromatic pine oil applications do not remain in the soil for long periods, and reports suggest pine oil residues disappear by evaporative and soluble processes within 72 hours. This fact, together with findings made by (Duke *et al*., 2002), means that this essential oil can be used as a viable weed control technology in organic farming systems without leaving residues.

In conclusion, this study suggests that essential oils from aromatic plants at different concentrations have adverse effects on the seed germination and seedling emergence of the *G. pubescens* plant, where pine oil is found to significantly arrest the seed germination of the target seedbank as well as reducing the size of the local seedbank. Such a tool could be very beneficial for rehabilitation of grasslands severely impacted by *G. pubescens* and could be used to provide an ecological window for actively sowing beneficial species to enable their establishment with reduced competition from *G. pubescens* seedbank.

Chapter 5: Investigation of Different Control Techniques for the Invasive Species *Galenia pubescens* in a Degraded Ex-grassland Reserve Southeast of the Werribee River, Victoria, Australia

5.1 Summary

Invasive species cause ecosystem degradation, leading to the decrease of native plant density and diversity, and major changes in ecosystem structure and function. Woody weed herbs (such as *G. pubescens*) often grow faster than native species, produce larger and/or more seed, and out-compete other mid- and under-storey species for light and other resources in invaded habitats. The Victorian State Government has moved to acquire lands to the south east of Melton and west of Werribee in southern Victoria, Australia, and to form the Western Grassland Reserves. Its aim is to protect some of the most important native grassland communities in the state. *G. pubescens* is a significant weed within the area, and its invasion of these reserves seriously threatens the ecological integrity of the native grasslands.

This study aims to assess the control of *G. pubescens* using five different control strategies. These are: herbicide control with glyphosate, organic herbicide control with pine oil, the application of mulch, and the addition of native seeds to the available seedbank. All possible combinations of these techniques were tested.

The results demonstrate that any one single treatment of a *G. pubescens* infestation without undertaking a secondary treatment is insufficient to control this weed. Further, there is limited regeneration of native vegetation unless additional techniques, such as direct seeding, are applied. There was a strong indication that a combined strategy employing all the aforementioned techniques is more effective than any single technique treatment. However, full regeneration of the area may not be possible

unless further restoration programs are instituted after the full cycle of *G. pubescens'* treatment has been completed.

5.2 Introduction

In the course of recent decades, the decay and destruction of native grasslands in Australian territories has expanded (Williams *et al*., 2005). In Victoria, Australia, lowland native grassland is one of the state's most endangered vegetation communities (Stuwe, 1986; Calder *et al*., 1992; Barlow & Ross, 2001). It is listed as a threatened community under Victorian legislation, and has been nominated as a critically endangered community under Federal legislation. In western Melbourne, 1670 ha (23%) of the 7230 ha of native grassland present in 1985 has been damaged by development, and a further 1469 ha (21%) had been degraded to non-native grassland by 2000. In the remaining areas of native grassland, fewer patches and greater distances between patches were recorded in 2000 than in 1985, showing that fragmentation of these areas has intensified (Williams *et al*., 2005).

Alarm at the loss of temperate native grasslands in Victoria is not new. For example, Sutton (1916), a prominent naturalist of the early 1900s, reported that the grasslands west of Melbourne while not being favoured for residential purposes and not being much built over, has been devoted so completely to pastoral and farming uses, that barely any part remains in a virgin condition. Since the 1980s, Melbourne has experienced a period of managed development characterised by new residential and industrial development and high rates of population growth (State of Victoria Department of Infrastructure, 2000). As well as the threats posed by such development, native grasslands are being degraded due to significant and rapid weed invasion.

Various factors appear to give non-indigenous species a competitive advantage over indigenous grassland species. These include the disbursement of supplement-rich water from neighboring farming or urban areas, and the soil-unsettling practices connected with vehicle access, as well as the activities of road and rail management agencies and of utility companies (Kirkpatrick *et al*., 1995). Invasive species are also favoured by inappropriate land management (Lunt & Morgan, 1999a,b), particularly a failure to remove biomass by burning or grazing, leading to an increase of dead plant material whose decomposition releases nutrients that further support weed expansion (Morgan & Lunt, 1999). The remaining grasslands will continue to be threatened by weed invasion and other degradation processes. Many highly invasive alien species, particularly *Nassella neesiana* and *G. pubescens* present a major threat to Victorian native grasslands (Carr *et al*., 1992; McLaren *et al*., 1998; Morgan, 1998).

The current options available to control *G. pubescens* infestation in Australia are restricted to herbicide treatments and mechanical removal (Cook, 2013), which limits the success of control options in many situations. Reliance on a few synthetic herbicides has resulted in environmental and human health issues, and is leading to an increasing incidence of herbicidal resistance among many weed species. Furthermore, options for chemical weed control in non-arable situations are limited to very expensive spot treatments and blanket coverage, which is non-selective. In addition, intensive use of synthetic herbicides can result in soil and groundwater contamination. Warnock (2012) theorised that generally, herbicide application was only effective in the short-term, and that the bare soil resulting from the herbicide treatments will assist in the recruitment of new seedlings from pre-existing seeds in the following year. Several authors suggest that this phenomenon is common to many weed species. Herbicide treatment aimed at achieving short-term goals can result not

only in reinvasion, but can increase weed abundance to a level greater than that which existed prior to treatment, directing the vegetation community further away from the desired outcome (Burke & Grime, 1996; Sheley *et al*., 2006). In addition, herbicide as a control method may have several possible side-effects, including spray drift, for example, which can impact on surrounding areas, particularly with aerial application (Robinson *et al*., 2001). Any areas that are missed may represent a source of seed for rapid re-colonisation. A complicating factor is that some herbicides, such as glyphosate, are not species specific, therefore non-target species are likely to be inadvertently affected (Cox & Anderson, 2004).

There are also several disadvantages associated with mechanical control methods. They may not always be applied when required, as the ground could be too wet, steep or stony for machinery to operate successfully, or the area of infestation may be too large to be covered in a reasonable time, and in situations where machinery can be used, their use may damage soil structure and encourage erosion.

According to the report from the Weed Society of Victoria, there are (in Australia) no biological controls available for *G. pubescens* (Australia's Virtual Herbarium, 2007). One study to investigate options for chemically controlling this species has recently been undertaken (Cook, 2013). At this time, there are no known organic control agents available for this species. Therefore, efforts to develop alternative means of weed control that are not only eco-friendly, but also cost effective and biologically robust, are required. This study aims to provide evidence to inform long term management solutions intended to swing the competitive balance towards native grasslands species by: (i) selectively killing the mature *G. pubescens* plants through spot applications of glyphosate, (ii) removing or reducing the *G. pubescens'* seedbank through application

of an essential oil (pine oil), (iii) the application of mulch (sugar and sawdust) to increase microbial drawdown of soil nitrogen to reduce competitiveness of nitrogen-loving colonising weeds such as *G. pubescens,* and (iv) the introduction of native grassland species to colonise bare ground created by the treatments and to provide competition for *G. pubescens.* If these techniques are effective, new rehabilitation methodologies may be developed to accelerate restoration efforts in these degraded grasslands, and innovative seedbank control techniques, such as that proposed in this investigation, may also become an important tool for eradication and control of other priority weed species in the future.

5.3 Materials and methods

5.3.1 Study site

The experiment reported in this chapter was conducted to assess the effectiveness of potential management treatments for *G. pubescens* in one of the proposed areas which will comprise the Western Grassland Reserves. This trial site is approximately 12 km from Werribee, just off the Ballan Road and is representative of the distribution of *G. pubescens* south east of the Werribee River, Victoria. It is located at 37º 49' 5.63" S 144º 34' 58.77"E, at an altitude of 66 m (Figure 5.1). Prior to experiment in 2013, soil test data was collected for high, medium and low density *G. pubescens* infestations. High cover sites generally had higher potassium, sulphur, and pH (acidic) (See Appendix B for more details).

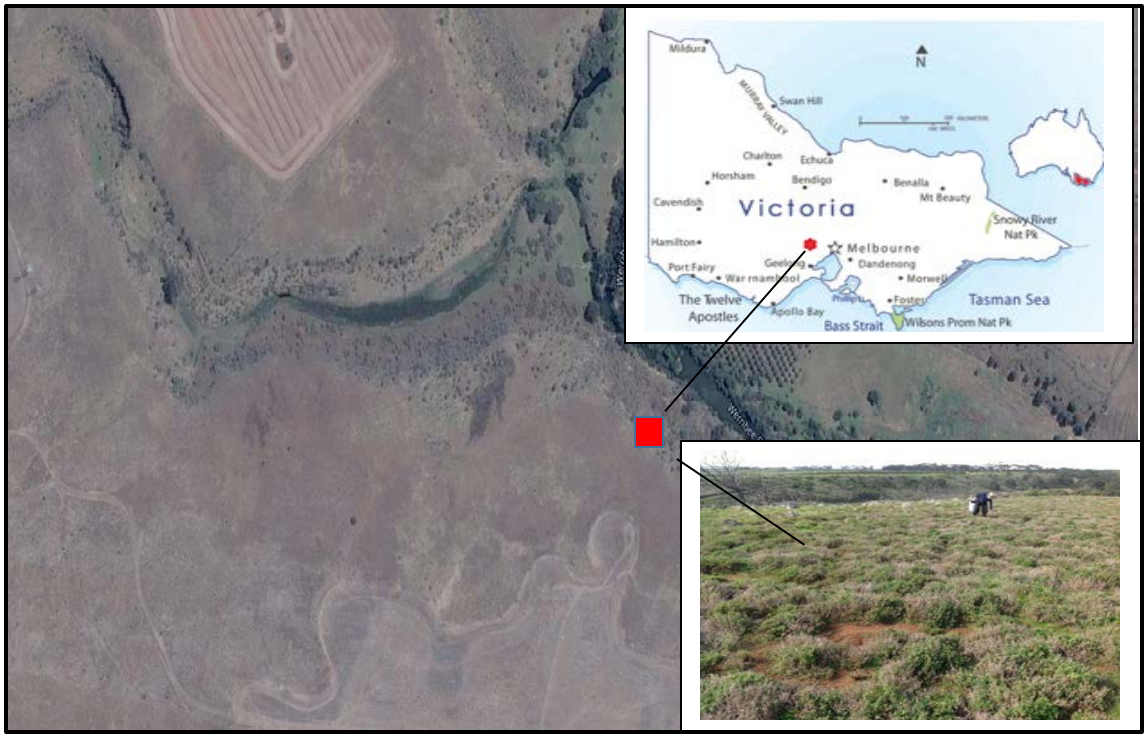

Figure 5.1. Study site (■) at grassland southeast of the Werribee River, Victoria, Australia.

5.3.2 Experimental design and treatments

This study was carried out in four 96 m x 96 m study-blocks (Figure 5.2), each block consisting of 16, 6mx6m subplots with almost identical infestation levels of *G. pubescens*. The trial involved 16 treatments, and was conducted in a completely randomized block-design (RCBD) replicated four times. Treatments included: control (no treatment), chemical herbicide (glyphosate), essential oil (pine oil), mulch (sawdust + white sugar) and seed addition (mixture of native grass seeds) delivered as separate treatments and also in combinations (Figure 5.3). Each treatment plot was

permanently marked with numbered fence droppers and was individually labelled. Pickets were painted in different colors to differentiate the treatments.

Figure 5.2. Four 96 m x 96 m study blocks in the Cobbledick's Ford area

Block 1	G+P	G+P+M+S	M+S	P	P+S	No treatment	M	P+M
	G + S	P+M+S	S	G+M	G+M+S	G	G+P+S	G+P+M
Block 2	P+M+S	M+S	G+M	M	G	G+P+S	P+M	G + S
	No treatment	G+M+S	P	G+P	P+S	S	G+P+M+S	G+P+M
Block 3	G+P+M	G + S	P+M	G+M	P+M+S	M+S	P	M
	No treatment	G+M+S	G	G+P+M+S	S	G+P+S	P+S	G+P
Block 4	P+M	G+P+M	M	G+P+M+S	No treatment	P	G + S	G+M+S
	P+S	G	P+M+S	G+P+S	G+P	S	M+S	G+M

Figure 5.3. Randomized treatment distribution within each 96 m by 96 m study-blocks. Control (No treatment); p = pine oil; G = glyphosate; S = addition of native seed; M = mulch (sawdust +sugar); G+P = glyphosate + pine oil; G+M = glyphosate + mulch; G + S = glyphosate + seeding; P+M = pine oil + mulch; P+S = pine oil + seeding; M+S = mulch + Seeding; G+P+M = glyphosate + pine oil + mulch; G+P+S = glyphosate + pine oil + seeding; G+M+S = glyphosate + mulch + seeding; P+M+S = pine oil + mulch + seeding; G+P+M+S = glyphosate + pine oil + mulch + seeding.

5.3.3 Application of treatments

Glyphosate application

A new generation of glyphosate, called Sipcam Raze Herbicide, was used in the glyphosate application treatments. It is a member of the glycine group of herbicides. This product is a water-soluble liquid herbicide and it is non-selective, broad spectrum, systemic and will control a wide range of emerged annual and perennial weeds (Pozo *et al*., 2005). It is absorbed by plant leaves and green stems and is then translocated throughout the plant to the root system. The product inhibits a plant enzyme (5-enolpyruvylshikimate-3-phosphate (EPSP) synthase), causing a breakdown in the metabolic photosynthesis pathway which leads to the death of the plant (Zabalza & Royuela, 2014). Application of this herbicide occurred at the test site in early March 2014. The rate used was 90 ml of herbicide per 10 L of water. Plots receiving selective glyphosate treatment were sprayed using a solo backpack sprayer model 425 with 90 psi using a piston pump with flat spray nozzles (Figure 5.4). The entire surface area of the plots were sprayed. The temperature was 30˚C during the application and there was no wind.

Figure 5.4. Glyphosate application trial

Pine oil application

Pine oil is an essential oil (see Chapter 4; section 4.2) and was selected for this research as it is widely available commercially. This treatment was applied using a solution of 20,000 L/ha using a watering can. The entire surface area of each of the relevant plots was treated in April 2014, and it was applied immediately after rainfall to avoid any significant evaporation effect. Each 6 m x 6 m plot required 72 L of pine oil at a concentration rate of 10% (Figure 5.5).

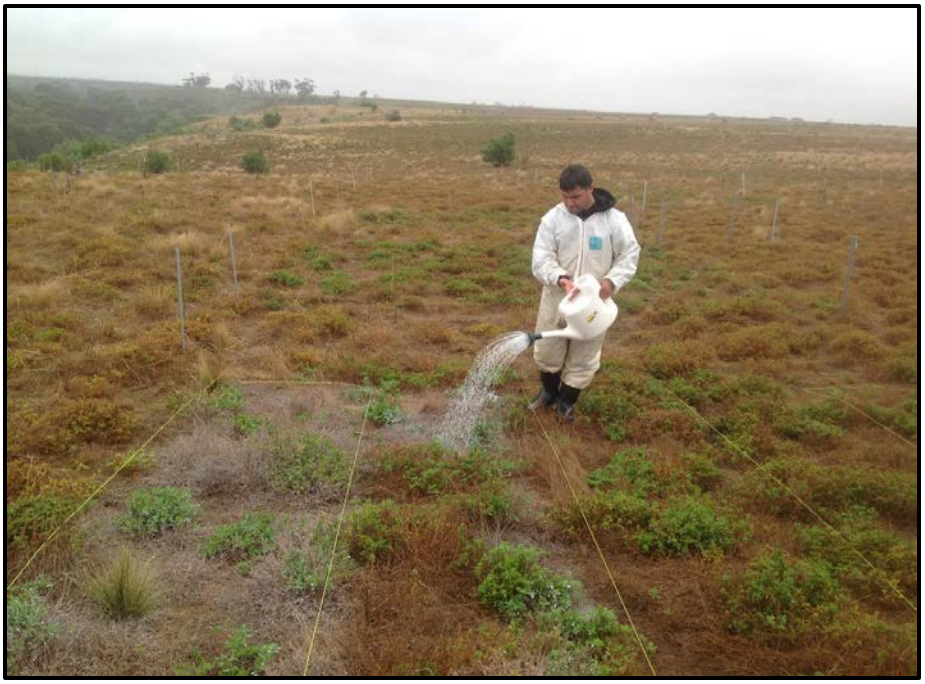

Figure 5.5. Pine oil treatment being applied

Mulch treatment

Mulch (white sugar plus sawdust), was added to the soil in order to increase microbial populations and CO_2 production, while also reducing soil nitrogen (Jonasson *et al*., 1996; Eschen *et al*., 2007). Plots undergoing the mulch treatment were treated as follows: first, 12.5 kg of white sugar was applied to each 6 m x 6 m plot, and then 20 kg of sawdust was added to a depth of approximately 5 cm.

Seed addition trial

The addition of native plant seeds was achieved by broadcasting a native plant seed mix of four native grass species onto the surface of plots in order to provide competition to *G. pubescens.* Seed addition occurred one month after herbicide application and two weeks after pine oil applications and one week after sugar applications. This timing was chosen to permit the applications to achieve their optimal effect. In the plots which required a Mulch + Seeding application, the addition of

sawdust occurred after seed addition in order to protect seeds and to provide some warmth to the soil.

