Factors affecting recruitment in populations of Spiny Rice-flower (*Pimelea spinescens* Rye subspecies *spinescens*) in Victoria's natural temperate grasslands: relationships with management practices, biological and ecological characteristics.

By

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Abstract

"According to Darwin's *Origin of Species*, it is not the most intellectual of the species that survives; it is not the strongest that survives; but the species that survives is the one that is able best to adapt and adjust to the changing environment in which it finds itself"

Leon Megginson (1963)

Pimelea spinescens Rye subspecies *spinescens* is an endemic subshrub found within temperate grasslands of the Victorian volcanic plains. It is listed as critically endangered under the Federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). Efforts to bolster populations using horticultural techniques have been largely unsuccessful. For long-term survival it is necessary to successfully germinate seed and employ methods which encourage wild populations to reproduce *in situ*.

The aim of this research was to identify which factors of the biology, ecology and management of *P. spinescens* populations, significantly affect the species' *in situ* recruitment potential.

This research developed both laboratory and field methods for the collection of demographic data, assessments of viability, germinability, *in situ* germination and survival. These components were used as measures of recruitment potential which were assessed for

relationships with a range of environmental and management variables. Field assessments were undertaken at 16 sites over a two year period.

Environmental influences on the annual seed production and quality were found to be likely but require further investigation. *Pimelea spinescens* seed displays an endogenous non-deep physiological dormancy (Type II), which was partially overcome *in vitro* using gibberellic acid treatments.

In situ germination and survival appeared to be the most critical stages to the recruitment success of *P. spinescens* populations. Population density and specifically female density were found to positively influence germinant recruitment. But overwhelmingly the recruitment potential of *P. spinescens* populations was most strongly associated with regimes of biomass reduction events. That is, the production of germinants decreased with increasing time since a biomass reduction event. Biomass reduction events are required at intervals no greater than four years,

Reduced litter, weeds, greater bare soil, a high indigenous species diversity, all contributed to a high site condition score and were positively associated with the survival of *P. spinescens* germinants. The, demographic parameters such as the proportion of mature flowering plants and mature plant survival were also found to be positively associated with regimes of biomass reduction.

Student declaration

I Deborah Michelle Reynolds declare that the PhD entitled "Factors affecting recruitment in populations of Spiny Rice-flower (*Pimelea spinescens* Rye subspecies *spinescens*) in Victoria's natural temperate grasslands: relationships with management practices, biological and ecological characteristics" is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or part, for award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Deborah Michelle Reynolds 26th of June 2013

Acknowledgments

This research was designed to learn more about the ecology and biology of this critically endangered plant to promote *Pimelea spinescens* long term survival and ensure that future funding and the corresponding allocation of resources are based on sound scientific knowledge.

Many people have encouraged, guided and supported me throughout this project and our understanding of the biology of *Pimelea spinescens* has increased but we have only just scraped the surface. My hope is future research will occur and is supported and funded as I have been.

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Chapter 1 General introduction

"Conservation biology differs from most other biological sciences in one important way: it is often a crisis discipline. Its relation to biology, particularly ecology, is analogous to that of surgery to physiology and war to political science. In crisis disciplines, one must act before knowing all the facts; crisis disciplines are thus a mixture of science and art, and their pursuit requires intuition as well as information."

Michael E. Soule (1985)

1.1 Conservation biology

Conservation biology is both the pure and applied science of preserving the world's biological diversity. It has been described as a crisis discipline (Soule, 1985), which deals with the 'science of scarcity' (Soule, 1986a). Indeed, scarcity is a rapidly growing global phenomenon, with about twenty-five percent of the world's vascular plant species predicted to become extinct in the next forty years (Schemske, Husband *et al.*, 1994). The Australian Bureau of Statistics reports that there has been a steady decline in Australia's floral biodiversity and that the number of threatened plant species rose from 1,143 in 2000, to 1,344 in 2012 which is an increase of ~20 % (Department of Sustainability Environment Water Population and Communities, 2009, Australian Bureau of Statistics, 2010, 2012, Moncrief, 2012). Identification of threats and reasons for the current status of rare species is not difficult (Burgman, Kieth *et al.*, 2007), as the spaces they occupy conflict with the current economic and political demands of human society. Allocating conservation funds and resources has become the real battle ground, and due to the scale of potential losses, not all the remaining populations of each species can be conserved (Meneges, 1990, Shaffer, 1990, Pavlik, 1994, Pearce and Moran, 1998, Thrall, Burdon *et al.*, 2000).

Embedded within conservation biology is population biology – the study of the regulation of populations of organisms (Feidler and Kareiva, 1998, Smith and Smith, 1998, Attiwill and Wilson, 2003). Benchmark information such as population size, structure and critical life-stage processes are essential to begin to understand the conservation status of a species and initiate effective crisis management in an informed manner (Soule, 1987, Pavlik, 1994). This research is the beginning of the informed conservation efforts for the critically

endangered species *Pimelea spinescens* subspecies *spinescens* (Australian Federal Government, 2007).

1.2 Genus *Pimelea*

The family Thymelaeaceae is found throughout South America, Asia, South Africa, Australia and New Zealand. Within this family, the genus *Pimelea* contains 108 species which are distributed across Australia, Lord Howe Island, Chatham Island, New Zealand, Timor and New Guinea. Ninety of these species are endemic to Australia (Threlfall, 1982, Walsh and Entwisle, 1996). Representatives of the genus are distributed across all Australian states and territories (Threlfall, 1982, Centre for Australian National Biodiversity Research and Australian National Herbarium, 2008). In 1995, sixteen (18 %) of Australia's *Pimelea* species were listed as rare or threatened (Briggs and Leigh, 1996). In contrast, only nine species were listed as rare or threatened in 1981 (Leigh, Briggs *et al.*, 1981).

The name *Pimelea* comes from the Greek word *pimele* which means soft, fat or lard. This is in reference to the oily nature of its seed or possibly the presence of succulent cotyledons (Walsh and Entwisle, 1996).

1.3 The subspecies of *Pimelea spinescens*

Pimelea spinescens exists as two subspecies, *spinescens* and *pubiflora.* They are distinguished from other *Pimelea* species by their subshrub habit and having spinescent stems (Figure 1A) (Commonwealth of Australia, 2009a). Both subspecies are only found in Australia, endemic to Victoria and are easily differentiated by their flower. Subspecies *spinescens* has flowers which are glabrous on their outer surface (Figure 1B), whereas the

flowers of *pubiflora* are pubescent (Figure 1C) (Walsh and Entwisle, 1996, Walsh and Stajsic, 2007, Threatened Species Scientific Committee (TSSC), 2009).

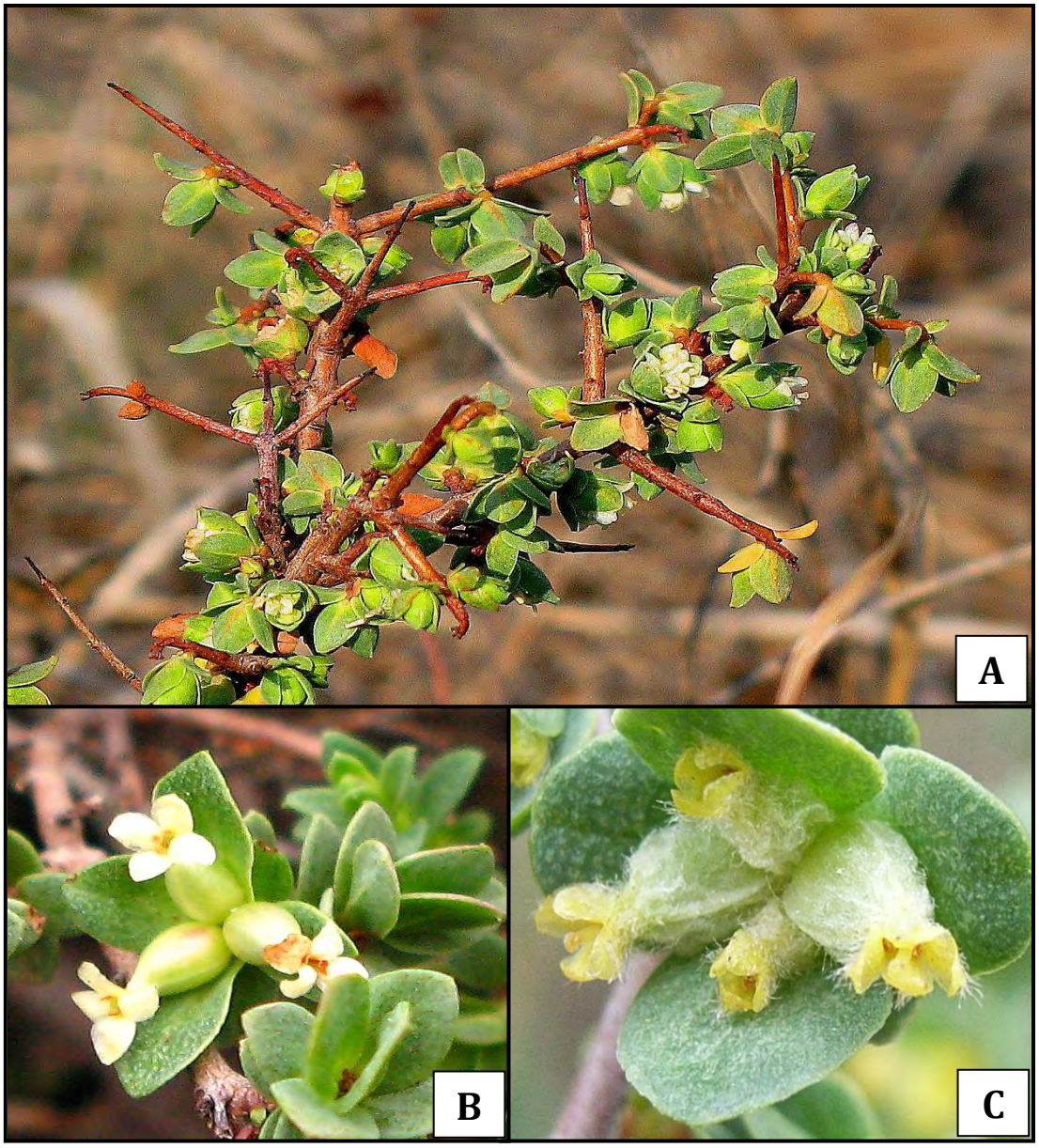


Figure 1 – The morphological characteristics of the two subspecies of *Pimelea spinescens*: A - The spinescent branches of *Pimelea spinescens* sbsp. *spinescens*. B - The glabrous flowers of *Pimelea spinescens* sbsp. *spinescens.* C - The pubescent flowers of *Pimelea spinescens* subsp. *pubiflora.*

1.4 *Pimelea spinescens* subspecies *spinescens*

Pimelea spinescens Rye subspecies *spinescens* (Rye, 1990) (from here-on referred to as *P. spinescens* or *Pimelea spinescens*) is commonly known as “Spiny Rice-flower”. Other less frequently used common names include Plains Rice-flower and Prickly Pimelea. The small form, crowded leaves and spinescent nature of its stems distinguishes it from all other *Pimelea* species (Walsh and Entwisle, 1996). *Pimelea spinescens* is considered long-lived and slow growing (Mueck, 2000). It is classified as a stunted subshrub usually forming a dense tuft of short spinescent divaricate branches (stems) and growing to between 5 and 30 centimetres in height (Walsh and Entwisle, 1996, Victorian Government Department of Sustainability and Environment, 2008). Fresh stem growth is not spiny but soft, smooth and almost herbaceous (Figure 2). As the stems age the ends become hard, leafless and form a spiny tip (spinescent). The leaves are a uniform dull green colour; decussate in arrangement along the stem; have short petioles; elliptic in shape; measure two to ten millimetres in length; and are one to three millimetres in width (Walsh and Entwisle, 1996).

The plant produces a taproot root system which has been documented to extend up to one metre or more in to the soil. Over time, the taproot contracts below the soil surface and underground stems develop which form nodes of regeneration following the loss of all above ground biomass (PsRT, 2013).

Figure 2 – A mature *P. spinescens* plant with new growth seven months after a fire.

The flowers are produced in a terminal compact head. There are between six and twelve flowers per inflorescence. The pale-yellow to white flowers are glabrous (hairless) and have four rounded, petal-like lobes about two millimetres long. Unlike the majority of other grassland plants *P. spinescens* flowers over winter from April through to August depending on the prevailing seasonal conditions (Walsh and Entwisle, 1996).

Pimelea spinescens is mostly dioecious (i.e. plants are either female or male). Female flowers have a protruding style which is shorter than the ovary and small non-functional anthers (Figure 3A). Male flowers have two anthers with abundant bright orange pollen (Figure 3B). The fruit is ellipsoid, two to three millimetre long and has a thin, initially fleshy,

layer around a slightly woody 'stone' that encloses the single, oily seed (Walsh and Entwisle, 1996, Victorian Government Department of Sustainability and Environment, 2008).

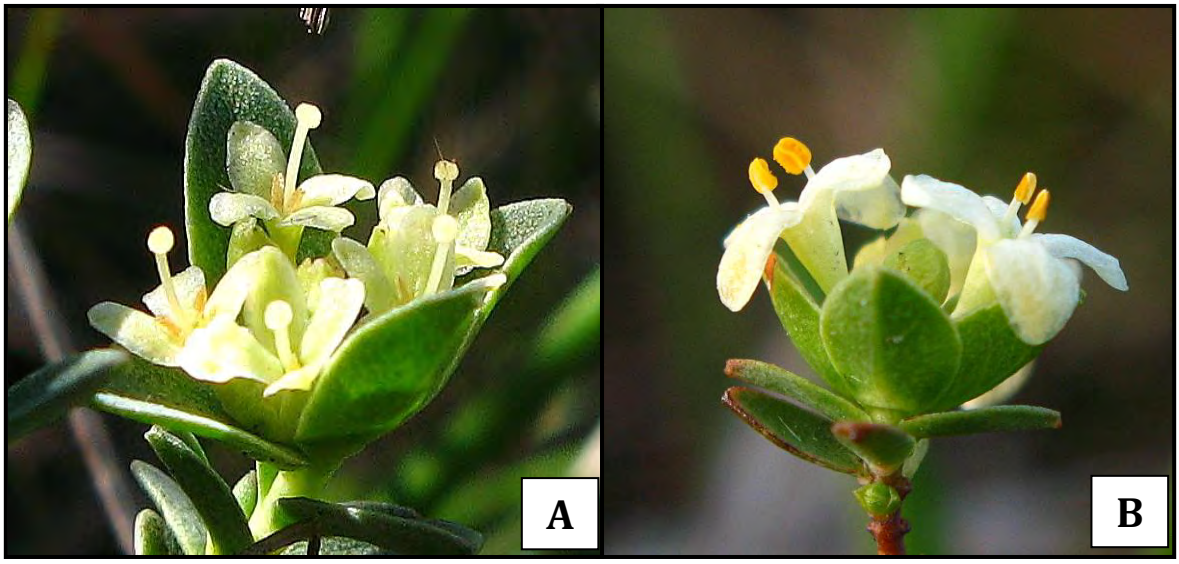


Figure 3 – Sexual morphology of *P. spinescens* flowers: A - Female flowers have an obvious stigma and insignificant stamens with no pollen. B - Male flowers have obvious stamens with distinctive orange pollen.

1.4.1 *Distribution*

The most easterly extent of the range of *P. spinescens* is the western suburbs of Melbourne. From this point the distribution extends 190 kilometres in a northerly direction to Echuca; 229 kilometres in a westerly direction to Dunkeld; and 122 kilometres in a south-westerly direction to Cressy (Figure 4).

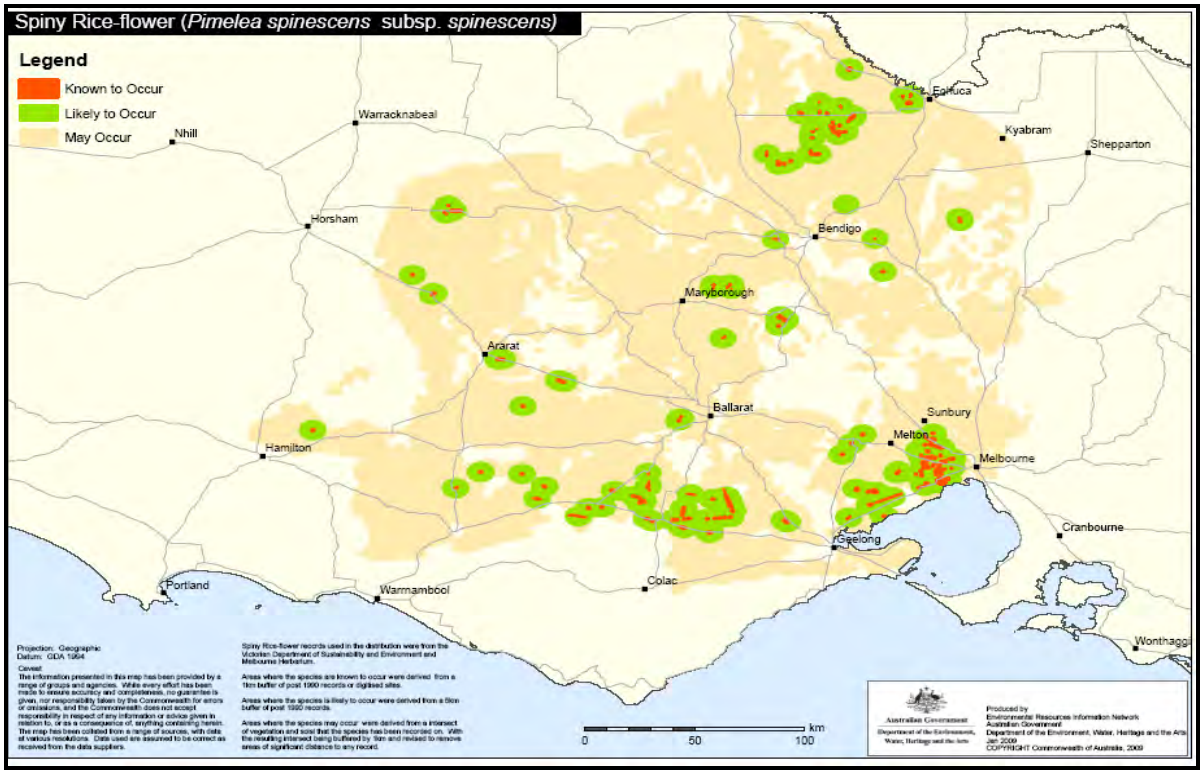


Figure 4 – The distribution of *P. spinescens*. Red indicates known locations. Green and beige indicate areas where it was likely to occur or may occur, respectively (Commonwealth of Australia, 2009b).

Biogeographic regions (bioregions) capture the patterns of ecological characteristics in the landscape and have been described for all parts of Australia (Department of Natural Resources and Environment, 1997b, pp 26). There are twenty-one bioregions recognised in the state of Victoria (The State of Victoria, 2011). The distribution of *P. spinescens* occurs across three of these bioregions: Victorian Volcanic Plain, Victorian Midlands (Goldfields) and Victorian Riverina (Department of Environment and Heritage, 2000).

Within these bioregions, the species is found predominantly in the ecological vegetation class (EVC) of 'lowland grasslands' but is also found in 'grassy woodlands' and 'open

shrublands' (Threatened Species Scientific Committee [TSSC], 2003, Department of the Environment Water Heritage and the Arts (DEWHA), 2009). Ecological vegetation classes are basic mapping units used by managers for biodiversity planning and conservation assessment at landscape and regional scales. They attempt to group plant communities that occur in the same types of environments and respond in a similar manner to ecological processes (Department of Sustainability and Environment, 2004, The State of Victoria, 2008). The EVCs in which *P. spinescens* occurs are associated with the basalt derived black or grey clay soils to the west of Melbourne and alluvial soils across the north-west of Victoria (Carter and Walsh, 2006, Victorian Government Department of Sustainability and Environment, 2008).

1.4.2 *The grassland habitat of Pimelea spinescens*

The biology of *P. spinescens* is strongly associated with evolution of the temperate grasslands of Victoria. These grasslands are relatively treeless areas dominated by perennial forbs, small shrubs and grasses. They are a product of the combined factors of geology and climate and are maintained by seasonal events such as droughts, floods, and frequent fire which inhibits tree establishment (Smith and Smith, 1998, Attiwill and Wilson, 2003). Additionally, many of the plants in these grasslands are adapted to grazing (Webb, 1977, 1978).

The grasslands of the Victorian volcanic plain occur on a series of lava flows that erupted between 5 million and 4,000 years before present (Society for Growing Australian Plants, 1995, Australian Plants Society Keilor Plains, 2012) forming a flat undulating expanse (McDougall, 1987). It is one of the largest volcanic plains in the world covering approximately

2.3 million hectares and extending from Melbourne, west to Portland, south to Colac and north to Beaufort (Department of Natural Resources and Environment, 1997a, Threatened Species Scientific Committee, 2008a). The lava of these flows cooled and hardened to form basalt rock which has subsequently weathered to form heavy clay soils that are fertile, with poor drainage (Conn, 1993, Society for Growing Australian Plants Maroondah Inc, 2001). From a biological perspective, an important characteristic of these soils is their capacity to shrink and expand with moisture availability. In summer the moisture is slowly lost from the upper levels and the clay soils crack, becoming hard like concrete. During rainfall events, the soils rapidly soak up the moisture, expanding and closing the cracks (McKenzie, Jacquier *et al.*, 2004). Plants which survive in this environment must be able to survive these constantly changing soil conditions and the associated mechanical forces.

Climatic conditions in Victoria are driven by variations in the temperature of both the Southern and Pacific oceans, giving rise to a periodic cycle of drought and flood (Attiwill and Wilson, 2003). The Victorian natural temperate grasslands usually receive an annual rainfall of 500mm to 700mm which is spread evenly throughout the year, with peaks in winter and spring (Lunt, Barlow *et al.*, 1998). Summer thunderstorms with heavy downpours are not uncommon, for example in Keilor, 138 mm of rain fell over a 24 hour period during February 2005 (Lunt, Barlow *et al.*, 1998, Commonwealth of Australia, 2012). Extended dry periods often occur in summer which is generally hot, with occasional dry northerly desert winds (Walker, 1981). Winters are cold and frosty. Grasslands in northern Victoria are subject to slightly warmer temperatures and less rainfall than the western areas of the volcanic plain (Commonwealth of Australia, 2012).

Speciation in the majority of Australian plant genera has been driven by historical changes in climate (Barlow, 1994). Due to their sessile nature, plants have little capacity to escape their environment but with a slow rate of environmental change, natural selection enables plants to adapt, reproduce and flourish (Horn, 1979). The northward progression of the Australian landmass in conjunction with global cooling over the last 40 million years (Attiwill and Wilson, 2003) has caused a gradual climate change, resulting in 'the browning of Australia' (White, 1994); a concept which describes the gradual transition from the dominance of rainforest vegetation to sclerophyllous, grassy and arid vegetation types.

With the drying of the Australian continent, fire became a feature of the landscape, driving an evolution towards the dominance of fire tolerant vegetation (Noble, 1986, Bradstock, Williams *et al.*, 2002). Charcoal sediments indicate that fires were regularly sparked by lightning strikes prior to the arrival of humans on the Australian landmass (Kemp, 1981, Singh, Kershaw *et al.*, 1981). Following this, these same sediments suggest a rapid increase in the fire frequency across the continent around 40,000 years ago, which approximately corresponds with the arrival of the aboriginal people (Kemp, 1981, Singh, Kershaw *et al.*, 1981).

Australia's indigenous ancestors observed the effects of repetitive fire on the landscape and learned to use it for their benefit (Bowler, 1970, du Cros, 1990). Aborigines managed fire with precision, burning areas with high biomass in a mosaic pattern, with some areas rarely burnt and others regularly fired (Stokes, 1846, Thompson, 1949, Gott, 1992). The effect of these mosaic burning patterns was to keep sections of the landscape clear of tall vegetation. This opened up areas for easy passage, promoted the growth of food sources and allowed seedlings to germinate for future crops. These open areas quickly developed fresh new

growth, which enticed grazing animals in, making them easy prey for hunting (Christensen and Burrows, 1986, Gott, 1992, Jones, 1992, Aplin, 1999, Gott, 2005).

As a consequence of natural fire and deliberate and regular burning by humans, fire has become an essential process that influences and in many ways maintains many of the ecosystems of Australia (Heywood, 1989, Knox, Ladiges *et al.*, 2001, Attiwill and Wilson, 2003). In Australian grasslands the flora has evolved under these burning regimes and now requires fire for rejuvenation. Indeed, many plants, such as *Themeda triandra* (Kangaroo Grass), encourage fire by accumulating biomass. Others, such as *Microseris* sp. 1 (Murnong), *Ptilotus spathulatus* (Pussy Tails) and all Orchid spp., shed all above ground biomass seasonally and retreat to below ground storage organs that are protected from the effects of fire (Mutch, 1970, Walker, 1981, Attiwill and Wilson, 2003).

When Europeans first sighted the volcanic plains of Victoria (1802), it was reported that there appeared to be thousands of acres of fertile soil with few trees and instead grassy plains filled with wildflowers (Jones, 1992). This gave future landholders adequate enticement and hope for prosperity, as it was billed as perfect land ready for stock or agriculture (Brown, 1977, Hellyer, 1987 [1861], Jones, 1992, Rolls, 1999). Since European occupation (from 1837) of Victoria's volcanic plains, many novel processes have been introduced to the ecosystem, including hard hoofed grazers, agricultural cultivation, roads, fences, rabbits, weeds and altered fire regimes (Billot, 1979, Jones, 1992, Wheeler, Jacobs *et al.*, 2002). Over the last 175 years these factors have contributed to the decline of native grasslands, leaving the isolated and fragmented remnants that occur today (Miller, 2000). Habitat fragmentation was not random, with remnant vegetation often being left in areas unsuitable for other, especially agricultural, land uses (Lindenmayer and Fischer, 2006).

For plants such as *Pimelea spinescens*, a reduction in the area of habitat and population size can result in a decline in essential ecosystem processes and species function. Some effects included reduced: pollinator visitation; genetic diversity; seed production; and seed quality (Allen and Antos, 1993, Rathcke and Jules, 1993, Steffen-Dewenter and Tscharntke, 1999, Aizen and Feinsinger, 2002, Duncan, Nicotra *et al.*, 2004, Morgan and Scacco, 2006, Wenzel, Schmitt *et al.*, 2006). It is probable that many of the current remnant populations of *P. spinescens* occur at sites with characteristics that are not typical of the species' former distribution.

Pimelea spinescens occurs in association with vegetation usually dominated by *Themeda triandra* (Kangaroo Grass), with *Austrostip*a spp. (Spear-grasses) or *Austrodanthonia* spp. (Wallaby-grasses) as co-dominant species. Various herbs such as *Chrysocephalum apiculatum* (Common Everlasting), *Eryngium ovinum* (Blue Devil), *Velleia paradoxa* (Spur Velleia), *Acaena echinata* (Sheep's-burr), *Plantago varia* (Variable Plantain), *Ptilotus erubescens* (Hairy Tails), *Schoenus apogon* (Common Bog-sedge) and *Calocephalus citreus* (Lemon Beauty-heads) are often also found in close proximity (Carter and Walsh, 2006). In the absence of biomass reduction, the dominant species found in Victoria's natural temperate grasslands is *T. triandra,* which can rapidly outcompete other species through abundant leaf litter production (>11 tonnes/ha over a period of 10 years) (McDougall, 1989). The lack of inter-tussock gaps and large amounts of accumulated grass biomass, limits the availability of light and moisture to other grassland species, while preventing germination and recruitment (Morgan, 1999b, 2001). Therefore the floristic diversity of such grasslands is dependent on regular fire and/or grazing, to reduce the biomass (Johnson, Burbidge *et al.*, 1989, Tremont and McIntyre, 1994, Lunt and Morgan, 2002).

Pimelea spinescens is often described as a cryptic species (Victorian Government, 2010a). Within the grassland environment the fine delicate form can be difficult to detect, even when the species is known to exist at a site (Figure 5A and 5B). This is particularly a problem in the non-flowering spring to summer period, which is the traditional time for botanical surveys in the grasslands of the Victorian volcanic plains (Walsh and Entwisle, 1999, Carter and Walsh, 2006, Commonwealth of Australia, 2009a, Garrard, 2009).

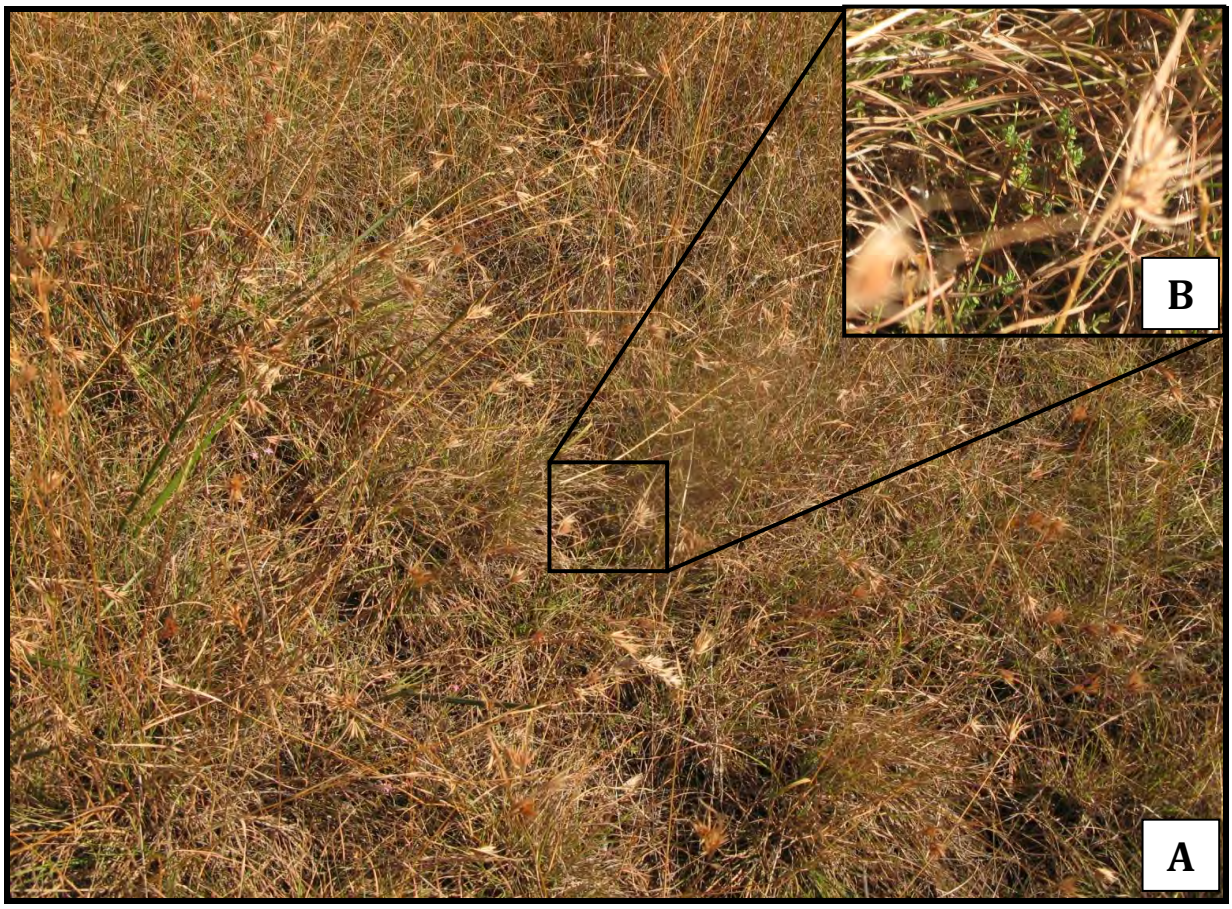


Figure 5 - The difficulty of detecting *P. spinescens* during spring/summer flora surveys is illustrated with these photographs: A – Typical *P. spinescens* habitat during summer (January) with one plant *in situ*. B – Close up branches of the individual *P. spinescens* plant.

1.4.3 *Population size*

For *P. spinescens*, a population has been described as “a collection of individual plants occurring close together but separated geographically from other such collections” (Commonwealth of Australia, 2009a); and, “at least 20 individual plants where there is no more than 100 metres distance between any two plants, and between 40 % and 60 % of the plants are male” (Victorian Government, 2010b).

Current and historic records of *P. spinescens* are maintained via the Victorian Biodiversity Atlas (VBA) (Department of Environment and Sustainability, 2012). In 2012, this database contained records for the species from 208 sites, including population size estimates for 179 of these sites. Of these, over half (53 %; 95 sites) of the sites supported 50 individual plants or less, and an additional 35 % (63 sites) of the sites were estimated to support 500 individuals or less.

Based on VBA records, it is estimated that there are more than 75,000 individual *P. spinescens* plants across the state. This number must be treated with caution however, as the data has not been collected in a standardised manner and may contain large inaccuracies. One source of inaccuracy was highlighted by Garrard (2009) who found that both the experience of the observer and the duration of time spent searching were important factors in the accurate detection of individuals, given the cryptic nature of the species (Victorian Government, 2010a). In an attempt to account for variations, the accuracy of records in the VBA database are graded on the basis of the field assessment method and time spent looking for plants.

Limited studies have been conducted to assess changes in population size over time. These include the work by Cropper at Denton Avenue Grassland (1999, 2000a, b) and the Western Water Treatment Plant (2002, 2005, 2006, 2007a, b, 2009) and also the studies by Foreman at various locations (2005, 2011).

At Denton Avenue Grassland St Albans, Cropper (1999, 2000a, b) initially estimated that the population of *P. spinescens* consisted of between 80 and 200 individuals in 1998. Following a burn in 1999, he was able to record 328 individuals in 2000.

In another study, Cropper (2003, 2007a) conducted two censuses of *P. spinescens* at the Western Water Treatment Plant. The first census was conducted three months after a burn, in 2003. The second census was conducted in 2007, six months following another burn event. Cropper (2007a) found that there was a high rate of population growth (17.8 %) within the first three years following the 2003 burn, and that the population grew at a slower rate (3.9 %) in the subsequent two years. Over the five year interval between censuses 22 plants died (4 %) and 224 (44 %) plants were recruited, indicating a trend of positive population growth. The response of the population was thought to be directly related to the fire event.

Foreman conducted an initial assessment of 16 populations in 2004, with follow-up monitoring in 2009. His work was based on a single 10 m x 10 m quadrat at each site (2005, 2011). He did not record any significant changes in population density over the five-year time-frame, despite finding a greater density of germinants on his return to these sites in 2009 (Foreman (2011).

1.4.4 *Population structure*

Within a population, individual *P. spinescens* plants have been categorised as: dead, senescent adult, mature adult (flowering), immature/juvenile (non-flowering), sucker, or germinant (Foreman, 2005, 2011). Under this system, mature adults were further categorised as male, female or unknown sex/bisexual. Analyses of population structure data found positive relationships between the numbers: of male and female plants; mature plants and immature plants; and female plants and germinants at each site (Foreman, 2011). Foreman (2005) also commented that the sites with the lowest mature plant densities had no germinants.

Cropper initially used a more simplified system than Foreman's and classified plants as being either established or recruits, based on their size (diameter at the widest point) (Cropper, 2003). In a 2007 census of the entire population at the Western Treatment Plant he found that 54 % of the plants were mature adults and 46 % of the population was estimated to be five years or younger (Cropper, 2007a).

1.4.5 *Breeding system*

Pimelea spinescens has been described as having unisexual flowers (Walsh and Entwisle, 1996, Carter and Walsh, 2006, Victorian Government Department of Sustainability and Environment, 2008), with a dioecious breeding system (Commonwealth of Australia, 2009a, Mueck, 2009). Dioecious species consist of individual plants which exhibit either only male functional flowers or only female functional flowers (Sakai and Weller, 1999). In contrast, a gynodioecious population contains plants which are functionally female, as well as plants which are hermaphroditic - no true males exist (Sakai and Weller, 1999).

In the genus *Pimelea,* dioecy has evolved from gynodioecy (Ross, 1970), which is the cited breeding system for a quarter of all *Pimelea* species found throughout New Zealand (Burrows, 1960, Merrett, 2007, Burrows, 2009) and Australia (Bentham and von Mueller, 1873, Rye, 1999).

Foreman (2005), described the sex ratio of *P. spinescens* populations in terms of females and males. He noted however, that frequently individuals were not exclusively dominated by flowers of one sex, with some of the female plants displaying a scattering of male flowers. Cropper (2005) used a more elaborate system by identifying the following four flower types that can occur on the branches of a single plant:

I. Perfect flowers - anthers and ovary normal;

II. Staminate flowers - anthers normal, pollen abundant, ovary shriveled and non-functional;

III. Staminate flowers - anthers half size, pollen present but not abundant, and ovary shriveled and non-functional; and

IV. Pistillate flowers - ovary normal.

Based on these flower types Cropper (2005) divided the study population into two broad groups: female plants which were dominated by type four flowers, but occasionally type two or type three flowers and rarely type one flowers; and male/hermaphrodite plants which predominately had type two and three flowers but infrequently type one and four flowers. Finally, he used the below functional groups based on observed flower types to classify individual plants in the study:

- Male - type II and III flower types;
- Mixed male - type I, II, II and IV flower types;
- Female – type IV flowers;
- Mixed female – type IV, I, II and III flowers; and
- Mixed hermaphrodite – type I and II flowers.

Using the categories of male and female plants, both Foreman (2005, 2011) and Cropper (2005) report an overall unbiased sex ratio. Often plants that were probably hermaphrodites were recorded as 'unknown sex' but were reported to occur in low numbers by both authors (Cropper, 2005, Foreman, 2005, 2011).

In contrast, a recent survey of a population destined for destruction found twice as many female to male individuals (J MacDonald, 2008 pers. comm. November). For *P. spinescens* it is unclear what factors are driving such variation in sex ratios or whether this is a limiting factor for recruitment within populations.

1.4.6 *Flowering and pollination*

Pimelea spinescens is reported to flower between April and August (Walsh and Entwisle, 1996, Carter and Walsh, 2006), although personal observations have noted flowers as late as October. Flowering appears to be stimulated by the onset of colder nights in autumn (D. Reynolds 2008, pers. obs. June), usually between late March and early April depending on seasonal conditions.

Due to its dioecious nature, *Pimelea spinescens* is assumed to be an obligate out-crosser and requires visitation by a suitable pollinator (Rathcke and Jules, 1993, Alonso and

Herrera, 2001). In contrast, Foreman (2011) suggested that the species might be self-compatible, at least to some extent. This notion is based on the observation that some sites had an apparent lack of pollinators yet produced viable seed and significant numbers of germinants.

Butterflies (Merrett, 2007), moths, flies (Burrows, 1960, Hingston and McQuillan, 2000, Dawson, Rapson *et al.*, 2005) and bees (Hogbin, 2006) have all been observed foraging on *Pimelea* species in Australia and New Zealand. For *P. spinescens* anecdotal observations have included visitation by lycaenid butterflies (Foreman, 2005), beetles (Cropper, 2004) and flies (Cropper, 2005). In addition to these observations, insects from the taxonomic orders Diptera (Figure 6A, B and C) and Hymenoptera (Figure 6D, E and F) were noted as frequent visitors in the current study (D. Reynolds 2009, pers. obs. May to October).

Following pollination, seeds develop slowly and are usually held on the plant until late into the season. The warmer period of spring appears to facilitate maturation, when the seed falls from the plant (D. Reynolds 2008, pers. obs. November).

No literature regarding seed dispersal for this species or any other species of *Pimelea* has been published.

The relationship between pollinator presence, success rates of pollination, seed viability and the final production of germinants are important aspects of the recruitment biology of *P. spinescens* that are poorly understood.

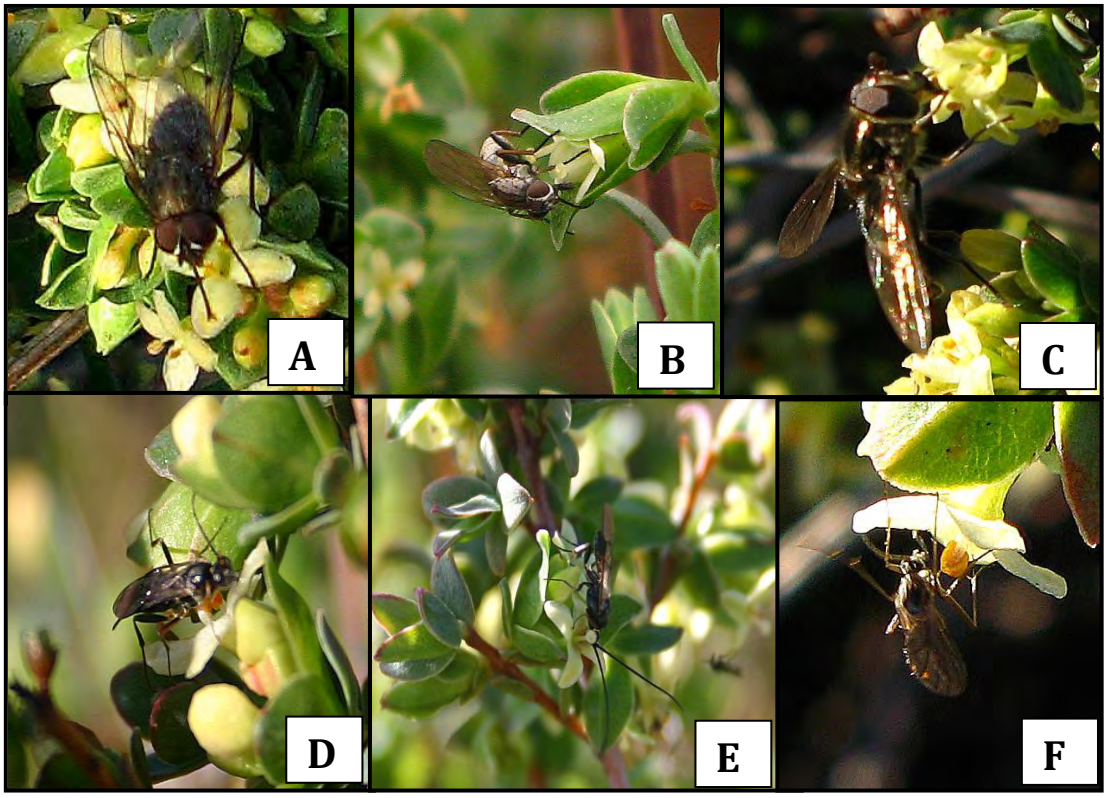


Figure 6 – Potential pollinators of *P. spinescens*: Flies (A, B, and C) and Wasps (D, E and F) observed on *P. spinescens* in the field during 2009.

1.4.7 *Seed germination*

Very little is known about the conditions that trigger the germination of seed for any species in the genus *Pimelea* and this is also true for *P. spinescens*. Across the genus, seed is thought to remain viable for up to four years in the field (Keighery and Dixon, 1984). Additionally, at least some species appear to undergo a period of seed dormancy, with various studies reporting delayed germination (at least 30 days and up to one year) at low and erratic rates under both laboratory and nursery conditions (Wittwer, 1965, Keighery and Dixon, 1984, Fox, Dixon *et al.*, 1987, Roberts, 1990, MacPhee, 1998, Willis, McKay *et al.*,

2003, Gibson Roy, Delpratt *et al.*, 2007b). A range of treatments have been trialled to trigger germination for various species of *Pimelea* (Roberts, 1990, Dixon, Roche *et al.*, 1995, MacPhee, 1998, Clarke, Davison *et al.*, 2000, Willis, McKay *et al.*, 2003, Gibson Roy, Delpratt *et al.*, 2007a). Of these, the greatest success has been achieved for *P. sylvestris* via cool smoke, using a smoke fumigation tent treatment. This resulted in a germination rate of 32 % (Dixon, Roche *et al.*, 1995). Leaching has also been reported as a useful seed pre-treatment (Keighery and Dixon, 1984).

For *P. spinescens*, efforts to germinate seed using horticultural techniques in nursery settings have been largely unsuccessful (Mueck, 2000, Thomas, 2008a). Under these conditions, and using smoke water as a treatment, germination occurred sporadically but the results were inconsistent and unreliable (I Taylor, 2011 pers. comm. November).

Reports of *in situ* recruitment of *P. spinescens* have been rare and knowledge about the timing, duration and microsite location of germination is in its infancy. Foreman (2005, 2011) noted the following characteristics of germination: it occurs in July; at sites recently burnt; there is a possible association with increased autumn rainfall; and a positive association with mature female plant density. Cropper also reported the presence of germinants at an intensively managed site that was regularly burnt, with ongoing weed control and surrounded by a rabbit-proof fence (Cropper, 2007a, 2009). Further analytical study of the germination ecology of *P. spinescens* is required.

1.4.8 *Conservation status*

Prior to 2003 *P. spinescens* was federally listed as a Vulnerable species under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).

Following a review of its conservation status (Department of the Environment & Heritage, 2003), the species was transferred to the listing of Critically Endangered, as it has been deemed to be at high risk of extinction due to the ongoing reduction in both population size and area (NSW Parliamentary Counsel's Office, 2010).

Currently *P. spinescens* and its associated ecological community, the Natural Temperate Grassland of the Victorian Volcanic Plain are both listed as Critically Endangered on the federal EPBC Act (Threatened Species Scientific Committee [TSSC], 2003, Australian Federal Government, 2007, Threatened Species Scientific Committee, 2008b). The species and community (listed as Western (Basalt) Plains Grasslands Community) are also recognised as being threatened with extinction under the Victorian *Flora and Fauna Guarantee Act 1988* (FFG Act) (Department of Sustainability & Environment [DSE], 2007a). As a result of these listings, any action which would lead to an adverse impact on *P. spinescens* populations requires a referral to the Australian Federal Government for approval by the Environment Minister (Australian Federal Government, 2009, pp 10). Commonly, mitigation conditions imposed for approved actions to remove *P. spinescens* populations include the translocation of individual plants, and the securing of offsets with funding for active management of sites containing existing populations of *P. spinescens*.

1.4.9 *Threats*

Habitat loss is widely recognised as the greatest contributing factor to the loss of biodiversity (Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2005, Lindenmayer and Fischer, 2006, Williams, Morgan *et al.*, 2006). The Natural Temperate Grasslands of the Victorian Volcanic Plain, which are the stronghold of *P. spinescens*, have undergone substantial

reduction and modification, with an estimated 2 % of its pre-1750 extent remaining (Department of Natural Resources and Environment, 1997b, 2002a). Such a large reduction in habitat has resulted in similar reductions in the number and size of populations of *P. spinescens*, in rural and metropolitan areas, and across different land tenures (Mueck, 2000, MacDonald, 2008, Department of Environment Water Heritage and the Arts, 2009, Kellogg Brown & Root Pty Ltd, 2010). Habitat loss is an ongoing threat to this species (DSE 2007b) due to the expected expansion of the Melbourne Urban Growth Boundary (Victorian Government, 2009a), and evidenced by a 30 % decrease in the total area of grassland remnants since the 1980's (Threatened Species Scientific Committee [TSSC], 2003, Williams, Morgan *et al.*, 2005). The process of habitat loss and fragmentation is ongoing (Victorian Environment Assessment Council, 2011).

The threatening factors that are cited within the *P. spinescens* National Recovery Plan (Carter and Walsh, 2006) are:

- Ongoing population fragmentation, leading to small populations;
- Weed invasion;
- Road and rail maintenance activities;
- Grazing;
- Inappropriate fire regimes; and
- Changing land use.

The threat of habitat loss is a significant issue for populations in close proximity to cities and regional centres, which are subject to the impacts of urban expansion (Williams *et al.* 2005a,

Williams *et al.* 2005a). Seventy percent of the sites supporting *P. spinescens* are along thin linear road or rail reserves which are subject to high levels of edge effects and therefore have a greater probability of degrading over time (Williams, McDonnell *et al.*, 2005). In rural areas, habitat loss is more inadvertent and is often associated with the installation or maintenance of infrastructure, such as roads and railways. Recent examples of impacts on the species in rural areas include expansion of the Cressy-Shelford Rd, Cressy in 2008; and a disturbance caused by the expansion of a rail access track at the Mitiamo Rail Reserve in 2007, which resulted in the destruction of 38 *P. spinescens* plants. Both of these deleterious actions resulted in enforceable undertakings to provide remedial work and compensation (Australian Government, 2009).

Weeds that have been noted to have a particular impact on *P. spinescens* include *Phalaris aquatica* (Canary grass) and *Lophorpyrum ponticum* (Tall Wheat-grass). They are considered to be invasive species because they have the ability to grow quickly and are long-lived. In effect, they limit the recruitment potential of native plants by occupying space (Carter and Walsh, 2006, Thomas, 2006, Victorian Government Department of Sustainability and Environment, 2008, Mueck, 2009). Unless carefully implemented, measures to control such weeds can further impact on individual *P. spinescens* plants which are difficult to detect (Commonwealth of Australia, 2009a). Weed control (spray drift) has been documented to have a negative effect on another species in the genus, *P. spicata* (Matarczyk, Willis *et al.*, 2002).

Populations of *P. spinescens* have been located on private properties and roadsides, where a regime of light grazing has been implemented (Carter and Walsh, 2006, L Woodward 2010, pers. comm. October). It appears that the species can tolerate some grazing but little

is known about the impacts of different types of grazing regimes and the critical point beyond which the species is lost from the landscape is unknown. There are many examples of grazed properties that do not support *P. spinescens*, despite the presence of large populations on adjacent roadsides.

Given the evolutionary history of *P. spinescens* and its associated ecosystem, the species is well adapted to surviving fires (Mueck, 2000, Carter and Walsh, 2006, Thomas, 2006). Indeed, since European settlement, many populations of the species continue to persist along railway lines where embers from steam engines or friction on dry grass have ignited fires. More recently, the biomass along railway lines has been deliberately managed by regular fuel reduction burns (Thomas, 2006). Burns in these grasslands are usually conducted in late summer when the process of flowering to seed fall is complete (Lunt and Morgan, 2002, Thomas, 2006), although spring and autumn burns are not uncommon. There is little understanding about the impacts of fire frequency, timing and intensity for *P. spinescens*.

1.4.10 *Conservation actions*

Two significant documents have been prepared as a result of *P. spinescens* being listed under commonwealth and state legislation: the *National Recovery Plan for the Spiny Rice-flower Pimelea spinescens subspecies spinescens* (Carter and Walsh, 2006), and the *Flora and Fauna Guarantee Action Statement for Spiny Rice-flower Pimelea spinescens subsp. spinescens* (Victorian Government Department of Sustainability and Environment, 2008). The overarching objective of the Recovery Plan is to “minimise the probability of extinction of *Pimelea spinescens* sbsp. *spinescens* in the wild and to increase the probability of important

populations becoming self-sustaining" (Carter and Walsh, 2006). There are seven specific objectives and aims to:

1. Acquire accurate information for conservation status assessments;
2. Identify habitat that is critical, common or potential;
3. Ensure that all populations and their habitat are legally protected;
4. Manage threats to populations;
5. Identify key biological functions;
6. Determine the growth rates and viability of populations; and
7. Build community support for conservation.

In 2009, an agreement was reached between the commonwealth and state governments which outlined prescriptions (Victorian Government, 2010b) to be used at sites containing *P. spinescens* approved for development within Melbourne's expanded urban growth boundary (Department of Natural resources and Environment, 2002b, Victorian Government, 2009b, 2010a). Application of these prescriptions is hinged upon the quality of the site and the numbers of individual plants present (Victorian Government, 2010b). Consequently, the importance of correctly identifying *P. spinescens* and accurately recording numbers present at a site is critical for its survival (Garrard, 2009).

Conservation and threat amelioration responses for *P. spinescens* losses have included the development of a seed collection protocol (Thomas, 2008b) and a translocation protocol (PsRT, 2013). Since 1999, considerable amounts of funding (Commonwealth of Australia, 2005) have been, allocated to various translocation methods including: cuttings (Mueck,

2000); direct seeding (P Wlodarczyck, 2010, pers. comm. November); nursery plants grown from seed (B Thomas, 2010, pers. comm. June & S Bretherton 2011, pers. comm. April); and whole mature plants (Mueck, 2000, S Bretherton 2011, pers. comm. April). This is despite a poor understanding of the species' life history, population biology and ecology. Reports of mortality and survival rates following the translocations have been difficult to obtain and at best only reported for the first year. Assessments of the long-term success for any of these methods are either in progress, unknown or access to the information is limited.

The Recovery Plan advocates for the development of management plans for all *P. spinescens* sites (Carter and Walsh, 2006). In 2012, a total of 18 plans, including six translocation management (Wlodarczyck, 2005, Mueck, 2006, MacDonald, 2008, Thomas, 2008a, Kellogg Brown & Root Pty Ltd, 2010, Hindell, 2011) and 12 site management plans (Mueck, Larwill *et al.*, 1998, Cropper, 1999, 2004, 2005, Robertson and Fitzimons, 2005, Cropper, 2006, Leversha, 2006, Cropper, 2007b, Griffith, 2008, Cropper, 2009, Culter and Mueck, 2009, Just and Francis, 2010), had been prepared since 1998. With the exception of Thomas (2008a) and Cropper (, 2000a, b, 2002, 2005, 2006, 2007a, b, 2009), reports pertaining to the outcomes for the subject populations are not readily available for public access.

The *P. spinescens* National Recovery Plan was published in 2006 and is currently under review. The review will include an update on the state of knowledge for the species and an evaluation of the achievements against stated objectives.

The Royal Botanical Gardens Melbourne (RBGM) is currently undertaking work to identify and quantify the pattern of genetic variation across the current range of *P. spinescens* (James, 2011). Results to date have found that the Volcanic Plains *P. spinescens*

populations sampled appear to have genetic connectivity, which is likely to be reflecting the historical gene flow found prior to the current fragmentation (James, 2013).

1.5 Research significance

Most of the information on *P. spinescens* is anecdotal, coming from a single site (Cropper, 2002, 2003, 2004, 2005, 2006, 2007b, a, 2009), two commissioned reports (Foreman, 2005, 2011) and plant salvage reports produced by ecological consultants such as Mueck (1998, 2000), Wlodarczyck (2005), MacDonald (2008) and Kellogg (2010). Given that efforts to bolster populations of *P. spinescens* using horticultural techniques in nursery settings have been largely unsuccessful (I Taylor, 2011 pers. comm. November), it is apparent that the best hope for the long-term survival of this species is to employ methods that encourage wild populations to successfully reproduce *in situ.* As a general guide, the literature on plant population ecology suggests that the following factors are required to achieve the goal of self-sustaining populations: different aged plants (including germinants), effective pollinators (Waser and Price, 1983, Olsen, 1997, Merrett, Robinson *et al.*, 2007), genetic fitness (Silvertown and Charlesworth, 2001, Holmes, James *et al.*, 2009) and the production of viable seed (Faegri and van der Pijl, 1979, Caswell, 2001, Eckstein, 2005, Fenner and Thompson, 2005, Griffiths, Wessler *et al.*, 2008). Additionally, specific environmental conditions and a management regime that facilitates a population's ability to recruit are also required (Lunt, 1997a, Morgan, 1998a, 2001).

Successful management of fragmented populations of *P. spinescens* that promotes autogenic recovery is reliant on knowledge of several key aspects of the species biology, ecology and habitat. These include:

- The characteristics of the structure, genetics and demography of individual populations;
- The importance of site characteristics, historical environmental events and management regimes;
- An understanding of predator and pollinator ecology; and
- An understanding of the ecology of reproduction.

1.6 Thesis aims and outline

This research was designed to learn more about the ecology and biology of the critically endangered species *Pimelea spinescens* sbsp. *spinescens*, in an attempt to promote its long term survival. Such knowledge will ensure that future funding and the corresponding allocation of resources have a sound scientific basis. The identification of significant characteristics of the species' reproductive biology and reproductive potential will enable land and wildlife managers to implement appropriate actions that will lead to germination and promote recruitment of populations *in situ*.

Four of the seven objectives within the *P. spinescens* 2006 Recovery Plan (Carter and Walsh, 2006) that are partially addressed by this research are:

- Objective one - to acquire baseline data in relation to population estimates of numbers, size and structure and a measure of population change;
- Objective two - requires the identification of environmental and habitat features that are essential for all life history stages of *P. spinescens,* including recruitment;

– Objective five - to evaluate the reproductive status, longevity, fecundity and recruitment of *P. spinescens* populations by conducting laboratory trials to determine seed germination requirements; and

– Objective six - requires comparison of census data in relation to site management histories.

The principal aim of this research was to identify which factors of the biology, ecology and management of *P. spinescens* populations significantly affect the species' *in situ* recruitment potential. In order to achieve the principal aim, these specific aims were addressed in the following chapters:

Chapter 2

1. Describe the size, composition and structure of a sub-sample of each of the target populations of *P. spinescens*;
2. Identify any relationships between recruitment potential and sub-sample population size, composition or structure.

Chapter 3

3. Formulate a method to assess the seed production of *P. spinescens* populations;
4. Formulate a method to assess the viability of *P. spinescens* seeds;
5. Assess various treatments for the ability to consistently germinate *P. spinescens* seed and use this method to calculate a germinability score;
6. To assess the recruitment potential of individual populations using measures of seed production, seed viability, seed germinability, and germination *in situ*; and

7. To derive a suitable measure/s of recruitment potential that could be used to evaluate the impacts of environment and habitat management.

Chapter 4

8. Evaluate the influence of environmental variables such as rainfall and temperature on recruitment potential.

Chapter 5

9. To evaluate the influence of past management practices on recruitment potential.

Chapter 6 will provide a summary of the results and interrelatedness of various factors found in this research. It will also assess the methods used in this thesis and include recommendations for future research which will inform and guide future management practices.

Chapter 2 Characteristics of *Pimelea spinescens* populations

"The investigation of nature is an infinite pasture-ground, where all may graze, and where the more bite, the longer the grass grows, the sweeter is its flavour, and the more it nourishes"

Thomas H. Huxley (1904)

2.1 Introduction

To ensure the long-term future of an endangered species such as *Pimelea spinescens* and to manage that species effectively, it is important to understand the biological functioning of its discrete populations (Godt, Hamrick *et al.*, 1990, Rymer, Morris *et al.*, 2002). To achieve these goals, basic demographic and phenological data are required – specifically, the number of extant populations, their size, spatial arrangement, structural characteristics and genetic composition (Begon, Harper *et al.*, 1996, Holsinger, 2000, James, 2011). The collection and interpretation of such demographic and phenological data is listed as the first objective of the *Pimelea spinescens* National Recovery Plan (Carter and Walsh, 2006).

Populations are dynamic and affected by both deterministic and stochastic factors that interact spatially and temporally (Silvertown and Charlesworth, 2001, Attiwill and Wilson, 2003). Deterministic factors are those that have a constant effect on the population in proportion to its size. Whilst deterministic factors can lead to population decline, it is often the complex association of stochastic factors that significantly influence population viability. Stochastic events occur randomly and the effects on the population are uncertain (Van Dyke, 2008). Sources of stochastic variation arise from a wide range of demographic, genetic and environmental characteristics, as well as catastrophic events (Shaffer, 1981, Gilpin and Soule, 1986, Silvertown and Charlesworth, 2001).

Random variations in the biotic and abiotic components of the environment can strongly influence the present and future success of a species, particularly in small and fragmented populations (Holsinger, 2000, Burgman, Kieth *et al.*, 2007), and for species with low rates of recruitment (Menges, 1992). For a species such as *P. spinescens*, population loss is an

ongoing phenomenon and the rate of recruitment is thought to be very low (Carter and Walsh, 2006). In order to understand the impacts of stochastic factors, it is important to establish baseline data which will enable the estimation of population changes that are associated with these complex and interacting factors.

2.1.1 *Population size*

A population is a group of individuals of the same species residing within the same geographical area that are able to interbreed to produce fertile offspring; a population is reproductively isolated from other groups of the same species (Smith and Smith, 1998, Solomon, Berg *et al.*, 1999). To be successful, a population requires enough individuals to recover from deleterious stochastic events and to maintain sufficient genetic diversity to be able to cope with possible future environmental changes or events.

A minimum viable population (MVP) has tentatively been defined by Shaffer (1981) as "the smallest isolated population having a 99 % chance of remaining extant for 1000 years despite the foreseeable effects of demographic, environmental and genetic stochasticity and natural catastrophes". Populations of fewer than 100 individuals (Falconer, 1989, pp 438) have been found to be more exposed to the risk of genetic failure due to inbreeding or the accumulation of deleterious genes (Gilpin and Soule, 1986, Ellstrand and Elam, 1993, Lynch, Conery *et al.*, 1995, Sherwin and Moritz, 2000, Silvertown and Charlesworth, 2001). However, MVPs are species specific and reports for various floristic taxa range from as low as 50 individuals to as high as 50,000 individuals (Franklin, 1980, Brown and Morgan, 1981, Shaffer, 1981, Soule, 1986b, Falconer, 1989, pp 438, Billington, 1991, Nunney and Elam, 1994). For dioecious species such as *P. spinescens,* which have complex life histories,

including a time lag before functional reproduction, it has been suggested that populations with fewer than 50 vagile individuals were in immediate danger of extinction and those that number less than 500 individuals were at long-term risk of extinction (Franklin, 1980, Shaffer, 1981, Soule, 1986b).

Various methods of estimating the size of plant populations have been used, the most common involving either a census or a sub-sampling process (Muir and Moseley, 1994, Elzinga, Salzer *et al.*, 2001). Due to their inherent variability, estimates based on sub-sampling usually rely on assigning populations to class sizes or categories (Muir and Moseley, 1994, Elzinga, Salzer *et al.*, 2001). For both types of estimates, care needs to be taken to clearly assess the 'effective' population size. The effective population consists only of those individuals that have the ability to actively contribute their genes to future progeny (i.e. flowering, gene contributing individuals) (Feidler and Kareiva, 1998). In dioecious plants such as *P. spinescens* the ability to produce offspring depends on a sufficient effective population of males and females being present to flower and seed set. Some authors suggest that the effective population size is often underestimated due to factors such as seed dormancy and persistence of seed banks (Soule, 1980, Meagher and Thompson, 1987).

From the current 208 sites that are known to support *P. spinescens* only 179 have population sizes recorded – in many cases these are roughly derived estimates. Fifty-three percent (95) of the 179 sites with population estimates support 50 individual plants or less. In total, 88 % of the sites with known population sizes have fewer than 500 *P. spinescens* plants (Department of Environment and Sustainability, 2012) and are likely to be at long-term risk of extinction (Franklin, 1980, Shaffer, 1981, Soule, 1986b). No sites have a population

greater than 50,000 individual plants and only three sites have population records of 5,000 individual plants or greater (Department of Environment and Sustainability, 2012). It is unclear what proportion of the plants at these sites represents an effective population.

2.1.2 *Population density*

Population size, while effective at determining numbers of individuals in a given area, is not informative for species where reproductive effectiveness is based on density-dependant factors, e.g. pollination (Brackenbury, 1995, Young, Boyle *et al.*, 1996, Silvertown and Charlesworth, 2001). Instead, population density, the distance between individuals, is often used as an index of abundance (Kunin, 1997). Generally, the size of a population is positively associated with the area occupied by the population. In some circumstances however, these two characteristics can be negatively related (Bruun, 2005). For example, a population may be numerically large even though the individuals within the population are sparsely dispersed over a large area (low density). Alternatively, a population may consist of few individuals that are aggregated together (high density).

Both population size and population density are important to the future success of a population. This is illustrated in studies of *Rutidosis leptorrhynchoides* (Button Wrinklewort), an herbaceous plant that is found in the Natural Temperate Grasslands of the Victorian Volcanic Plain. For this species, Morgan (1999b) found that populations of less than 30 individuals produced significantly fewer seed than populations of 500 to 5,000 individuals. This finding was supported by the genetic analysis of allozyme variation in 18 diploid populations, which found that those with fewer than 200 individuals had reduced allele richness, serving to decrease the effective population size and increase the chances of

genetic drift (Young, Brown *et al.*, 2000). Further to the importance of population size, population density was also found to have an influence on the reproductive success of the species. Research on rehabilitated populations of *R. leptorrhynchoides* found that those that were planted in high densities (0.5 m spatial intervals versus 4 m intervals) produced a relatively greater amount of seed (Morgan and Scacco, 2006). Similar findings have been reported for other species, where a smaller seed rain has been associated with smaller and more dispersed populations (Jennersten, 1988, Lamont, Klinkhamer *et al.*, 1993, Fischer and Matthies, 1998, Jacquemyn, Brys *et al.*, 2002). Greater seed set which resulted in positive population growth of some taxa was associated with high density populations (Brys, Jacquemyn *et al.*, 2004, 2005, Schleuning and Matthies, 2008).

Plant density may be particularly important for species that are dependent on animal pollinators. The ability of pollinators to find and move between plants determines the availability of an individual plant to potential mates and ultimately its reproductive success (Talavera, Bastida *et al.*, 2001). For example, the Australian plants *Acacia brachybotrya (*Grey Mulga*),* (Cunningham, 1999), *Banksia goodii* (Good's Banksia) (Lamont, Klinkhamer *et al.*, 1993) and *R. leptorrhynchoides* (Morgan, 1999b), were all unable to attract sufficient animals to effectively pollinate all available flowers within low density populations of these species. Such an effect is further compounded by the impacts of habitat fragmentation, which may negatively affect pollinator diversity and abundance (Steffen-Dewenter and Tscharntke, 1999, Duncan, Nicotra *et al.*, 2004, Wenzel, Schmitt *et al.*, 2006) and therefore the availability of insects to perform pollination (Powell and Powell, 1987, Aizen and Feinsinger, 1994). Without sufficient plant and pollinator density, both the quantity (Bosh and Waser, 1999, Steffen-Dewenter and Tscharntke, 1999, Mustajarvi, Pirkko *et al.*, 2001) and

quality (Newman and Pilson, 1997) of seed production has been found to be reduced in small populations.

Because *P. spinescens* is a dioecious obligate outcrosser (Commonwealth of Australia, 2009a, James, 2012), the inherent need to transport pollen between male and female plants (Rathcke and Jules, 1993, Alonso and Herrera, 2001, Aizen, Ashworth *et al.*, 2002, Rymer, Whelan *et al.*, 2005) makes it especially vulnerable to a lack of pollinators. *Pimelea spinescens* is pollinated by insects such as dipterans (flies) which are likely to have small home ranges and are only able carry pollen over finite distances (Powell and Powell, 1987, Morgan, 1995a). Therefore, the success of pollination for this species is likely to be influenced by the spatial distribution of individuals within a population, and also between populations. For *P. spinescens*, effective pollination and therefore production of sufficient quantity and quality of seed may be associated with both the size of the plant population and the density of the population (Steffen-Dewenter and Tscharntke, 1999, Mustajarvi, Pirkko *et al.*, 2001, Aizen, Ashworth *et al.*, 2002).

2.1.3 *Population structure and dynamics*

Ultimately, the population is defined by the rates of recruitment (natality and immigration) and departure (mortality and emigration). In plant populations, natality is a very difficult thing to measure because the essential unit of reproduction is a seed. The problem is that seed production varies between years and age-stage classes, and also that seeds can remain dormant in the soil for unknown periods of time (Hurtt and Pacala, 1986, Tilman, 1997, Smith and Smith, 1998, Silvertown and Charlesworth, 2001, Fenner and Thompson, 2005). Therefore, in plant demography it is of greater use to measure the germinant survival over

time as an indicator of natality. Plant mortality can be measured by quantifying the number of individuals lost over time.

