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Zygogramma bicolorata biology and its impact on the alien invasive weed *Parthenium hysterophorus* in South Africa.

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A dissertation submitted to the Faculty of Science,
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Master of Science,
Johannesburg, South Africa
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DECLARATION

I declare that this dissertation is my own work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted by me before for any other degree, diploma or examination at any other university or tertiary institution.



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22nd day of March 2018

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ABSTRACT

Parthenium hysterophorus L. (Asteraceae: Heliantheae) is an annual herbaceous plant native to the tropical regions of the Americas, which has become invasive in about 40 countries including India, Australia and South Africa. The weed causes negative health effects on people and animals, reduces crop and animal productions and reduces biodiversity conservation. Both chemical and mechanical control have some negative aspects, making biological control an attractive option.

Based on the biological control program in Australia, the leaf-feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) was first released in 2013 in South Africa. However; the beetle failed to establish at many release sites around South Africa, despite laboratory based research indicating that the climate of South Africa is suitable for the beetle. The level of control of the beetle on *P. hysterophorus* is not well understood at many release sites where populations have established. Additionally, it was not known if any biological factors were restricting establishment of the beetle. Furthermore, an integrated control program using a combination of biological control together with fire is of interest. The likelihood of the beetles surviving any burning regime was considered based on the results of this study.

The hypothesis that *Z. bicolorata* has the potential to control *P. hysterophorus* in South Africa was tested. A field study at selected sites indicated that when *Zygogramma bicolorata* successfully established, it reduced *P. hysterophorus* density (from 50 plants/m^2 to zero plants in some cases), and canopy volume (from 20 cm^3 to 2 cm^3 at best). However, the beetle did not affect flower production relative to plant size or the chlorophyll content of individual leaves on the plant.

The hypothesis that *Z. bicolorata* is affected by soil type and soil moisture due to larval pupation and adult diapause occurring in the soil was also tested. This study showed that *Z. bicolorata* struggles to establish at sites which have perennially dry soils i.e. below a volumetric soil water content of 10%, or at sites with soil saturated at 100% field capacity. Additionally soils which have high organic content (grey in colour) restrict the beetle.

The hypothesis that *Z. bicolorata* establishment is limited due to predation and parasitism of the eggs was tested. This study found that the eggs of *Z. bicolorata* experienced high predation rates (between 37.2% and 100%) at a field site. However, it is not known which predators attack the eggs of the beetle. No parasitoids emerged from the eggs.

The hypothesis that *Z. bicolorata* will be affected by the use of fire as a management tool was investigated by studying the diapause depth of the beetle in the soil. The shallow diapause depth of the beetle (less than 2 cm below the soil surface, with the majority of beetles diapausing at less than 1 cm below the soil surface) indicated that the beetles would likely be vulnerable to a burning regime. However, further study would be needed to determine whether lethal temperatures for the beetle are reached at 1 cm and 2 cm below the soil surface.

This study suggests that *Z. bicolorata* has the potential to control *P. hysterophorus* in South Africa if sites which match the biological needs of the beetle are targeted for releases. Sites with the correct soil moisture and soil texture should be selected. Additionally, release methods should be improved in order to lessen the impact of indigenous predators by releasing high numbers of beetles to saturate predator populations.

Keywords: Agent impacts; biological control; climatic suitability; field study; Parthenium; post-release; predation.

GLOSSARY OF TERMS AND ABBREVIATIONS

Allelopathy – The restriction of growth, germination or survival of one organism by another by bio-chemical means.

Autoclave – A process used for sterilization involving high temperatures and pressures.

Defoliation – The removal of leaves from a plant through feeding by an organism.

Desiccation – To dry up.

Diapause – A period of dormancy of an insect, usually in response to adverse environmental conditions, such as short day-length and low temperatures.

Fecundity – The ability of females to produce large amounts of offspring.

Field capacity - The amount of soil moisture retained in soil after excess water has been drained and the rate of downward movement has decreased.

Herbicide – A chemical toxin used to destroy plants.

Host-specificity – The ability of an organism to only feed or complete its life cycle on one, in some circumstances, a few closely related -species.

Phytophagous – A herbivorous (plant-feeding) organism.

Pupation – The life stage of an insect in which it undergoes metamorphosis from a larva to an adult.

Soil moisture - The quantity of water contained in soil.

SPAD – 'Single Photon Avalanche Diode'. A device which measures the leaf greenness of a plant by detecting the level of chlorophyll present in the leaf.

Vigour – Plant health and resistance to tough environmental conditions.

VSWC – 'Volumetric Soil Water Content'. A ratio of the volume of water per volume of soil.

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CHAPTER ONE

General Introduction and Literature Review

1.1. Impact of invasive alien organisms and biological control

Invasive alien organisms are one of the largest global threats to environmental and economic stability (Schaffner 2001; Vitousek *et al.* 1996). The invasive organisms can be particularly harmful to native plant and animal species, and the communities in which they are found, by changing community structures and displacing native species (Brooks *et al.* 2002; Lau 2008). This can result in transformed ecosystem dynamics, such as fire occurrence and frequency, or nutrient cycling in the environment (Lau 2008; Randall 1996). Although it can be argued that biological invasions are natural processes, the impact of humans on environmental stability through spreading and introducing exotic species on a global scale, by both deliberate and unintentional means, occurs on such a frequent and global scale that human introduction of alien species cannot be considered natural (Vitousek *et al.* 1996).

There are two main hypotheses which have been postulated to explain why some species become invasive in their introduced ranges: (i) abiotic conditions in the introduced range favour the exotic species, and (ii) the exotic species has an increased competitive ability due to there being fewer natural enemies (enemy release hypothesis) or there being fewer competitively superior organisms in the organisms' introduced ranges (Crawley *et al.* 1986; Hinz & Schwarzlaender 2004; Liu *et al*. 2006). Both of these postulated hypotheses are assumed to result in the exotic species' increased survival rates, establishment, vigour, and seed production (Hinz & Schwarzlander 2004). The enemy release hypothesis is the basis from which biological control stems. The hypothesis arises from the theory that the exotic organisms are released from the pressures of predation or herbivory by co-evolved natural enemies when they colonize an invaded range (Sax & Brown 2000). An organism in an invaded range will, instead, be interacting with species that have not specialised to attack the invading organism, resulting in the exotic organism having a greater competitive ability over native species (Sax &

Brown 2000). Introducing a co-evolved natural enemy from the exotic species' native range, attempts to restore the competitive balance in an invaded ecosystem.

Biological control is one of the only cost-effective, sustainable and long-term solutions to controlling invasive exotic species (De Lange & van Wilgen 2010). Classical biological control is the most used method when attempting to control an invasive alien weed (Bellows & Fisher 1999). Classical biological control involves introducing a host-specific, co-evolved herbivore (agent) from the invasive aliens' (target) native range into the aliens' introduced range in order to control the alien in its introduced range (Bellows & Fisher 1999; Hoddle 2004). This ideally results in the agent establishing and spreading in the introduced range while attacking and controlling the target which results in a self-regulating agent-target dynamic (McFadyen 1998).

Biological control is generally a successful control strategy, notably in South Africa, where 61% of weed biological control programs since 1913 are considered successful, resulting in 30 target species under complete or substantial control (SAPIA 2011; Zimmermann *et al.* 2004). However, many agents remain un-evaluated in their success in establishment and control of their target weed. Although there are often many releases of an agent into the field, 2/3 agents do not control their respective target weed (McFadyen 1998). There are many factors, both biotic and abiotic, which could limit an agent's ability to establish in the field, which would then affect the agent's ability to control the target weed. These factors include the number of individuals released, the release sites, season of release, climate, parasitoids, and predation (Goeden & Louda 1976; Paynter *et al.* 2010; Thrall & Burdon 2004). It is, therefore, important to evaluate the effectiveness of an agent once it has been released in the field.

1.2. *Parthenium hysterophorus* description and ecology

Parthenium hysterophorus L. (Asteraceae: Heliantheae), also known as parthenium, famine weed, bitter weed, carrot weed, broom-bush, congress weed, false ragweed, ragweed parthenium, whitetop, escobar weed and feverweed

(Brunel *et al.* 2014; Tamado & Milberg 2000) is an annual herbaceous plant (Fig. 1.1) and is considered one of the worst alien invasive weeds, due to its high invasiveness, negative impacts on human health, and negative agricultural and ecosystem impacts (Adkins & Shabbir 2014).



Figure 1.1. *Parthenium hysterophorus* in the field in Malelane, South Africa.

Parthenium can only reproduce via seeds, but is capable of germinating at any time of the year, provided that there is sufficient rainfall (Dhileepan 2012; Strathie *et al.* 2011) and temperatures are between 10 ºC and 25 ºC (McConnachie *et al.* 2010). Parthenium has been reported to grow in sandy and clay soil, but generally does best in clay (McConnachie *et al.* 2010). The plant can rapidly reach its maximum height of 2m after germination, and is capable of flowering four weeks after germination, provided it is in favourable soil (Navie *et al.* 1996; Reddy *et al.*

2007). Flowers have been observed in the field on plants as small as 41 mm tall (Fig. 1.2). The plant can remain in bloom for six to eight months (Jayachandra 1971), resulting in prolific seed production, with individuals producing up to 156768 seeds per plant (Dhileepan 2012). The seeds can remain viable for up to two years, provided they are buried, which results in a seed bank half-life of approximately six years (Navie *et al.* 1998; Tamado *et al*. 2002). The soil seed-banks are, consequently substantial, with between 914 and 95 800 seeds/m^2 being reported at various field sites in South Africa (Strathie *et al.* 2011). The plant does best in disturbed habitats where there is less intra-species competition (McConnachie *et al.* 2010).



Figure 1.2. *Parthenium hysterophorus* flowering at 41 mm tall in the field in Malelane, South Africa.

1.3. Origin and spread

Parthenium hysterophorus is native to the tropical regions of the Americas (Dhileepan 2012). There are two known races of parthenium (Rollins 1950). The white flowered race is the most common in its native range, and is invasive across much of the world, including South Africa (Strathie *et al.* 2011). The yellow flowered race is found to naturally occur in Argentina, Bolivia, Chile, Paraguay,

Peru, and Uruguay and is not invasive (Strathie *et al.* 2011). Apart from its native range, parthenium is found in three other continents, namely: Africa, Asia and Oceania (McConnachie *et al.* 2010). It has become invasive in 20 countries, including the African countries of South Africa, Swaziland, Mozambique, Zimbabwe, Kenya, Ethiopia, Eritrea, Somalia, and the islands of Mauritius, Reunion, Madagascar and the Seychelles (McConnachie *et al.* 2010). Parthenium is problematic in India and Australia. The weed was first recorded in Australia in Queensland in 1955 (Picman & Towers 1982). It was successfully eradicated in 1956, but was mistakenly reintroduced in contaminated pasture seed in 1958 (McFadyen 1992; Picman & Towers 1982). The plant now reportedly covers 170 000 km^2 of grazing country in Queensland (McConnachie *et al.* 2010). Parthenium was first recorded in India in 1956 (Picman & Towers 1982), and reportedly covers 5000 km^2 in and around Bangalore (Jayanth & Bali 1994). Parthenium was first recorded in South Africa in 1880 in Inanda, KwaZulu-Natal (Rollins 1950). Parthenium was not common in South Africa in 1977, but became widespread after Cyclone Demoina resulted in flooding over a large portion of the east coast of South Africa in 1984 (McConnachie *et al.* 2010). Parthenium is now invasive in KwaZulu-Natal, North West Province, Mpumalanga, and Limpopo (Strathie *et al.* 2011). It is believed to have dispersed primarily by the seeds being carried to new regions by vehicles, water, animals, farming machinery, and wind (McConnachie *et al.* 2010).

1.4. Impacts

Parthenium dominates agricultural areas, resulting in reduced yields (Dhileepan 2012; Tamado *et al.* 2002). In India, parthenium has resulted in a 40% decrease in agricultural yield, while uncontrolled parthenium populations have been found to decrease sorghum yield by 40- 97% in Ethiopia (Khosla & Sobti 1981; Tamado *et al*. 2002). Parthenium can also reduce the carrying capacity of pastures by up to 90% (Singh *et al.* 2004). In Queensland, Australia, *P. hysterophorus* reportedly covers large tracts of choice grazing land, resulting in a loss of approximately A$ 16.5 million to the pasture industry per annum (Chippendale & Panetta 1994).

Parthenium is also capable of disrupting natural ecosystems (Dhileepan 2007; Kanchan & Jayachandra 1980). This is mainly due to the plants' allelopathic character, which incorporates two groups of allelochemicals, namely: phenolics and sesquiterpene lactones (Picman & Towers 1982; Towers & Mitchell 1983). The main sesquiterpene lactone is parthenin which is found in the trichomes of the stems and the leaves, and the pollen of the plant; and can comprise approximately 8% of the plants' dry weight (Picman & Towers 1982; Towers & Mitchell 1983). Caffeic, vanillic, ferulic, chlorogenic and anisic acids are the main phenolics found in parthenium (Wakjira *et al.* 2009). Parthenium has also been found to release allelochemicals into the soil from its roots, primarily during its rosette and flowering stage (Kanchan & Jayachandra 1979). The allelochemicals reduce the germination and growth abilities of other plants (Kanchan & Jayachandra 1979; Reinhart *et al.* 2006). These allelochemicals affect cereals, vegetables, pasture grasses and many other plant species (Kanchan & Jayachandra 1979). This results in the displacement of native plant species, and the transformation of pristine grasslands, open woodlands, riverbanks, and floodplains into monocultures of parthenium (Dhileepan 2007).