Prior to applying the seeds, plots were lightly harrowed using a metal rake to aid seed penetration into the soil. Seed from four native grass species were hand-spread evenly across the 6 m x 6 m seed-addition plots. The seed mix used in this experiment comprised a mixture of *Rytidosperma acrosum* (vickery) seed at a rate of 20-30 kg/ha, *Bothrichloa macra* (red-leg grass) seed at 30-40 kg/ha, *Themeda triandra* (Kangaroo grass) seed at 40-50 kg/ha and *Austrostipa bigeniculata* (Spear grass) seed at 20-30 kg/ha. The seed was purchased from Flora Victoria, a registered producer of high quality seed in commercial quantities; the seed was delivered in a timely fashion, and was well-packaged.

Combined treatment applications

For the plots which received multiple treatments, the sequence of treatment application was as follows: for dual treatment applications; glyphosate was applied first. After two weeks, the second treatment was applied (pine oil, or mulch or native seeds).

For the triple treatment applications, and for the treatment which included all four applications, glyphosate was applied to the appropriate plots first. After two weeks, pine oil was applied where required, then after two weeks mulch was applied where required. Native seeds were scattered on designated plots one week after the mulch application. All treatments were complete by early-mid May 2014.

5.3.4 Measurements

Prior to the application of the treatments (in the summer of 2013), the foliage cover of the plant community was assessed within each of the treatment plots. These

community assessments formed a baseline against which post-treatment community compositions could be compared. The response of the plant communities within each treatment plot was determined for both the above and below-ground communities as follows:

Vegetation survey. To examine the aboveground vegetation cover before and after treatment applications, a baseline vegetation assessment was conducted during October 2013 (prior to any treatment application) with a follow-up assessment in spring 2014 (after 6 months) and again after 18 months of treatment application in 2015. The procedure for measuring the aboveground vegetation has been described in detail in Chapter 2, section 2.3.5.

Seedbank sampling and identification procedure. To determine the effect of treatments on seedbank composition, four soil samples were taken from each plot, once prior to treatment applications (October 2013) and twice after treatment applications in the following years (October 2014 and 2015). The procedure for measuring the composition percentage of the soil seedbank has been described in detail in Chapter 2, section 2.3.4.

5.4 Statistical analysis

Data were analysed using International Business Machines Statistical Package for the Social Sciences (IBM SPSS statistics 22); to determine overall changes in *G. pubescens* vegetation, cover percentage and seedbank abundance at the study site, a one-way ANOVA was used to measure the possible effect of time and treatments. In order to find out which treatment will be more significant, The Tukey Test was used for all pairwise comparisons of the mean responses to the different tratment groups, with a 0.05 level of significance.

5.5 Results

5.5.1 *Galenia pubescens* foliage cover percentage

Effects of time of observation

Overall cover percentages of *G. pubescens* were significantly affected by time under different experimental treatments (Figure 5.6). The R^2 adjusted value of 67.48% shows that almost 70% of the variation of the cover percentage of *G. pubescens* is explained by the treatments and time of observation. A one-way ANOVA showed that time of observation (before versus after treatment) had a significant (P<0.050) effect on the cover percentage of *G. pubescens* (F= 63.46; P=0.041). In 2013 (prior to applications), the main percentage cover of *G. pubescens* was recorded at 66.4% inside all the 64 study plots; in contrast, at the assessment point in 2014 (6 months after treatments were applied) the main cover percentage of *G. pubescens* within all the plots had been reduced dramatically to 23.3%. However, in the 2015 assessment (18 months after treatments were applied), the cover percentage of *G. pube*scens had increased to 35.5%, but the decrease still remained significant compared to the 2013 assessment.

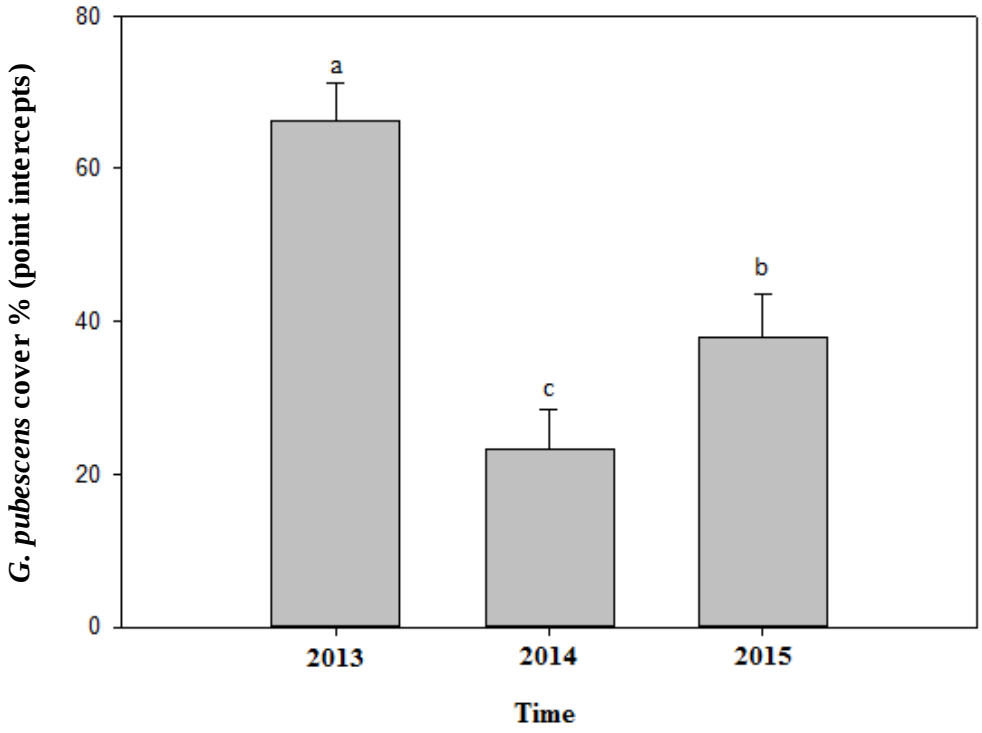

Figure 5.6. Effects of the time of observation on percentage cover of *Galenia pubescens* measured via point intercepts in 2013 (prior to treatment), 2014 (6 months after treatment) and 2015 (18 months after treatment) across all treatment plots. Vertical bars represent ± standard error from the mean. Different letters indicate a significant difference between years (Tukey's Multiple Range Test, $P< 0.05$).

Effects of treatments

The effects of individual treatments on the aboveground foliage cover of *G. pubescens* are shown in figure 5.7. The data show that there was no significant difference (P= 0.066) between plots in terms of the aboveground foliage cover (percentage foliage cover) of *G. pubescens* in the 2013 survey assessment (prior to treatments). In the plots which were selected for control purposes (no treatments), the average percentage cover of *G. pubescens* did not change significantly between the 2013 and 2015 assessments, that is, the average cover percentage remained at similar levels.

By contrast, the average percentage foliage cover of *G. pubescens* in the plots which were treated by single, dual or multiple treatments was significantly (P<0.05) reduced.

Pine oil application significantly reduced the percentage foliage cover of *G. pubescens* (F=12.42; P=0.000). The foliage cover of *G. pubescens* plants was measured at 61% prior to any treatment. By contrast, the 2014 survey showed that in the plots treated with a 10% pine oil solution, the foliage cover of alive *G. pubescens* was reduced to 15.7%. However, the 2015 assessment data show that *G. pubescens* had significantly reestablished itself, and the (reduced) cover percentage almost doubled when compared to the 2014 cover percentage.

Glyphosate application also significantly affected the percentage of foliage cover of *G. pubescens* after 6 months from the date of treatment. The data show that in the plots treated by glyphosate in 2013 the cover percentage was 73%, but this percentage was sharply reduced to 23%. However, after 18 months the cover percentage rose to 34%. The reduction in the percentage cover of *G. pubescens* was less in the mulch and seeding treatments compared to the pine oil and glyphosate treatments. However, when compared to the control cover percentage, the reductions were still significant (F= 63.46; P=0.041).

Regarding the effects of the combination treatments, compared to the control plots, all of the four dual treatments made a significant impact (P= 0.000) on the cover percentage of *G. pubescens* within two years from the date of application. In addition, the most effective dual treatment was that of glyphosate plus pine oil. In these treatments, the percentage cover of *G. pubescens* was reduced from 68% (prior to treatment) to 14% and 21% respectively by the 2014 and 2015 assessments. The

least effective combination effects were found in those plots which had received a mulch plus seeding treatment.

The impacts of multiple treatments on the cover percentage of *G. pubescens* are shown also in figure 5.7. The cover percentage of *G. pubescens* in the plots selected for multiple treatments was similar to that of the control plots in the 2013 survey. In contrast, the 2014 survey revealed that the percentage cover of *G. pubescens* had significantly changed in all plots which had been given multiple treatments. For example, the glyphosate + pine oil + mulch treatment reduced the cover percentage of *G. pubescens* vary dramatically from 67% (prior to treatment) to 10.5% six months after the date of application in 2014. Similar results were found for other multi-treatments. However, by 2015 the percentage cover can be seen to have increased slightly in all treated plots, but these still remained at lower levels when compared to the levels found in the control plots.

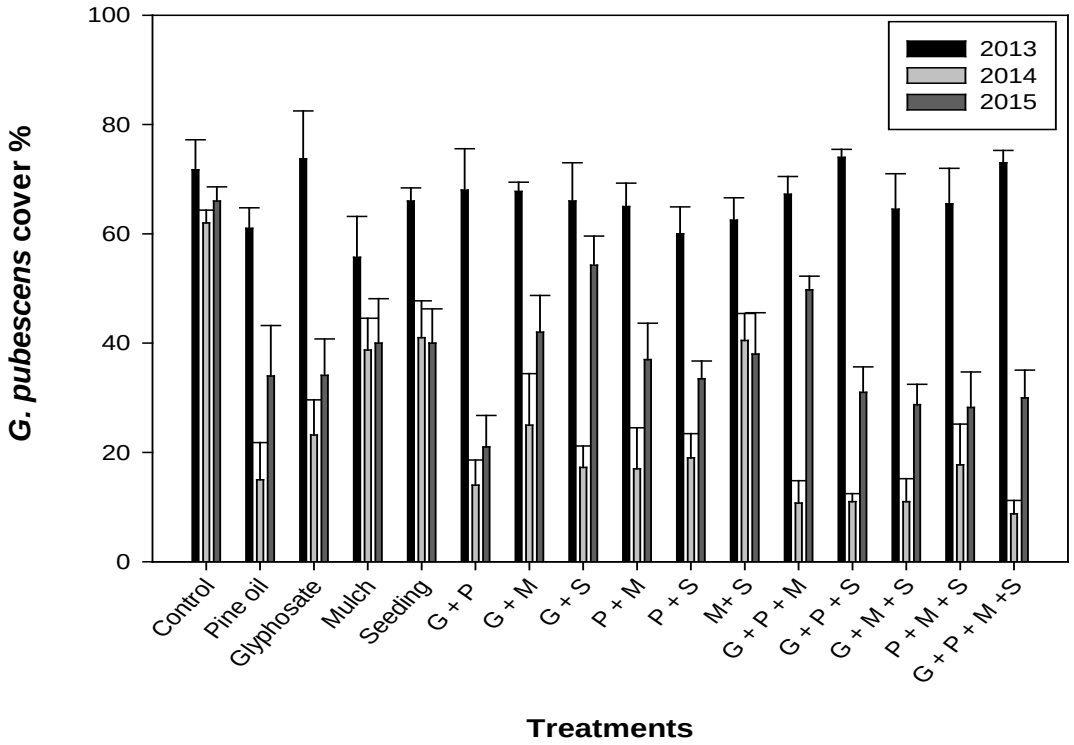

Figure 5.7. The effects of treatments on the cover percentage of *Galenia pubescens* from 2013 to 2015. Vertical bars represent ± standard error of the mean. Control = untreated; p = pine oil; G = glyphosate; S = addition of native seed; M = mulch (sawdust + sugar); G+P = glyphosate + pine oil; G+M = glyphosate + mulch; G+S = glyphosate + seeding; P+M = pine oil + mulch; P+S = pine oil + seeding; M+S = mulch + seeding; G+P+M = glyphosate + pine oil + mulch; G+P+S = glyphosate + pine oil + seeding; G+M+S = glyphosate + mulch + seeding; P+M+S = pine oil + mulch + seeding; G+P+M+S = glyphosate + pine oil + mulch + seeding.

5.5.2 *Galenia pubescens* seedbank abundance

Effects of time of observation

Figure 5.8 shows the changes in the average number of *G. pubescens* seedlings (the measure of seedbank abundance) from all study plots prior to treatments and at 6 months and 18 months post-treatment. These changes are regarded as significant (P<0.05). In 2013 (prior to treatments), the average number of seedlings of *G. pubescens* per plot was not significantly different among plots. The average was 86 seedlings/plot, but this number dramatically reduced to only 23 seedlings per plot 6 months (2014) after the application of treatments. By the 2015 assessment, the number of seedlings of *G. pubescens* had increased slightly; however, this increase was insignificant when compared to the numbers found during the 2013 assessment.

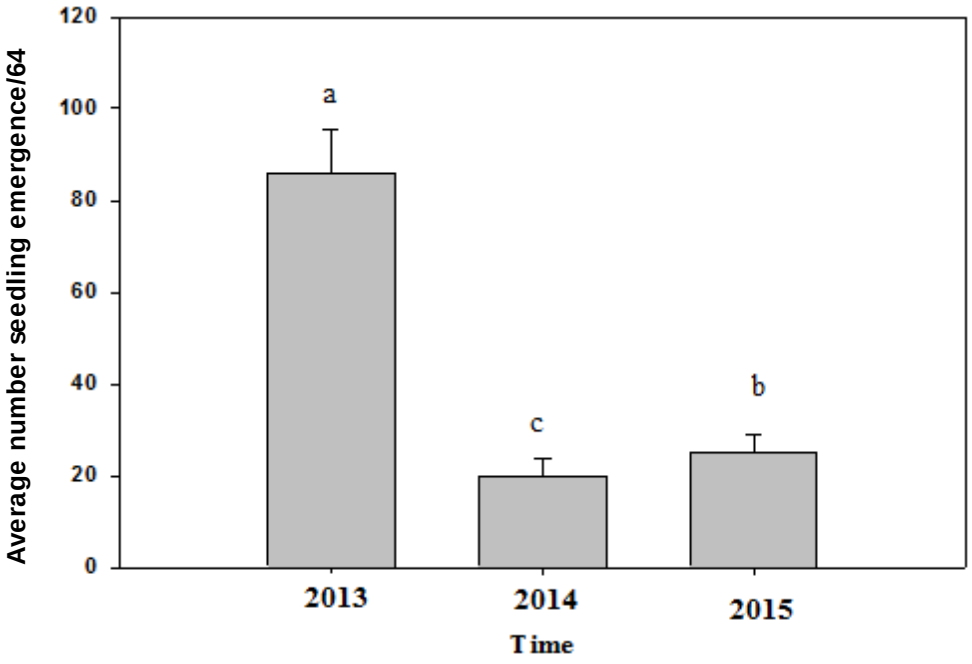

Figure 5.8. Average number of *Galenia pubescens* seedlings from soil samples taken in 2013 (prior to treatment), in 2014 (six months after treatment) and 2015 (18 months after treatment). Vertical bars represent ± standard error of the mean. Bars with different letters reveal that the means are significantly different at level 0.05.

Effects of treatments

The impact of the individual treatments on the *G. pubescens* seedbank composition is demonstrated in figure 5.9. In 2013 (prior to treatment), it can be observed that the mean number of *G. pubescens* seedlings across the four control plots was not statistically different between the 2013 and 2015 assessments (F=12.09; P=0.041). The mean number of *G. pubescens* seedlings in the control plots remained similar throughout the experiment, with the number of seedlings in 2015 being slightly higher compared to the results from 2013 and 2014. In contrast, the average number of *G. pubescens* seedlings significantly changed (F= 5.93; P = 0.000) in all other plots which received single treatments (of pine oil or glyphosate or mulch or seeding), dual treatments or multiple treatments.

In the case of single treatments, the most significant results were found in those plots which had received pine oil treatment. Prior to treatment, no significant difference was found between the control and the pine oil treatment plots. The average number of emergent seedlings in soil samples taken from control and pine oil-treated plots were 84.25 and 119.7 emergent seedlings per plot. In contrast, in 2014 (six months after the pine oil was applied) the average number of *G. pubescens* seedlings had fallen dramatically to 4.7 seedlings per plot in soil samples taken from plots which received an application of pine oil at 10% concentration. By 2015 (18 months after treatment) the germination rate of *G. pubescens* seedlings had increased again (34 ±2.8 mean seedlings per plot), but the average numbers remained significantly lower when compared to the numbers found in soil samples from control plots.

In regard to the glyphosate and mulch treatments, the results were found to be similar to those seen in the pine oil treatments (Figure 5.9). The least significant results were

recorded in the seeding treatments which showed little or no change from the 2013 to the 2015 assessments.