Plant population dynamics are unique to each species and not all individual plants make an equal contribution to the population growth rate (Crawley, 1990, Morris and Doak, 2002). Age structures can be used to describe populations, although for many taxa of flora, it is difficult to accurately determine age (Huenneke and Marks, 1987, Manly, 1990). Instead, life stages are often defined by sorting individuals within a population into either cohorts (groups of individuals germinated in the same period of time) or life stage categories such as pre-reproductive, reproductive and post-reproductive (Smith and Smith, 1998, Larcher, 2003, Lee, 2006). For practical purposes, plants are often categorised into stages such as germinants, juveniles, reproductively mature adults and senescent adults (Crawley, 1990, Silvertown and Charlesworth, 2001).

The categorisation of plants into life-stages allows for estimations of the population health in terms of long-term persistence. For example, the population structure of a perennial species may consist of one mature plant and 99 juveniles; which is very different to a population consisting of 99 mature plants and one juvenile (Attiwill and Wilson, 2003, Schleuning and Matthies, 2008). Both populations are the same size but depending on the survival rates for each life stage there will be different outcomes for these populations over time.

Temporal monitoring of the numbers of plants and duration in each life stage category is an important baseline for beginning to understand a population's life stage distribution and long term viability (Smith and Smith, 1998, Silvertown and Charlesworth, 2001, Morris and Doak, 2002, Attiwill and Wilson, 2003). By analysing changes in these life stage changes over time,

it is possible to develop an understanding of the natural growth rate of the population during the prevailing environmental and management conditions (Crawley, 1990, Silvertown and Charlesworth, 2001, Larcher, 2003).

A species survivorship curve is a graph plotting the proportion of individuals within a cohort that survive from germination to the maximum age reached by any one member of the cohort (Smith and Smith, 1998). It is plotted as log survival versus age and described as three different Deevey curves:

- Type I - has few deaths in early life but high deaths later in life e.g. humans;
- Type II - has a constant death rate throughout the life span e.g. songbirds; and
- Type III - has high mortality in early life followed by low mortality later in life E.g. insects.

These are idealised models and for most species survivorship curves are intermediates between these models (Fenner, Smith and Smith, 1998).

Long-lived species such as *P. spinescens* approximate a Deevey Type III curve, with individuals contributing many seed to the population via annual reproductive events. Deevey Type III populations consist of many older individuals but recruitment events are often episodic and result in the introduction of only a small number of individuals over time (Keeler, 1991, Menges and Dolan, 1998). This type of population structure allows for recently fragmented populations to appear stable and retain previous levels of genetic variation (Eriksson and Jakobsson, 1998). Yet, if no germinants survive to a reproductive age then eventually the population will cease to exist, when old plants senesce and die

(Colling and Matthies, 2006, Malanson, 2008). In such circumstances, only the seed bank harbours any chance for the species long-term existence (Stocklin and Fischer, 1999). Such a process has been referred to as an extinction debt and was strongly associated with poor dispersers inhabiting isolated sites, where individuals that die are not replaced to compensate for losses over time (Malanson, 2008, Cousins, 2009, Jackson and Sax, 2009, Cousins and Vanhoenacker, 2011).

Pimelea spinescens is thought to be long lived species (Mueck, 2000). Little is known about the longevity of individuals or population age structures, or how these factors contribute to the species' reproductive biology (Lunt, Barlow *et al.*, 1998). It is possible that the majority of plants in these populations are very old and may suddenly die *en masse*, leaving limited reestablishment ability. A longitudinal study to assess the survival of germinants over time and their contribution to the maintenance of viable and self-sustaining populations is required. To date, there have been no cohorts of *P. spinescens* germinants that have been tagged and temporally monitored; therefore the long-term cohort survival rate is unknown and the viability of populations is uncertain.

2.1.4 *Sex ratios*

Sex expression by plants is often highly variable in both time and space and does not fit into perfect categories. Furthermore, the categorisation of plant sex is not standardised as it can be applied to flowers, individual plants and entire populations (Sakai and Weller, 1999, Tanurdzic and Banks, 2004). Most flowering plants are hermaphroditic, with perfect flowers that are comprised of both male and female reproductive organs (Sakai and Weller, 1999, Silvertown and Charlesworth, 2001). In contrast, about 6 % of flowering plants exhibit a

dioecious condition in which plants produce flowers with the reproductive organs of only one sex (male or female) (Yampolsky and Yampolsky, 1922, Renner and Ricklefs, 1995, Barrett, 2002).

Dioecy has independently evolved many times and has been described for more than half of the families of flowering plants. Some dioecious species have also reverted back to hermaphroditism (Richards, 1997, Silvertown and Charlesworth, 2001, Barrett, 2002). Along the pathway to dioecy, dimorphic populations can be classified as gynodioecious, subdioecious or dioecious but often, depending on the numbers of different sexes found within the population, these sexual systems form a continuum with grey boundaries (Lloyd, 1976, Barrett, 1992). Sakai (1999) provides the following definitions for these systems:

- A gynodioecious population contains plants which are functionally female, as well as plants which are hermaphroditic. A hermaphroditic plant has perfect flowers that can carry out both male and female functions;
- A subdioecious population contains functional male plants, functional female plants and male plants with varying degrees of hermaphroditic flowers; and
- A dioecious population consists of functional male plants and functional female plants, with little occurrence of hermaphroditism.

Although not fully understood, the evolution of the dioecious condition is thought to have two driving factors which are both associated with environmental stress. Firstly, under stressful conditions such as water deprived environments, the energetics of a plant may not be able to support the production of both male and female reproductive characteristics and therefore only one sex is produced. Secondly, stressed environments tend to support smaller

populations and therefore the development of a dioecious breeding system ensures a process of obligate outcrossing in order to maintain genetic diversity (Charlesworth, 1999). Due to the biogeography and associated climates, both Australia (Adam and Williams, 2001, Williams and Adam, 2010) and New Zealand (Godley, 1979) have a high incidence of dioecious angiosperms.

Dioecious species are assumed to have 1:1 sex ratios (Rychlewski and Zarzycki, 1975, Webb, 1999, Beaumont, Edwards *et al.*, 2006). However, variation in sex ratios across the geographic distribution of a dioecious or gynodioecious species is not unusual (Dudle, Mutikainen *et al.*, 2001, Vaughton and Ramsey, 2002, Bailey and Delph, 2007). Environmental conditions appear to alter this ratio depending on the reproductive costs for the species at various sites across its range (Agren, Danell *et al.*, 1999, Delph, 1999, Geber, 1999, Sakai and Weller, 1999, Bailey and Delph, 2007). Locations with unfavorable conditions will hinder the survival of the sex incurring the highest stress, which over time can lead to an imbalance in the sex ratio (Lloyd and Webb, 1977, Bierzychudek and Eckhart, 1988). In addition to the environmental influences at the population level, in some species the individual plants are able to alter their sexual phenotype in response to resource availability (Stephenson, 1992). For a range of dioecious species, a biased sex ratio in favour of males has been documented in stressful habitats such as those with:

- High elevations (Grant and Milton, 1979, Hoffmann and Alliende, 1984);
- High levels of salinity (Freeman, Klikoff *et al.*, 1976, Vitale and Freeman, 1986);
- High pH (Cox, 1981);
- Greater plant densities (Onyekwelu and Harper, 1979);

- High phosphorus levels (Cox, 1981);
- Limited water availability (Freeman, Klikoff *et al.*, 1976, Fox and Harrison, 1981, Hoffmann and Alliende, 1984); and
- A greater level of general exposure (Lloyd and Webb, 1977).

The corollary of this is that greater numbers of female plants have been reported when habitat conditions are more favourable (less stressful) (Bierzychudek and Eckhart, 1988).

In contrast, recent research (Barrett, 1992, Wolf and Shmida, 1997, Ashman, 1999, Charlesworth, 1999, Ramsey and Vaughton, 2001, Delph, 2003) has found more females and a greater tendency towards true dioecy at drier sites. This trend was also noted by Darwin (1877) who found that females of the same species were more prevalent in low moisture sites. Therefore, in dioecious populations inhabiting stressed environments there are often more females than males and very few hermaphrodites but in favourable environments there are fewer females than males and many hermaphrodites.

It seems that imbalanced sex ratios have not been detected as a threatening process to dioecious plants, perhaps because they have some level of plasticity in their sexual expression (Delph and Wolf, 2005). In contrast, there are many examples where environmental change has lead to a deleterious skew in sex ratios in animal populations, including reptiles (Hawkes, Broderick *et al.*, 2007, Patino-Martinez, Marco *et al.*, 2012), amphibians (Blaustein and wake, 1990), birds (Thomas and Lennon, 1999), mammals (Hersteinsson and Macdonald, 1992) and butterflies (Parmesan, Ryrholm *et al.*, 1999).

Understanding the sex ratio of *P. spinescens* populations under various environmental conditions may provide important clues to successful management practices. Foreman

(2011) found that the average sex ratio across 16 populations was close to parity but did not conduct any more detailed analyses (Foreman, 2011). Although hermaphrodites in this species are thought to be rare, both Cropper (2005) and Foreman (2005, 2011) listed plants as having an 'unknown sex'.

2.1.5 *Chapter aims*

Successful management of fragmented populations of *P. spinescens* that promotes autogenic recovery is reliant on knowledge of several key aspects of the species biology, ecology and habitat. The first objective of the *P. spinescens* recovery plan is to acquire baseline data in relation to population estimates of numbers, size and structure and a measure of population change (Carter and Walsh, 2006). To address this, the objectives of this chapter are to:

1. Describe the size, composition and structure of a sub-sample of 16 targeted populations of *P. spinescens*; and
2. Identify any relationships between recruitment potential and sub-sample population size, composition or structure.

2.2 Methods

2.2.1 *Site selections*

For the purposes of accessibility and comparability, only those sites supporting *P. spinescens* populations on volcanic derived, black or grey clay soils were targeted for this study. Plants in the northern populations, on red clay complex soils, were not targeted for this study, as they have been described as more robust, exhibit a different structure and could possibly be a different sub-species (Walsh and Entwisle, 1996, Carter and Walsh, 2006).

In 2008, according to the VBA database, there were 48 sites listed as supporting *P. spinescens* on volcanic clay soils. These sites were categorised into four groups (Table 1) on the basis of the number of *P. spinescens* plants claimed to be present. The median four sites from each category were selected to capture a broad reflection of site conditions and population demographics. For the purposes of this study, each site is considered to be a discrete entity – that is, there is no interaction between sites. Thus, in total, 16 populations of *P. spinescens* were the focus of this study. Refer to Table 2 for information acquired from the VBA database in 2008, for the estimated number of individual plants at each site, as well as site size, elevation above sea level (ASL) (m), location and tenure. See figure 7 and 8 for a map of the geographic location of each of the 16 study sites across Victoria.

Table 1 - The study sites selected from the total number available on volcanic soils within each size class.

Population size class	Total number of sites on volcanic soils	Selected sites
>1,900	3	▪ Geggies Rd ▪ Mount Mercer-Shelford Rd (Mt Mercer Rd) ▪ McKenzie Rd* ▪ Poorneet West Rail Reserve (Poorneet WRR)
<1,900 - >300	13	▪ Christies Rd ▪ Cedarwoods Reserve (Cedarwoods R) ▪ Kirks Bridge Rd (Kirks BR) ▪ Glengower Rd (Glengower Rd)
<300 - >100	9	▪ Vite Vite Rail Reserve (Vite VRR) ▪ Ararat Airfield Rd (Ararat AR) ▪ Browns waterholes Bridge Rail Reserve (Brownswaterholes BRR) ▪ Baringhup West Rd (Baringhup WR)
<100 - >20	23	▪ Pitfield-Cressy Rd (Pitfield CR) ▪ Carisbrook-Baringhup Rd (Carisbrook BR) ▪ Calder Rise Rail Reserve (Calder RRR) ▪ Bannockburn Rail Reserve (Bannockburn RR)

* There were only six sites listed on the VBA database that had *P. spinescens* populations greater than 1,900, and only three of them were on volcanic soil. The McKenzie road site is located on the boundary of the soil bioregions classified as the Victorian Riverina and Goldfields. It was chosen for the study because it had a closer proximity to volcanic soil than the other two prospective sites.

Figure 7 - Map of Victoria, Australia, with inset defining the broad study region (State of Victoria, 2011).

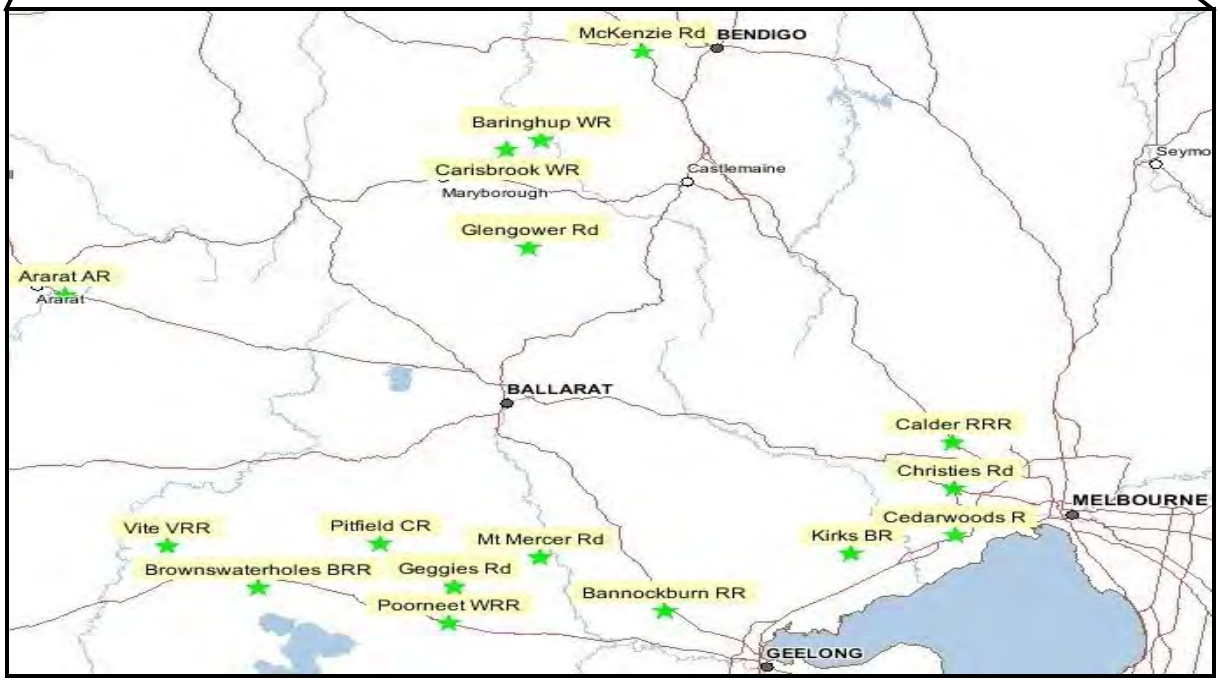


Figure 8 - The location of 16 *Pimelea spinescens* subsp. *spinescens* populations selected for this study (State of Victoria, 2011).

Table 2 – Information about each study site that was accessed from VBA records, organised into population size classes.

Population classes	No	SITE NAME	Elevation ASL (m)	Min No. of plants	Max No. of plants	Area (LxW)	Easting	Northing	Locality	Local Government Authorities	Land Tenure
> 1,900	1	Geggies Rd	167	2,068		3.1 kmx32 m	741128	5793313	Rokewood	Golden Plains	Roadside
	2	Mt Mercer SR	231	21,000		4 kmx32 m	756392	5800034	Shelford	Golden Plains	Roadside
	3	McKenzie Rd	198	4,734	5,000	800 mx40 m	243722	5926938	Marong	Greater Bendigo	Roadside
	4	Poorneet WRR	172	1,900		150 mx70 m	739977	5784014	Cressy	Colac Otway	Private rail Reserve
< 1,900 & > 300	5	Christies Rd	81	500		269.3 mx260 m	301162	5818420	Ravenhall	Melton	Reserve
	6	Cedarwoods R	24	453		375 mx260 m 330 mx280 m	301681	5806727	Laverton	Wyndham	Reserve
	7	Kirks BR	69	400		1.6 kmx20 m	283549	5801745	Little River	Wyndham	Roadside
	8	Glengower Rd	295	500		1.4 kmx13 m	757970	5877970	Campbelltown	Hepburn	Roadside
< 300 & > 100	9	Vite VRR	223	175		141 mx119 m x78 m	691271	5805590	Vite Vite	Corangamite	Railway Reserve
	10	Ararat AR	310	211		549 mx386 m	676009	5869354	Dobie	Ararat	Airfield roadside
	11	Brownswaterholes BRR	158	150	175	250 mx50 m	706757	5794579	Gnarput	Corangamite	Railway Reserve
	12	Baringhup WR	194	195	220	286 mx20 m	761127	5904500	Mount Alexander	Mount Alexander	Roadside
< 100 & > 20	13	Pitfield CR	200	50		2.8 kmx15 m	728575	5804832	Pitfield	Golden Plains	Roadside
	14	Carisbrook BR	196	20		500 mx6.5 m	755049	5902734	Baringhup West	Mount Alexander	Roadside
	15	Calder RRR	168	30		300 mx17 m	300551	5829939	Sydenham	Brimbank	Railway Reserve
	16	Bannockburn RR	130	31		3875 mx24 m	251098	5786613	Bannockburn	Golden Plains	Railway Reserve

2.2.2 *Pilot study*

A pilot study was conducted at nine of the study sites in 2008, in order to develop a robust and efficient method of assessment. The nine sites that were included in the pilot study were:

- Geggies Rd;
- Mt Mercer Rd;
- Christies Rd;
- Cedarwoods R;
- Kirks BR;
- Vite VRR;
- Ararat AR;
- Pitfield CR; and
- Bannockburn RR.

2.2.2.1 Establishing a study area

Within each *P. spinescens* site, a smaller, targeted study area was required to:

- Subsample the *P. spinescens* population at each site in an efficient and systematically robust manner;
- Enable an efficient method for estimating the population density of *P. spinescens*, for comparison between study areas; and

- Capture the variation of plant density for each study area, irrespective of the overall distribution across the entire site.

As part of the pilot study, a preliminary trial was conducted at Christies Rd, to determine the method for establishing a study area at each site. Christies Rd was opportunistically selected for this preliminary study due to its accessibility, recent biomass removal and a population of 788 plants that had been systematically tagged prior to the trial commencing (MacDonald, 2008). The population of *P. spinescens* at Christies Rd (and most likely all other populations) displayed a spatially aggregated distribution. Each individual plant had been flagged ensuring that the area selected contained areas of no individuals (>5m² that had no plants) as well as areas with a range of plant densities (< 0.5 plant/m² - > 1 plant/m²). A study area representing a typical part of the site containing *P. spinescens* plants was selected. The size of the study area was sufficiently large to ensure that it reflected the arrangement of the broader population by containing sections with high densities of plants, as well as sections that had no plants or low plant density.

The study area was measured out using a tape measure to form a rectangular polygon, enabling the area to be calculated (60 m x 25 m = 1,500 m²). With the intention of sub-sampling 10 % of the study area, the longest axis of the polygon was divided into 1m wide transect lines and six transect lines (6 x 1 m x 25 m = 150 m^2 = 10 %) were randomly selected for assessment, using a random number generator (see Figure 9 for a similar example).

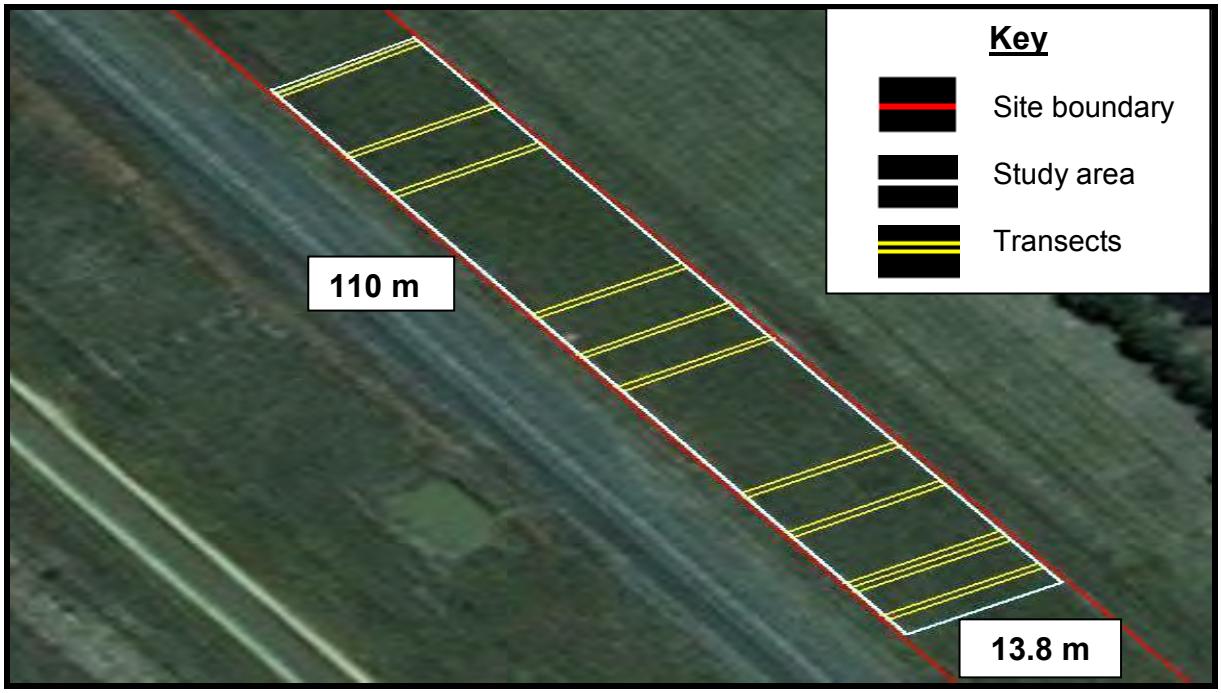


Figure 9 - An example of a study area (white outline) within a site (red outline). The polygon is 110 m long by 13.8 m wide (area = 1,518 m²), therefore eleven 1 m x 13.8 m transects (yellow outlines) were assessed to meet the 10 % sub-sampling requirement.

Within each transect, the number of *P. spinescens* plants were counted, tallied and extrapolated out to estimate the total number of plants that occurred within the study area. Additionally, the total number of *P. spinescens* plants within the entire study area were systematically counted to provide a comparison with the estimate derived from the transect data.

Assessment of the six randomly selected transects located 14 plants, which extrapolated out to 140 plants for the entire study area. In contrast, 150 plants were counted within the study area, indicating that the random transect method used to estimate the population of the study area had 93 % accuracy (see Table 3). To test for consistency of results, it may have

been beneficial to repeat this trial several times in populations where all individual plants had been identified and counted. However, given the constraints of time and resources, the results of this single trial were considered acceptable and the time taken to complete the survey was three hours, which would reasonably allow for two populations to be surveyed in a day. Thus, study areas of approximately 1,500 m² were established at all the other sites for the pilot study and also for the main study which commenced in 2009. Prior to study area selection a rapid on site assessment of the distribution of *P. spinescens* plants was conducted in order to select a study area that was representative of a typical part of the site's *P. spinescens* population.

Table 3 - Summary of preliminary site data collected.

Site	Population size class	Total area of site (m²)	Study area surveyed (m²)	No of transects	Estimated no of plants in study area	Actual no of plants in study area	Estimated plant density (plants/m²)
Christies Rd	<1,900 - >300	70,018	1,500	6	14 x10=140	150	0.093

Minimum sampling requirements

As part of the pilot study, a trial was conducted to determine the minimum numbers of plants required to represent the population within each study area. To do this, four 2 m² quadrats were selectively located in areas of varying densities (high/low) of *P. spinescens*, immediately outside the defined study area (as described above) at each of the pilot study sites.

Basal circumference was used as the feature for determining minimum sample size because it was found to be strongly correlated to the below ground measure of taproot circumference (D. Reynolds, unpublished data). Taproot circumference is thought to be somewhat indicative of the development of the individual plant over time. The variance of the basal circumference measure was found to be homogeneous between sites, suggesting that this is a potentially useful indicator of plant age that is not strongly influenced by biomass reduction events such as grazing, mowing or fire. Basal circumference was measured by firmly wrapping a piece of static string around the base of the plant, without constricting the stems. The length of the string was measured in centimetres.

During the pilot study, the basal circumference measurement was collected from between 23 and 67 plants within the quadrats at each site. Using data from the site with the largest sample size (67 plants at Ararat AR), a power analysis was conducted to estimate the minimum sample size required to represent a population. The power analysis indicated that a sample size of greater than 19 plants was required to achieve a 95 % confidence interval of no wider than 4 cm (d = +/-2 cm). Therefore, at least twenty plants were targeted for sampling from quadrats within each study area (see methods below) for the main study conducted throughout 2009 and 2010.

2.2.3 *Main study*

Between July 2009 and October 2010, each of the 16 sites were visited for data collection purposes five times. At each of these sites a study area was established by defining an area of approximately 1,500 m² that represented a typical part of the site containing *P. spinescens* plants (see above – section 2.2.2.1). Locational information such as longitude, latitude and

elevation ASL were acquired directly from a Global Positioning System (GPS) (Garmin GPSMAP® 60CSx) for each site.

2.2.3.1 Population estimation study

Within each of the defined study areas, randomly selected transect lines were located to enable 10 % of the area to be assessed. Within these transect lines the following data was recorded: number of plants; as well as the sex and flowering status of each individual. Plants were categorised as male, female, non-flowering or germinants. Males had flowers with stamens and the anthers carried conspicuous orange pollen (Figure 10). Female flowers were usually less obvious, with a single style with stigma, and anthers on the inconspicuous stamens did not bear orange pollen (Figure 11). Some individual plants displayed both male and female flowers (hermaphrodites) but were recorded as males. Non-flowering plants had no flowers. Germinants were identified by the presence of their cotyledons (first two leaves) signifying recent germination and they often had a small central stem, which was usually red in colour (Ralph, 2003) (see Figure 12).

Figure 10 - Male *P. spinescens* flowers had obvious stamens with distinctive orange pollen on the anthers.

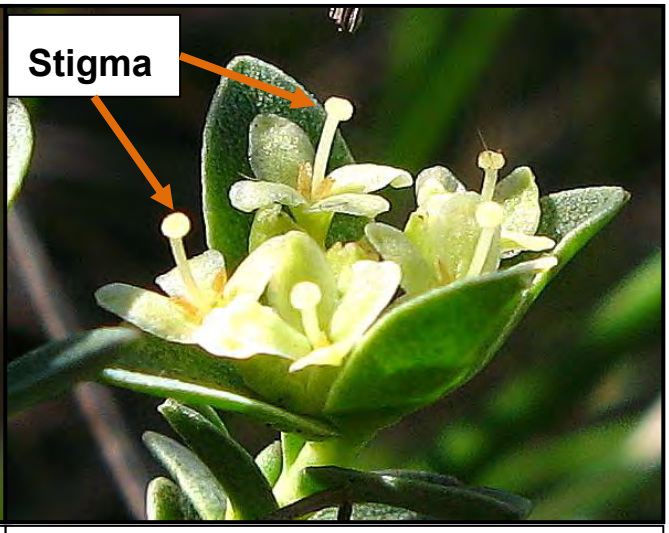


Figure 11 - Female *P. spinescens* flowers had an obvious stigma and small stamens with no pollen.

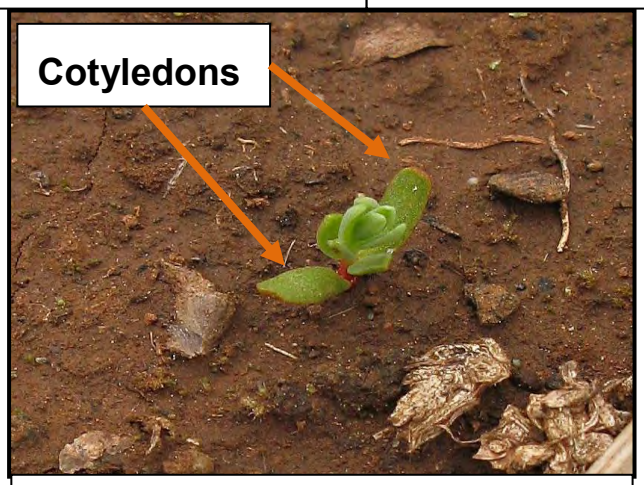


Figure 12 - A *P. spinescens* germinant with cotyledons and a small central stem.

Data analysis

Population size and density

For each study area, the number of plants (excluding germinants) found within the transects was multiplied by ten to obtain an estimate of the total number of plants. The population density was calculated by dividing the number of plants (excluding germinants) recorded in the transect lines by the total area (square metres) assessed in the transect lines.

Population structure and flowering status

Within each study area the estimated proportion of females, males and non-flowering plants was calculated by dividing the number of plants found in each category, respectively, by the total number of plants recorded in the transects (excluding germinants), and multiplying by one-hundred.

Sex ratios

The sex ratio was calculated by dividing the number of males by the number of females found within the transects of each study area.

The ratio of males to females at each study area was assessed using a chi-squared goodness of fit test to see if the ratio differed from the expected 1:1. To determine whether there was any difference in sex ratios between study areas, a heterogeneity chi-squared test was conducted (Zar, 1999).

2.2.3.2 Longitudinal study of selected plants

Assessment of transects within each study area (as described above) were used to provide a description of the general characteristics of populations of *P. spinescens*. A more targeted and longitudinal study of individual plants was conducted to assess the processes of natality and mortality. To determine natality/mortality, a series of monitoring quadrats containing at least one *P. spinescens* individual were established within each study area.

Within each study area, a minimum of three permanent quadrats were established in 2009 to enable repeated seed sampling from mature plants and to track the survival of mature and germinant individuals. Initially, a *P. spinescens* density-area curve was obtained to find the most appropriate quadrat size for sampling from within each study area (Cropper, 1993, Oostermeijer, 1994). Following this a random number generator (calculator) was used to randomly locate a grid position within the study area for the placement of the quadrats (Figure 13). If twenty plants were not found within the first three quadrats, then further quadrats were randomly located and assessed until this target sample size was achieved. Thus, the total number of quadrats; the size of the quadrats; and the number of female plants assessed, varied between study areas.

Figure 13 – A two metre squared random quadrat at Pitfield CR in 2009.

In 2009, all plants within the randomly located quadrats were individually tagged (Figure 14). The tags displayed individual numbers and were made from galvanized steel which was rust, fire and weather resistant. They were anchored by stainless steel orchid pins.

Figure 14 - Individually tagged plants located within a one metre squared quadrat at Brownswaterholes BRR in 2009.

For each tagged plant, the following data was recorded:

- Sex (as previously described – in section 2.2.3.1);
- Form (see descriptions below – in the 'plant form' section);
- Basal circumference (as previously described – in the 'minimum sampling requirements' section);
- Distance to nearest neighbour (the measurement (cm) to the closest *P. spinescens* plant);
- Flowering status (flowering or non-flowering); and

- Life-stage (see descriptions below - in the 'life stage and flowering status' section).

Plant form

The typical *P. spinescens* plant was about 5 - 10 cm high by 20 – 25 cm wide but in the field it was noted that there was a high level of variability in size, stature and leaf coverage. A classification system of plant forms was employed to capture these variations. Eight different forms were described for plants in the randomly located permanent quadrats, using a combination of the following characteristics:

- Small (<15 cm height or width) or large (>15 cm height or width);
- Upright or prostrate (in stature); and
- Compact (<50 % leaf coverage of entire plants branches) or open (<50 % leaf coverage of entire plants branches).

Pictorial examples of each of the forms are presented in figures 15 A, 15 B, 16 A, 16 B, 17 A, 17 B, 18 A and 18 B.

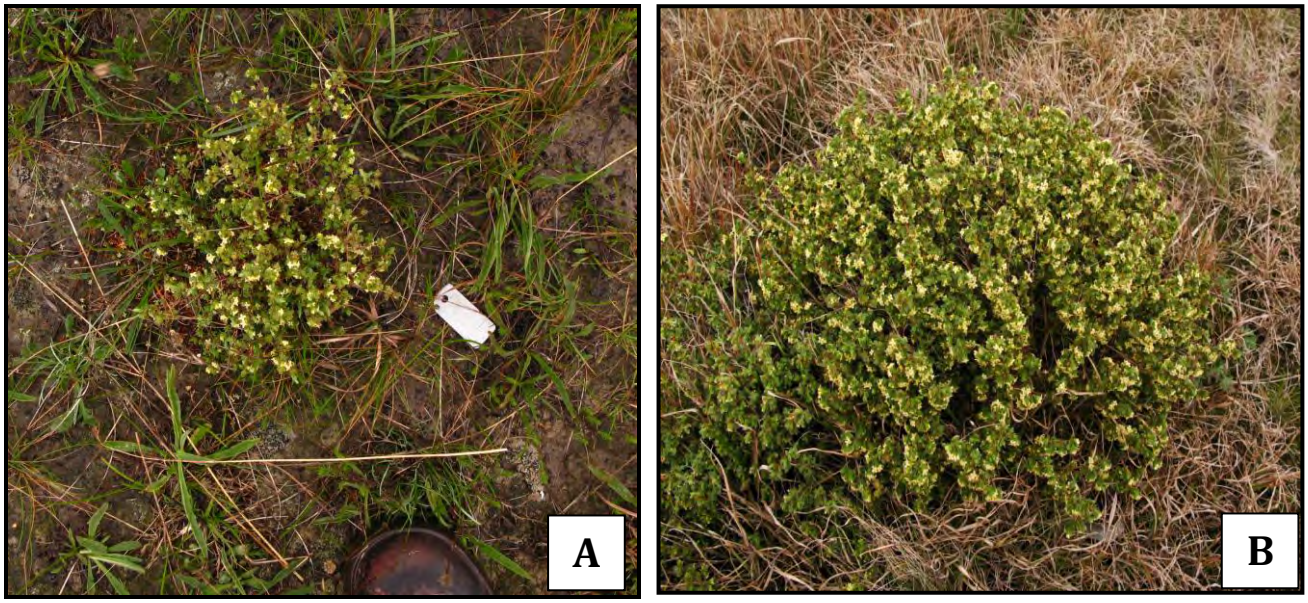


Figure 15 A - Small upright compact individual. B. Large upright compact individual.

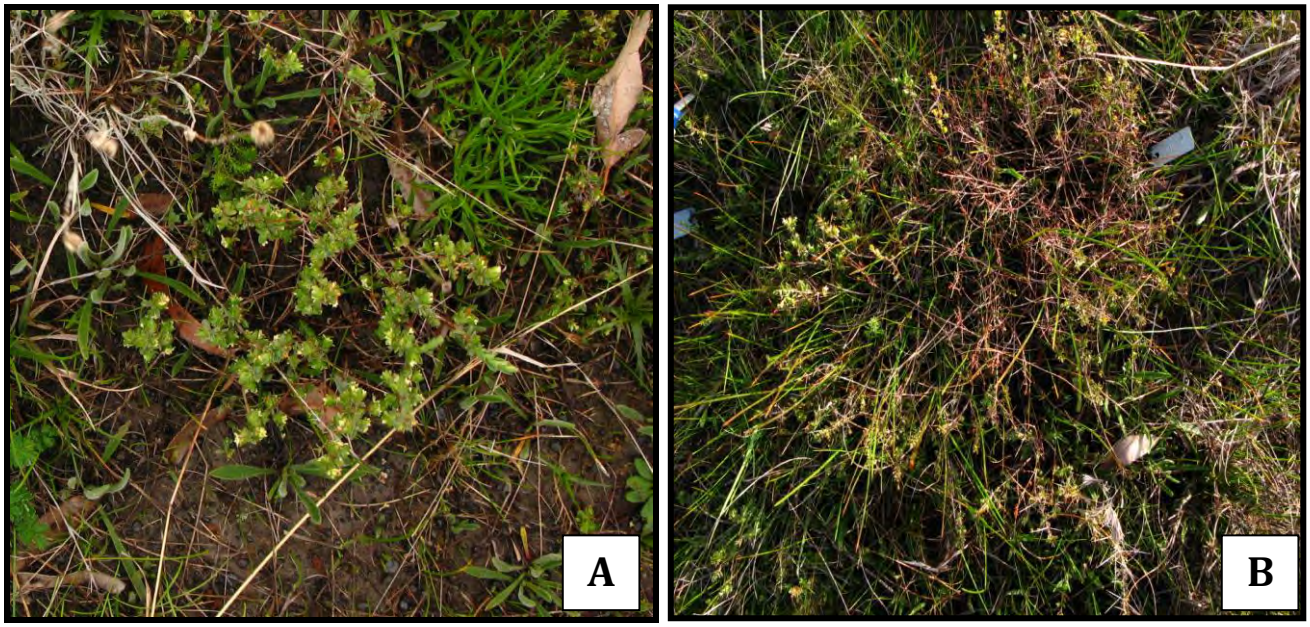


Figure 16 A - Small upright open individual. B. Large upright open individual.

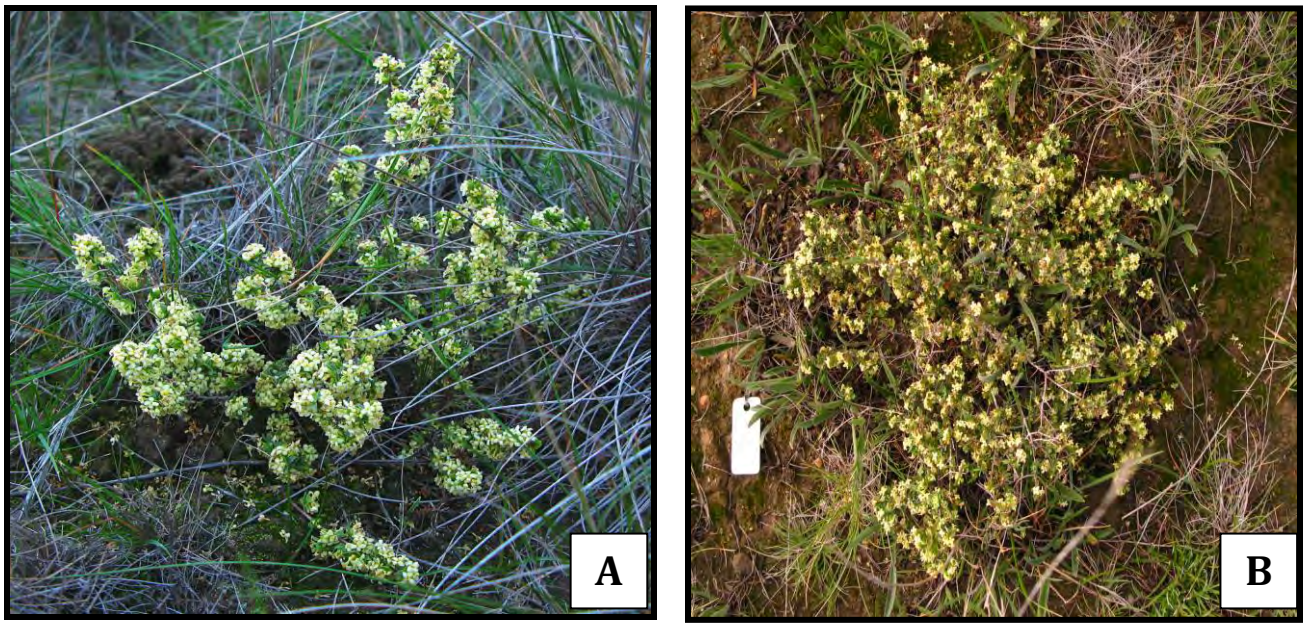


Figure 17 A - Small prostrate compact individual. B. Large prostrate compact individual.

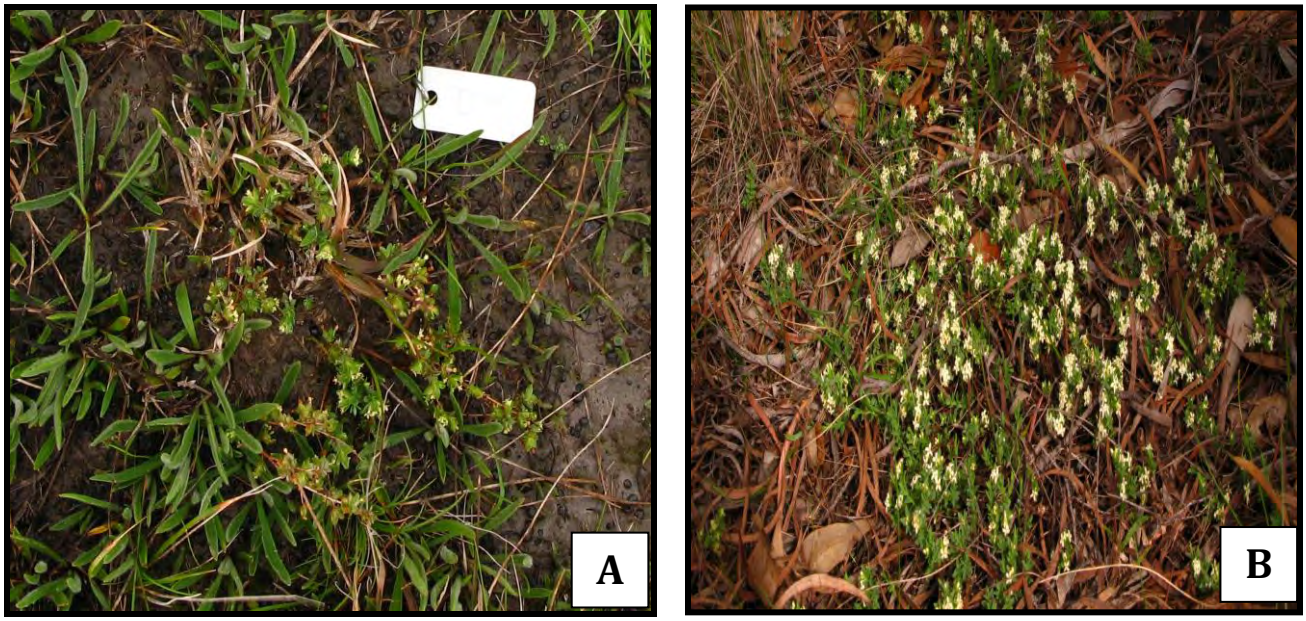


Figure 18 A - Small prostrate open individual. B. Large prostrate open individual.

An additional form was also described; a single stemmed individual with or without cotyledons that was either flowering or non-flowering (see Figure 12, 19a and b).

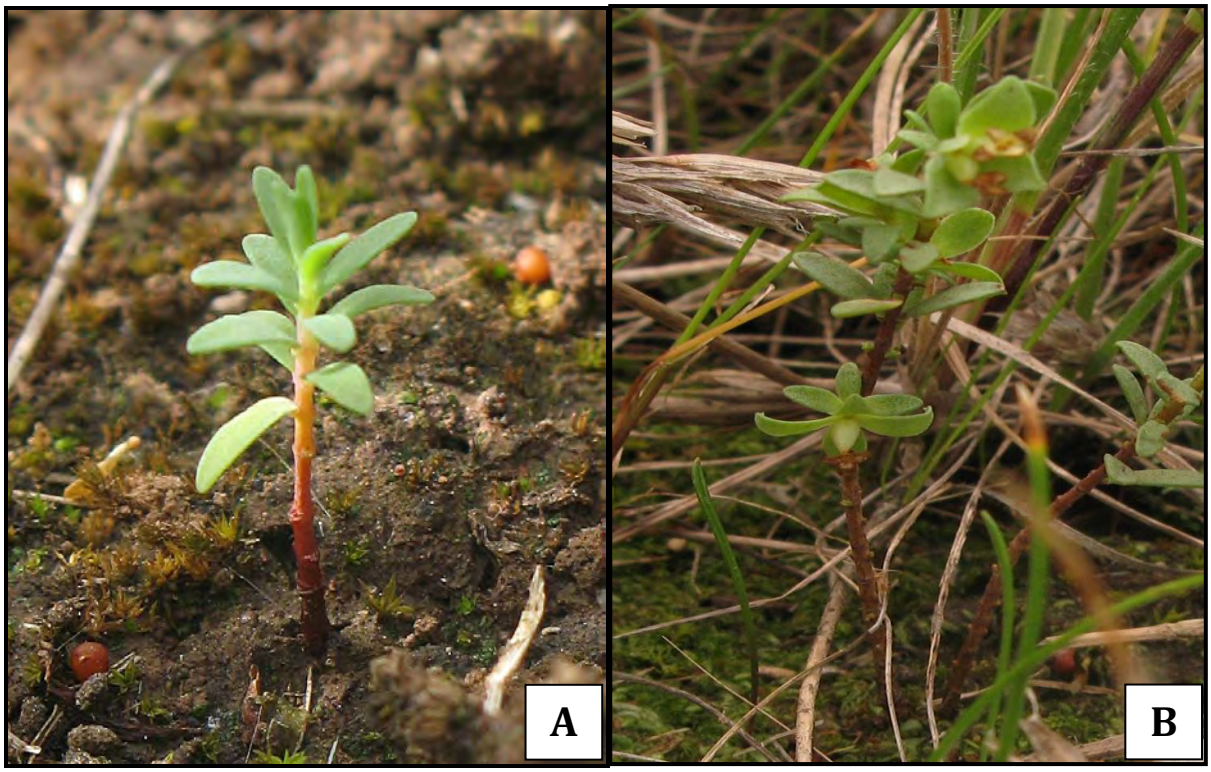


Figure 19 – A. A single-stemmed juvenile *P. spinescens* . B. A first year seedling *P. spinescens* with evidence of flowering.

Life-stage and flowering status

Plants within the randomly located quadrats were categorised into the following life stages:

- Juvenile plant - a single stemmed individual; has only one stem exiting the ground, although it may have many branches; fresh supple growth; the central stem can be

woody but other stems are not woody; and observed with or without cotyledons (Figure 19a and 19b). Juvenile plants were then considered as either:

- A first year flowering seedling – observed flowering or displayed evidence of flowering during the flowering season (Figure 19b); or
- A non-flowering juvenile – not observed flowering during the flowering season.

- Mature plant - more than one stem exiting the ground; older rigid growth; stems and branches were woody. Mature plants were then considered as either:
  - Flowering mature – observed flowering during the flowering season, then further catergorised as either male or female (see method section 2.2.3.1 above); or
  - Non-flowering mature – not observed flowering during the flowering season.

The height of juveniles greater than 0.2 cm was measured to determine:

- Whether there was a minimum height that an individual plant must obtain before flowering can occur; and
- If the sexual phenotype displayed is influenced by height.

Data analysis

For each study area, data from the randomly located quadrats was pooled. The proportion of plants for each life-stage category was calculated by dividing the number of plants in each respective category, by the total number of plants found within the combined quadrats and multiplying by one-hundred.

The height data for first year flowering seedlings was combined for all study areas. An independent sample *t*-test was used to compare the height of males and females.

Within each quadrat the density was calculated by dividing the number of plants (excluding germinants) recorded in the quadrats by the total area (square metres) assessed of the quadrat.

Mature plant mortality

All the mature plants that were located within quadrats and tagged in 2009, were identified the following year (2010) to assess for survival. For each study area, mature plant mortality was calculated in 2010, as a proportion of the total number of mature plants present in 2009. Descriptive statistics were used to assess the rate of mortality for each of the categories: male; female; and non-flowering plants.

Natality

In situ germinant production and survival

Each quadrat was assessed twice in 2009 and twice in 2010 for the presence of germinants (Figure 20). Any germinants that were located were counted and permanently tagged; some germinants appeared in clusters and only received one tag but all were counted. Germinants were able to be progressively monitored for survival throughout the study period.

Germinant survival was calculated by dividing the number of germinants relocated in 2010 from the numbers of germinants produced within the random quadrats of the study areas in 2009, then multiplying by one-hundred for a percentage of surviving germinants.

Figure 20 - An indication of the small size of two germinants.

Germinant density

For each of the main study years (2009 and 2010), the germinant density for each study area was calculated by dividing the number of germinants recorded within the quadrats by the total area (metres squared) assessed in the quadrats.

Data analysis

A Paired sample *t* test with an α of 0.05 was use to compare the germinant densities of all 16 study areas for the years 2009 and 2010. A Cohen's *d* value was used to assess the size of the effect (Cohen, 1988). A simple multiple regression analysis was conducted using SPSS (Version 18) to identify the study area population variable (estimated population size, population density, percentage of females, percentage of males, female density, male density and sex ratio) which had the greatest influence on germinant density. Using the R package `lme4` (Bates, Maechler *et al.*, 2011), a generalized linear mixed model with a Poisson family and log link function (Poisson regression) was used to identify which significant variable had the greatest influence on germinant density.

Rate of population growth

The annual or finite rate (FRI) of population increase was determined by the ratio of the number of individual plants counted in the quadrats in 2009 divided by the number of individual plants present in the same quadrats in 2010. To obtain the intrinsic rate of increase or the exponential growth rate, the FRI value was transformed into its natural logarithm.

Statistical analysis

For all 16 study areas the demographic data recorded from the transects and from the quadrats were correlated using Microsoft Excel against: the geographical attributes of each site (location and elevation); all potential recruitment measures (see the introduction of Chapter 3); individual plant measures (basal circumference and distance to nearest

neighbour); and the longitudinal study quadrat density. Bivariate analyses were conducted via SPSS (Version 18) to assess the direction and strength of any associations. Data that was normally distributed underwent a Pearson's product-moment correlation (r). Where data did not conform to the requirements of normality, data transformations were conducted according to Zar (1999). If the data obtained was logarithmic and included zero values a value of one was added to the raw data before $\log_{10}$ transformation. When normality could not be achieved, a Spearman's rho analysis (r_s) was conducted. Some graphs were obtained using "R" via the "RStudio" version. All graphs where study areas are represented are organised in descending order of the preliminary population size classes derived from the VBA database.

2.3 Results

2.3.1 *Population estimation study*

2.3.1.1 Population size and density

The mean size of the 16 study areas was 1,485 m². The smallest study area was 650 m² in size and all others ranged between 1,256 m^2 and 1,760 m^2. Carisbrook BR was estimated to have the smallest number of plants (70) with Poorneet WRR having the largest (5,250) and the average across all sites was 1,127.

Although the 16 sites had been selected from the VBA database on the basis of population size categories, the number of plants and corresponding densities within the defined study areas did not follow a similar pattern. That is, sites in large population size categories did not necessarily support the largest number of plants and greatest plant densities within the respective study area, and vice versa. Two of the study area populations displayed very high densities of *P. spinescens* (Table 4). These were Poorneet WRR (2.98 plants/m²) which was selected from the largest size class (>1,900 plants) and Brownswaterholes BRR (2.8 plants/m²) which was selected from the third-largest size class (<300 & >100 plants). The density of *P. spinescens* plants across the study areas ranged from 0.05 to 2.98 plants/m², with an average density of 0.65 plants/m².

Table 4 – Estimates of the number of individuals and plant density in each of the 16 study areas, arranged by preliminary population size classes.

Site	Population size class of entire site	Study area (m^2)	Estimated no of plants in the study area	Plant density (plants/m^2)
Geggies Rd	**> 1,900**	1350	710	0.52
Mt Mercer SR		1700	740	0.43
McKenzie R		1575	1540	0.97
Poorneet WRR		1760	5250	2.98
Christies Rd	**< 1,900 & > 300**	1610	260	0.16
Cedarwoods R		1608	130	0.08
Kirks BR		1510	1170	0.77
Glengower Rd		1560	610	0.39
Vite VRR	**< 300 & > 100**	1256	90	0.07
Ararat AR		1610	630	0.4
Brownswaterholes BRR		1740	5010	2.8
Baringhup WR		1600	310	0.19
Pitfield CR	**< 100 & > 20**	1540	330	0.21
Carisbrook BR		1300	70	0.05
Calder RRR		650	740	1.14
Bannockburn RR		1518	440	0.29

2.3.1.2 Population structure and flowering status

All study areas except Vite VRR contained a proportion of non-flowering individuals. Excluding Vite VRR, the proportion of non-flowering plants ranged from 4 % to 66 % of the total numbers, with an average of 26 % (Figure 21). All study areas had both male and female flowering plants. The proportion of male flowering plants ranged from 11 % to 89 %

of the total numbers, with an average of 40 %. While the proportion of female flowering plants ranged from 8 % to 77 % of the total numbers, with an average of 32 %.

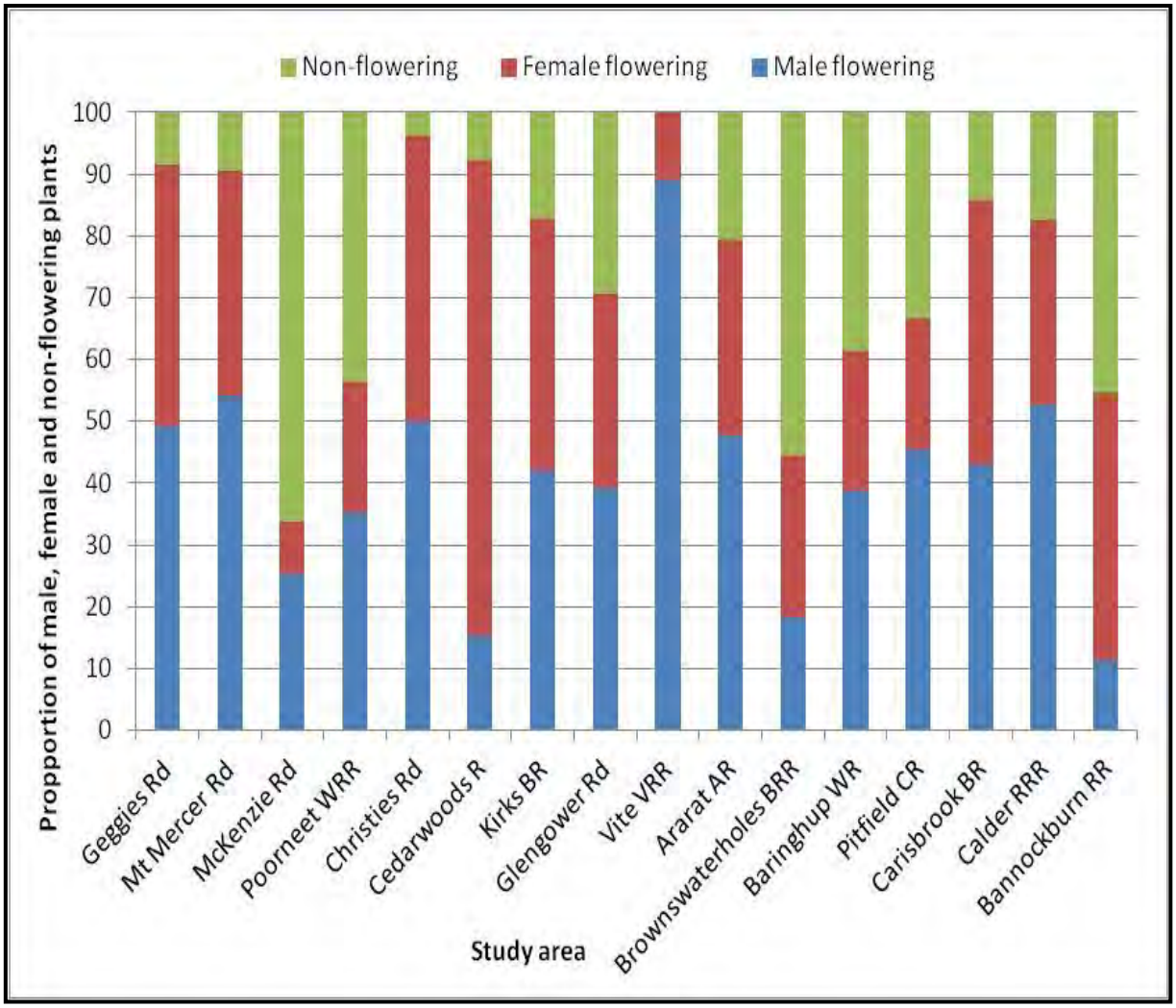


Figure 21 –The proportion of male, female and non-flowering *P. spinescens* plants at each study area (assessed in 2009).

2.3.1.3 Sex ratios

A chi-squared goodness of fit test revealed that the sex ratio at six of the study areas was significantly different from parity. The assumptions of expected frequencies have not been violated as only 12.5 % of expected frequencies were less than 5 which is below the 20 % violation threshold. A heterogeneity chi-squared analysis indicated that significant differences occurred in the sex ratios between study areas $\chi^2 = 50.9$, $df = 15$, $p < 0.001$. At Cedarwoods R, Brownswaterholes BRR and Bannockburn RR significantly fewer male than female plants were observed, whereas Calder RRR, McKenzie Rd and Poorneet WR exhibited the opposite, having significantly more male than female plants (Table 5).

Table 5 – The sex ratio of flowering plants within each study area. Six of the study areas were significantly different from parity, as indicated by a significant *p* value (*).

	Number of plants (M+F+N/F)	Male	Female	Percentage of females	Sex ratio M:F	Chi-square value	*df*	*p*
Geggies Rd	71	35	30	42	1:0.86	0.55	1	>0.75
Mt Mercer Rd	74	40	27	36	1:0.675	2.14	1	>0.25
McKenzie Rd	154	39	13	8	1:0.33	13	1	<0.001*
Poorneet WRR	525	185	111	21	1:0.6	18.5	1	<0.001*
Christies Rd	26	13	12	46	1:0.92	0.0083	1	>0.09
Cedarwoods R	13	2	10	77	1:5	5.3	1	<0.025*
Kirks BR	117	49	48	41	1:0.97	0.02	1	>0.75
Glengower Rd	61	24	19	31	1:0.79	0.37	1	>0.5
Vite VRR	9	8	1	11	1:0.125	1.64	1	>0.25
Ararat AR	63	30	20	31	1:0.66	2	1	>0.1
Brownswaterholes BRR	501	92	131	26	1:1.42	6.85	1	<0.01*
Baringhup WR	31	12	7	22	1:0.58	1.9	1	>0.25
Pitfield CR	33	15	7	21	1:0.46	2.8	1	>0.05
Carisbrook BR	7	3	3	42	1:1	0	1	>0.25
Calder RRR	74	39	22	29	1:0.56	5.3	1	<0.025*
Bannockburn RR	44	5	19	43	1:3.8	4.41	1	<0.025*

2.3.1.4 The influence of location on sex ratios

Further analysis of the sex ratio data revealed that, a greater proportion of females compared with males were present at study areas with a lower elevation within the landscape. A bivariate analysis indicated the presence of a positive correlation between the sex ratio (male/female) and elevation above sea level of the respective study area $r = 0.562$, $p = 0.023$, n = 16 (Figure 22).

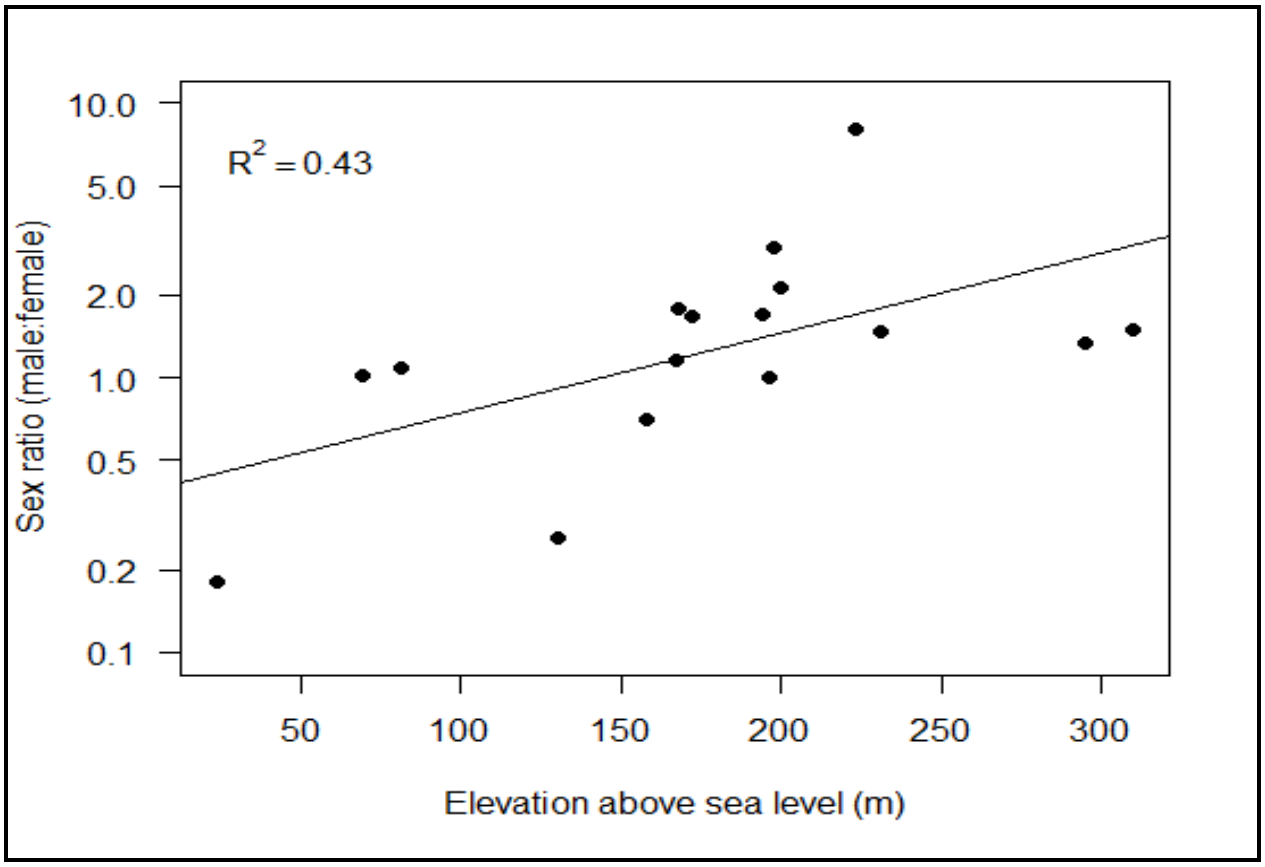


Figure 22 – More females than males were found at study areas with a lower elevation above sea level (n = 16). The y axis is a $\log_{10}$ scale.

There appeared to be no other general trends found for sex ratios across the study areas but there was an association between the geographic location and the density of females. A Spearman's rho analysis indicated a significant negative correlation between the density of females in each study area and the respective distance from the equator (latitude) for the site r_s = -0.576, p = 0.019, n = 16. Thus, the density of female plants was greater at sites with more southerly geographic locations (Figure 23).

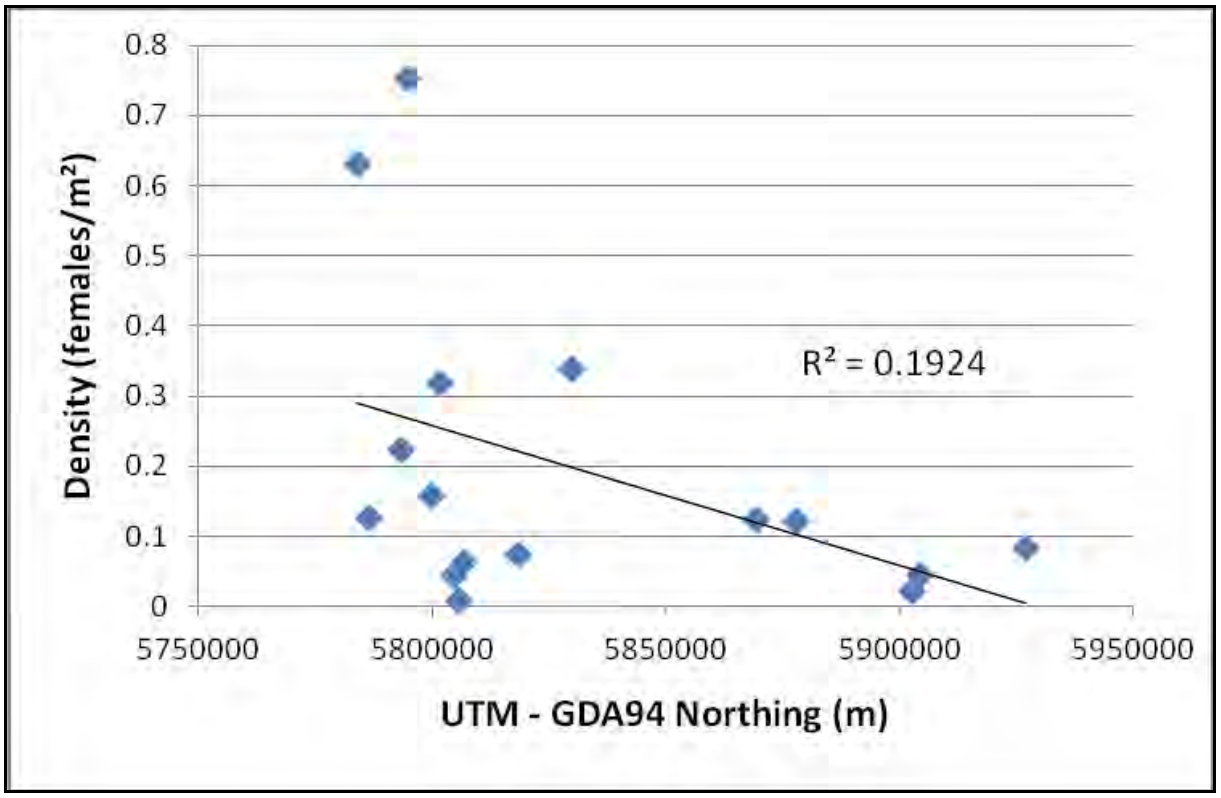


Figure 23 – Study areas at more southerly geographic locations had a greater density of female plants (n = 16).

2.3.2 *Transects versus quadrats*

On average, plant density within quadrats was positively and significantly associated with the plant density recorded in the transects r_s = 0.859, p < 0.001, n = 16 (Figure 24). The quadrats had approximately 4.5 times greater plant density than the transects (y = 4.56x + 0.8376).

The quadrats show a bias towards larger plant density because the criteria (see section 2.2.3.2 above) for selecting quadrats was that they contain at least one *P. spinescens* plant.

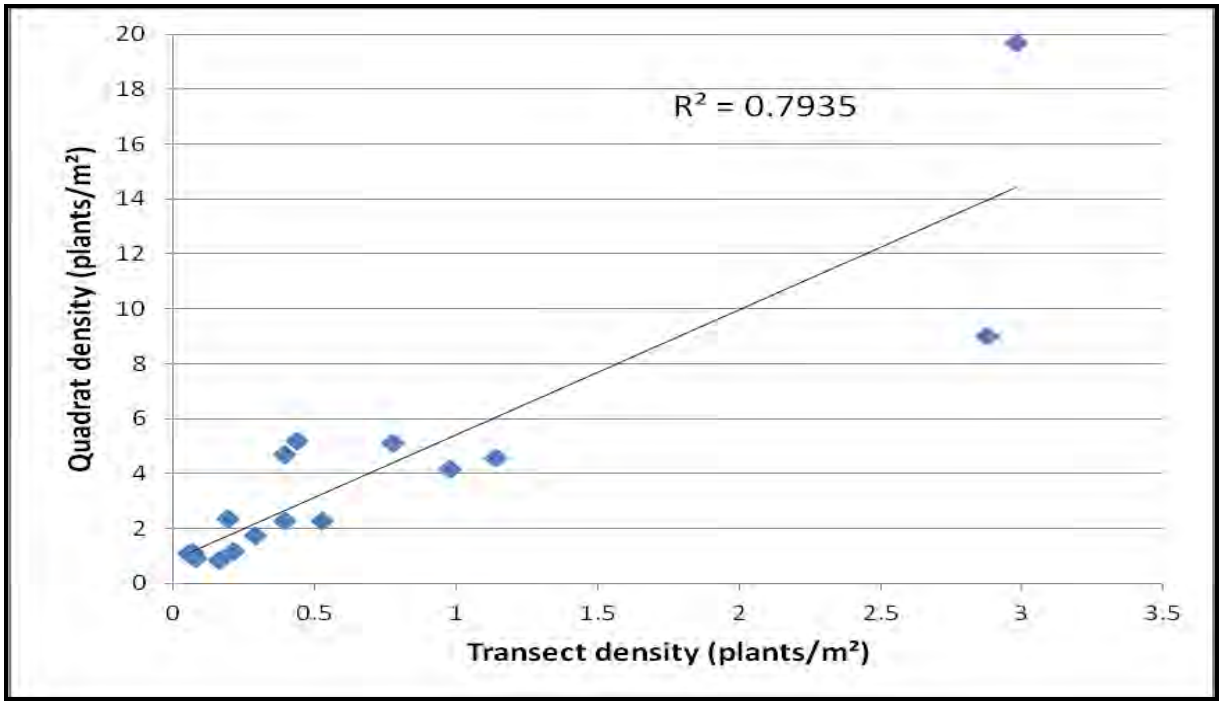


Figure 24 – Transect density is positively associated to quadrat density within the study area (n = 16).