Parthenium is capable of producing large quantities of pollen, with an average of 624 million pollen grains per plant (Towers & Mitchell 1983). Heavy pollen deposition by parthenium can result in interference of fruit setting in certain crops (Towers *et al.* 1977). The artificial dusting of pollen on the stigma of eggplant, tomato, and paprika has resulted in reduced fruit development of those plants (Towers *et al.* 1977). In addition, maize has been found to have a 40% reduction in grain filling when parthenium pollen occurs on maize stigmas (Towers *et al.* 1977).

Parthenium taints the milk and meat of domestic animals, resulting in a reduced value of the product (Beyene *et al*. 2013). When contaminating animal feed, parthenium can cause dermatitis and skin lesions in livestock (Beyene *et al*. 2013). The presence of parthenium also reduces the value of honey from honey

bees, as bees that collect parthenium pollen produce honey with an undesirable taste (Beyene *et al*. 2013).

Rural homes are often surrounded by dense fields of parthenium, which results in local people being in direct and frequent exposure to parthenium (McConnachie *et al.* 2010). This is worrying as parthenin can result in allergic contact dermatitis in humans from direct contact with the plant, as well as airborne contact dermatitis from exposure to the plants' pollen (Towers & Mitchell 1983). It has been suggested that parthenium can escalate allergic reactions in individuals who are immuno-compromised by diseases such as HIV or tuberculosis (Evans 1997). These diseases are common in African populations (Dolin *et al.* 1994; Mukadi *et al.* 2001; Pettifor *et al.* 2005). Parthenium negatively affects African subsistence farms. These negative impacts result in the need to control *P. hysterophorus*.

1.5. Control of *Parthenium hysterophorus*

1.5.1. Chemical and mechanical control

Effective chemical control of parthenium has been achieved using herbicides (Reddy *et al.* 2007). However the use of herbicides is not a cost-effective method of control, which is problematic in rural areas and areas which require repeat applications of the chemicals (Hobbs & Humphries 1995). Successful mechanical control of weeds requires knowledge about the weed that is being controlled; timing, in particular is important (Gunsolus 1990). In addition, the mechanical control of weeds is not favourable, as the practice is time-consuming and is labour intensive (Armstrong *et al.* 1968). The health problems associated with parthenium also make the use of mechanical control of the weed undesirable (Muniyappa *et al.* 1980; Wakjira *et al.* 2009). This makes other control methods desirable.

1.5.2. The biological control of *Parthenium hysterophorus*

A biological control program for parthenium was first attempted in Australia in 1977 (Dhileepan & McFadyen 2012). From 1977 to 1994, Australian scientists conducted surveys in the native range of parthenium in search of suitable control agents (Dhileepan & McFadyen 2012). This resulted in 159 phytophagous arthropod species from North and Central America being catalogued on parthenium (McClay *et al.* 1995). 144 species were associated with feeding on parthenium during at least one stage of their life-cycle, and of these species, 13 were found to feed only on Ambrosiinae (Asteraceae) (Dhileepan & McFadyen 2012). At present, there are nine insect species and two rust-fungi species that have been released in Australia in an attempt to control parthenium (Dhileepan & McFadyen 2012). They have had limited success in Australia due to the agents only having short outbreaks of large numbers, but the outbreaks result in significant damage to the weed (Dhileepan & McFadyen 2012).

South Africa started a biological control program against parthenium in 2003 (Strathie *et al.* 2005). Based on the Australian biological control program, three insects: *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), *Listronotus setosipennis* (Hustache) (Coleoptera: Curculionidae), and *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae); and a rust fungus, parthenium summer rust, *Puccinia xanthii* var. *parthenii-hysterphorae* Seier, Evans and Romero (Uredinales: Pucciniaceae) were selected as being suitable for host-specificity testing in South Africa (Strathie *et al*. 2005). Additionally another rust fungus, parthenium winter rust *Puccinia abrupta* var. *partheniicola* Parmelee (Uredinales: Pucciniaceae) has been found to occur on South African populations of parthenium (Wood & Scholler 2002). In 2013 host-specificity testing on *Z. bicolorata* and *L. setosipennis* was completed and the insects were released into the South African populations of the weed (McConnachie 2015).

1.6. The biological control agent *Zygogramma bicolorata*

1.6.1. Description of *Zygogramma bicolorata*

Zygogramma bicolorata is a leaf-feeding beetle native to Mexico, however, the specimens used in the South African biological control program were imported from Australia (Strathie *et al.* 2005). The beetle was imported from Mexico into Australia in 1977 after host-specificity tests found that it was capable of developing on only two species of *Parthenium* and *Ambrosia* other than *P. hysterophorus* (McFadyen & McClay 1981). The adult and larval stages of the beetle feed on the leaves of parthenium and prefer young leaves (Dhileepan & McFadyen 2012). Adults oviposit several eggs onto the leaves, flower heads, stems, terminal buds, and axillary buds of parthenium (Dhileepan & McFadyen 2012). The larvae feed on the leaves of the plant until they reach their final (fourth) instar, at which point they burrow into the soil where they pupate (Dhileepan & McFadyen 2012). The pupal stage can last up to two weeks, but can be shorter (Dhileepan & McFadyen 2012). The beetle can complete its life-cycle in six to eight weeks, resulting in the population achieving up to four generations per annum, although this fluctuates with rainfall and food availability (Dhileepan & McFadyen 2012). An adult beetle can live for up to two years (Dhileepan *et al.* 2000). The adult beetles have been found to begin diapause in autumn, and can stay in this state for up to six months (Dhileepan *et al.* 2000). The beetles diapause in the soil, and it has been found that diapause is triggered by cool temperatures and a short day-length (Dhileepan & McFadyen 2012). The beetles can, therefore, be prevented from diapausing by keeping the temperature at 25 ºC and keeping the beetles on a 12:12 hour day:night cycle.

The adults emerge from diapause during early spring with the arrival of increased temperatures, rainfall and an increase in day-length (Dhileepan & McFadyen 2012). The beetles have a high fecundity of about 836 eggs per female (McClay 1985). The eggs also have a high viability with about 85%-91% hatching success (McClay 1985). This allows the population to increase rapidly once the beetle becomes established, showing the potential to be a successful biological control agent (Cowie *et al.* 2018; McClay 1985). Defoliation of parthenium by *Z.*

bicolorata has been found to reduce the plants' height and ability to produce flowers in Australia (Dhileepan *et al.* 2000). Additionally, the beetle is capable of dispersing by flight, and has been found up to 50 000 km^2 from its release sites in India (Jayanth & Visalakshy 1994), making it a suitable self-dispersing biological control agent.

Establishment of *Z. bicolorata* on parthenium in the field in Australia was initially slow, with only two of the 20 initial release sites supporting beetles twelve months after their release (Dhileepan & McFadyen 2012). This was attributed to very dry summers during the release stages (Dhileepan & McFadyen 2012). The two release sites where the beetle was seen twelve months after its release were both located along a creek (Dhileepan & McFadyen 2012). Ten years after the initial release, large outbreaks of the beetle occurred during the summer, which resulted in the complete defoliation of small patches of parthenium within an area of 200 km^2 of the creek sites (Dhileepan & McFadyen 2012). It is suspected that the long time it took for the beetles to establish was due to the beetles struggling to adapt to a dry habitat (Dhileepan & McFadyen 2012). The beetle is now responsible for an area of defoliation of approximately 12 000 km^2 of *P. hysterophorus* in Australia, due to natural spread and deliberate redistribution by people (Dhileepan & McFadyen 2012). Outbreaks of the beetle now occur in Australia and result in the reduction of parthenium vigour, reproductive output, weed density, and soil seed banks (Dhileepan *et al*. 2000, Dhileepan 2003).

1.6.2. Host specificity and release of *Zygogramma bicolorata*

Trials were conducted to determine the host specificity of *Z. bicolorata* in India, Australia, and Mexico. Very few non-target plants were attacked, however, the agriculturally important plant, cultivated sunflower, *Helianthus annus* L. (Asteraceae) was found to suffer minor feeding damage from the beetle (McFadyen & McClay 1981; Jayanth *et al.* 1998). The beetle is unable to complete its life-cycle on this plant, because the young larvae are unable to feed on the sunflower, despite the beetle being able to oviposit on the plant (Jayanth *et al.* 1998). Adults were also found to be unable to oviposit after only one day of

feeding on sunflower (Jayanth *et al.* 1998). Field testing revealed that adult beetles only fed on sunflowers if the sunflower had been treated with crude aqueous extract from *P. hysterophorus* or if the sunflowers had been dusted with *P. hysterophorus* pollen grains (Jayanth *et al.* 1998). It was therefore concluded that the beetles are able to detect the allelochemical parthenin, which is unique to parthenium and occurs in parthenium leaves and pollen and the beetles mistook the sunflowers for parthenium (Jayanth *et al.* 1998). The incidence of feeding of the adults was also found to be significantly lower on sunflower than on parthenium when considering the high population density of 2000 adults/m^2 at the field sites (Jayanth *et al.* 1998). A study by Jayanth and Visalakshy (1994) found that 90% of 500 marked adult beetles that were released into a sunflower field moved away from the field within 24 hours of release and that all beetles had moved away by the seventh day of release. Whilst both sunflower and parthenium are native to Mexico, *Z. bicolorata* has never been observed feeding on sunflower in Mexico (McFadyen & McClay 1981). *Zygogramma bicolorata* was therefore presumed to be safe for release and was released in Australia in 1980 and India in 1983 (Dhileepan & McFadyen 2012; Jayanth *et al.* 1998).

Host specificity testing conducted in South Africa focused on cultivated, native, and exotic Asteraceae species in South Africa as most of the host range testing on species outside the family had been conducted in Australia and India (McConnachie 2015). Twelve of the most widely grown and economically important sunflower varieties found in South Africa were tested (McConnachie 2015). The tests found that the beetle has a low risk (<0.2%) of feeding successfully on non-target plants, and the beetle was approved for release in South Africa in August 2013 (McConnachie 2015).

1.7. Rationale

Zygogramma bicolorata is considered the most promising agent for biological control of parthenium, however it has failed to establish at many release sites around South Africa, and has had little apparent impact on the target weed. Why has *Z. bicolorata* not had any noticeable effect on *P. hysterophorus* at most field

sites in the field in South Africa? Post release evaluations of biological control agents on weeds have shown that agents that look promising in laboratory trials do not always establish (Morin *et al.* 2006). When agents do establish in the field they do not always have a noticeable impact on the target weed (Morin *et al.* 2006). Reasons that have been suggested for the lack of establishment and impact of the agent on the target weed in the field include climatic incompatibility, disturbance of overwintering sites, parasitism of the agent by native parasitoids, predation of the agent, host plant quality and burning of the weed with no management plan (Wheeler 2001). Lack of knowledge regarding the agents' biological needs, has resulted in some agents being released in unsuitable environments, leading to the failure of the biological control program (Byrne *et al.* 2002). A thorough laboratory investigation of *Z. bicolorata*'s thermal and humidity level limits were conducted in South Africa (King 2008). That study concluded that *Z. bicolorata* was highly likely to establish in South Africa as most of the country had highly favourable climatic conditions for the beetle (King 2008). The beetle has, however, failed to establish in high numbers at most release sites, indicating that factors other than temperature and humidity are affecting *Z. bicolorata* populations in the field in South Africa. This project will survey *Z. bicolorata* populations and feeding damage on parthenium at South African release sites. In addition it will investigate the influence of soil type, soil moisture level and native predators and native parasitoids on *Z. bicolorata* growth and development as well as the likelihood of an integrated system of *Z. bicolorata* and a burning regime being able to control *P. hysterophorus*.

1.8. Study aims and objectives

The aim of this study is to determine whether biological aspects, such as soil field capacity, soil type, and predators and parasitoids, are affecting the ability of *Zygogramma bicolorata* to establish in the field in South Africa. This study will also attempt to measure the level of feeding damage and the effect of the beetle's feeding damage on *Parthenium hysterophorus* flower production, leaf greenness, canopy volume, and density in the field in South Africa. Finally, the study will determine the diapause and pupation depth of *Z. bicolorata* in order to determine

the likelihood of integrated control with a burning regime as a viable option. These are embodies in five objectives.

Objective 1: Investigate the level of damage to parthenium caused by *Z. bicolorata* in the field in South Africa.

Objective 2: Investigate the effect of the beetle's feeding on the level of parthenium flower production, leaf greenness, canopy volume and density in the field in South Africa.

Objective 3: Discover if predators or parasitoids attack the egg stage of *Z. bicolorata* in the field in South Africa.