The statistical analysis of the effects of dual treatments on the total emergence of *G. pubescens* seedlings is shown also in figure 5.9. It is apparent that the 2014 assessment showed significant reductions of *G. pubescens* seedling emergence in all dual treatments. The best results were found in plots which had been treated with pine oil + mulch or pine oil + seed addition. By the time of the 2015 assessment, there was a slight increase observed in the average number of emergent *G. pubescens* seedlings in all treated plots, but the numbers still remained significantly different (F= 5.93; P= 0.000) to the average number of seedlings found in the untreated (control) plots.

In the case of the influence of multiple treatments on the total emergence of *G. pubescens* seedlings, the data show that, while all plots selected for multiple treatments had a *G. pubescens* seedling-count similar to the control plots as assessed in 2013, the average number of *G. pubescens* seedlings per plot was significantly lower in plots which received multiple treatments in the 2014 surveys. For example, in the plots where a glyphosate + pine oil + mulch treatment was given, the average number of *G. pubescens* seedlings reduced dramatically from 101 seedlings per plot (prior to treatment) to only 3 seedlings per plot in 2014, 6 months after the treatment application. Similar results were found for other treatment combinations. Eighteen months later the average number of emergent seedlings per plot had slightly increased in all plots, but still remained significantly lower than the average number of seedlings emerging from the control plots. This slight increase has some management implications –for example, the need to check and re-treat controlled areas as necessary.

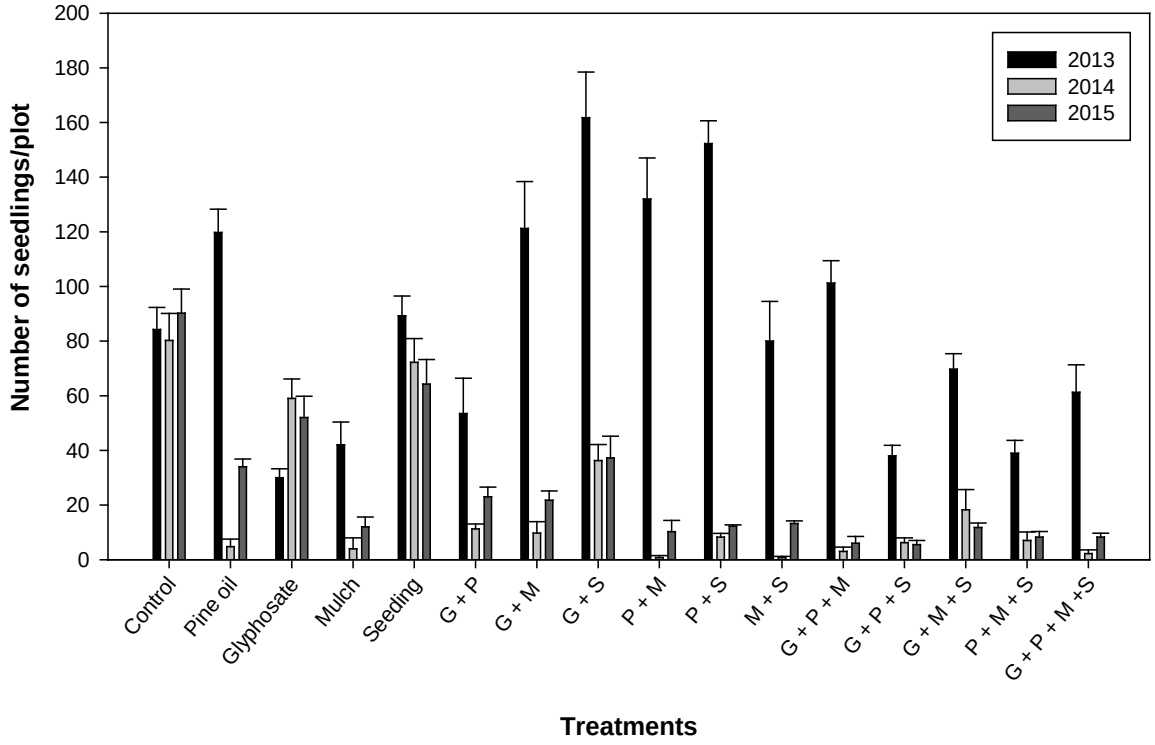

Figure 5.9. The effects of different treatment applications on the *Galenia pubescens*' seedbank composition from 2013 to 2015. Vertical bars represent ± standard error of the mean. (Control = untreated; P = pine oil; G = glyphosate; S = addition of native seed; M = mulch (sawdust + sugar); G+P = glyphosate + pine oil; G+M = glyphosate + mulch; G+S = glyphosate + seeding; P+M = pine oil + mulch; P+S = pine oil + seeding; M+S = mulch + seeding; G+P+M = glyphosate + pine oil + mulch; G+P+S = glyphosate + pine oil + seeding; G+M+S = glyphosate + mulch + seeding; P+M+S = pine oil + mulch + seeding; G+P+M+S = glyphosate + pine oil + mulch + seeding.

5.6 Discussion

This study shows that the management techniques examined had a significant effect on both the cover percentage and number of viable *G. pubescens* seeds stored in the soil in the experimental plots after 18 months of various treatments.

Regarding the effect of time of observation upon the changes to cover percentage and composition of the soil seedbank, the outcomes clearly showed that all the treatments tested were successful when measured 6 months after the date of application as compared to the figures relating to the untreated plots. However, the effectiveness of the treatments declined at 18 months from the date of application, implying that in situations of heavy weed infestations, one round of control strategies might not be enough to kill all the mature plants or deplete seed numbers in soil stored seedbanks. *G. pubescens* plants are highly capable of producing large numbers of seeds annually and of re-establishing themselves from undamaged parts of the treated plant (García-de-Lomas *et al*., 2010).

Regarding the combinations of the effectiveness of the treatment in reducing foliage cover percentage and density or quantity of soil seedbank of *G. pubescens,* single element treatments and combinations of two, three or four elements appeared significant for stats testing within just a few months. Not all treatments were equally effective however. Single element treatments (for example pine oil alone, or mulch alone) were less effective than dual, or multiple combinations which provided the most comprehensive and effective control, and are recommended because they are less costly over the long-term (Paynter & Flanagan, 2004; DiTomaso *et al*., 2006). A study of other invasive weedy species also found that greater control was achieved when different treatments were combined rather than being applied separately (Renz & DiTomaso, 2004). While the study was conducted over 18 months, its relevance to

long-term control may perhaps be extrapolated from this period of time: the management advice, if followed, will significantly reduce the dominance of *G. pubescens* in the long term. In the case of pine oil application which was the novel method of this experiment in that this is the first time it has been used in the field as a tool for the control of the weed soil seedbank, the outcomes showed that this treatment is very effective as a means to reduce the density of *G. pubescens* seedling emergence. Previous studies in pot trials (Chapter 4) indicated that the essential oil, such as pine oil, destroys seed viability by 'cracking' the outer coat of the seeds, causing cell damage and desiccation (Matthews *et al*., 2006; McLaren *et al*., 2012). In addition, Kremer and Spencer (1989) stated that mechanical degradation of seeds, particularly to the seed coat, makes the seeds more susceptible to attack by microorganisms, which suggests that may have microbial attack was partly responsible for the reduction in seedbank germination observed for the pine oil treatment in this trial. The changes in the seedbank composition of *G. pubescens* demonstrates that pine oil applications may play an important role in the control of weed seedbank for biodiversity conservation. The results indicate that if applications of pine oil are made regularly in areas of heavy weed infestation a significant shift in seedbank composition will be achieved. Further, if the pine oil treatment is followed by an application of native plant seeds, after a with-holding period of two weeks, then the control will be more effective, because the increased native plant population will reduce the incidence of bare soil, compete directly with weed-re-establishment and decrease the possibility of soil erosion.

In the case of glyphosate application, this nonselective herbicide was the only chemical method used either independently or in combination to reduce foliage cover and seedbank density of *G. pubescens*. It was found that this treatment, besides not

being eco-friendly, was only effective in the short-term in the reducing foliage cover of mature plants. However, in this study it was found that there was considerable regrowth of *G. pubescens* six months after the treatment. This finding is in line with Cook (2013), who showed that glyphosate application was not an effective control of *G. pubescens* especially if the treatment was delivered in spring, but he found that glyphosate application was moderately effective if made in early and mid-autumn. It was suggested that the poor efficacy of application in spring was compounded by early signs of moisture stress, thus this 'timing' effect needs to be re-investigated without the interference of such secondary effects.

The other effective method found by this study to control *G. pubescens* was mulch application, the mulch being a combination of white sugar and sawdust. It was found that this treatment had a positive effect in reducing the infestation of *G. pubescens,* and at the same time it also inhibited seedling emergence. There are a number of plausible reasons for plant decreases within mulched areas. Mulch generally makes a layer on the ground and prevents airborne or dropped seeds from reaching the surface, whilst also smothering mature plants and preventing their growth. Previous studies also found that mulches are an effective technique to control weeds (Hywood, 1999; Bond & Grundy, 2001; Coleman *et al*., 2015; Khan *et al*., 2016). Another advantage of mulch relates to its promotion of the activity of microorganisms in the soil, which then absorb soil nutrients as they grow, reducing their availability for weeds (Reicosky *et al*., 1995; Chen *et al*., 2014). The relative lack of nutrients will inhibit weed growth while simultaneously providing a better soil environment for native plants, which can grow well in low nutrient conditions (Nottingham *et al*., 2009). In addition, certain organic mulches, especially sawdust or wood chips, may control weeds chemically through the leaching of allelopathic chemicals naturally occurring in the

wood (Griffiths *et al*., 2003). In another study, Greenly and Rakow (1995) stated that organic mulches are most effective in weed control when applied at a sufficient depth. Mulch treatment reduces light, which will stress existing weeds and prevent the germination of many weed species, especially those with small seeds such as *G. pubescens*. However, mulch also presented a germination barrier to herbaceous and small-seeded plants, where researchers have found that small-seeded Aizocease species can survive for more than two years in the soil seedbank, and can re-emerge when conditions are suitable (Wiegand *et al*., 1995; De Abreu, 2011).

Seeding treatment with mixed native grass seeds was used to introduce or recover native grassland species so as to enable them to compete with *G. pubescens* in treated plots. The results indicate that the use of seeding alone in areas of heavy infestation by *G. pubescens* did not significantly reduce the cover percentage and seedbank composition. This was due in part to the massive biomass of *G. pubescens* and its ability to produce large number of seeds. However, in plots where seeding treatments were combined with other control techniques, the results were greatly promoted native species to grow.

Previous studies have also shown that seeding alone is not a recommended solution (Milton & Dean 1995; Viser *et al*., 2004) as seeding alone was not able to increase the density of native species. It is possible that better results may have been obtained if the introduction of the native seed into the degraded area was made one to three years after herbicide or mulch treatments. Many authors suggest that for ecosystems in a highly degraded state, rehabilitation efforts should first focus on removing the infestation of invasive species by using applications of herbicides or by biological controls. Once this intervention has reduced the degradation, the introduction of direct

seeding of indigenous plants should then take place (McEvoy & Coombs, 1999; Mendez *et al*., 2008).

It can be concluded that the highest percentage of mature plants and highest number of viable *G. pubescens* seeds in the study area was found before the application of treatments, and that these treatments were most effective in reducing the population of *G. pubescens* when applied in combination. While our results did not assess the management costs associated with the various control practices, it is strongly suggested that a combination of treatments can improve the control of *G. pubescens,* and that it also has the potential to become a useful management consideration for other perennial invasive weedy species.

Chapter 6: The Use of Prescribed Fire to Control *Galenia pubescens* in the Western Grassland Reserve in Victoria, Australia

6.1 Summary

The Western Grassland Reserves are located on the volcanic plains near Werribee, west of Melbourne, Victoria, and provide typical examples of small, highly modified and fragmented patches of natural temperate grassland threatened by the invasion of exotic weeds. A particularly invasive weed of these reserves is carpet weed (*Galenia pubescens*), as discussed at length in previous chapters. This study describes the assessment of attempts to modify a low rainfall (<400 mm/year) grassland region that having been invaded by *G. pubescens*, was treated by combinations of herbicides and a late spring prescribed burn in an attempt to control its aboveground cover and its seedbank. In assessing the burn effects on the aboveground vegetation of *G. pubescens,* results have shown that a combination of chemical control and late-spring burning has reduced the foliage cover of non-native species such as *G. pubescens,* suggesting that this would be a useful tool in its management. This is supported by findings which are based on several treatments of an area where an 80% *G. pubescens'* untreated basal cover was measured before treatment applications. First, following treatment with no burn plus glyphosate, the basal cover was found to be reduced to 28%. Second, under a burn plus glyphosate treatment, the basal cover was measured at 25%. Third, following burn plus Grazon™ Extra, the cover was found to be 23%. In addition, the results also show significant decreases in *G. pubescens'* seed viability of more than 70% in the burial seeds after exposure to a prescribed grassland burn. In conclusion, we suggest that the outcomes provide evidence that late-spring burning reduces the cover of non-native species, thus fire may be an effective tool for

increasing mortality of *G. pubescens* seeds, and contribute to its management across a broad range of habitats.

6.2 Introduction

Exotic plants are now present in the vast majority of temperate and low rainfall Australian grasslands. They are represented by a wide diversity of annual, biennial and perennial forbs, and annual and perennial grasses (Kirkpatrick *et al*., 1995; Sharp, 1997; Groves & Whalley, 2002; Carter *et al*., 2003; Dorrough *et al*., 2004; Fry *et al*., 2014). Lowland grassland in Victoria was considered by Faithfull (2012) to be among the weediest of the broad vegetation formations in Victoria, with 344 exotic taxa, 87 of which were considered to be very serious weeds. Between one quarter and one third of the flora in grasslands in Victoria consists of exotics, and weed invasion is a major threat to the survival of native flora (Kirkpatrick *et al*., 1995; Groves *et al*., 2004).

Fire is one of the most established tools utilised by human communities to manage vegetation (Pyne & Vale, 2003). Such events are believed to have made many indigenous grassland species well adapted to a regular fire history. More recently, prescribed fire has been used to reduce fuel loads, restore historical disturbance regimes, improve forage and habitat, and promote biodiversity (Armatas *et al*., 2016). Fire also has been used to manage invasive plant species, either directly or as part of an integrated approach (Wiens & Hobbs, 2015). In Australia, prescribed fire events have been widely used to manage weed infestation generally across Australian grasslands (Russell-Smith *et al*., 2000; Goergen *et al*., 2006). Observations from hot fire events have suggested that *G. pubescens* does not recover well, and therfore fire might be a useful management tool for containing *G. pubescens* and to help re-establish healthy grasslands on the Victorian Volcanic Plains. However, because fire increases the surface temperature of the soil matrix and produces significant levels of

smoke, it is also thought that these two components, either separately or together, may serve as germination cues and/or significantly reduce the activity of weed seedbanks (Tieu *et al*., 2001; Gashaw & Michelsen, 2002; Clarke & French, 2005; Wright & Clarke, 2009). In this respect, smoke has been widely acknowledged as a key agent in the release of seed dormancy for some plant species and may positively interact with heat to promote this release (Smith *et al*., 1999). De Lange and Boucher (1990) investigated plant-derived smoke as a germination cue and found that smoke products can stimulate germination in many South African species. In addition, seed morphology may facilitate germination after fire because seeds are more likely to survive when buried in the soil. Therefore, it is essential to understand the combined effect of heat and smoke on both seedbank and vegetation cover of *G. pubescens* before attempting to institute fire control techniques as a control mechanism. (Mahmood *et al.*, 2016)

In addition, because few invasive woody weeds are effectively managed by a single year of prescribed burning, it is often necessary to incorporate other control methods into a long-term management strategy (Kyser & DiTomaso, 2002). These methods can include mechanical, cultural, biological, and chemical options. For example, a study of another invasive weedy perennial *Lepidium latifolium* (pepperweed), found that greater control over the foliage cover was obtained when mechanical and chemical treatments were combined rather than being applied separately (Renz & DiTomaso, 2004).

This chapter presents the results of a study to determine the effects of a late-spring prescribed fire on *G. pubescens* and other proximal weed species at a site near Werribee, west of Melbourne, Victoria, (Figure 6.1). The specific objective of the

prescribed fire was to reduce the dominance of *G. pubescens* by reducing its basal cover and reducing the viability of its seedbank. It is hypothesised here that post-fire weed control activities at the burn site might result in reduced weed-species seed richness, abundance and cover, with a possible and corresponding increase in native species over time.

6.3 Materials and Methods

6.3.1 Study site and experimental design

Research was conducted at the same site (but in different plots) as described in detail in Chapter 2, section 2.3.1. The property was formally a cattle-grazed pasture composed predominately of *Lolium rigidum* (annual rye grass) but is now being managed for rehabilitation of indigenous grasslands. This site is now dominated by *Galenia pubescens* and *Nassella trichotoma* (serrated tussock) that are threatening the grassland rehabilitation process.