2.3.3 *Longitudinal study of selected plants*

2.3.3.1 Population structure and dynamics

Across all 16 study areas, the average area surveyed using quadrats was 17 m², with the smallest being 3 m² and the largest 42 m². For all quadrats per study area combined, the number of plants tagged in 2009 ranged from 25 to 336 individuals across all study areas. The average number of plants tagged per study area in 2009 was 76 individuals (Table 6). In total, 1,220 plants were tagged in 2009 and follow-up monitoring was conducted on one occasion in 2009 and two occasions in 2010.

Table 6 – The study area quadrat sizes, numbers of plants and life-stages.

	Quadrat size (m²)	Number of Quadrats	Area assessed (m²)	Number of juvenile plants	Number of mature plants	Total number of plants
Geggies Rd	4	5	20	49	38	87
Mt Mercer Rd	2	5	10	64	27	91
McKenzie Rd	1	6	6	1	24	25
Poorneet WRR	1	3	3	43	46	89
Christies Rd	6	7	42	9	25	34
Cedarwoods R	6	6	36	1	33	34
Kirks BR	2	6	12	21	42	63
Glengower Rd	2	7	14	2	32	34
Vite VRR	5	6	30	6	29	35
Ararat AR	2	5	10	12	35	46
Brownswaterholes BRR	1	4	4	71	36	107
Baringhup WR	1	9	9	49	20	69
Pitfield CR	4	6	24	2	27	29
Carisbrook BR	5	5	25	310	26	336
Calder RRR	4	3	12	12	48	60
Bannockburn RR	4	5	20	53	27	80

Life-stage and flowering status

Within the randomly located quadrats, the greatest proportions of flowering plants were mature individuals and the greatest proportions of non-flowering plants were juveniles, yet both categories contained proportions of both flowering and non-flowering individuals. On average, flowering mature plants accounted for 56 % (range 7 – 96 %) of all individuals in the quadrats, across the 16 study areas (Figure 25). Non-flowering juveniles comprised an average of 36 % (range 3 – 92 %) of plants within the quadrats. Further to this:

- Ten study areas had quadrats that contained an average of 8 % (range 0.5 – 19 %) non-flowering mature plants;
- Twelve study areas had quadrats that contained an average of 3.3 % (range 0.2 - 8.5 %) first year flowering seedlings;
- Non-flowering juveniles (n = 165) ranged in height from 0.2 cm to 9.5 cm, with an average height of 2.1 cm; and
- First year flowering seedlings (n = 25) ranged in height from 1.5 cm to 15 cm, with an average height of 6.3 cm.

There is no clear height threshold at which plants commence flowering.

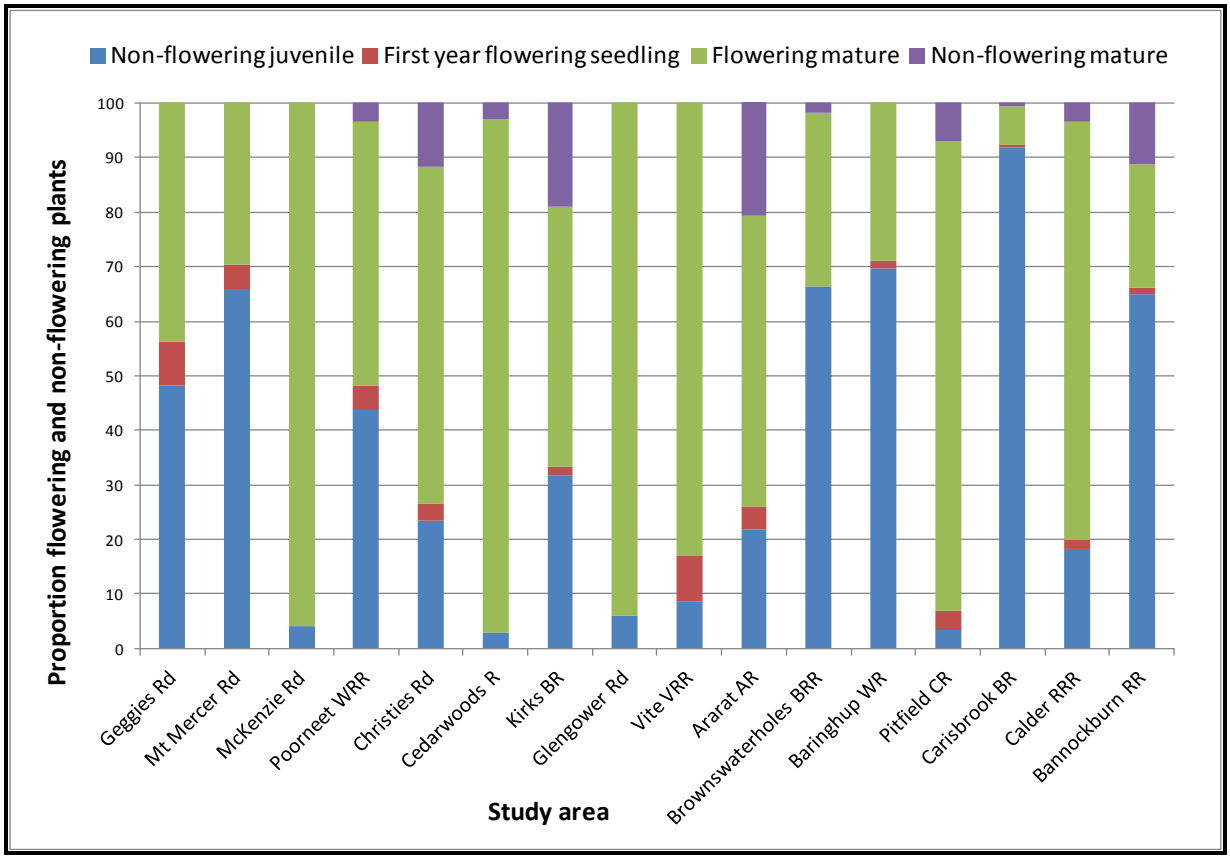


Figure 25 – Proportions of plant life-stages in relation to flowering (assessed in 2009).

The sex of the first year flowering seedlings were represented by both male (n = 10) and female (n = 15) plants. It was found that the height of the first year flowering seedlings of males and females were not found to be significantly different t (10, 15) = -0.451, p = 0.657. The height threshold at which the plants flower is not influenced by the sex.

Mature plant mortality

Between October 2009 and October 2010, the average rate of mature plant mortality in quadrats across all 16 study areas was four percent. However, mortality of tagged mature plants was recorded at only half of these study areas (eight), with the greatest rate of mortality (36 %; 9 plants) recorded at Christies Rd. Of the 25 mortalities that were observed, over half of them (52 %) had been recorded as non-flowering plants and over a quarter (28 %) were recorded as males in 2009. Five female plants made up the remaining 20 % of mortalities and four of these were from Christies Rd (Figure 26).

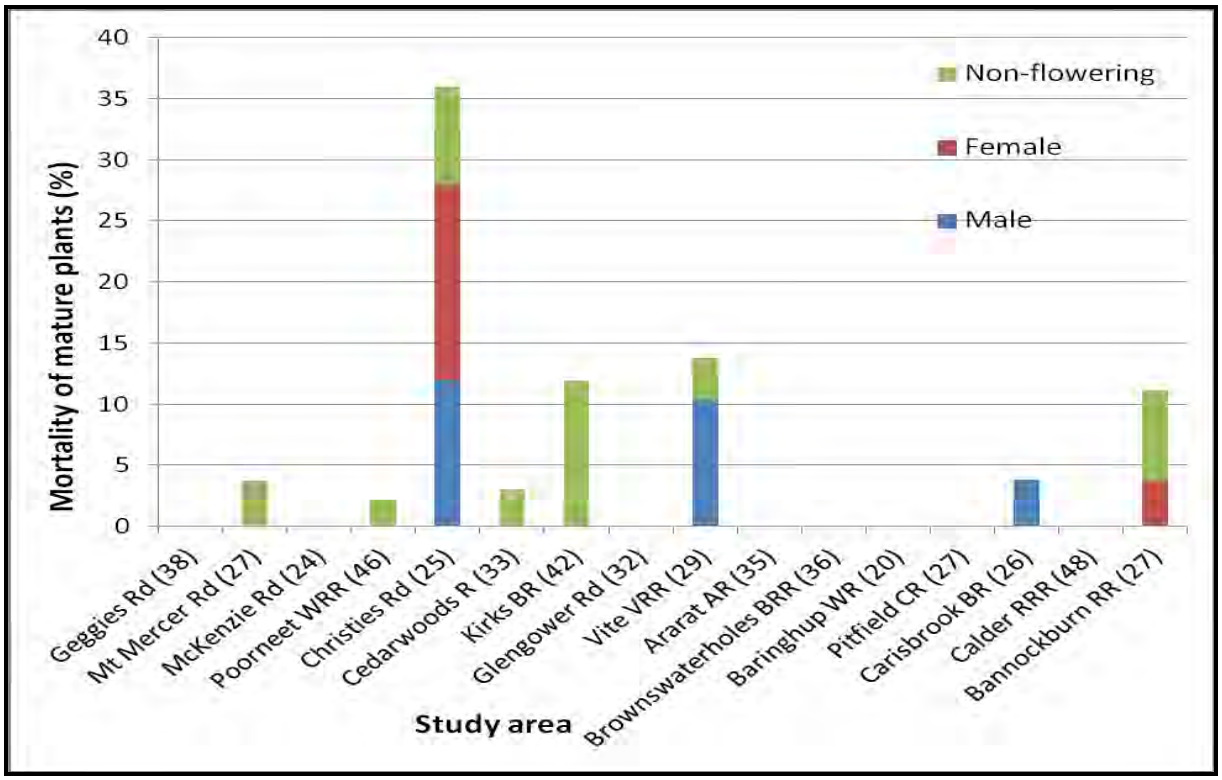


Figure 26 – Mortality of tagged mature plants at each study area between 2009 and 2010. Figures in brackets are the sample sizes.

The greatest mature plant mortality occurred in quadrats at study areas where the average distance between plants was large r_s = 0.572, p = 0.021, n = 16. This effect was observed at study areas where the average distance to the nearest neighbour approximated 40 cm or greater (Figure 27).

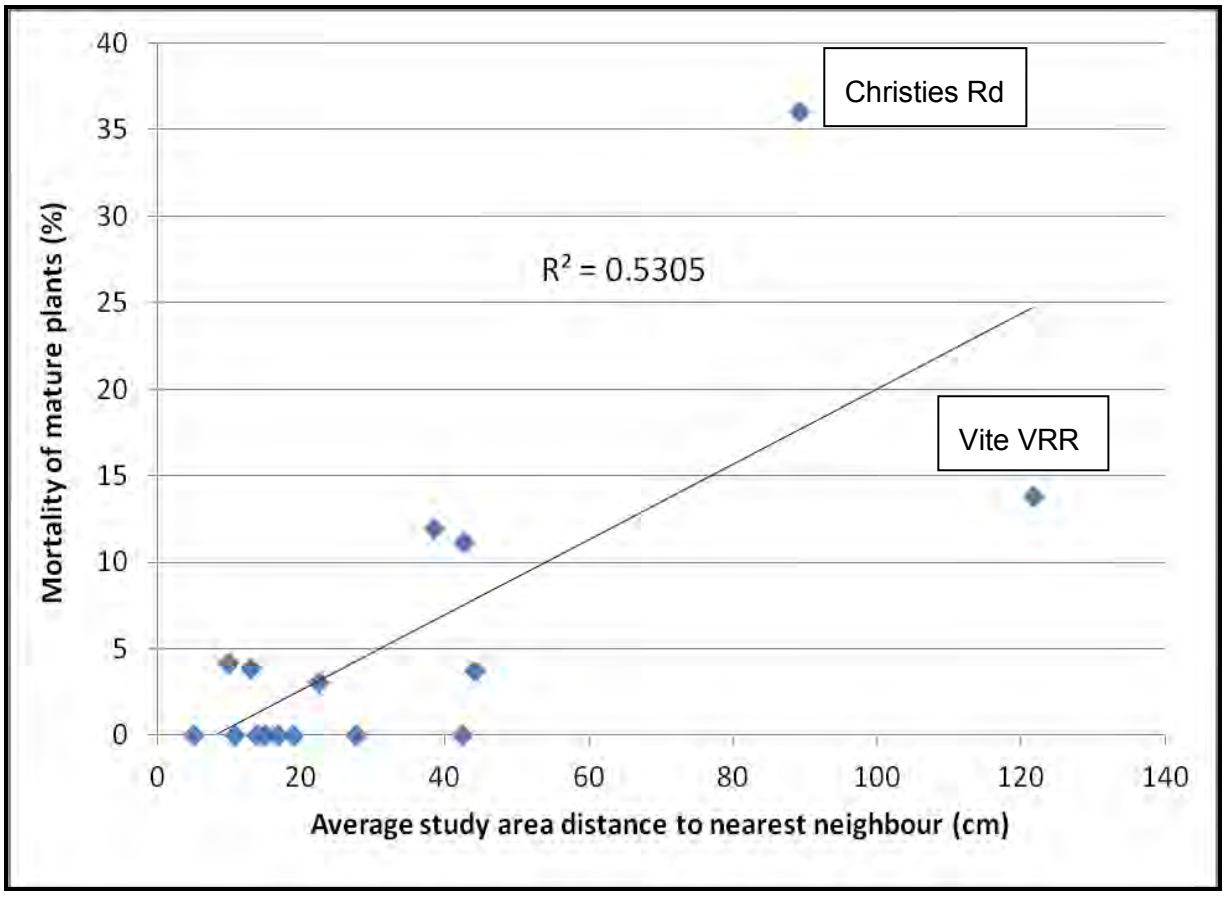


Figure 27 – The mortality of mature plants between 2009 and 2010 is greater when there is a larger average distance between plants in a study area (n = 16).

Natality

In situ germinant production and survival

In August 2009, germinants were detected in quadrats in 13 of the 16 study areas, totalling 798 individuals. In one of the study areas (Carisbrook BR) 389 germinants were observed, but six or fewer germinants were detected in six of the study areas and within three study areas (McKenzie Rd, Christies Rd and Glengower Rd) no germinants were observed. By October 2009, 77 % of these germinants had survived.

In August 2010, 116 (14.5 %) of the original 798 germinants had survived at 11 of the study areas. The survival of germinants across the study areas was not proportional to the original number of germinants. That is, survival was not necessarily greatest in study areas that produced larger numbers of germinants.

In 2010, 91 new germinants were found within quadrats, the majority of which were recorded in four study areas (Table 7). Only half (eight) of the study areas produced germinants in both 2009 and 2010 and all but two (Christies Rd and Glengower Rd) produced germinants in either 2009 or 2010.

Germinant density

Across all study areas, in 2009 the germinant density ranged from 0 – 14.7 germinants per square metre, with an average of 4.2 germinants per square metre. Seven of the sites had greater than one germinant per square metre. In contrast for 2010, the greatest density recorded was only 3 germinants per square metre at Baringhup WR, with an average of 0.6 germinants per square metre across all study areas. In 2010, only two study areas had

greater than one germinant per square metre (Table 7). A paired samples *t* test revealed a significant difference in the density of germinants produced between 2009 and 2010 t (11) = 2.43, p = 0.028 (95 % CI 0.475 to 7.24), with a large Cohen effect size d = 0.99 (Cohen, 1988). On average the quadrats in study areas in 2009 produced seven times the density of germinants when compared to the same quadrats in 2010.

Table 7 – Germinant production, density and survival in 2009 and 2010.

	2009			2010	
	Germinants	**Germinant density (m²)**	**Survival after 1 year (%)**	**Germinants**	**Germinant density (m²)**
Geggies Rd	50	2.5	26	1	0.05
Mt Mercer Rd	62	6.2	55	9	0.9
McKenzie Rd	0	0	-	2	0.3
Poorneet WRR	29	9.6	21	2	0.66
Christies Rd	0	0	-	0	0
Cedarwoods R	1	0.02	0	2	0.05
Kirk's BR	5	0.41	20	0	0
Glengower Rd	0	0	-	0	0
Vite VRR	1	0.03	0	0	0
Ararat AR	2	0.2	100	0	0.2
Brownswaterholes RR	59	14.7	52	1	0.33
Baringhup WR	122	13.5	4	28	3.11
Pitfield CR	1	0.04	100	0	0
Carisbrook BR	389	15.56	1.5	0	0
Calder RRR	6	0.5	50	42	3.5
Bannockburn RR	71	3.55	20	4	0.2

The influence of population density on *in situ* germinant production

A greater density of germinants was detected in study areas where there was a greater density of mature plants within the quadrats. A significant correlation between these two variables was detected when 2009 and 2010 data was combined, despite different levels of germination between these years $r_s = 0.548$, $p = 0.002$ and n = 30. Note that the 2009 data for the Carisbrook BR and Baringhup WR study areas were removed from the Spearman's rho analysis because there were strong land management influences affecting these sites. The influences of land management practices on germinant production will be explored in Chapter 5.

Although some mature plants died between the two sample periods (as described previously in results – 'mature plant mortality' section), these deaths would not have affected the germinant density as:

- The study area (Christies Rd) with the greatest female plant deaths (four) produced no germinants in either 2009 or 2010; and
- The only other female plant death occurred at the Bannockburn RR study area which has an estimated population of 440 plants and of which 43 % are female. Of the 107 mature plants recorded in quadrats at this study area, the loss of one plant would be inconsequential.

The influence of population structure on *in situ* germinant production

Germinant density is not only related to population density but also to population structure. At study areas with a high female plant density, there was a significant positive relationship

with the density of germinants recorded in the quadrats, for 2009 and 2010 data combined r_s = 0.564, p = 0.001, n = 30 (Figure 28). Again, the Spearman rho analysis was conducted without the Carisbrook BR and Baringhup WR study area data for 2009. At densities approximating 0.5 female plants per square metre a larger density of germinants was observed.

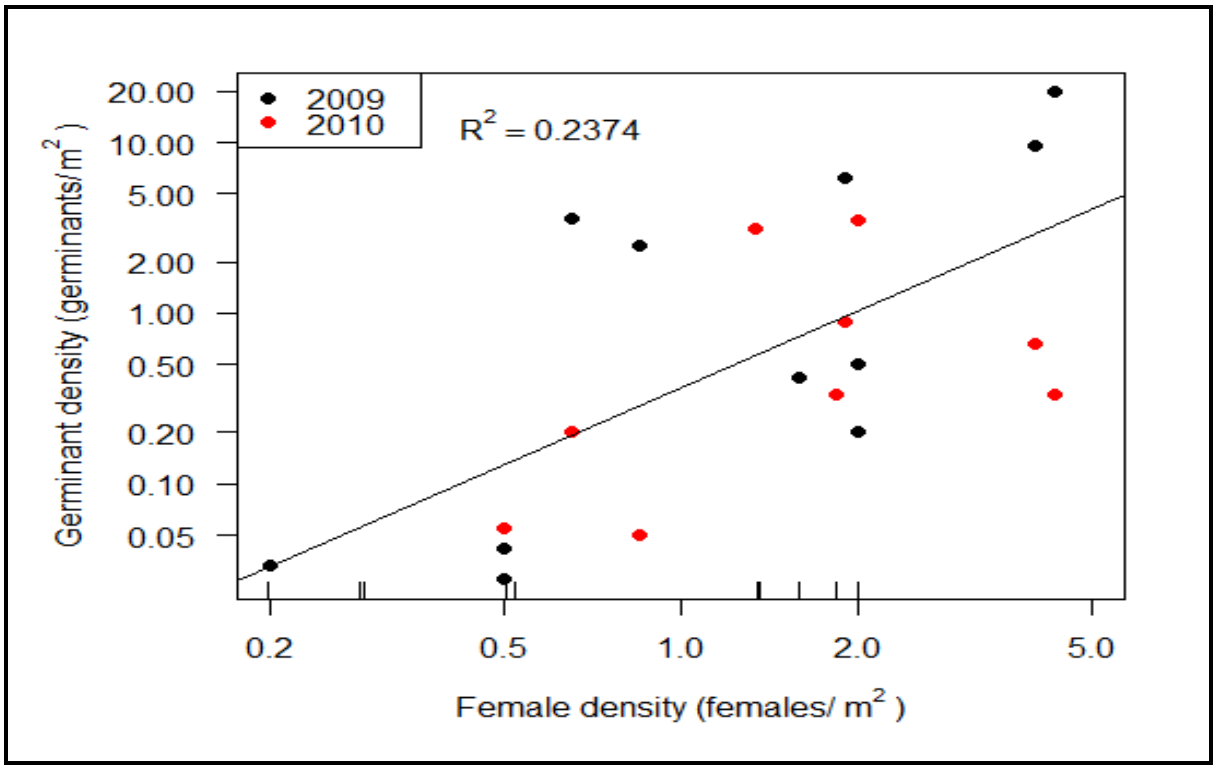


Figure 28 - Germinant density in 2009 and 2010 is positively correlated with female density at 14 study areas in 2009 and 16 study areas in 2010 (n = 30). Lines on the x axis represent zero values and both axes are log_{10} scale.

To determine whether the population density or female density was the stronger predictor of germinant density, a Poisson regression analysis was performed. The Poisson regression analysis was selected because the germinant density data was not normally distributed and separate regressions were conducted because the independent variables were significantly

correlated and confounded the results. The Poisson regression model found that germinant density was significantly associated with population density z of 3.024, $p = 0.0025$, n = 30 but that female density was the stronger predictor z of 4.125, $p < 0.0001$, n = 30.

Rate of population growth

There was no consistent trend in the rate or direction of population growth amongst the study areas between 2009 and 2010. The rate of population change ranged from an 84 % increase to a 26 % loss, with an average of 13 % positive population growth across all study areas (Table 8).

Table 8 – Summary of population change from 2009 to 2010 within the study areas from greatest to the least (FRI = finite rate of increase, ln (FRI) = intrinsic rate of increase).

Study area	FRI (λ)	ln (FRI)	% change
Mt Mercer Rd	1.8461538	0.613104473	84
Brownswaterholes BRR	1.7073171	0.534923175	70
Geggies Rd	1.2826087	0.248896047	28
Bannockburn RR	1.255814	0.227783931	25
Baringhup WR	1.2380952	0.2135741	23
Carisbrook BR	1.1034483	0.098440073	10
Poorneet WRR	1.0869565	0.083381609	8
Calder RRR	1.0545455	0.053109825	5
Ararat AR	1.0416667	0.040821995	4
Pitfield CR	1.0322581	0.031748698	3
Glengower Rd	1	0	0
Cedarwoods R	0.9705882	-0.029852963	-3
McKenzie Rd	0.96	-0.040821995	-4
Kirks BR	0.9193548	-0.084083117	-8
Vite VRR	0.8666667	-0.143100844	-13
Christies Rd	0.7352941	-0.3074847	-26

2.3.3.2 Relationships between demographic characteristics and other measures of recruitment potential

The data presented in this chapter found a relationship between the demographic characteristics of populations of *P. spinescens* and one potential measure of recruitment – germinant density. Chapter 3 introduces methods of evaluating other measures of recruitment potential, including seed production, seed viability, seed germinability, the viability adjusted germination score and germination *in situ*. Chapter 3 also assesses the value of these measures to evaluate environmental and management effects on the reproductive capacity of populations of *P. spinescens*. All of these measures of recruitment potential were assessed for relationships with the demographic characteristics of the populations but only the germinant production measure was found to be significant.

2.4 Discussion

2.4.1 *Population size and density within study areas*

The sub-sampling approach used in this study enabled a reasonable estimate of the number of *P. spinescens* plants within each study area but could not be used to estimate the size of the population across the entirety of each site. At six sites it was apparent that the estimated number of plants within the study area exceeded the numbers that had previously been estimated for the entire site, according to the VBA records. This suggests that previous attempts to estimate the minimum number of plants at some sites were an underestimate or that the population had increased since last estimate. Foreman (2005) had previously come to the same conclusion at many of the *P. spinescens* sites he studied.

Several studies on species within the genus *Pimelea* have undertaken a plant population census at each site. These include studies on the New Zealand *Pimelea arenaria* (Sand Daphne) (Dawson, Rapson *et al.*, 2005, Merrett, 2007) with relatively small populations numbering up to 118 individuals; and a census of up to 518 individual *P. spinescens* plants distributed across a 44.3 hectare study area (Cropper, 2003, 2007a). Whilst a census of a small population can be easily achieved, it is more difficult, costly and time-consuming to accurately census larger populations. Indeed, Cropper (2003) claimed that it took 34 hours to search, tag and measure each of the 518 individual plants. Because of the cryptic nature of *P. spinescens*, variability in the identification skills of the observer, and time restrictions in the field, Garrard (2009) suggests that there would always be a margin of error when monitoring for this species. At large sites, it seems that it would be impossible to account for

every individual or search the entire area and accurately repeat the exercise (Bunnefeld, Hoshino *et al.*, 2011).

Rather than conducting a census, a process of sub-sampling is often employed in population studies (Cropper, 1993, pg 38, Elzinga, Salzer *et al.*, 2001). Using a single 10 m x 10 m quadrat, Foreman (2005, 2011) sub-sampled 16 discrete populations of *P. spinescens*. However, given the non-uniform distribution of the species, it is not possible to extrapolate sub-sample population estimates unless the pattern of distribution of plants across a site is clearly understood or there is a sufficient level of replication of sub-sampling within a site. Even through the extrapolation of three 20 m x 20 m replicated quadrats within a site, Cropper (2004, 2005, 2006, 2007b, 2009) was not able achieve an accurate reflection of the growth of the entire *P. spinescens* population over a five year period.

The approach taken in this study involved the establishment of a discrete study area (polygon) within the study site; further sub-sampling was conducted through the use of randomly located transects and quadrats. Quadrats consistently led to an overestimate of population size due to the inherent bias of their selection – that is, only quadrats containing *P. spinescens* were assessed. In contrast, the transect method accounted for spatial variations in the presence, absence and density of the species, resulting in a population estimate that was representative of the study area. The transect approach used to estimate a population size was relatively quick, repeatable and delivered a greater accuracy than the quadrats.

Plant densities recorded across the study areas were highly variable but were within the ranges reported by Cropper (2004, 2005, 2006, 2007b, 2009) and Foreman (2005). Indeed, four of the sites had previously been assessed by Foreman and density measures at two of

these sites were within 0.1 plants per square metre of the measures recorded for those sites in the current study. This also suggests that the measure of density obtained from the transect method could be useful as a comparison between sites and across time.

The transect approach developed in this study could be used in future projects to provide an achievable, efficient and repeatable method of estimating population size and density within the defined study area. At large sites, it is possible that an approach involving the statistical replication of study areas (ie sub-sampling) across the site would result in an estimate of the total population. This method would increase the accuracy of the current VBA records and enable population trends to be monitored through an adaptive management model.

2.4.2 *Sex ratios*

Dioecious species are generally assumed to have a 1:1 sex ratio (Rychlewski and Zarzycki, 1975, Webb, 1999, Beaumont, Edwards *et al.*, 2006), however this does not appear to be a standard condition for *P. spinescens*. Across the 16 study sites, variations from sex ratio parity were observed, with skews towards both males and females, implying that there is not normally a balance within the species' sexual breeding system. Similar inconsistent departures from parity have been documented for other dioecious species, where environmental conditions appear to alter the ratio depending on the reproductive costs at individual sites (Agren, Danell *et al.*, 1999, Delph, 1999, Geber, 1999, Sakai and Weller, 1999, Bailey and Delph, 2007).

The literature recognises at least five possible evolutionary pathways to gender dimorphism which fall in to two groups. The dioecious condition arises either from:

1. A mutant in which one set of reproductive organs are sterile, allowing the alternative set of reproductive organs to proliferate into a population, thus establishing gender dimorphism (Lewis, 1941, Delph, 2003); or

2. Selective pressures (resource availability) acting on the existing dimorphism in a co-sexual species (Ross, 1970, 1982, Webb, 1999).

In theory, the evolutionary development of functionally female plants must provide an overall advantage to the population [either through greater seed production (Delph and Carroll, 2001, Shykoff, Kolokotronis *et al.*, 2003) or greater seed viability (Lewis, 1941, Bailey and Delph, 2007)], that allows the dioecious forms to survive and proliferate over time. Where these changes are not as beneficial, degrees of the hermaphroditic condition will remain and could facilitate a return to the previous breeding condition (Richards, 1997, Silvertown and Charlesworth, 2001, Barrett, 2002). In circumstances that promote the dioecious condition, the seed fecundity of the surviving hermaphrodite plants are not as vital to the survival of the population (Delph, 1990, Barrett, 1992, Webb, 1999), and either through reduced performance or genetic pressures, these plants functionally become more male (Lewis, 1941, Ross, 1982, Delph, 2003).

Under the resource compensation model, theories on the evolution of dioecy and variations in sex expression of a species across its distribution range suggest that locations with unfavorable conditions will hinder the survival of the sex (male, female or hermaphrodite) incurring the highest stress. Over time, such pressures can lead to a skew in the sexual presentation of individuals in discrete populations (Lloyd and Webb, 1977, Bierzychudek and Eckhart, 1988). However, the advantage is not necessarily obvious and only through

research have the fecundity advantages of a plant's sex lability been exposed (Crawley, 1990, Shykoff, Kolokotronis *et al.*, 2003). A study of the dioecious desert shrub *Ochradenus baccatus* (Taily Weed) found that female frequency in a population is negatively correlated with males producing seed (hermaphrodites) and positively associated with males producing pollen (Wolf and Shmida, 1997). A study of the gynodioecious mountain wildflower *Silene acaulis* (Moss Campion) by Delph *et al.* (2001), found that female frequency in a population is negatively correlated with hermaphrodites producing quality seed. Similarly, female plants of the Australian herb *Wurmbea dioica* (Early Nancy) had a greater production of seed than hermaphrodites, and male plants produced more pollen than hermaphrodites (Ramsey and Vaughton, 2001). In Spain eighteen populations of the *Ecballium elaterium* (Squirting Cucumber) were categorised as having either a dioecious or monoecious (individual plants have both male and female flowers) breeding system; it was found that dioecious populations contained greater genetic heterozygosity, whereas the monoecious populations were highly inbred (Costich and Meagher, 1992). A meta-analysis of studies published over a 20 year period and involving 23 different dioecious species, found that female plants had greater fruit set, greater total seed production and produced heavier seeds which germinated in greater numbers, when compared with the hermaphrodite plants found within the same population (Shykoff, Kolokotronis *et al.*, 2003).

A dioecious breeding system has been maintained in most populations of *P. spinescens* but resource variability (environmental conditions) has also allowed hermaphrodites to continue to exist. The most probable term which best describes the breeding system of *P. spinescens* is subdioecious (Sakai and Weller, 1999, Ramsey and Vaughton, 2001, Cropper, 2005). This current study adopted Cropper's system (Cropper, 2005) of describing populations based on

two broad flower types that essentially resulted in a male to female sex ratio. However, all study areas contained plants that appeared to be hermaphroditic. The identification of hermaphrodites within a population is important, as these plants are likely to have a different reproductive potential to plants that are strictly male or female. Several studies have documented variations in seed production found between hermaphrodites and female plants of *Schiedea adamantis* (Diamond Head Schiedea) (Sakai, Weller *et al.*, 1997), (Delph and Carroll, 2001) and *Ochradenus baccatus* (Taily Weed) (Wolf and Shmida, 1997). For *P. spinescens*, it is important to identify the occurrence of hermaphrodites across the species range and evaluate their fitness and contribution to discrete populations. This should be the subject of future research.

Over a flowering season the sex of some individual plants appeared to change. The pattern noted for these individual plants was to exhibit female flowers during the initial visit and varying proportions of male flowers later in the flowering season. Thus, the timing of examination for the sexing of individual *P. spinescens* plants requires some caution and definition, as many plants appeared to become functional hermaphrodites during their flowering season rather than the sex originally displayed when first encountered. Burrows (1960) also noted this phenomenon for *P. traversii* in New Zealand. Future research should target labile individuals across several populations and intensively (fortnightly) monitor the proportion of flower types that are displayed throughout a season. This will enable the clarification of the existence of change in sexual expression if it is occurring, and provide an optimal timing window for future sex classification surveys.

For some dioecious species, plant size, age or the prevailing local conditions appears to dictate which sex is able to be expressed. Within the genera *Arisaema* (Hotta, 1971),

Guraniinae (Cucurbitaceae) (Condon, 1988) and the species *Panax trifolium* (Dwarf Ginseng) (Schlessman, 1991), male flowers are produced until a size threshold is reached, after which the plants are able to change sex and became female; this is often a long term transition (years). A large body of literature found 119 plant species that appeared to be able to change or revert back from female to male due to stress in regards to factors such as: increasing age, temperatures, dry soil, light intensity and trauma (Freeman, Harper *et al.*, 1980).

For *P. spinescens*, plants were able to commence flowering for the first time when they were still small, a short period after germination. Both females and males were well represented within the samples at this life-stage. It appears that *P. spinescens* individuals do not need to obtain a specific size before being able to function as a female.

2.4.2.1 The influence of location on sex ratios

Pimelea spinescens study areas with increased topographical elevation and at greater distances from the equator were significantly correlated with a greater male to female sex ratio and greater female density, respectively. These geographical characteristics have previously been reported to correlate with variations in sex ratio for other species of dioecious plants (Grant and Milton, 1979, Hoffmann and Alliende, 1984, Alonso and Herrera, 2001, Merrett, 2007).

In relation to elevation, previous studies on *Laretia acaulis* (Cushion Plant) of the Andes (Hoffmann and Alliende), *Populus tremuloides* (Quaking Aspen) of North America (Grant and Milton) and *Daphne laureola* (Spurge Laurel) in Spain (Alonso and Herrera) all reported a similar trend of fewer females at sites of greater elevation. The underlying causes of such

trends may be varied but in the case of *D. laureola*, Alonso *et al.* (2001) suggested that the association may be driven by water availability. Female plants of *D. laureola* appeared to be better suited to the frequent droughts that occur at lower elevations in the Mediterranean area. Additionally, in contrast to the progeny of hermaphrodites, the progeny of female *D. laureola* plants were found to cope better in these environments, due to guaranteed heterozygosity (Alonso and Herrera, 2001). It should be noted however, that these studies were conducted on species distributed across an elevation range of at least 1,000 metres, whereas sites in the current study were distributed across an elevation range of only 250 metres. It is unclear what underlying cause(s) may be driving the association between sex ratio and elevation in *P. spinescens.*

In some dioecious species, the distance from the equator appears to be associated with variations in the sex ratio of discrete populations. In New Zealand, female plants of the gynodioecious species *Pimelea arenaria* (Sand Daphne) were found at greater frequencies than hermaphrodites at more northerly latitudes (Merrett, 2007). The direction of these associations was opposite to that observed for *P. spinescens* in this study, where a greater density of females was observed at more southerly latitudes. A previous study on *P. spinescens* was also suggestive of bias towards females at southerly latitudes. Foreman (2010) reported that “on average there were 26 % more female plants – a bias that was particularly pronounced on the volcanic plains”, implying that he did not find such a strong trend at the sites he assessed in northern Victoria. Associations between latitude and sex ratio are not apparent for all dioecious species, as illustrated by studies on the dioecious shrub *Oemeria cerasiformis* (Osoberry or Indian Plum) (Allen and Antos, 1993). With so little research available regarding this phenomenon in land plants it is difficult to postulate a

rationale, especially when results are conflicting. Further research on *P. spinescens* and other species within the genus is required in this area.

The sex ratio of dioecious plants seems to be dependent on environmental conditions and the associated reproductive costs (Rychlewski and Zarzycki, 1975, Agren, Danell *et al.*, 1999, Geber, 1999, Sakai and Weller, 1999, Webb, 1999, Beaumont, Edwards *et al.*, 2006, Bailey and Delph, 2007). Various studies have found that female plant frequency is often greater when site conditions were more stressful in regards to soil moisture (Barrett, 1992, Alonso and Herrera, 2001, Delph, 2003). In contrast, various studies have also found that sites at lower elevations tend to be drier and warmer and that plants in these situations limit resource allocation to essential functions, forcing hermaphrodite individuals to become more male in function, further leading to the development of a dioecious breeding system (Sakai and Weller, 1991, Delph and Lloyd, 1996, Charlesworth, 1999). An example is provided by Ramsey *et al.* (2001) who investigated *Wurmbea dioica* across 24 populations during two flowering seasons and found that 71 % of males were labile in their sex expression and 30 % changed to hermaphrodites following a winter of plentiful rainfall.

Based on these reports, it seems that soil moisture availability is a contributor to sexual expression and therefore the sex ratio of many dioecious plant populations. Because soil moisture availability is influenced by geographic factors such as elevation and latitude, it seems that such factors may be indirectly associated with the sex composition of populations. As a result, populations may be described as subdioecious, gynodioecious or dioecious, with sex ratio frequencies that are significantly different from parity. For *P. spinescens*, populations with more female plants than male plants and greater densities of female plants were associated with study areas that had greater average rainfalls and lower

maximum temperatures, as result of elevation and latitude (Commonwealth of Australia, 2012). Given that the male category used in this study included hermaphrodites, the observed skews towards a greater proportion of females at lower altitudes are realistic, whereas skews towards more males at higher elevations may be influenced by the inclusion of hermaphrodites into this category.

2.4.3 *Population structure and dynamics*

2.4.3.1 Flowering status

In dioecious species, plants which are non-flowering are often categorised as being immature or juvenile (Gibson and Meneges, 1994, Foreman, 2005, Merrett, 2007). That is, it is assumed that non-flowering plants are not reproductively mature (Crawley, 1990, Larcher, 2003). Foreman (2005, 2011) categorised non-flowering *P. spinescens* plants as immature and recorded them in large numbers. In the current study, non-flowering plants were represented by germinants, first year seedlings, juveniles and large multi-stemmed plants that were many years old.

Because juvenile plants are unlikely to have accumulated or accessed sufficient resources to enable flowering (Klinkhamer, Jong *et al.*, 1987, Crawley, 1990), it is not surprising that juvenile *P. spinescens* plants made the greatest contribution to the total number of non-flowering plants within a study area. However, at every site a proportion of these juvenile plants were categorised as first year flowering seedlings, indicating that plants have the potential to flower within the first year, if site microsite (Tirado and Pugnaire, 2003) and prevalent seasonal conditions are suitable.

Although the non-flowering status of an individual plant is associated with reproductive life-stage, it is likely to also be associated with individual site stress factors which may act on plants in all life stage categories (Nicotra, 1998, Matsushita, Nakagawa *et al.*, 2011). For example, a study of the population structure of the dioecious species *Ilex montana* (Mountain Holly) found many non-flowering individuals in each mature age category (Cavigelli, Poulos *et al.*, 1986).

The stress factor which is most likely to influence the flowering status of mature *P. spinescens* individuals is water availability. Prior to 2010, Victoria had experienced 13 years of drought (below average rainfall) (National Meteorological Service, 2006, 2008, National Climate Centre, 2009, Commonwealth of Australia, 2012) and mature plants at all sites would have had access to only limited water resources and depleted energy reserves. Under these conditions, the plants are likely to have been functioning in survival mode, rather than expending resources on reproduction (Larcher, 2003). Indeed, many mature plants which were classified as non-flowering in 2009, flowered in 2010 [an average to greater than average rainfall year (National Climate Centre, 2010, Commonwealth of Australia, 2012)] and were subsequently able to be sexed.

Adoption of standardisation categories of the life-stages and flowering status of *P. spinescens* plants is required. This would enable the identification of temporal/seasonal patterns of flowering and associations with environmental influences.

2.4.3.2 Mature plant mortality

Mature plant mortality was not a prominent feature throughout this study. Presumably in self-sustaining populations, low levels of mature plant mortality are replaced by the entry of germinants into the population.

Although non-flowering mature plants accounted for an average of 8.5 % of the plants at the sites at which they were found, this category accounted for more than half of the mortalities recorded. Non-flowering plants may be indicative of a component of the population that is in the natural process of senescence, leading to mortality. Because long-lived dioecious species tend to occur in harsh environments (Weller, Sakai *et al.*, 1990, Barrett, 1992, Bailey and Delph, 2007), they have evolved to modify their reproductive output in response to stressful conditions (Lloyd, 1980, Charlesworth, 1999, Delph, 2003). The resource intensive process of flowering is temporarily halted and survival becomes the strategy (Bierzychudek and Eckhart, 1988, Larcher, 2003). The presence of non-flowering mature plants within a population may be an indicator of environmental stress. Presumably, these will be the first plants within the population to die if stress factors continue unabated.

The spatial distribution of *P. spinescens* at some study areas may provide further evidence of environmental stress. In the plant kingdom the majority of species exhibit a clumped distribution in space as a consequence of limited seed dispersal distance, clonal growth and competition from other species (interspecific competition). This is termed intraspecific aggregation and reduces the degree of contact between different species and allows the formation of spatial refugees where only the peripheral individuals are competing with other species (Silvertown and Charlesworth, 2001). This describes the spatial distribution of *P. spinescens*.

At study areas where high mature plant mortalities were recorded, the level of intraspecific aggregation appears to be reduced and there was an increased distance between neighbouring plants. It is unclear whether a large spatial distribution was a contributing factor to mature plant mortality, or whether it was a symptom of high rates of mature plant mortality. That is, it is possible that some study areas had experienced high levels of mature plant mortality in the years prior to and during this study (due to stressors such as drought), resulting in the large distances between nearest neighbours that were observed at these study areas. If the rate of recruitment was not equal to or greater than the rate of mature plant mortality, then such a process of mortality would lead to a more dispersed population.

2.4.4 *Natality*

2.4.4.1 *In situ* germinant production and survival

Reports of recruitment of *P. spinescens* are rare, yet this study demonstrates that most populations have at least some capacity for reproduction, although this may vary between seasons. The infrequency in reporting of recruitment is most likely due to the cryptic nature of the species (Garrard, 2009) and poor descriptions of the germinants, leading to a lack of identification in the field. The few details that are available come from Foreman (2005, 2011) who noted that germination was often observed in July, often at sites recently burnt, and at northern sites it was possibly associated with an increased autumn rainfall. Cropper (2007a, 2009) demonstrates evidence of germinant survival over time but the proportion of surviving germinants has not previously been assessed.

In this study, reasonable densities of germinants were detected in roughly half of the study areas in 2009. The densities of germinants were much fewer in all study areas in 2010. Such

variability in the production of germinants over time is a common feature in the plant kingdom (Morgan, 1995b, Yates and Ladd, 2004, de la Bandera and Traveset, 2006) and has previously been reported for *P. spinescens* (Cropper, 2003, 2004, 2005, Foreman, 2005, Cropper, 2006, 2007b, a, 2009, Foreman, 2011).

The number of germinants that survived over a one year period was not necessarily proportional to the respective levels of germination that were initially recorded in each of the study areas. That is, large numbers of germinants did not translate into a high rate of seedling survival. Similar inequalities between germinant production and germinant survival have been reported for the European shrub *Thymelaea velutina* (de la Bandera and Traveset, 2006), which is also in the family Thymelaeaceae, and *Verticordia staminosa* sbsp. *staminosa* (Wongan Featherflower) of Australia (Yates and Ladd, 2004).

Long-lived plants such as *P. spinescens* usually exhibit a Deevey type III curve which means they have high seedling mortality followed by a safer adulthood, so that the long-term survival is represented by only a small proportion of the seedlings that survive the first year (Fenner, 1987). Thus, the survival of seedlings during the first year after germination is important but does not necessarily translate to long-term survival (Traveset, Riera *et al.*, 2003, Yates and Ladd, 2004). In the above studies on *T. velutina* and *V. staminosa* sbsp. *staminosa*, it was found that seedling survival was positively correlated to the availability of soil moisture (Yates and Ladd, 2004, de la Bandera and Traveset, 2006). For *P. spinescens*, *s*urvival of germinants is clearly not dependant on the germinant numbers produced but is likely to be influenced by site characteristics, such as environmental variables and management practises. These will be addressed in Chapters 5 and 6. Other factors affecting

survival would include the genetic make-up of seedlings, competition, predation and microsite suitability (Grubb, 1977, Fenner, 1987, Fenner and Thompson, 2005).

Although germination was recorded in all populations except Christies Rd, in either 2009 or 2010, only 14 % of these germinants survived for a period of one year. This suggests that despite the occurrence of germination events, successful recruitment, where plants reach reproductive maturity, is quite low. The data for this present study was collected over two years and therefore provides only limited information about the long-term survival of *P. spinescens* seedlings. The ongoing collection of data is vital to confirm the long-term fate of germinants *in situ* and the rate of population recruitment.

2.4.4.2 The influence of population density on *in situ* germinant production

The factors affecting germinant production are likely to be a complex interaction between environmental conditions, habitat management practices and population demographics. All of these factors were found to be important in the germination of *P. spinescens* but aspects of environmental and habitat conditions will be discussed in Chapters 4 and 5, respectively. In terms of population demographics, the density of germinants was positively influenced by population density. At study areas where the density within the quadrats was greater than 0.2 *P. spinescens* plants per square metre, there was an increased likelihood of there being at least one germinant per square metre at 43 % of the sites. Similarly Foreman (2005, 2011) also detected a positive relationship between the density of mature and immature *P. spinescens* plants. The relationship between population density (or size) and recruitment has been recorded for other plant taxa. In Western Australia, the numbers of juvenile *V.*

staminosa sbsp. *staminosa* plants were highest in the larger populations and absent from medium-sized populations of this rare species (Yates and Ladd, 2004).

Intuitively, it makes sense that the more plants there are per defined area, the greater the likelihood that offspring will be produced within that area, simply as a result of the sheer number of seeds that are locally produced. However, it is worth understanding the underlying value of plant density and how this relates to the biology of the population. The literature reveals several reasons why plant density is associated with the capacity for germination. Such reasons include genetic fitness, pollination success and facilitation by nursery plants. The last two factors will be discussed as *P. spinescens* genetics were not investigated during this study.

The reproductive success of a plant population includes factors such as the capacity to attract pollinators and achieve successful pollination (Jennersten, 1988, Mustajarvi, Pirkko *et al.*, 2001, Jacquemyn, Brys *et al.*, 2002, Rymer, Whelan *et al.*, 2005, Hegland and Boeke, 2006). Both these factors are enhanced in denser populations and have been associated with both the production of germinants and germinant survival. Population density may influence the number of vectors available to provide pollination services (Hegland and Boeke, 2006), in turn influencing seed production and ultimately affecting rates of recruitment (Morgan and Scacco, 2006). Indeed, the density of plants was found to be more important than the overall size of the population in regards to pollination success for the Finnish perennial species *Lychnis viscaria* (Sticky Catchfly) (Mustajarvi, Pirkko *et al.*, 2001). In Australia, Morgan (2006) found that the planting density rather than population size affected the number of seed set in *R. leptorrhynchoides*.

Pollination is unlikely to be a limiting factor for *P. spinescens*, as field observations found that many species of Diptera (Flies) and Hymenoptera (Wasps) frequented flowers of both male and female plants (see Figure 6 in Chapter 1). *Pimelea spinescens* also has a potential advantage in attracting pollinators, as it is the primary source of pollen available in the grasslands during its flowering period of April to August. This is a time when few other grassland species are flowering.

Recently, the density of plants has been found to be associated with the creation of a nursery environment for the establishment of germinants (Bruno, Stachowicz *et al.*, 2003, Christian, Den Ouden *et al.*, 2008). Nursery environments are often associated with mature plants that have a clumped spatial distribution next to which there is increased soil moisture and reduced thermal evapotranspiration due to shading, as well as increased nutrients due to the decay of plant litter (Nobel, 1989, Barnes and Archer, 1999, Ibanez and Schupp, 2001, Pugnarie and Luque, 2001, Bruno, Stachowicz *et al.*, 2003, Tirado and Pugnaire, 2003, Corinna, Milton *et al.*, 2005, Christian, Den Ouden *et al.*, 2008). Termed positive density dependence, this process of facilitation has been documented for shrubs which act as nurse plants to a range of germinating species in various harsh environments such as deserts (Went, 1942, Tielborger and Kadmon, 1995, Suzan, Nabhan *et al.*, 1996, Foreman, 2011), dry forests (Arriaga, Maya *et al.*, 1993) and the arctic (Brooker and Callaway, 1998).

Pimelea spinescens is a low stature, insect pollinated plant, with an aggregated spatial distribution (Cropper, 2007a). There is limited capacity for its seed to be dispersed far from the maternal plant. Over summer, the grassland environment of this species is harsh and the shading effect of mature plants would create microclimates in which seeds would have a greater opportunity for germination and survival. Observation of germinants occurring in

close proximity to other *P. spinescens* plants (D. Reynolds 2009, pers. obs., August) and the greater production of germinants in study areas with higher mature plant densities suggest that the process of facilitation is occurring. Further investigation of the process of facilitation in this species is required. This would involve the study of germinant production and survival under various population densities and spatial distributions, and a greater understanding of the microclimate under which germination occurs.

2.4.4.3 The influence of population structure on *in situ* germinant production

Germinant density of *P. spinescens* was positively associated with the density of female plants. Additionally, germinants were often located within close proximity of what was presumed to be the maternal plant. A female density within quadrats of greater than 0.5 *P. spinescens* plants per square metre increased the likelihood of getting at least one germinant per square metre at 43 % of the sites. Foreman (2005, 2011) found a similar, yet stronger relationship between female density and germinant density at his study sites.

In subdioecious species, it has been found that because female plants are obligate outcrossers, they produce seed of a better genetic quality than hermaphrodites (Shykoff, 1988, Wolf and Shmida, 1997, Morris and Doak, 1998, Alonso and Herrera, 2001). Therefore populations with a female biased sex ratio are more likely to produce greater quality seed, resulting in potentially higher rates of germination (Darwin, 1877, Shykoff, 1992, Shykoff, Kolokotronis *et al.*, 2003). No literature was found regarding female densities and germinant relationships but a female biased sex ratio in the dioecious shrub *Ceratiola ericoides* (Florida Rosemary) was positively associated with germinant density. Across four

populations of *C. ericoides* it was found that the density of juveniles increased exponentially with an increase in female frequency and that the juveniles aggregated within one metre of the female plants (Gibson and Meneges, 1994). The distance between germinants and female *P. spinescens* plants was not measured for this study but could be the subject of future research.

The observed intraspecific aggregated arrangement of plants, combined with the positive relationship identified between germinants and female individuals in part explains the evolutionary development of the spatial distribution within a *P. spinescens* population. Environmental factors and land management practices are also likely to be influencing the production of germinants and need to be considered in association with the influence of demographic factors that have been identified (see Chapters 4, 5 and 6 for further discussion).

2.4.5 *Rate of population growth*

Ten of the 16 study areas had an increasing population with the average growth rate of 13 % in the brief window between 2009 and 2010. Previously, both positive and negative population growth has been described for several *P. spinescens* populations (Cropper, 2007a, Foreman, 2011). At the intensively studied Western Treatment Plant, the rate of growth appeared to decrease from 19.7 % in 2003, to 3 % in 2007 (Cropper, 2007a).

2.4.6 *Summary*

Sub-sample assessments using randomised transects within a defined polygon presented an accurate, efficient and repeatable method for assessing *P. spinescens* populations.

Standardised methods for categorising the life-stages, sex and flowering status of *P. spinescens* plants, and their frequency of occurrence is required. To quantify change and understand its causes, effective temporal monitoring must occur both before and after management interventions, and through different environmental conditions (Field, O'Connor *et al.*, 2007, Lindenmayer and Likens, 2009). This type of monitoring will highlight the risks associated with management practices whilst taking into account the local environmental conditions (Mackenzie and Keith, 2009). From this information, population trends can be identified, allowing management practices to be adapted accordingly. Experimental hypothesis testing studies are required to provide greater clarity about specific population/habitat management practices.

The breeding system presently displayed by *P. spinescens* is highly likely to be subdioecious with the reproductive contribution of hermaphrodite individuals requiring both qualitative and quantitative assessments within discrete populations. The sex phenology of this species was not found to be governed by plant size but the presentation of sexual lability requires further investigation.

Mature plant mortality was detected and associated with large distances between congeners.

Most populations of *P. spinescens* appear to have the ability to produce germinants. Population density and specifically female density was positively associated with germinant density, although germinant survival was low. It is possible that mature plants provide a level of facilitation to the production and survival of germinants.

Chapter 3 Measuring the recruitment potential of *Pimelea spinescens* populations.

"Every item of natural history is both a joy to behold and an instrument of our potential enlightenment"

Stephen J. Gould (1998)

3.1 Introduction

Reports of *in situ* recruitment of *P. spinescens* have been rare and knowledge about the timing, duration and microsite location of germination is in its infancy (Mueck, 2000, Foreman, 2005). Although germinants have been observed in some *P. spinescens* populations (Foreman, 2005, Cropper, 2007a, 2009, Foreman, 2011), the natural progression of events leading to the stimulation of recruitment is unknown. Concern about the reproductive capacity of *P. spinescens* has been raised in the wake of research that has found that recruitment is a limiting factor for the continued persistence of several other species of Australian grassland flora (Cropper, 1993, Gilfedder and Kirkpatrick, 1993, 1994a, Morgan, 1998c, 2001, Clarke and Davison, 2004).

Successful recruitment requires both sufficient quality and quantity of seed, as well as suitable environmental conditions to trigger germination coupled with a microsite location that supports continued survival (Peart, 1989, Primack and Miao, 1991, Morgan, 1995a, Eriksson and Jakobsson, 1998, Zobel, Otsus *et al.*, 2000, Mouquet, Leadly *et al.*, 2004, Eckstein, 2005, Fenner and Thompson, 2005, Gibson Roy, Delpratt *et al.*, 2007a). The Macquarie Dictionary (1982) defines recruitment as meaning a newly secured member of a body or class but because restrictions to recruitment can occur at any stage of the plant reproductive cycle (Chambers and MacMahon, 1994, Fenner and Thompson, 2005), a range of measures are commonly used to assess recruitment potential. Factors that are considered as contributors to recruitment potential include flower production, seed production, seed viability, seed germinability, germination *in situ* and germinant survival (Grubb, 1977, Spears Jr, 1983, Leishman, 1999, Fontaine, Dajoz *et al.*, 2006, Merritt, 2006, Poorter, 2007, Reynolds and Fenster, 2008).

To study the influence of biotic and abiotic factors on the recruitment of *P. spinescens*, it is necessary to assess various stages of the reproductive cycle for their contribution to the overall recruitment potential. This chapter has been divided into four sections, each focusing on one recruitment measure. This study develops the methods to be employed and assesses the value of the following measures of recruitment potential:

- Seed production (Chapter 3a);
- Seed viability (Chapter 3b);
- Seed germinability (Chapter 3c);
- Germination *in situ* (Chapter 3d and see Chapter 2, 'Natality' section); and
- Germinant survival (see Chapter 2).

3.1.1 *Chapter aims*

This chapter addresses objective five of the *Pimelea spinescens* Recovery Plan (Carter and Walsh, 2006), which is to:

- Evaluate the reproductive status, longevity, fecundity and recruitment levels of *P. spinescens* populations; and
- Determine seed germination requirements by conducting laboratory and field trials aimed at identifying key stimuli.

The specific aims addressed in this chapter are to:

1. Formulate a method to assess the seed production of *P. spinescens* populations;

2. Formulate a reliable, cheap and simple method to assess the viability of *P. spinescens* seed;
3. Assess various treatments for the ability to consistently germinate *P. spinescens* seed and use this method to calculate a germinability score;
4. To assess the recruitment potential of individual populations using measures of seed production, seed viability, seed germinability, and germination *in situ*; and
5. To derive a suitable measure/s that could be used to evaluate the impacts of environment and habitat management on recruitment potential.

Chapter 3a - Measuring the recruitment potential of *Pimelea spinescens* populations: seed production

3a 1 Introduction

Seed rain is vital for the regeneration of plant populations, as it provides fresh seed for colonisation and adds to the soil seed bank for the future (Morgan, 2001, Fenner and Thompson, 2005). In grasslands, a large seed rain is essential for the maintenance of many species (Graham and Hutchings, 1988, Grime, 1998, Stocklin and Fischer, 1999, Soons and Heil, 2002, Williams, Morgan *et al.*, 2005). Indeed, some studies suggest that seed rain (seed production) may be a limiting factor to recruitment in grasslands. For example, studies in Estonia (Zobel, Otsus *et al.*, 2000) and the United States (Tilman, 1997) have shown that the addition of seed to the naturally occurring seed rain resulted in a significant increase in both the cover and the number of seedlings in a plot when compared with no seed addition.

Seed production has been identified as a limiting factor for many species in the natural temperate grasslands of Victoria (Morgan, 1995a, Gibson Roy, Delpratt *et al.*, 2007a). This is partly because the soil seed bank of many species does not persist beyond the first year (Lunt, 1990b, Morgan, 1998b). The annual contribution of seed rain is therefore a critical factor in the recruitment of grassland species when conditions are favourable (Lunt, 1990a, Scarlett and Parsons, 1990, Scarlett and Parsons, 1992, Morgan, 2000, Clarke and Davison, 2004).

Seed production in *Pimelea spinescens* has not previously been assessed. The species flowers sequentially over an extended period and produces many flowers per inflorescence,

along each stem. It is unclear whether the number of seed produced is a factor that is limiting the future recruitment capability of populations of this species.

3a 2 Methods

3a 2.1 *Plant selection*

A pilot study was conducted to determine the minimum number of plants from which seed should be sampled at each study area to maintain sufficient statistical power. The pilot study was conducted in 2008, ahead of the main study which was conducted in 2009 and 2010.

3a 2.1.1 2008 Pilot study

During the pilot study described in Chapter 2, section 2.2.2, seed was collected from all female plants identified within the selected quadrats, with at least seven and up to sixteen plants accessed at each of the nine sites. Based on these samples, a power analysis was conducted to ascertain the minimum number of female plants needed to represent the population within each study area.

For the measure 'seeds per stem' (see section 3a 2.2 for method), a population mean of 34.4 and standard deviation (s^2) of 31.7 was obtained from data collected across the nine pilot study sites. Via a power analysis, a Ø of 6.5 could be achieved by using seven plant samples from nine populations, resulting in a < 1 % chance of making a Type II error (Zar, 1999).

3a 2.1.2 2009 and 2010

In 2009, using the randomly selected quadrats (as described in Chapter 2, section 2.2.3.2), a minimum of seven and up to ten female plants were targeted for seed collection within each of the 16 study areas (as described in Chapter 2, section 2.2.2.1). Sample plants were clearly marked using individually numbered fire proof and weather proof stainless steel tags,

which were attached to the ground by small stainless steel orchid pins. Seed from these plants was collected again in 2010 (see below section).

3a 2.2 *Seed collection technique*

Seed collection bags were placed on target plants, after the majority of flowering had occurred and seed was forming, around late August and early September each year. The bags were made from nylon panty hose (stockings) cut into sections that were 10 cm – 15 cm in length. One end of each section was closed off using a small piece of plastic coated wire to form a small bag. The bags were stretched out over seed bearing stems of the plant, ensuring that all observable immature seeds were enclosed (Figure 29). Another piece of plastic coated wire was then used to secure the bag to the base of the stem/s and stop any seed fall from escaping. Between late October and early November the bags, containing all the seed produced by the target stems, were removed as per the seed collection protocol (Thomas, 2008b).

Seeds per stem were selected as the unit of measurement because the sequential flowering of the plant would have made it difficult and time-consuming to collect data for the more traditional measure of seeds per inflorescence. Thus, seed was collected from a minimum of three stems per female plant, forming a subset of the total seed production per plant, for each of the study years. Each subset of seed is hereafter referred to as a 'batch' of seed. At the time of collection, each batch of seed was placed in a paper bag and labelled, identifying the date; plant species; collector; site; unique plant tag number; and the total number of stems from which collection occurred.

Figure 29 – A *P. spinescens* plant with three seed collection bags attached and also showing an individually numbered plant tag.

3a 2.3 *Measuring seed production*

The reproductive output of each plant was measured as the number of seed produced per stem. This was calculated using the following method:

- In the laboratory, each batch of seed was weighed;

- The average weight per seed (+/-0.0001 gm) in each batch was calculated by weighing a random subset of 60 seed and dividing by 60;
- An estimate of the total number of seed in each batch was calculated by dividing the weight of the batch by the average weight of a single seed. Batches with less than 60 seed were simply counted; and
- The total number of seed per batch was divided by the total number of stems from which seed was harvested.

3a 2.3.1 Data analysis

For each year, 2009 and 2010, seed production data was analysed using a generalised linear mixed model with the R package `lme4` (Bates, Maechler *et al.*, 2011). The seeds per stem for the ith plant in the jth study area were modelled as a Poisson variable with mean mu_{ij} where log(mu_{ij})=log(nstems_{ij})+a_j+p_{ij} where p_{ij} was assumed to follow a normal distribution with mean 0 and variance sigma^2 to be estimated from the data. There is an a_j for each study area, with a_1 set to zero, and hence a_2 to a_16 can be used to test whether study areas 2 to 16 respectively are different to study area 1 in terms of seed production. In addition the difference between the estimated a_2 and a_3, for example, can be used to test whether study area 2 and 3 are different in terms of seed production and similarly for the other pairs of study areas 2 to 16. The *p* value was Bonferroni adjusted, in order to decrease the risk of making a Type I error.

To compare the seed production between years, an analysis of deviance compared the 'no interaction' model (yearseeedstem) to the 'interaction' model (yearseedstemint). Assuming that the null hypothesis (yearseedstem) is true, the difference in twice the log likelihood (the

deviance) follows a chi squared distribution with degrees of freedom equal to the additional number of parameters in the interaction model. This model was used to detect any differences in seed production, allowing for site effects to differ between years.

Within each year, the data for each study area was assessed for homogeneity of variance via a Levene's test. Where no significant differences were found between plants within study areas, a nested ANOVA was conducted, followed by a variance component analysis. This provided an indication of the proportion of variance attributed to the year and to the study area.

3a 3 Results

3a 3.1 *Seed availability*

In 2009, the minimum seed sampling target of seven plants per study area was achieved for all sites except Cedarwoods R. Although the number of seeds in each batch varied, the required number of seed (min. 60) to conduct both seed viability (refer to section 3b 2.2.2) and germination testing (refer to section 3c 2.2) was obtained in 2009.

In 2010, the seed availability was much reduced, with a number of plants failing to produce any seed. As a result, although it was possible to measure the number of seeds per stem (= 0) for these plants, it was no longer possible to measure the seed viability. Table 9 provides details of the number of plants assessed annually for each of the recruitment potential measures: seeds per stem, seed viability (target = min. 15 seeds per batch) and seed germinability (target = min. 45 (15 x 3) seeds per batch).

Table 9 – A summary of the number of plants sampled for each of the measures of recruitment potential, across sites and years (ns = not sampled).

	Number of plants sampled					
	2008	2009			2010	
	Seed Viability	Seed per stem	Seed Viability	Seed germination	Seed per stem	Seed Viability
Geggies Rd	7	7	7	7	7	7
Mt Mercer SR	7	8	8	7	8	5
McKenzie Rd	ns	8	8	7	8	0
Poorneet WRR	ns	8	8	7	8	6
Christies Rd	10	7	7	6	7	4
Cedarwoods R	7	6	6	4	6	5
Kirks BR	7	7	7	7	7	7
Glengower Rd	ns	10	10	7	10	4
Vite VRR	7	9	9	7	9	8
Ararat AR	7	8	8	6	8	8
Brownswaterholes BRR	ns	9	9	7	9	7
Baringhup WR	ns	8	8	7	8	6
Pitfield CR	7	8	8	7	8	9
Carisbrook BR	ns	7	7	7	7	4
Calder RRR	ns	9	9	7	9	0
Bannockburn RR	7	7	7	5	7	7

3a 3.1 *Seed Production*

3a 3.1.1 2009

In 2009, seed was sampled from at least 3 stems and up to 14 stems, with an average 4.5 stems per plant. The seed production ranged from 247.5 seeds per stem at Carisbrook BR to 14.6 seeds per stem at Christies Rd, with an overall average of 52.5 seeds per stem.

The generalised linear mixed model analysis (Figure 30) found that seed production was significantly different between study areas. Thus, the seed production variable is a measure of recruitment potential that is useful for comparing associations with environmental and habitat management predictor variables between study areas (Table 10). For the full results see Appendix 1.

Seed production at Carisbrook BR was an outlier and was found to be significantly different to thirteen of the remaining 15 study areas in 2009. The results of these comparisons are given in Appendix 2. The study areas Carisbrook BR, Glengower Rd, McKenzie Rd and Baringhup WR were significantly different to many of the other 12 study areas, but were not significantly different to each other (see Table in Appendix 2). Interestingly, Carisbrook BR, Glengower Rd, McKenzie Rd and Baringhup WR are geographically grouped to the north of the other study areas (see Figure 8 in Chapter 2).

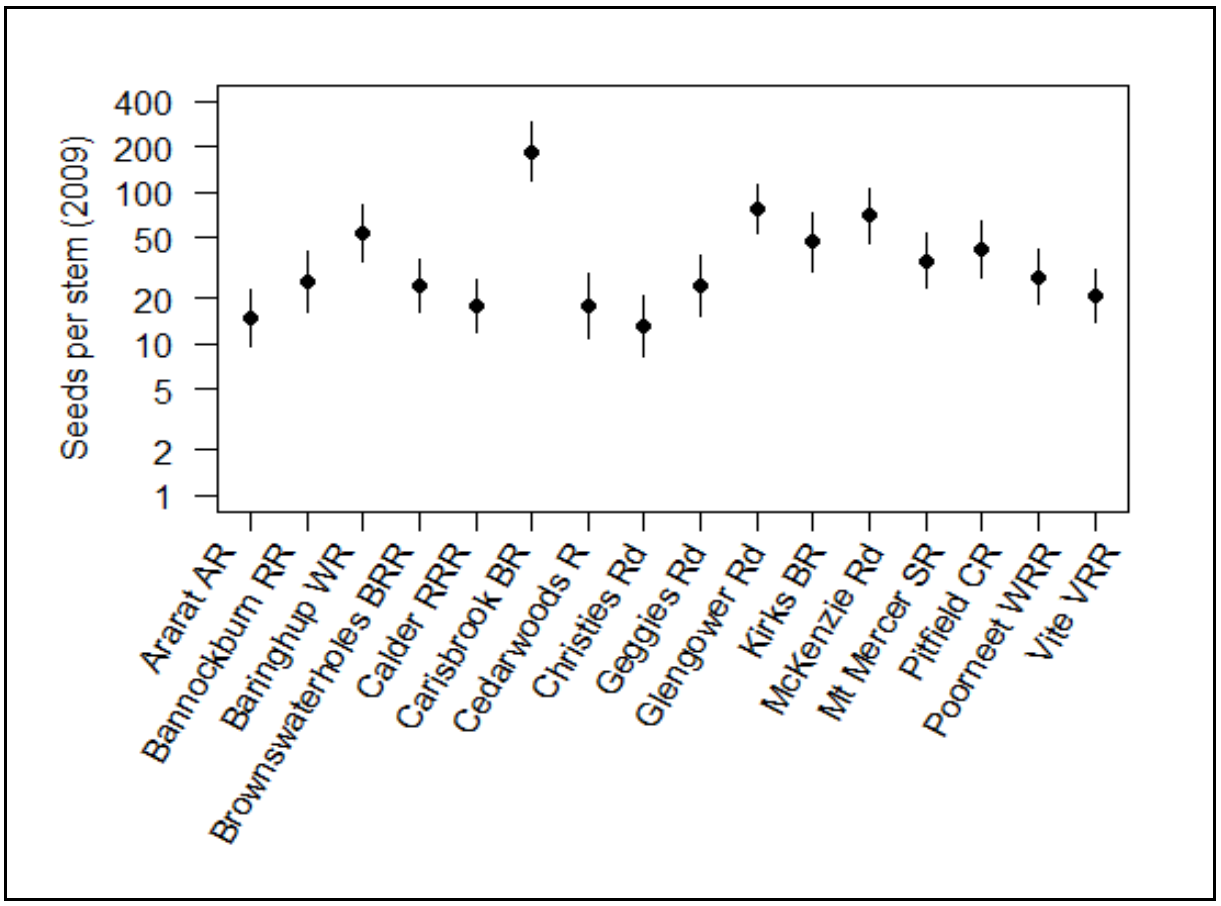


Figure 30 – The seed production of *P. spinescens* plants at all 16 study areas in 2009, (n = 126). The y axis is a $\log_{10}$ scale and the error bars are ±2SE.