Objective 4: Assess the effect of soil type and soil field capacity on the beetle's pupation and diapause.

Objective 5: Discover at what depth the beetle pupates and diapauses in the soil.

1.9. References

ADKINS, S.W. & SHABBIR, A. 2014. Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.). *Pest Management Science* **2014**.

ARMSTRONG, D.L., LEASURE, J.K. & CORBIN, M.R. 1968. Economic comparison of mechanical and chemical weed control. *Weed Science* **16**: 369-371.

BELLOWS, T.S. & FISHER, T.W. 1999. *Handbook of Biological Control: principles and applications of biological control.* Academic Press, California, United States of America.

BEYENE, H., GEBREHIWOT, L., NIGATU, L., ZEWDIE, K., REGASSA, S., TADESSE, B., DEJEN, A. & MERSIE, W. 2013. Impacts of parthenium (*Parthenium hysterophorus*) on the life of Ethiopian farmers. *Ethiopian Journal of Weed Management* **6**: 1-14.

BROOKS, T.M., MITTERMEIER, R.A., MITTERMEIER, C.G., DA FONSECA, G.A.B., RYLANDS, A.B., KONSTANT, W.R., FLICK, P., PILGRIM, J., OLDFIELD, S., MAGIN, G. & HILTON-TAYLOR, C. 2002. Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* **16**: 909-923.

BRUNEL, S., PANETTA, D., FRIED, G., KRITICOS, D., PRASAD, R., LANSINK, A.O., SHABBIR, A., & YAACOBY, T. 2014. Preventing a new invasive alien plant from entering and spreading in the Euro-Mediterranean region: the case study of *Parthenium hysterophorus. EPPO Bulletin* **44**: 479-489.

BYRNE, M.J., CURRIN, S. & HILL, M.P. 2002. The influence of climate on the establishment and success of the biocontrol agent *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biological Control* **24**: 128-134.

CHIPPENDALE, J.F. & PANETTA, F.D. 1994. The cost of parthenium weed to the Queensland cattle industry. *Plant Protection Quarterly* **9**:73-76

COWIE, B.W., WITKOWSKI, E.T.F., BYRNE, M.J., STRATHIE, L.W., GOODALL, J.M. & VENTER, N. 2018. Physiological response of *Parthenium hysterophorus* to defoliation by the leaf feeding beetle *Zygogramma bicolorata*. *Biological Control* **117**: 35-42

CRAWLEY, M.J., KORNBERG, H., LAWTON, J.H., USHER, M.B., SOUTHWOOD, R., O'CONNOR, R.J. & GIBBS, A. 1986. The population biology of invaders [and discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **314**: 711-731.

DE LANGE, W.J. & VAN WILGEN, B.W. 2010. An economic assessment of the contribution of biological control of the management of invasive alien plants and the protection of ecosystem services in South Africa. *Biological Invasions* **12**: 4113-4124.

DHILEEPAN, K. 2003. Seasonal variation in the effectiveness of leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Curculionidae) and stem-galling moth *Epiblema strenuata* (Lepidoptera: Tortricidae) as biocontrol agents on the weed *Parthenium hysterophorus* (Asteraceae). *Bulletin of Entomological Research* **93**: 393-401.

DHILEEPAN, K. 2007. Biological control of parthenium (*Parthenium hysterophorus*) in Australian rangeland translates to improved graas production. *Weed Science* **55**: 497-501.

DHILEEPAN, K. 2012. Reproductive variation in naturally occurring populations of the weed *Parthenium hysterophorus* (Asteraceae) in Australia. *Weed Science* **60**: 571-576.

DHILEEPAN, K & MCFADYEN R.C. 2012. *Parthenium hysterophorus* L. - parthenium. In: JULIEN, M., MCFADYEN, R. & CULLEN, J. (eds) Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne. Pp. 448-462.

DHILEEPAN, K. SETTER, S. & MCFADYEN, R.E. 2000. Impact of defoliation by the introduced biocontrol agent *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on parthenium weed in Australia. *Biocontrol* **45**: 501-512.

DOLIN, P.J., RAVIGLIONE, M.C. & KOCHI, A. 1994. Global tuberculosis incidence and mortality during 1990-2000. *Bulletin of the World Health Organisation* **72**: 213-220.

GOEDEN, R.D. & LOUDA, S.M. 1976. Biotic interference with insects imported for weed control. *Annual Review of Entomology* **21**: 325-342.

GUNSOLUS, J.L. 1990. Mechanical and cultural weed control in corn and soybeans. *American Journal of Alternative Agriculture* **5**: 114-119.

HINZ, H.L. & SCHWARZLAENDER, M. 2004. Comparing invasive plants from their native and exotic range: What can we learn for biological control? *Weed Technology* **18**: 1533-1541.

HOBBS, R.J. & HUMPHRIES, S.E. 1995. An integrated approach to the ecology and management of plant invasions. *Conservation Biology* **9**: 761-770.

HODDLE, M.S. 2004. Restoring balance: Using exotic species to control invasive exotic species. *Conservation Biology* **18**: 38-49.

JAYACHANDRA. 1971. *Parthenium* weed in Mysore State and its control. *Current Science* **40**: 568-569.

JAYANTH, K.P. & BALI, G. 1994. Biological control of *Parthenium hysterophorus* by the beetle *Zygogramma bicolorata* in India. *FAO Plant Protection Bulletin* **42**: 207-213.

JAYANTH, K.P. & VISALAKSHY, P.N.G. 1994. Field evaluation of sunflower varieties for susceptibility to the parthenium beetle *Zygogramma bicolorata. Journal of Biological Control* **8**: 48-52.

JAYANTH, K.P., VISALAKSHY, P.N.G., CHAUDHARY, M. & GHOSH, S.K. 1998. Age-related feeding by the parthenium beetle *Zygogramma bicolorata* on sunflower and its effect on survival and reproduction. *Biocontrol Science and Technology* **8**: 117-123.

KANCHAN, S.D. & JAYACHANDRA. 1979. Allelopathic effects of *Parthenium hysterophorus* L. I. Exudation of inhibitors through roots. *Plant and Soil* **53**: 27-35.

KANCHAN, S.D. & JAYACHANDRA. 1980. Pollen allelopathy-a new phenomenon. *The New Phytologist* **84**: 739-746.

KHOSLA, S.N. & SOBTI, S.N. 1981. Effective control of *Parthenium hysterophorus* L. *Pesticides* 15: 18-19.

KING, H. 2008. Thermal physiology and predicted distribution of *Zygogramma bicolorata* (Chrysomelidae), a promising agent for the biological control of the invasive weed *Parthenium hysterophorus* in South Africa. Unpublished. Masters diss., Faculty of Science and Agriculture, University of KwaZulu-Natal, 2008.

LAU, J.A. 2008. Beyond the ecological: Biological invasions alter natural selection on a native plant species. *Ecology* **89**: 1023-1031.

LIU, H., STILING, P., PEMBERTON, R. W. & PEÑA, J. 2006. Insect herbivore faunal diversity among invasive, non-invasive and native Eugenia species: Implications for the enemy release hypothesis. *The Florida Entomologist* **89**: 475-484.

MCCLAY, A.S. 1985. Biocontrol agents for *Parthenium hysterophorus* from Mexico. In: DEL FOSSE, E.S. (ed) Proceedings of the Sixth International Symposium on Biological Control of Weeds. Ottawa, Canada. Pp. 19-25.

MCCLAY, A.S., PALMER, W.A., BENNETT, F.D. & PULLEN, K.R. 1995. Phytophagous arthropods associated with *Parthenium hysterophorus* (Asteraceae) in North America. *Environmental Entomology* **24**: 796-809.

MCCONNACHIE, A.J. 2015. Host range and risk assessment of *Zygogramma bicolorata,* a defoliating agent released in South Africa for the biological control of *Parthenium hysterophorus. Biocontrol Science and Technology* **25**: 975-991.

MCCONNACHIE, A.J., STRATHIE, L.W., MERSIE, W., GEBREHIWOT, L., ZEWDIE, K., ABDUREHIM, A., ABRHA, B., ARAYA, T., ASAREGEW, F., ASSEFA, F., GEBRE-TSADIK, R., NIGATU, L., TADESSE, B. & TANA, T. 2010. Current and potential distribution of the invasive plant *Parthenium hysterophorus* (Asteraceae) in eastern and southern Africa. *Weed Research* **51**: 71-84.

MCFADYEN, R.E. 1992. Biological control against parthenium weed in Australia. *Crop Protection* **11**: 400-407.

MCFADYEN, R.E. 1998. Biological control of weeds. *Annual Review of Entomology* **43**: 369-393.

MCFADYEN, R.E. & MCCLAY, A.S. 1981. Two new insects for the biological control of parthenium weed in Queensland. *Proceedings of the Sixth Australian Weed Conference* **1**: 145-149.

MORIN, L., EVANS, K.J. & SHEPPARD, A.W. 2006. Selection of pathogen agents in weed biological control: critical issues and peculiarities in relation to arthropod agents. *Australian Journal of Entomology* **45**: 349-365.

MUKADI, Y.D., MAHER, D. & HARRIES, A. 2001. Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa. *Aids* **15**: 143-152.

MUNIYAPPA, T.V., PRASAD, T.V.R. & KRISHNAMURTHY, K. 1980. Comparative efficacy and economics of mechanical and chemical methods of control of *Parthenium hysterophorus* Linn. *Indian Journal of Weed Science* **12**: 137-144.

NAVIE, S.C., MCFADYEN, R.E., PANETTA, F.D. & ADKINS, S.W. 1996. The biology of Australian weeds 27. *Parthenium hysterophorus* L. *Plant Protection Quarterly* **11**: 76-88.

NAVIE, S.C. PANETTA, F.D., MCFADYEN, R.E. & ADKINS, S.W. 1998. Behaviour of buried and surface-laying seeds of parthenium weed. (*Parthenium hysterophorus* L.) *Weed Research* **38**: 338-341.

PAYNTER, Q., FOWLER, S.V., GOURLAY, A.H., GROENTEMAN, R., PETERSON, P.G., SMITH, L. & WINKS, C.J. 2010. Predicting parasitoids accumulation on biological control agents of weeds. *Journal of Applied Ecology* **47**: 575-582.

PETTIFOR, A.E., REES, H.V., KLEINSCHMIDT, I., STEFFENSON, A.E., MACPHAIL, C., HLONGWA-MADIKIZELA, L., VERMAAK, K. & PADIA, N.S. 2005. Young people's sexual health in South Africa: HIV prevalence and sexual behaviours from a nationally representative household survey. *Aids* **19**: 1525-1534.

PICMAN, A.K. & TOWERS, G.H.N. 1982. Sesquiterpene lactones in various populations of *Parthenium hysterophorus. Biochemical Systematics and Ecology* **10**: 145-153.

RANDALL, J.M. 1996. Weed control for the preservation of biological diversity. *Weed Technology* **10**: 370-383.

REDDY, K.N., BRYSTON, C.T. & BURKE, I.C. 2007. Ragweed parthenium (*Parthenium hysterophorus*) control with preemergence and postemergence herbicides. *Weed Technology* **21**: 982-986.

REINHART, C., VAN DER LAAN, M., BELZ, R.G., HURLE, K. & FOXCROFT, L. 2006. Production dynamics of the allelochemical parthenin in leaves of P*arthenium hysterophorus* L. *Journal of Plant Diseases and Protection* **XX**: 427-433.

ROLLINS, R.C. 1950. The guayale rubber plant and its relatives. *Contributions from the Gray Herbarium of Havard University No. 172*. Gray Herbarium of Harvard University, Cambridge, Massachusetts, U.S.A.

SAPIA. 2011. South African Plant Invaders Atlas, SAPIA News No. 18. ARC-Plant Protection Research Institute, Pretoria, South Africa.

SAX, D.F. & BROWN, J.H. 2000. The paradox of invasion. *Global Ecology and Biogeography* **9**: 363-371.

SCHAFFNER, U. 2001. Host range testing of insects for biological weed control: How can it be better interpreted? Data on the host range of biocontrol candidates are particularly relevant in assessing potential detrimental effects to nontarget organisms. *BioScience* **51**: 951-959.

SINGH, S., YADAV, A., BALYAN, R.S., MALIK, R.K. & SINGH, M. 2004. Control of ragweed parthenium (*Parthenium hysterophorus*) and associated weeds. *Weed Technology* **18**: 658-664.

STRATHIE, L.W.; MCCONNACHIE, A.J. & RETIEF, E. 2011. Initiation of biological control against *Parthenium hysterophorus* L. (Asteraceae) in South Africa. *African Entomology* **19**: 378-392.

STRATHIE, L.W., WOOD, A.R., VAN ROOI, C. & MCCONNACHIE, A. 2005. *Parthenium hysterophorus* (Asteraceae) in southern Africa, and initiation of biological control against it in South Africa. In: RAMACHANDRA PRASAD, T.V. (ed) Proceedings of the Second International Conference on Parthenium Weed Management. Bangalore, India. Pp. 127-133.