A total of ten 1 ha plots were mapped out for large scale treatment applications during 2013 (Figure 6.1). Within each of these blocks, a permanently-marked monitoring plot measuring 20 m x 20 m was selected (Figure 6.2), giving 10 such monitoring plots. One metre long metal dropper posts with a permanent label were used to mark each corner of all those plots. To collect seasonal vegetation data, five 20 m transects were arranged at 4 m intervals across each monitoring plot. The transects were offset from the parallel edges of the corner posts by 2 m to avoid 'edge effects' from nutrients caused by birds using the marker posts as roosts. Using the point intercept method (Dix, 1961; Mueller-Dombois & Ellenberg, 1974; Rodriguez & Jacobo, 2010), observations were taken at points located every 20 cm along each transect, there being 100 points per transect and 500 intercept points for each plot. Observations

included whether there were plants, bare ground or rock at each intercept point. Where a plant was recorded, its species was noted along with its status as 'live' or 'dead'. The point intercept method is used to characterise percentage cover by species. For a particular plot, the point scores for a particular species are presented as a percentage of the total number of point scores for the plot.

To assess the effects of a combination of chemical control and fire on *G. pubescens,* ten 1 ha plots were marked out; five were treated with glyphosate and three were treated with Grazon™ Extra and two were left untreated (controls). The treatment protocol and treatment replications is described in Table 6.1. This site selection was based on the high density of *G. pubescens* infestation and upon the logistical capacity to effect and control the burn.

Herbicides were applied using 10 L "solo" backpack sprayers fitted with flat spray nozzles which applied water at a rate of 1500 L ha^{-1}. The sprayers used a piston pump. Both herbicides (glyphosate and Grazon™ Extra) were applied directly to individual *G. pubescens* plants. The teams of contractors would move systematically across each targeted 1 ha block attempt to treat each individual *G. pubescens* plant. Treatment plots were numbered from 1 to 8 with two additional plots (9, 10) reserved as control plots: no chemical treatments and no burning took place upon these plots (Figure 6.1). Due to *G. pubescens* regrowth, follow-up treatments using glyphosate and Grazon™ Extra were applied as outlined in Table 6.1. Glyphosate was applied to plots 7 and 8 without burning and applied to 1, 3 and 6 followed by burning. Grazon™ Extra was applied to plots 2, 4 and 5 during a hot spell in December 2013 (with applications being halted several times as a consequence of the hot weather). Consequently, burning treatment took place in spring 2014.

To provide competition for herbicide treated weeds while also providing extra grass biomass for the proposed spring 2014 burn, *Lolium rigidum* seed was applied during autumn 2014 across plots 1, 2, 3, 4, 5, and 6. Seed was spread by hand across each of these plots at a rate of 2.8 kg/ha.

Figure 6.1. The ten 1 ha plots at Cobbledicks Ford Rd site. These were numbered 1 to 10: (9, 10 were reserved for control treatment; 7, 8 for glyphosate treatment without burning; 1, 3, 6 for glyphosate + burning; 2, 4, 5 for Grazon™ Extra + Burning. Each plot measured 1 ha.

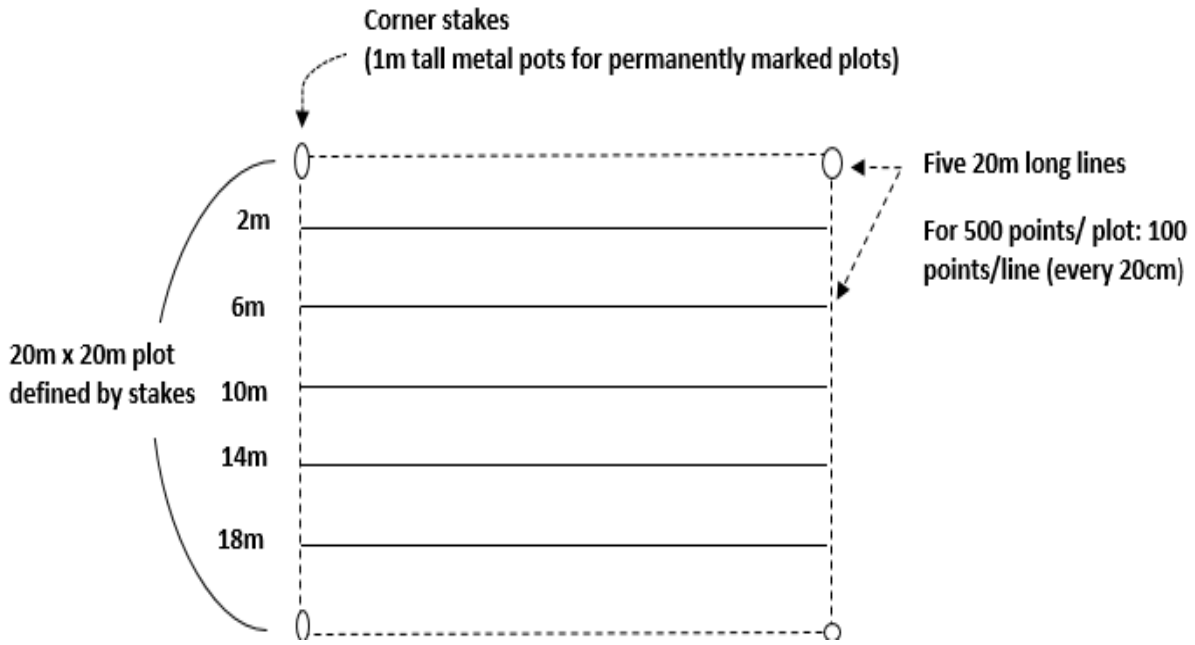

Figure 6.2. Sampling plot design for conducting detailed vegetation assessments.

Table 6.1. Details of individual plot treatment and plan applications for management of *G. pubescens*.

Treatment name	Plot number	Product rate	Date applied
Control (no burn; no herbicide)	9,10	Not applicable	Not applicable
Glyphosate + no burn	7, 8	13 ml/L	Spring 2014
Glyphosate + Wimmera rye grass + Burn	1, 3, 6	13 ml/L, 2.8 kg/ha, burn	Spring 2013, Autumn 2014, Spring 2014
Grazon Extra™ + Wimmera rye grass + Burn	2, 4, 5	5 ml/L 2.8 kg/ha, burn	Spring 2013, Autumn 2014, Spring 2014

6.3.2 Set up of data logger

To monitor burn temperatures and temperature effects on *G. pubescens* seeds, each plot had a data logger (DS1922L Thermochrom iButton high resolution data logger button) and one muslin packet of 25 *G. pubescens* seeds buried in the soil.

To bury the data loggers and seed bags, a 10 cm diameter auger was used to cut a circular section of soil approximately 3 cm deep. One data logger was placed at the

bottom of each hole and the circular section of soil-core was then replaced. The seed bags were then dug in so that they were placed just under the soil surface of each of the holes with approximately 0.5-1 cm of soil covering the seed bag. Each seed bag was located 2.5 cm directly above the data logger and 0.5 cm below the soil surface. Care was taken to ensure that similar amounts of trash/litter/vegetation occurred on top of the soil core as was to be found in the immediate vicinity. Data loggers and seed bags were retrieved two days after the fire event. Temperature data was captured from the data loggers using Thermodata viewer software version 3.2.3, and recorded onto *Excel* formatted charts via that program.

6.3.3 Vegetation survey

A baseline vegetation assessment was undertaken during winter 2013 (before application of treatments) with follow-up assessments being made in winter 2014 (that is, 1 year after treatments), winter 2015 (2 years after treatments) and spring 2015 (2 years and 3 months after treatments). Vegetation monitoring was undertaken according to the methods described in a previous section (6.3.1).

6.3.4 Seed germination assessments

Seeds of *G. pubescens* that had been exposed to fire were retrieved 2 days after the prescribed fire treatment and taken to the Seed Ecology laboratory at Federation University Australia. Seed were surface-sterilised by rinsing in 1% w/v sodium hypochlorite for 1 minute, and placed into labelled 10 cm diameter Petri dishes (25 seeds per dish) lined with moistened filter paper to aid seed germination. Two or three replicates were made, depending on the treatment: Control: 2 replicates; glysophate + no burn: 2 replicates; glysophate + burn: 3 replicates; Grazon ™ Extra + burn: 3 replicates. These dishes were individually placed into a constant temperature cabinet equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux

of 40 µmol m ¯2s ¯1 and set to an optimal alternating temperature of 17/7°C and a 12:12 h light/dark photoperiod (Mahmood *et al*., 2016). A seed was considered to have germinated when the protruding radicle achieved the length of 2 mm beyond the seed coat (Amri, 2010). Daily observations were taken of seed germination for a period of 30 days from the date of sowing and final germination percentage were recorded. Results were also expressed as a viability percentage, which was defined as the percentage of live seeds that had germinated at the end of the test, plus seeds that were assessed as alive using the Tetrozolium test (Saatkamp *et al*., 2011; Mahmood, *et. al*., 2016).

6.4 Statistical analysis

Prior to statistical analysis, the basal cover data of each site in autumn 2015 was angularly transformed. For each measurement, the data was analysed as an analysis of variance, without blocking, and using an experimental plot as the unit of analysis. As preliminary analysis indicated that burning had a major effect on germination, treatment effects were divided into (a) the effects of burning (i.e. a. combination of the non-burnt treatments to the 2 burnt treatments), and (b) any effect of herbicide within treatments with same burning regime (i.e. a combination of the effect of no herbicide versus glyphosate with no burning and the effect of glyphosate versus Grazon™ Extra with burning). The residual degree of freedom was 5 for the germination and temperature measurements, and 6 for the basal cover measurements.

For seed viability results, data were converted to percentages. A one-way Analysis of Variance (ANOVA), was used for comparing all treatment effects. Means were compared using Tukey's HSD comparison. All analyses were conducted using GenStat 16 (Payne, 2013).

6.5 Results

The effect of the spring 2014 burn on *G. pubescens* populations at Cobbledick's Ford Rd is shown in Figure 6.3., and the statistical analysis of herbicide and burn treatments is shown in Table 6.2. It is apparent that both the glyphosate and Grazon™ Extra treatments produced significant reductions (P = 0.0013) in *G. pubescens* % basal cover but no significant (P = 0.13) differences were seen between herbicides with and without burning. However, as expected, the burn did create significant differences with respect to litter reduction (P = 0.002), extent of bare ground (P = 0.003) and increased presence of broadleaf weeds (P = 0.01).

The Grazon™ Extra treatment completely killed all the foliage for the *G. pubescens'* plants after six months from application date (Plots 2, 4 and 5) while glyphosate also achieved good results in all plots except plot 6, which was again being rapidly re-invaded by *G. pubescens* by Spring 2015 (Figure 6.3). It is also apparent that *G. pubescens* populations had grown back strongly after the spring burn across all treatments by autumn (Figure 6.3). However, *G. pubescens* had also increased its populations in the no-burn glyphosate and control plots at this location (Figure 6.3). Of particular note was the observation that after the burn, the larger *G. pubescens* plants had not burned through, and remained green in the center (Figure 6.4B).

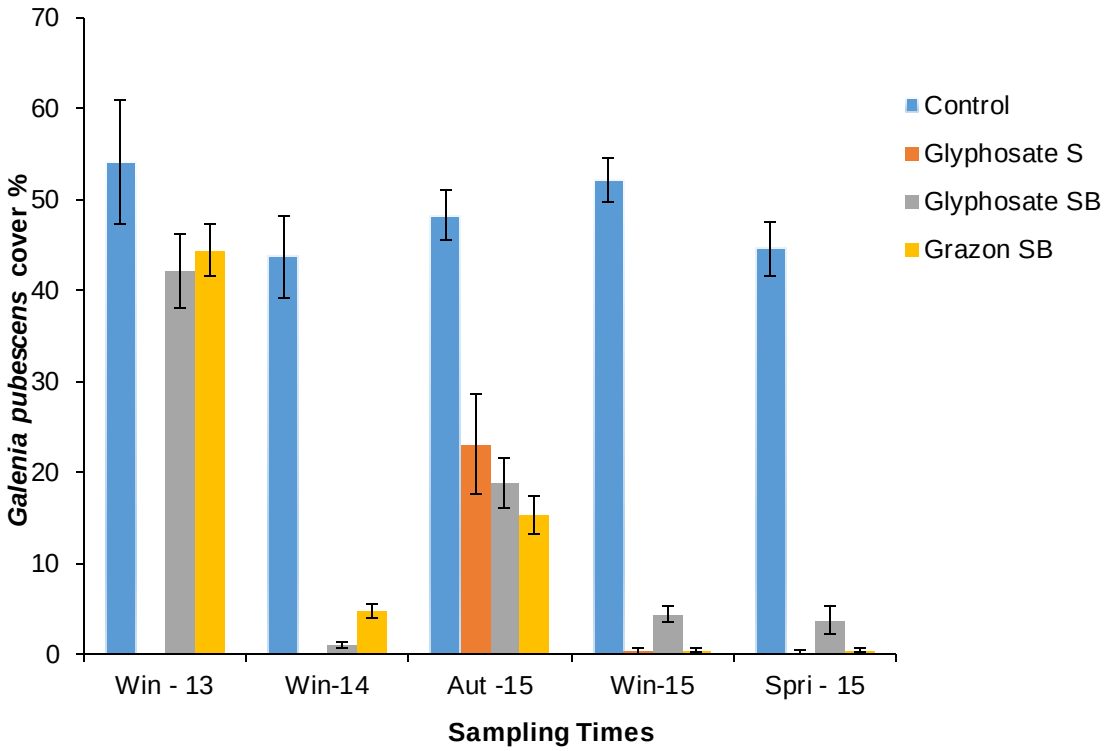

Figure 6.3. Effect of treatments on live *Galenia pubescens* percentage cover. Herbicide applications: Grazon™ Extra (applied spring 2013); Glyphosate (applied autumn 2014) (S = autumn 2014 rye grass seed application; B = Burning).

Table 6.2. Effect of burning and herbicide treatment on basal cover. P values are calculated for (a) effect of burning (No Burn + No Herbicide, No Burn + Glyphosate vs Burn + Glyphosate, Burn + Grazon) and (b) any effect of herbicide within either of two burning status (No burn or Burn). Residual degree of freedom is 5.

	No Burn		Burn		SED			P values	
	No Herbicide	Glyphosate	Glyphosate	Grazon	No burn v Burn in Glyphosate	Min Replication n=2 vs n=2	Max Replication n=3 vs n=3	Burn v No Burn	Herbicide within Burn Status
Angularly transformed basal cover(degrees) – Autumn 2015									
Bare Ground	12	14	38	40	7.6	8.3	6.8	0.003	0.97
Litter	37	38	19	16	5.2	5.7	4.7	0.002	0.74
Broadleaf Weeds	7	6	22	13	4.4	4.8	3.9	0.011	0.15
Native Grass	8	10	11	15	7.8	8.5	7.0	0.47	0.82
Alive *Galenia pubescens*	45	28	25	23	6.8	7.5	6.1	0.04	0.13
Alive Serrated Tussock	7	7	2	15	6.0	6.6	5.4	0.74	0.13
Replication (n, same in all species)	2	2	3	3					
Backtransformed basal cover (%) – Autumn 2015									
Bare Ground	5	5	38	41					
Litter	36	39	11	7					
Broadleaf Weeds	2	1	15	7					
Native Grass	2	3	4	7					
Alive *Galenia pubescens*	51	22	18	15					
Alive Serrated Tussock	2	1	0	7					

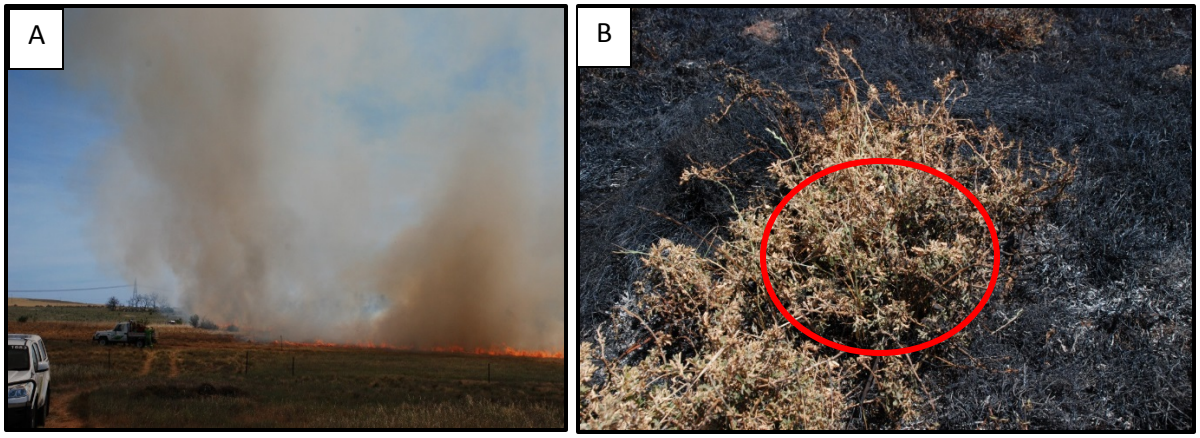

Figure 6.4. (A) Fire team burning plots. (B) Mature *Galenia pubescens* plants did not burn thoroughly and remained green in the center.