Table 10 – The study areas found to be significantly different from any of the other 15 study areas in 2009.

Site	Number of significantly different study areas
Geggies Rd	3
Mt Mercer Rd	1
McKenzie Rd	7
Poorneet WRR	2
Christies Rd	6
Cedarwoods R	4
Kirks BR	3
Glengower Rd	9
Vite VRR	3
Ararat AR	5
Brownswaterholes BRR	3
Baringhup WR	5
Pitfield CR	2
Carisbrook BR	13
Calder RRR	2
Bannockburn RR	2

3a 3.1.2 2010

In 2010 seed was sampled from at least 3 stems and up to 9 stems with an average 3.5 stems per plant, across all study areas. The average seed production ranged from 32.5 seeds per stem at Pitfield CR to 3.6 seeds per stem at Calder RRR, with an overall average of 15 seeds per stem.

Following a generalized linear mixed model analysis (Figure 31), no significant differences in seed production between study areas was detected in 2010. The results of these comparisons are given in Appendix 3.

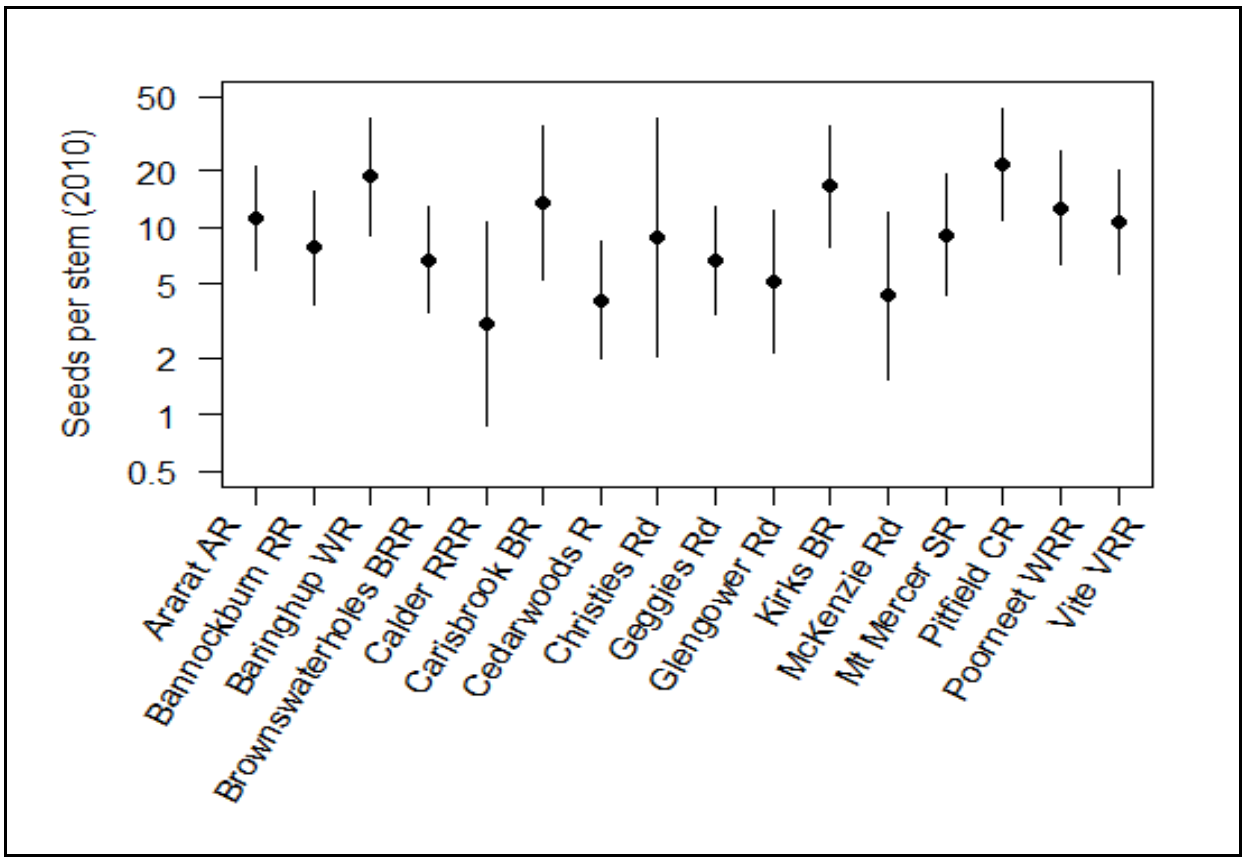


Figure 31 - The seed production of *P. spinescens* plants at all 16 study areas in 2010 (n = 126). The y axis is a $\log_{10}$ scale and the error bars are ±2SE.

3a 3.1.3 Comparisons 2009/2010

An analysis of deviance comparing the no interaction model to the interaction model for seed production found the difference in chi squared was 2484.7 $p < 0.0001$. The production of seed was significantly different between years for 3 of the 16 study areas (Figure 32): Carisbrook BR, Glengower Rd and McKenzie Rd (all with $p < 0.001$). The causes of this difference will be investigated further in Chapters 4 and 5. The results of these comparisons are given in Appendix 4.

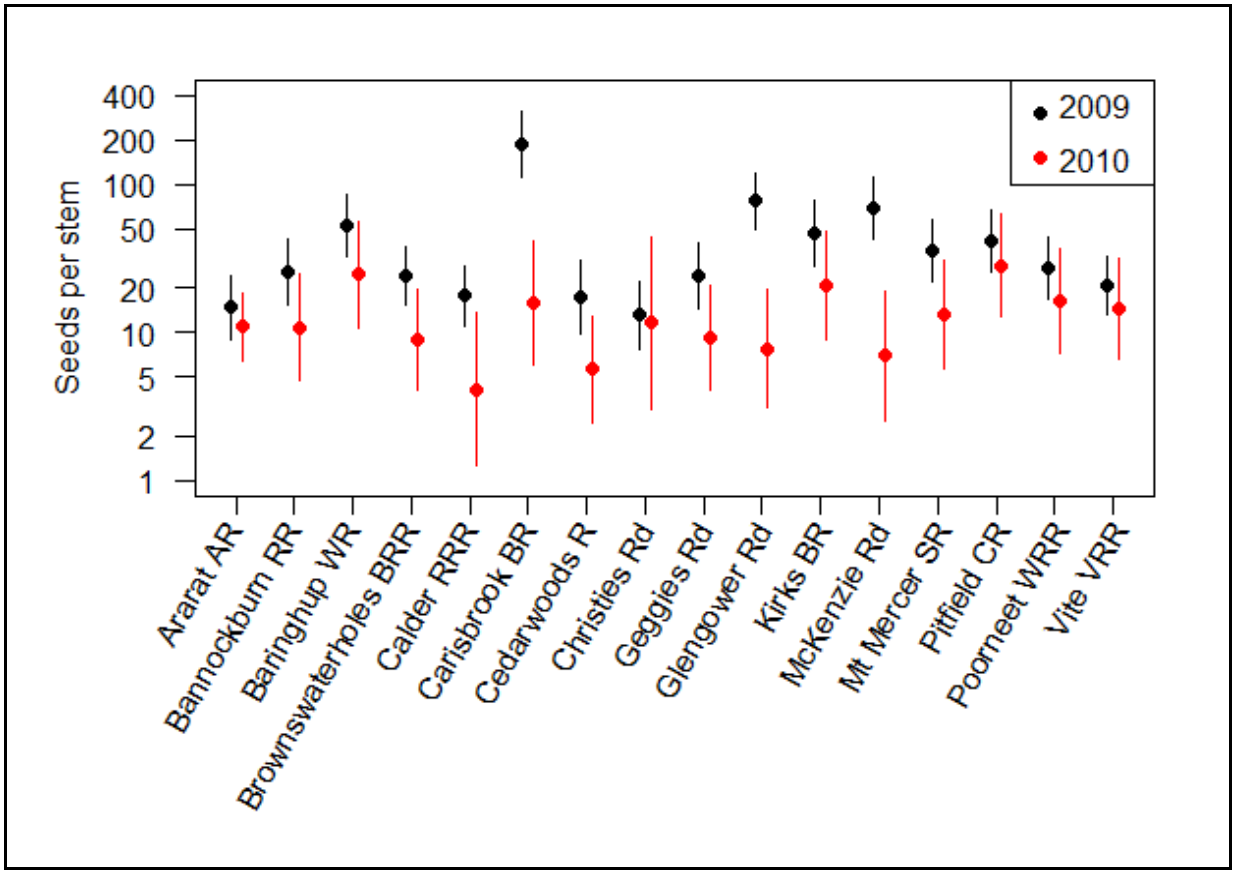


Figure 32 – Seed production for each study area in 2009 and 2010 (n = 252). The y axis is a $\log_{10}$ scale and the error bars are ±2SE.

Although a Levene's test found a high variability between plants within study areas, this variability was consistent and resulted in a homogeneity of variance between study areas for both years F = 1.1178, df = 15, p = 0.349 (2009) and F = 1.169, df = 15, p = 0.306 (2010). Following this, a nested ANOVA found a significant difference in the seed production of any given plant between years (F = 11.93, df = 1, p = 0.002) and that seed production varied between plants within a given study area (F = 16.46, df = 30, p < 0.001). The variance estimate of each component shows that there was a low error (15.2 %), that the year accounted for a fifth of the variance in seed production (21.5 %) and that within a year the study area (63.3 %) had the most effect on the seed production (Figure 33).

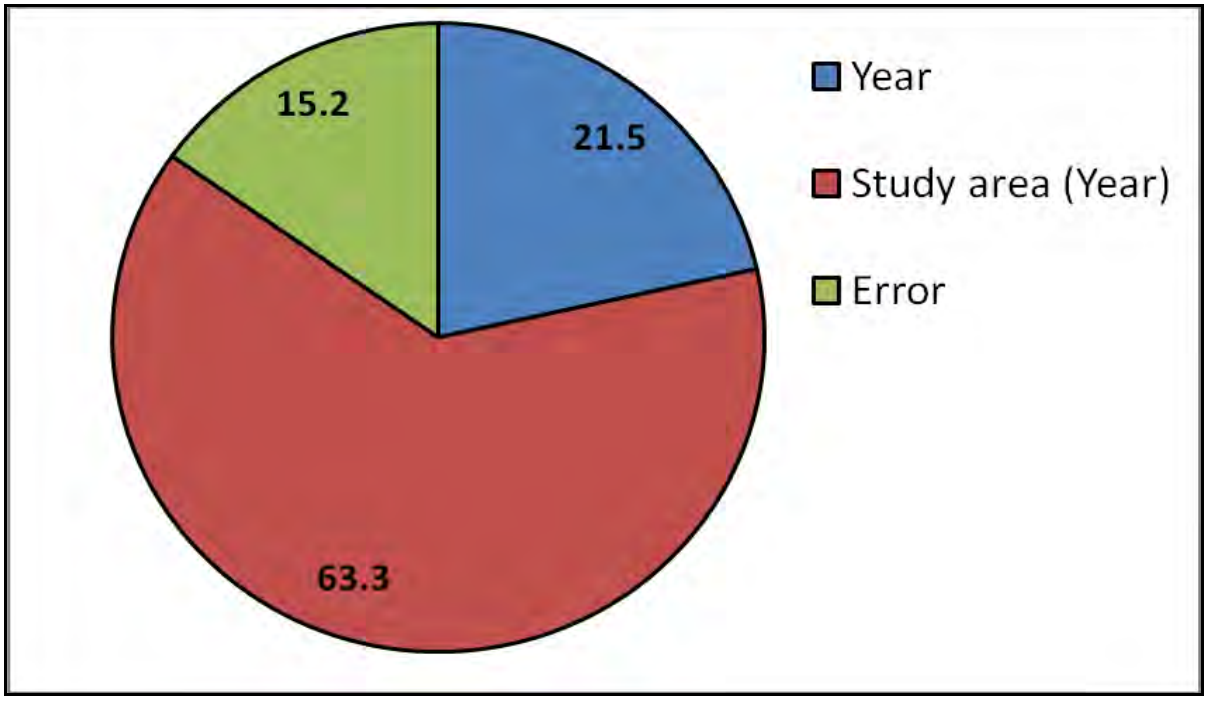


Figure 33 - The contribution of the effects of year and study area within a year on *P. spinescens* seed production.

3a 4 Discussion

Seed production was a useful measure of reproductive output during a season, as it has exhibited variability at two levels (spatial and temporal), possibly reflecting underlying influencing factors. The method of measuring seed production was successfully applied to all sites and the data showed variability between study areas in 2009 and between years for some study areas. Future hypothesis testing could be conducted using this variable in relation to assessing different environmental conditions and management practises.

The greatest variability in seed production was attributed to the study area which is indicative of the influence of local factors. Approximately a fifth of the variability was attributed to the year, suggesting that annually changing environmental and/or management factors may have some influence on the overall level of seed production within a study area. Interestingly, the four study areas with the greatest level of seed production in 2009 (Carisbrook BR, Glengower Rd, McKenzie Rd and Baringhup WR), were the most northerly located sites in this study, although statistical analysis did not detect a linear relationship between seed production and latitude (refer to Chapter 2).

Three of these four study areas were also significantly different between years. In 2009, a very large seed production was recorded at these four study areas, whereas in 2010 the seed production was similar to the other 13 study areas, with no significant differences found. The discrete nature of this study provides only a snapshot of the seed production capacity of *P. spinescens* and it is not possible to determine whether the measure of seed production is typical or abnormal for either of the study years.

The seed of *P. spinescens* has not previously been collected for the purposes of an ecological study. Therefore, the methods employed to assess seed production were experimental and designed to be achieved in a timely manner by two individuals. In the field of botany, the common practise is to count seeds per inflorescence and then count the number of inflorescences per plant (Oostermeijer, van Eijck *et al.*, 1994, Fischer and Matthies, 1998, Kery, Matthies *et al.*, 2000, Mustajarvi, Pirkko *et al.*, 2001, Jacquemyn, Brys *et al.*, 2002, Merrett, 2007, Tomimatsu and Ohara, 2010). There are also examples of ecological studies where seed has been collected from whole plants, e.g. *Rutidosis leptorrhynchoides* (Morgan, 1995b, 2000). Because *P. spinescens* produces between one and six small flowers per inflorescence that bloom sequentially throughout the season (over 5 - 6 months), it would have been difficult and time-consuming to use the measure 'seeds per inflorescence'. Instead, the measure 'seeds per stem' was adopted as an achievable and standardised method for assessing seed production between study areas and years. The limitation to this method was that plants varied in size and probably also stem length, which may have influenced the amount of seed that they were capable of producing. However, the variability in plant size, as measured by the basal circumference, was not found to be significantly different between sites (refer to Chapter 2, 'minimum sampling requirements' section). That is, plants within a study area varied in size but this level of variability was consistent between study areas. Therefore, it was assumed in this study that the variability in stem length was also consistent between sites and that the measure 'seeds per stem' would allow for comparisons between study areas over time.

Associations between seed production and environmental and/or management variables, will be further explored in Chapters 4 and 5.

Chapter 3b - Measuring the recruitment potential of *Pimelea spinescens* populations: seed viability

3b 1 Introduction

Although a plant may be capable of producing large quantities of seed, the quality of the seed may also be an important factor in the recruitment potential of a population (Murdock and Ellis, 2000, Merritt, 2006). High quality seeds are viable because they contain a living embryo and sufficient nutrient resources which provide the potential for germination (Sweedman and Merritt, 2006). For the majority of species, germination is generally used as the definitive test for seed viability. In genera that have a seed dormancy such as *Pimelea* (Bell, Plummer *et al.*, 1993, Copeland and McDonald, 2001, Merritt and Rokich, 2006) other methods must be explored to measure seed quality (Kearns and Inouye, 1993, Merritt, 2006).

Determining whether seed viability is a limiting factor for germination in the field can be assessed by testing the quality of individual seed batches (Baskin and Baskin, 2001). A viability test is an estimate of whether the seed is alive or dead, therefore providing its germination potential (Merritt, 2006). As seed viability had not previously been assessed for *P. spinescens*, the optimal method was unknown. The four commonly used seed viability testing methods are: cut, float, chemical (tetrazolium) and squash (McMillan Browse, 1980, Kearns and Inouye, 1993, Merritt, 2006).

- The cut test involves cutting the seed in half to reveal the integrity of the embryo. A viable embryo looks plump and fills out the seed case (Ooi, Auld *et al.*, 2004, Merritt, 2006).

- The float test places the seed in a container of water. After a period of time (depending on the seed properties) the seeds either sink or float. Seeds that sink are considered viable (Association of Official Seed Analysts, 1990, International Seed Testing Association, 1999).
- Tetrazolium staining involves exposing an imbibed seed to tetrazolium [according to standard procedures recommended by the International Seed Testing Association (1999)] for a period of time (usually greater than 24 hours, depending on the seed's uptake of the chemical). The seed is then cut open to reveal the staining. The embryo of a viable seed will stain red due to cellular respiration and a non-viable seed will not stain.
- In the squash test, a seed is placed between layers of oil absorbent paper and enough pressure is applied to squash the seed. Viable seed will leave an oily stain but there is no stain left from non-viable seed.

The difference between seed production and seed quality is dependent on how the plant allocates resources (Fenner and Thompson, 2005). Where these resources are limited, there is a trade-off between the number of seed and the size of seed (Galen, Plowright *et al.*, 1985, Jennersten, 1991). Therefore, seed weight can also be used as a measure of seed viability. For example, Soons (2002) determined a threshold weight below which the seeds of two grassland species in Spain were never viable.

Seed viability has been carried out on six species of *Pimelea* using either the cut (Dixon, Roche *et al.*, 1995, Roche, Dixon *et al.*, 1997, Willis, McKay *et al.*, 2003) or tetrazolium method (MacPhee, 1998, Clarke, Davison *et al.*, 2000, Gibson Roy, Delpratt *et al.*, 2007a).

To undertake tetrazolium staining on two species of *Pimelea*, Gibson Roy (2007) found that the seedcoat required piercing prior to the uptake of the stain.

Using the cut method, analysis revealed a 50 % viability of one batch of *P. spinescens* subsp*. pubiflora* seed at RBGM (M. Hurst 2008, pers. comm. June). The cut method is the only method currently practiced at the RBGM as they have found it the most reliable for the majority of their seed testing.

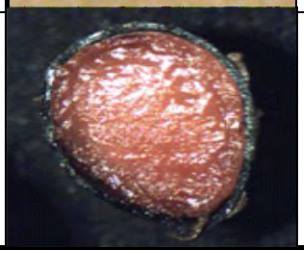
It is surprising that there are no documented cases of the squash test being used for this genus which has previously been noted and named for its oily seeds; *pimele* being the Greek word for fat or lard. No assessment of the viability of *P. spinescens* subsp. *spinescens* seed has been undertaken. Adoption of the most reliable method for assessing seed viability in *P. spinescens* will enable investigation into the demographic/environmental/management factors associated with variations in this measure of recruitment potential. Ideally the method adopted will be simple, cheap and effective.

3b 2 Methods

3b 2.1 *Selecting a method for assessing seed viability*

There are four different methods that are commonly selected for assessing the viability of seed: cut test; float test; squash test; and tetrazolium staining. To determine which was the more reliable and efficient method for assessing the viability of *P. spinescens* seeds, an experiment was conducted. A sub-sample of 25 seeds per treatment was randomly selected from one batch of seed, collected in 2008 (as described in section 3a 2.2) from four different study areas (Pitfield CR, Christies Rd, Vite VRR and Geggies Rd). Each sub-sample was subjected to the four different treatments (cut, float, squash and tetrazolium staining) as described in Table 11.

Table 11 – Methods for assessing seed viability. Images show the appearance of viable seed for each method.

Treatment		Treatment method
Cut		Seed was longitudinally sectioned through the middle and visually inspected. Viable seed were plump and firm, with a clear to white embryo which filled the black pericarp. Non-viable seed were shrivelled, with decayed and discoloured tissue (Ooi, 2004, Merritt, 2006).
Float		Seed was placed in a beaker of cold tap water for 24 hours. Seed that floated were considered not viable and seed that sank were assumed to be viable (Merritt, 2006).
Squash		Seed was placed between two layers of absorbent paper and enough pressure was exerted on the seed to squash it. Viable seed left an oily stain on the paper. Non-viable seed did not leave a stain (Ralph, 2003).
Tetrazolium		Tetrazolium staining was conducted according to standard procedures recommended by the International Seed Testing Association (1999). Viable seed respires and absorbs tetrazolium, staining red. Non-viable seed will not stain.

3b 2.1.1 Data analysis

An ANOVA was used to detect any differences between viability test treatments.

3b 2.2 *Seed viability testing*

3b 2.2.1 2008 Pilot study

In 2008, the cut method was used to assess the viability of *P. spinescens* seed, as recommended by MRBG.

As per the methodology for investigating seed production as a measure of recruitment potential, power analyses were also conducted on the viability of seed samples taken during the 2008 pilot study to determine the minimum number of female plants and number of seed required to represent the population within each study area.

For the measure 'seed viability', a population mean of 59.35 and s^2 of 20.5 was obtained across the nine pilot study sites. Via a power analysis, a Ø of 7.8 could be achieved by seven plant samples (seed batches) collected from nine populations, resulting in a < 1 % chance of making a Type II error (Zar, 1999).

To determine the number of seed required to represent the viability of seed within a batch, a power analysis was conducted, based on data obtained across the nine pilot study sites. With a population mean of 12.2 viable seed per batch and a standard deviation (s^2) of 4.7, a Ø of 6.09 could be achieved by using 20 seed from seven plants, resulting in a < 1 % chance of making a Type II error (Zar, 1999).

3b 2.2.2 2009 and 2010

Given that there was no significant difference in the detection of viable seed between the four test treatments (refer to results, section 3b 3.2), a method had to be selected for testing viability. The squash test was selected as the preferred method for this study, as it was cheap, quick and the easiest to perform of all four methods.

In 2009 and 2010, many plants failed to produce seed and many did not produce the minimum number required to conduct viability testing with sufficient statistical power (see above section 3a 3.1 'Seed availability').

A further power analysis, to find the minimum number of female plants and a sample size test to find the minimum number of seed per batch that were required to represent the population within each study area was conducted.

A power analysis using three plants from nine populations (2008 pilot study), obtained a population mean of 58.3 and a s^2 of 19.437, which gave a Ø of 7.2, resulting in a < 1 % chance of making a Type II error.

A sample size test found that using a standard deviation of $s^2 = 4.7$ (2008 pilot study) and a confidence interval of no wider than 4 seed (d = +/-2 seed) with a confidence level at 95 %, a sample size of 15 seed or more was required.

Therefore in 2009 and 2010, a minimum of 15 seeds per batch, from at least three plants (seed batches) per study area was aimed for to evaluate the seed viability characteristics of a study area. When seed batches yielded less than 15 seeds, all the available seed was used for the viability testing.

Data analysis

The 2009 and 2010 seed viability data was analysed using a generalised linear mixed model with the R package `lme4` (Bates, Maechler *et al.*, 2011). The number of viable seed from the number of seed tested for the ith plant in the jth study area were modelled as a binomial variable with mean mu_{ij} where log(mu_{ij})=log(nstems_{ij})+a_j+p_{ij} where p_{ij} was assumed to follow a normal distribution with mean 0 and variance sigma^2 to be estimated from the data. There is an a_j for each study area, with a_1 set to zero, and hence a_2 to a_16 can be used to test whether study areas 2 to 16 respectively are different to site 1 in terms of seed viability. In addition the difference between the estimated a_2 and a_3 can be used to test whether study area 2 and 3 are different in terms of seed viability, and similarly for the other pairs of study areas 2 to 16. The *p* value was Bonferroni adjusted, in order to decrease the risk of making a Type I error.

To compare the seed viability between years, an analysis of deviance compared the no interaction model (yearviability) to the interaction model (yearvibilityint). Assuming that the null hypothesis (yearviability) is true the difference is twice the log likelihood (the deviance) and follows a chi squared distribution with degrees of freedom equal to the additional number of parameters in the interaction model. This model was used to detect any differences in seed viability allowing for site effects to differ between years. For each year, the study areas were assessed for homogeneity of variance via a Levene's test.

Seed viability and seed weight were correlated against the other measures of recruitment potential (seed per stem, seed germinability, *in situ* germination and germinant survival) for

possible associations using Microsoft Excel. Bivariate analyses were conducted via SPSS (Version 18) to assess the direction and strength of any associations.

Data that was normally distributed underwent a Pearson's product-moment correlation (r). Where data did not conform to the requirements of normality, data transformations were conducted according to Zar (1999). If the data obtained was logarithmic and included zero values a value of one was added to the raw data before $\log_{10}$ transformation. When normality could not be achieved, a Spearman's rho analysis (r_s) was conducted. Some graphs were obtained using "R" via RStudio version.

3b 3 Results

3b 3.1 *2008 Pilot study*

The seed viability across the nine sites ranged from 28 % to 77 %, with an overall average of 59 %. From the 1,260 seed tested for viability, 11 (0.87 %) seeds were found to have no embryo when cut open. Seeds that were unviable contained varying degrees of dehydrated embryos (Figure 34).

Figure 34 – Examples of unviable *P. spinescens* seeds (2008).

3b 3.2 *Selecting a method for assessing seed viability*

There was no significant difference in the proportion of viable seed detected by each of the four methods tested, $F = 0.494$; $df = 3, 12$; $p = 0.693$ (Figure 35). The float test consistently produced the highest number of viable seed (percentage) and the tetrazolium treatment

gave the highest variability (range). The cut method was time consuming. Thus, the squash test was selected as the preferred method for all further testing conducted during this study, as it clearly confirmed the seed's oil content, was simple and effective.

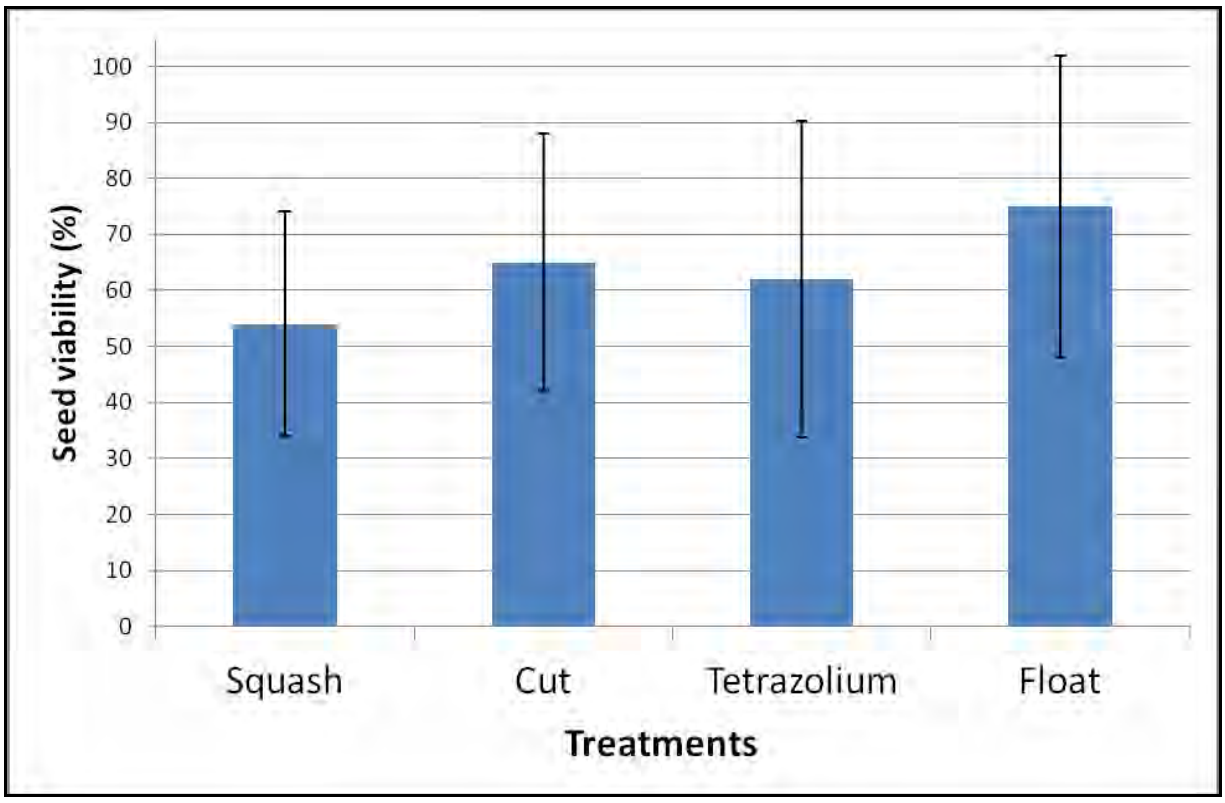


Figure 35 – Comparisons of the treatments for assessing seed viability (n = 25x4x4 = 400). The error bars represents ±1SE.

Seed viability was significant and positively related to seed weight (milligrams) for all the batches of seed that were harvested in 2008 (r_s = 0.683 p < 0.001, n = 64), 2009 (r_s = 0.631 p < 0.001, n = 126) and 2010 (r_s = 0.422, p < 0.001, n = 84). The overall trend found in all three years was for a heavier seed to have a greater viability (r_s = 0.581 p < 0.001, n = 274), and therefore it was likely to contain a sufficient nutritional source to support the development of the embryo (Figure 36).

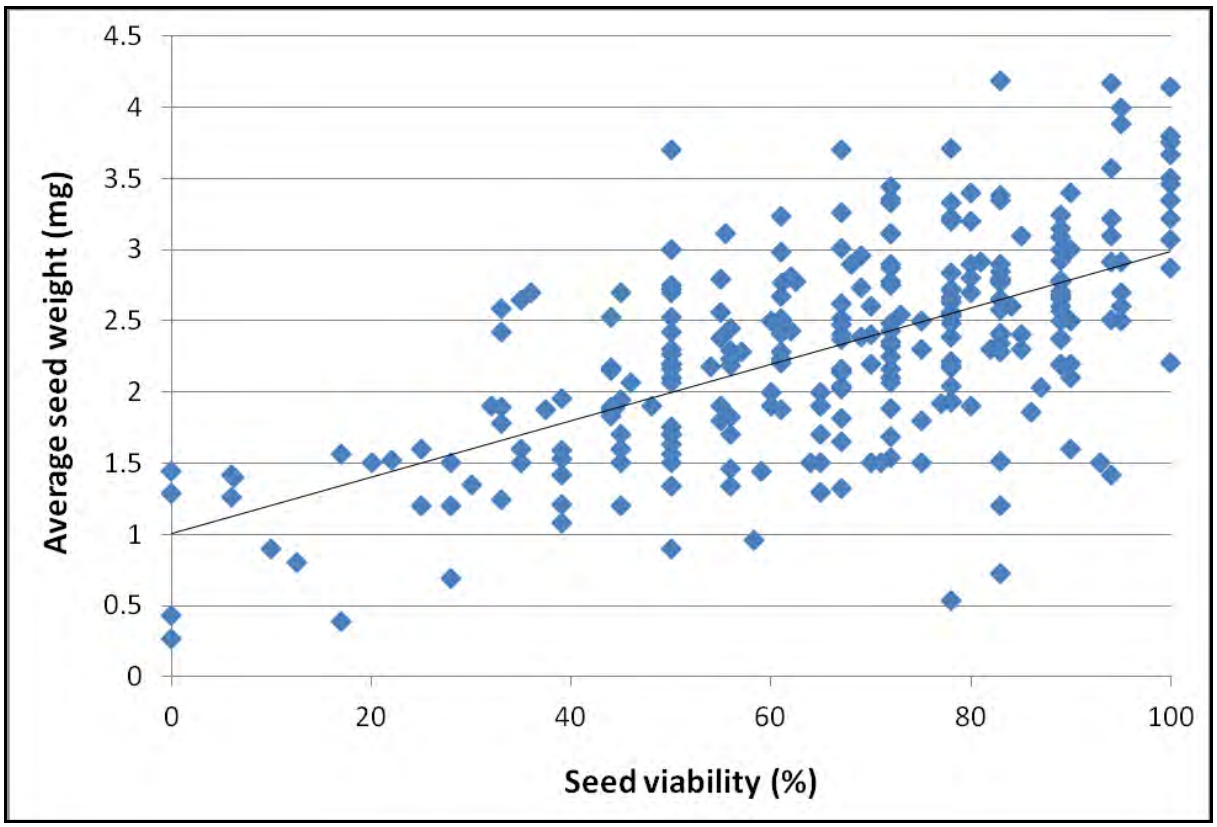


Figure 36 – In 2008, 2009 and 2010 (n = 274) the average seed weight was positively and significantly correlated to seed viability.

3b 3.3 *Seed viability testing*

3b 3.3.1 2009

In 2009, seed was collected in sufficient quantities from at least six *P. spinescens* plants per study area. The mean seed viability across study areas ranged from 39 % to 82 %, with an overall average of 65 %.

Following a generalised linear mixed model analysis (Figure 37), six study areas were found to be significantly different (Table 12). The study area which achieved the greatest seed viability, Brownswaterholes BRR, was found to be significantly different to three study areas,

while McKenzie Rd with the lowest seed viability was significantly different to Brownswaterholes BRR and an additional two study areas. The results of these comparisons are given in Appendix 5.

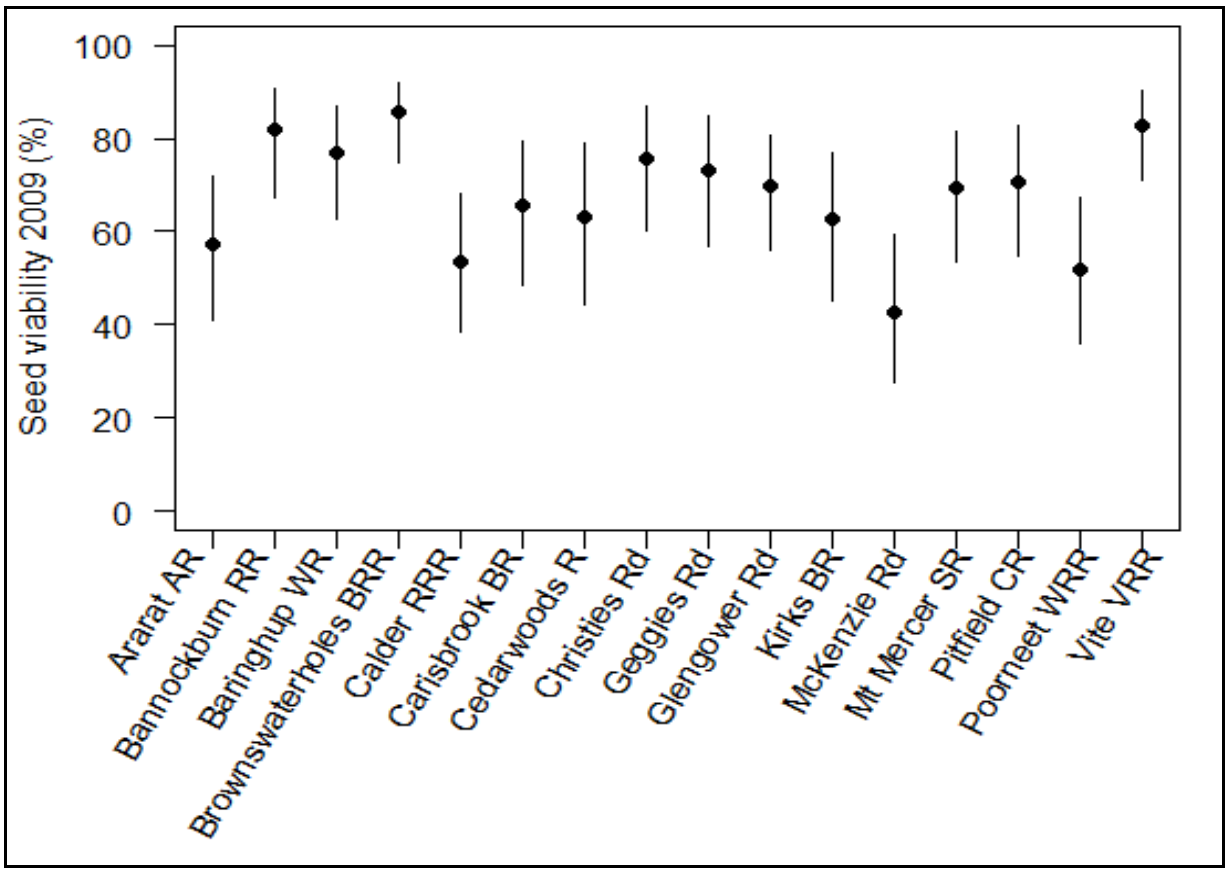


Figure 37 – Average viability of *P. spinescens* seed at all 16 study areas in 2009 (n = 126). The error bars are ±2SE.

Table 12 – Study areas which were significantly different in relation to seed viability in 2009.

Site	Number of significantly different study areas
Bannockburn RR	1
Brownswaterholes BRR	3
Calder RRR	1
McKenzie Rd	3
Poorneet WRR	1
Vite VRR	1

3b 3.3.2 2010

In 2010, seed was able to be collected in sufficient quantities from at least four *P. spinescens* plants per study area and 14 study areas were included in the analysis with Calder RRR and McKenzie Rd study areas being omitted. The mean seed viability across the 14 study areas ranged from 59 % to 92 %, with an overall average of 72.4 %.

The generalized linear mixed model analysis found that there was no significant difference in seed viability between study areas (Figure 38). The results of these comparisons are given in Appendix 6.

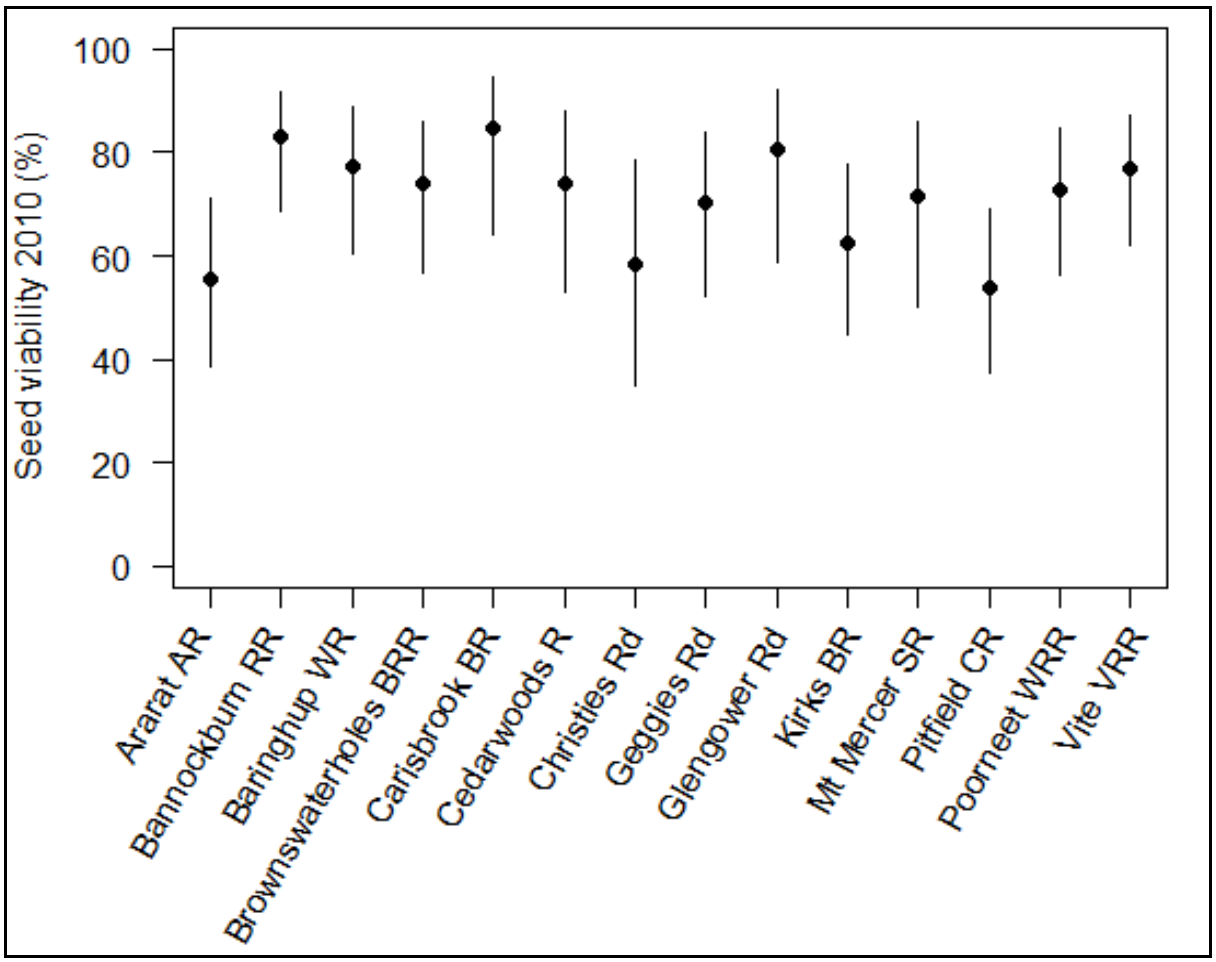


Figure 38 – Average viability of *P. spinescens* seed at 14 study areas in 2009 (n = 88). The error bars represent ±2SE.

3b 3.3.3 Comparisons 2009/2010

Repeated sampling was used to compare the seed viability in study areas between 2009 and 2010. Such a comparison could only be assessed for 12 study areas because reduced seed production for some plants resulted in small sample sizes and insufficient statistical power. The data for Calder RRR, Carisbrook BR, Christies Rd and McKenzie Rd study areas were removed from the analyses.

Although high variability was found between plants within study areas, homogeneity of variance was achieved between study areas for each year $F = 1.4604$, $df = 11$, $p = 0.1753$ (2009) and $F = 1.8252$, $df = 11$, $p = 0.0732$ (2010). An analysis of deviance comparing the no interaction model to the interaction model found the difference in chi squared was 13.881, $p < 0.2397$, indicating that there was no significant difference between study areas or between years (Figure 39). The results of these comparisons are given in Appendix 7.

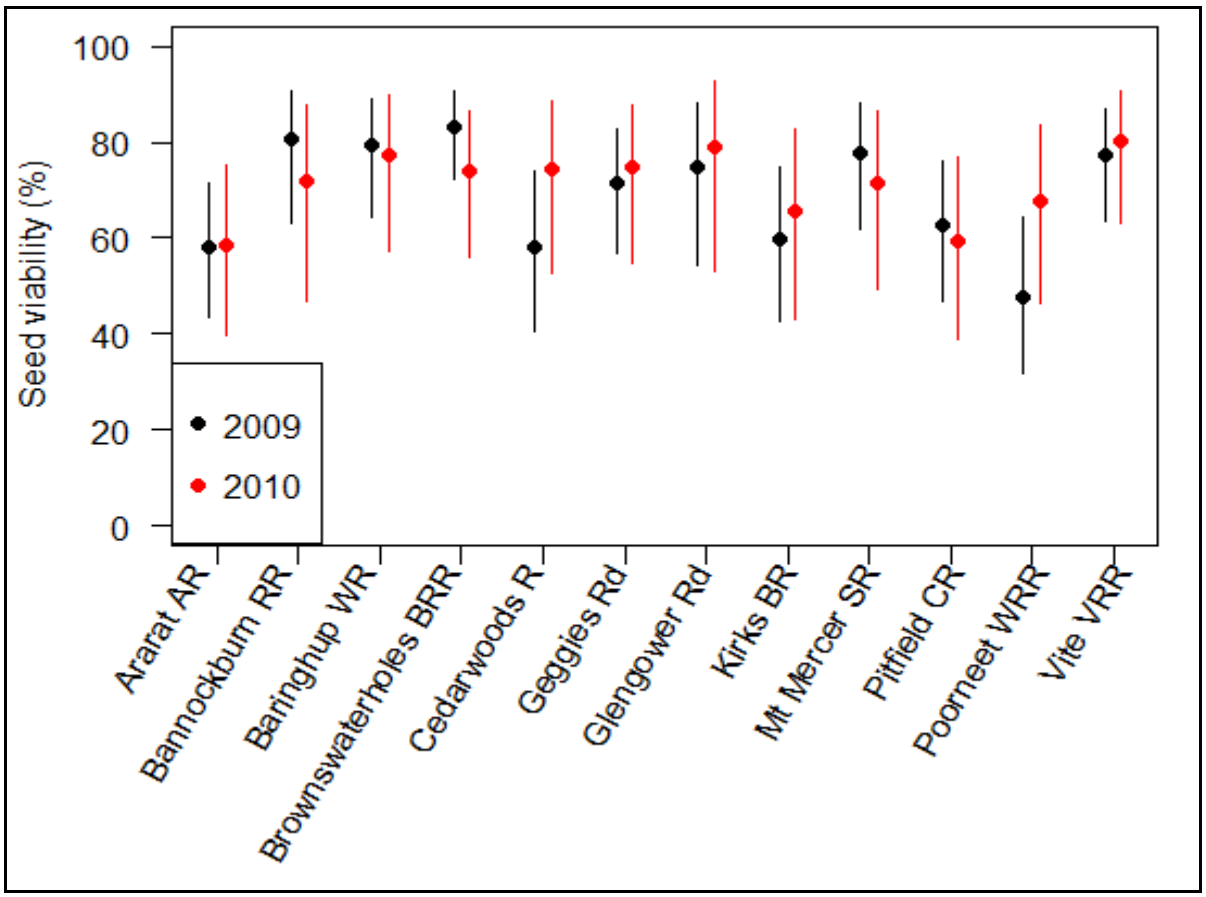


Figure 39 – Seed viability for 12 study areas in 2009 and 2010 (n = 128). The error bars represent ±2SE.

3b 3.4 *Seed production and seed viability*

In 2010, seed production was found to have a significantly negative relationship with seed viability r = -0.572, p = 0.033, n = 14 but not in 2009 (n = 15, outlier Carisbrook BR removed). When the seed production and seed viability data was combined for both years r = -0.433, p = 0.019, n = 29 (Figure 40) the trend was maintained. Fewer seeds produced in a study area was associated with greater seed viability.

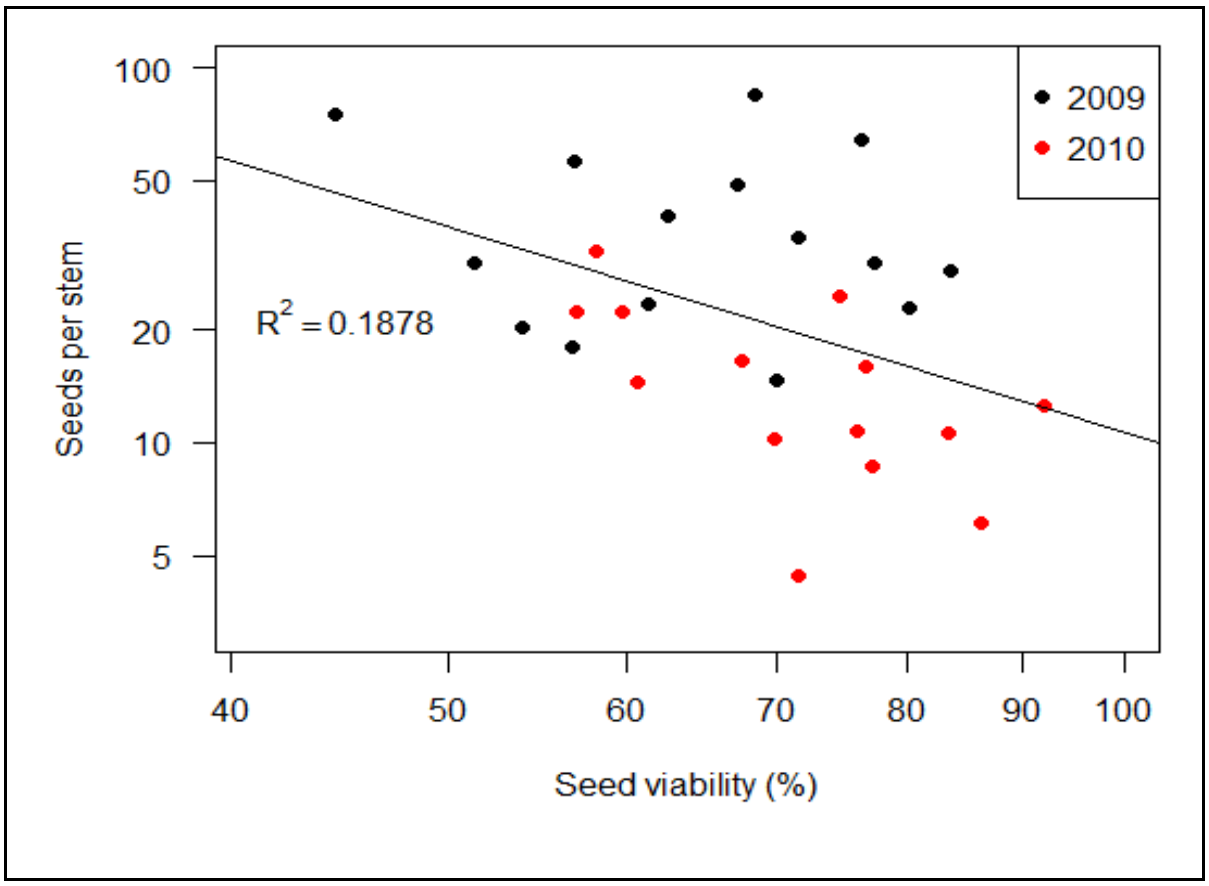


Figure 40 – When greater numbers of seeds were produced a lower seed viability was found (n = 29). Both axes are $\log_{10}$ scale.

3b 4 Discussion

3b 4.1 *Seed viability testing*

All of the four commonly recommended methods for testing seed viability (cut, float, chemical [Tetrazolium] or squash) are suitable for assessing the seed of *P. spinescens.* However, only the squash test was selected for this study as it was a relatively quick, easy, cheap and reliable method when applied to this species. Each of the methods, including the squash test has limitations, yet any of them could reliably be used to assess the viability of *P. spinescens* seed.

Although the cut test is a simple method, it is better suited to large seed. This method was used to assess the viability of *P. spinescens* seed in 2008. Because the seed were small it was difficult to physically cut and visually inspect them. This method was time consuming.

The float test is a simple method for assessing seed viability but was the least accurate of the four methods trialled. In a study on another species within the genus *Pimelea*, this method was found to be less reliable as some seeds sink or rise regardless of their viability (Merritt, 2006).

Tetrazolium staining is the most commonly used method for assessing seed viability and is considered to be relatively quick and accurate (Vankus, 1997, Copeland and McDonald, 2001). Using this method on *P. spinescens*, it was difficult to conclusively determine viability, as some seed stained pale pink rather than red or were only patchily stained. Similar problems have been experienced in other species (Lush, 1982), particularly those that exhibit seed dormancy (Bell, Vlahos *et al.*, 1987, Vankus, 1997). Physiological dormancy is thought to be associated with decreased gas permeability of the seed's embryo covers and

thus could affect the detection of cellular respiration and the resulting tetrazolium staining pattern (Lunt, 1995, Nikolaeva, 2004).

Tetrazolium testing has been described as subjective (Thompson, Adkins *et al.*, 2001, Merritt, 2006), difficult to interpret (Lush, 1982, Bell, Vlahos *et al.*, 1987), and variable in the amount of time required for completion (Gravina and Bellairs, 2000, Moles, Warton *et al.*, 2003). In Western Australia, Bell *et al.* (1995) found in at least two species (*Acacia pulchella* and *Regelia ciliata*) that tetrazolium viability results were questionable because germination was significantly greater than the viability testing results suggested.

Squash testing is recommended by Flora Bank (Australian Tree Seed Centre and Mortlock, 1999) and is suitable for oily seed only. It can be performed quickly and the results are immediate (Sawma and Mohler, 2002, Borza, Westerman *et al.*, 2007). It is particularly suitable for species in the genus *Pimelea* which have previously been noted for the oily nature of their seeds (Vickery, 1980). A fame analysis, independently conducted by ACS Laboratories (Australia), confirmed the presence of several fatty acids in *P. spinescens* seed, mainly Palmitic acid, some Linolenic acid and Oleic acid (see Appendix 10). The presence of these oils guarantees an available source of nutrition to the developing embryo and therefore indicates that the seed is viable for germination.

Research in the United States on a range of agricultural weeds species compared tetrazolium and squash testing methods for seed viability (Sawma and Mohler, 2002, Borza, Westerman *et al.*, 2007). It was found that the variance was insignificant (Sawma and Mohler, 2002) and the results were strongly and significantly correlated (Borza, Westerman

et al., 2007). The findings of Sawma *et al.* (2002) and Borza *et al.* (2007) are in keeping with the observations of this study on *P. spinescens.*

3b 4.2 *Seed viability analysis*

Using the squash test, some differences in seed viability were detected between study areas in 2009. However, these differences were not maintained in 2010 and the analyses did not reveal any significant difference between years. Within a given study area, the variation in the viability of seed between individual plants was large. This variation was relatively consistent between study areas and also between years. The consistency in seed viability limits the usefulness of this measure for hypothesis testing but it could be a valuable variable for relationship testing.

Seed collection for this species is time consuming due to the fiddley application of stockings and time required to wait for seed fall (Thomas, 2008b). When collecting seed for propagation purposes, it is important that the seed is viable and of high quality. The squash test proved useful in the laboratory and is a method that could easily be used in the field prior to wholesale seed collection, to ensure collection from individual plants that produce high quality seed.

One of the drawbacks of the squash test is that it destroys the seed, which is problematic when working with a critically endangered species.

3b 4.3 *Seed production, seed viability and seed weight*

In addition to identifying measures of recruitment which could be used as dependant variables, each of the measures was assessed for interrelatedness. Seed viability was found to be negatively associated with seed production but positively associated with seed weight.

Further investigation could lead to the development of a seed weight range that is indicative of seed viability for *P. spinescens*. Such an approach has previously been described for the grassland forbs *Centaurea jacea* (Brown Knapweed) and *Cirsium dissectum* (Meadow Thistle) (Soons and Heil). This method would avoid destructive viability testing, whilst ensuring the collection of high quality seed.

Using seed weight or seed viability as indicators of seed quality, it appears that as the production of seed increases the quality of seed decreases, and vice versa. This type of negative relationship has been described for a range of other species (Harper, Lovell *et al.*, 1970, Lloyd, 1987, Shipley and Dion, 1992, Fenner and Thompson, 2005). The association was more pronounced during the drought conditions of 2009 when a greater level of seed production occurred, even though the resources that were available for reproduction may have been more limited (this will be discussed further in Chapter 4), resulting in lower quality seed.

Chapter 3c – Measuring the recruitment potential of *Pimelea spinescens* populations: seed germinability

3c 1 Introduction

The process of germination transforms a dormant, partially hydrated embryo (seed) with a barely detectable metabolism into one that is fully hydrated with a vigorous metabolism culminating in growth (Bewley and Black, 1994). Germination depends on the ability of viable seed to respond to water and other environmental cues including an optimum temperature regime and sufficient moisture, oxygen and light (De Jong and Klinkhamer, 1988, McIntyre, 1990, Probert, 1992, Chambers and MacMahon, 1994, Morgan and Lunt, 1994, Olff, Pegtel *et al.*, 1994, Morgan, 1998a, Pons, 2000, Eckstein, 2005). Germination testing experiments can determine both the proportion of seeds that will germinate under the provided conditions and the optimum combination of conditions that are required to maximise the germination of seed (Willis and Groves, 1991).

For example, a study of 64 species that occur in the natural temperate grasslands of Victoria found that about a third of these had very high rates (51 - 100 %) of germination with unspecialised germination triggers immediately following harvest (Gibson Roy, Delpratt *et al.*, 2007a). Of the remaining species, it is unclear whether low rates of germination are the result of poor seed quality or inadequate germination conditions. Therefore, it is important to determine the optimum germination conditions for each species.

Germination tests on a range of species within the genus *Pimelea* have shown highly variable results (Keighery and Dixon, 1984, Dixon, Roche *et al.*, 1995, MacPhee, 1998, Willis, McKay *et al.*, 2003, Gibson Roy, Delpratt *et al.*, 2007b), with the greatest rate of 32 %

being recorded for *P. sylvestris* (Dixon, Roche *et al.*, 1995). Germination tests conducted with *Pimelea spinescens* sbsp. *pubiflora* and *Pimelea axiflora* at RBGM achieved rates of only 1 % and 4 % germination, respectively (M Hurst, 2010, pers. comm. June). Past efforts to germinate *P. spinescens* sbsp. *spinescens* have had inconsistent results (I Taylor, 2010, pers. comm. November). It is assumed that the species, like many in the genus, (Wittwer, 1965, Keighery and Dixon, 1984, Fox, Dixon *et al.*, 1987, Roberts, 1990, MacPhee, 1998, Willis, McKay *et al.*, 2003) has a dormancy which is limiting germination. To be able to reliably test germinability, it is necessary to overcome seed dormancy and provide the optimum conditions for germination.

3c 1.1 *Seed dormancy*

Seed dormancy is an important survival mechanism which enables a species to persist and adapt to an environment. Seed dormancy is an inherited trait and its intensity (level) is modified by the local environmental conditions during seed development (Naylor, 1983, Copeland and McDonald, 2001). A dormant seed is one that is viable but fails to germinate within a reasonable timeframe when subjected to a perceived range of optimal environmental conditions such as temperature, moisture, air and light for radicle emergence (Baskin and Baskin, 2004b, Sweedman and Merritt, 2006). Germination in dormant seeds can only occur once a series of pre-determined conditions are satisfied (McMillan Browse, 1980).

Dormant seeds are not simply resting awaiting optimal environment conditions but are prevented from germinating despite suitable environmental conditions (Copeland and McDonald, 2001). During dormancy seeds are sensitive to local environmental conditions

and adjust their level of dormancy to optimise their chance of completing their life-cycle successfully, once germinated (Vleeshouwers, Bouwmeester *et al.*, 1995).

Seed are considered to have either primary and/or secondary dormancy. Primary dormancy occurs during seed formation on the mother plant. The seed is shed without the ability to germinate immediately. Secondary dormancy is induced following dispersal due to adverse environmental conditions (Merritt and Rokich, 2006).

Primary dormancy is the most common form of dormancy and there are two types: exogenous and endogenous (Murdock and Ellis, 2000, Copeland and McDonald, 2001, Baskin and Baskin, 2004b). Exogenous dormancy involves some characteristics of the seed's structure which prevents the seed from germinating. For example, the seed coat prevents the seed from imbibing water and requires either a chemical or mechanical action to occur, as once the seed coat is nicked or cracked, the dormancy is broken (Baskin, Baskin *et al.*, 2000). Endogenous dormancy involves a characteristic of the seed's embryo that prevents germination. Endogenous dormancy can be further categorised into the classes of morphological dormancy, physiological dormancy and a combination of both.

Morphological dormancy is characterised by the presence of an underdeveloped embryo. Physiological dormancy is characterised by an embryo with the presence of physiological mechanisms of different depths or levels, which inhibit germination. Physiological dormancy is maintained by chemical inhibitors that restrict the metabolic processes (i.e. respiration) of the embryo. Despite seemingly favourable conditions (e.g. high temperatures), the breaking of a physiological dormancy requires a specific progression of environmental stimuli to commence the production of growth promoting chemicals that will overcome any growth

inhibiting chemicals. Once physiological dormancy has been broken, gas exchange (oxygen and carbon dioxide) and metabolic processes increase within the embryo, allowing germination (Nikolaeva, 1977, Copeland and McDonald, 2001).

Physiological dormancy is the most phylogenetically widespread class of seed dormancy and has three levels: deep, intermediate and non-deep. Baskin *et al.* (2004b) separated non-deep physiological dormancy in to five types according to biogeographic regions in which the plants are found; and the combination of chemical and temperature conditions that are required to overcome the effects of the inhibiting chemicals, enabling germination.

The goal of the seed ecologist is to break dormancy by finding the correct sequence of conditions essential to overcome the inhibitor and induce sufficient growth potential in the embryo for germination (Merritt and Rokich, 2006).

3c 1.2 *Breaking seed dormancy*

In order to achieve germination, dormancy, if it exists in the seed, will need to be overcome. Baskin *et al.* (2004) have developed a method for determining the conditions under which dormancy is broken. The “Double-germination phenology technique” or “Move along experiment” was designed to be used when there is limited seed and little is known about the germination ecology or life cycle of a species. The experiment is designed to be adaptive to local conditions and should progress through the appropriate simulated seasonal patterns of a species to discover what environmental patterns will effectively overcome dormancy. A dichotomous key is provided to utilise and assist in the identification of the type of dormancy, depending on the conditions under which the seed germinates.

Many different pre-treatments have been trialled to overcome seed dormancy and promote germination in Australian species (Ralph, 2003). Predictable and common environmental cues such as temperature and rainfall conditions from the species native habitat have been trialled with varying success (Bell, Rokich *et al.*, 1995, Bell, 1999). Also, due to Australia's long fire history, treatments to simulate the corresponding direct and indirect effects of fire have been trialled, including: smoke; heat shock; denaturing of allopathic agents (McPherson and Muller, 1969); increased nutrient levels via formation of an ash bed (Siddiqi, Carolin *et al.*, 1976); changes in soil pH (Warcup, 1981); and increased light and temperatures at ground level (Whelan, 1995, Auld and Bradstock, 1996). All these treatments or combinations of them have successfully facilitated germination for many species (Keith, 1997, Dixon and Barrett, 2003).

Germination in some species of *Pimelea* has been found to be successful by pre-treating the seed using: smoke (Dixon, Roche *et al.*, 1995, Willis, McKay *et al.*, 2003); smoke followed by one year of soil storage (Roche, Dixon *et al.*, 1997); leaching (MacPhee, 1998); cold stratification (Metcalf, 1995); and high temperatures (Dawson, Rapson *et al.*, 2005). In *Pimelea spicata* Willis *et al.* (2003) found that fresh seed appeared dormant but this disappeared following three months of storage, and germination was promoted by a specific temperature regime (20°C/10°C, day/night cycle). Germination trials on *Pimelea spinescens* sbsp. *pubiflora* yielded only 1 % success using smoke water as a pre-treatment (M Hurst, 2010, pers. comm. June). Overall, the germination successes from these various treatments have been few and inconsistent (Keighery and Dixon, 1984, Dixon, Roche *et al.*, 1995, Clarke, Davison *et al.*, 2000). Because of the poor results of previous trials other pre-treatment methods must be considered such as: plant hormones in the form of gibberellic

acid (Bell, Rokich *et al.*, 1995, Plummer and Bell, 1995); an after-ripening period (Bell, 1999); and passage through a birds gut (simulated chemical treatments) (Baker, 1988, Traveset, 2001, Dawson, Rapson *et al.*, 2005, Robertson, Trass *et al.*, 2006).

Although *Pimelea spinescens* sbsp. *spinescens* has yielded 25 - 30 % germination using a smoke water pre-treatment, there has been poor consistency with repeated attempts (I Taylor, 2010, pers. comm. November). This research will test a range of dormancy breaking methods in order to discover the cues that break the dormancy of *P. spinescens* seed, leading to a more reliable treatment which could provide consistent germination.

3c 1.3 *Viability adjusted germination*

Germination testing is usually carried out to test the quality of a seed batch throughout storage, in order to determine the likelihood of a plant's ability to contribute to future generations and to obtain a method to reliably stimulate germination (Baskin and Baskin, 2001, Merritt, 2006). Seed viability provides an estimate of whether a seed is alive or dead while seed germination provides an estimate of the number of viable seed which are able to germinate. Only viable seed have the ability to germinate, therefore when determining the percentage germination, seed viability should be incorporated into the calculations. The viability adjusted germination score provides a greater accuracy to the results, as it is a measure of the percentage of viable seed that germinated (Merritt, 2006, Cromer, 2007, Martyn, Seed *et al.*, 2009). This method was employed to assess *P. spinescens* seed germinability.

3c 2 Methods

3c 2.1 *Seed germination*

A seed was considered to have germinated when the radical emerged from the seed.

3c 2.1.1 Selecting a method for assessing seed germinability

To successfully germinate a dormant seed, it is important to know which type of dormancy is present. An initial experiment was conducted to determine if *P. spinescens* seed had an exogenous (physical) or endogenous dormancy (Baskin and Baskin, 2001). Following this, a dormancy breaking preliminary trial and dormancy breaking experiment were consecutively conducted on *P. spinescens* seed to find the most suitable method for germination. Once a suitable method was found, testing was conducted to evaluate the germinability of each batch of seed collected from each of the study areas in 2009.

Exogenous dormancy test

To test seed for water permeability, five seed batches were randomly selected from seed collected during the 2008 pilot study (refer to section 3a 2.1.1). From each batch, 10 seed were randomly selected and weighed as a group. The seed were then placed on moist filter paper for 24 hours at room temperature. Then the group of 10 seeds were blot dried and reweighed. An increase in weight meant that the seed had a permeable coat and were able to imbibe water; therefore they did not have a physical/exogenous dormancy (Baskin and Baskin, 2001, 2004a).

3c 2.1.2 Dormancy breaking preliminary trial (2008 seed)

Three plants from three different study areas (Christies Rd, Vite VRR and Bannockburn RR with seed viability of 70 %, 60 % and 75 % respectively) in the 2008 pilot study (refer to Chapter 2, section 2.2.2) provided large amounts of seed. These seed batches were utilised in a dormancy breaking preliminary trial. The trial used a 'move along' experimental design in which seeds were placed in incubators simulating the average light and temperature regimes in a seasonal progression (Baskin and Baskin, 2004a).