TAMADO, T. & MILBERG, P. 2000. Weed flora in arable fields of eastern Ethiopia with emphasis on the occurrence of *Parthenium hysterophorus*. *Weed Research* **40**: 507-521.

TAMADO, T., SCHÜTZ, W. & MILBERG, P. 2002. Germination ecology of the weed *Parthenium hysterophorus* in eastern Ethiopia. *Annals of Applied Biology* **140**: 263-270.

THRALL, P.H. & BURDON, J.J. 2004. Host: pathogen life history interactions affect biological control success. *Weed Technology* **18**: 1269-1274.

TOWERS, G.H.N., MITCHELL, J.C., RODRIGUEZ, E., BENNETT, F.D., SUBBA RAO, P.V. 1977. Biology and chemistry of *Parthenium hysterophorus* L.: a problem weed in India. *Journal of Scientific and Industrial Research* **36**: 672-684.

TOWERS, G.H.N. & MITCHELL, J.C. 1983. The current status of the weed *Parthenium hysterophorus* L. as a cause of allergic contact dermatitis. *Contact Dermatitis* **9**: 465-469.

VITOUSEK, P.M., D'ANTONIO, C.M., LOOPE, L.L. & WESTBROOKS, R. 1996. Biological invasions as global environmental change. *American Scientist* **84**: 468-478.

WAKJIRA, M., BERECHA, G. & TULU, S. 2009. Allelopathic effects of an invasive alien weed *Parthenium hysterophorus* L. compost on lettuce germination and growth. *African Journal of Agricultural Research* **4**: 1325-1330.

WHEELER, G.S. 2001. Host plant quality factors that influence the growth and developement of *Oxyops vitiosa*, biological control agent of *Melaleuca quinquenervia. Biological Control* **22**: 256-264.

WOOD, A.R. & SCHOLLER, M. 2002. *Puccinia abrupta* var. *Partheniicola* on *Parthenium hysterophorus* in Southern Africa. *Plant Disease* **86**: 327.

ZIMMERMANN, H.G., MORAN, V.C. & HOFFMANN, J.H. 2004. Biological control in the management of invasive alien plants in South Africa and the role of the Working for Water programme. *South African Journal of Science* **100**: 34-40.

CHAPTER TWO

***Zygogramma bicolorata* (Pallister) (Coleoptera: Chrysomelidae) Establishment and Effectiveness on *Parthenium hysterophorus* (L.) (Asteraceae: Heliantheae) in the Field in South Africa**

2.1. Abstract

Zygogramma bicolorata is a biological control agent which has been released to control *Parthenium hysterophorus* in South Africa. The beetle has had a limited number of successful establishments and persistence at release sites around South Africa, resulting in an overall low impact on the weed. A post-release survey was conducted on *Z. bicolorata* to establish whether the beetle has the potential to control the weed, and to establish whether there are any factors which limit the potential establishment of the beetle. While the beetle reduces the plant's density (50 plants/m^2 to 0 plants/m^2 at some sites) and canopy volume (20 cm^3 to 2 cm^3 at its best), it did not effectively reduce the chlorophyll content of the plants' leaves or flower production. However; the beetle cannot tolerate soils that are drier than a volumetric soil water content of 10%. The beetles' eggs are subject to high predation rates (between 37.2% and 100% of all eggs were prey on). Therefore, in order to maximise the chances of beetle establishment, it is suggested that only adult beetles be released into the field in high numbers.

Keywords: Canopy volume; chlorophyll content; egg predation; feeding damage; flower production; post-release evaluation; soil moisture.

2.2. Introduction

2.2.1. The effects of agents on target weeds in the field

Post-release evaluations, which consist of establishment surveys, biological control agent incidence rates and damage assessments of the target weed, are an important part of determining the success of a biological control project (Morin *et al.* 2009). These evaluations take place after the agent has been released in order to assess whether it has an impact on the target weed in the introduced range (Thomas & Willis 1998). The main factors which post-release evaluations

typically focus on are: the damage to the target weed by the agent, agent prevalence, and change in target weed density (Briese 2004; Carson *et al.* 2008). A review of 61 studies on the effectiveness of biological control agents against their target weeds by Clewley *et al*. (2012) found that agents can reduce target weed size ($\overline{x}$ = 28 ± 4% reduction), target weed flower production ($\overline{x}$ = 35 ± 13% reduction), and target weed density ($\overline{x}$ = 56 ± 7% reduction). The most effective agents are found in the families Chrysomelidae (Latreille) (Coleoptera) and Curculionidae (Latreille) (Coleoptera) (Clewley *et al*. 2012).

Zygogramma bicolorata (Pallister) (Coleoptera: Chrysomelidae) was released into South Africa in 2013, in order to control the alien invasive weed, *Parthenium hysterophorus* (L.) (Asteraceae: Heliantheae) (McConnachie 2015).The success of *Z. bicolorata* in South Africa has yet to be determined, although the beetle has reduced *P. hysterophorus* density, height and total reproductive output in Australia (Dhileepan *et al.* 2000; Dhileepan 2003). This study measured the effects of the beetle on *P. hysterophorus* density, canopy volume, flower production, and chlorophyll content in the field in South Africa.

2.2.2. Constraining factors on agent establishment in the field

Local climate is thought to be a dominant factor for agent dispersal and establishment (Robertson *et al.* 2008). It is estimated that up to 65% of all biological control agents fail to establish on their target weeds in the field (Julien *et al.* 1984) and 44% of agents are estimated to have failed because of climatic unsuitability (McEvoy & Coombs 1999). Additionally, poorly matched climates have been found to reduce the agent's impact on the target weed (Freckleton 2000; Norris *et al.* 2002) and unsuccessful establishment or inadequate control are often caused by climatic incompatibility (Byrne *et al.* 2002). A variety of factors may be responsible for climatic incompatibility; however, studies typically focus on temperature (King 2011). A thorough investigation of *Z. bicolorata*'s thermal and humidity level limits under laboratory conditions was conducted (King 2008). The study concluded that *Z. bicolorata* was likely to establish in South Africa as most of the country had favourable temperature and humidity conditions for the beetle

(King 2008). The beetle has, however, established in low numbers at most release sites in South Africa, indicating that additional factors other than temperature and humidity affect *Z. bicolorata* populations in the field. The beetle pupates in the soil during the wet seasons of spring and summer, emerging as an adult. Soil is, therefore, an important factor in the beetle's lifecycle, and one that has been overlooked so far. This is a knowledge gap that this study will fill. Soil is considered a safe haven for organisms as it typically has a relatively stable and high humidity (Barbercheck 1992). Hawley (1949) found that the population of the Japanese beetle *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae) was high in years with high rainfall, but was low in years when the rainfall was not substantial, due to the soil-inhabiting life stages of the beetle dying off in years with low rainfall. It is important to document whether *Z. bicolorata*'s ability to establish in the field could be limited by the moisture of the soil in which it is pupating and diapausing.

Predators and parasitoids are another factor which may reduce the effectiveness of a biological control agent (Gupta *et al*. 2004; Paynter *et al*. 2010). Lawton (1985) suggested that agents should ideally be taxonomically distinct and feed differently to native species in order to avoid the agent being targeted by local parasitoids. However, it is not always possible to successfully avoid entire taxonomic groups. It has been found that parasitoid richness in localised areas increases as the time an agent has been present in the area increases (Paynter *et al*. 2010). Hill and Hulley (1995) found that susceptible weed biological control agents can rapidly accumulate parasitoids. However, no study has been conducted on the incidence of parasitoid attacks on *Z. bicolorata*. This is an additional knowledge gap that this study will fill.

Predators of *Z .bicolorata* have been found in India (Gupta *et al*. 2004). *Andrallus spinidens* Fab. (Hemiptera: Pentatomidae) and *Cantheoconidea furcellata* Wolf (Hemiptera: Pentatomidae) were observed attacking *Z. bicolorata* larvae, while *Sycanus pyrrhomelas* Walker (Hemiptera: Reduviidae) was observed attacking *Z. bicolorata* adults and larvae (Gupta *et al*. 2004). These predatory bugs were only

found in areas where the *Z. bicolorata* population was high, where *P. hysterophorus* plants were nearly defoliated (Gupta *et al*. 2004). No predators were found in areas where *Z. bicolorata* was in the process of establishing (Gupta *et al*. 2004). No study of this kind has been conducted on *Z. bicolorata* in South Africa. This is another knowledge gap which this study will address.

2.3. Aims

The aim of this study was to measure if and how *Z. bicolorata* affects *P. hysterophorus* in the field in South Africa. This study will also attempt to measure whether soil moisture affects the control potential of *Z. bicolorata* in the field and discover if any predators or parasitoids attack *Z. bicolorata* eggs at selected field sites in South Africa. The study objectives were as follows:

Objective 1: Determine the level of feeding damage on parthenium.

Objective 2: Determine whether the beetle's feeding damage affects *P. hysterophorus* density, canopy volume, flower production and leaf chlorophyll content.

Objective 3: Determine whether soil moisture affects *Z. bicolorata*'s influence on *P. hysterophorus*.

Objective 4: Determine if any predators or parasitoids attack *Z. bicolorata* eggs in the field.

2.4. Materials and methods

2.4.1. Study site description

Three field sites were selected near Malelane, Mpumalanga for these experiments, namely Ivaura farm (25°32' 46"S; 31°38' 51"E), Mauricedale game ranch (25°31'46"S; 31°36'38"E) and an abandoned cane field (25°29'21"S; 31°31'38"E). The town of Malelane is located 255 m above sea level and has a lowveld subtropical climate. Generally, the wet season occurs from September to April (Fig. 2.1) and the dry season from May to August. On average, the lowest quantity of rainfall occurs in the month of June (6.25 mm), while the highest mean rainfall occurs in January (146.22 mm) (SASRI Weather Web 2017). The mean annual precipitation is 768.76 ± 139.97 mm (SASRI Weather Web 2017). The

hottest month of the year, on average, is February (25.6 °C) (Fig. 2.2), while June (16.5 °C) is the coldest (Climate-Data.org 2017). The variation of average monthly temperature is 9.1 °C (Climate-Data.org 2017).

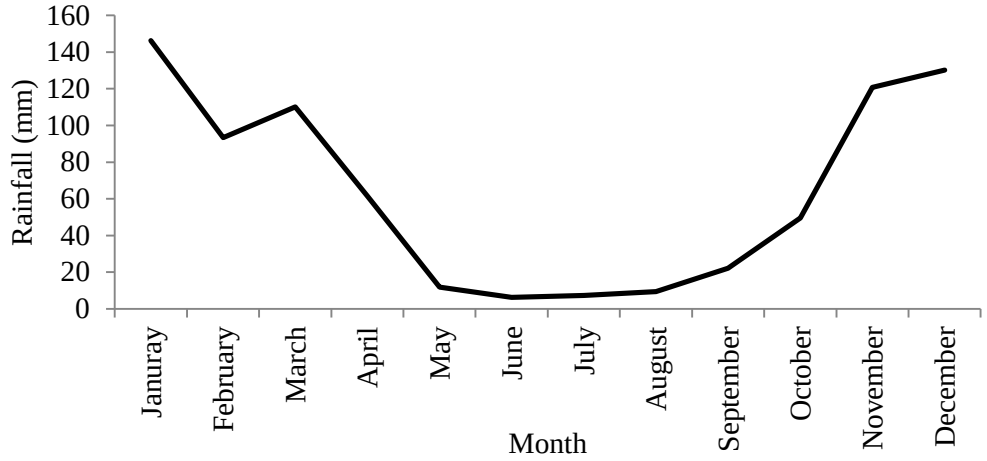


Figure 2.1. Mean monthly rainfall (mm) from 1997 to 2017 recorded at Malelane during a year (SASRI Weather Web 2017).

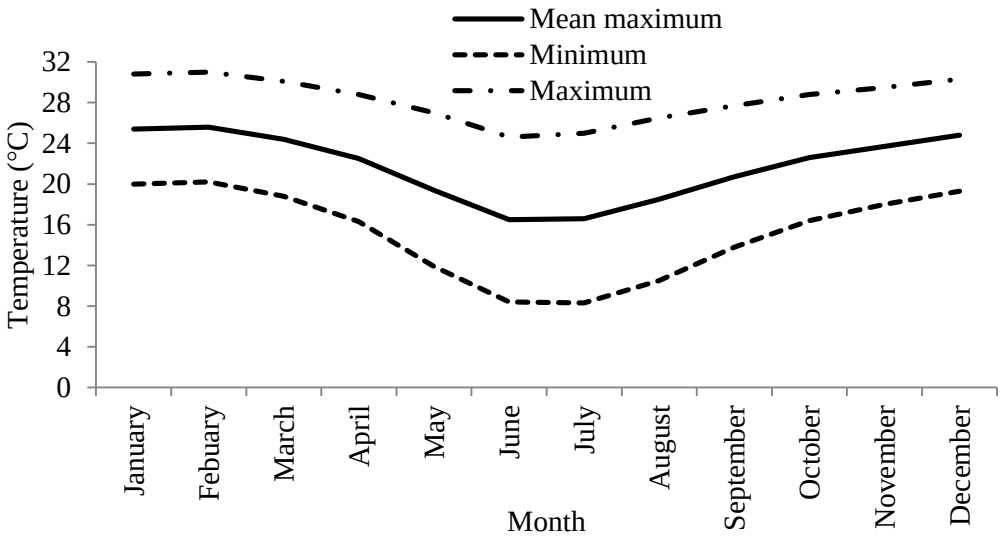


Figure 2.2. Mean maximum, minimum, and maximum monthly temperature (°C) from 1997 to 2017 recorded at Malelane during a year (Climate-Data.org 2017).