Burn effects on Galenia pubescens' seed viability

The observation that *G. pubescens'* seeds recovered after the burn indicates that at a depth of 0.5-1 cm their viability was reduced by approximately 70% when the burn was combined with chemical treatments (Figure 6.5 and 6.6); the statistical analysis is shown in Table 6.3. These results correlate closely with those found in the heat-shock treatments presented in Chapter 3.5.2. The fact that the burn heated the soil to temperatures of greater than 40 °C for 2.5 hours at 5 cm depth at one of the plots suggests that at 1 cm depth temperatures may have been higher than 40 °C.

Plot 8, which was selected for only glyphosate treatment, was not scheduled to be burnt but it was affected by fire when the fire went out of control. This plot was just on the burnt edge of the fire. Interestingly, the seed germination rate of this plot was higher than the other burn plots but lower than the non-burn plots (Figure 6.6).

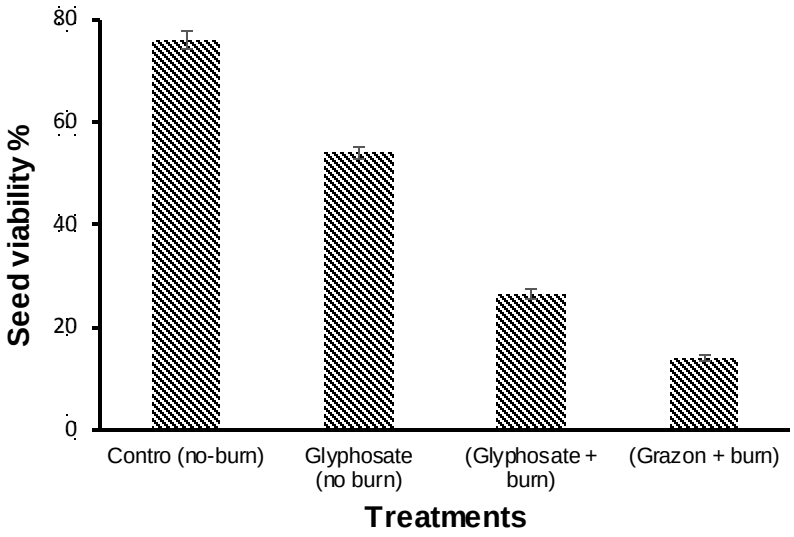

Figure 6.5. *Galenia pubescens* seed viability following exposure to a burn and no burn event and in combination with herbicides. Vertical bars represent ± standard error of the mean.

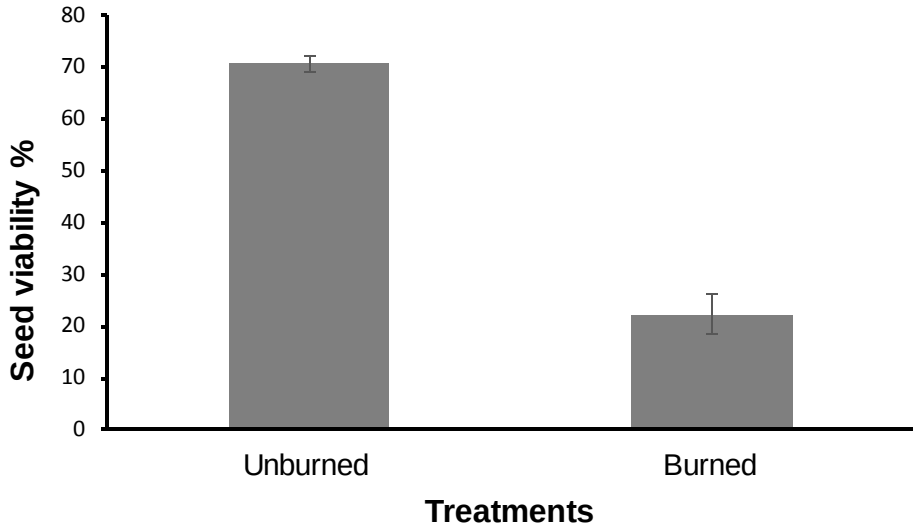

Figure 6.6. Average *Galenia pubescens* seed viability % across burnt and unburnt plots. Vertical bars represent ± standard error of the mean.

Table 6.3. Effect of burning and herbicide treatments on seed germination and temperature. P values are calculated for (a) effect of burning (No Burn + No Herbicide, No Burn + Glyphosate vs Burn + Glyphosate, Burn + Grazon) and (b) any effect of herbicide within either burning status (No burn or burn). Residual degree of freedom is 5.

	No Burn		Burn		SED			P values	
	No Herbicide	Glyphosate	Glyphosate	Grazon	No burn v Burn in Glyphosate	Min Replication n=1 vs n=2	Max Replication n=3 vs n=3[a]	Burn v No Burn	Herbicide within Burn Status
Germination Rate of seeds									
Mean	76	60	23	11	8.1	8.6	5.7	0.00011	0.094
Replication (n)	2	1	3	3					
Maximum Temperature									
$Log_{10}(y-21.4)$ transformed	0.3	0.2	0.3	0.3	1.04	1.04	0.73	1.00	1.00
Replication (n)	2	1	2	4					
Back Transformed	23.4	23.1	23.3	23.3					

6.6 Discussion

It was hypothesised that post-fire weed control activities in the burn site of Cobbledick's Ford would result in reduced weed-species richness, abundance and cover, with a corresponding increase in native species richness, abundance and cover. This hypothesis has been partly supported; significant decreases in *G. pubescens* foliage cover were identified post fire, but no significant increases in native grasses were evident to visual inspection. The fire also resulted in significantly more bare ground and less litter in the burn plots which will have increased the exposure of surface seeds to direct light post-fire.

Grazon™ Extra was applied to plots 2, 4 and 5 during a hot spell during December 2013, with applications being halted several times as a consequence of the hot weather. Grazon ™ Extra needs significant amounts of water during application to be effective (Dow AgroSciences, 2016); evaporation during these warmer conditions may have reduced the effectiveness of this herbicide on these plots as a consequence.

The major interest was upon the effects of fire on soil seedbank viability, as the management of *G. pubescens* seed density is crucial to the control of this weed. In this respect, a large proportion of seeds are located near the soil surface for this weed, and this positioning may mean that the exposed seeds will be readily manipulated by fire. Morgan (1999) found that soil surface temperatures during a fire event can be substantial (98 - 458 °C), whilst soil temperature at 10 mm depth never increased by a factor of 10 °C. It was shown that *G. pubescens* was able to germinate over a broad range of temperatures, but short bursts (5 minutes) of high temperatures (80 °C to 120 °C replicating possible exposures to a fire) reduced seed germination (Mahmood *et al*., 2016). As a consequence, it is suggested that surface seeds will most likely be

killed when exposed to fire, but fire was unlikely to damage seeds buried at a depth of 10 mm or more. Whilst actual soil temperatures will depend on the fuel load, soil type and soil moisture in addition to weather conditions during the fire, the results of this study predict that there may well be a large numbers of *G. pubescens* seeds which can remain alive in the soil at depths of 5 mm or more.

It is generally accepted that a single fire event is unlikely to eradicate many invasive species because they often produce abundant seeds and some will undoubtedly escape fire (Smith *et al*., 1999). *G. pubescens* is one of these woody invasive species that can grow sexually and asexually, so it is most likely that it will be difficult to control with a prescribed single burn since the unburnt branches may supply additional seeds. Previous studies (Morgan, 1999; Clarke & French, 2005) found that fire is most effective on annual species, in both grasses and broadleaf weeds. Herbaceous perennial grasses and forbs survive for more than two years, and are more difficult to control with fire than invasive annuals. This is particularly true for perennial forbs, since most of the problematic invasive woody species re-sprout from the base when these are injured. These species are difficult to control with prescribed burning and often require very hot burns, multiple burns, or a combination of management tools to be successful (Clarke *et al*., 2005; DiTomaso *et al*., 2006).

It was found that fire did not have the same impact across all experimental sites having regard to seedbank mortality. This seems to be due to the existence of different micro-conditions in the grassland plots, which compromised the degree of effectiveness of fire as a management tool. For example, in patches where the surface ground was not covered by litter or dry plants, and which consequently represent cool spots, it is suggested that the fire might not have reached the temperature needed to kill the seeds in the soil (Mahmood *et. al*., 2016). Seeds will thus remain viable and will

germinate when exposed to favorable weather conditions. In contrast, those patches that were covered by relatively high amounts of litter or dry plants, which consequently represent hot spots, would experience fire-temperatures sufficient to kill seeds in the soil seedbank to a depth of at least 5 mm.

Another observation of note is that some mature plants of *G. pubescens* were not burned completely (Figure 6.4) in plots that were only treated by fire without using herbicides in combination. This is largely due to the plants' prolific leaf production (Ross, 1994). This implies that green and mature plants of *G. pubescens* are difficult to control by fire alone. Indeed, a historical review (Kleinkope *et al*., 1976) indicated that the main reason for the introduction of this species into the USA was to reduce fire hazards in grasslands. It was noted in this early work that this species has prolific leaves and was known to tolerate fire, thus encouraging the introduction of the use of herbicides before a prescribed burn to facilitate desiccation and increase the flammability of these exotics that can be difficult to burn (such as *G. pubescens)*. It was subsequently found that in this investigation, plots where fire treatment was combined with chemical herbicides, the resultant reduction of *G. pubescens* cover percentage was higher, suggesting that the application of chemical herbicides to increase dry materials significantly increased the fire burn temperature which gave good results.

It has been reported that whilst control of some species has been minimal with biological, chemical, mechanical, or fire treatments being applied separately, these methods become more effective when combined (Paynter & Flanagan, 2004). Subsequent fire treatment provides additional opportunities to disrupt the life cycle of invasive species through direct seed mortality, whereas it seems that single methods

often affect only established individuals. Manipulating fire behavior to promote high seed mortality may then hasten and improve restoration efforts if coordinated with management strategies that target invasive individuals surviving fire or at different stages of their life cycle.

In regard to fire effectiveness on native species, previous studies commonly observed that fire events stimulate native species, particularly when the infestation of invasive weeds was removed or significantly reduced. This could be due to native seeds having a strong dormancy stage which is broken by fire, giving a population of new seedlings. In addition, the fire will clean litter from the ground, giving seedlings better light exposure (Ooi *et al*., 2006).

In conclusion, the results obtained in this research indicate that post-fire management of priority weed species has benefits that are achievable in the short-term. The significant reduction in the cover of *G. pubescens* in test plots that receive herbicide applications plus burning indicates the effectiveness of this combination of control methods. This reduction of the *G. pubescens* may enhance native species recruitment, leading in turn to improvements in the floristic and vegetative community structure in the study area in the future.

Chapter 7: Investigations into the Effects of Elevated Carbon Dioxide and Drought on the Growth and Physiology of the Invasive Weed *Galenia pubescens*

This Chapter has been published as a Conference paper:

Mahmood, A. H., Florentine, S., Fernando, N., McLaren, D. A., Wright, W., Palmer, G. C., & Sillitoe, J. (2016). Investigations into the effects of elevated carbon dioxide and drought on the growth and physiology of carpet weed (*Galenia pubescens* Eckl. & Zeyh.), *20th Australian Weeds Conference,* Perth, September, 2016.

A copy of this paper is provided in the Appendix **C** of this thesis.

7.1 Summary

The rise in atmospheric carbon dioxide (CO_2) concentration and it's interaction with water availability have been predicted to change the future distribution of invasive species. The present study aimed to examine the interactive effects of elevated atmospheric CO_2 concentration and drought stress on the growth and some of the physiological processes of *G. pubescens*. One month old *G. pubescens* plants from the glasshouse were transferred to growth chambers of ambient (400 ppm) and elevated (700 ppm) CO_2 concentrations for 60 days. Day/night temperatures were maintained at 22°C and the photoperiod was 12 hour of the chambers. Prior to the experiment, all the pots were watered to field capacity. Drought condition was induced for half plant under both CO_2 concentrations before the start of CO_2 measurements. The photosynthetic rate of plants were seen to increase under elevated CO_2 concentration, however simulated drought condition caused significant reduction in net photosynthetic rate by (45% in 400 ppm CO_2) and (27% in 700 ppm CO_2) after five days when compared with well-watered plants. Plants grown under elevated CO_2 level with ample water, produced a greater biomass (17.5$\pm$ 0.5 g per plant) compared to the plants which were grown under the ambient CO_2 concentration in both well-watered (15.7 g $\pm$ 0.7 g per plant) and drought treatments (12.9 $\pm$ 0.3 g per plant). Results of

this study indicate that *G. pubescence* plants may have an advantage in responding directly to increasing atmospheric CO_2 concentration in the future if the annual precipitation rate is adequate.

7.2 Introduction

Plant invasion can be developed or hindered as a result of changes in both the climate and ecological processes. Changes in climate include change in precipitation patterns, rise in atmospheric temperature and the onset of elevated CO_2 levels. In addition, climate change facilitates deposition of nitrogenous material and can stimulate fire regime changes that can lead to changes in resource availability (Miri *et al*., 2012; Sheppard & Stanley, 2014). Biological changes caused by heightened levels of carbon dioxide and variations in temperature and precipitation patterns will have major impacts on the distribution and population levels of invasive species, in addition to affecting the composition and relationships between species in ecosystems (Chornesky & Randall, 2003). Temperature changes can change the phenology and subsequently alter the life cycles of species, which may lead to longer growing seasons in mid and high latitudes. Other outcomes are that a species' range shifts towards the poles, and consequent establishment at higher altitudes leads to changes in the reproductive cycles of plant and animals (Parmesan, 2006). Also, drought may become more common under the projected climate change (Perry *et al*., 2013), and evidence to date shows that plant growth and productivity responses to elevated CO_2 are controlled by drought, depending on its severity and duration as well as on the plant species (Xu *et al*., 2013).

The current observations of the effects of climate change also include an increase in the amount of CO_2 concentration in the atmosphere. Some reports predict that CO_2

concentrations in the air may increase from the present-day 380 ppm (present day) to 550 ppm by 2050 (Franks, 2013) and then to surpass 700 ppm to 1000 ppm before the end of the 21st Century (Dietz & Stern, 2009; IPCC, 2015) This increase in atmospheric CO_2 concentration will affect plant growth in a variety of ways (Dukes, 2011; Plett *et al*., 2015), as it can have a direct impact upon photosynthesis and metabolism (Sage *et al*., 1995).

The rise in CO_2 availability directly impacts photosynthetic processes, evoking a wide range of physiological and morphological responses in plants (Plett *et al*., 2015). These vary among species, depending on differences in photosynthetic pathways, intrinsic growth rates, and other properties. Common responses include changes in growth rates (Poorter, 1993), allocation patterns (Bazzaz, 1990), water use efficiency (Eamus, 1991), and nutrient uptake rates (BassiriRad *et al*., 1996; Jackson & Reynolds, 1996). Carbon dioxide is essential for plant growth as a source of carbon necessary for photosynthesis, and it influences the regulation of stomata which in turn influence water vapour and gas exchange (Mishra *et al*., 1999; Chaves *et al*., 2009). Elevated CO_2 levels may enhance plant diversity and productivity in an entire ecosystem by decreasing stomatal conductance (gs) and consequently increasing water use efficiency (WUE) and soil water availability (Nelson *et al*., 2004; Morgan *et al*., 2011).The stimulation of plant growth by elevated CO_2 may be weakened under drought, and even prohibited under severe drought. Nevertheless, compared to the well-watered condition, final plant biomass shows decreases with drought, even under elevated CO_2 (Poorter & Pe´rez- Soba 2001; Perry *et al*., 2013).

Additionally, a study by Kohli *et al*., (2006) noted that the growth of an exotic species *Pathenium hysterophorus* (ragweed) will be significantly enhanced in the future where there is an enriched atmospheric CO_2 concentration, producing taller plants with a

greater biomass, and presumably with a higher seed output. Another important issue to note is CO_2 fertilization, which can be defined as enhancement of plant growth or net primary production by CO_2 enrichment that could occur in natural or agricultural systems as a result of an increase in the atmospheric concentration of CO_2 (Lobell & Field, 2008). It has been shown that CO_2 fertilization intensifies the weed problem. Interestingly, because plants, in the process of photosynthesis, convert CO_2 into oxygen, it is sometimes argued that such "CO_2 fertilization" could potentially provide a strong negative response on changing CO_2 concentrations. This mainly happens in grazed rangelands where it was noted that there was an increase in woody weeds (Berry & Roderick, 2002). Another factor that may facilitate woody weed invasion is the presence of C_4 photosynthetic pathways' species. These species use less water to make dry matter and may therefore make the extra water become available for woody weeds to invade (Hsiao & Acevedo, 1974). Importantly, it has been estimated that climate change potentially can lead to the spread of at least 20 weeds which are important to the Australian economy. This was confirmed by use of climatic models on the major invasive plants of economic importance (Aghighi, 2014) including *Rubus allegheniensis* (blackberry), *Mimosa pigra* (mimosa), and *Asparagus asparagoides* (bridal creeper).

The future potential of growth characteristics of *G. pubescens* under an elevated atmospheric CO_2 concentration and drought are unknown. Hence, the objective of this study was to assess the effects of elevated CO_2 and water availability on the physiology and growth on the exotic weed *G. pubescens* in order to improve management.