Pre-treatments

Based on a review of the relevant literature (Roberts, 1990, Dixon, Roche *et al.*, 1995, MacPhee, 1998, Clarke, Davison *et al.*, 2000, Willis, McKay *et al.*, 2003, Gibson Roy, Delpratt *et al.*, 2007a), five commonly used dormancy breaking pre-treatments and nine combinations of those pre-treatments were trialled. The pre-treatments were as follows:

- Water;
- Smoke. Seeds were soaked in smoke water for 24 hours;
- Gibberellic acid. Seeds were placed on a filter paper disc (Waltham size 4) saturated with 0.1 % (1,000 parts per million [ppm]) gibberellic acid (GA_3 minimum 90 % of total gibberellins);
- Heat shock. Seeds were dipped in near boiling water for ~2 minutes;
- Cold storage. Seeds were refrigerated at 4°C for 1 month; and
- Various combinations of the selected treatments:

- No sterilisation (see 'pre-experimental seed preparation' below) and cold storage;
- Smoke and gibberellic acid;
- Heat shock and gibberellic acid;
- Heat shock and smoke;
- Heat shock, smoke and gibberellic acid;
- Cold storage and heat shock;
- Cold storage, heat shock and smoke;
- Cold storage, heat shock and gibberellic acid; and
- Cold storage, heat shock, smoke and gibberellic acid.

For each pre-treatment there were two Petri dishes per batch of seed. One of each pair of Petri dishes was assigned to commence either in winter conditions or summer conditions (see 'incubator set-up' below). Thus, in total the preliminary trial consisted of 84 Petri dishes (14 treatments x 2 incubators (summer/winter) x 3 batches of seed = 84 Petri dishes in total). With the exception of ten Petri dishes containing seed from the Bannockburn RR batch, each Petri dish contained 25 seeds. Due to a limited number of seeds, the Bannockburn RR Petri dishes contained only 20 seeds.

Pre-experimental seed preparation

All seed was surface-sterilised prior to the experiment using 1 % strength calcium hypochlorite ($Ca(ClO)_2$) solution (Sweet and Bolton, 1979). The randomly selected seed

from each batch was placed in a tea infuser and submerged in the hypochlorite solution for five minutes, then placed in distilled water and agitated for five minutes. The distilled water process was repeated twice more. The seed was then towel dried and placed in Petri dishes which contained moistened paper discs on top of one layer of kitchen felt sponge, to help maintain moisture. The seeds were arranged in five rows of five (or four rows of five for 20 seeds).

Incubator set-up

Incubators were set to the average seasonal temperatures for the Victorian volcanic plains over the preceding five years (Commonwealth of Australia, 2012), as indicated in Table 13. One incubator commenced under summer conditions and the other under winter conditions, both running for 52 weeks.

Table 13 – The seasonal conditions simulated within the incubators.

Season	Summer	Autumn	Winter	Spring	Lights
Day temperature (°C)	26	20	13	20	on
Night temperature (°C)	10	10	5	8	off
Day/night length (hours)	12	12	12	12	on/off
Weeks	12	8	12	8	

The experiment commenced in March 2009 (31.03.2009) (approximately six months after harvest) and ran until March 2010 (23.03.2010). Water was added as required to maintain adequate moisture over time and the Petri dishes were hermetically sealed and labelled.

Each Petri dish was examined on a weekly basis, to record germination events and determine if changing temperature conditions over time (12 months) promoted seasonal flushes of germination.

Data analysis

The percentage of germinants for each replicate was transformed using an arcsine-square root transformation to normalise the data (Sokal and Rohlf, 1981). Data was initially analysed for differences between incubators, seed batches and treatments using a nested ANOVA. Because no significant differences were detected between incubators *(F* (1, 15) = 1.11, $p > 0.05$), the incubator data was combined with respect to the seed batch. A nested ANOVA was then conducted to detect any significant differences between seed batches and treatments, with a post hoc Tukey's test selected to detect differences between treatments.

Dormancy breaking experiment (2009 seed)

In a further attempt to find the natural cues for germination, a second germination experiment was conducted. The literature revealed that dormancy breaking and germination had been achieved in other species of the genus *Pimelea* through leaching (MacPhee, 1998); and in other plant species via different chemical washes or a combination of chemical treatments (Cohn and Hilhorst, 2000, Perveen, INaqvi *et al.*, 2008, Sakhanokho, 2009). During 2009, field observations also revealed plump fruit on the plants (Figure 41) which may have been attractive to ground birds or reptiles, and thus treatments to simulate the chemical passage through the gut of a frugivore were also included (McMillan Browse, 1980, Baker, 1988, Robertson, Trass *et al.*, 2006). The dormancy breaking preliminary trial (described previously) suggested that seed germination was cued by changing temperatures

and so this aspect was also included in the second germination experiment. Seed used in this experiment was collected in 2009 as per section 3a 2.2.

Figure 41 – The plump fruit of *P. spinescens* produced at the Baringhup WR study area in 2009.

Pre-treatments

In total, twelve different pre-treatments (or combinations of pre-treatments) were trialled for the second germination experiment (Table 14). These were as follows:

Gibberellic acid (Control)

1. Filter paper was saturated with 0.1 % (1,000ppm) gibberellic acid (GA_3).

Temperature changes

2. Seed was subjected to a 'summer temperature' regime which included:
   - Five days at 40°C for 12 hours (day simulation) and 10°C for 12 hours (night simulation), followed by;
   - Two days at cooler temperatures (25°C day and 10°C night); and
   - Two weeks repetition of this temperature regime before seed was rested at room temperature for two weeks.

Leaching with temperature changes

3. Seed was bagged in nylon stockings and placed in the toilet cistern for four weeks. The toilet was flushed at least daily.
4. Seed was subjected to a 'summer temperature' regime (as described above). Following this, seed were then leached (see above No 3).
5. Seed was subjected to a 'summer temperature' regime (as described above). Following this, the seed was subjected to a cold period (12°C day and 0°C night) for two days and then leached (see above No 3).
6. Seed was subjected to a 'summer temperature' regime (as described above). Following this the seed was subjected to a cold period (12°C day and 0°C night) for two days and leached (see above No 3). A subsequent 'summer temperature' regime followed (as described above).

Chemical pre-treatments

Following the breakdown of the seed coat, the highly corrosive nature of chemicals such as strong acids and bases have the potential to damage the seed embryo if the duration of application is too long (Cavanagh, 1987, Baskin and Baskin, 2001, Crawford, Cochrane *et al.*, 2004).

Timing

To determine the optimum application time of chemical pre-treatments, a trial was conducted using the strongest chemical trialled in this study. Five seeds were dipped into 98 % sulphuric acid (H_2SO_4) (pH 1 – 0) for periods of 1, 2, 5, 10, 15, 20, 25 and 30 minutes (Crawford, Cochrane *et al.*, 2004).

At 15 minutes the treatment did not seem to have sufficiently penetrated the seed coat but after 25 minutes the seed coat and pericarp appeared thin and had disintegrated. Thus, a 20 minute pre-treatment per chemical was selected.

Acid

7a. The seed was dipped into 50 % sulphuric acid (H_2SO_4) pH of 0 - 1 (Roberts, 1990, Mandujano, Montanta *et al.*, 2005, Sakhanokho, 2009). The seed was rinsed three times in distilled water.

7b. The seed was dipped into 98 % sulphuric acid (H_2SO_4) pH 0 - 1 (Muhammad and Amusa, 2003, Mandujano, Montanta *et al.*, 2005). The seed was rinsed three times in distilled water.

Alkaline

8. The seed was dipped into 0.4 % calcium hydroxide ($Ca(OH)_2$) pH 12 (Perveen, INaqvi *et al.*, 2008). The seed was rinsed three times in distilled water.

Acid followed by Alkaline

9a. The seed was dipped into 98 % sulphuric acid (H_2SO_4) pH 0 - 1 (Roberts, 1990, Mandujano, Montanta *et al.*, 2005, Sakhanokho, 2009). The seed was then rinsed with water, dipped into 0.4 % calcium hydroxide ($Ca(OH)_2$) pH 12 (Perveen, INaqvi *et al.*, 2008), and rinsed three times in distilled water.

9b. The seed was dipped into 50 % sulphuric acid (H_2SO_4) pH 0 - 1 (Muhammad and Amusa, 2003, Mandujano, Montanta *et al.*, 2005). The seed was then rinsed with water, dipped into 0.4 % calcium hydroxide ($Ca(OH)_2$) pH 12 (Perveen, INaqvi *et al.*, 2008), and rinsed three times in distilled water.

Alcohol

10. The seed was dipped into ethanol (C_2H_5OH) 0.2M pH 7.8 (Corbineau, Gouble *et al.*, 1991) and then rinsed three times in distilled water.

Table 14 - A summary of the treatments in the second dormancy breaking experiment.

No	Pre-treatments
1	GA_3 (0.1 %) only
2	40°C daily / 10°C night for 2 weeks, rest 2 weeks
3	4/52 leach
4	40°C daily / 10°C night for 2/52 weeks, rest 2 weeks, leach 4/52
5	40°C daily / 10°C night for 2/52, rest 2/52, cold snap 2/7, leach 4/52
6	40°C daily / 10°C night for 2/52, rest 2/52, cold snap 2/7, leach 4/52, 40°C daily / 10°C night for 2 weeks, rest 2 weeks
7a	H_2SO_4 (50 %)
7b	H_2SO_4 (98 %)
8	$Ca(OH)_2$
9a	H_2SO_4 (98 %) followed by $Ca(OH)_2$
9b	H_2SO_4 (50 %) followed by $Ca(OH)_2$
10	Ethanol (0.2 M)

Due to limitations with seed availability, only one batch of seed (collected in 2009) from one plant at the Vite VRR study area was used in the dormancy breaking experiment. Each pre-treatment consisted of three replicates, with 20 seed per Petri dish. The seed was arranged in Petri dishes, in four rows of five on moistened paper discs, which had one layer of felt sponge underneath to help maintain moisture. Water was added as required to maintain adequate moisture over time and the Petri dishes were hermetically sealed and labelled.

All replicates were placed in a single incubator at winter temperatures (13°C day and 5°C night), which oscillated between a 12/12 hour daylight/night cycle respectively, throughout the experiment. The experiment commenced on the 25th of May 2010 (approximately six months after harvest) and continued until the 6th of September 2010 (14 weeks).

Data Analysis

A Kruskal-Wallis ANOVA test was performed to detect if any dormancy breaking treatments were significantly different.

3c 2.2 *Seed germinability testing*

Seed germinability testing was conducted to assess the germination potential of the population of *P. spinescens* at each study area. On the basis of both the 'dormancy breaking preliminary trial' (see results below) and the 'dormancy breaking experiment' (see results below), the seed treatment of 0.1 % gibberellic acid (1,000ppm) was selected.

Using seed collected in 2009 only, three subsets of 15 seed were randomly selected from each batch and treated with gibberellic acid, as described in above section 'gibberellic acid'. In cases where fewer than 45 (3 x 15) seeds were harvested, all the available seed was divided equally to form three replicates. Replicates for each batch were distributed across three incubators set to the winter temperatures of 13°C (max.) and 5°C (min.) which oscillated between a 12/12 hour daylight/night cycles respectively, throughout the experiment. The germinability tests commenced on the 10th of August 2010 and ceased after 17 weeks on the 17th of December 2010. Each Petri dish had one layer of felt sponge within,

was hermetically sealed, labelled and checked weekly for seed germination. Water was added as required to maintain adequate moisture over time.

3c 2.2.1 Data analysis

The germination data was initially analysed using a generalised linear mixed model with the R package `lme4` (Bates, Maechler *et al.*, 2011). The number of germinants per number of seed assessed for the ith plant in the jth study area were modelled as a binomial variable with mean mu_{ij} where log(mu_{ij})=log(nstems_{ij})+a_j+p_{ij} where p_{ij} was assumed to follow a normal distribution with mean 0 and variance sigma^2 to be estimated from the data. There is an a_j for each study area, with a_1 set to zero, and hence a_2 to a_16 can be used to test whether study areas 2 to 16 respectively are different to study area 1 in terms of germinants. In addition the difference between the estimated a_2 and a_3, for example, can be used to test whether study areas 2 and 3 are different in terms of germination, and similarly for the other pairs of study areas 2 to 16. The *p* value was Bonferroni adjusted, in order to decrease the risk of making a Type I error. For each study area, data was assessed for homogeneity of variance via a Levene's test.

In order to take into account the proportion of viable seed produced from each individual plant, a "viability adjusted germination score" was calculated by generating a generalised linear mixed model for each of the seed viability scores and germination scores and then tested using a Markov chain Monte-Carlo methodology. From the posterior distribution, 1000 observations were generated for both the seed germination and seed viability data using the `sim` function from the R package `arm` (Gelman, Su *et al.*, 2012).

The ratio of these two sets of generated data (seed viability score and germination score) formed the viability adjusted germination score, which was calculated with 95 % confidence intervals using the 2.5^{th} percentile and the 97.5^{th} percentile. In addition empirical *p* values were calculated from the generated ratios from each site. The *p* value was estimated using $2 \times \Phi(-|\text{mean}(x)/\text{sd}(x)|)$ where x is equal to the difference between the site i to site j parameter and Ø is the standard normal distribution. The *p* value was Bonferroni adjusted in order to decrease the risk of making a Type I error.

3c 3 Results

3c 3.1 *Seed germination*

3c 3.1.1 Selecting a method for assessing seed germinability

Exogenous dormancy test

The average increase in weight of the group of 10 seeds after exposure to moisture ranged from 0.71 mg to 2.05 mg, with an overall average of 1.3 mg. All seed groups tested were able to imbibe water (Figure 42), indicating that *P. spinescens* seed is not exhibiting a physical or exogenous dormancy.

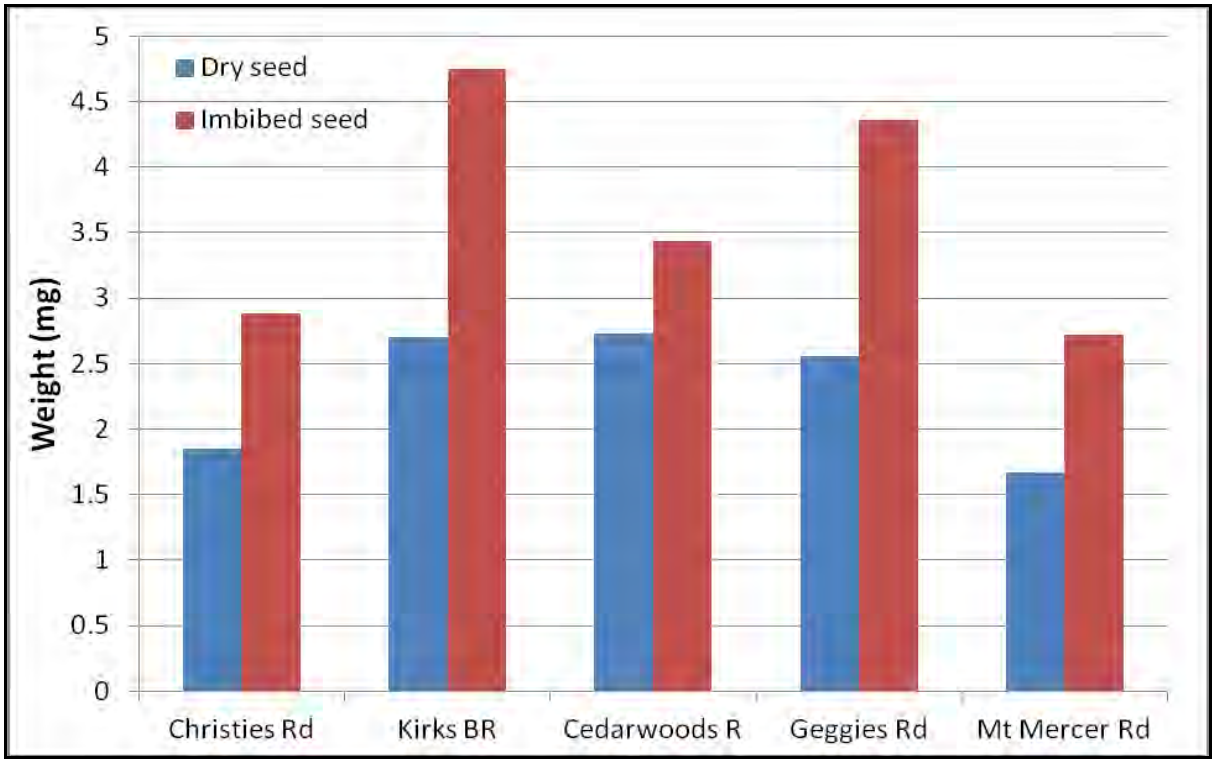


Figure 42 – Weight of a group of 10 seeds from five 2008 batches before and following immersion in water for 24 hours.

Dormancy breaking preliminary trial (2008 seed)

Germination of *P. spinescens* seed appears to occur more readily under autumn, winter and spring temperature regimes. Germination occurred less readily under summer temperature regimes.

In the winter incubator the first germinant appeared at week four and germination peaked at week eight. Regular germination events continued through winter and spring temperatures but declined to low numbers and ceased eight weeks after the incubator moved into summer. Autumn temperatures initiated another germination pulse, which peaked at week two during the second winter, then germination declined over the following ten weeks of winter.

In the summer incubator, the first germination events occurred in late summer at week nine, with the start of a pulse germination event in week five of autumn continuing until the first week of spring. Two germinants emerged in late spring with none occurring during the second summer (Figure 43).

Three germination spikes occurred between three and five weeks after moving from a warmer temperature to a cooler temperature.

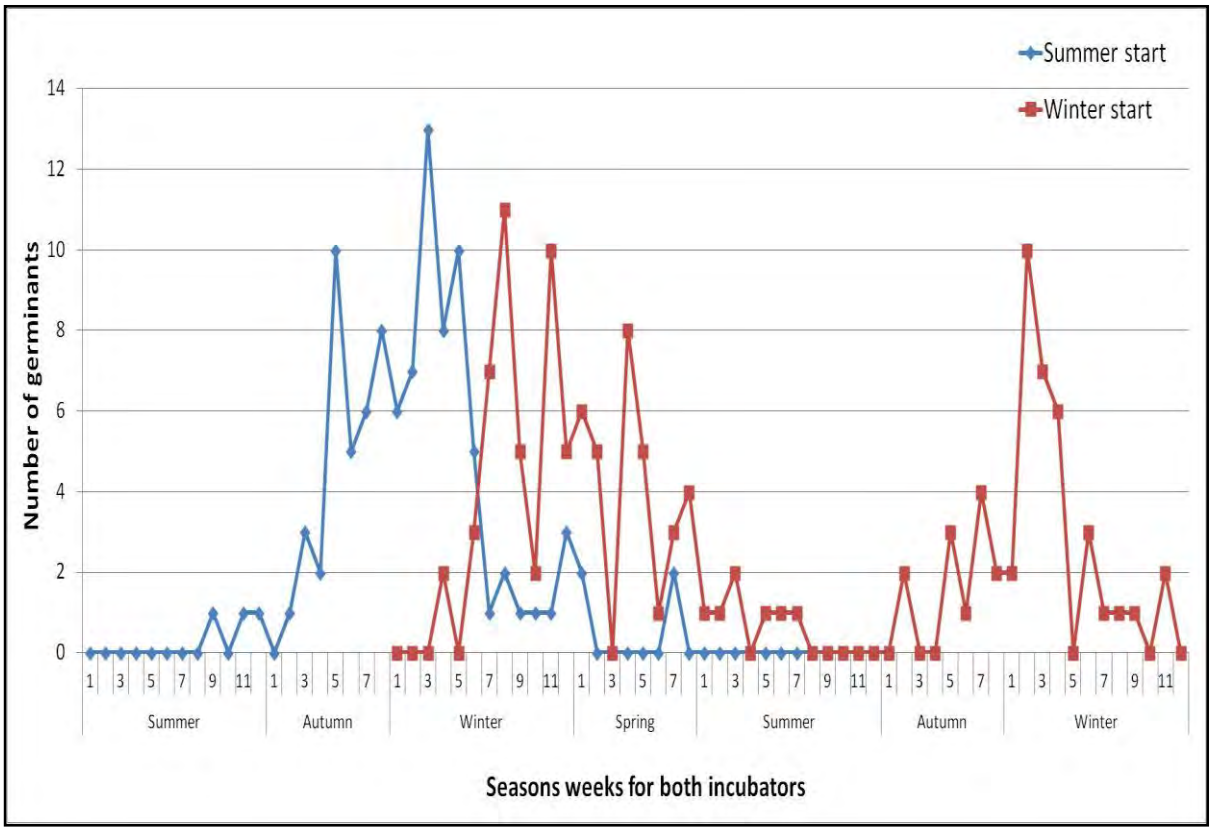


Figure 43 - The numbers of germinants produced per week in the winter and summer commencement incubators (n = 2,000).

Of the 2,000 seed that were used in the dormancy breaking preliminary trial, only 229 (11.45 %) germinated over 52 weeks. A nested ANOVA comparing the germination (%) per seed batch and per treatment detected a significant difference between all parameters, including an interaction (Table 15). Differences in the germinability between seed batches could be attributed to variations in the proportion of viable seed (differences ranged between 5 % and 15 %). However, because seed from each batch contributed to each treatment, the experiment is balanced and therefore a significant finding for the treatments is valid.

Table 15 - The results of a nested ANOVA that tested the effects of dormancy breaking treatment and seed batch on the dependent variable 'percentage germination'.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.871[a]	41	0.143	14.83	<0.001
Intercept	5.590	1	5.590	578.959	<0.001
Treatment	4.267	13	0.328	33.999	<0.001
Seed batch	0.223	2	0.112	11.557	<0.001
Treatment (Seed batch)	1.381	26	0.053	5.501	<0.001
Error	0.405	42	0.010		
Total	11.866	84			
Corrected Total	6.277	83			

a. R Squared = .935 (Adjusted R Squared = .872)

Following the nested ANOVA, a post-hoc Tukey's test assigned three homogenous subsets to the treatments. The subsets included:

1. All treatments containing gibberellic acid;
2. The treatment 'heat shock and smoke' and all treatments that included the use of gibberellic acid, with the exception of the two best germination treatments (cold storage, heat shock, smoke and GA_3; and GA_3); and
3. The seven lowest yielding treatments, none of which included a gibberellic acid treatment (Table 16).

A graphical representation of these homogeneous subsets is presented in Figure 44, where subset three can be clearly differentiated from subsets one and two.

Table 16 – Homogeneous subsets of dormancy breaking germination treatments, as defined by a Tukey's (HSD[a]) test.

Treatment	N	Subset for alpha = 0.05		
		1	2	3
No sterilisation and cold storage	6	<0.001		
Cold storage	6	<0.001		
Cold storage and heat shock	6	<0.001		
Water	6	0.033		
Cold storage, heat shock and smoke	6	0.033		
Smoke	6	0.105		
Heat shock	6	0.125		
Heat shock and smoke	6		0.343	
Cold storage, heat shock and GA_3	6		0.428	0.428
Heat shock and GA_3	6		0.451	0.451
Heat shock, smoke and GA_3	6		0.457	0.457
Smoke and GA_3	6		0.468	0.468
GA_3	6			0.579
Cold storage, heat shock, smoke and GA_3	6			0.589
Subset Sig. (*p* value)		0.632	0.624	0.245

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

The vast majority of germination events (88 %) occurred in treatments that included the presence of gibberellic acid. The only treatment that produced a notable number of germinants without the presence of gibberellic acid was the 'heat shock and smoke' treatment (Figure 44).

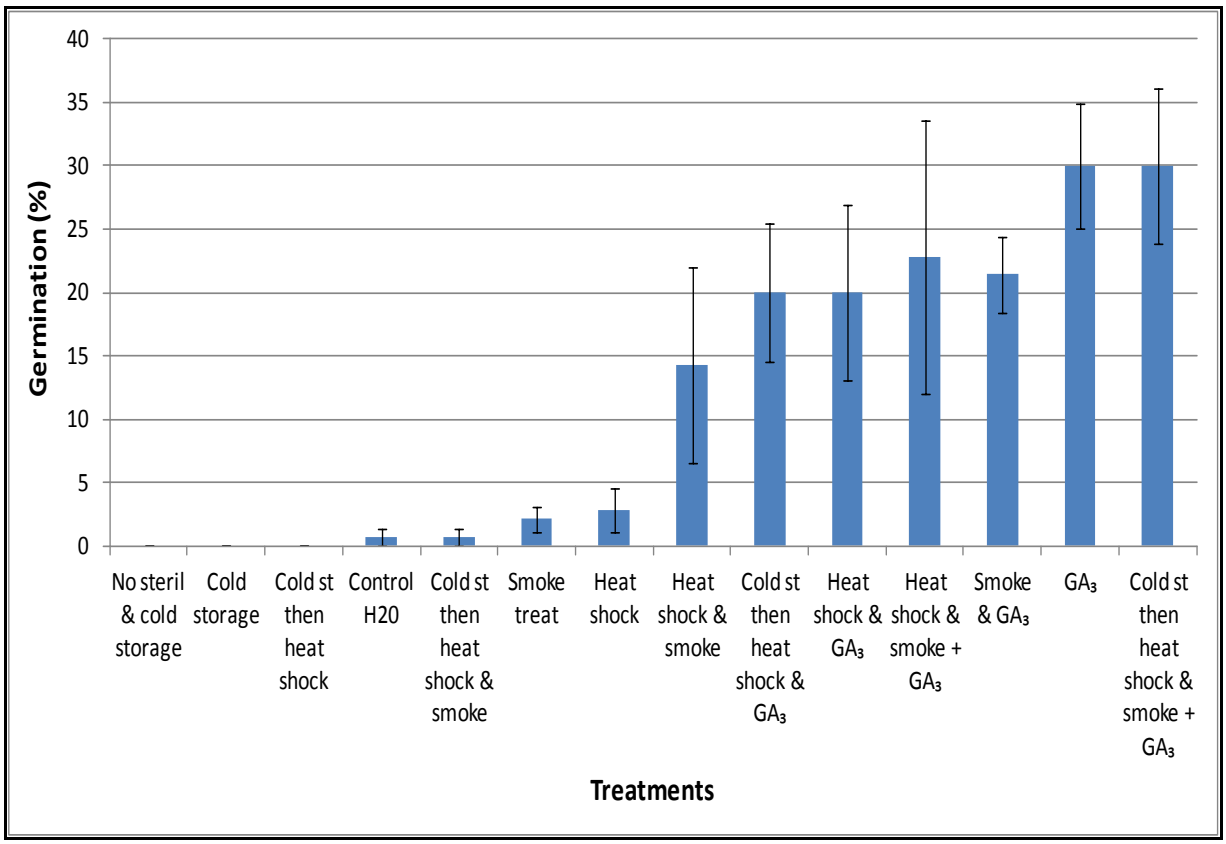


Figure 44 – The proportion of germinants produced in each dormancy breaking treatment (n = 2,000). The error bars represent ±1SE.

Even though the seed were sterilised prior to commencement of the germination trials, some fungal growths occurred in the moist conditions of the Petri dishes over the year of the experiment. Despite the presence of fungal growths, many seed were able to successfully germinate (Figure 45 A, B, C and D).

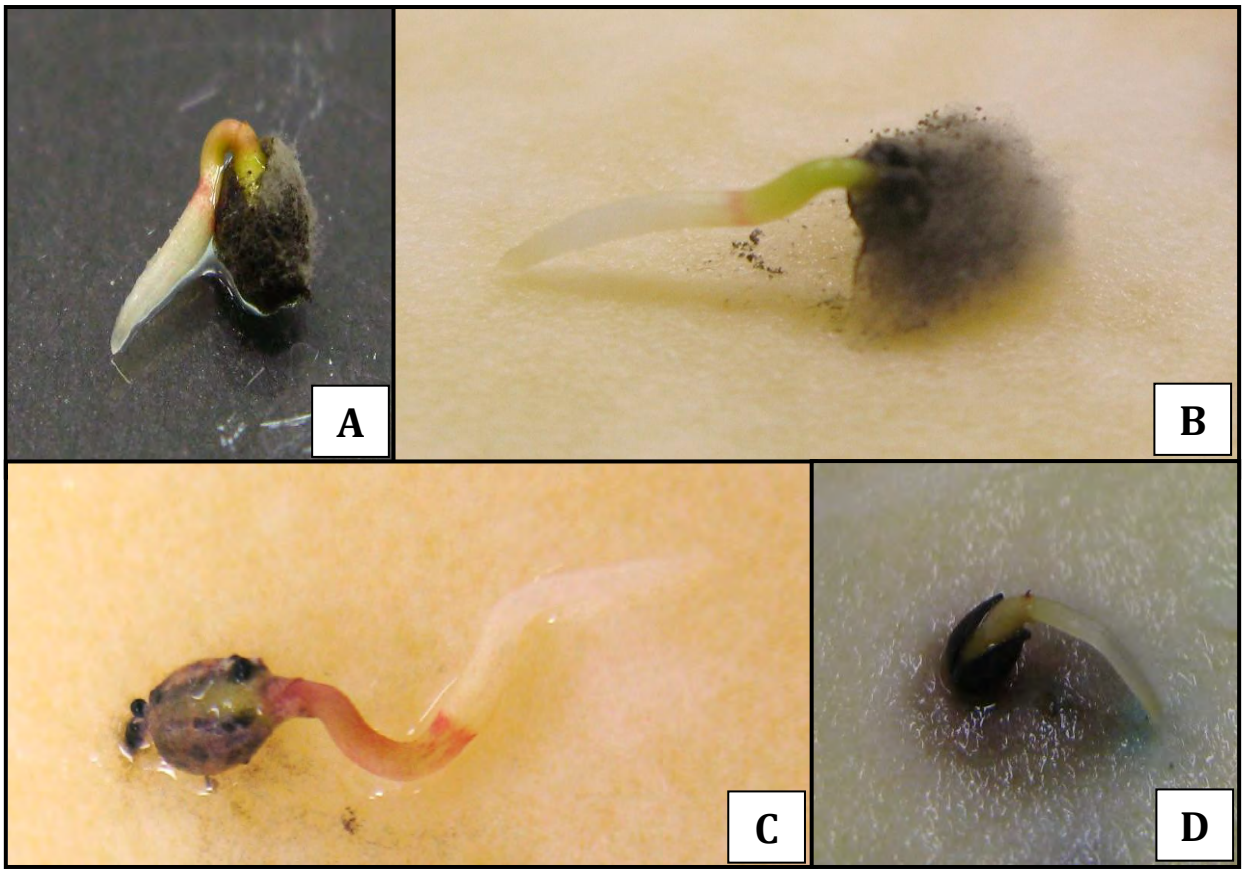


Figure 45 - A, B, C and D – Fungal growth found on and with *P. spinescens* germinants during the dormancy breaking preliminary trial.

Dormancy breaking experiment (2009 seed)

Of the 720 seed that were used in the dormancy breaking experiment, only 18 (2.5 %) seed germinated, 67 % (12) of which occurred in the presence of gibberellic acid. A small number of germinants (Figure 46) were also observed in some of the other treatments: summer temperature regime followed by leaching; leaching and 98 % sulphuric acid followed by alkaline. Germinants produced under the acid/alkaline treatment, formed stunted radicles and died shortly after germination (Figure 47 B). The control (GA_3) was the best treatment to

yield a higher proportion of germinants. The treatment data was not normally distributed so a Kruskal-Wallis ANOVA test was performed, indicating a significant difference between treatments $\chi^2 = 8.726$, df (3), $p = 0.033$.

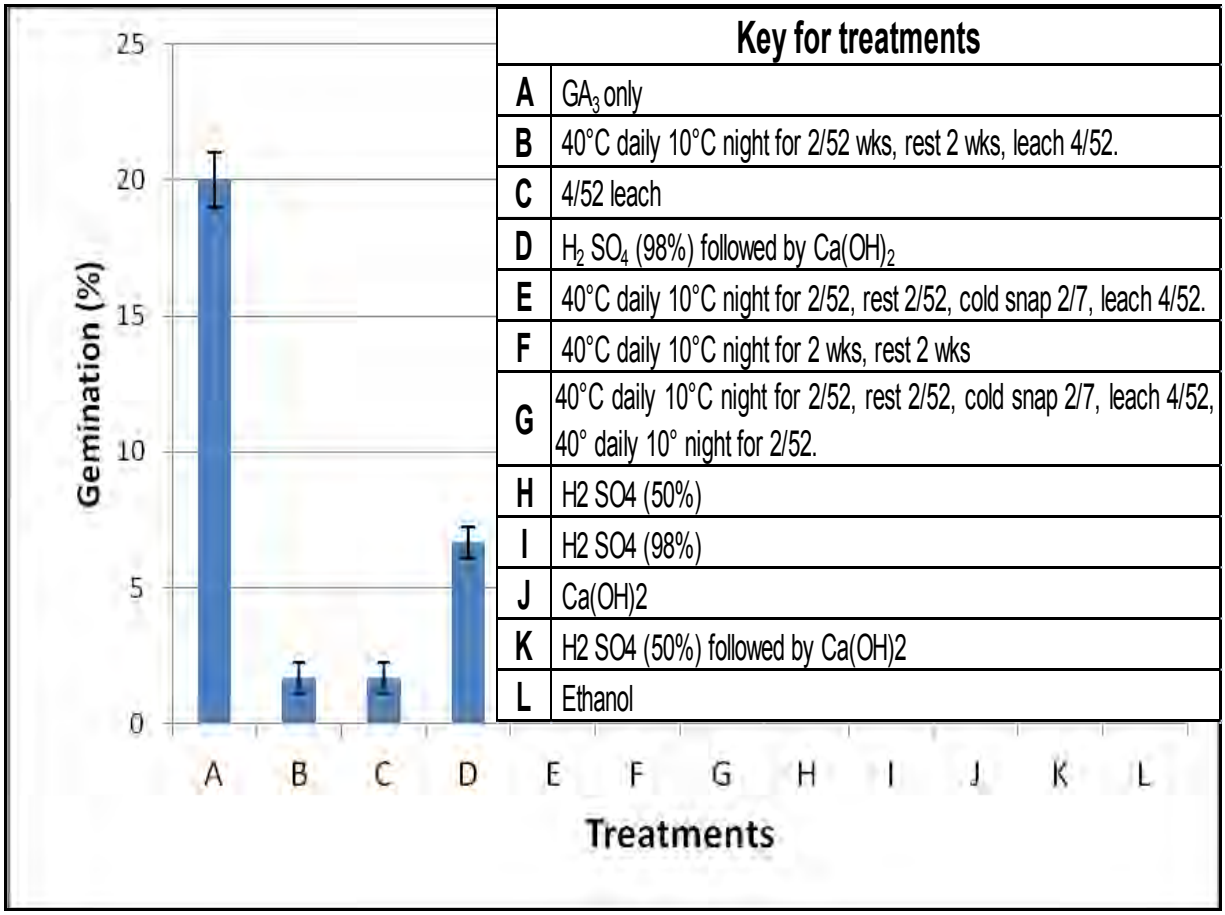


Figure 46 – The proportion of seed that germinated during the dormancy breaking experiment. The error bars represent ±1SE.

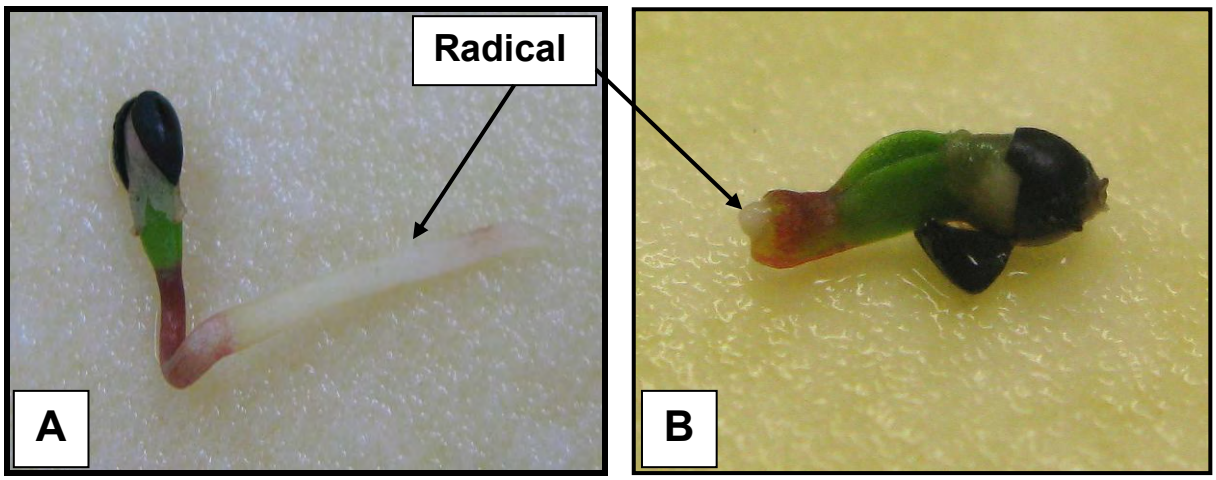


Figure 47 – A - A normal germinant; B - A germinant produced via an acid and base treatment.

Overall, between the dormancy breaking preliminary trial and the dormancy breaking experiment, 25 germination treatments were trialled. Of these, germination was consistently greatest in the presence of gibberellic acid. Thus, the gibberellic acid treatment was selected for germinability testing of seed collected from all study areas during 2009, enabling the calculation of a viability adjusted germination score (%) for each of the 16 study areas and their plants.

3c 3.2 *Seed germinability testing*

The seed from a total of 105 *P. spinescens* plants across the 16 study areas were assessed for germinability (Table 17). After 17 weeks a total of 775 seed had germinated (~14.6 %). The pattern of germination was similar to that observed in the dormancy breaking preliminary trial. Starting with winter temperature regimes, the first germinants appeared in week three, with increasing rates of germination to a peak in week ten, followed by a gradual decline. Of the total germinants produced, 14 % had germinated by week five and 62 % had

germinated by week ten (Figure 48). A slow rate of germination appeared to be ongoing at the conclusion of the experiment (week 13 produced 4 % per week dropping to 2 % by week 17).

Table 17 – The number of plants and seeds per study area used for germination testing and the number of germinants produced from the seed used per study area.

Study area	No of plants	No of seed tested	No of Germinants (%)
Geggies Rd	7	318	50 (15)
Mt Mercer SR	7	364	73 (20)
McKenzie Rd	7	376	24 (6.4)
Poorneet WRR	7	377	21 (5.5)
Christies Rd	6	261	32 (12.3)
Cedarwoods R	4	196	49 (25)
Kirks BR	7	377	34 (9)
Glengower Rd	7	375	105 (28)
Vite VRR	7	378	17 (4.5)
Ararat AR	6	268	26 (9.7)
Brownswaterholes BRR	7	356	83 (23.3)
Baringhup WR	7	378	66 (17.5)
Pitfield CR	7	376	41 (11)
Calder RRR	7	367	39 (10.6)
Bannockburn RR	5	245	35 (14.3)
Carisbrook BR	7	369	80 (21.6)
Total	**105**	**5,381**	**775 (14.6)**

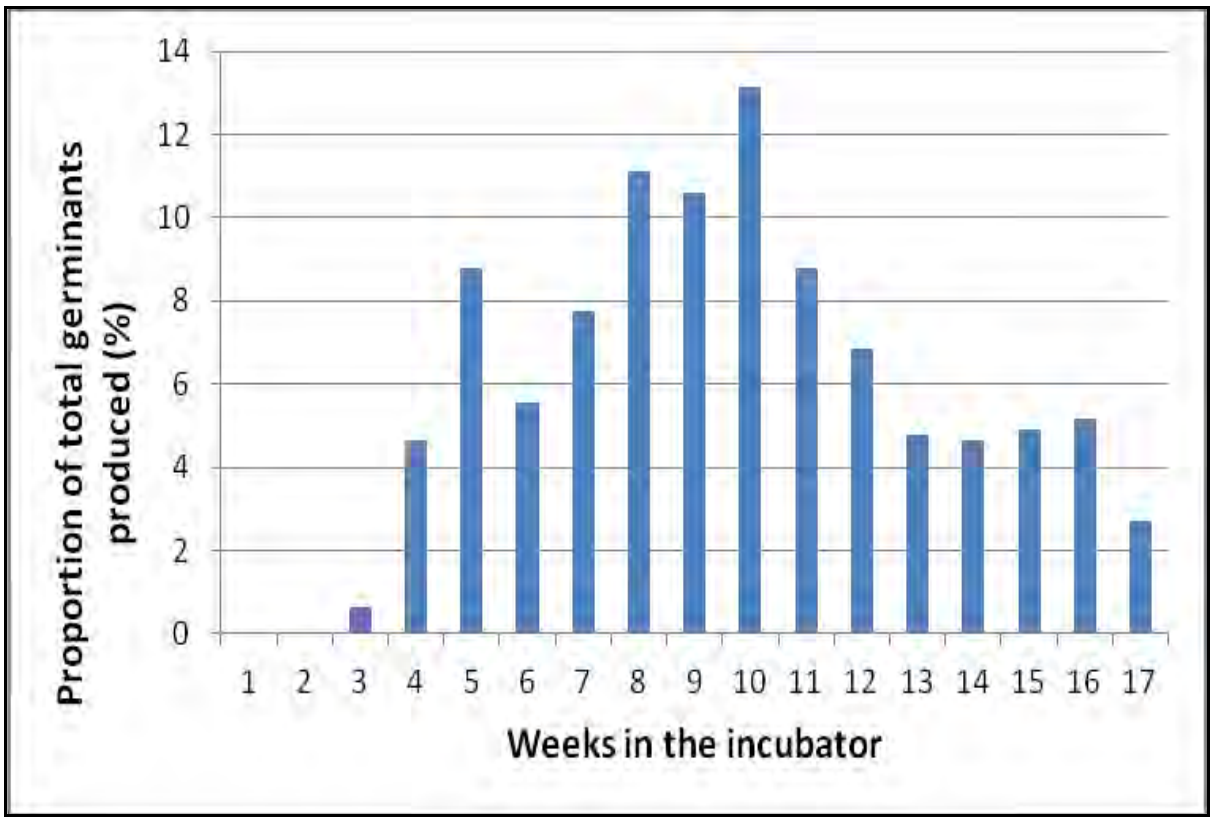


Figure 48 - The pattern of germination over time from all seed batches combined in 2009 (n = 5,381).

3c 3.3 *Viability adjusted germination score*

No significant difference in the viability adjusted germination score was detected between study areas (Figure 49). The results of each of the generalised linear mixed models are given in Appendix 5 and 8 with the Markov chain Monte-carlo summary in Appendix 9. Homogeneity of variance was not violated as the Levene's test was not significant. High variability was found between plants within study areas but there was no significant difference in the variance of the seed germination data for all plants between study areas $F = 1.3091$, $df = 15$, $p = 0.2138$.

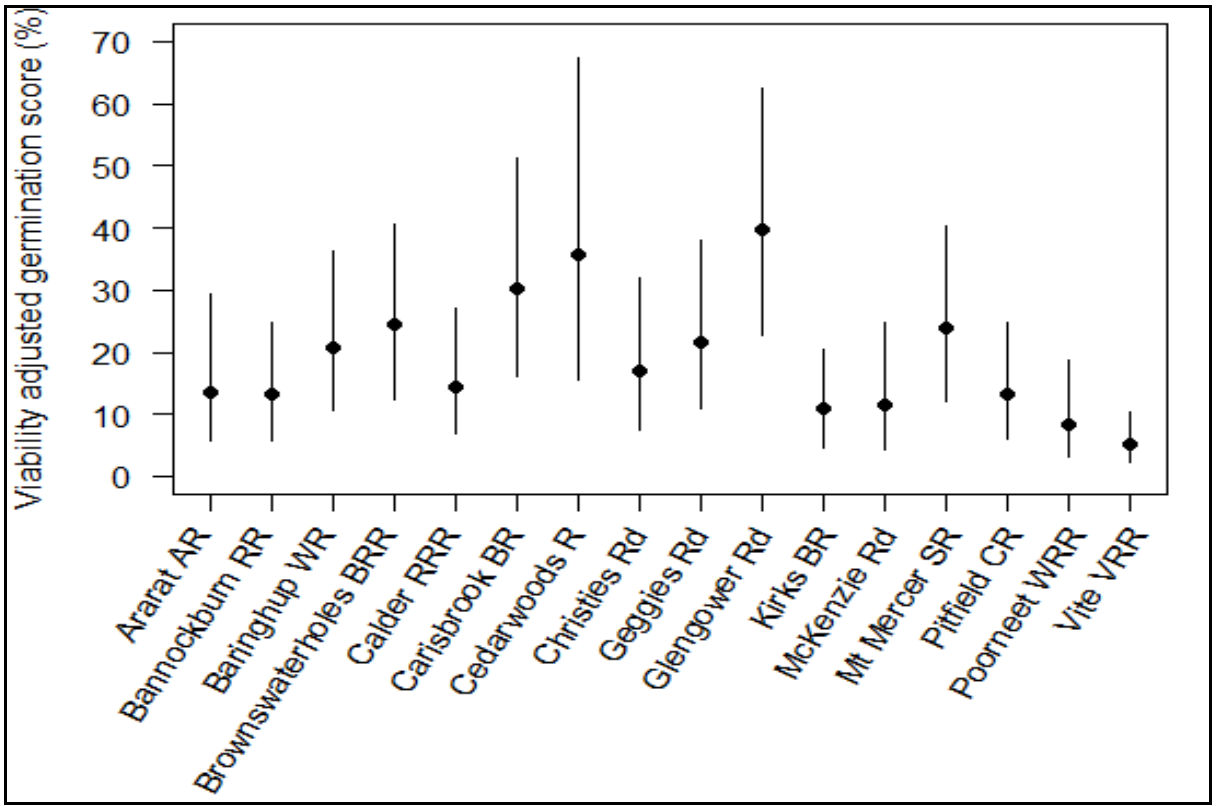


Figure 49 – The viability adjusted germination score of seed collected at each study area (n = 105). The error bars represent ±2SE.

3c 4 Discussion

3c 4.1 *Breaking seed dormancy*

Using the dichotomous key for the "Double-germination phenology technique", the seeds of *P. spinescens* were found to have an endogenous non-deep physiological dormancy (Type II) (Baskin and Baskin, 2004a). Species which exhibit this type of dormancy are often distributed in temperate zones; break dormancy in cool conditions; germinate under a wide range of temperatures; and germinate in the presence of gibberellic acid (Baskin and Baskin, 2001, Baskin and Baskin, 2004b). In this study, *P. spinescens* seed germinated after a period of dry storage (three months) followed by an exposure to cold temperatures for at least a period one month or greater, and exhibited a positive response to gibberellic acid.

For plants with seed that exhibit an endogenous non-deep physiological dormancy, germination cannot occur until inhibiting chemicals that prevent germination are turned off or lost from the seed (Smith, Coupland *et al.*, 2010). When the levels of the inhibiting chemicals have been reduced, germination conditions become possible and germination is promoted by an increase in the production of gibberellins within the seed (Fenner and Thompson, 2005). The gibberellins are a class of phytohormones that control plant growth by elongating cells, mobilising endosperm stores and promoting embryonic tissue growth (Jones and Stoddard, 1977, Smith, Coupland *et al.*, 2010). Although the production of gibberellic acid naturally occurs following the removal of germination inhibiting chemicals, horticulturalists have found that the artificial application of gibberellic acid has the action of overcoming these inhibiting chemicals and thus breaking seed dormancy.

Treating a seed with gibberellins is not a simple task as there are over fifty different naturally occurring variants and to achieve germination may require two or more types (McMillan Browse, 1980). The most commonly used and available type (gibberellic acid – GA_3) was applied to *P. spinescens* seed with success but a different type or combination might prove a better growth promoter. This could be the focus of future germination research.

Different strengths of gibberellic acid have been trialled in many species, including *Pimelea*. For example, applications of 50 ppm, 500 ppm and 1000 ppm gibberellic acid were trialled on seed of three species in the genus *Dirca* (Thymelaeaceae), finding that 1,000 ppm was optimal (Schrader and Graves, 2005). Similarly, 100 % germination has been achieved by soaking *Astroloma xerophyllum* seed in 1,000 ppm gibberellic acid (Turner, Commander *et al.*, 2009). In contrast, treatment of *Pimelea axiflora* (Bootlace Bush) with 250 ppm gibberellic acid was not successful (M. Hirst, 2012, pers. comm. June). Nor was the germination of *Pimelea lehmanniana* and *Pimelea suaveolens* (Scented Banjine) successful following soaking in 200 ppm of gibberellic acid, although *Pimelea ciliata* (White Banjine) achieved 4.7 % germination with the same treatment during seed testing conducted by Alcoa in Western Australia (Cromer, 2007). In keeping with these findings, the larger dose of 1,000 ppm gibberellic acid, combined with a suitable temperature regime, allowed for an improved rate of germination of *P. spinescens* seed in this study.

The influence of gibberellic acid provided germination results but it is not the natural stimulus for germination and did not lead to the germination of all viable seed through a single application within the defined study period. It is apparent that many seed did not respond despite suitable environmental conditions and in the presence of growth stimulating hormones at a sufficient level to stimulate many seed. Some seed might have been in a

deeper level of dormancy and the gibberellic acid exposure was unable to overcome the inhibitor's effect/level (Vleeshouwers, Bouwmeester *et al.*, 1995).

Seed dormancy is a natural defence mechanism developed to spread the risk of seedling death associated with a high probability of diverse and often adverse environmental conditions between years (McMillan Browse, 1980). The delay in the germination of *P. spinescens* seed, possibly for as long as two years, as a result of its non-deep physiological dormancy is likely to be an inherited long-term survival strategy.

3c 4.1.1 Seasons

Pimelea spinescens is a winter active species, during which time it establishes new growth both above and below ground. The water and nutrient resources that are accumulated and stored during the active growth period help to ensure survival during the harsh Victorian summer, a period of reduced activity for *P. spinescens*. The active growth period is also the time during which seed germination is most likely to occur. During this study new *in situ* germinants were found in July through to October, corroborating the observations made by Foreman (2005).

In the laboratory, the majority of seed germinated under autumn, winter and spring temperatures, and appeared to be triggered by changes in temperature regimes. In contrast, summer temperatures appeared to promote ongoing seed dormancy. Clearly, cooler temperatures are, at least in part, an important stimulant. Germination in response to transitioning through identified temperature patterns and time periods with or without the presence of gibberellic acid, is a feature of seeds with endogenous physiological dormancy (Baskin and Baskin, 2001, Nikolaeva, 2004). According to Baskin *et al.* (2001), physiological

dormancy is broken either by progression through summer (heat) to germinate in early autumn when temperatures drop, or to go through winter (cold) and germinate in early spring as temperatures rise. The ability of *P. spinescens* seed to germinate under simulated autumn and winter conditions is reflective of this species capacity for vegetative growth throughout winter. The rapid and active growth of seedlings during this period would enable roots to access moist soil layers below the surface and to accumulate sufficient nutrient and water resources prior to the onset of harsh summer conditions.

Germination mostly occurred three to four weeks following a transition to cooler stimulating temperatures. The time to germination for *P. spinescens* is in keeping with other species in the genus, which has been described as slow (45 to 60 days) (Wittwer, 1965). Roberts (1990) found in *Pimelea axiflora* (Bootlace Bush) that the time to germination took 31 days, while Roche *et al.* (1997) found *Pimelea ciliata*, *Pimelea imbricata*, *Pimelea leucanthea* and *Pimelea suaveolens* took 33, 42, 45 and 45 days to germinate, respectively. In the field, having a time lag before germination would be advantageous as it prevents growth during brief seasonal anomalies where the temperature conditions may be appropriate for germination but are quickly followed by longer periods of the prevalent and unfavourable conditions (e.g. a brief cold spell in mid-summer). A germination lag period has been found for other grassland species (Liliaceae) which was combined with low germination rates. The ecological implications are that it is assumed that these species are likely to form a persistent seed bank and have complex dormancy mechanisms (Morgan, 1998a).

For germination to be successful, the exposure of *P. spinescens* seed to suitable temperatures needs to occur for at least four to six weeks to allow the seed to break dormancy. However, *Pimelea spinescens* seed can remain dormant and viable in favourable

conditions for an extended period of time. This has been found for a range of species (Copeland and McDonald, 2001), including several species within the genus *Pimelea* (Keighery and Dixon, 1984, Gibson Roy, Delpratt *et al.*, 2007b). Future germination research, trialling at least four week periods that transition through each of the seasonal temperatures commencing in separate summer, autumn and winter incubators, with winter temperatures as a control, could identify an improved *P. spinescens* germination method.

3c 4.2 *Seed longevity*

Seed dormancy allows seed to remain viable in the seed bank for an extended time (Baskin and Baskin, 2001, Fenner and Thompson, 2005). *Pimelea spinescens* seed was found to be able to germinate at least one year and up to two years following production (seed produced in 2008, germinated in 2010). Gibson Roy (2004) found that the seed of *Pimelea curviflora* (Curved Rice-flower) remained viable in the soil seed bank, as germination occurred more than a year following being sown. Seed of other species in the genus have been found to survive for at least one or two years and successfully germinate (Keighery and Dixon, 1984, Clarke, Davison *et al.*, 2000, Gibson Roy, Delpratt *et al.*, 2007b). It is likely that *P. spinescens* seed is viable for much longer *in situ* (Fenner and Thompson, 2005), making the seed bank an important resource for future recruitment within a population.

3c 4.3 *Natural dormancy breaking cues*

The germination of many Australian shrubs species occurs following a fire (Dixon, Roche *et al.*, 1995, Keith, 1997, Clarke, Davison *et al.*, 2000, Dixon and Barrett, 2003, Baker, Steadman *et al.*, 2005, Thomas, Morris *et al.*, 2007). In some cases the heat of a fire breaks the seed coat, overcoming exogenous seed dormancy. However, the chemical reactions associated with an increase in temperature, as well as the presence of smoke and chemicals released during a fire can also play a part in breaking endogenous seed dormancy (Copeland and McDonald, 2001), and may be effective in the germination of *P. spinescens*.

3c 4.3.1 Heat shock and smoke treatment

Heat shock followed by smoke was the only treatment without gibberellic acid to overcome the endogenous dormancy of *P. spinescens* and approximate the germination results achieved when gibberellic acid was applied to the seeds. This treatment not only simulates the effects of fire on seed but in a natural environment would also be associated with the liberation of space, light and resources (Grubb, 1977, Grime, 1979), thereby providing a more favourable environment for the growth of seedlings.

In a commercial nursery, smoke water treatments have been applied to a single batch of *P. spinescens* seed over two years. In the first year, good levels of germination (25 – 30 %) were achieved, followed by a lack of germinants in the second year, despite seed being stored in a quality controlled environment in the intervening time (I Taylor, 2011, pers comm. December). In the closely related *Pimelea spinescens* sbsp. *pubiflora,* germination trials at the RBGM achieved only 1 % germination in the presence of smoke water (M. Hirst, 2012,

pers. comm. May). The results suggest that the combination of heat followed by smoke is likely to be an important combination for stimulating germination naturally.

3c 4.3.2 Simulated ingestion treatment

The dormancy breaking experiment was undertaken to find the natural cues for breaking the non-deep physiological dormancy displayed by *P. spinescens* seed. Whilst the passage of seed through a bird gut is usually associated with breaking the seed coat, the chemical effects of this process may also have an effect on the endogenous dormancy of some seeds. Many studies show seed germination is more successful following ingestion by a frugivore (mostly birds). However, timing, seed age, retention time in the gut and seed size, appear to produce variable results when compared with seed not germinated via ingestion (Traveset, 1998, 2001).

Victoria's natural temperature grasslands have many endemic birds (*Coturnix pectoralis* [Stubble Quail], *C. Ypsilopora* [Brown Quail], *C. Chinenis* [King Quail], *Pedionomus torquatus* [Plains Wanderer], *Anthus novaeseelandiae* [Richards Pipit], *Neochmia phaeton* [Zebra Finch]) and a lizard (*Tiliqua scincoides* [Common Blue-tongue]) which potentially could ingest *P. spinescens* seed (Baker, 1988, Pizzey and Knight, 1997, Olesen and Valido, 2003, Nogales, Padilla *et al.*, 2007). During fieldwork in 2009, the author observed a pattern of increased occupancy of *P. spinescens* occurring under fence lines throughout the study areas. Fence lines are often used as perches and landing spots by most bird species which inhabit an area and are frequently used as defecating points by birds (Hollander, 2007). *Pimelea spinescens* seed is nutritious (see Appendix 10), palatable and produced during winter when minimal food supplies are available. It is likely that frugivores are instrumental as dispersers and as a stimulant to the germination of *P. spinescens* seed.

The simulation of passage through a bird's gut (acid followed by base) produced some germinants but they were malformed. The acid/base treatment used to promote germination was a crude simulation of what may occur in nature and a more finely tuned approach may produce improved results. Future research might find a more appropriate acid/base treatment that enables the germination of healthy seedlings.

3c 4.3.3 Fungal associations

Despite efforts to sterilise the outer surface of the seed and run dormancy-breaking and germination trials under aseptic conditions, fungal growths of various types formed on many of the seeds, apparently without hindering germination. Although no efforts were made to identify these fungi or their source of introduction to the Petri dishes, their presence and the continued germination of the seed, raises the prospect of *P. spinescens* having a mycorrhizal association. This is a mutually beneficial symbiotic association between a vascular plant and a fungus which effectively increases the surface area of the roots of the plant, therefore improving root function (Smith, Coupland *et al.*, 2010).

A survey of 659 published papers (since 1987) found mycorrhizal associations in 80 % of 3,617 land plant species (92 % of plant families) (Wang and Qiu, 2006), including several species from the family Thymelaeaceae (Maremmani, Bedini *et al.*, 2003, Turjaman, Tamai *et al.*, 2006). In Australia, although few species had been found to have mycorrhizal associations prior to Warcup's (1980) research, more recent studies have identified an increasing number of such relationships. Three species within the genus *Pimelea* have been found to have mycorrhizal associations: *P. linifolia* (Slender Rice-flower) (Bellgard, 1991); *P. imbricata* (Brundrett, 1991); and *P. glauca* (Smooth Rice-flower) (McGee, 1986).

Interestingly, *P. glauca*, which has a distribution that includes the Victorian volcanic plains, was found in association with *P. spinescens* at eight of the 16 study areas.

There is a possibility that *P. spinescens* has a mycorrhizal association that may assist with the process of seed germination and is also providing benefits to mature plants. Indeed, during opportunistic root analyses conducted as part of this study (but not presented in this thesis), many types of fungi were observed in association with the taproots. Future research should determine the presence of a mycorrhizal association and, if present, explore its role in the germination biology and survival of *P. spinescens.*

3c 4.4 *Seed germination*

Three different germination experiments were conducted over three different periods of time within this study: 52, 14 and 17 weeks. The longest running experiment which closely simulated the natural progression of the seasons yielded the greatest germination rate (30 %). It seems that time is a key component in overcoming the dormancy of *P. spinescens* seed and requires further investigation.

Germinability results were affected by the non-deep physiological dormancy of *P. spinescens* seed. Although seed viability provided a measure of the potential number of seed per batch which might germinate, this did not translate into the germination of a high proportion of the seeds, despite the application of the best available treatment. A similar failure to germinate, despite a high level of viability has been found with other Australian species. Roche *et al.* (1997) collected data on 165 Western Australian species finding that the species that were difficult to germinate had a mean viability of 67 % but only a mean maximum germination percentage of 13 %.

The variables of ‘seed germination’ and ‘viability adjusted germination score’ are not reliable measures of the full recruitment potential of a population of plants within a given study area. Although the viability adjusted score may provide information about the recruitment potential of individual plants, it was not a useful score for temporally evaluating entire populations or assessing the influence of environmental and habitat or management variables.

Chapter 3d – Measuring the recruitment potential of *Pimelea spinescens* populations: germination *in situ*

3d 1 Introduction

The combinations of factors that lead to germination *in situ* are complex and may vary over space and time. A snapshot assessment of *in situ* germination may provide a coarse indication of a species ability to undergo recruitment at a given location and clues to factors that influence the recruitment potential of a population. However, without long-term monitoring, *in situ* germination does not provide a true reflection of recruitment, given that many germinants may not survive to reproductive maturity.

In Victoria's natural temperate grasslands, the germination of herbaceous species is considered a sporadic and rare event during small windows of optimal conditions, even in areas with large or dense mature populations (Lunt, 1990a, Scarlett and Parsons, 1990, Scarlett and Parsons, 1992, Morgan, 2000, Lunt and Morgan, 2002, Clarke and Davison, 2004). Only a few assessments of species specific germination events have been conducted. For *Rutidosis leptorrhynchoides*, germinants appeared in the autumn break when soil moisture was above 20 %, and 87 % of germinants were located within 20 cm of an established plant (Morgan, 1995b). Another study found that 67 % of the indigenous species re-introduced as part of revegetation program germinated within two years and increased in numbers either through seedling or vegetative recruitment (Gibson Roy, Delpratt *et al.*, 2007b). Further studies are required to understand the recruitment capacity of naturally occurring populations of herbaceous flora in the natural temperate grasslands of Victoria.

Research in Western Australia has reported juvenile plants were highest in larger populations studied for the rare species *Verticordia staminosa* subsp. *Staminosa* (Yates and Ladd, 2004). Similarly, both the population density and the female density of *P. spinescens* have been demonstrated to have a positive relationship with the density of germinants found in the study areas, as described in Chapter 2 section '*The influence of population density on germinant production*'. These observations are also supported by Foreman (2005, 2011) who documented a positive relationship between female density and germinant density. Intuitively, this makes sense in that the more plants there are per defined area, the greater the likelihood that offspring will be produced within that area.

Long-lived plants such as *P. spinescens* usually exhibit a Deevey Type III curve which means they have high seedling mortality followed by a safer adulthood. Long-term survival of the species is represented by only a proportion of the seedlings that germinate in the first year (Fenner, 1987). There are few records of recruitment of *P. spinescens* and little knowledge of long term survival of the germinants that are produced. It is important to be able to quantifying the occurrence of germinants in a way that can be associated with site characteristics, leading to an improved understanding of the factors affecting the germinant potential of a site (Meneges, 1986, Menges, 2000, Baxter, 2008).

3d 2 Methods

3d 2.1 *Density of germinants in situ*

To take into account the finding that the density of germinants had a significant positive relationship with the density of female plants (as described in Chapter 2 section '*Natality - The influence of population structure on germinant production*'), the germinant density data presented (see Chapter 2 section '*Natality - Germinant density*') was adapted to be described as a proportion of the density of mature female plants (expressed as 'germinants/female plant').

3d 2.1.1 Data analysis

For each year (2009 and 2010) the germinant density data (germinants/female plant) was analysed to determine if there were differences between study areas. Statistical comparisons between years were not possible because of a high proportion of zero (0) count data in 2010.

For 2009, germinants density data (germinants/female plant) was analysed using a generalised linear mixed model with the R package `lme4` (Bates, Maechler *et al.*, 2011). The proportion of germinants per female plant for the ith quadrat in the jth study area were modelled as a Poisson variable with mean mu_{ij} where log(mu_{ij})=log(density of female plants_{ij})+a_j+p_{ij} where p_{ij} was assumed to follow a normal distribution with mean 0 and variance sigma^2 to be estimated from the data. There was an a_j for each study area, with a_1 set to zero, and hence a_2 to a_16 could be used to test whether study areas 2 to 16 respectively were different to study area 1 in terms of the density of germinants/female plant. In addition the difference between the estimated a_2 and a_3, for example, could be

used to test whether study area 2 and 3 were different in terms of the density of germinants/female plants and similarly for the other pairs of study areas 2 to 16. The *p* value was Bonferroni adjusted, in order to decrease the risk of making a Type I error.

No comparisons of the *in situ* germinant density/production was able to be conducted between years due to a paucity of germinants produced in 2010 resulting in a data set composed almost entirely of zeros (0) for that year (see Chapter 2 section '*Natality - in situ germinant production and survival*').

3d 3 Results

3d 3.1 *Density of germinants in situ*

The results for the density of germinants found in study areas during 2009 and 2010 have been presented in Chapter 2, section '*Natality*'. Summarised here:

- In 2009:
    - 13 study areas produced germinants with an average of 4.2 germinants per square metre; and
    - Carisbrook BR recorded the greatest density (15.5 germinants per square metre);
- In 2010:
    - 9 study areas produced germinants with an average of 0.6 germinants per square metre; and
    - Baringhup WR recorded the greatest density (3.11 germinants per square metre).

3d 3.1.1 2009

In 2009, the density of germinants ranged from an average of 0 – 13.25 germinants/female plant across all study areas. Combining all study areas, the overall average germinant density was 2.5 germinants/female plant. At three of the study areas (Christies Rd, Glengower Rd and McKenzie Rd) there were no germinants produced in any of the quadrats, thus these study areas were not included in the generalised linear mixed model analyses. At six study areas the density of germinants/female plant for all quadrats was less

than one. Post a generalised linear mixed model analysis, the density of germinants/female plant was not found to be significantly different between study areas (Figure 50, for full results see Appendix 11). However, Figure 50 suggests that study areas might be placed into three broad groups:

- Study areas with an average germinant density that is greater than one germinant/female plant, and little variability between replicated quadrats;
- Study areas with an average germinant density that is less than one germinant/female plant, and large variability between replicated quadrats; and
- Study areas with zero germinants.

It seems that a high level of variability in the germinant density within study areas has prohibited the detection of any significant differences between study areas by a generalised mixed model analysis.

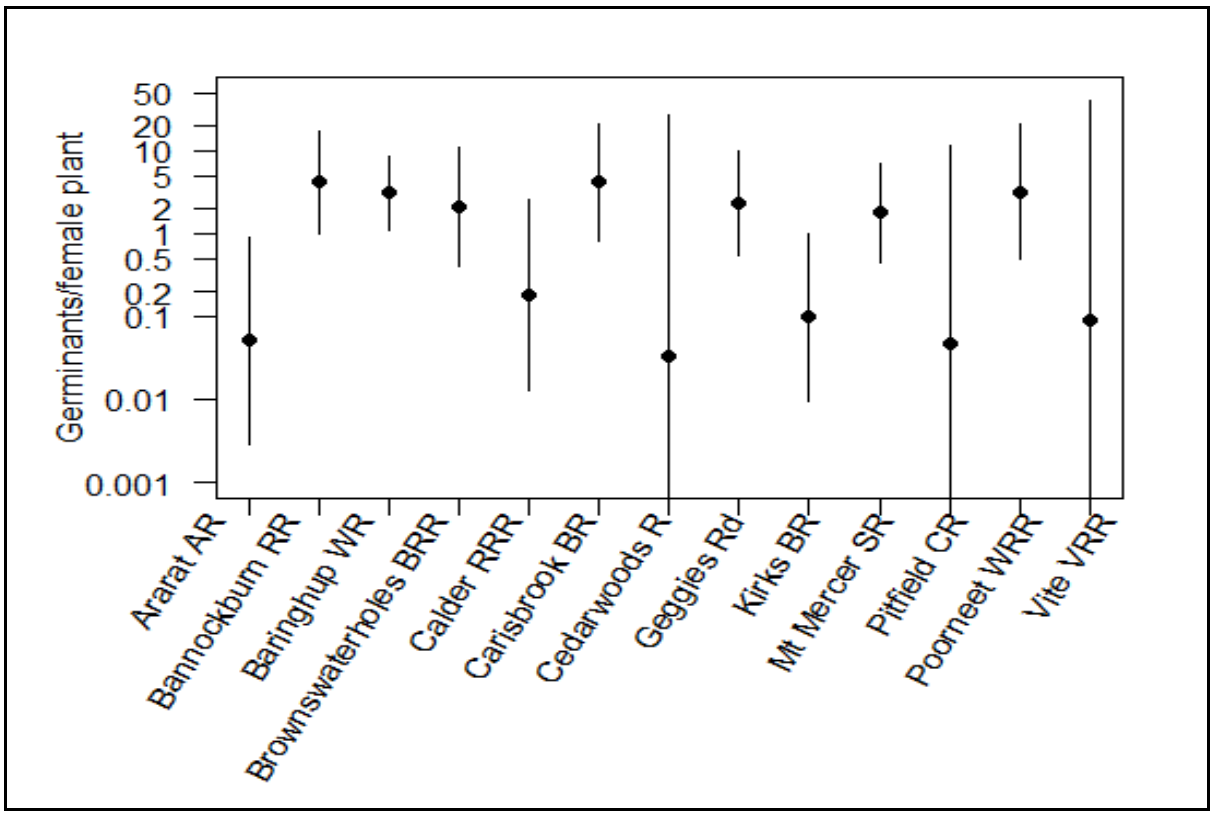


Figure 50 – The density of germinants at 13 study areas in 2009 (n = 53). The y axis is a $\log_{10}$ scale. The error bars represent ±2SE.