The Ivaura site is a former cattle farm that was converted to a game farm. *Parthenium hysterophorus* is suspected to have invaded the property through seeds in the cattle feed. The areas that *P. hysterophorus* has invaded are the grass pastures of the farm, while the woodland areas are un-invaded. The grass pastures were burnt in the winter of 2015 before the experiments were started, but were not

burned during the experiments. The Mauricedale site is a game farm, and has a mixture of grass pastures and woodland. *Parthenium hysterophorus* has invaded the grass pastures of the game farm, whilst the weed is absent from the woodland areas of the property. However, it is not known how the weed invaded the property. No burning has been conducted on the property. The third site is an abandoned cane field, where native grass has re-established along with *P. hysterophorus*. No management activities occur on this site, so no burning has taken place since the agricultural activities at this site have ceased.

2.4.2. Release and monitoring of *Z. bicolorata* on *P. hysterophorus*

The purpose of this experiment was to measure the effect of *Z. bicolorata* on *P. hysterophorus* density, canopy volume, flower production and chlorophyll content of the leaves in the field. Invaded areas in Ivaura and in Mauricedale were selected for this experiment. Two cages of 2.5 X 2.5 m in basal area and 2 m in height covered with thrips gauze screening, and zips at one end to allow access to the cages were placed at each site over patches of established *P. hysterophorus*. Beetles were released into the cages and confined so that their effect on *P. hysterophorus* could be measured ("beetles present" treatment). A permanent plot of 2.5 X 2.5 m was marked out in each field in order to measure plant dynamics where no beetles were present in similar environmental conditions ("beetles absent" treatment). One hundred adult *Z. bicolorata* beetles were placed onto *P. hysterophorus* plants in each of the experimental cages at the start of the experiment, and were replaced if no evidence of any beetles in the experimental cages was found on the following sampling occasions. If no evidence of beetles were found in the cages, they were presumed to be dead. The replacement of beetles only occurred during the first season of sampling. Evaluations took place every 5 weeks over a two-and-a-half year period at both sites from November 2015 to May 2016 and from December 2016 to May 2017. Evaluations did not take place between May 2016 and December 2016 as there were no plants present at either site during this time. Each cage and marked-out plot was divided into a grid of 0.5 X 0.5 m quadrats (n=25 quadrats per plot) to aid random selection of plants during sampling. Ten representative quadrats for each cage and control plot

were chosen by generating a random number, related to a specific 0.5 X 0.5 m quadrat of each cage. When possible, ten *P. hysterophorus* plants from each quadrat were selected as representatives, as follows: the two tallest plants in the quadrat, the two shortest plants in the quadrat, while the other six plants selected were of average height for that quadrat.

The level of feeding damage on each *P. hysterophorus* plant was estimated by giving it a rating of 0-7 according to the Walker eight point scale (Table 2.1) (Walker 1976). The plant canopy volume, chlorophyll content of the leaves, and flower production of the same *P. hysterophorus* plants per quadrat was assessed on each occasion. The control plots at each site were checked for beetle presence to confirm that no beetles were present. The sampling method was the same for the "beetles present" and "beetles absent" treatments. The number of open flowers and flower buds on each of the selected *P. hysterophorus* plants was counted, if there were fewer than 100 flowers. If there were more than 100 flowers on a single plant, three representative branches were selected and their flowers were counted and multiplied by the total number of branches to estimate the total number of flowers on the plant.

Table 2.1. The Walker eight point scale rating (Walker 1976) used to estimate percentage of feeding damage by *Z. bicolorata* per *P. hysterophorus* plant.

rating	% feeding damage
0	0
1	1-9
2	10-25
3	26-50
4	51-75
5	76-90
6	91-99
7	100

The volume of each plant was calculated by measuring to the nearest millimetre the height (Ht) of the plant; the widest diameter of the plant (D_1), and the diameter of the plant at right angles to the widest width of the plant (D_2). The volume of the plant was calculated based on:
Volume = $(4/3)\pi r^3$ = Volume= $(4/3)\pi$ $(Ht/2)(D_1/2)(D_2/2)$.

From March 2016 to May 2017, the photosynthetic rates of the plants were estimated by measuring chlorophyll content, as an increase in chlorophyll content is associated with an increase in a plant's photosynthetic rate (Eaton 1931; Emerson 1929). A Single Photon Avalanche Diode (SPAD 502 Chlorophyll Meter (Minolta, Osaka 542, Japan)) was used to measure the chlorophyll content of the largest leaf of each randomly selected plant. *Parthenium hysterophorus* density was calculated by counting the number of *P. hysterophorus* plants in each of the randomly selected quadrats within each 2.5x2.5 m plot/cage.

2.4.3. The effect of soil moisture on *Z. bicolorata* establishment.

The abandoned cane field site was used to assess the effect of soil moisture on *Z. bicolorata* because the beetles had established at this site as a result of previous field releases since 2013. Additionally, a stream was present on the edge of one end of the field, creating a natural moisture gradient. A 100 m transect was set out perpendicular to the stream and replicated for each field trip. Measurements of *Z. bicolorata* incidence, feeding damage scores, and soil moisture were taken every 10 m starting next to the stream and ending in the field. A 0.5 X 0.5 m quadrat was placed every 10 m. A 12 cm HS2 Hydrosense II (Campbell Scientific, Inc. Utah 84321-1784), which measures the volumetric soil water content (VSWC), was used to measure the soil moisture every 10 m along the transect. A 12 cm probe was used because the *P. hysterophorus* roots do not penetrate deeper than 8 cm below the surface of the soil (Fig. 2.3). Beetle feeding damage and the number of *Z. bicolorata* adults, larvae, and eggs per quadrat were measured in order to determine beetle incidence. These measurements were then compared against the VSWC.



Figure 2.3. Root lengths of *P. hysterophorus* grown in the abandoned cane field experimental area.

2.4.4. Vulnerability of *Z. bicolorata* eggs to predation and parasitoids

This assessment of the vulnerability of *Z. bicolorata* eggs to predation and parasitism took place between December 2015 and March 2017 at the Ivaura field site. Beetles in the laboratory culture were allowed to lay eggs on six whole potted parthenium plants one day before the eggs were released into the field. The eggs on each plant were counted before being placed in the field. Three (control) of the six plants had a 2 cm wide line of ant tack ("All Trap", Jumbo Cash & Carry (Pty) Ltd) placed around the stem, 5 cm above the soil, so that no crawling insects could access the plant foliage. The other three plants (treatment) had no ant tack placed on them so that crawling insects could climb the plant. All six plants had the leaves protruding from the lowest 5 cm of the stem removed, to prevent any potential crawling predators from bypassing the ant tack. Holes were dug and the potted plants were then placed into the *P. hysterophorus* stand at the Ivaura field site by burying the pots into the ground to the point where the soil level in the pot matched the ground level. Any tall grass, sticks and branches of other plants within a 0.5 m circumference of the buried pots were cleared away in order to prevent an alternative avenue of access to the eggs other than the *P. hysterophorus* stem. The *P. hysterophorus* plants were placed in treatment-control pairs, no more than 1 m away from each other, to minimise differences in microclimate between treatments. The plants were left in the field for five days, and watered daily in

order to avoid them drying out. Then the plants were collected and the number of eggs remaining on the plants was counted to estimate the extent of predation of *Z. bicolorata* eggs and compare between treatment and control plants. Absent eggs were assumed to be predated. The remaining eggs were removed from their respective plants and placed in separate Petri dishes per plant with damp tissue paper in order to prevent the eggs from becoming dehydrated and labelled accordingly. The eggs remained in the Petri dishes for eight days and were checked daily for hatching or parasitoid emergence. The percentage of *Z. bicolorata* eggs parasitized was then calculated.

2.4.5. Statistical analysis

The differences between *P. hysterophorus* plant parameters and feeding damage between the "beetles present" and "beetles absent" treatments were tested using ANOVA on Microsoft Excel 2010, with a Tukey HSD post-hoc test when needed, using R 3.4.3. The effect of VSWC on *Z. bicolorata* feeding damage level was compared using regression analysis. Regressions were compared by ANCOVA (Microsoft Excel 2010), with a Tukey HSD post-hoc test on R 3.4.3. *Zygogramma bicolorata* egg predation between experimental and control treatments for each field trip was compared using an ANOVA test on Microsoft Excel 2010. Data sets which contained no data, i.e. a cage of *P. hysterophorus* which had no flowers when analysing the flower production of the plants, were excluded from statistical tests.

2.5. Results

2.5.1. Rainfall over the study period, recorded by SASRI Weather Web

The rainfall remained lower than the average throughout the season one sampling period (Fig. 2.4A). The lowest recorded monthly rainfall during this sampling period occurred in May 2016 (2.4 mm), whilst the highest recorded monthly rainfall occurred in December 2015 (113.7 mm). Both of these monthly values were lower than their respective long term means (May: 11.9 mm; December: 130.12 mm), and no recorded monthly rainfall value was higher than the long term mean during this time. Rainfall during the season two sampling period was

higher, with the majority of months having an equal or higher monthly rainfall than the respective month in the long term mean (Fig. 2.4B). Even though there were drier periods during the season two sampling period, the higher overall rainfall indicated that this was a wetter season than the previous one.

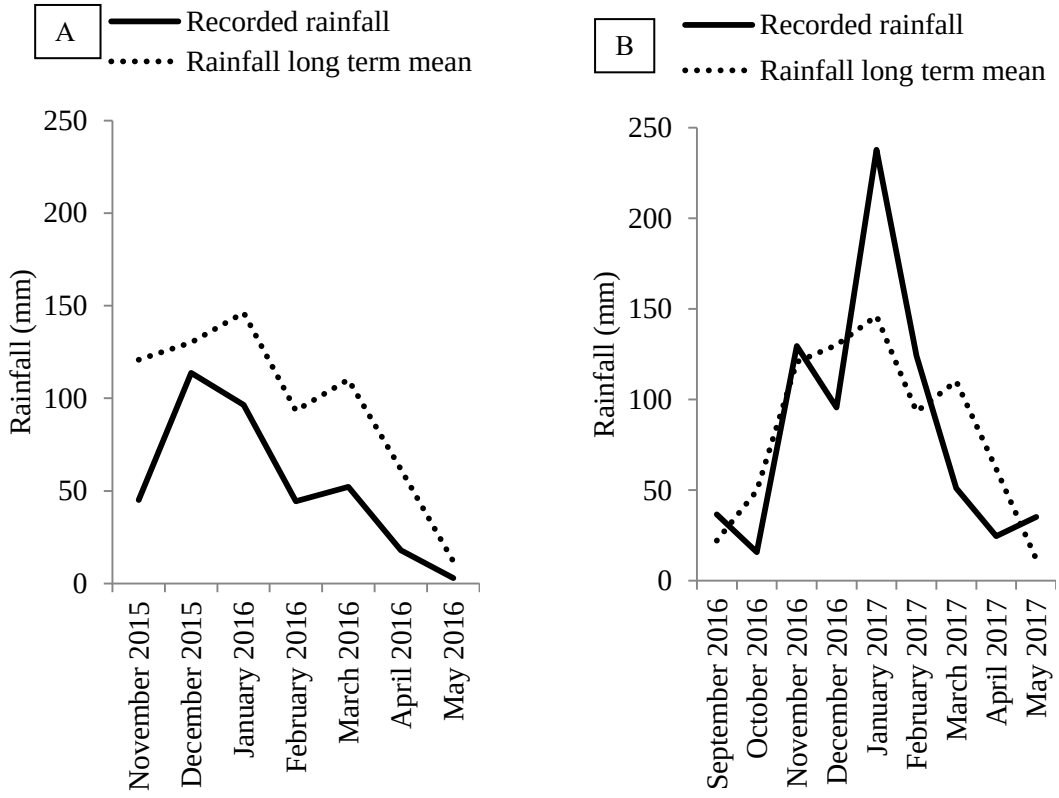


Figure 2.4. The recorded monthly rainfall and the long term mean monthly rainfall during season 1 (A) and season 2 (B) over the study period between November 2015 and May 2017 (SASRI Weather Web).