7.3 Materials and methods

7.3.1 Seedlings establishment

Mature *G. pubescens* seeds were collected from several populations of mature plants in the Werribee region (37º 49' 5.63" S 144º 34' 58.77" E), and cleaned seeds were placed in an airtight container until needed.

Soil used for this trial was collected from the same above mentioned location. All vegetative materials such as leaves, rhizomes and litter were separated from soil samples using a sieve (4 mm mesh). Approximately 25 kg of cleaned soil was then placed in three biohazard bags and incubated in the Autoclave (Horizontal, bench-top Autoclave Systec DX–90, Germany) using a wet cycle run for a period 2.5 hours at 250 °C to kill any viable seeds or propagules. The sterilized soil was then air dried in the lab before use.

In the glasshouse, 20 plastic pots (13 cm diameter and 13 cm high) were prepared as follows: for soil moisture movement and aeration, the sterilised industrial, seed-free sand soil was placed to a depth of 1 cm lined with paper towel. One kg of well-mixed dry sterilised soil was placed into each pot. On the top of each pot ten *G. pubescens* seeds were sown. Pots were labelled and placed into large white trays (44 cm x 36 cm x 7 cm) to facilitate watering from below, which ensured minimal disturbance of seeds and later, of germinants. Following emergence (4-6 leaf growth stage), seedlings were thinned to one per pot and transferred to CO_2 chambers on 9th November 2015.

7.3.2 Setting-up CO_2 chambers and growth conditions

Two identical *Steriudium e2400* model chambers were used for this experiment. These chambers make it possible to control temperatures, relative humidity, light and CO_2 concentration. Temperature, relative humidity and light settings were maintained at

the same levels inside both cabinets in order to maintain the same growing conditions. Day/night temperatures were 22°C and the photoperiod was 12 hours. Photosynthetic photon flux density during the light period was maintained at 1000 ± 50 µmol $m^{-2}s^{-1}$ with sodium vapour and metal halide lamps. Day/night relative humidity was approximately 50/70%.

Each chamber was set-up with a different CO_2 concentration. One of the chambers was set at an ambient atmospheric CO_2 concentration (400 ppm), simulating the present concentration of CO_2 in the atmosphere. The second chamber was placed at elevated atmospheric CO_2 concentration (700 ppm), simulating the likely future concentration of atmospheric CO_2 (by the end of this century) as predicted in climate change scenarios (Franks, 2013).

Thereafter, 10 pots (randomly chosen) were placed in one growth chamber (with a CO_2 concentration of 400 ppm). Another 10 pots (randomly chosen) were placed into the other growth chamber (with a CO_2 concentration of 700 ppm). All pots were watered to field capacity based on need for water. One week prior to the taking of measurements in each CO_2 chamber, half of the plants from each chamber (randomly chosen) were kept well-watered and the other half were allowed to dry out slowly until visible wilting occurred.

7.3.3 Plant measurements

Physiological parameters

The net photosynthetic rate (A; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}) and stomatal conductance (gs; mol H_2O m^{-2} s^{-1}) were measured using a portable gas exchange system (Li-6400XT, Li-Cor, Lincoln, NE, USA), following methods described by Le *et al*., (2016) with the following adjustments: mass flow rate 0.33 mol

m^{-2} s^{-1}, atmospheric pressure 96.5 kPa, photosynthetically active radiation 1000 µmol m^{-2} s^{-1}, water vapour pressure at the outlet of leaf chamber ranged from 1.9 to 3.7 kPa, temperature of the ambient air in the leaf chamber ranged from 21-23 °C, and temperature of leaf ranged from 21-24 °C, with the block temperature and relative humidity of the chamber being 20 °C and 55%, respectively. The physiological parameters were recorded at four times. The first measurement began at 60 days after emergence (DAE) and continued four times with an interval of 7 days. The CO_2 concentrations were adjusted to 400 ppm and 700 ppm for plants grown in separate CO_2 cabinets. The infrared gas analyzer (IRGA) was matched every time after changing the CO_2 concentration. The measurements were recorded between 10:00 am to 12:00 pm and one healthy, fully extended leaf was selected from each plant for measurement. Finally, the water use efficiency (WUE; µmol CO_2 mmol-1 H_2O), which is defined (Eamus, 1991) as the measure of a cropping system's capacity to convert water into plant biomass or grain, was measured. This includes both the use of water stored in the soil and rainfall during the growing season, and was determined by dividing the net photosynthetic rate value by the transpiration rate value (Shabbir *et al*., 2014).

Vegetative parameters

After the completion of the trial, which corresponded approximately to the end of the vegetative stage and at the beginning of flowering, the following morphological parameters were measured: The number of branches that were present on each plant was recorded. Then the plants were harvested from the soil surface and the stems and leaves were separated and kept in labelled paper bags and oven dried at 70 °C for 48 hours. The root system from each plant was removed from the soil and washed thoroughly, were put in labelled paper bags separately and oven dried at 70 °C for 48

hours. The dry leaves were added to the dry branches, to determine individual dry biomass. The root/shoot ratio was measured by dividing the root dry weight to the branch dry weight.

7.4 Experimental design and data analysis

In order to statistically account for any potential influence of CO_2 concentration and drought treatments on plant responses, a randomized complete design was used with two treatments and five replications. Data were analysed using a general linear model (GLM). The GLM was set up with two atmospheric CO_2 concentrations, two soil moistures and the interaction as the main factor. An analysis was then undertaken using an adjusted sum of squares approach using 95.0% confidence intervals. A two-way analysis of variance tests was run for each parameter separately to detect differences caused by each factor using the Statistix 8.1 Regd package. Means were compared by Tukey's test at a 5% level of probability.

7.5 Results

7.5.1 Physiological parameters

Figure 7.1 A shows the effects of atmospheric CO_2 concentration and water availability on the net photosynthesis rate of *G. pubescens* seedlings at 60 days after the placement of plants in CO_2 chambers, with measurements being taken four times (with 48 hours between each recorded date). It should be noted that the plants under elevated CO_2 concentration recorded significant ($F= 9.60$; $P < 0.05$) increases in the mean net photosynthesis rates (21.3 μmol mol^{-1}) on all recorded date when compared with the plants in ambient atmospheric CO_2 concentration (13.3 μmol mol^{-1}) under the well-watered treatment. While, moisture stress significantly decreased the rate of net photosynthesis, the decrease was greater in plants that were grown under elevated CO_2 conditions.

The average transpiration rate was slightly higher in plants that were grown under ambient CO_2 level when watered well as compared with those grown under elevated CO_2 conditions. However, data indicated that this rate was not significantly different (F= 0.87; P= 0.478). The drought treatment affected the transpiration rate under both CO_2 concentrations, with data showing that the duration of drought significantly reduced transpiration rates in both CO_2 concentration (Figure 7.1 B).

An estimate of the instantaneous water use efficiencies of the plants was made by dividing the net photosynthetic rate by the average transpiration rate. The water use efficiency was significantly different (F= 17.10; P=0.000010) and data showed an approximate doubling in the instantaneous water use efficiency between ambient and elevated CO_2 from average 4.3 to 7.6 respectively in well-watered treatments. However, the duration of drought did not result in significant differences in either CO_2 levels or water availability treatments (Figure 7.2 A).

In the well-watered treatment, stomatal conductance rates were not significantly different (F= 1.187; P= 0.175) in plants in the elevated CO_2 concentration compared to ambient CO_2 concentration. However, there was an overall significant reduction in stomatal conductance in the plants with continued drought treatment ($P< 0.05$), though this was not significant at any of the individual sample times between both CO_2 levels (Figure 7.2 B).

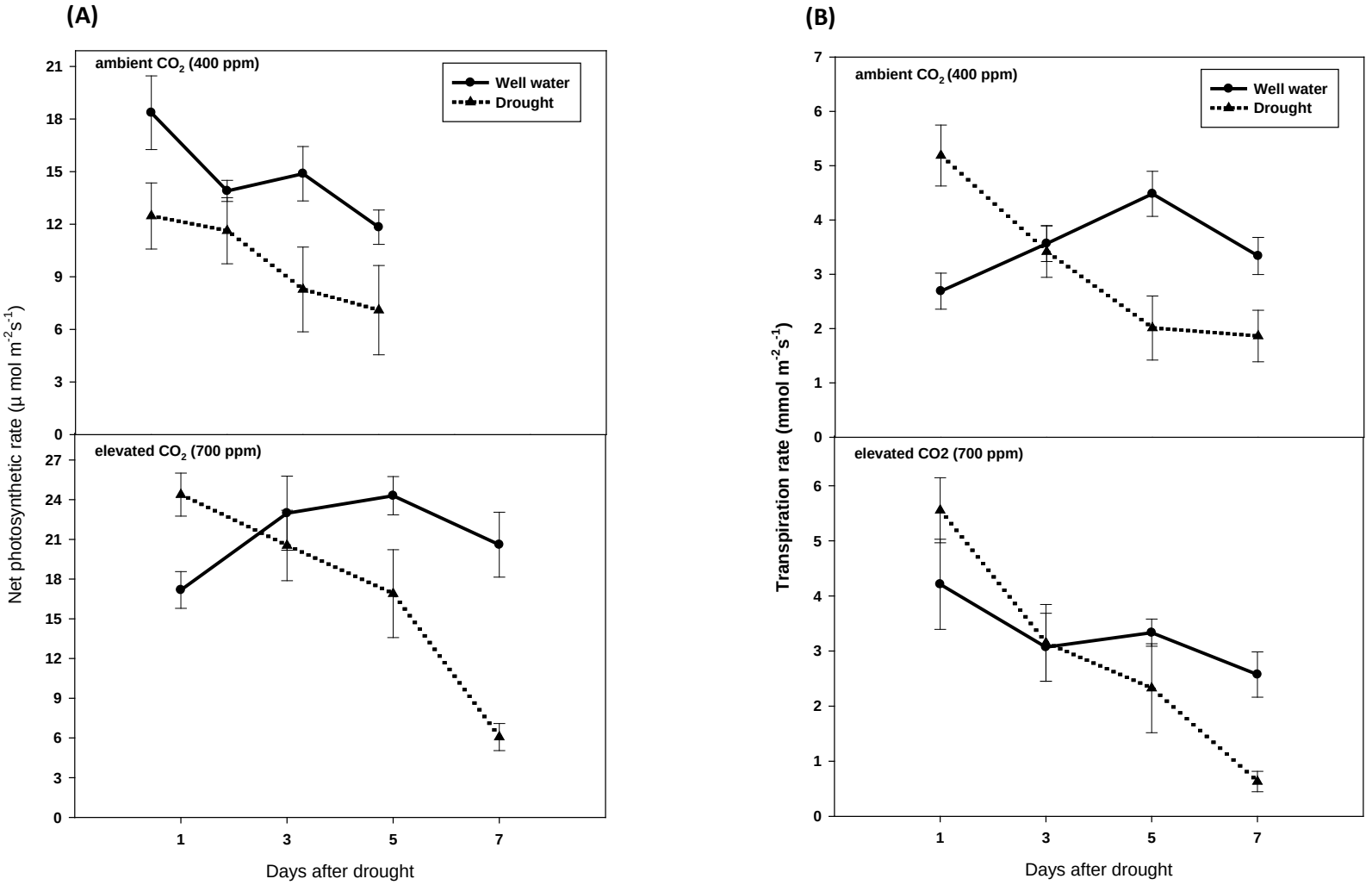

Figure 7.1. Effect of ambient (400 ppm) and elevated (700 ppm) CO_2 concentrations on net photosynthetic rate (µmol $mol^{-2}S^{-1}$) (**A**) and transpiration rate (mmol $mol^{-2}S^{-1}$) (**B**) of *Galenia pubescens* plants growing under well-watered and drought conditions. Vertical bars show standard errors of the mean value derived from the five replications.

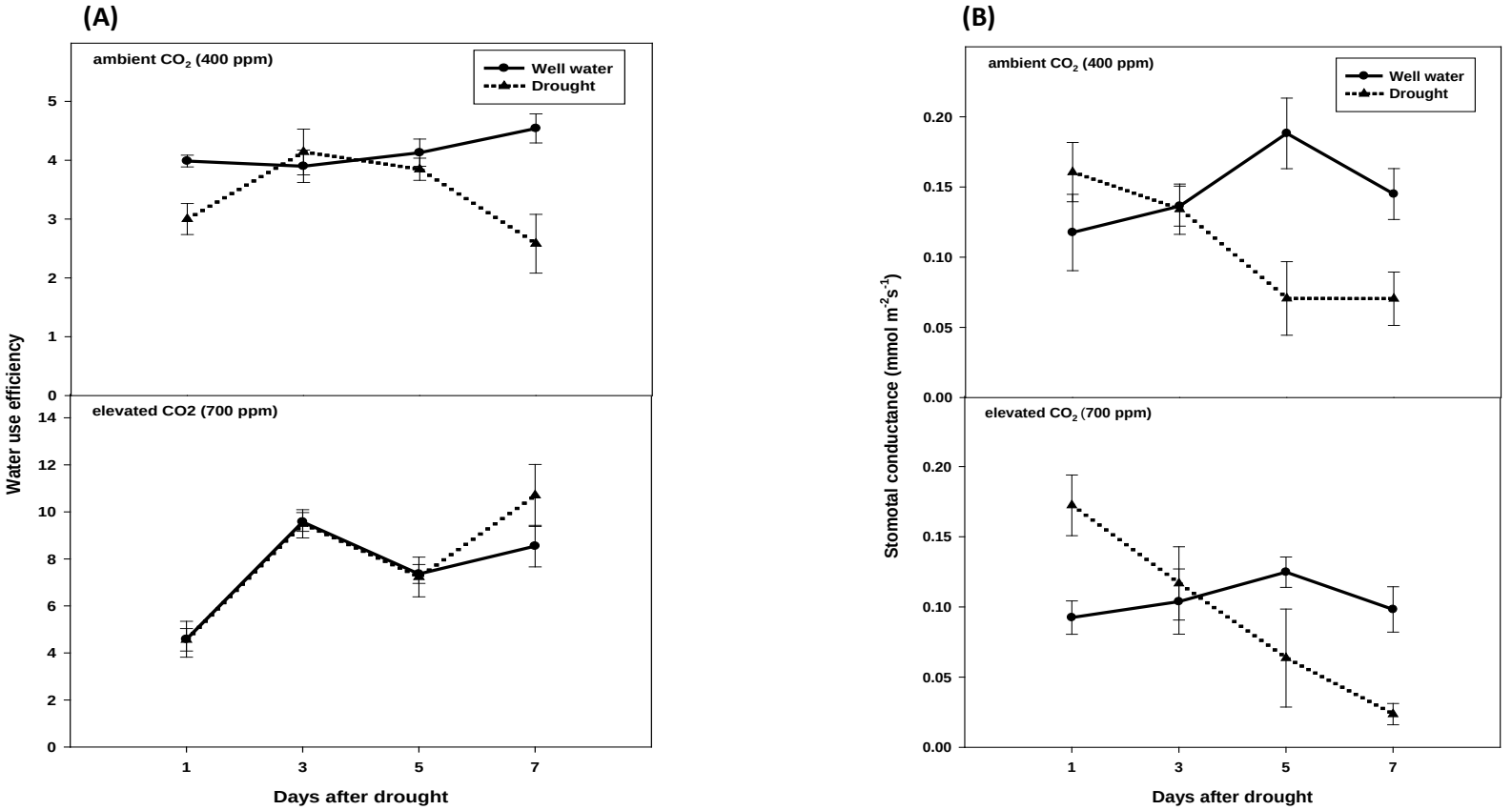

Figure 7.2. Effect of ambient (400 ppm) and elevated (700 ppm) CO_2 concentrations on of the water use efficiency (**A**) and the stomatal conductance (mmol $mol^{-2}S^{-1}$) (**B**) of *Galenia pubescens* plants growing under well-watered and drought conditions. Vertical bars show standard errors of the mean value derived from the five replications.

7.5.2 Vegetative parameters

The *G. pubescens* plants did not differ significantly (F = 1.68; P = 0.2132) in their production of dry biomass under changed atmospheric CO_2 concentrations. However, when water availability was considered, the dry biomass was significantly different for plants in well-watered and drought treatments (Figure 7.3 A). Similarly, elevated CO_2 concentration did not have a significant effect on root shoot ratio; however, data showed that the ratio was slightly decreased by increasing CO_2 concentrations in both well-watered and drought treatments (Figure 7.3 B). Regarding dry root weight, data showed no significant difference (F = 2.26; P = 0.152) among plants under both CO_2 levels and water availability treatment (Figure 7.3 C). Dry leaf and dry shoot weight were affected by changing CO_2 levels; in contrast, water availability significantly affected dry leaf and dry shoot weight (Figure 7.3 D and E). The number of branches per plant was not significantly affected (F= 2.69; P= 0.1203) by changing atmospheric CO_2 concentration. However, the number of branches per plant was different under well-watered and drought treatments (Figure 7.3 F).