3d 3.1.2 2010

In 2010, the density of germinants ranged from an average of 0 – 3.1 germinants/female plant across all study areas. Combining all study areas, the overall average germinant density was 0.3 germinants/female plant. At seven study areas (Ararat AR, Carisbrook BR, Christies Rd, Glengower Rd, Kirks BR, Pitfield CR and Vite VRR) there were zero germinants recorded and at only one study area (Baringhup WR) was the average germinant density was greater than one germinant/female plant. Thus, the data collected in 2010 was insufficient to perform a generalised linear mixed model analysis.

3d 3.1.3 Comparisons 2009/2010

A generalised linear mixed model analysis comparing the density of germinants between years was not possible due to a paucity of germinants in 2010. Nonetheless, there is an obvious difference between years, with overall germinant production about eight times greater in 2009 (Figure 51).

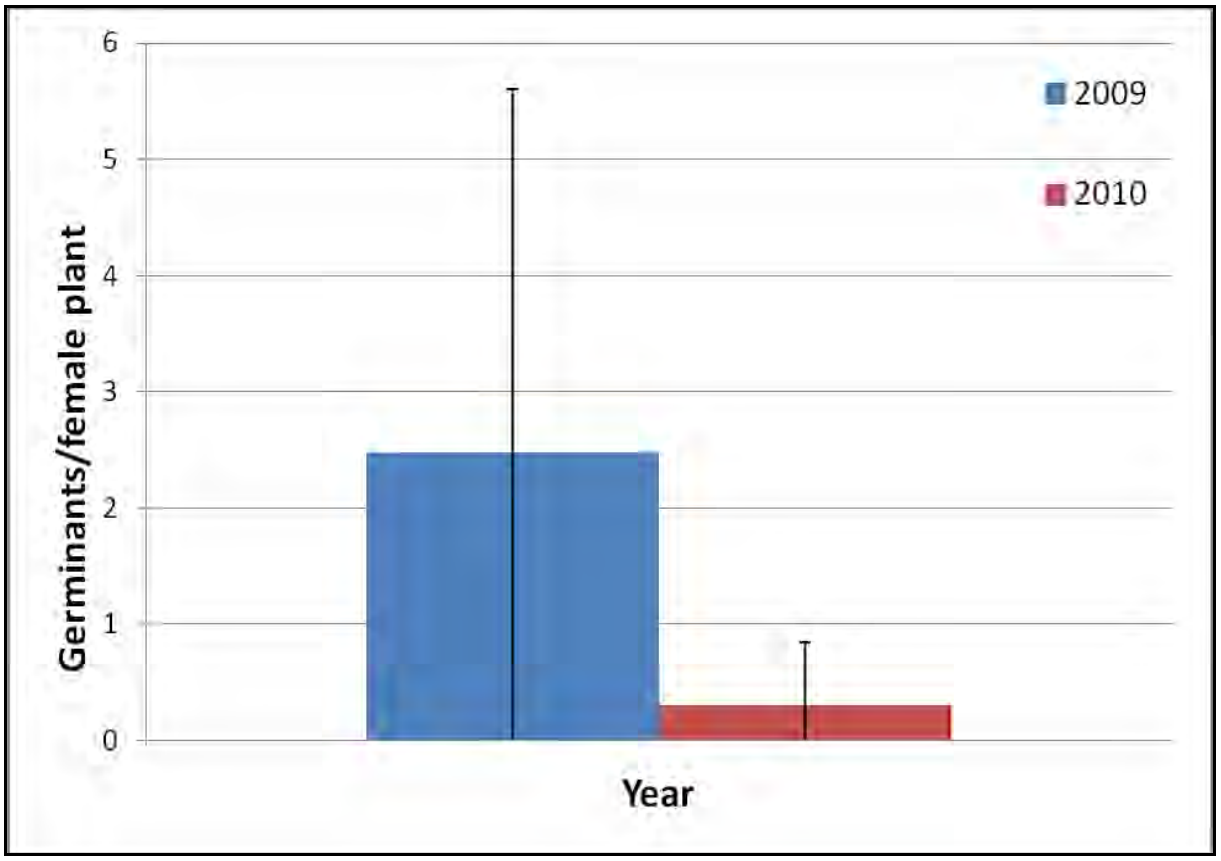


Figure 51 – The proportion of germinants/female plant across all study areas in 2009 and 2010 (n = 32). The error bars represent ±2SE.

3d 4 Discussion

Based on the finding that the number of germinants produced within a given area was a function of the number of female plants within the defined area, this study used a ratio of the density of germinants to the density of females (expressed as 'germinants/female plant') as a measure of recruitment potential. However, it is possible that *in situ* germination may also be a function of a range of other factors that should be investigated further, including:

- The annual seed production (load) falling to the ground;
- The annual quality (viability) of the seed produced (assessed in this study);
- The dispersal ability of the seed once fallen;
- The amount of seed predated; and
- The longevity (maintenance of viability) of the seed in the seed bank.

(Crawley, 2000, Fenner and Thompson, 2005, Merritt and Rokich, 2006)

Whilst this study was able to demonstrate that *in situ* germination is occurring in many of the study areas, there was a great deal of variability in the measure 'germinants/female plant', both within study areas and between study areas. It is likely that the variability recorded in this study is realistic and reflective of the amount of contribution that individual mature plants make to the recruitment potential of a site. The results suggest that there are small proportions of plants (or clusters of plants) that make a large contribution to the overall site recruitment and a much larger proportion of mature plants that contribute very little. A similar observation was made in terms of seed production, and it is intuitive that plants that produced large quantities of seed would have a corresponding likelihood of having large numbers of germinants in their immediate vicinity. However, it is unclear whether the

significant contributors to the recruitment potential of a study area are consistent, or whether individual plants vary in their level of contribution over time. This is worthy of further investigation, as are the elements that 'make' a good contributor.

Clearly, the seed from which germinants arise was produced in a previous season/s (Thomas, 2008b). If the plants that make significant contributions to the recruitment potential within a given year vary over time, then it would be important to take into account the seed production of individual plants in the years prior to germination events.

It appears that there was a difference in the *in situ* production of germinants between years and possibly also between study areas (the causes of such differences will be further examined in Chapters 5 and 6). Although 'germinants/female plant' may be a valid measure of *in situ* germination, the variability between quadrats and sites that was recorded in this study made the results difficult to interpret. The issue of high variability might be resolved through increased sample sizes or perhaps a more targeted assessment strategy (Elzinga, Salzer *et al.*, 2001). Future assessments should also include quadrats that do not contain any mature *P. spinescens* plants, in order to make comparative analyses and evaluate the capacity for recruitment in these spaces.

The measure 'germination *in situ*' has the potential to serve as a dependent variable for assessing the influence of demographic, environmental and habitat management factors on the recruitment potential of *P. spinescens* populations. The approach taken in this study provides a snapshot of localised recruitment contributing events. Long-term monitoring is required to assess the fate of germinants and their capacity to contribute to the overall success in populations of *P. spinescens.*

3.2 Conclusions and future research/directions/implications

This chapter assessed four measures of recruitment potential: seed production, seed viability, seed germinability and germination *in situ*, for their value as dependant variables to detect associations with environmental factors (Chapter 4) and management practices (Chapter 5) that may be influencing the reproductive capacity of *P. spinescens* populations.

Of the measures assessed, the following appear to be useful as dependant variables for assessing differences between both populations and also between years:

- Seed production - exhibited sufficient significant spatial and temporal variability and could be used for hypothesis testing to identify which environmental conditions and management practices are associated with seed production.
- Germination *in situ* - when expressed as the ratio 'germinants/female plant' this measure exhibited significant temporal variability, and may also be useful for assessing spatial variability. This measure is useful as a predictor of environmental factors and management practices.

There was limited variability in seed viability between study areas or years. It is unlikely that this measure would be useful for hypothesis testing but it may be useful for assessing trends and associations.

Of these measures of recruitment potential, seed production and seed viability did not appear to be limiting the recruitment capacity of *P. spinescens* populations. Individually tagged plants were able to produce seed in each year of the study and the level of seed

viability was maintained throughout this time period. While there are sufficient numbers of reproductively mature plants that are able to flower and set seed, there is the potential for ongoing recruitment within *P. spinescens* populations.

This study successfully used the squash test to assess the viability of *P. spinescens* seed but also demonstrated that seed viability was positively associated with seed weight. Using the weight of batches of seed presents a non-destructive method for assessing the viability of the seed of this threatened species.

Pimelea spinescens seed was found to have an endogenous non-deep physiological dormancy (Type II) which was overcome by winter temperatures in the presence of gibberellic acid. Although these conditions could stimulate germination, there were many viable seed that remained dormant, suggesting that refinement of this method is required to enhance germination levels. Germination was also found to occur during the treatments of 'heat shock and smoke' and simulated ingestion (acid followed by base), as well as in the presence of fungi.

The persistent dormancy of *P. spinescens* seed in the laboratory suggests that in the field only a small proportion of the available viable seed would germinate each year. Further work is required to investigate the longevity of soil stored seed, which may act as an important future source of population recruitment.

Although difficult to analyse, the measure germinants *in situ* displayed a great deal of variability both spatially and temporally. Temporal difference suggest that monitoring of the recruitment potential of *P. spinescens* populations should be based on data collected over several years, taking into account environmental factors and management practices (see

Chapters 4 and 5 respectively). The method for monitoring germinants *in situ* requires further refinement. Spatially, *P. spinescens* populations could be categorised into three groups based on the density of germinants (germinants/female plant):

- Populations with consistently high densities of germinants;
- Populations with variable but on average low densities of germinants; and
- Populations with no germinants.

'Germinants *in situ*' represents a measure of recruitment potential that may be useful for conducting hypothesis testing and assessing associations with environmental factors and land management practices. When using this measure of recruitment potential, future studies should increase the sample size and also incorporate spaces within the *P. spinescens* population that do not contain mature female plants.

Chapter 4 Environmental factors associated with the recruitment potential of *Pimelea spinescens*

"The protection of an animal or of a plant will be ineffectual so long as we do not also preserve that organism's conditions of life"

Franz Kuhnert (as cited in Diole, 1974)

4.1 Introduction

Regional climatic conditions are important for plant growth as they govern the extent of a species' distribution and influence survival (Larcher, 2003). High levels of environmental variability at the regional level can lead to increased probabilities of extinction and have been identified as a major influence on birth and death rates, especially for species with low rates of recruitment (Menges, 1992).

The cost of reproduction borne by a parent plant may be strongly affected by localised environmental conditions, depending on the species life history (Copeland and McDonald, 2001, Fenner and Thompson, 2005, Gutterman, 2010). For annual plants, the cost of reproduction is solved by a short growth period during favourable environmental conditions that culminates with a relatively high reproductive output and death of the parent plant. However, for long-lived perennials reproduction is not usually attempted until the requirements of survival such as growth, storage and defence are met (Crawley, 1997, Larcher, 2003). Both life history strategies are subject to the effects of environmental variability but the perennial life-history provides the opportunity for delayed or modified levels of reproduction during times of extreme environmental conditions (Fenner and Thompson, 2005, Bazzaz, Ackerly *et al.*, 2010).

The two main stages in the reproductive cycle of a plant that can be strongly influenced by environmental variations are: processes leading to the formation of a viable embryo (seed); and processes that influence the germination of the seed and survival of the offspring (Copeland and McDonald, 2001, Schultze, Beck *et al.*, 2002, Larcher, 2003). A range of environmental factors may influence success at each of these stages but precipitation tends

to be the strongest driver (De Jong and Klinkhamer, 1988, Lavorel, 1999, Knapp, Fay *et al.*, 2002, Laporte, Duchesne *et al.*, 2002, Larcher, 2003, Eckstein, 2005).

4.1.1 *Environmental effects on seed quantity and quality*

Rainfall variability has been found to be associated with the reproductive output of many species (Crawley, 2000, Larcher, 2003). In simulated greenhouse rainfall treatments, the flowering duration of *Avena barbata* was extended and seed production and quality improved when above average and average rainfall was compared to below-average rainfall (Olivares, Johnston *et al.*, 2009). Similarly, an increase in rainfall has been associated with increased flowering in the African grassland species *Stipagrostis uniplumis* (Zimmermann, Higgins *et al.*, 2008). An increase in seed production has been demonstrated in water addition experiments for *Themeda triandra* (O'Connor, 1996) and also for *Delphinium nelsonii* (Low Larkspur) (Zimmerman, 1983). Increased availability of moisture has a positive relationship with the flowering, seed quantity and seed quality of these species.

Reduced soil moisture has been associated with a decrease in the number of seed produced. In an experiment conducted with the Australian grassland species *Leucochrysum albicans* (Hoary Sunray), comparisons of the effects on seedlings were made between the treatments control, drought and wet conditions. The drought treatment was found to prevent seedlings from producing any flowers (Gilfedder and Kirkpatrick, 1994b). In agricultural species the timing of reduced soil moisture has been found to strongly influence seed development (Copeland and McDonald, 2001). Soil moisture deficits prior to flowering reduced seed numbers (Meckel, Egli *et al.*, 1984) and when the deficits occurred during flowering a reduction in seed size was the result (Andrews, Collins *et al.*, 1977, Sionit,

Hellmers *et al.*, 1980, Wright, Shokes *et al.*, 1984, Eck, 1986). Reduced soil moisture negatively affects both seed quantity and quality.

4.1.2 *Environmental effects on germination*

In Australia, rainfall variability (moisture availability) has also been associated with germination and germinant survival. Andersen (1989) found that seedling establishment of long-lived perennials in a Victorian woodland environment, was significantly and positively affected by periods of favourable rainfall. In the temperate grasslands of New South Wales (N.S.W.) the addition of water promoted recruitment of an unusually high number of rare species (McIntyre and Lavorel, 1994b). A study of the Victorian grassland forb *R. leptorrhynchoides* found that autumn rains, which increased soil moisture levels to above 20 % by weight, was the trigger for germination (Morgan, 2001). In these cases in south-eastern Australian grasslands and grassy woodlands, increased rainfall positively affected germination and seedling establishment.

4.1.3 *Environmental effects on recruitment potential in the genus Pimelea*

In New Zealand, it is thought that the point of failure in the recruitment of *Pimelea arenaria* probably occurs during the seedling establishment stage, rather than being associated with imbalanced sex ratios, poor seed set or germination failure. Environmental or predation pressures are most the most likely factors to affect the successful recruitment of *P. arenaria* (Dawson, Rapson *et al.*, 2005, Merrett, 2007).

For *P. spinescens*, Foreman (2005, 2011) suggested that germination at study sites in northern Victoria was driven by autumn rainfall, although this trend was not observed at the

southern sites on volcanic soils. Cropper (2009) attributed above average rainfall in the seasons of winter, spring and summer to germinant survival at a site on the Victorian volcanic plain (southern Victoria) with an increasing *P. spinescens* population. Whilst seasonal rainfall appears to have an influence on the germination of *P. spinescens*, further investigation is required to describe the nature of the relationship and to determine the influence of environmental variables (rainfall and temperature) on the other measures of recruitment potential.

4.1.4 *Chapter aim*

Successful management of fragmented populations of *P. spinescens* that promotes autogenic recovery is reliant on an understanding of the environmental factors that affect population recruitment.

This chapter addresses the second objective of the *P. spinescens* Recovery Plan (Carter and Walsh, 2006), which aims to identify environmental and habitat features that are essential for all stages of the species life history, including recruitment. The objective is addressed by:

- Evaluating the influence of environmental variables such as rainfall and temperature on recruitment potential.

4.2 Methods

4.2.1 *Measures of recruitment potential*

Using plants in the randomly located quadrats (longitudinal study) within each of the 16 study areas described in Chapter 2, and the methods of assessment described in Chapter 3, environmental data was assessed for associations with the following measures of recruitment potential:

- Seed production (seeds per stem);
- Seed viability;
- Germination *in situ* (germinants/female plant); and
- Germinant survival *in situ* (as described in Chapter 2, 'Natality' section).

4.2.2 *Environmental data*

Rainfall and temperature data was obtained via the Bureau of Meteorology website link to historical records for the closest weather station to each site (Commonwealth of Australia, 2012). Rainfall data was collected from discrete weather stations near each site, with the exception of data for Geggies Rd and Pitfield CR, which was sourced from a single weather station (Table 18). It was not possible to obtain temperature data that could uniquely represent each study area, and in some cases a single weather station was accessed to provide data for multiple sites (Table 19).

4.2.3 *Rainfall data*

Rainfall data was analysed for correlations with all four measures of recruitment potential. The rainfall measures that were used for analyses were:

- Annual rainfall (winter 2008 – autumn 2009);
- Annual rainfall (winter 2009 – autumn 2010);
- Total seasonal rainfall (spring; summer; autumn; or winter) between spring 2008 and winter 2010;
- All possible combinations of cumulative seasonal rainfall between spring 2008 and winter 2009: For example, 'spring 2008 + summer 2008/2009', 'spring 2008 + summer 2008/2009 + autumn 2009', 'summer 2008/2009 + autumn 2009', 'autumn 2009 + winter 2009, 'spring 2008 + autumn 2009', 'spring 2008 + winter 2009', 'summer 2008/2009 + winter 2009'; and
- All possible combinations of cumulative seasonal rainfall between spring 2009 and winter 2010 (see above example and replicate for the 2009 to 2010 seasons).

Table 18 – Rainfall weather stations.

Sites	Weather station name	Station No.
Ararat AR	ARARAT PRISON	89085
Bannockburn RR	BANNOCKBURN	87009
Baringhup WR	CAIRN CURRAN RESERVOIR	88009
Brownswaterholes BRR	LISMORE (POST OFFICE)	89018
Carisbrook BR	CARISBROOK	88013
Calder RRR	MELBOURNE AIRPORT	86282
Cedarwoods R	LAVERTON RAAF	87031
Christies Rd	ROCKBANK (MELTON)	87121
Geggies Rd	ROKEWOOD (WURROOK SOUTH)	89068
Glengower Rd	CAMPBELLTOWN	88011
Kirks BR	LITTLE RIVER	87033
McKenzie Rd	WOODSTOCK-ON-LODDON (ALEXANDRA PARK)	81100
Mt Mercer SR	WARRAMBINE NO 2	89092
Pitfield CR	ROKEWOOD (WURROOK SOUTH)	89068
Poorneet WRR	CRESSY	89010
Vite VRR	DERRINALLUM (POST OFFICE)	89074

4.2.4 *Temperature data*

Temperature data (autumn 2008 – winter 2010) was analysed for correlations with all four measures of recruitment potential. The temperature measures that were used for analyses were:

- Daily mean maximum temperature for each season (spring, summer, autumn and winter). This is the average of all daily maximum temperatures recorded throughout a given season;
- Monthly mean maximum temperature for each season (spring, summer, autumn and winter), was calculated by averaging the highest temperature recorded for each of the three months of the season. For example, monthly mean maximum temperature autumn = (March highest temperature + April highest temperature + May highest temperature)/3 ; and
- Monthly mean minimum temperature for each season (spring, summer, autumn and winter), was calculated by averaging the lowest temperatures recorded for each of the three months of the season. For example, monthly mean minimum temperature autumn = (March lowest temperature + April lowest temperature + May lowest temperature)/3.

Table 19 – Temperature weather stations.

Sites	Weather station name	Station No.
Ararat AR	ARARAT PRISON	89085
Bannockburn RR	BANNOCKBURN	87009
Baringhup WR	CAIRN CURRAN RESERVOIR	88009
Brownswaterholes BRR	LISMORE (POST OFFICE)	89018
Carisbrook BR	CARISBROOK	88013
Calder RRR	MELBOURNE AIRPORT	86282
Cedarwoods R	LAVERTON RAAF	87031
Christies Rd	ROCKBANK (MELTON)	87121
Geggies Rd	ROKEWOOD (WURROOK SOUTH)	89068
Glengower Rd	CAMPBELLTOWN	88011
Kirks BR	LITTLE RIVER	87033
McKenzie Rd	WOODSTOCK-ON-LODDON (ALEXANDRA PARK)	81100
Mt Mercer SR	WARRAMBINE NO 2	89092
Pitfield CR	ROKEWOOD (WURROOK SOUTH)	89068
Poorneet WRR	CRESSY	89010
Vite VRR	DERRINALLUM (POST OFFICE)	89074

4.2.5 *Statistical analysis*

The rainfall and temperature data for the 16 sites was correlated using Microsoft Excel against identified measures of recruitment potential (see section 4.2.1) for possible associations. A paired sample *t* test was used to compare the influence of the annual rainfall between years. Bivariate analyses were conducted via SPSS (Version 18) to assess the direction and strength of any associations. Where data did not conform to the requirements of normality, data transformations were conducted according to Zar (1999). If the data obtained was logarithmic and included zero values a value of one was added to the raw data before $\log_{10}$ transformation. Data that was normally distributed underwent a Pearson's product-moment correlation (r) and all other data underwent a Spearman's rho analysis (r_s). Some graphs were obtained using "R" via R Studio version.

Differences in measures of recruitment potential between years were assessed by an ANOVA. Significant environmental variables were checked to determine whether they were correlated to each other and if so, a series of simple linear regressions was conducted using SPSS (Version 18) to find the best predictor variable/s. Then a stepwise linear regression analysis was conducted with all possible predictor variables to find the best predictor model for the measure of recruitment potential.

4.3 Results

4.3.1 *Observed rainfall patterns*

Historically (statistics for all years of data collection records), the average annual rainfall recorded at weather stations representing all 16 study areas was between 452 mm (McKenzie Rd) and 624 mm (Vite VRR). During the period of this study annual rainfall was generally lower than average for most sites during 2008 (mean = 387 mm) and 2009 (mean = 299 mm) but approximating average or above average for 2010 (mean = 595 mm) (Figure 52). A paired sample *t* test for all study areas found that the total annual rainfall (2008 – 2009) was significantly different to the total annual rainfall (2009 – 2010), t (15) = 9.407, p < 0.001 and had a large effect (Cohen's d = 41). On average sites received an additional 296 mm (95 % CI of 229 to 363) rainfall for the annual period from winter 2009 – autumn 2010, when compared with the same period in 2008 - 2009 (Cohen, 1988).

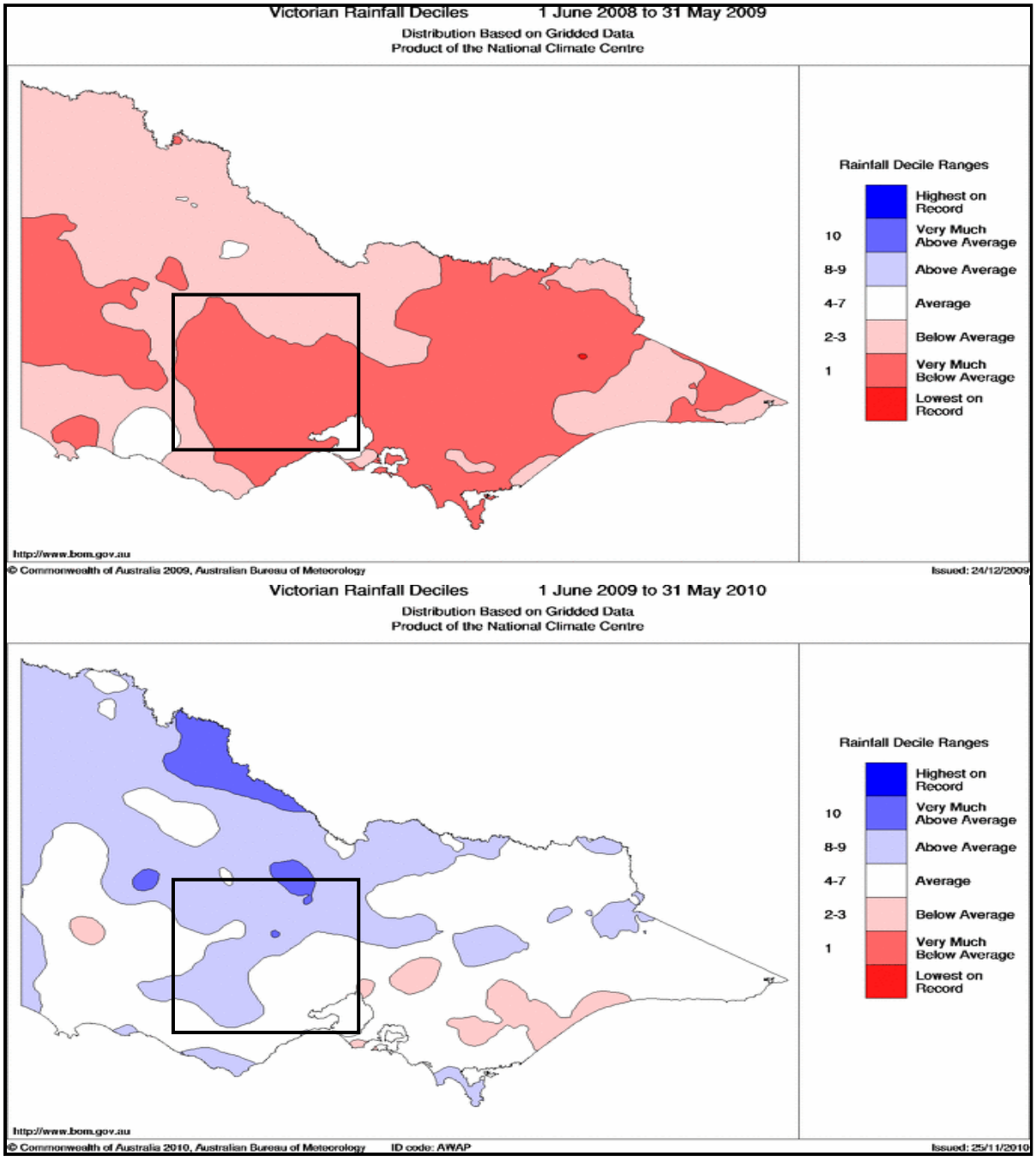


Figure 52 - Victorian rainfall deciles from spring 2008 until autumn 2009 and spring 2009 until autumn 2010 (Commonwealth of Australia, 2012). The boxed area contains all study areas.

4.3.2 *Observed temperature patterns*

Since the 1960's the average annual temperature for all of Australia has been increasing. Leading into this study, the decade from 1998 to 2007 was exceptionally dry and hot with the daily mean maximum temperature 0.6°C above the 30 year average (1961 - 1990) (Victorian Government Department of Sustainability and Environment (DSE), 2008). Throughout the period of this study the years 2008 and 2009 were warmer than 2010. For each year, the daily mean maximum temperatures in the region of Victoria in which this study was conducted were as follows:

- 2008 – 'above average' to 'very much above average' (Bureau of Meteorology, 2009);
- 2009 – 'very much above average' (Bureau of Meteorology, 2010);
- 2010 – 'above average' (Bureau of Meteorology, 2011).

4.3.3 *Seed production*

As presented in Chapter 3 section 3a 3.1.1, seed production was significantly different between many of the study areas in 2009, although this was not the case in 2010. For three study areas (Carisbrook BR, Glengower Rd and McKenzie Rd) there was a significant difference in seed production between years. In terms of the analysis of the seed production data, Carisbrook BR was an obvious outlier in 2009 (Figure 53) and was therefore removed from the data set for weather-related analyses. Explanation for the high level of seed production at Carisbrook BR in 2009 will be presented in Chapter 5.

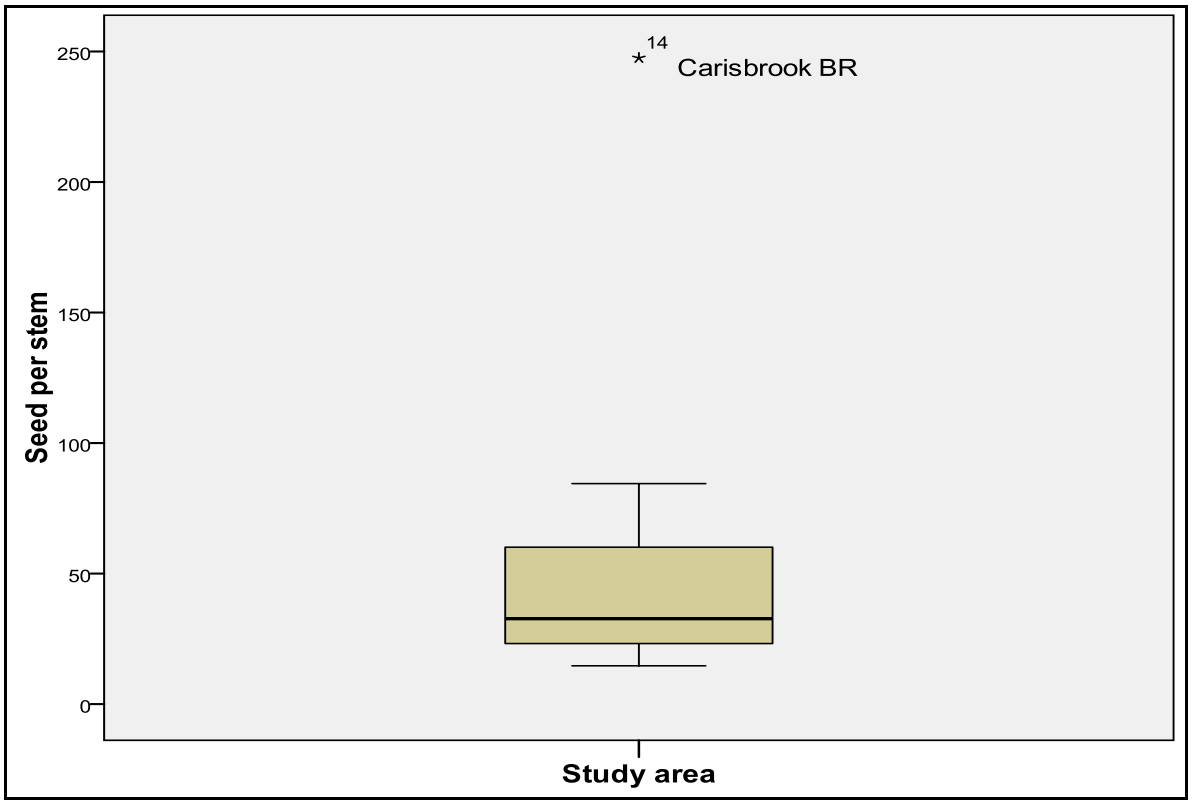


Figure 53 – The distribution of seeds production data for 16 sites in 2009, showing that study area 14 - Carisbrook BR was an outlier.

4.3.3.1 Rainfall and seed production

The correlation analyses identified four significant associations with rainfall and seed production in the period winter 2008 to autumn 2009 but none were found for the period spring 2009 to autumn 2010. When each year was combined there were seven significant correlations identified (see section 4.3.3.3 below).

4.3.3.2 Temperature and seed production

The correlation analyses identified one significant association with temperature and seed production in the period winter 2008 to autumn 2009 but none were found for the period

spring 2009 to autumn 2010. When each year was combined there were four significant correlations identified (see section 4.3.3.3 below).

4.3.3.3 Environmental relationships with seed production

To determine which environmental factors were most strongly associated with seed production, the data for 2009 and 2010 were combined. Because each of the environmental variables were significantly correlated to each other and therefore confounding, a series of simple linear regressions were conducted for each of the seven rainfall variables and four temperature variables. Of the 11 simple linear regressions, only nine detected a significant association with seed production and are listed in order of the contribution that they made to the models (greatest to least):

- Cumulative rainfall for the spring, summer and autumn period (inclusive) preceding seed formation had a negative effect on seed production R^2 = 0.546, F = (1, 29) = 37.086, p < 0.001 (Mahalanobis distance = 5.241 < 13.816), predicting 54.6 % of the variance (Figure 54);
- Cumulative rainfall for the summer and autumn period (inclusive) preceding seed formation had a negative effect on seed production R^2 = 0.467, F = (1, 29) = 27.274, p < 0.001 (Mahalanobis distance = 5.719 < 13.816), predicting 46.7 % of the variance;
- Annual rainfall preceding seed formation had a negative effect on seed production R^2 = 0.461, F = (1, 29) = 26.706, p < 0.001 (Mahalanobis distance = 5.645 < 13.816), predicting 46.1 % of the variance;

- Total rainfall in the spring preceding seed formation had a negative effect on seed production R^2 = 0.455, F = (1, 29) = 26.018, p < 0.001 (Mahalanobis distance = 3.43 < 13.816), predicting 45.5 % of the variance;
- Total rainfall in the autumn preceding seed formation had a negative effect on seed production R^2 = 0.453, F = (1, 29) = 25.818, p < 0.001 (Mahalanobis distance = 3.341 < 13.816), predicting 45.3 % of the variance;
- Total rainfall in the summer preceding seed formation had a negative effect on seed production R^2 = 0.243, F = (1, 29) = 10.636, p = 0.003 (Mahalanobis distance = 6.41 < 13.816), predicting 24.3 % of the variance;
- Cumulative rainfall for the summer, autumn and winter period (inclusive) preceding and during seed formation had a negative effect on seed production R^2 = 0.227, F = (1, 29) = 9.82, p = 0.004 (Mahalanobis distance = 4.273 < 13.816), predicting 22.7 % of the variance;
- Monthly mean maximum temperature during the summer preceding seed formation had a positive effect on seed production R^2 = 0.25, F = (1, 29) = 10.99, p = 0.002 (Mahalanobis distance = 3.311 < 13.816), predicting 25 % of the variance; and
- Monthly mean maximum temperature during the winter flowering period had a positive effect on seed production R^2 = 0.158, F = (1, 29) = 6.611, p = 0.016 (Mahalanobis distance = 3.679 < 13.816), predicting 15.8 % of the variance.

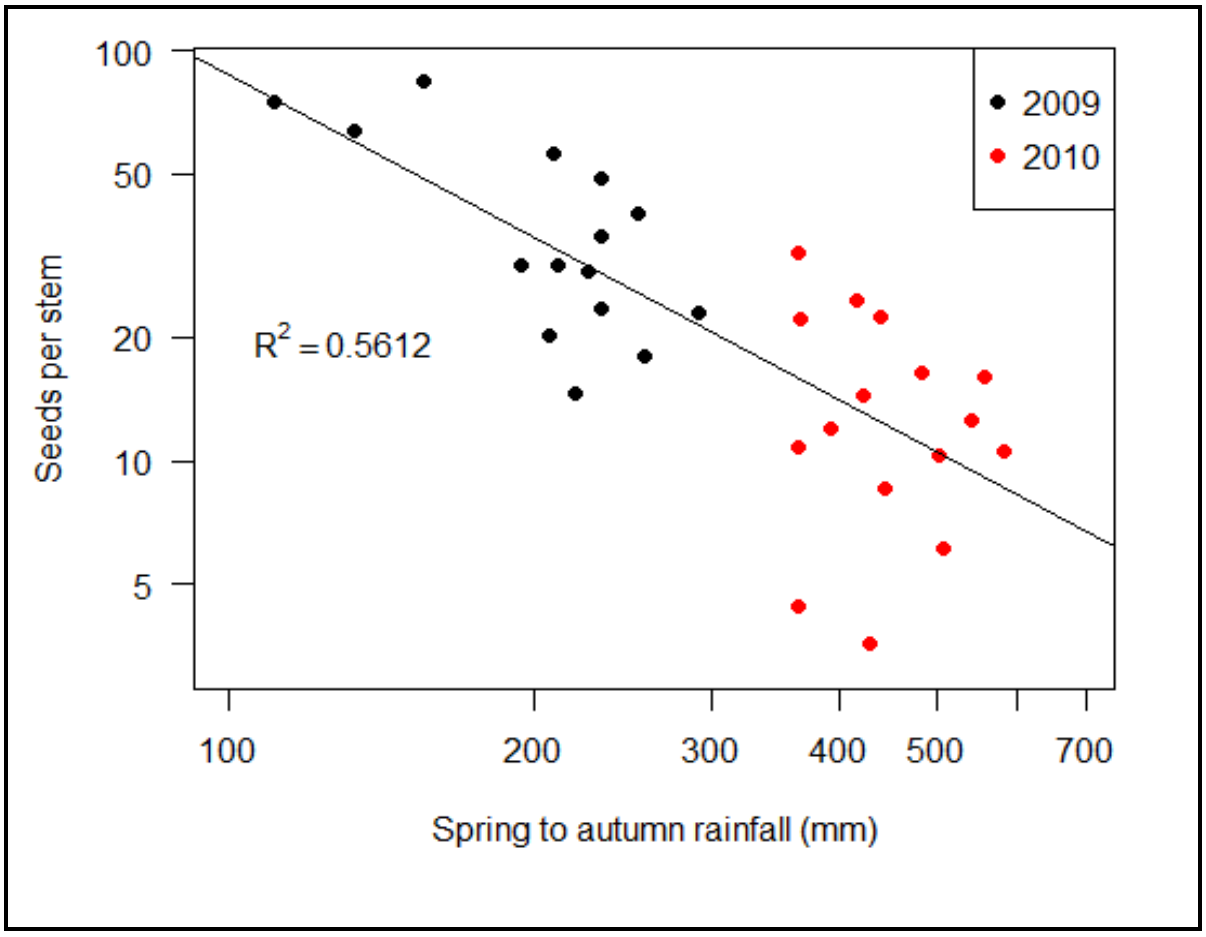


Figure 54 – Seed production was negatively correlated to the cumulative spring, summer and autumn rainfall prior to the period of seed formation for 2009 and 2010 combined (n = 31, Carisbrook BR 2009 data omitted). Both axes are log_{10} scale.

Of the models that included a rainfall variable, the predicted variances ranged from 22.7 to 54.6 %. Of these, the variable 'cumulative rainfall in the spring, summer and autumn period prior to the seed formation period' made the greatest contribution to the seed production model. In contrast, models that included a temperature variable were found to contribute no more than 25 % of the predicted variance.

In summary and generally speaking, a greater quantity of seed was produced when there was less rainfall coupled with higher average monthly maximum temperatures in the year preceding flowering and during seed formation.

4.3.4 *Seed viability*

Although there was no significant difference in seed viability between study areas or between years (see Chapter 3b 3.3.3), significant relationships with both rainfall and temperature variables were detected. As per Chapter 3b 3.3.2, in 2010 seed viability data from only 14 study areas were used in the analyses (McKenzie Rd and Calder RRR omitted).

4.3.4.1 Rainfall and seed viability

The correlation analyses identified four significant associations with rainfall and seed viability in the period winter 2009 to autumn 2010 but none were found for the period spring 2008 to autumn 2009. When each year was combined there were six significant correlations identified (see section 4.3.4.3 below).

4.3.4.2 Temperature and seed viability

For each of the individual seed viability data sets, 2009 and 2010, no relationships with any temperature parameters were detected. However, when the data sets were combined there were two significant negative correlations identified (see section 4.3.4.3 below).

4.3.4.3 Environmental relationships with seed viability

To determine which environmental factors were most strongly associated with seed viability, the data for both the 2009 and 2010 were combined. Because each of the environmental

variables was significantly correlated to each other and therefore confounding, a series of simple linear regressions were conducted for each of the six rainfall variables and two temperature variables. Of the eight simple linear regressions, all detected a significant association with seed viability and are listed in order of the contribution that they made to the models (greatest to least):

- Total rainfall for the autumn period preceding seed formation had a positive effect on seed viability R^2 = 0.239, F = (1, 28) = 10.101, p = 0.004 (Mahalanobis distance = 3.577 < 13.816), predicting 23.9 % of the variance (Figure 55);
- Cumulative rainfall for the summer, autumn and winter period (inclusive) preceding and during seed formation had a positive effect on seed viability R^2 = 0.239, F = (1, 28) = 10.093, p = 0.004 (Mahalanobis distance = 4.324 < 13.816), predicting 23.9 % of the variance;
- Cumulative rainfall for the summer and autumn period (inclusive) preceding seed formation had a positive effect on seed viability R^2 = 0.231, F = (1, 28) = 9.917, p = 0.004 (Mahalanobis distance = 5.163 < 13.816), predicting 23.1 % of the variance;
- Annual rainfall in the year preceding seed formation had a positive effect on seed viability R^2 = 0.191, F = (1, 28) = 7.848, p = 0.009 (Mahalanobis distance = 4.802 < 13.816), predicting 19.1 % of the variance;
- Monthly mean maximum temperature in the autumn preceding seed formation had a negative effect on seed viability R^2 = 0.186, F = (1, 28) = 7.619, p = 0.01 (Mahalanobis distance = 1.731 < 13.816), predicting 18.6 % of the variance;

- Cumulative rainfall for the spring, summer and autumn rainfall period (inclusive) preceding seed formation had a positive effect on seed viability R^2 = 0.184, F = (1, 28) = 7.520, p = 0.011 (Mahalanobis distance = 4.606 < 13.816), predicting 18.4 % of the variance;
- Daily mean maximum temperature in the winter during seed formation had a negative effect on seed viability R^2 = 0.12, F = (1, 28) = 4.941, p = 0.034 (Mahalanobis distance = 1.731 < 13.816), predicting 12 % of the variance; and
- Total rainfall in the spring preceding seed formation had a positive effect on seed viability R^2 = 0.110, F = (1, 28) = 4.599, p = 0.041 (Mahalanobis distance = 2.9 < 13.816, predicting 11 % of the variance.

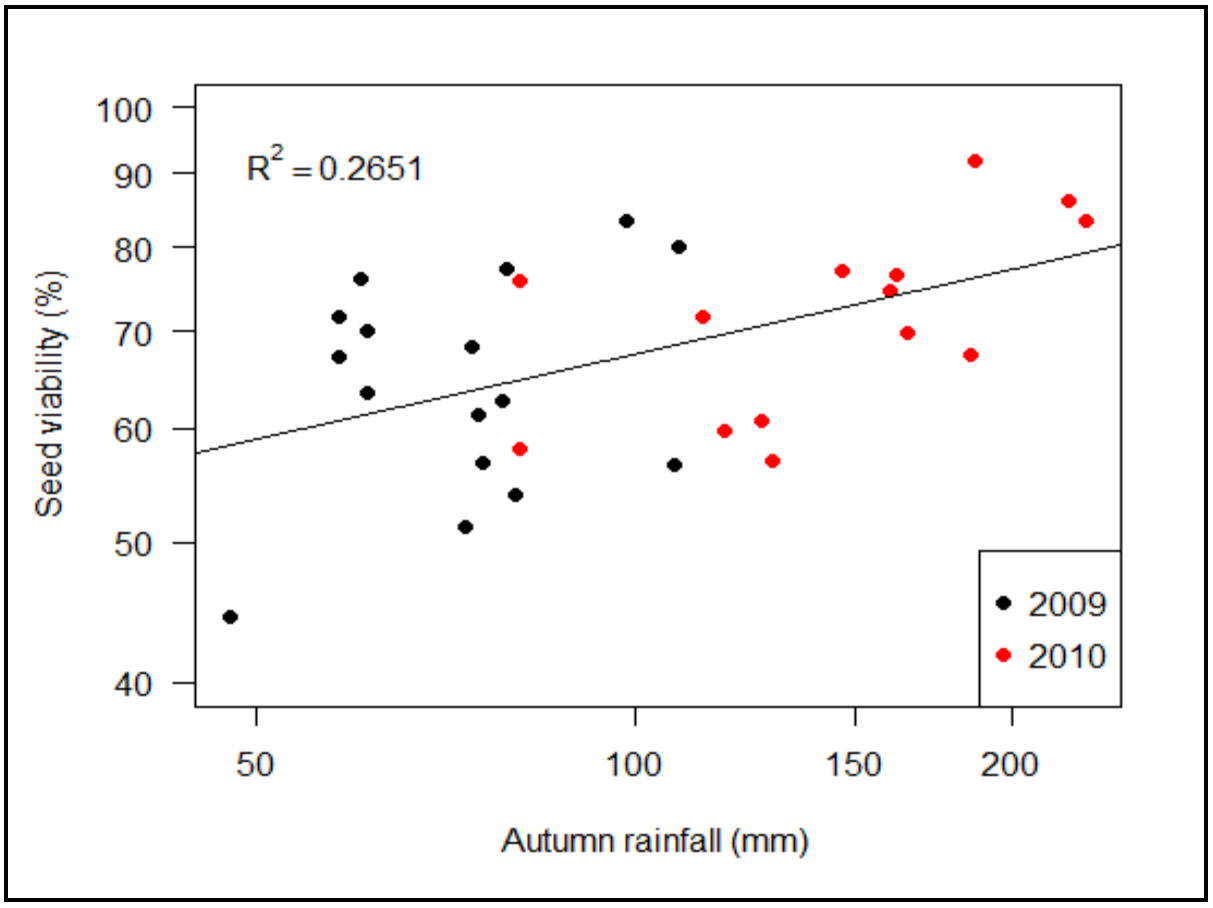


Figure 55 – Seed viability was greatest when the total rainfall during the autumn preceding and during flowering was high (n = 30). The x axis is a $\log_{10}$ scale.

Both the rainfall and temperature variables predicted similar variances in the models for seed viability, ranging from 11 % to 23.9 %. A stepwise regression analysis was performed using the most predictive variables from each environmental group (rainfall and temperature) and found that the variable 'total autumn rainfall' alone made the greatest contribution to the seed viability model (see section 4.3.4.3 above).

In short, seed viability is associated with the amount of rainfall in the period preceding seed formation. With greater rainfalls there is a corresponding lower temperature, and vice versa, which is positively associated with seed viability.

4.3.5 *Germinant recruitment measures*

The measures of recruitment potential 'germination *in situ* (germinants/female plant)' and 'germinant survival *in situ*' were analysed for relationships with all rainfall and temperature parameters. No significant relationships were detected.

4.4 Discussion

4.4.1 *Environmental data*

Victoria's climatic conditions are driven by cycles of *El Niño* events (negative Southern Oscillation Index [SOI]), which are characterised by below average rainfall and increased temperatures, and *La Niña* events (positive SOI) with greater than average rainfall and lower temperatures (Bjerknes, 1969). This study was undertaken at the interface of these two climatic patterns (National Climate Centre, 2009, 2010). Since 1996, the *El Niño* pattern had prevailed, resulting in a prolonged 12 year drought across the south-east of Australia. Measurements of the reproductive capacity of *P. spinescens* were first taken in 2009, a year accentuated by record high temperatures and extreme bushfire events across Victoria (National Meteorological Service, 2008, Fawcett, 2010). The second set of reproductive capacity measurements were taken in 2010, under the strong influence of *La Niña* conditions, highlighted by widespread flooding throughout parts of Victoria (National Climate Centre, 2010).

In this chapter, associations have been made between the reproductive capacity data collected from *P. spinescens* and the environmental variables of rainfall and temperature. However, it is unclear whether the reproductive output of the species for either of these years represents the typical situation. Encouragingly, the results demonstrate trends that span across the two years of data collection, although distinct contrasting results between years were also detected in some cases. Further investigation is required to determine whether such trends are persistent over time.

4.4.1.1 Rainfall

Pre-flowering rainfall between the study years was significantly different, with most sites receiving approximately double the rainfall in 2010. Despite such extreme differences in environmental conditions overall trends in measures of recruitment potential were maintained.

The rainfall data utilised in the study is reflective of regional weather patterns, rather than distinct site conditions. Rainfall data was sourced from the nearest public weather station. In some cases these weather observation stations were located several kilometres from the study sites. Future research into the effects of rainfall on *P. spinescens* would benefit from the placement of rain gauges at sites to gain a greater accuracy of actual rainfall.

4.4.1.2 Temperature

Temperature changes at the study sites and over time were much less volatile than rainfall, yet across the two study years, 2009 and 2010, temperature variations were associated with trends in the reproductive capacity for *P. spinescens*. The decade from 1998 to 2007 was exceptionally dry and hot with the average maximum temperature 0.6°C above the 30 year average (1961 – 1990) and the average minimum temperature 0.2°C warmer (Victorian Government Department of Sustainability and Environment (DSE), 2008). The year 2009 was the nation's second-warmest year on record, with an exceptional heat wave that broke a range of temperature records in Victoria during the pre-flowering months of January to February (Bureau of Meteorology, 2010). Although the *La Niña* event in 2010 resulted in the fifth-wettest year on record for Victoria, temperatures in the study region were average to above-average in the pre-flowering period (Bureau of Meteorology, 2011).

Within the study region, temperature data was not as widely recorded and available for public access. Therefore, only seven unique weather stations were found appropriate for the collection of temperature data to represent the 16 sites. In the western plains area of Rokewood which included six sites, temperature data was collected from a single weather station. A further two weather stations also represented three sites each. Therefore the temperature data is not site specific and only reflects a regions general temperature pattern. To validate the associations between temperature and reproductive capacity measures, future studies should collect site specific temperature data.

4.4.2 *Environmental relationships to seed production*

This study found that seed production was significantly and negatively correlated to cumulative rainfall, particularly in the spring to autumn period preceding flowering. Additionally, mean monthly maximum temperatures in the autumn preceding flowering were positively correlated to seed production, although the effect was not as strong. Essentially, during the year (2009) that was preceded by lower rainfall and greater temperatures, *P. spinescens* plants had a higher rate of seed production and vice versa in 2010 when a greater amount of rainfall was recorded in the pre-flowering period.

The relationship between rainfall preceding flowering and seed production has a linear distribution that is roughly located on a single gradient across the two years of this study. Curiously, the negative relationship (fewer seed produced during higher rainfall periods) is the opposite of what would be intuitively expected and what has been reported for numerous other species in the literature. Clearly, the *P. spinescens* populations throughout the

duration of this study did not undergo a process of resource matching in relation to water availability (Piovesan and Adams, 2005, Masaki, Oka *et al.*, 2008, Burns, 2012).

Despite the rainfall and seed production data for the combined years of 2009 and 2010 having a distribution along a single gradient, when these data were analysed for each discrete year the negative relationship was maintained only for the production of seed in 2009. No relationship was detected for these variables for the 2010 seed production data.

A possible explanation for the different quantities of seed produced and the potential differences in the relationship between seed production and rainfall for each of these years could be seed masting. A tendency for woody plants to alternate between high and low seed production years has been revealed by an analysis of 296 published and unpublished data sets (Herrera, Jordano *et al.*, 1998). Similarly, it has been found for many species that large seed crops occur at intervals of several years and in the intervening years seed production is considerably reduced (Shibata, Tanaka *et al.*, 2002, Fenner and Thompson, 2005). These types of variability in seed production may also occur for *P. spinescens*.

Within a plant population, the synchronised variation in seed production between years has been termed as “masting” or “mast seeding” (Wilson and Traveset, 2000, Fenner and Thompson, 2005). It is a phenomenon that has been mostly observed in temperate and tropical environments (Kelly, 1994, Herrera, Jordano *et al.*, 1998, Koenig and Knops, 2000, Norden, Chave *et al.*, 2007, Sanguinetti and Kitzberger, 2008). Mast seeding has mostly been associated with trees (Waller, 1993, Shibata, Tanaka *et al.*, 2002) but has also been documented for various species of shrubs (Selas, 2000) and grasses (Kelly, Harrison *et al.*, 2000). The phenomenon has also been found to be associated with long-lived species which

exhibit a high adult survivorship and a low population growth rate, as they are less affected by an absence of a reproductive effort in some years (Waller, 1979, Kelly, 1994).

Whilst it takes many years of study to determine whether or not a species undergoes mast seeding, the literature relating to this phenomenon may assist in providing some explanations as to the different relationships that were observed between *P. spinescens* seed production and rainfall across the two study years. Some possible explanations and/or triggers, which are not necessarily mutually exclusive, include:

- Seed predator satiation (Kelly, Harrison *et al.*, 2000, Espelta, Cortes *et al.*, 2008);
- Pollination efficiency (Norton and Kelly, 1988, Kelly, 1994, Shibata, Tanaka *et al.*, 1998);
- Temperature cues (either high or low) (Ashton, Givnish *et al.*, 1988, Rees, Kelly *et al.*, 2002);
- A reproductive output that is negatively correlated to vegetative growth (years of good vegetation growth are poor for reproductive output). This is a common occurrence in species that undergo mast seeding in the northern hemisphere (Koenig and Knops, 2000);
- Prediction of favourable conditions for establishment following environmental cues such as fire (Keeley, 1993, Kelly, 1994, Keeley and Bond, 1999) and/or drought (van Schaik, Terborgh *et al.*, 1993, Wright, Carrasco *et al.*, 1999, Williamson and Ickes, 2002, Piovesan and Adams, 2005); and

- Negative autocorrelation, which means that the current reproductive output is negatively related to the previous year/s reproductive output (Kelly and Sork, 2002).

Whilst any of the items in the above list (and possibly others) may contribute to the observed yearly variations in seed production of *P. spinescens*, a plausible explanation is derived from the last two points. Additionally, the physiology of the taproot is intrinsic to this explanation but requires further study. Initial investigations conducted as part of this project but not presented in this thesis suggest that the taproot of *P. spinescens* stores water and nutrients and has an outer layer of lignin at the proximal end which is impervious to water (D. Reynolds 2009, pers. obs., January).

At the end of 2009, following 12 years of drought, it is likely that the water and nutrient reserves within the taproot were becoming depleted and that the plants were suffering from severe water stress. In response to potential imminent death if the drought had continued (see table 1 in Williamson and Ickes, 2002), the plants underwent a process of mass flowering resulting in a high rate of seed production (Piovesan and Adams, 2005). Such a response to prolonged drought would ensure that a large volume of seed was available for germination when environmental conditions were more favourable (Kelly, 1994). Potentially, the space and nutrient resources occupied by any mature plants that succumbed to the drought would become available to germinating seed (Pugnaire, Hasse *et al.*, 1996, Walsh and Newberry, 1999, del Cacho and Lloret, 2010).

In late 2009, the drought broke, leaving in question the fate of the mature plants if the unfavourable drought conditions had continued. Instead, the majority of mature plants in this study survived.

The seed production of 2010 may have been a trade-off between fecundity and survival, as has previously been described for the tree *Quercus robur* L. (English Oak) (Crawley, 1985, Crawley and Long, 1995) and the herbaceous perennial *Lychnis flos-cuculi* (Ragged Robin) (Biere, 1995). A return to average rainfall in 2010, presented the opportunity for mature *P. spinescens* plants to replenish the store of water within the taproot. At this time additional energy resources would also have been directed to the taproot for storage as starch (D. Reynolds 2009, pers. obs., January), providing resources in future times of adversity. In comparison to 2009, fewer resources may have been directed towards reproductive output, although it is unclear whether the level of seed production observed in 2010 approximated an undefined average or whether it was depressed following the drought (Koenig and Knops, 2000).

Pimelea spinescens is likely to have adapted to survive in a harsh water stressed environment by accessing water and nutrients stored within the taproot and mast seeding when drought conditions become critical. When water becomes available, the plants allocate resources for storage in the taproot, therefore compensating for the previous year's reproduction output and ensuring the availability of nutrients and water in future times of adversity (Crawley, 1997, Larcher, 2003, Clary, Save *et al.*, 2004).

Mast seeding in response to drought conditions has been documented in several cases. Much of the work has been in tropical forests where it has been found that the climatic factors of irradiance level (solar radiation) and water availability play a major role in the flowering variation between seasons and years (van Schaik, Terborgh *et al.*, 1993). In the tropical forest of Panama (Barro Colorado Island), years of high fruit production have been associated with *El Niño* conditions, which are then followed by a recovery period resulting in

low fruit production (Wright, Carrasco *et al.*, 1999). Similarly, Curran (1999) found that 50 species of dipterocarp in Borneo mast seeded at times which coincided with four *El Niño* events in the 11 years from 1987 to 1998. Although *P. spinescens* is not a tropical species, its populations are subjected to *El Niño* Southern Oscillation (ENSO) cycles and may be responding in a similar way to the flora of Panama and Borneo, in terms of seed production events.

A further explanation for variation in seed production of *P. spinescens* between years is that the variation in weather conditions may have influenced the activity of pollinators. Bees and other insects do not tend to forage in the rain or when flowers are wet, limiting their pollination effectiveness during these conditions (Howe and Westley, 1997, Copeland and McDonald, 2001, Larcher, 2003, pg 309, Gonz´alez, Dalsgaard *et al.*, 2009). In comparison to 2009, the increased rainfall which occurred during 2010 could have negatively affected pollination success and impacted on seed production for *P. spinescens*. Although the presence of pollinators does not generally appear to be a limiting factor for *P. spinescens* (D. Reynolds 2009, pers. obs., July), further investigation of pollinator effectiveness under a range of environmental conditions is required.

The environmental variables studied in this chapter are only one source of influence on seed production. The low seed production in 2010 could also be the result of coinciding with a post-seed masting period in which you would normally expect reduced seed production (Kelly and Sork, 2002). *Pimelea spinescens* corresponds to the profile of a mast seeding species; it is long-lived and exhibits a Deevey Type III curve in relation to the population's survivorship. Further seed production monitoring is required to confirm if the trends and

associations continue. Other factors such as habitat and management associations will be investigated in Chapter 5.

4.4.3 *Environmental relationships to seed viability*

Whilst there was a negative relationship between seed production and rainfall, the opposite was true between seed viability and rainfall. Approximately 23 % of the variability in seed viability was due to rainfall, with other contributing factors being the sex ratio, number of females in a study area (see Chapter 2, discussion) and past management practices (this will be discussed in Chapter 5). With a greater rainfall prior to or during flowering, a consistently higher rate of seed viability was recorded.

During seed viability testing, unviable seed appeared to have dehydrated embryos (see Figure 34 in Chapter 3b 3.1), suggesting that water availability was an important factor in the production of quality seed. Using an experimental approach, a relationship between resource availability and seed quality has been documented for other species, including the chenopod *Sarcobatus vermiculatus* (Greasewood) in relation to fertiliser and water (Breen and Richards, 2008) and the Australian succulent *Maireana sedifolia* (Bluebush) in relation to water (Wotton, 1994). In both examples, the addition of these resources resulted in improved seed viability.

Seed viability is often only tested as a measure of the potential for germination and as a result there are few examples of comparisons between seed viability and a natural environmental factor. However, in the dioecious shrub species *Juniperus communis* (Common Juniper) differences in the levels of seed viability have been related to the climatic conditions in many of the harshest environments in which it is found. In the Arctic tundra,

with long cold winters, and the Mediterranean high mountains with cold winters and dry summers, the seed viability of *J. communis* was reduced when compared to sites with milder climates (Garcia, Zamora *et al.*, 2000). In *Avena fatua* L. (Wild oats) greater than one and half times more viable seed was produced in moist conditions as opposed to dry conditions (Peters, 1982).

Although an association has been found between the seed viability of *P. spinescens* and rainfall, the environmental conditions between the two years of this study are extremely contrasting and the association found is unlikely to be coincidental. Greater seed quality has previously been associated with more plentiful resources (Peters, 1982, Garcia, Zamora *et al.*, 2000) and this is likely to be true for *P. spinescens*. In this study, there appeared to be a trade-off between seed production and seed viability (Peters, 1982, Fenner and Thompson, 2005), reflecting a differential in the way resources are allocated and utilised under differing environmental conditions.

4.4.4 *Germinant recruitment measures*

Although germination occurred during both years of this study, such events did not appear to be directly related to weather conditions.

Of the germinants produced in 2009, 14.5 % survived across eleven study areas until the final assessment in October 2010. By comparing germinant survival between study sites during this period, no associations could be made with any of the environmental variables assessed. This small time frame is insufficient to make any assumptions about the effects of any environmental variables on the long-term survival of germinants. The monitoring of

germinants in relation to environmental conditions over time should be the subject of future research.

Ten of the 16 study areas had an increasing population with the average growth rate of 13 % from 2009 to 2010. Positive population growth has previously been described for several *P. spinescens* populations (Cropper, 2007a, Foreman, 2011). At the intensively studied Western Treatment Plant, the rate of growth appeared to decline during the period of the drought from 19.7 % in 2003 to only 3 % in 2007 (Cropper, 2007a). It is possible that the period from the end 2009 to 2010 which was the start of the drought breaking period, that a 13 % growth rate is an exceptional recruitment achievement for *P. spinescens* populations. Future research is required into temporal monitoring of population growth rates and environmental conditions at sites.

4.4.5 *Summary*

The study was undertaken during two years of extremely contrasting weather conditions. The first year was associated with the end of a 12 year drought (limited rainfall and high temperatures). The second year was highlighted by drought breaking rains and lower temperatures, associated with *La Niña* conditions. Rainfall was found to have relationships with both seed production (negative) and seed viability (positive). Temperature relationships were less prominent but supported the rainfall associations. Greater seed production was recorded in conditions with lower rainfall, higher maximum temperatures. In contrast, greater rainfall combined with lower temperatures was associated with greater seed viability. Overall, the seed viability of *Pimelea spinescens* was high, suggesting that the species appears to have had sufficient resources (taproot storage) to maintain seed quality throughout the long-term drought conditions.

There is a possibility that *P. spinescens* mast seeds in response to drought conditions but the differences observed in 2010 may also be related to the energetics of the plant, rather than the climatic conditions during that year. Further research over a longer time period is required to clarify the relationship between rainfall and the production and viability of *P. spinescens* seed.

Chapter 5 The effects of past management practices on site recruitment potential

"While eagerly pressing forward in the search after the secrets of nature, we are apt to keep the eye too constantly fixed on the way that has to be travelled, and lose sight and remembrance of the paths already trodden"

Sir Archibald Geikie (1897)

5.1 Introduction

Grasslands exist within the landscape as part of a dynamic continuum that is maintained by a range of environmental factors (Attiwill and Wilson, 2003, Carter, Murphy *et al.*, 2003) and management processes (Lunt, Prober *et al.*, 2012). They are disturbance communities that persist partly as a result of digging by animals (Pyrke, 1994, Claridge and Barry, 2000, Garkaklis, Bradley *et al.*, 2004) and humans (Curr, 1883, Presland, 1980, Gott, 1982, 1983, Clarke, 1985, Gott, 1992), grazing (Belsky, 1992, Hobbs and Huenneke, 1992, McIntyre, Huang *et al.*, 1993, McIntyre and Lavorel, 1994a, McIntyre, Lavorel *et al.*, 1995, Lunt, 1997a, Verrier and Kirkpatrick, 2005) and fire (Hobbs and Huenneke, 1992, Lunt, 1997a, Morgan, 1998c, Carlsen, Menke *et al.*, 2000, Morgan, 2001, Jutila and Grace, 2002, Keeley, 2002, Clarke and Davison, 2004, Gott, 2005). Such disturbance processes have led to the maintenance of grassy environments by limiting the capacity for recruitment of woody species, whilst making resources such as space, light and nutrients accessible to grassy and herbaceous life forms (Solomon, Berg *et al.*, 1999, Attiwill and Wilson, 2003).

The natural temperate grasslands of the Victorian volcanic plains have evolved under a regime of frequent fire events and soil disturbance from animals (Pyrke, 1994, Lunt, 1997a, Claridge and Barry, 2000, Morgan, 2001). For at least the last 30,000 years (Presland, 1983), there has been a greater frequency of biomass removal as a result of burning and soil disturbance by aborigines as a means to maintain their food supply (Kohen, 1995, Keith, Williams *et al.*, 2002, Gott, 2005). Over the last 200 years and particularly since the 1930's, these processes have declined due to fire suppression activities, the loss of native peoples and animals within the landscape. Today there is less than five per cent left in its natural state (representing a landscape before European influences) and less than 0.1 % is

considered high quality (Australian Government, 2011, Victorian Environment Assessment Council, 2011).

5.1.1 *The importance of grassland management*

Apart from the direct effects of habitat loss, the arrival of Europeans introduced novel types of disturbance and altered patterns of biomass reduction to the Natural Temperate Grasslands of the Victorian Volcanic Plain (Billot, 1979, Jones, 1992, Wheeler, Jacobs *et al.*, 2002). These altered disturbance regimes resulted in the degradation of almost the entire grassland community (Stuwe and Parsons, 1977, Morgan, 1998d, Wong and Morgan, 2007). Today, the last remaining intact remnants are found on: private land which has been lightly grazed by domestic animals (Stuwe and Parsons, 1977, Victorian Environment Assessment Council, 2011); roadsides that have been periodically burnt and grazed (Morgan, 1998e, d); and railway verges which were frequently burnt (Stuwe and Parsons, 1977, Raynor, Marsh *et al.*, 1984, Williams, McDonnell *et al.*, 2005).

More recently, it has become apparent that an absence of biomass reduction practices or changes in the type or frequency of management can result in significant degradation of grassland remnants (Morgan, 1998c, Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2006). For example, following the corporatisation and then privatisation of Victoria's railways in the 1980's and 1990's, the management of railway reserves shifted from a regular burning regime to management using ploughed fire breaks, herbicide control, mowing and slashing (Raynor, Marsh *et al.*, 1984, Lunt, 1992, Williams, McDonnell *et al.*, 2005). Research assessing the state of these railway reserves has found that these management changes have facilitated weed invasion, increased the fire risk and reduced the

native species richness in these grasslands (Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2005, Williams, Morgan *et al.*, 2006, Australian Government, 2011).

Although the destruction of the Natural Temperate Grasslands of the Victorian Volcanic Plain is ongoing, there is a persistent threat of degradation in the remaining remnants as a result of poor or insufficient management practices (Victorian Environment Assessment Council, 2011). In 2011, the Victorian Environment Assessment Council found that in Victoria the greatest threat to the remaining native grasslands and their species diversity is lack of active management (Victorian Environment Assessment Council, 2011).

5.1.2 *Pimelea spinescens and habitat management*

Depending on their inherent ecology, dominance in the community and anatomical features, each plant species responds to the disturbance of biomass loss differently (Huston, 2004). Following a biomass reduction event which removes all exposed vegetation, *P. spinescens* re-sprouts from under-ground stems which are attached to a taproot (Mueck, 2009, also observed by D. Reynolds during root excavations in 2009). As has been described for a range of other species with taproot root systems (Bell, Pate *et al.*, 1996, Schultze, Beck *et al.*, 2002), the survival of a mature *P. spinescens* plant following biomass reduction is dependent on the reserves stored within the taproot.

In general, the strongholds of *P. spinescens* are on remnants which occur on roadsides, railway reserves, cemeteries and airports (Carter and Walsh, 2006). These are all areas that historically would have had some form of regular biomass management, although the type and frequency of management would have varied between sites (Stuwe, 1986, Kirkpatrick, McDougall *et al.*, 1995).

There is some evidence of the impacts of habitat management from a long-term study of the species at the Western Water Treatment site (Cropper, 2003, 2004, 2005, 2006, 2007b, a). During the first three years following a spring burn the rate of population growth of *P. spinescens* was reported to be as high as 17.8 %. By the fifth year, the rate of population growth had declined to 3.9 %. With annual weed control and follow-up biennial burns, the population was found to have doubled by year five (Cropper, 2007a, 2009).

At sites on the Volcanic plains, Foreman (2005, 2011) found that the occurrence of germinants was higher when burns had occurred less than 3½ years previously and when the percentage cover of bare soil was greater than 5 % but less than 20 %. An increased fire frequency was also found to be associated with a lower cover of exotic species and a greater indigenous species density. It is evident that populations of *P. spinescens* are able to persist and thrive when intensive but appropriate management actions are implemented and favourable environmental conditions exist.

Although the strongholds for *P. spinescens* are on sites that are considered to be high quality temperate grassland remnants, populations have been found in low quality remnants with poor species richness (Foreman, 2011). As an inter-tussock species, it unclear which habitat attributes that are readily modified by management actions influence the survival and reproductive success of *P. spinescens,* including the measures of recruitment potential such as seed production and quality, and germinant production and survival.