2.5.2. The effectiveness of *Z. bicolorata* on *P. hysterophorus* in the field

It initially took two months (until January 2016) to find beetles persisting (i.e. establish) at the Ivaura site during the first field trip of the first season (Fig. 2.5A, C). In the first months after establishment (January – March 2016) the level of feeding damage was not high (<10% defoliation), and did not result in any significant differences of *P. hysterophorus* density between the "beetles present" and "beetles absent" treatments. However; the feeding damage in April 2016 was significantly high (100% defoliation) in both of the "beetles present" treatments ($F_{10, 309}$=0.55; P<0.05). This high level of feeding damage resulted in the *P.*

hysterophorus density being significantly lower in the “beetles present” treatment than the *P. hysterophorus* in the “beetles absent” treatment ($F_{18, 422}$=1.78; P<0.05). Following the high feeding damage in May 2016, no *P. hysterophorus* was found in either of the “beetles present” treatments, while the plant was still present in the “beetles absent” treatment.

During the second season (Fig. 2.5B, D) *P. hysterophorus* was absent during September 2016 from both treatments. Between November 2016 and December 2016 it rained heavily, which resulted in high *P. hysterophorus* density in the “beetles absent” treatment. However; the “beetles present” treatments both had significantly lower plant densities, despite the feeding damage recorded on the plants in those treatments remaining below 10% defoliation. The following sample showed that one of the “beetles present” cages had no *P. hysterophorus* plants, while the other had a significantly lower *P. hysterophorus* density than the “beetles absent” treatment. The “beetles present” treatment which had plants present also had a significantly higher feeding damage score. In March 2017 *P. hysterophorus* was only found in the “beetles absent” treatment. In May 2017, no *P. hysterophorus* was found in either treatment, indicating that the plant had died back, likely due to climatic factors.

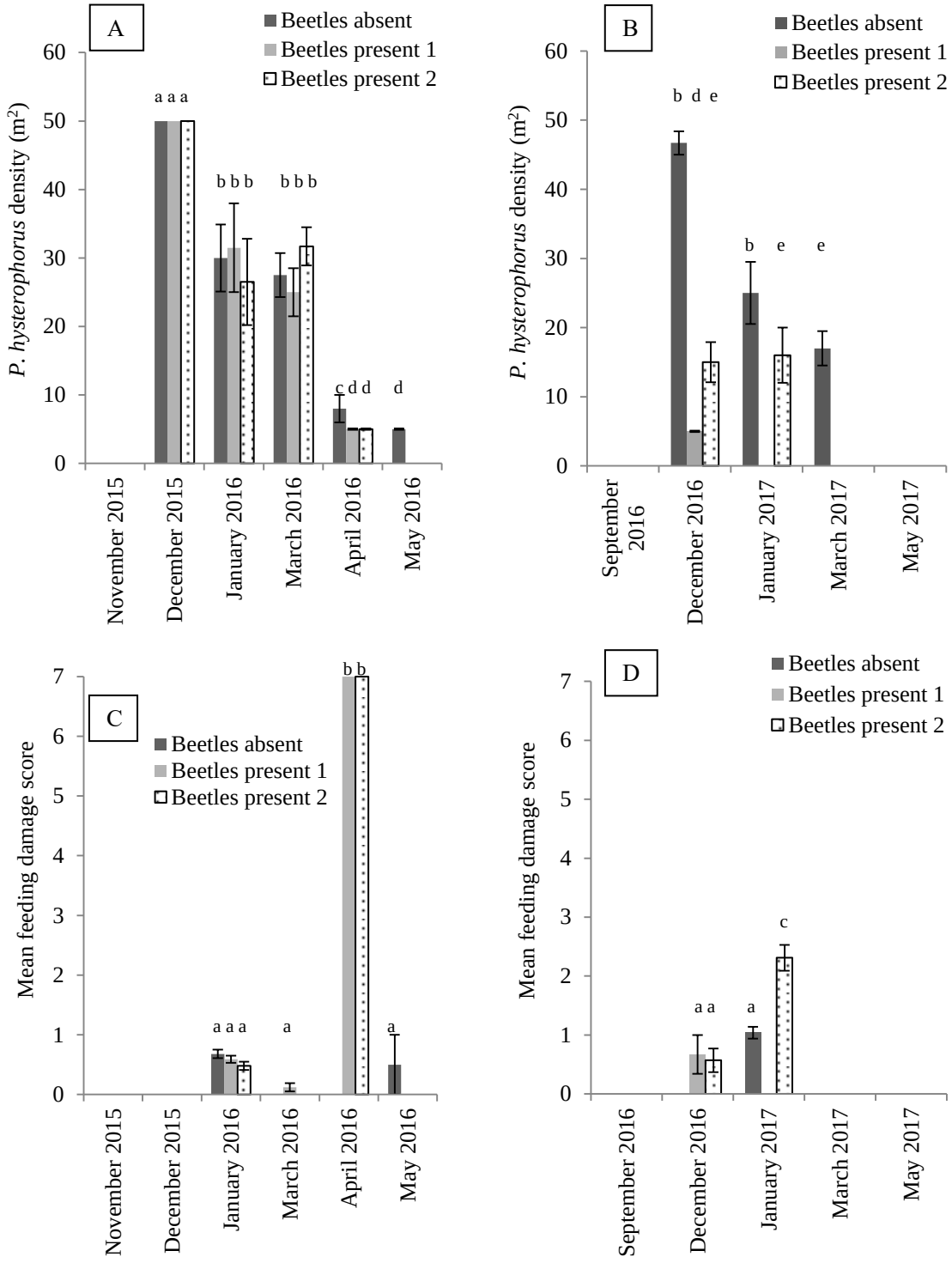


Figure 2. 5. The effect of season and rainfall on *Parthenium hysterophorus* density (mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{18, 422}$=1.78; P<0.05) and *Zygogramma bicolorata* feeding damage (mean ± S.E.) during season 1 (C) and season 2 (D) ($F_{10, 309}$=0.55; P<0.05) in two plots where *Z. bicolorata* beetles were present and one plot where beetles were absent at Ivaura. Different letters above bars indicate significant differences between treatments and time periods (P<0.05, Tukey).

Similarly to the Ivaura site, it initially took two months (until January 2016) to find beetles persisting (i.e. establish) from the first field trip of the first season at the Mauricedale site (Fig. 2.6A, C). The feeding damage was significantly higher in the "beetles present" treatments than the "beetles absent" treatment in January, March and April 2016 ($F_{15, 759}$=6.45; P<0.05). However; this did not result in any significant differences in the *P. hysterophorus* density between the treatments during these months. Feeding damage was not high during the first season, with the majority of mean feeding damage scores less than one (<10% defoliation). However, the mean feeding damage score increased to between one and two (10-25% defoliation) in both the "beetles present" treatments in April 2016. During May 2016 one of the "beetles present" treatments contained no *P. hysterophorus*, while the other's mean of 30 plants/m^2 was much lower than that of the plants in the "beetles absent" treatment (50 plants/m^2), despite there being no significant difference between the feeding damage scores during this time period. However, the high variation of densities in the quadrat sub-samples resulted in there being no significant difference between the treatments.

During the second season (Fig. 2.6B, D) *P. hysterophorus* was absent during September 2016 from both treatments. Heavy rain between November 2016 and December 2016 resulted in high *P. hysterophorus* density in the "beetles absent" treatment. However, the "beetles present" treatments both had significantly lower plant densities ($F_{19, 796}$=3.98; P<0.05), despite the feeding damage recorded on the plants in those treatments remaining below 10% defoliation. During January, March and May 2017 no *P. hysterophorus* was found in any of the "beetles present" treatments, while the plant was found in the "beetles absent" treatment during this time.

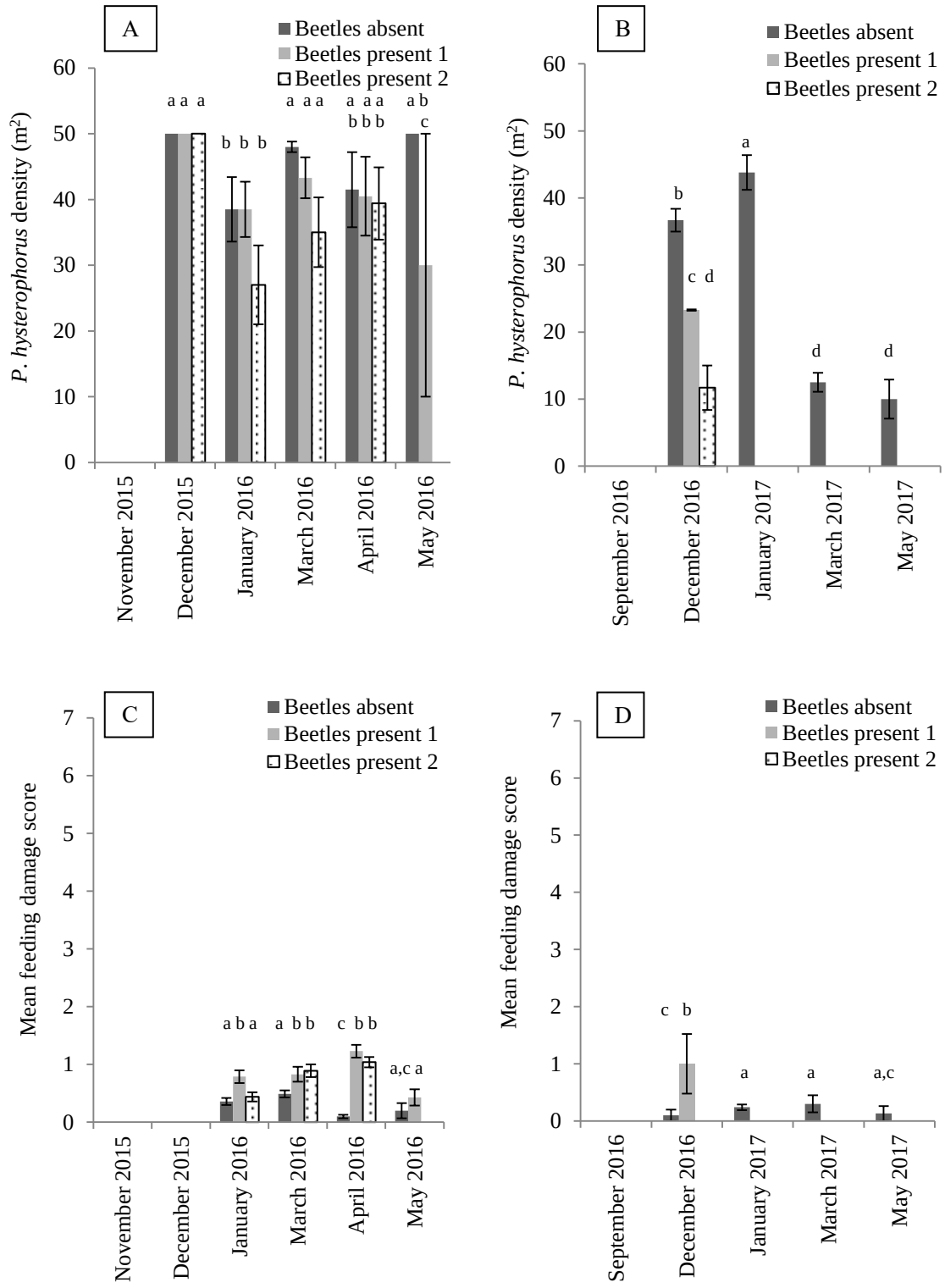


Figure 2.6. The effect of season and rainfall on *Parthenium hysterophorus* density (mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{19, 796}$=3.98; P<0.05) and. *Zygogramma bicolorata* feeding damage (mean ± S.E.) during season 1 (C) and season 2 (D) ($F_{15, 759}$=6.45; P<0.05) in two plots where *Z. bicolorata* beetles were present and one plot where beetles were absent at Mauricedale. Different letters above bars indicate significant differences between treatments and time periods (P<0.05, Tukey).

No significant differences ($F_{19, 422}$= 0.45; P>0.05) were found between treatments for *P. hysterophorus* canopy volume, regardless of feeding damage score during the entirety of season one or season two at the Ivaura site (Fig. 2.7). However, no *P. hysterophorus* plants were found in the "beetles present" treatments during most field trips of the second season, despite being present in the "beetles absent" treatment.