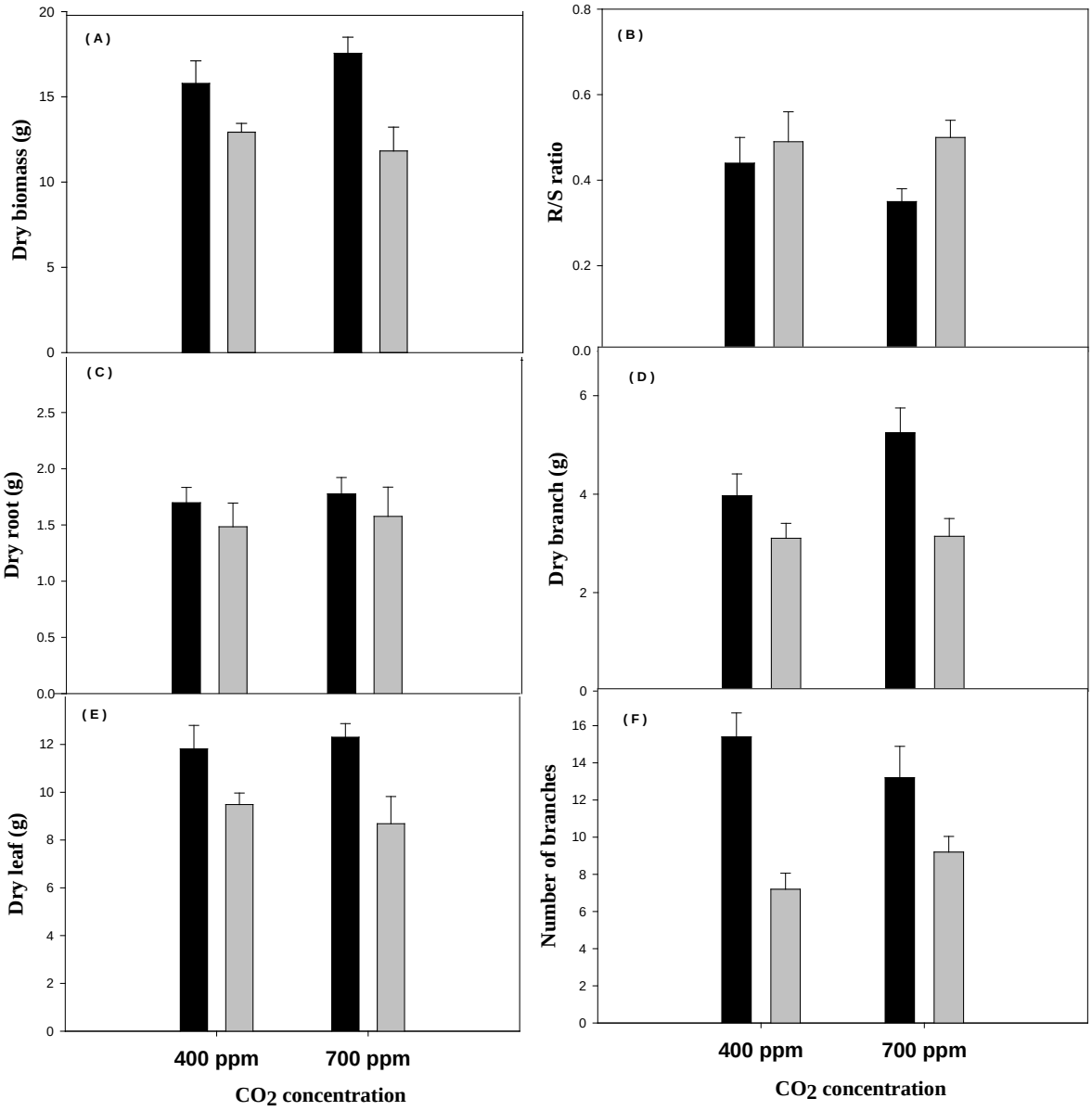

Figure 7.3. The effect of atmospheric CO_2 concentrations on vegetative growth of *Galenia pubescens* plants growing under well-watered (■) and drought (▭) treatments. (A) dry biomass, (B) root/shoot ratio, (C) dry root, (D) dry shoot, (E) dry leaf and (F) number of branches. Vertical bars show standard errors of the mean value derived from the five replications.

7.6 Discussion

Elevated CO_2 is beneficial for *G. pubescens* growth under well-watered conditions. The photosynthesis rate and the percentage of dry biomass were increased subsequently, whereas under drought conditions, elevated CO_2 markedly showed reductions in the photosynthesis rate and dry biomass of the plants. Similar results were obtained by Erice *et al*., (2007), who reported that plant biomass increased under elevated CO_2 under well-watered conditions, but under drought, CO_2 concentrations had no significant effects on the leaf, shoot, and root dry weight in either CO_2 concentration. These responses are in contrast with those for many herbaceous plants (Farrar & Williams, 1991). From previous research, it was expected that the elevated atmospheric CO_2 concentration might stimulate the growth of plants, either directly through enhanced photosynthesis or indirectly, through reduced plant water consumption (Morgan *et al*., 2004). Similar stimulations in growth and photosynthesis were observed for *Cirsium arvense* (thistle) as a recognised invasive weed, under an elevated atmospheric CO_2 concentration (Ziska, 2002).

The water use efficiency (WUE) of *G. pubescens* plants was enhanced under the elevated atmospheric CO_2 concentration. As is well known, WUE increases under higher CO_2 levels, especially in moderately dry conditions (Housman *et al*., 2006; Kim *et al*., 2006). Leaky *et al*., (2009) proposed that the potential for increased growth and yield of C_4 plants at elevated CO_2 concentrations relies on the decrease in water use and reduction caused by drought stress, and not a direct effect of increased photosynthesis. Huxman *et al*., (1998) reported that increases in the maximum net photosynthetic rate and WUE of *Larrea tridentate* (creosote bush) seedlings under a high CO_2 concentration with a short period of drought disappeared as the severity of the drought increased. Furthermore, severe water stress also damaged the

photosynthetic apparatus, which may indicate that WUE does not increase with elevated CO_2 (Zhao *et al*., 2004). Moreover, severe drought may induce stomatal closure (Warren *et al*., 2011). Shabbir *et al*., (2014) documented that plants exposed to elevated CO_2 concentrations lose less water via transpiration and as a result water use efficiency increases as the atmospheric CO_2 content rises. Similarly Garcia *et al*., (1998) found a one-third increase in water use efficiency of wheat plants grown at 550 ppm CO_2, as compared with the ambient CO_2 concentration. This enhancement in water use efficiency under elevated atmospheric CO_2 concentrations has important implications for the future abundance and spread of *G. pubescens,* and for the control program that will need to be applied to manage it in a changing climate.

Past studies have shown that stomatal conductance frequency decreased with increasing CO_2 concentration (Woodward, 1987; Clifford *et al*., 1995; Ghannoum *et al*., 1997), although in this study it appears that the stomatal conductance reduced only slightly with increasing atmospheric CO_2, but not significantly so. Furthermore, in a study conducted in an open-top chamber by Adams *et al*., (2000), a doubling of atmospheric CO_2 concentration was shown to cause consistent reductions in stomatal conductance and transpiration water losses in the dominant C_4 plant *Andropogon geradii* (tallgrass prairie), thus contributing to significant increases in water use efficiency.

Thus, under the predicted future climatic condition in many semiarid regions worldwide (Rogeli *et al*., 2012), plant productivity may decline under a combination of elevated CO_2 with severe drought compared to the combination of elevated CO_2 and well-watered conditions that may take place in these low-latitude semi-arid regions (Leakey *et al*., 2012).

It can be concluded, that this study supported the view that the growth of *G. pubescens* will be significantly enhanced in a future climate with an enriched atmospheric CO_2 concentration, producing taller plants with a greater biomass, and presumably with a higher seed output under well-watered conditions. These changes will have important implications for management of this noxious weed in the future. The required actions in terms of management are twofold: firstly, given the future vulnerability of large tracts of Victorian land in terms of CO_2 concentrations, water availability and ambient temperature, then the movement of *G. pubescens* must be carefully monitored. This monitoring should extend beyond the observations of locals landowners or users, and become the formal responsibility of such government bodies as the Department Environment, Land, Water and Planning. Secondly, action to control and then eliminate the current infestations of this weed is urgent, because on predictions based on current modelling, time is on the side of the weed since delay will only be to its advantage. It is therefore recommended that such management and elimination programs as described in pervious chapters be implemented as soon as is practically possible, and that this approach to the problem become part of larger and broader management program which would ensure that *G. pubescens* does not become an even greater threat under climate change in the future.

Chapter 8: General conclusion

8.1 Introduction

In part one, this thesis set out to extend what is known about *Galenia pubescens* in order to better understand the ecological niche occupied by this plant. Such knowledge will assist in understanding the ecological reasons for the success of *G. pubescens* in the grasslands of western Victoria, Australia, where its presence at infestation levels has severely reduced the biodiversity of the local environment and compromised agricultural activity. A series of experiments was conducted, targeting the capability of *G. pubescens* seeds to germinate under various environmental conditions. In part two, a range of strategies to counter the threat posed by this invasive species was developed and investigated to determine those management options which would most effectively reduce the populations of mature plants and decrease seedbank composition.

This chapter revisits the key findings (Figure 8.1) in order to elucidate the ecological reasons for the success of *G. pubescens* and to set out some management principles for its effective control. Finally, future research priorities in this and related areas are described.

8.2 Research findings

There are several ecological characteristics of *G. pubescens* that contribute to its increasing dominance in the western grassland regions of Victoria, Australia. Various environmental factors acting on seed germination of *G. pubescens* have been set out in chapter 3, where a summary of the ecological characteristics of *G. pubescens,* as identified by this research, is to be found.

Temperature requirements for the germination of *G. pubescens* demonstrated the potential of its seedbank to germinate all year round in western Victorian conditions. Germination occurred between 7°C and 30°C. The temperatures in western Victorian grasslands are suited to this range; seeds of *G. pubescens* are therefore able to germinate at any time in the annual cycle. Light was found to be essential for the germination of *G. pubescens* seeds. A 12:12 light/dark photoperiod system showed significant increases in germination percentages compared to 24 hour dark conditions. *G. pubescens* seeds are capable of germination under low to medium levels of salinity stress, but are quite sensitive to osmotic potential. *G. pubescens* was shown to have high germination rates on the soil surface. The field seed-burial trial showed that seed viability declined significantly after 30 days. This suggests that, if *G. pubescens* propagules can be properly managed, the seedbank will decline. Such management should therefore contribute to the long-term control of *G. pubescens.*

Findings in regard to the management practices for Galenia pubescens

Galenia pubescens is a perennial broadleaf weed which produces massive amounts of seeds every year; effective long-term management therefore needs to target the seedbank. Such management would aim to achieve both a reduction in the seedbank and the minimization of future seedbank inputs. The success of any strategy to reduce the seedbank through the destruction of stored seeds in soil is in turn dependent on the effective control of new seedling emergence. This involves the prevention of native new plant numbers, the reduction of existing high plant numbers, and the promotion of species to provide competition. These measures are designed to prevent or substantially limit the seedbank replenishment abilities of *G. pubescens*.

Given the ability of *G. pubescens* seeds to germinate virtually the whole year round, there is a requirement for monitoring and control be undertaken on a perennial basis also.

With regard to the management strategies, it was found that in extremely degraded grasslands infested by noxious weeds such as *G. pubescens,* there is also an accompanying absence of desirable and competitive native plants. In such areas, grassland weed control measures are often only of short term efficacy because native species are not available to occupy those niches opened up by the weed control procedures. Introducing and establishing competitive plants is essential for the successful management of weed infestations and the restoration of desirable plant communities. However, this re-vegetation procedure is often not included in a weed management plan because of its relatively high cost, and the high risk of failure.

The long-term management of *G. pubescens* requires an integrated approach to weed management – that is, the implementation of programs that involve the simultaneous application of a combination of control measures rather than the reliance upon any single-control method. These control methods may include chemical herbicides, organic herbicides, burning and physical removal. Combining the factors discussed above will have wide applications for the types of grassland areas so far studied. Cost-effective and reliable methods need to be developed to more satisfactorily achieve the establishment of native species; these ends will best be achieved by applying the various control methods in a single pass.

The use of herbicides, even when used in combination with non-chemical options, must be carried out in ways that minimise the negative impacts of these chemicals

upon the environment and to minimise the plant's opportunity to build resistance to a single herbicide.

This research provided key new knowledge for the use of organic herbicides such as pine oil as a means to reduce the weed seedbank in the soil in a wide range of grasslands.

The other effective method found by this study to control *G. pubescens* was mulch application, the mulch used here being a combination of white sugar and sawdust. It was found that this treatment had a positive effect in reducing the infestation of *G. pubescens* and also inhibited seedling emergence. Another advantage of mulches relates to their promotion of the activity of microorganisms in the soil, which then absorb soil nutrients as they grow, reducing the availability of those nutrients for weeds. The relative lack of nutrients will inhibit weed growth while simultaneously providing a better soil environment for native plants, which can grow well in low nutrient conditions.

It was also found that limiting control methods to the broadcasting native seeds alone in areas of heavy infestation by *G. pubescens* did not significantly affect the cover percentage and seedbank composition; however, in plots where seeding treatments were combined with other control techniques, the results were significant.

The results in Chapter 6 showed that the combination of chemical control and late spring burning reduced the cover of non-native species, such as *G. pubescens,* suggesting that this could be a useful tool in its management. The combination of these (well-known) control measures is central to success here.

It is recognised that a relatively small and short-term project, such as this investigation has necessarily been, cannot answer all the questions that need to be asked and answered about weed ecology and weed control. However, it has been useful with

respect to certain strategies in the management of *G. pubescens*, and has opened up areas of potential interest for future researchers.

8.3 Future Research

With regard to the ecological part of this thesis, the influence of environmental factors was only investigated as far as they related to the life-stages of the *Galenia pubescens* seed, and then only under laboratory conditions. Further research is therefore required to investigate the influence of the same factors upon other stages of the life-cycle of this species, and it is recommended that these investigations be made under field conditions given that this species is a perennial and that its seeds can be viable for several years in the ground.

The time of emergence of seedlings is the most vulnerable period in the life cycle of most plants, including *G. pubescens*. In the context of weed control, the ongoing investigation of growing stages may achieve more precise information of weed species within their environment, and this focus may reveal when and how these species may best be targeted for control. It is suspected that the earlier in its life-cycle a species can be controlled, then the lower are its chances of reaching reproductive maturity. This can serve to reduce the risk of infestation in the long term.

Most plants exhibit allelopathic effects on seed germination, growth and development of other plants by releasing allelochemicals into the soil, either as exudates from living organs or by decomposition of plant residues. Although rapid growth and prolific reproduction of *G. pubescens* clearly contribute to its successful invasion, it might have strong allelopathic effects beside the physical competition capability on native plants. It may therefore be of interest to test and check the allelopathic effect on different seeds (from native and exotic species) which commonly associate with *G. pubescens* in the native Victorian volcanic plains grasslands. This will further extend

and clarify our knowledge about this biological aspect of the *G. pubescens* species, which may in turn open up the possibility of another organic management strategy. Indeed, at this point it seems that the suite of options for integrated weed management will only be extendable to biological control agents if, and when, more research is done upon this aspect of *G. pubescens*.

Plant invasion can be developed or hindered as a result of numerous changes in the climate and ecological processes. Changes in climate include; change in precipitation pattern, rise in temperature, CO_2 levels and deposition of nitrogenous material, and fire regime changes that can lead to changes in resource availability. Our work in ecological restoration recognized that beside habitat deterioration and the establishment of invasive species, climatic change are believed to be most important threats to biodiversity. The Western Grassland Reserve occurs within a rain shadow and therefore the climate-sensitive nature of the region requires particular consideration with respect to management of invasive exotic weeds such as *G. pubescens*, therefore, a further avenue for future research are bioclimatic mapping studies, which would look at the future distribution of *G. pubescens* under climate change conditions. While, in this study the responses of *G. pubescens* to rising CO_2 levels were investigated, the future distribution of this species with potential increasing mean annual temperature in Australia and other parts of the world still remain unknown. An understanding of the responses of *G. pubescens* to climate change will help relevant agencies to develop informed policies whose implementation may reduce the threat currently posed by this species, and prevent its possible invasion into new areas under new conditions.

8.4 Conclusion

The findings presented in this thesis have significantly extended our current knowledge of aspects of the ecology of *G. pubescens.* On the basis of these insights, it has been possible to explain some of the ecological reasons for the success of this species within the western Victorian grasslands. This has opened the possibility of the development of management methods specifically designed to curtail the spread of this species over the short and long term. These methodologies, developed in this study, should be useful in the prediction of future weed problems as well as of species shifts within these semi-arid grasslands. Strategies for potential management of this species have been summarized in flow-chart form in Figure.8.2.

Investigations into the ecology and management of the invasive weed (*Galenia pubescens*)

Seed ecological trials

Effects of temperature regime and photoperiod

- Greater than 90% seeds germinated in wide range of temperature regimes, but the optimum (T°) was found 17/7 °C 12:12 Light /dark.
- Seed germination was positively favoured by light.

Effects of heat and smoke water

- Short bursts (5 minutes) of heat (80 to120 °C) significantly reduced seed germination by more than 80 %.
- This indicated that fire will be an effective tool to reduce viability of seeds.

Seed age and Longevity trial

- Field burial trial showed that seed viability % declined to more than 70% after 1 year from burial.