5.1.3 *Chapter aims*

Successful management of fragmented populations of *P. spinescens* that promotes autogenic recovery is reliant on knowledge, including an understanding of the importance of site characteristics and management regimes.

This chapter addresses the sixth objective of the *P. spinescens* Recovery Plan (Carter and Walsh, 2006), which is to acquire historical data in relation to site management histories. The objective is addressed by:

- Evaluating the influence of past management practices on recruitment potential.

5.2 Methods

5.2.1 *Measures of recruitment potential*

Using the sites, study areas and plants in the randomly located quadrats (longitudinal study) described in Chapter 2, and the methods of assessment described in Chapter 3, the effects of management and habitat characteristics were assessed for associations with the following measures of recruitment potential:

- Seed production (seeds per stem);
- Seed viability;
- Germination *in situ* (germinants/female plant); and
- Germinant survival *in situ* (as described in Chapter 2, 'Natality' section).

5.2.2 *Recent land management practices*

Information on land use and management practices was sought for all 16 sites. Information obtained was from landowners (or adjacent landowners), local government authorities, officers from the Department of Sustainability and Environment (DSE) and the Country Fire Authority (CFA). Information was related verbally and/or via CFA fire truck records. Where information was unavailable or recollections were uncertain, an educated guess was made about the management history, based on the information provided by local farmer and DSE officers, the observed state of the landscape and vegetation throughout the study period, and the presence of stock throughout the study period.

Biomass is all the organic matter (living and dead) produced within an ecosystem; it is also considered an energy source, as it can be converted into fuel (Miller, 2000, Attiwill and Wilson, 2003). In this study, biomass was considered as all the plant matter present within the grassland site study area. A biomass reduction event was any occurrence (see results section 5.3.1.1) that was known to have happened within the study area that had decreased the amount of biomass present during the period of a year (January to December).

The management information was compiled and used to define four parameters against which the measures of recruitment potential could be compared. These parameters were:

- The frequency of biomass reduction events over the five years preceding collection of measures of recruitment potential data;
- The frequency of biomass reduction events over the ten years preceding collection of measures of recruitment potential data; and
- The time (years and months) since the last biomass reduction event; and
- The number of burn events in a ten year period.

5.2.3 *Habitat condition*

The habitat condition data was collected using the Habitat Hectares method (Department of Sustainability and Environment, 2004); as well more refined methods to measure weed cover and bare soil (see sections 5.2.3.3 and 5.2.3.4 below). All habitat condition data was recorded in spring 2009, with the exception of the bare soil assessment which was measured in spring 2009 and spring 2010.

5.2.3.1 Habitat Hectares

The quality of the vegetation within each study area was assessed by applying the Habitat Hectares method as described in the Vegetation Quality Assessment Manual (Department of Sustainability and Environment, 2004). The Habitat Hectares method took into account both the quality and quantity of a defined patch of vegetation. The quality of the vegetation was assessed by combining a 'site condition score' and a 'landscape context score', resulting in a 'final habitat score' out of 100. The final habitat score was then multiplied by the size of the defined vegetation patch, to calculate the Habitat Hectares score. The method can be applied to any patch of terrestrial vegetation and uses standardisers to maintain equivalence between vegetation types.

To apply the Habitat Hectares method, reference to a pre-defined benchmark of high vegetation quality for the relevant Ecological Vegetation Class (EVC) was required. Although *P. spinescens* occurs in a range of EVC's, the benchmark considered most appropriate for all 16 study areas was EVC 132_61: Heavier-soils Plains Grassland as it most clearly approximated the conditions found at the majority of the sites.

To apply the Habitat Hectares method a species area curve was obtained within each study area to determine the optimum quadrat size for assessment (Table 20). All components (Table 21), as required by the benchmark, of the Habitat Hectares assessment were conducted at each study area, to achieve the 'final habitat score'. For the 'lack of weeds', 'recruitment' and 'organic litter' scores respectively, an average was derived from the quadrats assessed within each study area.

The sub-score for each component of the Habitat Hectares assessment was used as an independent variable for correlations with the measures of recruitment potential for *P spinescens*.

Table 20 – The number and size of habitat condition quadrats and the size of the total area assessed for each study area.

	No. of quadrats assessed	Quadrat size (m^2)	Total area assessed (m^2)
Ararat AR	5	5	25
Bannockburn RR	4	4	16
Baringhup WR	4	4	16
Brownswaterholes BRR	6	3	18
Calder RRR	4	4	16
Carisbrook BR	4	3.5	14
Cedarwoods R	4	4	16
Christies Rd	4	4	16
Geggies Rd	6	1	6
Glengower Rd	4	4	16
Kirks BR	4	4	16
McKenzie Rd	8	2	16
Mt Mercer Rd	6	5	30
Pitfield CR	4	5	20
Poorneet WRR	5	4	20
Vite VRR	5	4	20

Table 21 – Explanation of the components of a Habitat Hectare scoring system (Department of Sustainability and Environment, 2004).

Habitat Hectare scoring system	
Components	**Definitions and explanation of the components**
Understorey	An assessment of the number of life forms present and the respective percentage cover, compared with the EVC benchmark.
+ Lack of weeds	An estimate of the percentage cover of weeds and graded according to the presence (%) of any high threat weed species.
+ Recruitment score	An estimate of recruitment potential by assessing the percentage of bare ground available for recruitment, which is further graded by the diversity of herbaceous species present to provide seed for colonisation.
+ Organic litter	Assessments of the percentage cover of organic litter, which is further graded as being indigenous or exotic.
x standardiser 1.36	This is a multiplier which is required to standardise the score, as treeless vegetation has fewer components for assessment than other EVC's.
= SITE CONDITION SCORE	A sum of the understory, lack of weeds, recruitment and organic litter scores.
Patch size	An area of native vegetation that is continuous with the area being assessed. In this study, the patch size was defined by the entire site, rather than the study area.
+ Neighbourhood	Scores the amount and configuration of native vegetation surrounding the assessment area, on the basis of three radii (100m, 1km and 5km).
+ Distance to core area	An estimate of the distance to a core area (an area of any EVC type comprising of native vegetation greater than 50 hectares) and graded by the quality of vegetation in that core area.
= LANDSCAPE CONTEXT SCORE	A sum of the patch size, neighbourhood and distance to core area scores.
FINAL HABITAT SCORE	**= SITE CONDITION SCORE + LANDSCAPE CONTEXT SCORE**
HABITAT HECTARES	**= FINAL HABITAT SCORE X PATCH SIZE (ha)**

5.2.3.2 Species diversity assessment

The species diversity was measured as part of the understory component of the Habitat Hectare scoring method. It is the total number of different indigenous species that were found within the quadrats per study area.

5.2.3.3 Weed cover assessment

The lack of weed score was measured using the Habitat Hectare scoring method and was further refined to provide a 'weed cover' score. This was done by the addition of the percentage cover of weeds found per quadrat, divided by the number of square metres assessed to gain a percentage of weed cover per square metre at each study area for comparison.

5.2.3.4 Bare soil assessment

In 2009, the study area percentage bare soil was obtained via a section of the understory component of the Habitat Hectare score. In 2010, every quadrat within all study areas was visually assessed for the percentage of bare soil available within the quadrat. The total of each quadrat percentage of bare soil was then divided by the total number of square metres assessed for each study area.

5.2.4 *Statistical analysis*

For all 16 sites the management and habitat condition data was correlated using Microsoft Excel against identified measures of recruitment potential (see section 5.2.1) and identified demographic characteristics of the populations (see Chapter 2, results) for possible associations. Bivariate analyses were conducted via SPSS (Version 18) to assess the direction and strength of any associations. Where data did not conform to the requirements

of normality, data transformations were conducted according to Zar (1999). If the data obtained was logarithmic and included zeros a value of one was added to the raw data before $\log_{10}$ transformation. Some graphs were obtained using "R" via RStudio version. Data that was normally distributed underwent a Pearson's product-moment correlation (r) and all other data [including ordinal data (Allen and Bennett, 2008)] underwent a Spearman's rho analysis (r_s).

If two or more variables were found to have significance for a measure of recruitment potential, a stepwise linear regression analysis was conducted using SPSS (Version 18) to identify the variable/s predictability. But if the variables were significantly correlated, a series of simple linear regressions were first conducted to find the best predictor variable/s. Then stepwise or enter/simple linear regressions were conducted with all possible predictor variables to find the best predictor model for the measure of recruitment potential. The resulting standardised residuals were evaluated against standardised predicted values for assumptions of normality, linearity and homoscedasticity.

5.3 Results

5.3.1 *Recent land management practices*

5.3.1.1 Management practises

Management of remnant grasslands has generally consisted of various forms of biomass reduction and ongoing efforts to limit weed infestations (Eddy, 2002, Verrier and Kirkpatrick, 2005, Wong and Morgan, 2007). The types of biomass reduction activities that occurred across the 16 study areas could be grouped into the following categories:

- Opportunistic (animals in the adjoining paddock eating the roadside reserve) and planned periodic or seasonal grazing;
- Controlled and accidental burning;
- Mowing; and
- Weed control.

For data analysis purposes, all forms of biomass reduction were collectively grouped except for the number of burns in a ten year period parameter. However, to accommodate the various degrees of biomass reduction that were associated with each category, a system of weighting was applied. Burning and mowing were considered to have resulted in almost the complete removal of above-ground vegetation, and land managers revealed that the level of biomass reduction was very high at the sites where weed control occurred. In contrast, grazing was most likely sporadic in nature, highly localised and not as complete in the removal of above-ground vegetation. For this reason a weight of 0.5 was given to the

analysis each time grazing was found to occur at a site, whereas a weight of 1 was used for all other forms of biomass reduction.

5.3.1.2 Site histories

Because study areas were selected from a range of jurisdictions and land tenures, the management regimes and associated record-keeping were unique to each site. Table 22 provides information about the source of land management records while table 23 provides a timeline of the management events at each study area between 1999 and 2010.

Table 22 – The source of information about recent land management practices at each study area.

Study area	Source of information about recent land management practices
Ararat AR	Verbal history from airfield owner and council Officer.
Bannockburn RR	CFA truck history. Verbal history from DSE Officer.
Baringhup WR	Verbal history from adjacent landholder. Site observations of plants and local conditions.
Brownswaterholes BRR	Verbal history from adjacent land holder.
Calder RRR	Verbal history from Parks Victoria staff.
Carisbrook BR	Verbal history from adjacent landholder. Site observations of plants and local conditions.
Cedarwoods R	Verbal history from on-ground works contractor.
Christies Rd	Verbal history from on-ground works contractor and local residents.
Geggies Rd	CFA truck history.
Glengower RR	Council's written historical documents and verbal history from the manager of the Council works crew.
Kirks BR	Verbal history taken from a contractor's written historical documents. Contractor managing the site accounts of work completed.
McKenzie Rd	Verbal history from local DSE Officer. Site observations of plants and local conditions.
Mt Mercer Rd	CFA truck history. Verbal history from adjacent landholder.
Pitfield CR	CFA truck history.
Poorneet WRR	Verbal history from the landowner and a CFA member.
Vite VRR	CFA truck history. Verbal history from adjacent landholder.

Table 23 – The management history of each study area over a twelve year period from 1999 until 2010, inclusive.

Site	Years											
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Ararat AR												
Bannockburn RR								20.03.06				
Baringhup WR												
Brownswaterholes BRR	Dec		Dec		Dec		Dec			Autumn		
Calder RRR									Autumn			
Carisbrook BR												
Cedarwoods R		Feb		5.04.02								
Christies Rd								Dec				
Geggies Rd	7.2.99	6.2.00			12.3.03	24.2.04			21.2.07	.4.08		
Glengower Rd	Dec	Dec	Dec	Dec	Dec	Dec	Dec	Dec				
Kirks BR												
Mt Mercer SR	Winter		Winter	March	Winter	March	Winter	March	Winter		Winter	
McKenzie Rd												
Pitfield CR								Autumn				
Poorneet WRR			Mar					12.04.06				
Vite VRR			April		March	March			Nov			

Key	
Type	Weighting
Fire	1
Graze	0.5
Mow	1
Weed control	1

An analysis of the temporal distribution of biomass reduction events found that in the nine years from 1999 to 2007, an average of 5.8 biomass reduction events per year occurred across the 16 study areas. This trend tapered to an average of only 1.6 biomass reduction events per year across the 16 study areas during the last three years 2008 - 2010 (Figure 56).

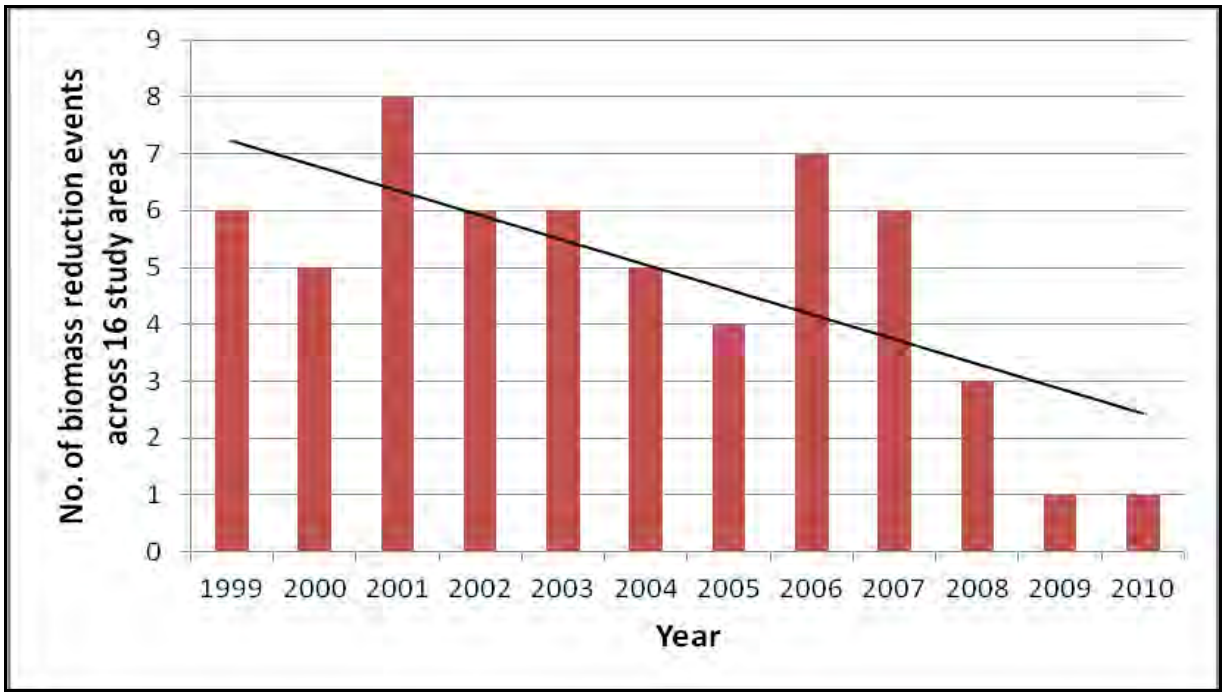


Figure 56 - The number of biomass reduction events occurring across the 16 selected study areas has declined in the last three of twelve years (1999 to 2010).

5.3.2 *Habitat condition*

The condition of vegetation was variable across the 16 study areas. 'Final habitat scores' ranged from 11 to 67, with an average of 55 (Table 24). The two sites which have the lowest scores have had frequent grazing pressure with no burns recorded in the last ten years.

Table 24 – The Habitat Hectare component scores for each of the study areas.

	Ararat AR	Bannockburn RR	Baringhup WR	Brownswaterholes BRR	Calder RRR	Carisbrook BR	Cedarwoods R	Christies Rd	Geggies Rd	Glengower Rd	Kirks BR	McKenzie Rd	Mt Mercer Rd	Pitfield CR	Poorneet WRR	Vite VRR
Understory	20	20	5	20	15	5	15	15	20	15	15	10	15	15	20	20
Lack of weeds	13	11	4	11	13	0	6	11	15	6	7	15	13	13	9	15
Recruitment	6	6	3	10	6	0	6	3	6	10	6	0	10	10	6	6
Organic litter	5	2	4	2	5	2	4	4	0	2	4	3	3	3	3	3
x standardiser 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36	x 1.36
SITE CONDITION SCORE (SCS)	59.84	53.04	21.76	58.48	53.04	9.32	42.16	44.88	55.76	44.88	43.5	38.08	55.76	55.76	51.68	59.8
Patch size	1	6	1	2	1	1	4	4	6	1	4	2	6	2	2	1
Neigbourhood	4	2.4	1.2	0.6	3.2	0	1	4.6	1.4	0.6	9.4	4.2	1.2	0.6	3.8	2.4
Distance to core area	1	4	3	2	4	1	4	4	4	3	5	5	4	3	5	1
LANDSCAPE CONTEXT SCORE (LCS)	6	12.4	5.2	4.6	8.2	2	9	12.6	11.4	4.6	18.4	11.2	11.2	5.6	10.8	4.4
FINAL HABITAT SCORE = SCS + LCS	66	65	27	63	61	11	51	57	67	49	62	49	67	61	62	64

5.3.3 *Mature plant mortality*

In the year 2009 – 2010, mature plant mortality (refer to the results in Chapter 2) was significantly and negatively correlated to the proportion of bare soil recorded at each of the study areas in spring 2010 r_s = -0.592, p = 0.016, n = 16 (Figure 57).

The average rate of mortality across all 16 study areas was four per cent between 2009 and 2010. A paired samples t test revealed a significant difference in the study area density of mature plants between 2009 and 2010 t (15) = 2.232, p = 0.041 (95 % CI 0.00089 to 0.03874), but it was a small effect size of less than one plant lost per site d = 0.05 (Cohen, 1988).

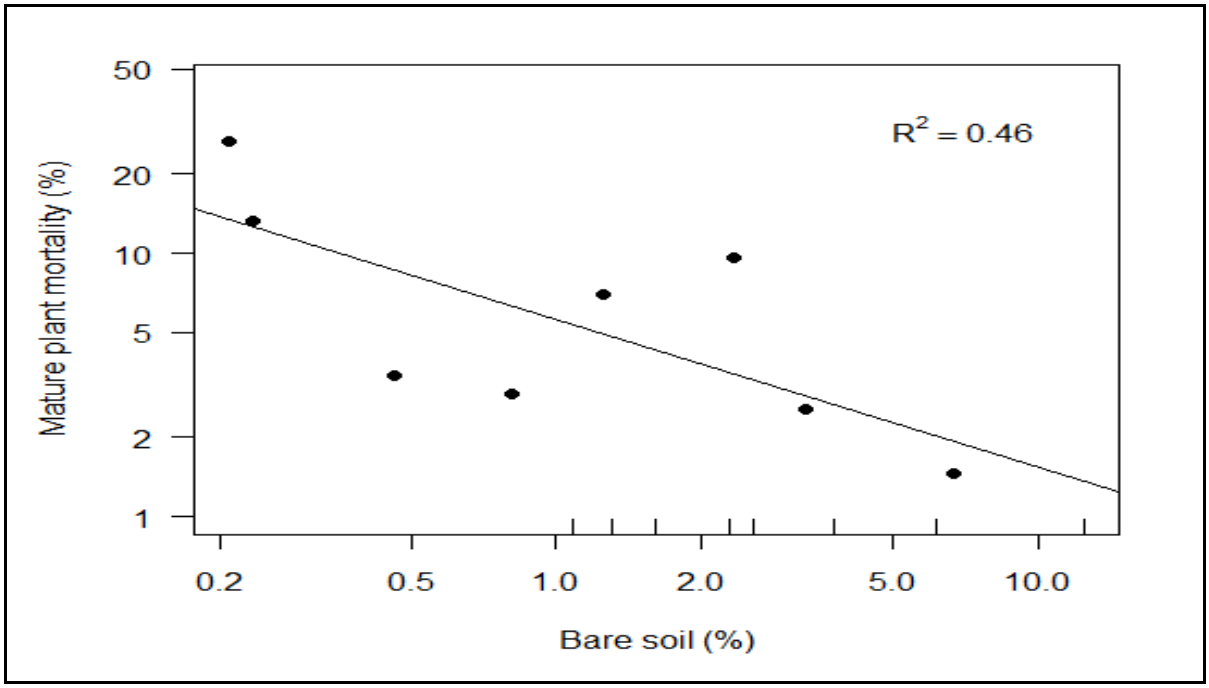


Figure 57 – The mortality of mature plants between 2009 and 2010 was less when there was a greater proportion of bare soil (%) at a study area (n = 16). Lines on the x axis represent zero values and both axes are $\log_{10}$ scale.

5.3.4 *Seed production*

All historical biomass reduction and habitat condition scoring parameters were analysed for relationships with seed production in the years 2009 and 2010. No significant relationships were detected.

The population structure was divided into life-stages and then flowering status which are presented in the results of Chapter 2. It was interesting to note that the proportion of non-flowering mature plants in quadrats was significantly greater in study areas that had fewer biomass reduction events within a five year period r_s = -0.542, p = 0.03, n = 16 (Figure 58).

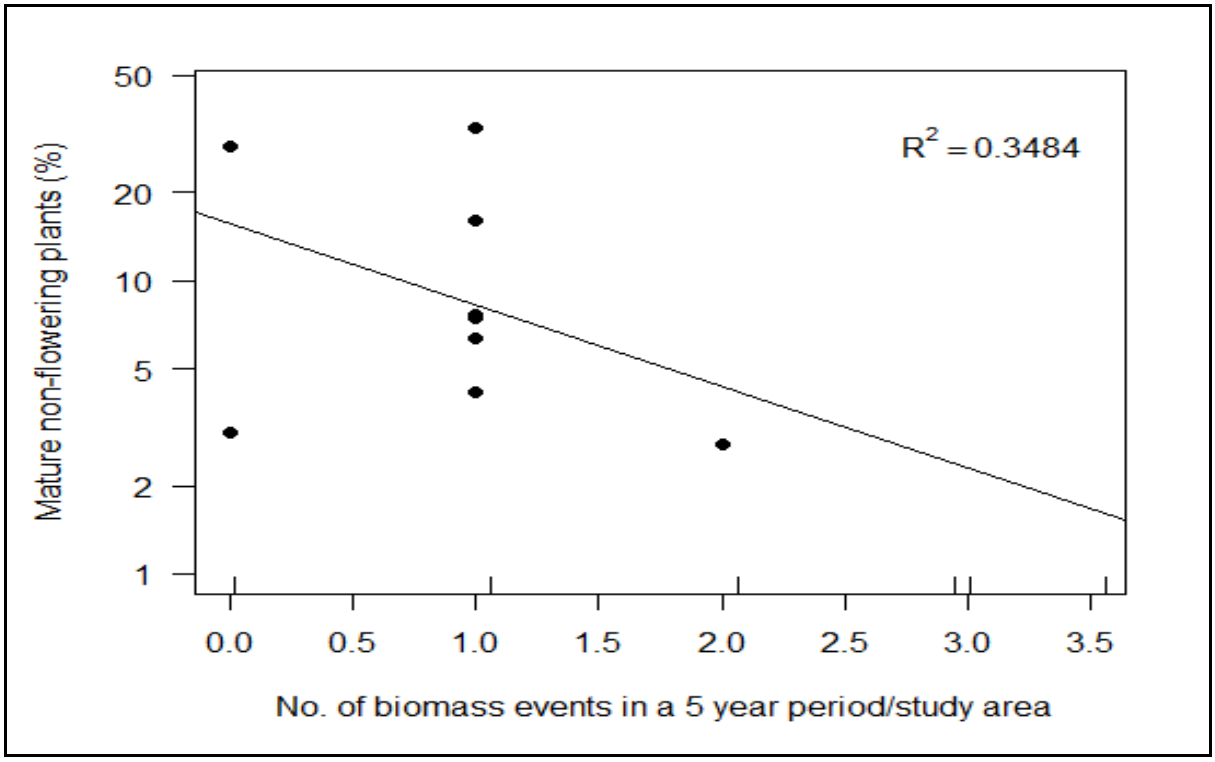


Figure 58 – Less frequent biomass reduction events are correlated with an increase in the proportion of non-flowering plants (n = 16). Lines on the x axis represent zero values for those particular study areas and the y axis is $\log_{10}$ scale.

5.3.5 *Seed viability*

For each year (2009 and 2010), the quality of seed produced was correlated to both the frequency of biomass reduction events and the number of years since biomass reduction. Greater seed viability was found at sites with an increased frequency of biomass reduction events within a ten year period for both 2009 r = 0.658, p = 0.006, n = 16 and 2010 r = 0.619, p = 0.018, n = 14 (Figure 59). Further to this, there was a significant negative correlation between seed viability and the time since biomass reduction events for both 2009 r = -0.613, p = 0.012, n = 16 and 2010 r = -0.542, p = 0.045, n = 14.

The seed viability data for both 2009 and 2010 was combined and entered as the dependent variable in a series of simple linear regressions for each of the two significant management variables. Of these, the model for the number of biomass reduction events in a ten year period was the best predictor of seed viability, accounting for 27.1 % of the variance R^2 = 0.271, F = (1, 28) = 11.761, p = 0.002 (Mahalanobis distance = 5.054 < 13.816).

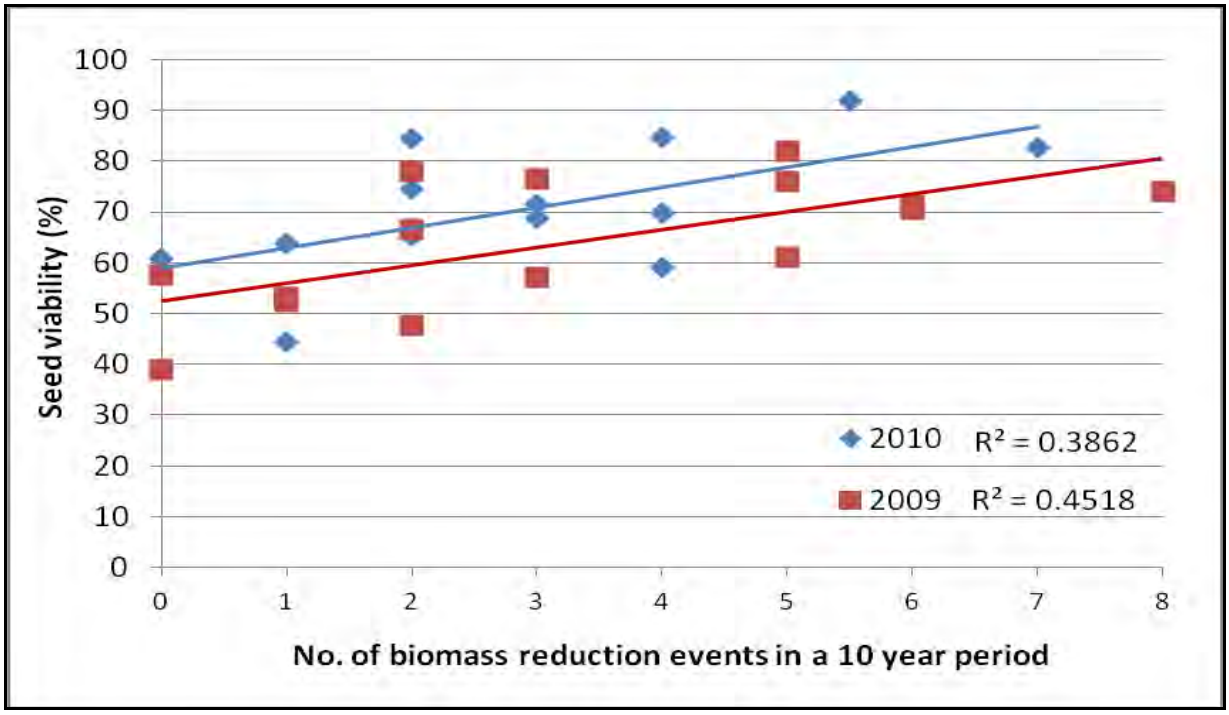


Figure 59 – A greater frequency of biomass reduction is associated with improved seed viability in 2009 (n = 16) and 2010 (n = 14).

As an indicator of seed viability, seed weight from 2009 samples was also found to be significantly and positively correlated to the number of burns in a ten year period r_s = 0.721, p = 0.002, n = 16 (Figure 60) and negatively associated with an increasing time since biomass reduction r_s = -0.53, p = 0.035, n = 16.

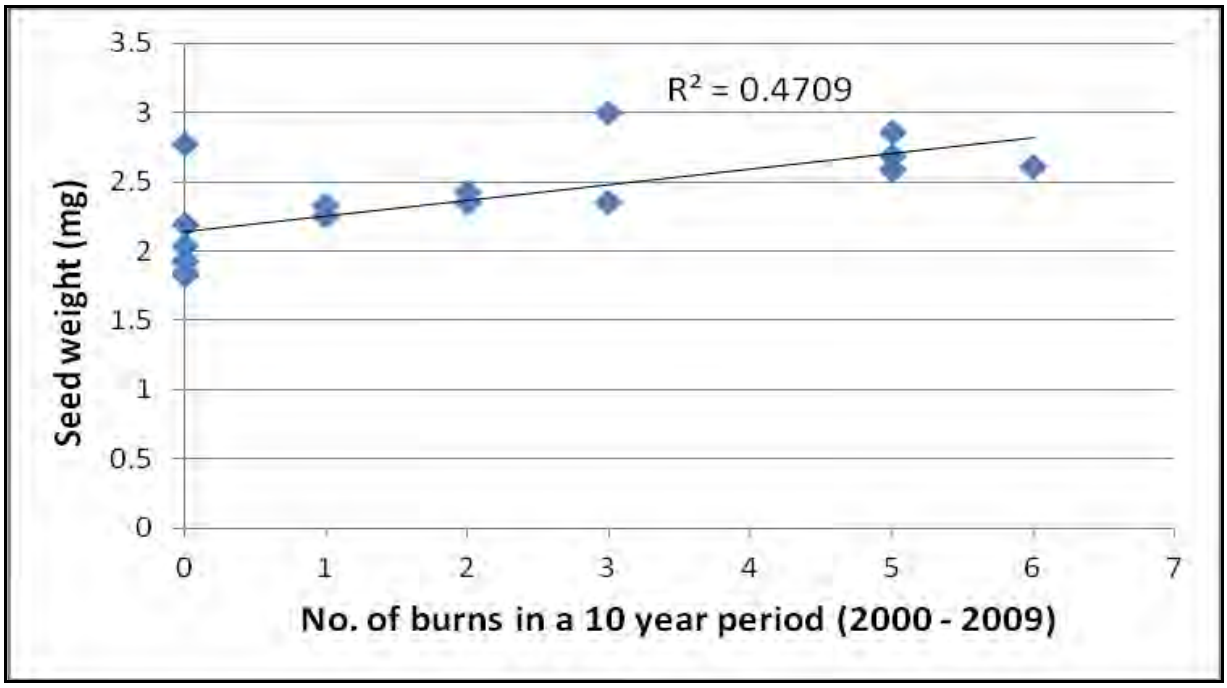


Figure 60 - A greater number of burns in a ten year period is associated with a greater seed weight (n = 16).

5.3.6 *Natality*

5.3.6.1 Germinant production

Of all the parameters assessed, the only one that was found to be associated with the *in situ* production of germinants was the time since biomass reduction events. In 2009, the production of germinants was negatively correlated to time since biomass reduction r_s = -0.531, p = 0.034, n = 16, although a similar trend was not observed in 2010. When the data was combined for both years a significant negative association was maintained r_s = -0.529, p = 0.002, n = 32. A rapid decline in germination density was observed at study areas where the time since biomass reduction events was greater than four years (Figure 61).

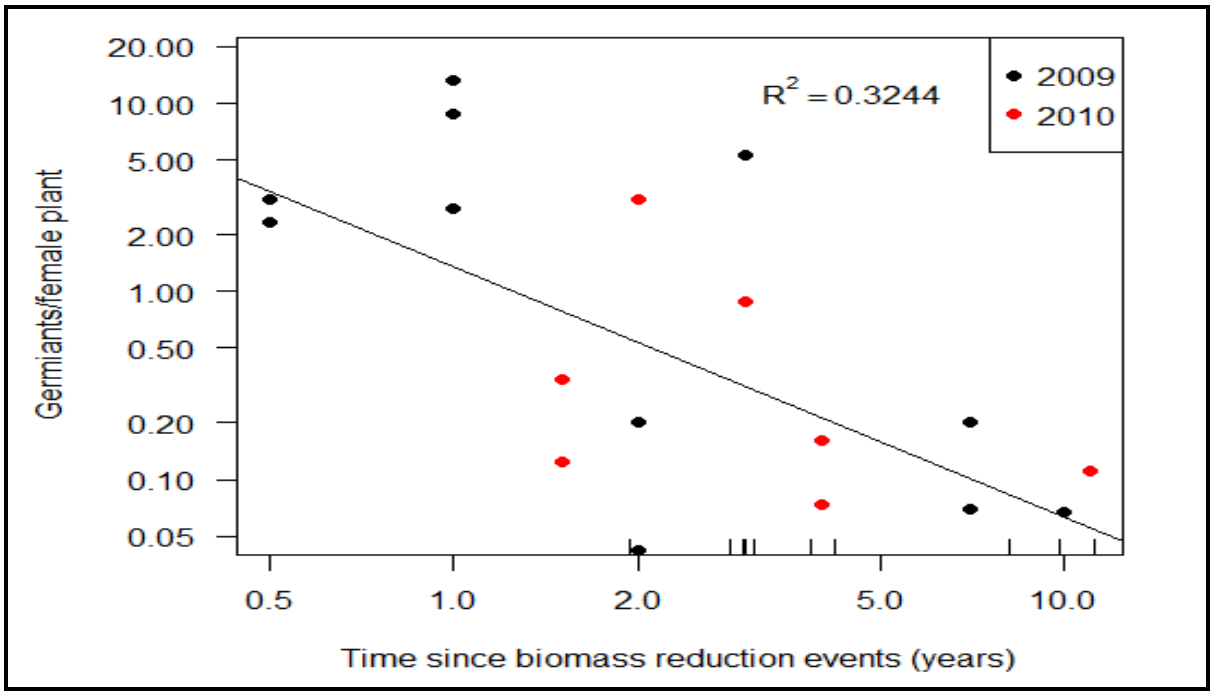


Figure 61 – Germinants production was greater at sites where biomass reduction had occurred recently (n = 32). Lines on the x axis represent zero values and both axes are $\log_{10}$ scale.

5.3.6.2 Germinant survival

In study areas where germination was recorded, the survival of germinants between spring 2009 and spring 2010 correlation analyses, identified significant associations with two of the Habitat Hectare components, the recruitment score and site condition score (SCS) and three other habitat condition measures, species diversity, weed cover and organic litter. These variables all relate to microsite conditions.

Each of the Habitat Hectare components and other habitat condition measures were significantly correlated to each other and therefore confounding. To determine which of the Habitat Hectare components or other habitat condition measures were most strongly associated with germination survival a series of simple linear regressions were conducted for each significant habitat variable identified. Of the five simple linear regressions, all detected a significant association with germination survival and are listed in order of the contribution that they made to the model (greatest to least):

- Site condition score had a positive effect on germination survival R^2 = 0.883, F = (1, 10) = 67.926, p < 0.0001 (Mahalanobis distance = 5.301 < 13.816), predicting 88.3 % of the variance (Figure 62);
- Recruitment score had a positive effect on germination survival R^2 = 0.746, F = (1, 10) = 26.461, p = 0.001 (Mahalanobis distance = 4.268 < 13.816), predicting 74.6 % of the variance (Figure 63);
- A greater weed cover had a negative effect on germination survival R^2 = 0.724, F = (1, 10) = 23.621, p = 0.001 (Mahalanobis distance = 2.369 < 13.816), predicting 72.4 % of the variance (Figure 64);

- A greater diversity of species had a positive effect on germination survival R^2 = 0.65.4, F = (1, 10) = 17.031, p = 0.003 (Mahalanobis distance = 2.889 < 13.816), predicting 65.4 % of the variance (Figure 65);and
- The presence of organic litter had a negative effect on germination survival R^2 = 0.633, F = (1, 10) = 15.508, p = 0.003 (Mahalanobis distance = 5.375 < 13.816), predicting 63.3 % of the variance (Figure 66).

All habitat condition variables predicted similar variances in the models for germinant survival, ranging from 63.3 % to 88.3 %. Considering that the variables of species diversity, organic litter, recruitment score and to a degree weed cover are all components of the site condition score, it would seem that they are each making a contribution and that all the components of the overall site condition score are contributing to the germinant survival model.

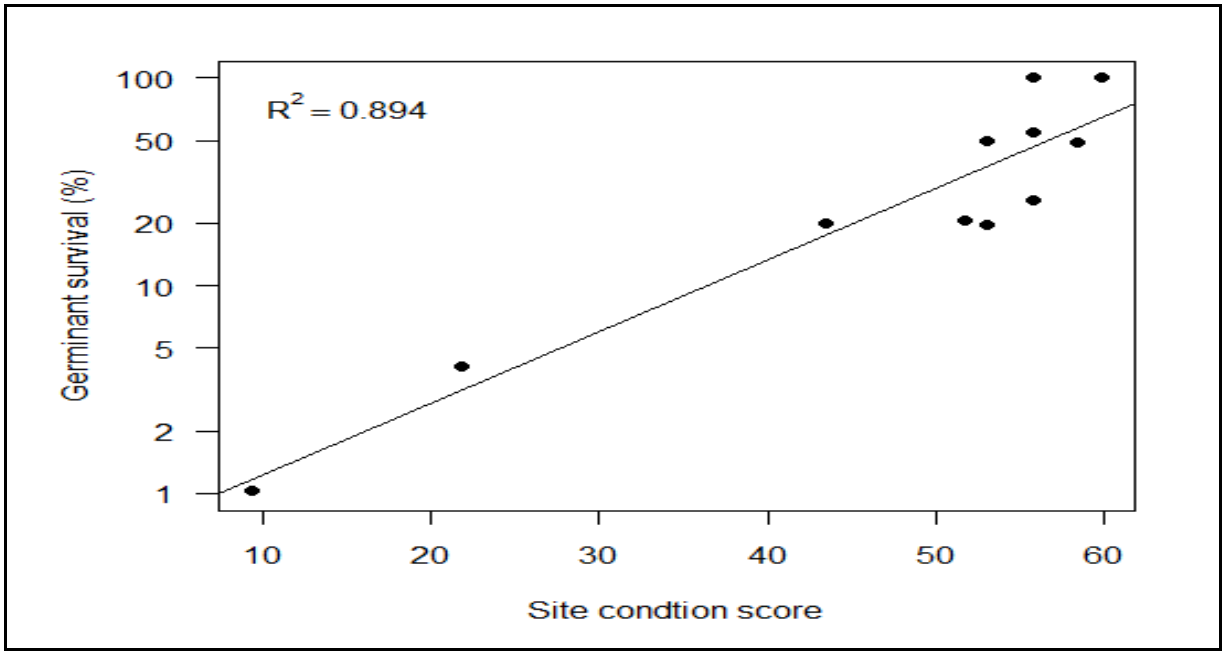


Figure 62 – Germinant survival was greater at sites which had a high site condition score (n = 11). The y axis is a log_{10} scale.

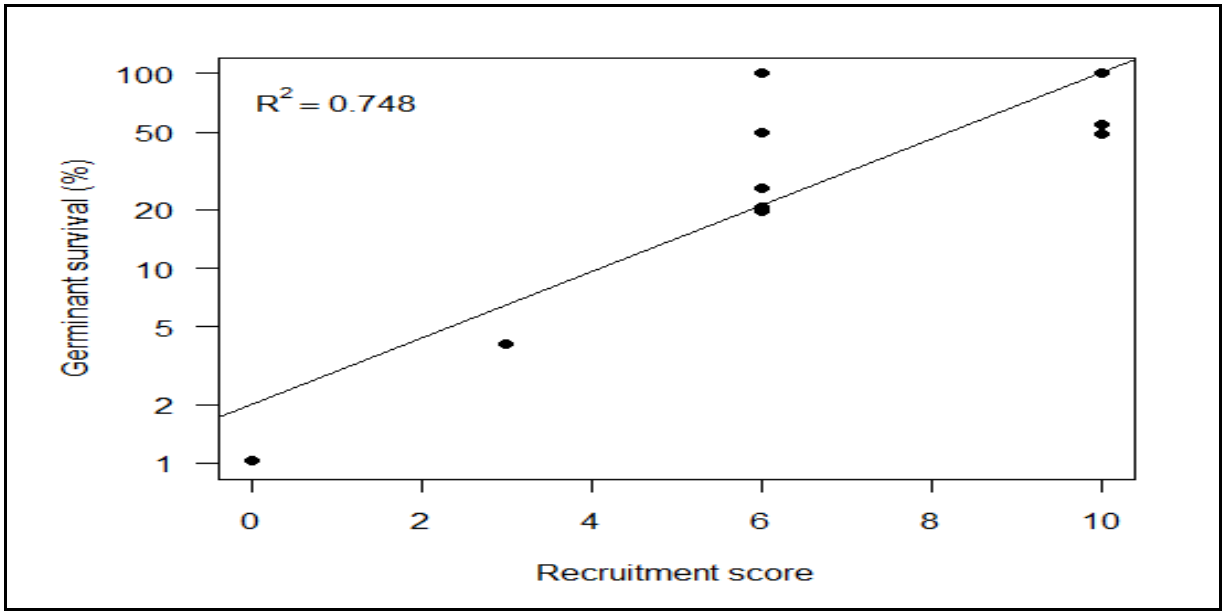


Figure 63 – Germinant survival was greater at sites which had a greater recruitment score (n = 11). The y axis is a log_{10} scale.

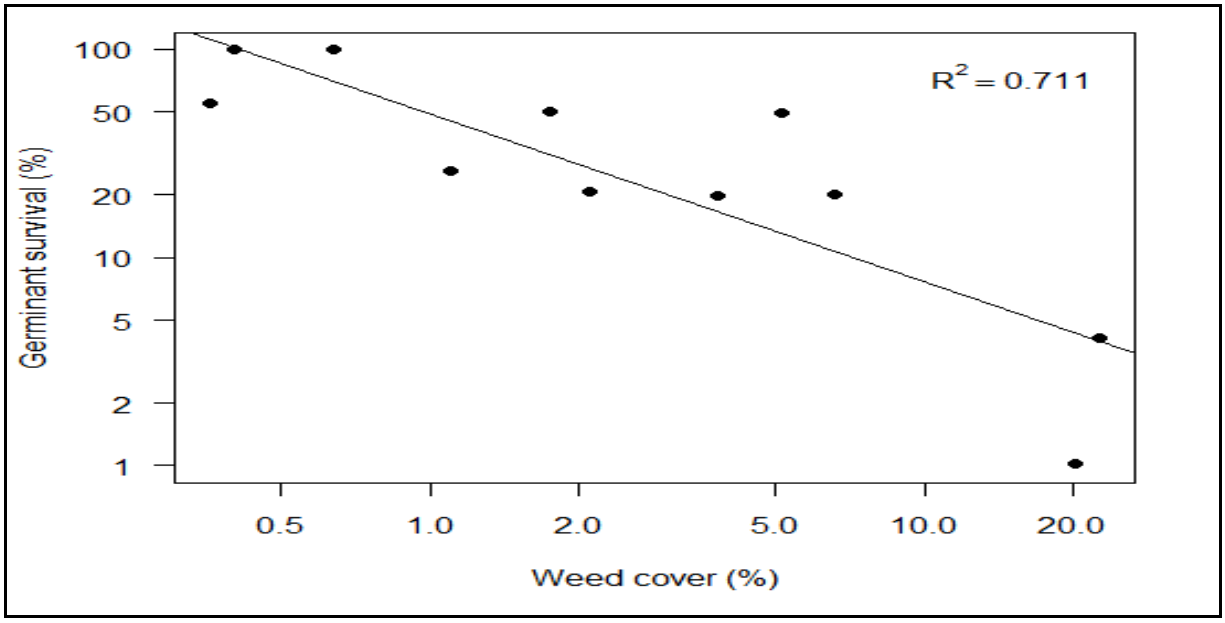


Figure 64 – Germinant survival was greater at sites which had less weed cover (n = 11). Both axes are log_{10} scale.

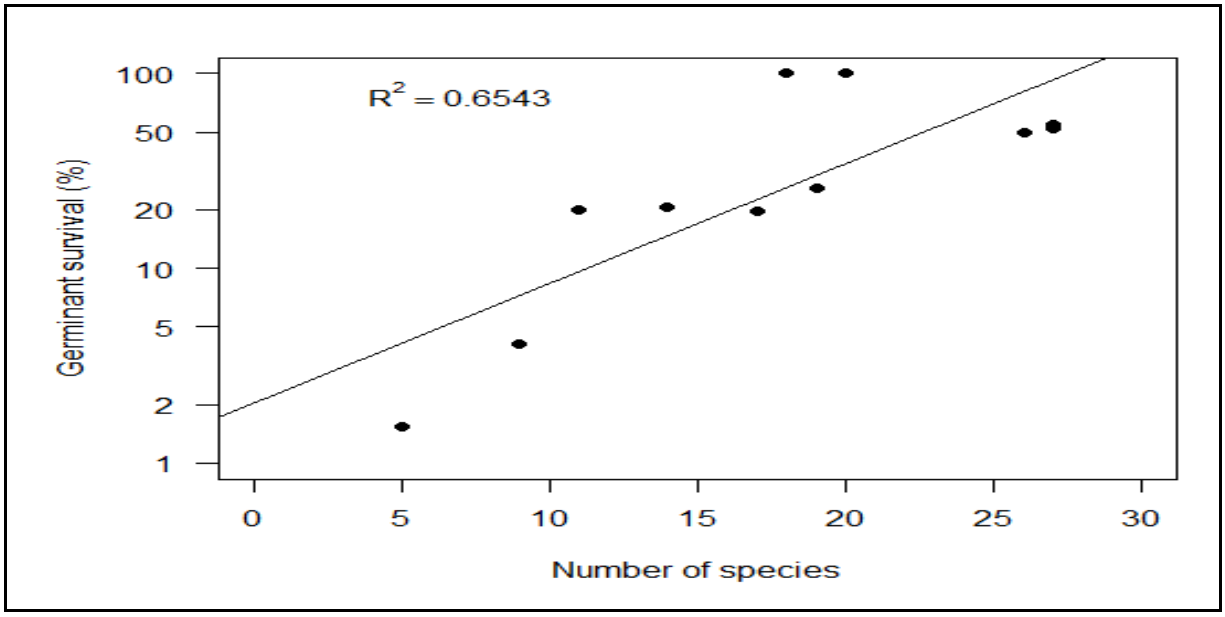


Figure 65 – Germinants survival was greater at sites which had a greater species diversity (n = 11). The y axis is a log_{10} scale.

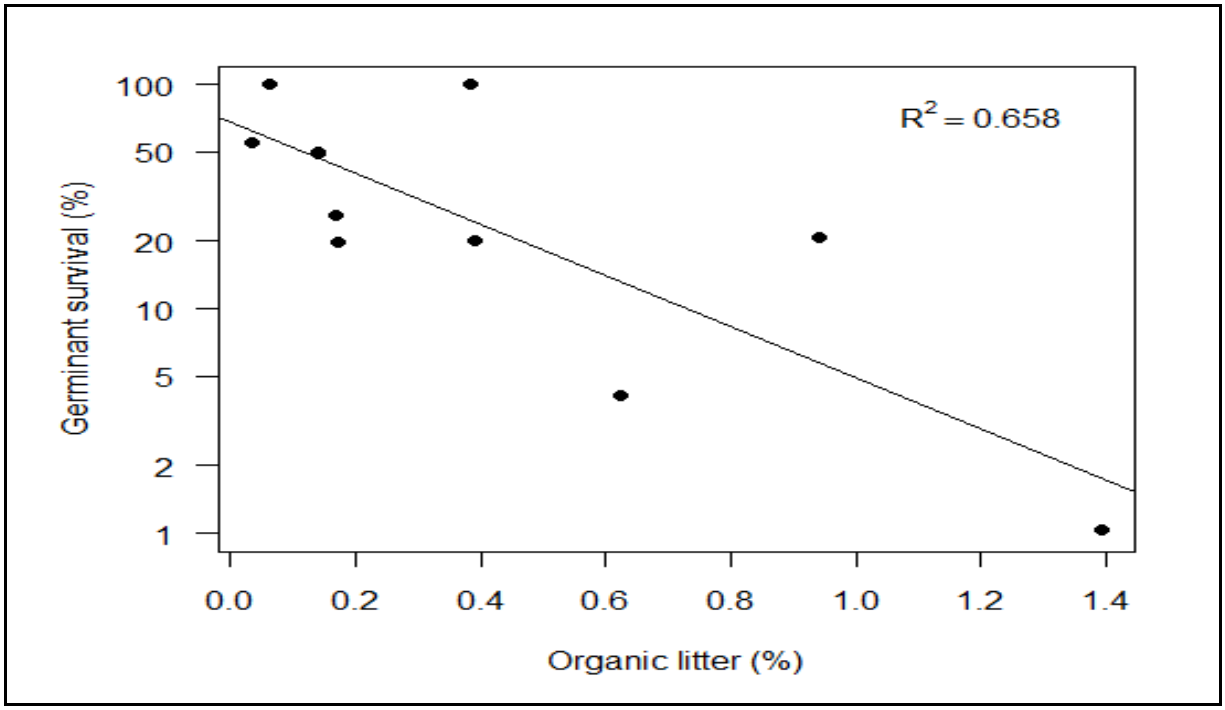


Figure 66 – Germinants survival was greater at sites which had a low organic litter cover (n = 11). The y axis is a $\log_{10}$ scale.

Although germinant survival was not correlated directly with any of the habitat management variables, the Habitat Hectare site condition score variable was found to be significantly and positively associated with the frequency of burn events in a ten year period $r_s = 0.652$, $p = 0.006$, n = 16.

5.4 Discussion

Habitat management appears to be an important feature in the reproductive capacity of *P. spinescens* populations. Some measures of recruitment potential were directly associated with the frequency of and time since biomass reduction events, whereas others were associated with some of the measurable effects of habitat management, such as increased bare soil, reduced litter, reduced weeds and an improved site condition.

5.4.1 *The function of biomass management*

Native species diversity is greatest in grasslands which have had regular biomass reduction events (Scarlett, Wallbrink *et al.*, 1992, McDougall and Kirkpatrick, 1994) and there is little research evidence of poor outcomes for native species at sites that have been biomass reduced by fire annually or biennially (Lunt and Morgan, 2002).

Whilst the benefits of grassland management have been recognised in the context of temperate Australia (McDougall, 1989, Lunt, 1997b, Craigie and Hocking, 1998, Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2005, Williams, Morgan *et al.*, 2006, Australian Government, 2011), there is evidence from around the globe that biomass reduction is important in the maintenance of grassland biodiversity (Knapp and Seastedt, 1986, MacGregor, 2005, Pärtel, Bruun *et al.*, 2005). For example, in grasslands in the Czech Republic it has been found that annual clipping or mowing of the dominant grass species *Festuca ruba* (Red fescue) enabled the inter-tussock species to increase in biomass and positively contributed to the maintenance of biodiversity (Herben, Krahulec *et al.*, 2003).

To understand the function of biomass reduction in the grasslands of temperate Australia requires knowledge of the composite life-forms and their function. In annually burnt Victorian

grasslands, Morgan (1999a) classified 64 % of the perennial species as resprouters (Bell, Hopkins *et al.*, 1984, McIntyre, Lavorel *et al.*, 1995), which undergo a process of vegetative regrowth following the removal of biomass (Lunt, 1990a, Morgan, 1996, Lunt and Morgan, 2002). Resprouters in the grassland can be further grouped into the dominant perennial tussock grasses and the inter-tussock species. The perennial tussock grasses tend to have shallow roots, whereas many inter-tussock species, such as *P. spinescens*, tend to be deep-rooted with underground storage organs such as taproots or tubers (Weaver, 1958, Sun, Coffin *et al.*, 1997).

Following a biomass reduction event, the starch and other nutrients within the storage organs of the inter-tussock species provide a temporary advantage over the grasses by enabling rapid vegetative growth (Pate, Froend *et al.*, 1990, Schultze, Beck *et al.*, 2002). During the initial recovery period the inter-tussock species are able to increase their photosynthetic surface area, occupy space within the environment and access other resources. The dominant shallow-rooted grasses (Sun, Coffin *et al.*, 1997), have little storage capacity and take longer to re-exert their influence. For example, immediately following a biomass reduction event (mowing) in grasslands in the Czech Republic, it was found that the roots of the inter-tussock species were able to temporarily dominate below ground space over the resident grassy species (Smilauerova and Smilaer, 2006).

In productive grassland ecosystems biomass is accumulated, with dead matter building up over time in the absence of any biomass reduction events (Morgan and Lunt, 1999, Prober, Thiele *et al.*, 2007, Schultz, Morgan *et al.*, 2011, Lunt, Prober *et al.*, 2012). Closed grass swards reduce the capacity for inter-tussock perennials such as *P. spinescens* to function by

limiting their access to space, light (PAR - Photosynthetic Active Radiation), moisture and nutrients (Grubb, 1977, Grime, 1979, Tremont and McIntyre, 1994, Morgan, 1999b, 2001).

Some examples of competitive exclusion processes are derived from the grasslands of the United States. Over-grazed American prairies had a high species diversity with the dominant grass species accounting for only 20 – 26 % of the biomass, in comparison to un-grazed prairies which had a low species diversity with the dominant grass species comprising 60 % of the standing biomass (Platt, 1975). In the grasslands of California, the native dicots were found to grow vigorously in the season following a biomass reduction event [fire] (Pollak and Kan, 1998). In subsequent years, the dominant grass *Andropogon gerardii* (Big bluestem) accumulated litter, reducing the availability of PAR, and thereby governing the energy flow of the whole tallgrass prairie system and its inter-tussock species responses (Knapp and Seastedt, 1986).

In the grasslands of the Victorian volcanic plain, the dominant species, *Themeda triandra* (Kangaroo grass), accumulates biomass of 4 ton ha^{-1} within a period of two to four years, which reduces PAR availability to the soil surface to less than 10 % (Lunt and Morgan, 2002). Effectively, *T. triandra* captures the resources of space, light, nutrients and moisture (Tremont and McIntyre, 1994). Accumulation of leaf litter not only restricts the capacity for the growth of inter-tussock species such as *P. spinescens* but eventually hinders new vegetative growth of the *T. triandra* tussock, leading to senescence. In long unburned grasslands (>11 years) up to 75 % of *T. triandra* tussocks have died as a result of this process, which is followed by a massive invasion of weeds (Groves, 1974, Lunt, 1995, Morgan and Lunt, 1999, Morgan, 1999b, 2001). Invasive species of weeds can also monopolise resources (such as water), competitively limiting the availability of these

resources to smaller inter-tussock species such as *P. spinescens* (Lenz and Facelli, 2005, Lunt, Prober *et al.*, 2012).

Incorporating regular biomass reduction events, in its many forms, into management practises is important for maintaining and promoting grassland biodiversity over-time.

5.4.1.1 Mature plant mortality

The mortality of mature *P. spinescens* plants was greatest at study areas that had a low coverage of bare soil. Under the Habitat Hectares system the proportion of bare soil is an indicator of recruitment potential, however the amount of bare soil could also be interpreted as an indicator of biomass management practices and is likely to be correlated to the cover of litter and biomass (not measured in this study). For example, in the grasslands of Australia, increased bare soil, exposed soil surface or gaps occur as a result of management practises such as frequent fire (Morgan, 1997, 1998c, Lunt and Morgan, 2002, Prober, Thiele *et al.*, 2007), and disturbances such as animal foraging (Gilfedder and Kirkpatrick, 1994d, Gilfedder and Kirkpatrick, 1994c, Pyrke, 1994, Clarke, Myerscough *et al.*, 1996).

At the study areas with greatest mortality of mature *P. spinescens*, it is probable that the low coverage of bare soil was a result of a combination of the frequency of biomass management activities and the associated biomass loads (not measured). Essentially, competition for the limited resources of light, nutrients and water, especially during the extended drought [from 1996 to the end of 2009 (National Meteorological Service, 2008)], might have depleted the reserves for some highly stressed mature *P. spinescens* individuals.

Lack of management and an extended maximum inter-fire interval of greater than five to six years have been found to result in the loss of grassland species over time (Lunt and Morgan, 1999, Morgan and Lunt, 1999, Williams, Morgan *et al.*, 2006). Further research on the causes of mature plant mortality and associations with biomass loads and management activities could confirm whether *P. spinescens* is similarly affected. Without recruitment, the death of mature *P. spinescens* plants will lead to a reduction in the size of the effective population over time, and therefore the reproductive potential of those populations.

5.4.1.2 Seed production and flowering

This study did not detect relationships between seed production and any of the management parameters assessed, or between seed production and the proportion of non-flowering individuals at a study area. However, a negative relationship was detected between the proportion of mature non-flowering plants and the frequency of biomass reduction. The less frequently biomass reduction occurred, the greater the proportion of mature non-flowering individuals. Intuitively, it would be expected that this would lead to an overall reduced rate of seed production within these populations because non-flowering plants are not reproductively functional (Hurtt and Pacala, 1986, Tilman, 1997). As suggested by Fenner (2005), increased flower production presumably provides opportunities for the increased production of seed for germination (Fenner and Thompson, 2005) but the proportion of flowering/non-flowering *P. spinescens* did not seem to be related to the seed production recorded for each study area.

This research found that within each study area there are a small proportion of female plants which are producing large quantities of seed, which promoted greater *in situ* germinants,

therefore making a greater contribution to the recruitment potential of the population than most of the other females. The superior productivity could be related to *P. spinescens* subdioecious breeding system (Lewis, 1941, Delph and Carroll, 2001, Shykoff, Kolokotronis *et al.*, 2003, Bailey and Delph, 2007). These high productivity plants could be true females (functionally only female) as opposed to the varying degrees of hermaphrodites which were labile in their presentation and inadvertently sampled. The importance of these high productivity females needs to be robustly evaluated, particularly in relation to management actions. Monitoring the flowering status of these particular individuals could identify important thresholds which will alert managers to the future decline of the *P. spinescens* population.

The importance of the relationship between biomass reduction and flowering capacity has been demonstrated in other studies of the flora of the temperate grasslands of Victoria (Lunt, 1994, Lunt and Morgan, 2002). An increased level of biomass by the dominant grass (*T. triandra*) was found to negatively affect the ability of inter-tussock species to access resources and led to a reduction in their ability to flower (Lunt and Morgan, 2002). Furthermore, it was demonstrated that a spring burn promoted flowering in many of these grassland species, although it is unclear whether this is a direct result of biomass reduction. In Canada, a positive association was detected between an open tallgrass prairie canopy and flowering individuals for the herb *Viola pedala* (Bird's-foot Violet), implying that the increased resources of light and possibly water had influenced the flowering capacity in this species (O'Dell, 1996, Thompson, 2006).

As with this study, Foreman (2005, 2011) also found many mature non-flowering individuals of *P. spinescens* at sites with no recent biomass reduction events. At sites supporting *P. spinescens*, the implementation of regular biomass reduction activities would, at the very

least, increase the opportunity for seed production as a result of all individuals having similar access to the available resources.

5.4.1.3 Seed viability

The contribution of greater quality seed to the seed bank has been found to improve a species long-term survival within a site (Stocklin and Fischer, 1999). The level of seed viability achieved by a plant is a reflection of many factors such as its genetics, pollination success, the population size/density, plant size/stage, the proportion of flowering plants, population structure, breeding system and environmental conditions (rainfall, temperature patterns) (Fenner and Thompson, 2005).

An additional factor that can influence seed viability is the access of the maternal plant to sufficient resources during seed development (Fenner and Thompson, 2005). This study found that although the seed viability of *P. spinescens* was consistent between study areas and study years, greater levels of viability were positively associated with the number of biomass reduction events that occurred in the preceeding years, suggesting that variations in seed viability are associated with resource availability.

Seed viability is rarely reported in research, except following germination experiments (Dixon, Roche *et al.*, 1995, Clarke, Davison *et al.*, 2000, Willis, McKay *et al.*, 2003, Gibson Roy, Delpratt *et al.*, 2007a) and in relation to seed bank studies (Lunt, 1995, Morgan, 1995a, Lunt, 1996). Few studies report on seed viability in relation to management practices. Research in the grasslands of California found that management practices improved the growing conditions of the maternal plants of *Nassella pulchra* (Bunchgrass), resulting in the production of greater seed viability. Removal of biomass via grazing improved seed viability

of this species but the complete removal of all biomass via fire stimulated the *N. pulchra* to produce an even greater quality seed the following season (Dyer, 2002).

Although seed viability does not appear to be a limiting factor in the reproductive ecology of *P. spinescens*, greater seed viability is likely to be associated with the maternal plant's ability to access resources, which might be improved as a result of frequent biomass reduction events.

5.4.1.4 Seed to germination

The production of *P. spinescens* germinants was negatively associated with the time since biomass reduction, as the greater the time period between biomass reduction events, the lower the density of germinants. A period of greater than four years appears to greatly diminish a population's capacity for the production of germinants. Similarly, Foreman (2011) only found germinants at sites where the time since the last burn was no more than 3½ years.

Because the recruitment of perennial inter-tussock species from seed is a rare occurrence in the temperate native grasslands of Victoria (Pyke, 1993, Morgan, 1998a, Morgan, 1999a), it is important to understand the conditions that provide the greatest opportunity for germination to occur. Previous research in these grassland communities has indicated that germination of inter-tussock perennials: is promoted by fire (Lunt, 1994, 1997b, Morgan, 1998c, 2001); requires an inter-tussock gap of greater that 300 cm² (Morgan, 1997, 1998c); and occurs in open areas of disturbed soil that provide favourable recruitment microsites (Robinson, 2003, Reynolds, 2006, Gibson Roy, Delpratt *et al.*, 2007b). Clearly biomass reduction is a significant contributor to these germination conditions.

Biomass reduction via fire has been found to induce an abundance of flowers and seed, leading to the germination of grassland flora in the bare soil or inter-tussock gaps the following year (Lunt, 1994, 1997b, Morgan, 1998c, 2001). Through this process, the following species have been documented to germinate in the inter-tussock gaps in the natural temperate grasslands of Victoria: *R. leptorrhynchoides* (Morgan, 1997), *Podolepis* sp. 1 (Basalt Podolepis), *Bulbine semibarbata* (Leek lily) (Robinson, 2003) and *Leucochrysum albicans* (Hoary Sunray) (Gilfedder and Kirkpatrick, 1994d, Gilfedder and Kirkpatrick, 1994c). Biomass reduction is thought to provide greater opportunities for the germination of inter-tussock species by creating soil microsites with available space, suitable surface moisture, a high soil/seed coverage, and a favourable light and temperature regime (Grubb, 1977, Primack and Miao, 1991, Pyrke, 1994, Morgan, 1995a, Mouquet, Leadly *et al.*, 2004, Fenner and Thompson, 2005). Although the *in situ* germination of *P. spinescens* was not found to be associated with any particular habitat characteristic, it seems that a frequent biomass reduction process is contributing to at least some of the conditions that promote the germination of this species. Given that *in situ* germination appears to be one of the most critical aspects of the reproductive ecology of *P. spinescens*, a program of regular biomass reduction is an important management feature in working towards the maintenance of flourishing and sustainable populations.

5.4.1.5 Germinant survival

For *P. spinescens* populations to be viable in the long-term, they must not only produce germinants but some must survive to become reproductive themselves (Falk, Miller *et al.*, 1996). This research has found that germinant survival for up to a year following germination was greatest at sites that had a high site condition score, as well as and including the

metrics of a high cover of bare soil, a low cover-abundance of weeds and a reduced amount of organic litter. In turn, these metrics can all be related to the regime of biomass management (Evans and Young, 1972, Fowler, 1988, Scarlett, Wallbrink *et al.*, 1992, McDougall and Kirkpatrick, 1994, Clements, Benoit *et al.*, 1996, Baar and Kuyper, 1998, Morgan and Lunt, 1999, Lofgren, Eriksson *et al.*, 2000, Prober, Thiele *et al.*, 2007, Schultz, Morgan *et al.*, 2011, Lunt, Prober *et al.*, 2012). Studies of other grassland flora have found that the likely causes of germinant mortality were water stress (Gilfedder and Kirkpatrick, 1994b, Morgan, 1995a), competition (Hitchmough, Curtain *et al.*, 1996, Huddleston and Young, 2005) and predation (Morgan, 1995a, Edwards and Crawley, 1999). Regular biomass management is likely to ameliorate some of these effects, resulting in improved germinant survival.

Site condition score and species diversity

Local habitat conditions appear to affect the survival of *P. spinescens* germinants, which was positively associated with the site condition score – a sub-component of the Habitat Hectares score. The "site condition score" included some of the metrics (species diversity, weeds, litter and bare soil) that were also found to be associated with germinant survival. None of the other sub-components, such as the "landscape context score" and "final habitat score", or the final value "Habitat Hectare score", appear to be related to the recruitment potential of a *P. spinescens* population.

The site condition score was also found to be positively associated to the frequency of burns in a ten year period. This is not surprising given that the metrics that contribute to the site condition score are likely to score higher when appropriate biomass management practices

are implemented (Tremont and McIntyre, 1994, McIntyre and Martin, 2001, Parkes, Newell *et al.*, 2003). For example, it has been demonstrated that a lack of biomass management can result in a loss of species over time (Williams, Morgan *et al.*, 2006). In contrast, sites (such as rail reserves) where the biomass was regularly reduced, maintained a greater species diversity which would positively contribute to the overall site condition score (Stuwe and Parsons, 1977, Lunt and Morgan, 2002).

This study found that the survival of *P. spinescens* germinants was positively associated with increased species diversity. Foreman (2011) also found that increased fire frequencies in *P. spinescens* populations were positively associated with increased numbers of indigenous species. It would appear that frequency of biomass management is associated with the maintenance of sites species diversity, including *P. spinescens*.

Rather than conducting a full Habitat Hectares assessment, future monitoring of *P. spinescens* populations should evaluate the metrics (species diversity, weeds, litter and bare soil) that are likely to be important for recruitment. These metrics may be used as indicators for applying the principals of adaptive management (Lindenmayer and Likens, 2009, Mackenzie and Keith, 2009) to biomass reduction practices at sites supporting populations of *P. spinescens.*

Bare soil

The survival of *P. spinescens* germinants was positively associated with a greater presence of bare soil. Bare soil or an increased grassland gap size has previously been found to be positively associated with improved germinant survival for many species in the grasslands of Victoria (Morgan, 1997, 1998c) and worldwide (De Jong and Klinkhamer, 1988, Peart, 1989,

Hobbs and Huenneke, 1992, Tilman, 1997, Turnball, Crawley *et al.*, 2000, Zobel, Otsus *et al.*, 2000, Wirth and Pyke, 2003, Hofmann and Isselstein, 2004, Eckstein, 2005). Bare soil is created and maintained by disturbance processes such as fire, animal diggings, mowing and weed control. For example, when sufficient moisture is available, successful recruitment of forbs in grasslands of the United States has been found to be associated with the creation of bare soil, either as a result of the digging activities of pocket gophers or by the removal of biomass through fire (Peart, 1989, Tilman, 1997). In Europe, soil disturbance and periodic removal of biomass via mowing or weed killing were the most significant factors in recruitment of forbs, providing that sufficient water was available over the summer months (De Jong and Klinkhamer, 1988, Hobbs and Huenneke, 1992, Turnball, Crawley *et al.*, 2000, Zobel, Otsus *et al.*, 2000, Wirth and Pyke, 2003, Hofmann and Isselstein, 2004, Eckstein, 2005).