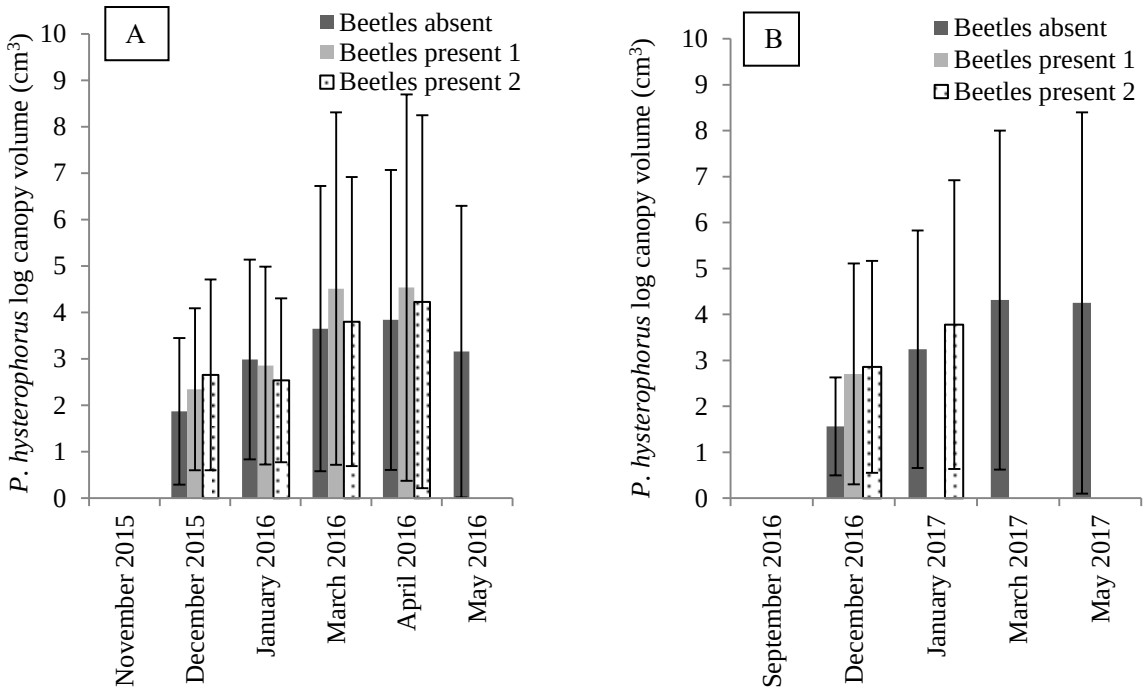


Figure 2.7 The effect of season and rainfall on *Parthenium hysterophorus* log canopy volume (mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{19, 422}$= 0.45; P>0.0 5) in two plots where *Zygogramma bicolorata* beetles were present and one plot where beetles were absent at Ivaura.

No significant differences were found between treatments for *P. hysterophorus* canopy volume, regardless of feeding damage score during the entirety of season one at the Mauricedale site (Fig. 2.8A). During December 2016 (Fig. 2.8B), it was found that the *P. hysterophorus* plants in one of the "beetle present" treatments (beetles present 1) had a significantly lower canopy volume ($F_{19, 796}$= 6.82; P<0.05) than the other two treatments, which occurred when the mean feeding damage score of that treatment was significantly higher than that of the other two treatments. During the remaining months, no *P. hysterophorus* plants

were found in the “beetles present” treatments, despite being present in the “beetles absent” treatment.

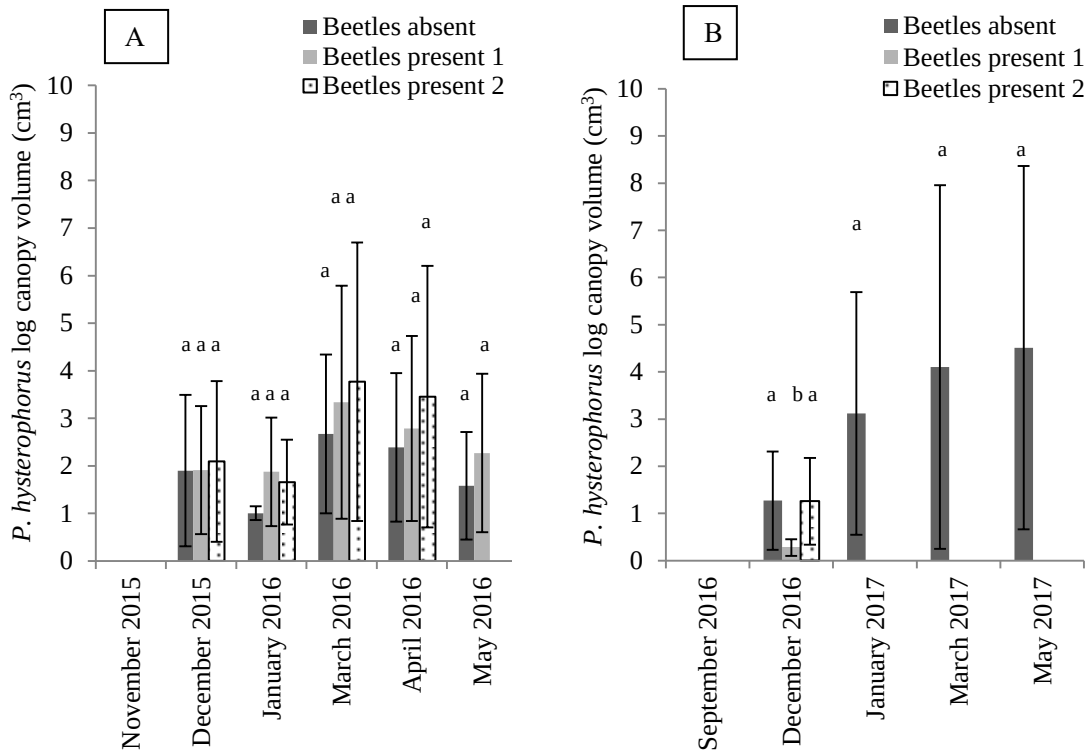


Figure 2.8. The effect of season and rainfall on *Parthenium hysterophorus* log canopy volume (mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{19, 796}$= 6.82; P<0.05) in two plots where *Zygogramma bicolorata* beetles were present and one plot where beetles were absent at Mauricedale. Different letters above bars indicate significant differences between treatments and time periods (P<0.05, Tukey).

No significant differences ($F_{12, 386}$= 0.55; P>0.05) were found between treatments for *P. hysterophorus* flower production, regardless of feeding damage score during the entire season one or season two at the Ivaura site (Fig. 2.9). However, this may be a result of the high level of variation in the data. When ignoring the variation around the means, the majority of *P. hysterophorus* plants had a lower flower production in the “beetles present” treatments than the “beetles absent” treatment. The flower production was lowered to nil during the April 2016, when defoliation was recorded at 100%.

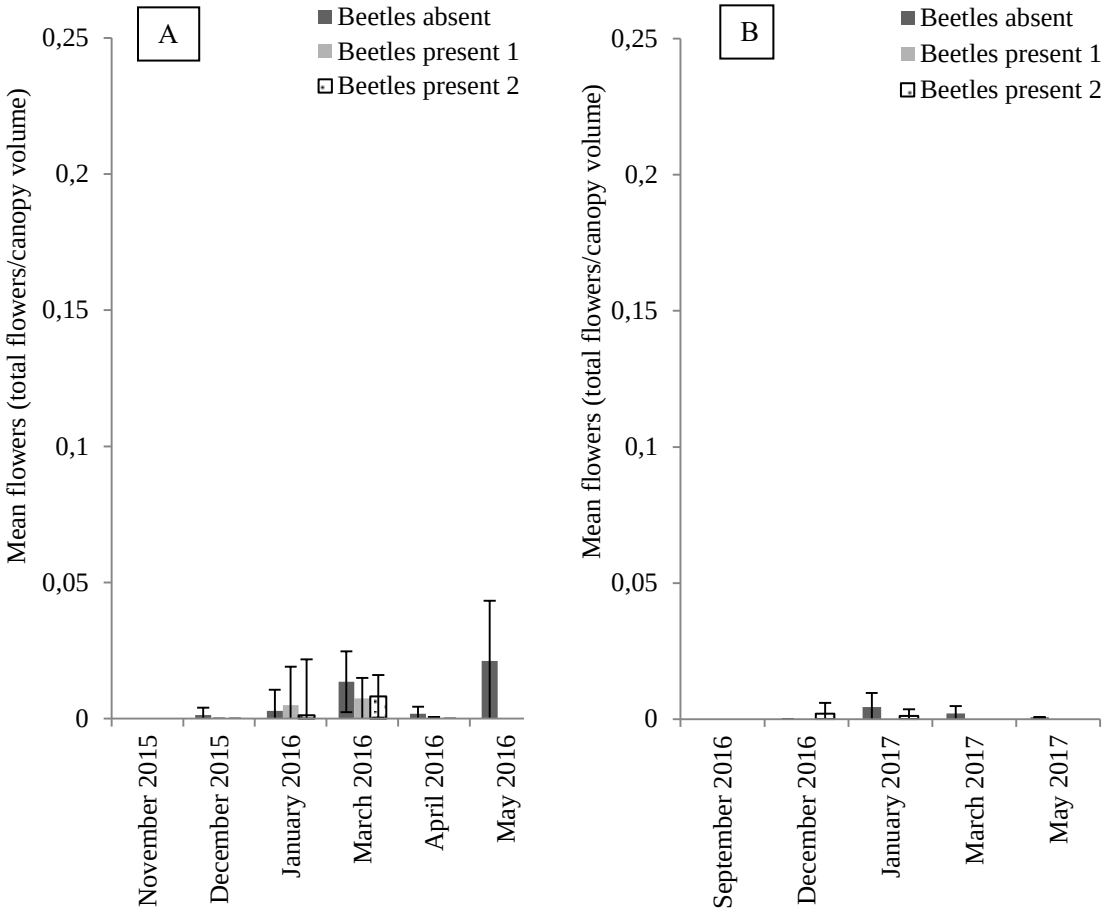


Figure 2.9. The effect of season and rainfall on *Parthenium hysterophorus* flower production (mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{12, 386}$= 0.55; P>0.05) in two plots where *Zygogramma bicolorata* beetles were present and one plot where beetles were absent at Ivaura.

As with the Ivaura site, no significant differences ($F_{9, 480}$= 1.34; P>0.05) were found between treatments for *P. hysterophorus* flower production, regardless of feeding damage score during the entire season one or season two at the Mauricedale site (Fig. 2.10). However, this may also be a result of the high level of variation in the data. When ignoring the variation around the means, the majority of *P. hysterophorus* plants had a lower flower production in the "beetles present" treatments than the "beetles absent" treatment. During March and April 2016, when the feeding damage was at its highest for the Mauricedale site, the mean flower production was particularly low in the "beetles present" treatment compared to the "beetles absent" treatment. It is important to note that the mean

flower production of *P. hysterophorus* was lower in season two than season one at both field sites in the "beetles absent" treatments.

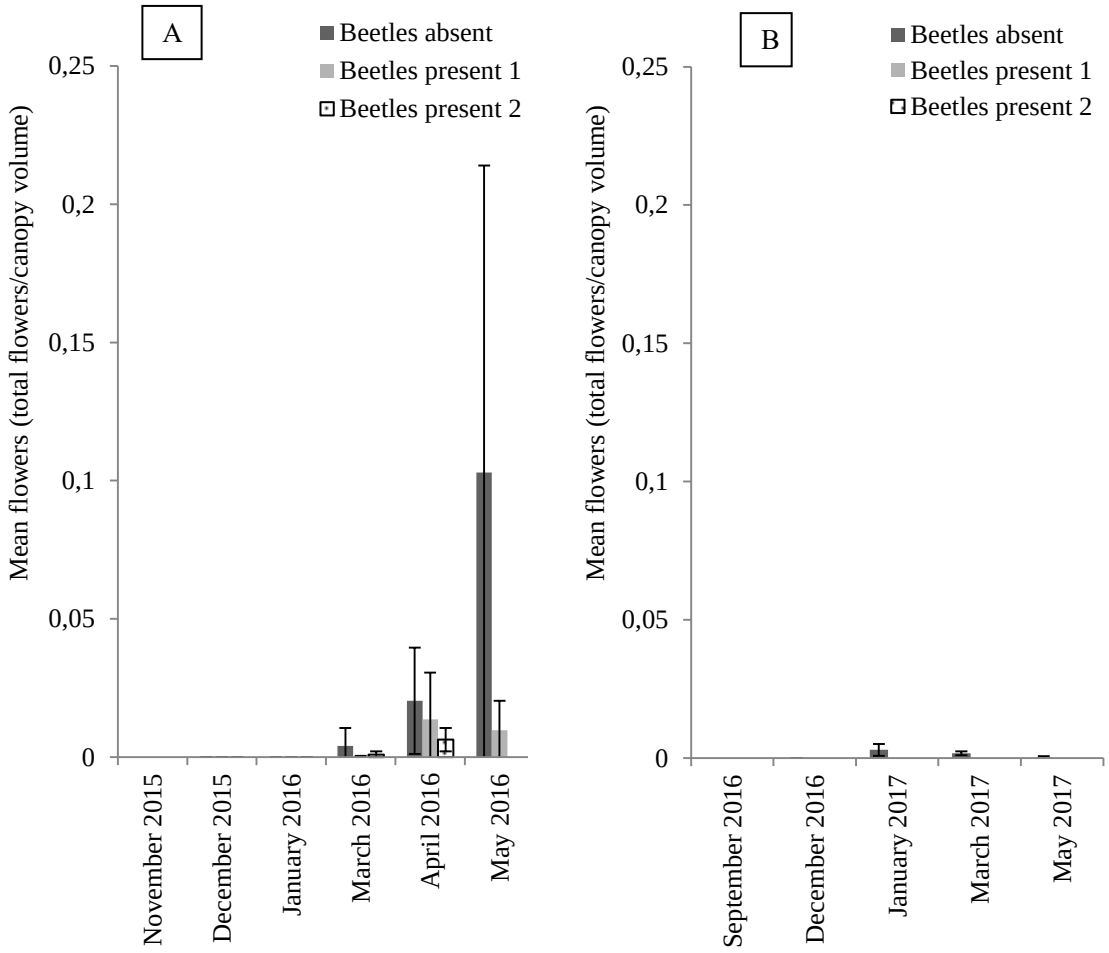


Figure 2.10. The effect of season and rainfall on *Parthenium hysterophorus* flower production (mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{9, 480}$= 1.34; P>0.05) in two plots where *Zygogramma bicolorata* beetles were present and one plot where beetles were absent at Mauricedale.