Effects of depth

- The emergence of seedlings decreased with increases in planting depth.
- No seedlings emerged from seeds placed at depths of 2 to 5 cm.
- This indicating that buried seeds will remain in a dormant state until disturbed.

Effects of salinity and water stress

- Seeds were moderately tolerated to salinity. Over 50% of seeds germinating at low level of salinity (60 mM NaCl).
- Water stress greatly reduced germination of seeds. More than 45% germinating reduced at -0.2 MPa.
- Germination was completely inhibited at -0.4 to -1.0 MPa.

Effects of pH levels

- pH levels from 4 (acidic) and to 10 (alkaline) did not affect germination percentage.
- This indicates that the seeds are capable of germination in different type of soil.

Management strategies

Do nothing (control)

Not effective

- The percentage foliage remained above 60% and the average soil seedbank composition was over 80 seedlings per plot after 2 years from monitoring.

Pine oil

Effective

- Foliage cover changed significantly. In 2013 prior treatment 61%; in 2014 reduced to 15% after 6 month from application.
- 5% or greater of pine oil concentration destroyed 100% of viability of the seeds.

Glyphosate

- Not effective in reduction of seedbank composition
- An effective technique to kill mature plants if repeated twice or three times a year.

Mulch application

- Effective technique to smother ground cover and inhibit seedling growth.
- Can increase microbial activities and reduce nitrogen in soil.

Broadcast seeding

- Ineffective method in heavily infested areas, but would be effective after removal of target weed.

Application of fire

- Ineffective technique for mature plants, but found to be a good technique to reduce viability % of seeds.

Combined treatments

- Found to be the most effective technique to reduce foliage cover and soil seedbank composition of *G. pubescens*.

Response to elevated CO_2 and drought

- Elevated CO_2 (700 ppm) found to be beneficial for growth and photosynthesis rate for *G. pubescens* seedlings under well watered conditions.
- This may indicate that in a future climate with an enriched atmospheric CO_2 concentration this species will be able to produce taller plants with greater biomass, presumably with a higher seed output.

Figure 8.1. Summary of key results from trials conducted from November 2013 - March 2016 into ecology and management control measures for *Galenia pubescens.*

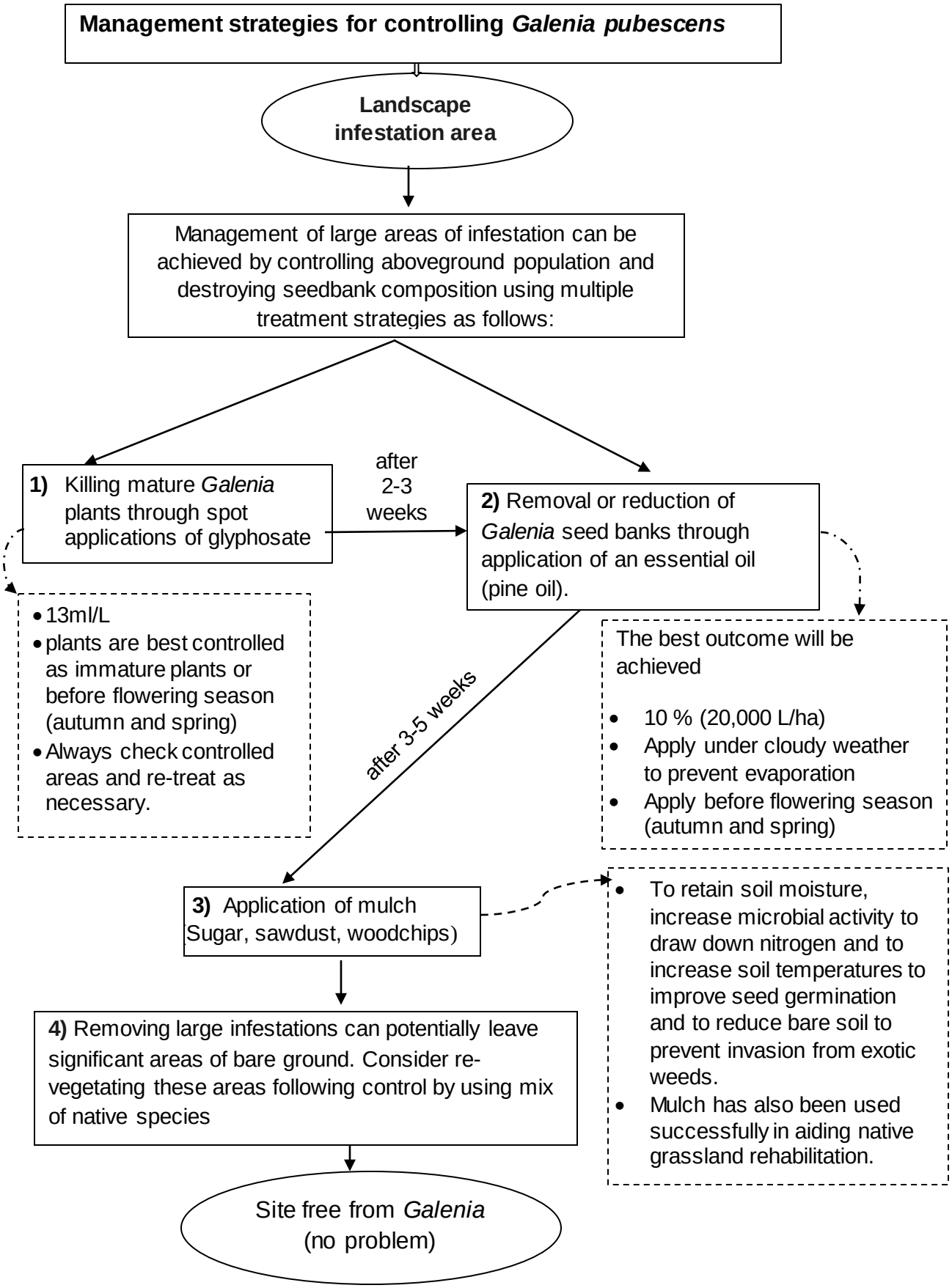

Figure 8.2. Potential recommendation to control aboveground (mature plants) and belowground (seedbank abundance) of *Galenia pubescens* in an infestation area.

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Appendix

Appendix A.

Appendix B. Soil analysis for the experimental site (high density G. pubescens infestations site), sampled 2013. Analysis by Australian Soil and Plant Advisory council.

Analysis	Laboratory Identification	Units
Phosphorus (Olsen)	9.0	Mg/kg
Potassium (Cowell)	521.0	Mg/kg
Sulphur (KCL40)	7.2	Mg/kg
Ammonium Nitrogen (KCl)	6.0	Mg/kg
Nitrate Nitrogen	< 1.0	Mg/kg
Copper (DTPA)	1.62	Mg/kg
Zinc (DTPA)	0.96	Mg/kg
Manganese (DTPA)	50.2	Mg/kg
Iron (DTPA)	91.2	Mg/kg
Boron (Hot CaCl2)	1.4	Mg/kg
Phosphorus (Colwell)	32.0	Mg/kg
pH (1:5 water)	6.2	
pH ($CaCl_2$)	5.2	
Soil Texture	Clay loam	
Salinity (EC) (1:5 water)	0.06	ds/m
Organic Carbon	2.98	%
Calcium (Amm-acet.)	9.98	Meq/100 g
Magnesium (Amm-acet.)	4.22	Meq/100 g
Sodium (Amm-acet.)	0.59	Meq/100 g
Aluminium (KCl)	0.03	Meq/100 g
Potassium (Exch)	1.33	Meq/100 g
Sum of cations (CEC)	16.15	Meq/100 g
Calcium/Magnesium ratio	2.4	
Sodium % of cations (ESP)	3.7%	
Aluminium % of cations	0.2%	

Appendix C.

Twentieth Australian Weeds Conference

Investigations into the effects of elevated carbon dioxide and drought on the growth and physiology of carpet weed (*Galenia pubescens* Eckl. & Zeyh.)

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Summary The present study aimed to examine the interactive effects of elevated atmospheric CO_2 concentration and drought stress on the growth and some of the physiological processes of *Galenia pubescens*. Photosynthetic rate of plants increased under elevated CO_2 concentration, however drought caused significant reduction in net photosynthetic rate by (45% in 400 ppm CO_2) and (27% in 700 ppm CO_2) after five days simulating the drought treatment when compared with well-watered plants. Plants grown under elevated CO_2 level and well-watered produced a greater biomass (17.5 ± 0.5 g per plant) compared to the plants which were grown under the ambient CO_2 concentration.

Keywords CO_2 concentrations, drought, weeds.

INTRODUCTION

Plant invasion can be developed or hindered as a result of numerous changes in the climate and ecological processes. Changes in climate include; change in precipitation pattern, a rise in temperature and CO_2 levels and deposition of nitrogenous material, and fire regime changes that can lead to changes in resource availability (Miri *et al*. 2012, Sheppard and Stanley 2014).

There has been a report that air CO_2 concentrations may be increased from the present-day 380 to 550 ppm by 2050 (Franks, 2013) and afterward to surpass 700 to 1000 ppm before the end of the 21st Century (Dietz and Stern 2009, IPCC 2015) This increase in atmospheric CO_2 concentration will affect plant growth in a variety of ways (Dukes 2011), as it can have a direct impact upon photosynthesis and metabolism (Plett *et al*. 2015).

The future potential of characteristics of *Galenia pubescens* under an elevated atmospheric CO_2 concentration and drought is unknown. Hence, the objective of this study was to assess the effects of elevated carbon dioxide and water availability on the physiology and growth on the exotic weed *G. pubescens* in order to improve management methods.

MATERIALS AND METHODS

Seedlings establishment Mature *G. pubescens* seeds were collected from several populations of mature plants in the Werribee region (37° 49' 5.63" S, 144° 34' 58.77" E), and cleaned seeds were placed in an airtight container until use.

In the glasshouse twenty plastic pots (13 cm diameter and 13 cm high) were prepared as follows: for soil moisture movement and aeration, the sterilised industrial, seed-free sand soil was placed to a depth of 1 cm lined with paper towel. One kg of well-mixed dry sterilised soil was placed into each pot. On the top of each pot ten *G. pubescens* seeds were sown. Pots were labelled and placed into large white trays (44 × 36 × 7 cm) to facilitate watering from below, which ensured minimal disturbance of seeds and later, germinants. One month old *G. pubescens* seedlings (4–6 leaf growth stage), were thinned to one per pot and transferred to CO_2 chambers.

Setting-up CO_2 chambers and growth conditions Two identical *Steriudium e2400* model chambers were used for this experiment. Each chambers was set-up with a different CO_2 concentration. One of the chambers was set at an ambient atmospheric CO_2 concentration (400 ppm), simulating the present concentration of CO_2 in the atmosphere. The second chamber was placed at an elevated atmospheric CO_2 concentration (700 ppm), simulating the likely future concentration of atmospheric CO_2 (by the end of this century) as predicted in climate change scenarios (Franks 2013).

Thereafter, 10 pots (randomly chosen) were placed in one growth chamber (with a CO_2 concentration of 400 ppm). Another 10 pots (randomly chosen) were placed into the other growth chamber (with a CO_2 concentration of 700 ppm). All pots were watered to field capacity based on their need for water. One week prior to the taking of measurements in each CO_2 chamber, half of the plants from each chamber

(randomly chosen) were kept well-watered and the other half were allowed to dry out slowly until visible wilting occurred.

Physiological parameters The net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}) and stomatal conductance (gs; mol H_2O m^{-2} s^{-1}) were measured using a portable gas exchange system (Li-6400XT, Li-Cor, Lincoln, NE, USA), following methods described by Le *et al*. (2016). The water use efficiency (WUE), was determined by dividing the net photosynthetic rate value by the transpiration rate value (Shabbir *et al*. 2014).

Plant biomass After the completion of the trial, approximately at the end of the vegetative stage and the beginning of flowering, the following morphological parameters were measured: The number of branches that were present on each plant was recorded. Then the plants were harvested from the soil surface and the stems and leaves were separated and kept in labelled paper bags and oven dried at 70°C for 48 hours. The roots were removed from the soil and washed thoroughly, were put in labelled paper bags separately and oven dried at 70°C for 48 hours. The dry leaves were added to the dry branches, to determine individual dry biomass. The root/shoot ratio was measured by dividing the root dry weight to the branch dry weight.

Data analysis In order to statistically account for any potential influence of CO_2 concentration and drought treatments on plant responses, a randomized complete design was used with two treatments and five replications. Data were analysed using a general linear model (GLM). The GLM was set up with two atmospheric CO_2 concentrations, two irrigation levels and interaction as the main factor. An analysis was then undertaken using an adjusted sum of squares approach using 95.0% confidence intervals. A two-way analysis of variance tests was run for each trait separately to detect differences caused by each factor using the Statistix 8.1 Regd package. Means were compared by Tukey's test at a 5% level of probability.

RESULTS

Figure 1 shows the effects of atmospheric CO_2 concentration and water availability of the net photosynthesis rate of *G. pubescens* seedlings at 60 days after the placement of plants in CO_2 chambers, and measurements were taken four times (48 hours were between each recorded date). It should be noted that the plants under elevated CO_2 concentration recorded significantly ($F = 9.60$, $P < 0.05$) increases in the mean net photosynthesis rates on all recorded dates (21.3 μmol mol^{-1}) when compared to the plants in ambient atmospheric CO_2 concentration (13.3 μmol mol^{-1}) under well-watered treatment. Whereas moisture stress significantly decreased the rate of net photosynthesis, the decrease was greater in plants that were grown under elevated CO_2 conditions.

The *G. pubescens* plants were not significantly different ($F = 1.68$, $P = 0.2132$) in their production of dry biomass by changing atmospheric CO_2 concentrations. However, the plants were significantly different between well-watered and drought treatments in producing dry biomass (data not shown). Similarly, elevated CO_2 concentration did not have a significant effect on root shoot ratio; however, data showed that the ratio was slightly decreased by increasing CO_2 concentration in both well-watered and drought treatments. Regarding dry root weight, data showed no significant difference ($F = 2.26$, $P = 0.152$) among plants under both CO_2 levels and water availability treatment. Dry leaf and dry shoot weight were also found not significantly affected by changing CO_2 levels; in contrast, water availability significantly affected dry leaf and dry shoot weight. The number of branches per plant was found not to be significantly affected by changing atmospheric CO_2 concentration. However, the number of branches per plant was significantly different under well-watered and drought treatments.

DISCUSSION

Elevated CO_2 is beneficial for *G. pubescens* growth under well-watered conditions. The photosynthesis rate and the percentage of dry biomass were increased subsequently, whereas under drought conditions, elevated CO_2 markedly showed reductions in the photosynthesis rate and dry biomass of the plants. Similar results were obtained by Erice *et al*. (2007), who reported that plant biomass increased under elevated CO_2 under well-watered conditions but not under drought. CO_2 concentration had no significant effects on the leaf, shoot, and root dry weight in either CO_2 concentrations. These responses are in contrast with those of many herbaceous plants (Farrar and Williams 1991, Ziska 2002).

It can be concluded, that this study supported the view that the growth of *G. pubescens* will be significantly enhanced in a future climate with an enriched atmospheric CO_2 concentration, producing taller plants with a greater biomass, and presumably with a higher seed output under well-watered conditions.

These changes will have important implications for management of this noxious weed in the future.

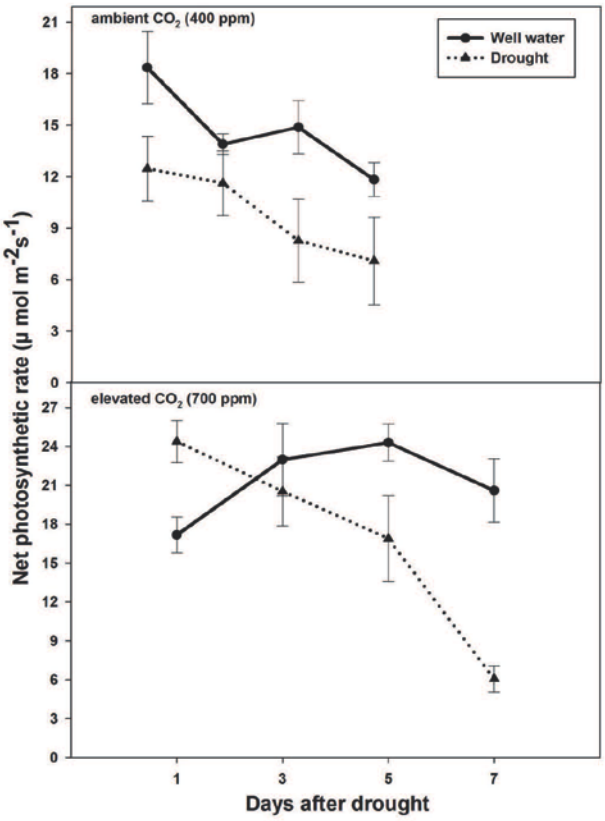

Figure 1. The effect of atmospheric CO_2 concentrations (a) ambient CO_2 (b) elevated CO_2 of the net photosynthetic rate (μmol $mol^{-2}s^{-1}$) of *G. pubescens* plants growing under well-watered and drought conditions. Vertical bars show standard errors of the mean from five replications.

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