In New South Wales (Clarke, Myerscough *et al.*, 1996) and Tasmania (Pyrke, 1994), it was found that diggings by small mammals created shallow holes and mounds that were significantly associated with the recruitment of local forbs, at the expense of perennial grasses. Forb seedlings were more prevalent on mounds, which were characterised by having a lack of litter, warmer soil, greater light penetration and less above and below ground competition from surrounding plants (Platt, 1975, Collins, 1989, Reader and Buck, 1991). It is possible that a similar set of characteristics associated with the maintenance of bare soil are important to the survival of *P. spinescens* germinants.

Weed cover

Weeds are exotic dominant species that can out compete indigenous species for space and other resources (Carter and Walsh, 2006, Thomas, 2006, Victorian Government Department of Sustainability and Environment, 2008, Mueck, 2009). Once weeds are established they can be superior to indigenous species due to their invasive nature, short growth period and high seed production (Carr, Yugovic *et al.*, 1992). In both Europe and Australia, weeds have been found to negatively affect both *in situ* germination (Pywell, Bullock *et al.*, 2002) and establishment (Waters, Whalley *et al.*, 2001, Cole and Lunt, 2005) of grassland species. Many of the weeds found in Australia are of European origin and have a relatively short but rapid growth period leading to maturity in the Australian winter/early spring (Hobbs and Hopkins, 1990, Richardson, Richardson *et al.*, 2006). The growth period of these weeds coincides with the timing of germination and active growth of *P. spinescens* seedlings, placing them in direct competition with each other for resources. Foreman's study (2011) found that increased fire frequencies (biomass reduction events) were negatively associated with exotic cover. Without active management, weeds are likely to persist and compete with *P. spinescens* for resources, negatively impacting on the survival of germinants and ultimately the future recruitment potential of populations of this species.

Litter

The survival of *P. spinescens* germinants was negatively associated with the amount of organic litter at each study area. Litter has been found to reduce the survival rates of seedlings of several inter-tussock forb species in American woodlands, (Fowler, 1988, Baar and Kuyper, 1998). Similar effects have been documented in temperate grasslands (Morgan, 1995a, Hitchmough, Curtain *et al.*, 1996), although Bosy (1995) suggested that the effects are likely to be species specific. *Pimelea spinescens* appears to be one of the species which is negatively affected by the presence of litter, which reduces the amount of bare soil, occupies space, reduces sunlight penetration, reduces water evaporation and adds nutrients to the soil (Morgan, 1999b, 2001, Ashman and Puri, 2002, Brearley, Press *et al.*, 2003, Murphy, Lodge *et al.*, 2004). Because the temperate grasslands of Victoria have evolved under a regime of regular fire (Mutch, 1970, Walker, 1981, Bradstock, Williams *et al.*, 2002, Attiwill and Wilson, 2003), large amounts of organic litter would have been an uncharacteristic feature. It is postulated that indigenous grassland species such as *P. spinescens* have not adapted strategies to cope with the competitive effects of large amounts of organic litter.

In summary, germinant survival is associated with a range of habitat characteristics that can be maintained by regular biomass reduction practices, even though the direct measures of biomass reduction used in this study were not found to be significantly associated with germinant survival. Only 14 % of germinants survived their first year *in situ* and it is unknown how many continued to survive and are contributing to the reproductive potential of the population. The ability of a germinant to survive is vital knowledge that is currently lacking,

which could provide information about the future potential sustainability of a *P. spinescens* population.

5.4.2 *Case study - Carisbrook BR*

During 2009, plants at the Carisbrook BR study area were found to exhibit a very large seed production (at least 60 to 424 seeds per stem), resulting in numerous germinants (389 germinants, comprising 49 % of the total germinants produced by all study areas). The bulk of this production was from three contributing plants that each produced in excess of 349 seeds per stem, a rate that was unrivalled by any of the other plants in this study (the next greatest seed production was on a plant at the Glengower Rd study area which produced 150 seed per stem). One of these females was surrounded by 120 (31 %) of the germinants produced in this study area. There are clearly some factors that are influencing seed and germinant production of some individual plants at this study area.

The following rationale for the high rate of seed production is speculative but does take into consideration the results of this research that found a positive association between germinant production and the time since biomass reduction.

At Carisbrook BR study area, the majority of the *P. spinescens* plants were found in close proximity to the adjacent landowner's fence-line. According to the local residents the paddock abutting the *P. spinescens* population regularly had cattle occupying it and they were often seen grazing close to the fence of the roadside reserve containing the *P. spinescens* plants. Consequently, these plants were within reach of the cattle and appear to have been regularly grazed but not trampled, as noted during site visits for this study (D. Reynolds, 2009 pers. obs. June and August).

Grazing is generally considered detrimental to the preservation of grasslands for ecological values, mainly attributed to the previous practices of over grazing and the compaction caused by large hoofed species (Kirkpatrick, 1999, Miller, 2000, Young, 2000, Attiwill and Wilson, 2003, Wong and Morgan, 2007). However, well-managed light grazing for short periods has been found to be beneficial to grassland conservation (Lunt, Eldridge *et al.*, 2007, Wong and Morgan, 2007). Long-term light grazing at a Northern plains site has also been found to contribute to an increase in *P. spinescens* growth rate over a period of five years (Foreman, 2011). The *P. spinescens* plants at Carisbrook BR appear to have responded well to the increased light, nutrients and seasonal water made available by the grazing, resulting in a profusion of flowers and a large seed crop.

If, as suggested by local residents, this management practice occurred frequently during the lifetime of these *P. spinescens* plants, it might be possible for large amounts of seed to accumulate within the immediate vicinity. This would ensure a good source of seed for germination when favorable germination conditions arise, possibly such as those experienced in 2009.

Unfortunately, upon return to the study area in August 2010, there was recent evidence of cattle trampling throughout the roadside verge. Only four germinants had survived from 2009 and the above ground foliage of many of the mature plants was damaged or removed. However, during the next visit in October 2010, the previously trampled mature plants were resprouting.

The Carisbrook BR study area highlights a specific case and provides speculative insights into the possible impacts that management events can have on the recruitment potential of a *P. spinescens* population. The frequent monitoring of the Carisbrook BR *P. spinescens* study

area over two years enabled a plausible explanation as to why the data for this study area was uniquely different (an obvious outlier) to all the other study areas in this project. For *P. spinescens*, the outcomes of seed and germinant production are likely to be site specific and associated with local management practices. This requires further monitoring and could be the subject of future research.

5.4.3 *The frequency of biomass reduction*

There appeared to be a trend towards less biomass reduction events across the 16 research sites. Historically, the western rural sites (Vite VRR, Pitfield CR, Geggies Rd, Mt Mercer Rd and Poorneet RR) were burned annually at the end of the spring harvest to provide a fire break for many properties (Morgan, 1999a, Lunt, Prober *et al.*, 2012). However, a decline in the number of farmers operating within the districts has limited the availability of manpower to continue to conduct burns in rural areas.

The management of rail reserves (Brownswaterholes BRR and Bannockburn RR) has changed from regular burning (Thomas, 2006) to ploughed fire breaks and herbicide treatments (Raynor, Marsh *et al.*, 1984, Lunt, 1992, Williams, McDonnell *et al.*, 2005). This has reduced the quantity, quality and diversity of these grassland remnants (Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2005, Williams, Morgan *et al.*, 2006, Australian Government, 2011, Lunt, Prober *et al.*, 2012). Of the known *P. spinescens* populations, approximately 11 % are found in rail reserves and are at risk of being lost due to the changes in management practices which do not allow for the regular creation of inter-tussock spaces.

Recommendations for the frequency of biomass reduction events ranges from annually up to once every five years (Robertson, 1985, Morgan, 1999a, Morgan, 2001, Lunt and Morgan, 2002, Wong and Morgan, 2007, O'Bryan, Prober *et al.*, 2009, Lunt, Prober *et al.*, 2012). However, the biomass loads in the temperate grasslands of Victoria are related to site productivity and the prevailing environmental conditions (Lunt, Prober *et al.*, 2012). As such, there is no single defined optimal frequency for biomass reductions that is appropriate for all sites on all occasions. The time period identified during this study appears to correspond to previous findings that a biomass reduction interval of greater than four years will hinder the germination of *P. spinescens* seed.

The trend for less biomass reduction events has been occurring throughout the 20th century according to Australia's palaeo-fire records (Kershaw, Clark *et al.*, 2002, Mooney, Harrison *et al.*, 2012) and this trend has also been found throughout the world (Marlon, Carcaillet *et al.*, 2008, Wang, Happellaz *et al.*, 2010). In 2006 and 2010 Williams highlighted an ongoing decline in 30 of Victoria's native grassland remnants due to fewer biomass reduction events and longer time intervals between biomass reduction events (fire) (Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2005, Williams, Morgan *et al.*, 2006). It seems *P. spinescens* is likely to be a casualty of such infrequent biomass management practices, with extinction debts potentially already occurring at many sites that support remnant populations of this species (Jackson and Sax, 2009, Cousins and Vanhoenacker, 2011).

5.4.4 *Type of biomass reduction*

The different types of biomass reduction events that occurred in this study were not adequately replicated across the 16 study areas to allow analysis according to type. As

such, the general term of biomass reduction was employed to cover all events regardless of their individual differences. Each different biomass reduction event was then weighted to sufficiently reflect the impacts that it was likely to produce. Although these weightings may not be truly representative of the differences between biomass management practices, it is clear that the impacts of any forms of biomass reduction when done frequently are better than no biomass reduction.

Interestingly, both seed weight (an indicator of seed quality) and the site condition score were found to be positively associated with the number of burns over the previous ten years. This suggests that burning as a form of biomass reduction might be superior to other forms of biomass reduction for the future reproductive capacity of populations of *P. spinescens*. Research in the grasslands of America comparing fire and grazing has found that fire is a more effective management strategy because it removes greater amounts of biomass, improves the light penetration to the soil (Dyer, 2002), and provides nutrients in the form of ash (nitrogen) (Knapp and Seastedt, 1986).

Biomass management is clearly an important feature in the recruitment ecology and status of *P. spinescens* populations. Future research should focus on the impacts and benefits of different types, timing and frequencies of biomass management.

5.4.5 *Summary*

Effective management, in the form of biomass reduction, appears to be a critical factor for various aspects of the reproductive ecology and survival of *P. spinescens.* Frequent biomass reduction events were associated with the capacity for the survival and positive growth of *P. spinescens* populations. Increased numbers of biomass reduction events were associated

with a greater a proportion of flowering individuals, greater seed viability, seed weight and a greater density of germinants. While we do not know why biomass reduction events are significant, the lack of these events within a four year period is likely to affect the future demographics of *P. spinescens* populations. The direct effects of biomass reduction events such as increased areas of bare soil, less weed cover, reduced litter and a high site condition were associated with a higher rate of germinant survival over the period of a single year following germination. Maintaining suitable habitat conditions may also reduce the rate of mortality of mature plants, thus providing opportunities for ongoing reproductive output. Clearly the habitat and microsite conditions are important factors in the production and survival of *P. spinescens* germinants, as well as maintaining the health of mature plants, which will ultimately lead to an improvement in the viability of the population.

This research was unable to conclusively determine which types of biomass management are best for the recruitment potential of *P. spinescens* but it is clear that a sensitive implementation of biomass reduction practices is better than no biomass management at all. Future research on the effects of different biomass reduction events on both mature plants and germinants, at suitably replicated sites, will assist in understanding the response of *P. spinescens* populations to management.

Chapter 6 Synthesis, conclusions and future research

"A little true science is better than a great deal of bad science. One is less liable to error by confessing one's ignorance than by fancying that one knows a great many things one does not"

Ernest Renan (1893)

6.1 Measures of recruitment potential

This research evaluated some of the factors that are associated with *in situ* recruitment potential in populations of *P. spinescens.* In keeping with similar research on various other flora (Faegri and van der Pijl, 1979, Waser and Price, 1983, Olsen, 1997, Caswell, 2001, Eckstein, 2005, Fenner and Thompson, 2005, Merrett, Robinson *et al.*, 2007, Griffiths, Wessler *et al.*, 2008), this project used a range of measures of recruitment potential to assess the reproduction of *P. spinescens* populations:

1. Seed production;
2. Seed viability;
3. Seed germinability;
4. Germination *in situ*; and
5. Germinant survival *in situ*.

Some additional factors of recruitment potential were found to be associated with flower production, and demographic parameters such as plant density.

Of the stages of recruitment potential that were assessed, *in situ* germination and germinant survival appear to be most critical to the reproductive potential of *P. spinescens* populations. Overall recruitment potential does not appear to be limited by either seed production or seed viability, as seed production occurred in both years of the study and consistent viability was detected. Seed germinability was difficult to evaluate due to a physiological dormancy and therefore is not useful as a measure of recruitment potential.

6.2 Biomass reduction is important for recruitment potential

Overwhelmingly, the recruitment potential of *P. spinescens* populations was most strongly associated with the regime of biomass reduction events. Of the recruitment potential measures assessed, seed viability and *in situ* germination were negatively associated with the time since biomass reduction and germinant survival was associated with a range of habitat parameters that are known to be influenced by biomass reduction processes (Sharp, 1994, Morgan, 1998c, Robinson and van Vuuren, 1998, Morgan, 2001, Williams, Morgan *et al.*, 2006, Gibson Roy, Delpratt *et al.*, 2007b, Lunt, Eldridge *et al.*, 2007, Wong and Morgan, 2007, Victorian Environment Assessment Council, 2011). Additionally, demographic parameters such as the proportion of mature flowering plants and mature plant survival were associated with regimes of biomass reduction.

This study demonstrated that although the density of *in situ* germinants was significantly different between the two study years, it was negatively associated with the time since biomass reduction and positively associated with the density of females. By way of comparing these independent variables, a Poisson regression analysis indicated that the year had the greatest association with germinant density ($z = -14.12$, $p < 0.0001$), followed by the time since biomass reduction ($z = -3.512$, $p = 0.0004$) and female density ($z = 2.73$, $p = 0.0054$). Although the variables that drive the difference in germinant production between years have not been identified in this study, the regime of biomass reduction is clearly an important contributor to the model. The three study areas that produced the greatest density of germinants in 2009, had all undergone a process of biomass reduction in the preceding year.

Although the time since biomass reduction appears to be an important factor in the *in situ* production of germinants, it is vital that a proportion of those germinants become effective members of the population by reaching reproductive maturity (Colling and Matthies, 2006). Germinant survival contributes to the long-term sustainability of the species by ensuring stable or growing populations (Falk, Miller *et al.*, 1996, Watkinson, 1997, Silvertown and Charlesworth, 2001, Meyer, Quinney *et al.*, 2006).

This study indirectly supports the idea that germinant survival was associated with biomass reduction, due to the strong associations that were detected between germinant survival and a high site condition score, high indigenous species diversity, increased bare soil, reduced litter and less weed cover. There is a wealth of studies and literature suggesting that, in temperate grasslands, the majority of these factors are all consequences of (or have been associated with) regular biomass management regimes (Stuwe and Parsons, 1977, Sharp, 1994, Tremont and McIntyre, 1994, Bosy and Reader, 1995, Morgan, 1995a, Hitchmough, Curtain *et al.*, 1996, Morgan, 1997, 1998c, Robinson and van Vuuren, 1998, McIntyre and Martin, 2001, Morgan, 2001, Lunt and Morgan, 2002, Robinson, 2003, Lenz and Facelli, 2005, Reynolds, 2006, Williams, Morgan *et al.*, 2006, Gibson Roy, Delpratt *et al.*, 2007b, Lunt, Eldridge *et al.*, 2007, Wong and Morgan, 2007, Victorian Environment Assessment Council, 2011).

Although the viability of *P. spinescens* seed was consistent between sites and across years, it had strong associations with the frequency of biomass reduction events and autumn rainfall. When both these variables were analysed using a stepwise linear regression they made a combined contribution of 48.2 % to the variance in seed viability $R^2 = 0.482$, $F(2, 27) = 14.493$, $p < 0.001$, Mahalanobis distance = 6.251 < 13.816 at $\alpha = 0.001$ and 51.8 % was

attributed to unknown causes. Clearly there are occasions when biomass management and seasonal conditions combine to have a strong association with seed viability.

Biomass management practices were also associated with the demographic characteristics of *P. spinescens* populations. A decline in the proportion of flowering plants was associated with less frequent biomass reduction events. Intuitively, this suggests that there may be negative consequences for population recruitment due to reduced opportunities for seed formation, even though no direct associations were detected in this study between seed production and biomass management. Further research is required to better understand the circumstances that give rise to non-flowering mature individuals, and the impact of this phenomenon on the population recruitment capacity.

Because the mortality of mature plants was negatively associated with the amount of bare soil, it seems an obvious and simple solution to implement management practices that ensures a less dense cover of vegetation. Although there was only a small level of mature plant mortality detected in this study, any scenarios involving the loss of large numbers of mature plants will presumably lead to a reduced recruitment potential and ultimately, population decline.

Pimelea spinescens is considered to be long-lived (Mueck, 2000), but how long is yet to be quantified. Loss of mature plants without sufficient compensation could be catastrophic for small and/or isolated populations (Watkinson, 1997). Large proportions (greater than 40 % of the population estimate) of non-flowering mature plants could serve as a warning sign for populations in distress. Furthermore, a low density of plants (less than 0.5 plants/m²) with large distances between individuals (greater than 80 cm) may be an indicator that a process

of population demise has commenced. In each of these circumstances the need for remedial action should be assessed and monitored as a matter of priority.

Biomass reduction events are recognised as essential management practices that promote germination, germinant survival and indigenous species diversity in the temperate grasslands of Victoria (Sharp, 1994, Morgan, 1998c, Robinson and van Vuuren, 1998, Morgan, 2001, Robinson, 2003, Reynolds, 2006, Williams, Morgan *et al.*, 2006, Gibson Roy, Delpratt *et al.*, 2007b, Victorian Environment Assessment Council, 2011). They are disturbance events, including low levels of soil disturbance, light grazing, weed control or fire (Lunt, Eldridge *et al.*, 2007, Wong and Morgan, 2007), that create inter-tussock spaces or gaps of bare soil by removing vegetation, litter and weed competition. The creation or maintenance of gaps is important because they provide opportunities for inter-tussock species to exploit and compete with the dominant grasses (Tremont and McIntyre, 1994, McIntyre and Martin, 2001).

In Victorian grasslands, high levels of biomass produced from weeds and the dominant grasses (such as *T. triandra*) negatively affect inter-tussock species by competitively limiting access to the essential resources of water, nutrients and light (Lenz and Facelli, 2005, Lunt, Prober *et al.*, 2012). Under favourable conditions, a reduction in biomass will promote flowering by grassland inter-tussock species (Lunt and Morgan, 2002) and make essential resources available at the soil surface where seed have fallen (Morgan, 1998c, Bullock, 2000), enabling the germination of inter-tussock species and allow for a greater rate of survival (Morgan, 1997, 1998c).

This research suggests that across the 16 study sites, the frequency and regularity of biomass reduction events has fallen dramatically in the three years of the study, when

compared with the previous nine years. As documented in previous research (Lunt and Morgan, 1999, Morgan and Lunt, 1999, Williams, Morgan *et al.*, 2006), infrequent biomass reduction events are likely to result in the loss of species diversity in native grasslands over-time. This study is consistent with the previous research (Tremont and McIntyre, 1994, Morgan, 1997, 1998c, McIntyre and Martin, 2001), as it seems that frequent and regular biomass reduction events are important in limiting the loss of mature *P. spinescens* plants and promoting germinant survival. Undertaking biomass reductions within a four year period is also likely to encourage germination of *P. spinescens* seed. Regular biomass reduction contributes to the demographic structure of *P. spinescens* populations and is clearly an important feature in various stages of the species reproductive ecology that can lead to stable or positive population growth.

6.3 The importance of spatial structure

In addition to biomass management, the spatial arrangement of plants was the other important factor in the recruitment potential of *P. spinescens* populations. Germinant production was strongly correlated to the density of female plants. Intuitively this would be expected because the seed rain is likely to be greater in areas with a greater female density. However, apart from seed load there are a range of other reasons why female density may be important to the production of germinants.

Previous research suggests that plants that exist in harsh environments, such as deserts, are more inclined to be spatially distributed in a clumped arrangement (Went, 1942, Hobbs and Hopkins, 1990, Tielborger and Kadmon, 1995, Suzan, Nabhan *et al.*, 1996). Such a spatial distribution enables large mature plants to act as nurse plants to juveniles, creating a

positive association between the age classes (Went, 1942, Arriaga, Maya *et al.*, 1993, Suzan, Nabhan *et al.*, 1996). In other species, mature plants that have a clumped spatial distribution have been shown to have increased soil moisture due to shading and reduced thermal evapotranspiration, as well as increased nutrients (Nobel, 1989, Barnes and Archer, 1999, Ibanez and Schupp, 2001, Pugnarie and Luque, 2001, Bruno, Stachowicz *et al.*, 2003, Tirado and Pugnaire, 2003, Corinna, Milton *et al.*, 2005, Christian, Den Ouden *et al.*, 2008). Termed positive density dependence, this process of facilitation has been documented for shrubs which act as nurse plants to germinants (Went, 1942, Arriaga, Maya *et al.*, 1993, Tielborger and Kadmon, 1995, Suzan, Nabhan *et al.*, 1996, Brooker and Callaway, 1998) and may be a feature of the ecology of *P. spinescens*. This is an area that requires further research.

In addition to the negative associations with bare soil, mature plant mortality was strongly associated with the spatial arrangement of the *P. spinescens* population. That is, a higher mature plant mortality was associated with an increase in the distance to nearest neighbour. In this study, it was not possible to determine whether a reduction in plant density contributed to mortality or whether there was an increased distance to nearest neighbour because the mature plants in between had already died as part of a process of population decline.

It is plausible that reduced habitat management leading to increased biomass loads has resulted in the mortality of mature plants at some study sites, ultimately leading to populations with a reduced plant density. This represents a positive feedback loop, in that a reduced plant density was found to be associated with reduced germinant production. Thus, study areas with lower plant densities may represent an extinction debt (Tilman, May *et al.*,

1994, Cousins and Vanhoenacker, 2011). It is important that plant density which is representative of the arrangement of plants is not confused with population size which relates to numbers of individual plants, as population size was not found to be associated with any identified variable. Nevertheless, population size should be considered an important attribute to maintaining the genetic diversity and long-term viability of populations of *P. spinescens*. Further research is required to understand the long-term impacts of spatial arrangement on population viability.

It may be possible to bolster the populations with a low female density in order to increase its future recruitment potential. This could be done using the techniques refined by this research to produce germinants, or through the translocation of plants and *in situ* germinants from sites that are scheduled for future development. Further work is required to determine the optimum spatial arrangement of plants used to augment existing or new populations.

6.4 Environmental associations with recruitment potential

The sites in this study were selected from a broad geographic area, representing a gradient of climates. Additionally, the study was conducted across two years with stark contrasts between the climatic conditions. Seed production of *P. spinescens* was associated with rainfall and also with temperature. In particular, seed production was significantly higher in the first year which had a low rainfall (drought). A significantly lower production of seed was recorded in the following year, which had a much greater level of rainfall. Based on these results, clearly rainfall is negatively associated with seed production and it is possible that *P. spinescens* mast seeds in response to drought. Further work is required to determine if mast seeding does occur and the long-term effects of this phenomenon. A greater understanding

of the influence of rainfall over a longer period of time will provide opportunities for better planning and management of *P. spinescens* populations.

Pimelea spinescens plants appear to be capable of accumulating sufficient resources to maintain seed quality throughout long-term drought conditions.

6.4.1 *Cause for concern*

It seems likely that the process of extirpation has commenced for some of the populations included in this study. In particular, the Christies Rd study area raises cause for concern because it:

- produced no germinants in either year;
- had the highest mature plant mortality;
- had the largest average distance between plants; and
- had the smallest proportion of bare ground available for colonisation.

The Vite VRR study area was also concerning in that it:

- produced one germinant in 2009 (it did not survive to 2010);
- had no germinants in 2010;
- had the second highest mature plant mortality;
- had the second largest average distance between plants; and
- had the second smallest proportion of bare ground available for colonisation.

Although limited to two replicated study areas, the features of both populations suggest a trend that may lead to extirpation if targeted and immediate remedial actions are not undertaken. A process of biomass reduction may provide a reprieve in inter-specific

competition, allowing an opportunity for potential *in situ* recruitment and positive population growth.

6.5 Empirical findings

6.5.1 *Demographic sampling*

6.5.1.1 Population size and density sampling

This research found that the approach of sub-sampling populations by defining a study area and randomly assessing ten per cent of the study area via transects was an achievable, efficient and repeatable method of assessing some of the demographic and reproductive attributes of *P. spinescens* populations. Such an approach may assist with ensuring that accurate and representative information is stored within databases that are used for future monitoring and decision-making.

Future longitudinal assessments of *P. spinescens* populations should be conducted using a standardised quadrat size. Initially, quadrats should be randomly located and clearly marked so that repeat sampling can be conducted over time. The random selection of quadrats is a balancing act in ensuring that there are a sufficient number of plants available to provide statistical rigor (at least 25 - 30 plants should be assessed and quadrats should be assigned until this is achieved), whilst maintaining a workload that is achievable and efficient. In this study, many of the randomly selected quadrats contained only a few plants but some quadrats contained numerous individuals that were extremely time-consuming to assess. The decision tree used in this study helped to achieve a balance between statistical requirements and an achievable workload. This study did not incorporate randomly selected quadrats that contained no *P. spinescens*, a feature that may have limited the capacity to

assess for colonisation of the species into these localised areas. Incorporation of quadrats where the species was absent may have also assisted with further refining which features are important to the survival and recruitment success of *P. spinescens* populations. It is important that at least some replicated quadrats with no *P. spinescens* plants present are included into future studies that monitor both the survival of mature plants and recruitment of new individuals into the population.

6.5.1.2 Breeding system

The breeding system of *Pimelea spinescens* was found to be representative of a subdioecious system. In this study all obvious female plants found within random quadrats were targeted for seed collection. If hermaphrodite individuals were inadvertently sampled during this research it is likely to have affected both the quality and quantity of seed produced, as seen in research on other flora (Delph, 1990, Barrett, 1992, Webb, 1999). The high variability in all recruitment potential measures that was recorded between plants within each study area could potentially be explained by a lack of consistency in sexual expression throughout a season, resulting in plants that were hermaphrodites being selected for this study. Further research is required to study the process of sequential flowering in labile individuals, and to comparatively quantify the contribution that females and hermaphrodites make to seed production and seed viability.

6.5.2 *Dormancy and germination*

This research has identified that *P. spinescens* seed displays an endogenous non-deep physiological dormancy (Type II) (Baskin and Baskin, 2004a). This dormancy was difficult to overcome in the seed germination experiments conducted during this research. Seed

dormancy is a mechanism to protect a species chance of survival by ensuring not all viable seed germinates during optimal conditions (Baskin and Baskin, 2001). If no progeny survive from the first germination event, this type of dormancy ensures that there is seed available for germination when the next period of suitable conditions occurs, potentially allowing for the survival of some resultant germinants (McMillan Browse, 1980, Vleeshouwers, Bouwmeester *et al.*, 1995, Copeland and McDonald, 2001). This suggests that *P. spinescens* has evolved to cope under variable and often unfavourable site conditions. Seed dormancy is an adaptive trait that has been successful at ensuring the long-term survival of this species.

The natural stimulus for the germination of *P. spinescens* seed was not identified but a treatment was found that obtained repeatedly consistent results. Seed treated with 0.1 % gibberellic acid under winter conditions will germinate at least a proportion of viable *P. spinescens* seed.

The only other germination treatment which produced germinants in numbers approaching any of the treatments which contained gibberellic acid was the combination of heat and smoke treatment. Thus, the use of fire as a biomass removal treatment is likely to be important for the natural germination of seed and recruitment potential of *P. spinescens* populations.

6.6 Limitations of study

For long-lived plants such as *P. spinescens*, two years of data is not enough time to study the processes or environmental conditions that promote *in situ* recruitment. The rate of germinant survival reported in this study is based on data collected over two years and

therefore the future survival of these germinants is unknown. Follow up monitoring is required to evaluate the fate of these germinants and to better understand the recruitment capacity of this species.

6.7 Recommendations for future research

In order to further advance the knowledge base about *P. spinescens* and maximise efforts to appropriately manage the species, experimental trials are required to better understand the impacts of a range of management practices on mature plant survival, reproductive capacity and recruitment of new individuals. Additionally, long-term monitoring of *in situ* individuals from all life-stages is essential. This research has initiated this process at 16 sites and opportunities now exist for future follow up on the fate of tagged individuals in relation to their management over-time. This is the only research on *P. spinescens* to assess most of the likely factors that may be limiting to the recruitment potential capacity of discrete populations. As such it is a great starting point for future research into the productive processes, thresholds and limitations of this species. Of all the different topics this research has highlighted which require further examination, the three outlined below are the ones that are likely to contribute the most to the future conservation management of this species:

1. An investigation of the impacts of biomass and biomass management on *P. spinescens*, in terms of:
    a. Germinant production and survival;
    b. Flower production; and
    c. Mature plant survival.

2. A detailed investigation of the breeding system of *P. spinescens*, with a focus on the occurrence of hermaphrodites and their contribution to discrete populations.
3. A study of the best spatial arrangement of *P. spinescens* plants for augmenting or reintroducing populations, in relation to individual survival and reproductive output.

6.8 Conclusions

Using 16 study sites that support populations of *P. spinescens*, this research revealed aspects of the species biology, ecology and response to management actions that are important factors contributing to the recruitment potential and long-term conservation success of its populations.

Regular active management in the form of biomass reduction events was found to be positively related to various stages of the reproductive cycle of *P. spinescens*. Conversely, a lack of active management over time, as is the current trend in Victoria's Natural Temperate Grasslands (Williams, McDonnell *et al.*, 2005, Williams, Morgan *et al.*, 2006, Victorian Environment Assessment Council, 2011), is very likely to reduce the capacity of *P. spinescens* populations to maintain mature plants and produce germinants, therefore limiting the capacity for future recruitment events.

This study used an investigative approach to assess a range of factors that may be influencing recruitment in *P. spinescens* populations. The study identified numerous topics for further research relating to the recruitment of *P. spinescens* and other aspects of its biology and ecology. Tagged plants at each of the 16 study sites provide an opportunity for

long-term monitoring of the fate of these individuals, and more importantly for the establishment of experimental hypothesis testing in relation to active management interventions.

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Appendix 1

Seed production

2009

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Generalized linear mixed model fit by the Laplace approximation
Formula: seedcount09 ~ offset(stems09) + (1 | plantno) + as.factor(Site)
   Data: thedata1
   AIC   BIC logLik deviance
 913.4 961.6 -439.7    879.4
Random effects:
 Groups  Name        Variance Std.Dev.
 plantno (Intercept) 2.9499   1.7175
Number of obs: 126, groups: plantno, 126

Fixed effects:
                             Estimate  Std. Error  z value  Pr(>|z|)
(Intercept)                  -0.12966    0.60935   -0.213  0.831492
as.factor(Site)Bannockburn RR  0.78177    0.89184    0.877  0.380716
as.factor(Site)Baringhup WR    1.51924    0.86082    1.765  0.077584 .
as.factor(Site)Browns WBRR    -0.31702    0.83673   -0.37   0.704781
as.factor(Site)Calder RRR     -0.80871    0.83695   -0.96   0.333915
as.factor(Site)Carisbrook BR   3.46026    0.89056    3.885  0.000102 ***
as.factor(Site)Cedarwoods R    0.42451    0.93094    0.456  0.648387
as.factor(Site)Christies Rd   -1.64571    0.89176   -1.845  0.064970 .
as.factor(Site)Geggies Rd     -1.29399    0.89125   -1.452  0.146535
as.factor(Site)Glengower Rd    1.96597    0.81650    2.408  0.016049 *
as.factor(Site)Kirks BR        1.63332    0.89094    1.833  0.066765 .
as.factor(Site)McKenzie Rd     2.88350    0.86083    3.350  0.000809 ***
as.factor(Site)Mt Mercer Rd    0.14631    0.86075    0.170  0.865022
as.factor(Site)Pitfield CR     0.70921    0.86071    0.824  0.409952
as.factor(Site)Poorneet RR     0.01765    0.86089    0.021  0.983640
as.factor(Site)Vite VRR       -1.05828    0.83680   -1.265  0.205989


Significant codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

* Note: the z values and associated p values are for the site parameter estimates compared to Ararat. In addition to these estimates, the differences in these parameter estimates are also of interest allowing us to compare sites. These difference estimates and their standard errors were calculated using the estimated parameter estimates and the associated variance co-variance matrix of the estimates.

Appendix 2

Study areas which were significantly different in relation to seed production in 2009.

Site	Site significantly different	Adjusted *p*-value
Carisbrook BR	Ararat AR	0.001
	Bannockburn RR	0.001
	Baringhup WR	0.001
	Brownswaterholes BRR	0.001
	Calder RRR	0.001
	Cedarwoods R	0.001
	Christies Rd	0.001
	Geggies Rd	0.001
	Kirks BR	0.001
	Mt Mercer Rd	0.001
	Pitfield CR	0.001
	Poorneet WRR	0.001
	Vite VRR	0.001
Glengower Rd	Ararat AR	0.001
	Bannockburn RR	0.01
	Brownswaterholes BRR	0.001
	Calder RRR	0.001
	Cedarwoods R	0.001
	Christies Rd	0.001
	Geggies Rd	0.001
	Poorneet WRR	0.01
	Vite VRR	0.001
McKenzie Rd	Ararat AR	0.001
	Brownswaterholes BRR	0.001
	Calder RRR	0.001
	Cedarwoods R	0.001
	Christies Rd	0.001
	Geggies Rd	0.04
	Vite VRR	0.001
Baringhup WR	Ararat AR	0.001
	Calder RRR	0.001
	Cedarwoods R	0.04
	Christies Rd	0.001
Kirks BR	Ararat AR	0.02
	Christies Rd	0.001
Christies Rd	Pitfield CR	0.01

Appendix 3

Seed production

2010

```
Generalized linear mixed model fit by the Laplace approximation
Formula: seedcount10 ~ offset(stems10) + (1 | plantno) + as.factor(Site)
   Data: thedata1
   AIC   BIC logLik deviance
 499.2 547.4 -232.6    465.2
Random effects:
 Groups  Name        Variance Std.Dev.
 plantno (Intercept) 2.4394   1.5619
Number of obs: 126, groups: plantno, 126

Fixed effects:
                                Estimate   Std. Error z value   Pr(>|z|)
(Intercept)                      0.228784   0.574251    0.398   0.6903
as.factor(Site)Bannockburn RR   -0.592817   0.846945   -0.700   0.4839
as.factor(Site)Baringhup WR     -0.002105   0.839904   -0.002   0.9980
as.factor(Site)Browns WBRR      -1.664494   0.811671   -2.051   0.0403
as.factor(Site)Calder RRR       -2.632063   1.081533   -2.434   0.0149
as.factor(Site)Carisbrook BR    -0.727043   0.926620   -0.785   0.4326
as.factor(Site)Cedarwoods R     -1.035847   0.868076   -1.193   0.2327
as.factor(Site)Christies Rd     -2.024053   1.165704   -1.736   0.0825
as.factor(Site)Geggies Rd       -0.567360   0.836696   -0.678   0.4977
as.factor(Site)Glengower Rd     -2.735437   0.951654   -2.874   0.0040
as.factor(Site)Kirks BR          0.272832   0.857067    0.318   0.7502
as.factor(Site)McKenzie Rd      -1.900852   0.991194   -1.918   0.0551
as.factor(Site)Mt Mercer SR     -1.312198   0.861211   -1.524   0.1275
as.factor(Site)Pitfield CR       0.510958   0.821195    0.622   0.5338
as.factor(Site)Poorneet WRR     -0.051409   0.827303   -0.062   0.9504
as.factor(Site)Vite VRR         -1.695782   0.810314   -2.093   0.0363


Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

* Note: the z values and associated p values are for the site parameter estimates compared to Ararat. In addition to these estimates, the differences in these parameter estimates are also of interest allowing us to compare sites. These difference estimates and their standard errors were calculated using the estimated parameter estimates and the associated variance co-variance matrix of the estimates.

Appendix 4

Seed production comparisons of 2009 to 2010

Site		Estimate	Std. Error	z value	Pr(>|z|)	Adjusted P-value
Ararat AR	as.factor(year)2010	-0.291	0.294	-0.987	0.323	1
Bannockburn RR	as.factor(Site)2:as.factor(year)2010	-0.863	0.434	-1.990	0.046	0.745
Baringhup WR	as.factor(Site)3:as.factor(year)2010	-0.779	0.436	-1.786	0.074	1
Brownswaterholes BRR	as.factor(Site)4:as.factor(year)2010	-0.989	0.412	-2.399	0.016	0.262
Calder RRR	as.factor(Site)5:as.factor(year)2010	-1.460	0.605	-2.409	0.016	0.256
Carisbrook BR	as.factor(Site)6:as.factor(year)2010	-2.449	0.501	-4.884	0	0
Cedarwoods R	as.factor(Site)7:as.factor(year)2010	-1.134	0.447	-2.538	0.011	0.177
Christies Rd	as.factor(Site)8:as.factor(year)2010	-0.118	0.686	-0.172	0.863	1
Geggies Rd	as.factor(Site)9:as.factor(year)2010	-0.972	0.427	-2.272	0.023	0.369
Glengower Rd	as.factor(Site)10:as.factor(year)2010	-2.297	0.468	-4.901	0	0
Kirks BR	as.factor(Site)11:as.factor(year)2010	-0.812	0.443	-1.829	0.067	1
McKenzie Rd	as.factor(Site)12:as.factor(year)2010	-2.307	0.519	-4.444	0	0
Mt Mercer Rd	as.factor(Site)13:as.factor(year)2010	-0.995	0.440	-2.259	0.023	0.382
Pitfield CR	as.factor(Site)14:as.factor(year)2010	-0.390	0.422	-0.923	0.355	1
Poorneet WRR	as.factor(Site)15:as.factor(year)2010	-0.513	0.426	-1.203	0.228	1
Vite VRR	as.factor(Site)16:as.factor(year)2010	-0.34	0.409	-0.838	0.402	1

Appendix 5

Seed viability

2009

```
Generalized linear mixed model fit by the Laplace approximation
Formula: cbind(seedvia09, seedtest09 - seedvia09) ~ -1 + (1 | plant) +
as.factor(site3)
   Data: thedata1
   AIC   BIC logLik deviance
 312.6 360.8 -139.3    278.6
Random effects:
 Groups Name        Variance Std.Dev.
 plant  (Intercept) 0.60403  0.77719
Number of obs: 126, groups: plant, 126

Fixed effects:
                             Estimate  Std. Error z value   Pr(>|z|)
as.factor(site3)Ararat AR     0.28996    0.32725   0.886   0.375591
as.factor(site3)Bannock RR    1.49675    0.39189   3.819   0.000134 ***
as.factor(site3)Baringhup WR  1.20747    0.34046   3.547   0.000390 ***
as.factor(site3)Browns WBRR   1.77324    0.34224   5.181   2.20e-07 ***
as.factor(site3)Calder RRR    0.14320    0.31035   0.461   0.644513
as.factor(site3)Carisbrook BR 0.64573    0.35651   1.811   0.070101 .
as.factor(site3)Cedarwoods R  0.54500    0.38861   1.402   0.160788
as.factor(site3)Christies Rd  1.14236    0.36723   3.111   0.001866 **
as.factor(site3)Geggies Rd    0.99611    0.35734   2.788   0.005310 **
as.factor(site3)Glengower Rd  0.83556    0.29607   2.822   0.004770 **
as.factor(site3)Kirks BR      0.51119    0.35072   1.458   0.144963
as.factor(site3)McKenzie Rd  -0.29061    0.33203  -0.875   0.381428
as.factor(site3)Mt Mercer SR  0.81329    0.33635   2.418   0.015607 *
as.factor(site3)Pitfield CR   0.87683    0.34146   2.568   0.010232 *
as.factor(site3)Poorneet WRR  0.07599    0.32464   0.234   0.814916
as.factor(site3)Vite VRR      1.55878    0.33350   4.674   2.95e-06 ***


Significant codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

* Note: the z values and associated p values are for the site parameter estimates. The differences in these parameter estimates and not the estimates themselves are the values of interest. These difference estimates and their standard errors were calculated using the estimated site effects and the associated variance co-variance matrix of the estimates.

Appendix 6

Seed viability

2010

```
Generalized linear mixed model fit by the Laplace approximation
Formula: cbind(seedvia10, seedtest10 - seedvia10) ~ -1 + (1 | plantnumber) +
as.factor(site4)
   Data: thedata1
   AIC   BIC logLik deviance
 223.6 260.7 -96.78    193.6
Random effects:
 Groups   Name        Variance Std.Dev.
 plnatnum (Intercept) 0.65685  0.81046
Number of obs: 88, groups: plantnumber, 88

Fixed effects:
                             Estimate   Std. Error z value   Pr(>|z|)
as.factor(site4)Ararat AR      0.2179     0.3416    0.638   0.523521
as.factor(site4)Bannock RR     1.5876     0.4020    3.949   7.83e-05 ***
as.factor(site4)Baringhup WR   1.2372     0.4099    3.018   0.002541 **
as.factor(site4)Browns WBRR    1.0416     0.3862    2.697   0.006993 **
as.factor(site4)Carisbrook BR  1.7226     0.5698    3.023   0.002500 **
as.factor(site4)Cedarwoods R   1.0537     0.4627    2.277   0.022763 *
as.factor(site4)Christies Rd   0.3312     0.4783    0.692   0.488663
as.factor(site4)Geggies Rd     0.8662     0.3891    2.226   0.026014 *
as.factor(site4)Glengower Rd   1.4221     0.5313    2.677   0.007435 **
as.factor(site4)Kirks BR       0.5193     0.3675    1.413   0.157591
as.factor(site4)Mt Mercer SR   0.9174     0.4540    2.021   0.043308 *
as.factor(site4)Pitfield CR    0.1487     0.3295    0.451   0.651776
as.factor(site4)Poorneet WRR   0.9902     0.3672    2.697   0.007002 **
as.factor(site4)Vite VRR       1.2108     0.3552    3.409   0.000652 ***


Significant codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

* Note: the z values and associated p values are for the site parameter estimates. The differences in these parameter estimates and not the estimates themselves are the values of interest. These difference estimates and their standard errors were calculated using the estimated site effects and the associated variance co-variance matrix of the estimates.

Appendix 7

Seed viability comparison of 2009 to 2010

Site		Estimate	Std. Error	z value	Pr(>|z|)	Adjusted P-value
Ararat AR	as.factor(year)2010	0.003049	0.266701	0.011432	0.9908786	1
Bannockburn RR	as.factor(Sites)2:year2010	-0.39036	0.515153	-0.75775	0.4485983	1
Baringhup WR	as.factor(Sites)3:year2010	-0.210533	0.450412	-0.46742	0.6401964	1
Brownswaterholes BRR	as.factor(Sites)4:year2010	-0.55685	0.425474	-1.30878	0.1906105	1
Cedarwoods R	as.factor(Sites)5:year2010	0.392515	0.435285	0.901742	0.3671939	1
Geggies Rd	as.factor(Sites)6:year2010	-0.038926	0.424027	-0.0918	0.9268556	1
Glengower Rd	as.factor(Sites)7:year2010	0.199722	0.535847	0.372723	0.7093545	1
Kirks BR	as.factor(Sites)8:year2010	0.237052	0.418287	0.566722	0.5709029	1
Mt Mercer Rd	as.factor(Sites)9:year2010	-0.343248	0.458837	-0.74808	0.4544103	1
Pitfield CR	as.factor(Sites)10:year2010	-0.132561	0.395093	-0.33552	0.7372343	1
Poorneet WRR	as.factor(Sites)11:year2010	0.806778	0.409185	1.971671	0.0486472	0.583
Vite VRR	as.factor(Sites)12:year2010	0.16408	0.429522	0.382005	0.7024577	1

Appendix 8

Seed germinability

```
Generalized linear mixed model fit by the Laplace approximation
Formula: cbind(noofgerms, numbofseed - noofgerms) ~ -1 + (1 | Plantid) +
as.factor(site2)                Data: thedata1
   AIC   BIC logLik deviance
 307.3 352.4 -136.6    273.3
Random effects:
 Groups  Name        Variance Std.Dev.
 Plantid (Intercept) 0.69668  0.83467
Number of obs: 105, groups: Plantid, 105

Fixed effects:
                            Estimate   Std. Error z value   Pr(>|z|)
as.factor(site2)Ararat AR    -2.5450     0.4232   -6.013   1.82e-09 ***
as.factor(site2)Bannock RR   -2.1314     0.4360   -4.889   1.02e-06 ***
as.factor(site2)Baringhup WR -1.7273     0.3495   -4.942   7.74e-07 ***
as.factor(site2)Browns WBRR  -1.3521     0.3450   -3.919   8.90e-05 ***
as.factor(site2)Calder RRR   -2.4585     0.3754   -6.549   5.78e-11 ***
as.factor(site2)Carisbrook BR -1.4382    0.3449   -4.170   3.05e-05 ***
as.factor(site2)Cedarwoods R -1.1897     0.4560   -2.609   0.00908  **
as.factor(site2)Christies Rd -1.9987     0.3921   -5.097   3.45e-07 ***
as.factor(site2)Geggies Rd   -1.7251     0.3550   -4.859   1.18e-06 ***
as.factor(site2)Glengower Rd -0.9987     0.3367   -2.966   0.00302  **
as.factor(site2)Kirks BR     -2.7582     0.3910   -7.054   1.74e-12 ***
as.factor(site2)McKenzie Rd  -2.9793     0.3975   -7.495   6.62e-14 ***
as.factor(site2)Mt Mercer SR -1.6470     0.3515   -4.685   2.80e-06 ***
as.factor(site2)Pitfield CR  -2.4253     0.3728   -6.506   7.74e-11 ***
as.factor(site2)Poorneet WRR -3.2906     0.4219   -7.800   6.18e-15 ***
as.factor(site2)Vite VRR     -3.2337     0.4101   -7.885   3.13e-15 ***
---
Significant codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

* Note: the z values and associated p values are for the site parameter estimates. The differences in these parameter estimates and not the estimates themselves are the values of interest. These difference estimates and their standard errors were calculated using the estimated site effects and the associated variance co-variance matrix of the estimates.

Appendix 9

Viability adjusted germination score

Based on Appendix 5 and 8.

	Estimate	Std. error	z value	Pr(>|z|)	
as.factor(site2)Ararat AR	13.44752	5.681115	2.367057	0.01793015	*
as.factor(site2)Bannock RR	12.93863	5.229369	2.474225	0.01335254	*
as.factor(site2)Baringhup WR	20.52237	6.43130	3.191015	0.00141773	**
as.factor(site2)Browns WBRR	24.48556	6.99306	3.501410	0.00046280	***
as.factor(site2)Calder RRR	14.59516	5.81387	2.510402	0.01205937	*
as.factor(site2)Carisbrook BR	30.39600	8.93145	3.403255	0.00066588	***
as.factor(site2)Cedarwoods R	36.35849	13.93853	2.608488	0.00909433	**
as.factor(site2)Christies Rd	16.83230	6.10462	2.757304	0.00582800	**
as.factor(site2)Geggies Rd	22.03148	6.96901	3.161347	0.00157041	**
as.factor(site2)Glengower Rd	40.25930	10.40862	3.867881	0.00010978	***
as.factor(site2)Kirks BR	11.13986	4.55696	2.444582	0.01450202	*
as.factor(site2)McKenzie Rd	11.38246	4.86719	2.338610	0.01935561	*
as.factor(site2)Mt Mercer SR	24.14111	7.62099	3.167711	0.00153644	**
as.factor(site2)Pitfield CR	13.30648	4.66357	2.853280	0.00432705	**
as.factor(site2)Poorneet WRR	8.43952	3.77280	2.236939	0.02529035	*
as.factor(site2)Vite VRR	5.28469	2.10788	2.507101	0.01217259	*

Significant codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

* Note: the z values and associated p values are for the site parameter estimates. The differences in these parameter estimates and not the estimates themselves are the values of interest. These difference estimates and their standard errors were calculated using the estimated site effects and the associated variance co-variance matrix of the estimates.

Appendix 10

27 April 2010 Report No: ACS104281

ACS Laboratories (Australia)
37 Stubbs St
Kensingham,
Victoria, 3031

Date of Sample Receipt: 1st April 2010

No. of Samples Received: 3

Report

RE: GCMS Chromatograms of *Pimelea spinescens* sbsp. *spinescens* seed extracts:

Fames Analysis

I have confirmed the presence of several fatty acids present in all seeds after methylation of the Hexane Extracts. Main fatty acid is Palmitic and with a small amount of Linolenic acid (C18:3) with both the n-3 and n-6 possibly being present. Smaller amounts of both Oleic acid (C18:1n9) and either Behenic or Erucic acids (C_{22}) may also be present. Significantly more C_{24} fatty acid may also be present in the seeds (Figure 7) as evidenced from the GCFID chromatograms but was not able to be confirmed by GCMS at this stage.

It is unclear from the data if we are looking the extraction of the seed oils (Seeds were not crushed) or some component of a triglyceride/ wax ester outer layer.

Significantly, the extract of seed 1 [Christies Rd (4281-1)] is different from seed 2 (Vite VRR) and 3 (Carisbrook BR) as evidenced from the GCMS chromatograms. Many of the other numerous baseline peaks are yet to be identified but appear to be straight and branched chain hydrocarbons (main Ions 57, 71 and 85 present.

Procedure

The samples (one half of provided seeds) were initially extracted in Hexane (2ml) after suitable untrasonication (30min).

One ml of the hexane extracts was the transferred to a 1ml vials after which it was evaporated under Nitrogen. 500ul of Hexane were added, capped and injected into the GCMS (Figures 4-6).

The remaining 1ml of the extracts was then evaporated and subject to esterification using a 14% BF3 in Methanol. The methylated extracts were then reconstituted in 500ul hexane and injected into both a GC/FID and GC/MS.

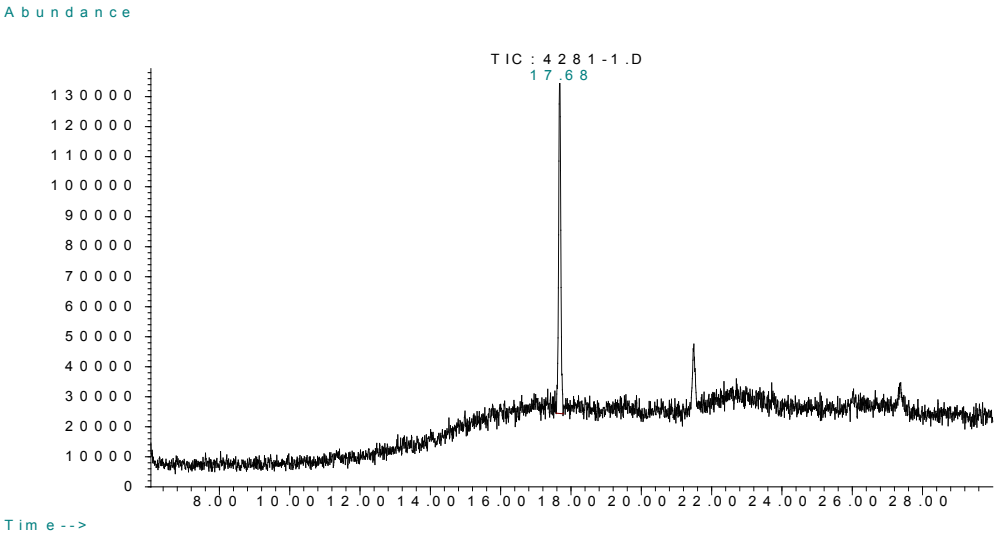
All underivitised extracts were run on:

1. *DB – 5MS 30m X 0.25um X 0.25mmID*

All Methylated Extracts were run on:

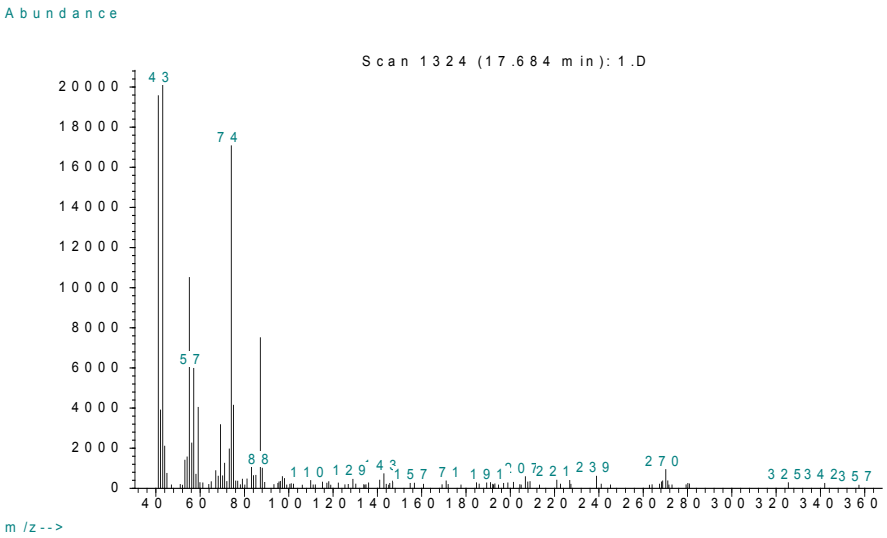
1. *ZB -624 X 1.2um X 0.25mm ID(GC/MS only)*
2. *BP X70 30m X 0.25um X 0.25mmiD (GC/FID Only)*

Figure 1 - Typical GCMS Chromatogram of Methylated Extracts (Seeds 1-3)



Column: ZB 624 – 30m X 1.2um X 0.25 mmID

Figure 2 - Mass Spectrum of Main Peak 17.68min



Mass Spectrum of peak at 17.68- Palmitic acid(>75/100)

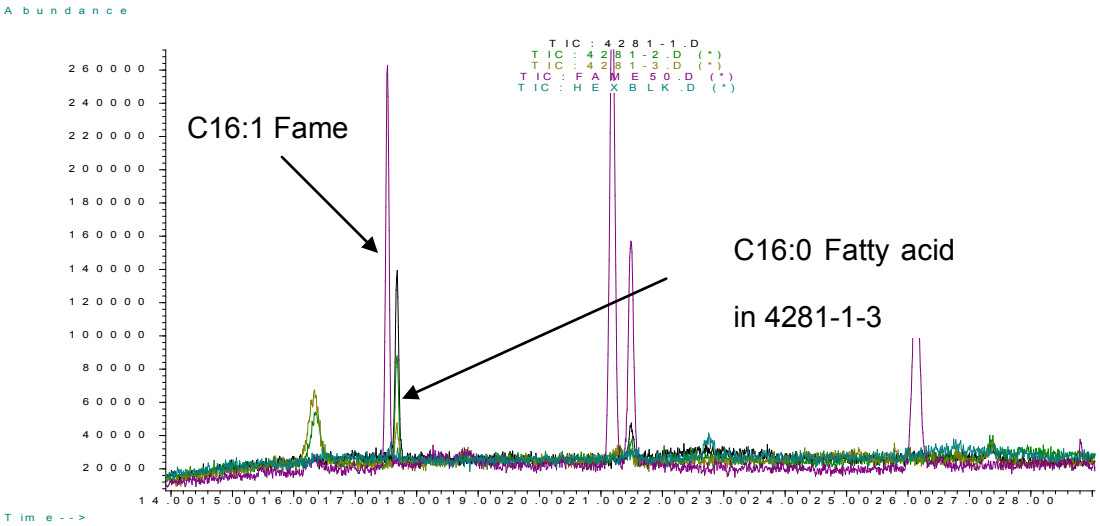
The Wiley Mass spectrum library match(>75/100) was also suggestive of *14- methyl, Pentadecanoic acid- Methyl Isopalmitate* but both GC/MS of the raw underivitised extracts and those of the methylated ones suggest the main acid is *Palmitic acid* (Both have MW of 270).

The limited Fames Standards used were:

Table 1

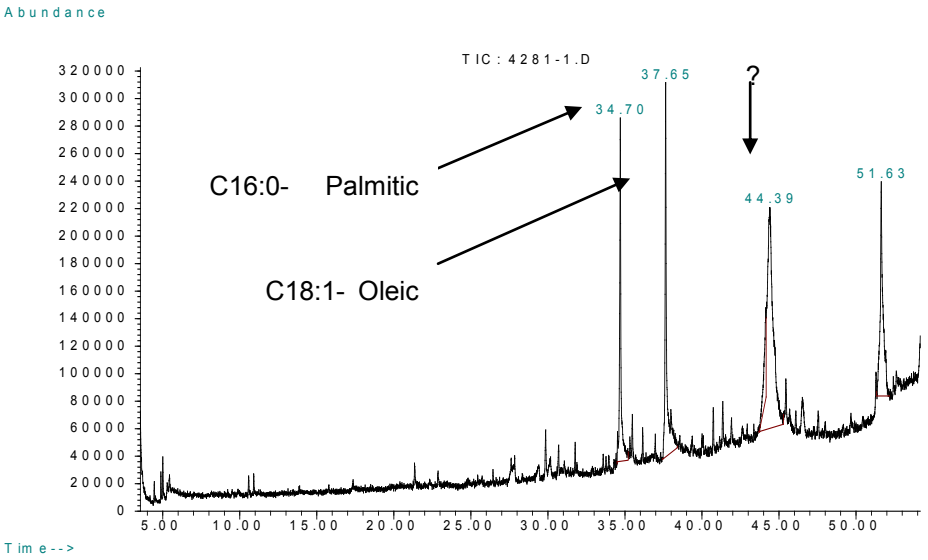
Fatty Acid(as Methyl Ester)	MW
Methyl Palmitoleate(C16:1)	268.43
Methyl Elaidate(C18:1- Trans)	296.0
Methyl Linoleate(C18:2- Cis)	294.47
Methyl Linolenate(C18:3- Cis)	292.46
Methyl Arachidonate(C20:0)	318.49

Figure 3 - GCMS of Methylated Seed Extract Chromatogram Overlaid with FAMES Standards



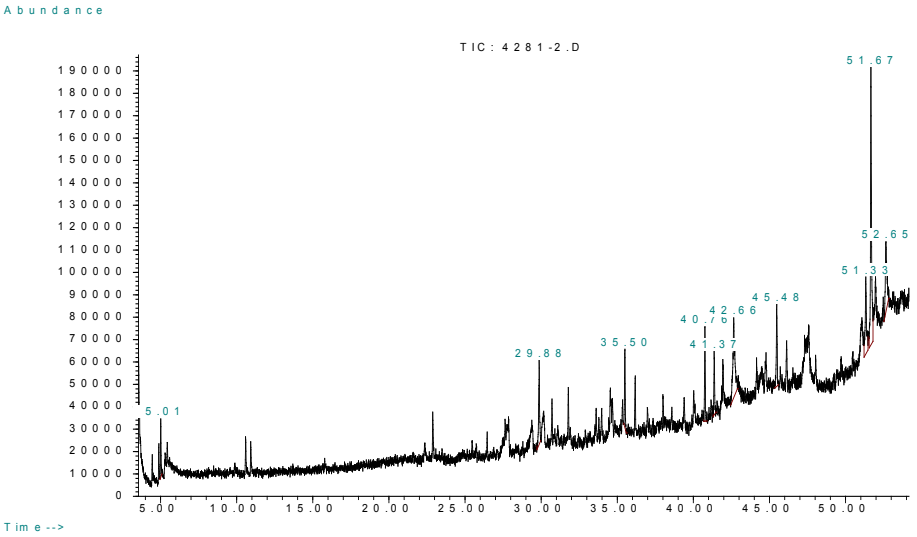
Column: ZB 624 – 30m X 1.2um X 0.25 mmID

Figure 4 - GCMS Chromatogram Seed 1 (Christies Rd): underivitised Hexane Extract 4281-1



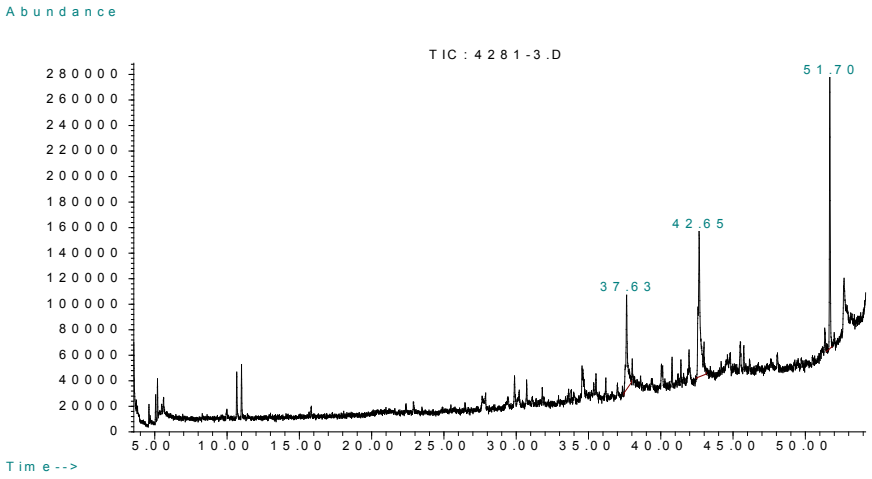
Column: DB5-MS – 30m X 0.25 um X 0.25 mmID

Figure 5 - GCMS Chromatogram Seed 2 (Vite VRR): Underivitised Hexane Extract 4281-2



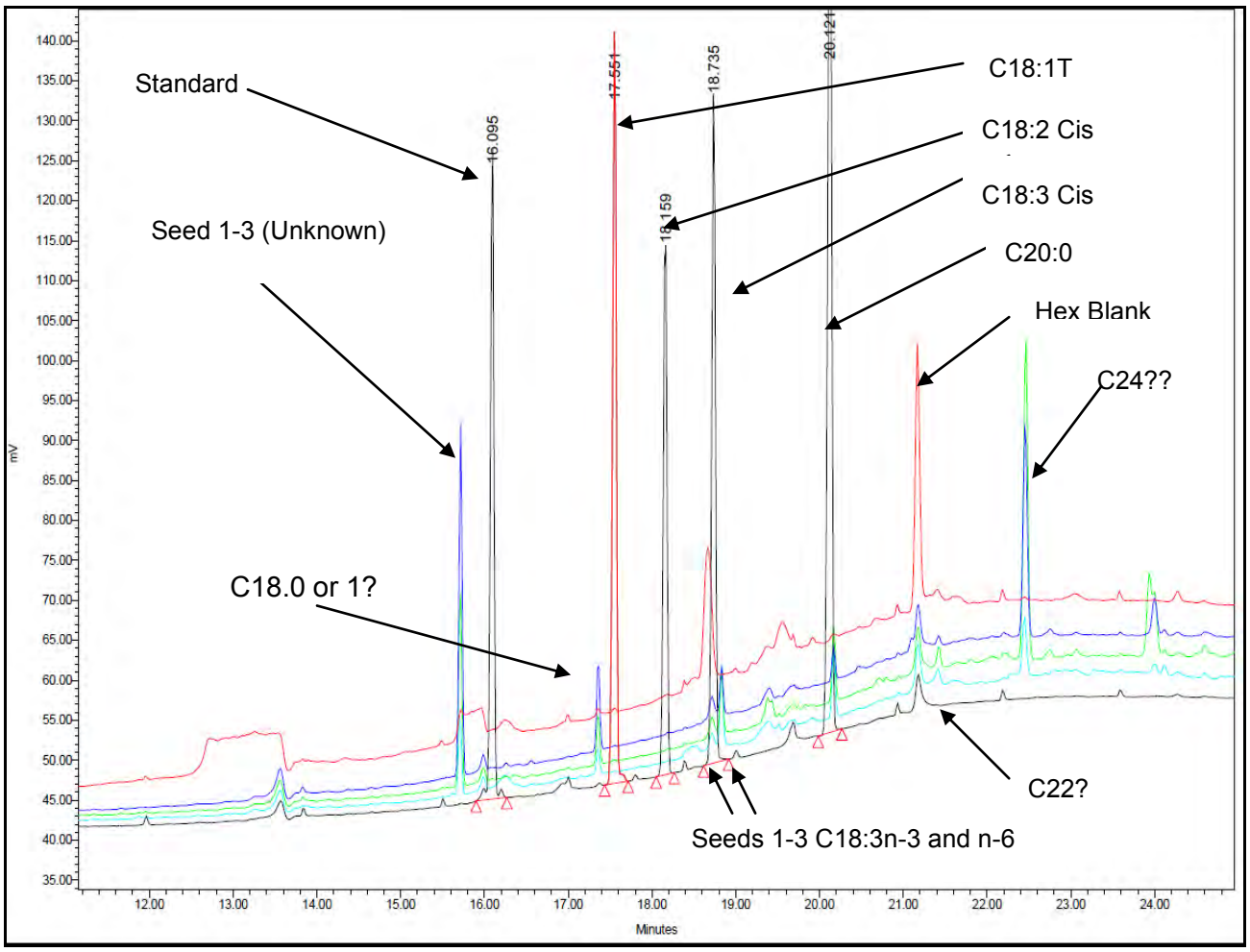
Column: DB5-MS – 30m X 0.25 um X 0.25 mmID

Figure 6 - GCMS Chromatogram Seed 3 (Carisbrook BR): Underivitised Hexane Extract 4281-3



Column: DB5-MS – 30m X 0.25 um X 0.25 mmID

Figure 7 - GC/FID Chromatogram of Seeds 1-3 Methylated Extracts with FAMES Standards



There is a peak in all three seeds extracts which is suggestive of a C_{22} and C_{24} fatty acid at ~ 21.2 and 22.4min respectively.

Interestingly we could not see the Methylated Oleic acid confirmed in the Underivitised Hexane extracts, in the GC/MS trace of the methylated extracts.

Yours faithfully,

Vince Murone (Principal Chemist)
ACS Laboratories (Australia)
ABN 85 708 233 006

Appendix 11

Germination *in situ* (germinants/female plant)

```
Generalized linear mixed model fit by the Laplace approximation
Formula: denofgerms ~ -1 + (1 | SitesQuads) + Sites + offset(log(denoffem))
   Data: test3
   AIC BIC logLik deviance
 138.5 166 -55.23    110.5
Random effects:
 Groups     Name        Variance Std.Dev.
 SitesQuads (Intercept) 1.7017   1.3045
Number of obs: 53, groups: SitesQuads, 53

Fixed effects:
                          Estimate Std. Error z value Pr(>|z|)
SitesArarat AR             -2.9773     1.4342  -2.076   0.0379 *
SitesBannockburn RR         1.4218     0.7087   2.006   0.0448 *
SitesBaringhup WR           1.1286     0.5152   2.190   0.0285 *
SitesBrownswaterholes BRR   0.7452     0.8150   0.914   0.3606
SitesCalder RRR            -1.6882     1.3258  -1.273   0.2029
SitesCarisbrook BR          1.4348     0.8109   1.769   0.0768 .
SitesCedarwoods R          -3.3906     3.3465  -1.013   0.3110
SitesGeggies Rd             0.8553     0.7228   1.183   0.2366
SitesKirks BR              -2.3241     1.1538  -2.014   0.0440 *
SitesMt Mercer SR           0.5707     0.6784   0.841   0.4002
SitesPitfield CR           -3.0400     2.7339  -1.112   0.2662
SitesPoorneet WRR           1.1560     0.9423   1.227   0.2199
SitesVite VRR              -2.4284     3.0668  -0.792   0.4285
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

* Note: the z values and associated p values are for the site parameter estimates. The differences in these parameter estimates and not the estimates themselves are the values of interest. These difference estimates and their standard errors were calculated using the estimated site effects and the associated variance co-variance matrix of the estimates.