No significant differences ($F_{11, 202}$= 0.26; P>0.05) were found between treatments for *P. hysterophorus* chlorophyll content, regardless of feeding damage score during the entire season one or season two at the Ivaura site (Fig. 2.11). However, no *P. hysterophorus* plants were found in the "beetles present" treatments during most of the second season, despite being present in the "beetles absent" treatment. It is important to note that there were no data recorded in the "beetles present" treatments in April 2016, because there were no leaves on the

plants present in the cage, due to the complete defoliation of the plants by the beetle.

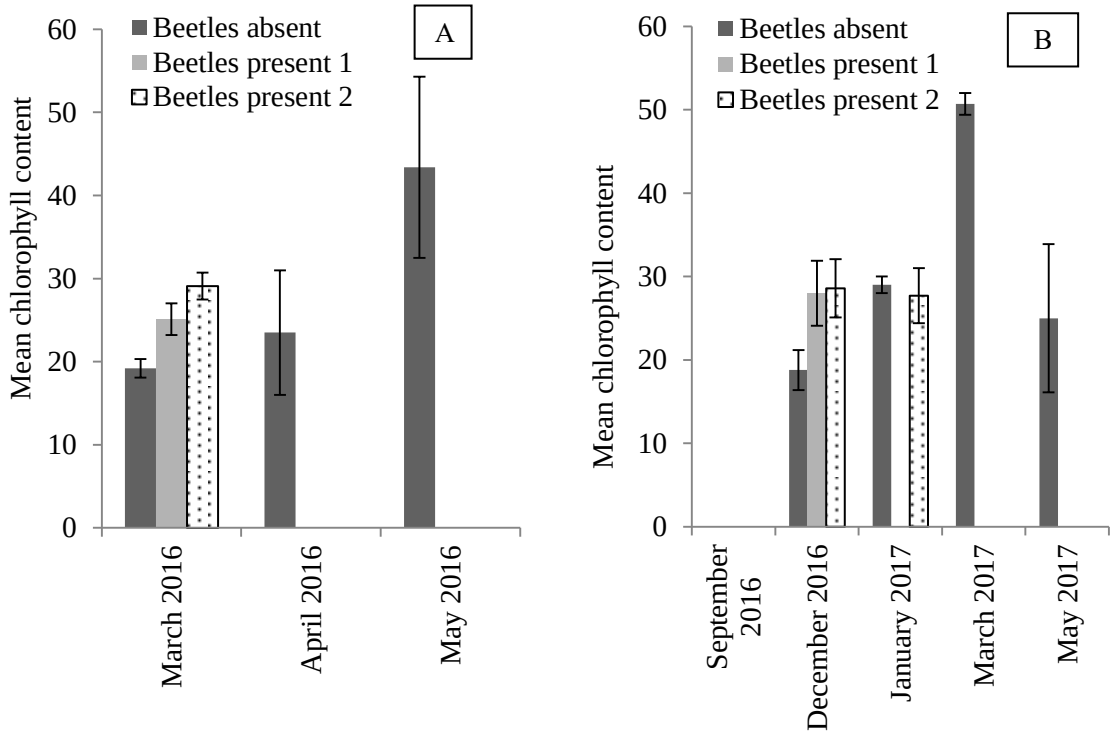


Figure 2.11. The effect of season and rainfall on *Parthenium hysterophorus* leaf chlorophyll content (SPAD values; mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{11, 202}$= 0.26; P>0.05) in two plots where *Zygogramma bicolorata* beetles were present and one plot where beetles were absent at Ivaura.

As with the Ivaura site, no significant differences ($F_{13, 557}$= 3.15; P>0.05) were found between treatments for *P. hysterophorus* chlorophyll content, regardless of feeding damage score during the entire season one or season two at the Mauricedale site (Fig. 2.12). However, no *P. hysterophorus* plants were found in the "beetles present" treatments during most of the second season, despite being present in the "beetles absent" treatment.

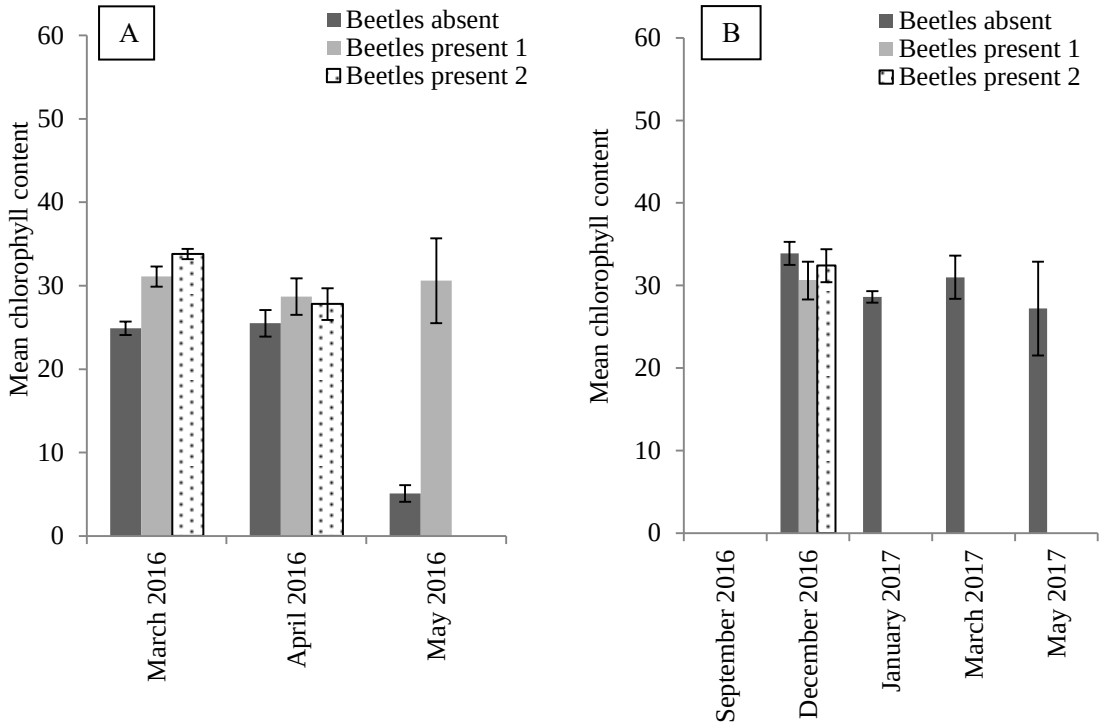


Figure 2.12. The effect of season and rainfall on *Parthenium hysterophorus* leaf chlorophyll content (SPAD values; mean ± S.E.) during season 1 (A) and season 2 (B) ($F_{13, 557}$= 3.15; P>0.05) in two plots where *Zygogramma bicolorata* beetles were present and one plot where beetles were absent at Mauricedale.

2.5.3. The effect of soil moisture on *Z. bicolorata* in the field

During season one of the field work, when rainfall was low, no beetles of any life stage were found along the transect (Fig. 2.13). However; during the second season, when the rainfall was higher, *Z. bicolorata* eggs, larvae and adults were found at the site. The mean number of beetles per plant was low throughout the season for all life stages. The adults ($\overline{x}$ = 0.41 ± 0.06 beetles per plant) were the most numerous life stage, followed by the eggs ($\overline{x}$ = 0.15 ± 0.1 beetles per plant). The beetle larvae ($\overline{x}$ = 0.01 ± 0.01) were the least numerous life stage.

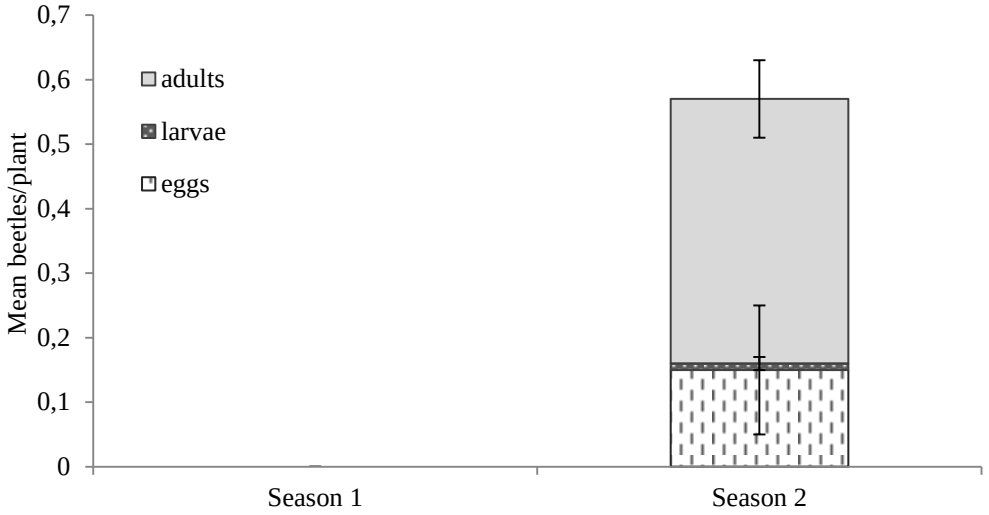


Figure 2.13. Number of *Zygogramma bicolorata* (mean ±S.E.) found per *Parthenium hysterophorus* plant at the abandoned cane field site in season 1 and season 2.

There were only two sampling events in the first season: one in May and the other in August 2016 (Fig.2.14). There was no significant difference in feeding damage between either of the sampling occasions ($F_{1, 6}$=6.91; P>0.05).

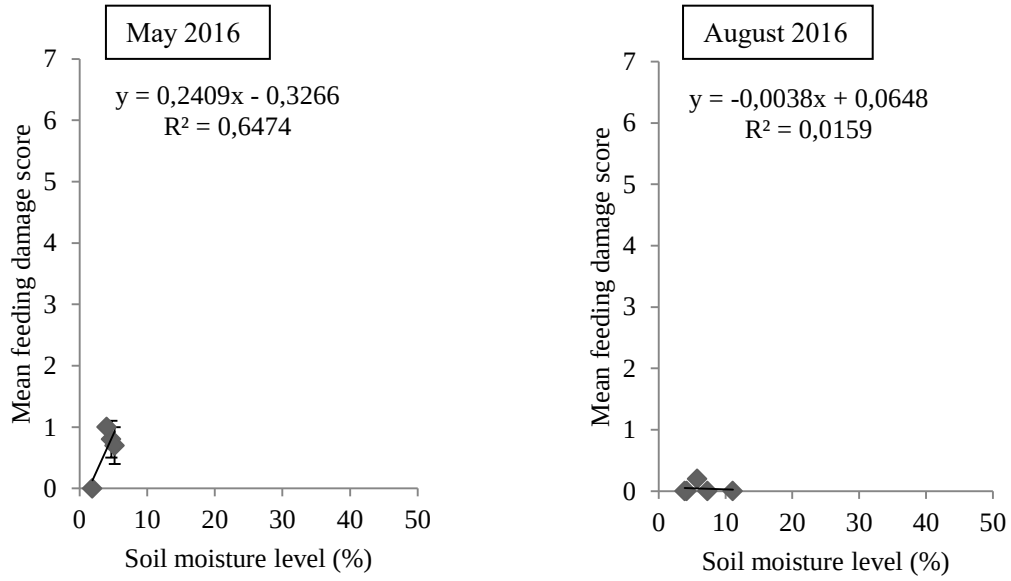


Figure 2.14. The effects of soil moisture upon *Zygogramma bicolorata* feeding damage (mean ±S.E.) on *Parthenium hysterophorus* along a soil moisture gradient during May 2016 and August 2016 at the abandoned cane field (Season 1) ($F_{1, 6}$=6.91; P>0.05).

The VSWC remained low from the previous season in September 2016 and November 2016 (Fig. 2.15); as a result the mean feeding damage was low, as with the first season of sampling. However, in December 2016, January 2017, and

March 2017 the VSWC increased, and with it came a significant increase in feeding damage ($F_{5, 27}$=1.87; P<0.05). The maximum mean feeding damage also got progressively higher as the months of high rainfall progressed, resulting in complete defoliation of some plants in March 2017. In May 2017 the VSWC had once again dropped below 10%, and the feeding damage dropped too (<10% defoliation), indicating that the beetles were no longer on the soil surface or on the plants, and had likely died off or were diapausing. The increase in VSWC above 10% resulting in high mean feeding damage scores, as well as the decrease in feeding damage scores when the VSWC level dropped below 10% indicates that the beetles require a VSWC of at least 10% in order to persist in the field and affect the weed.

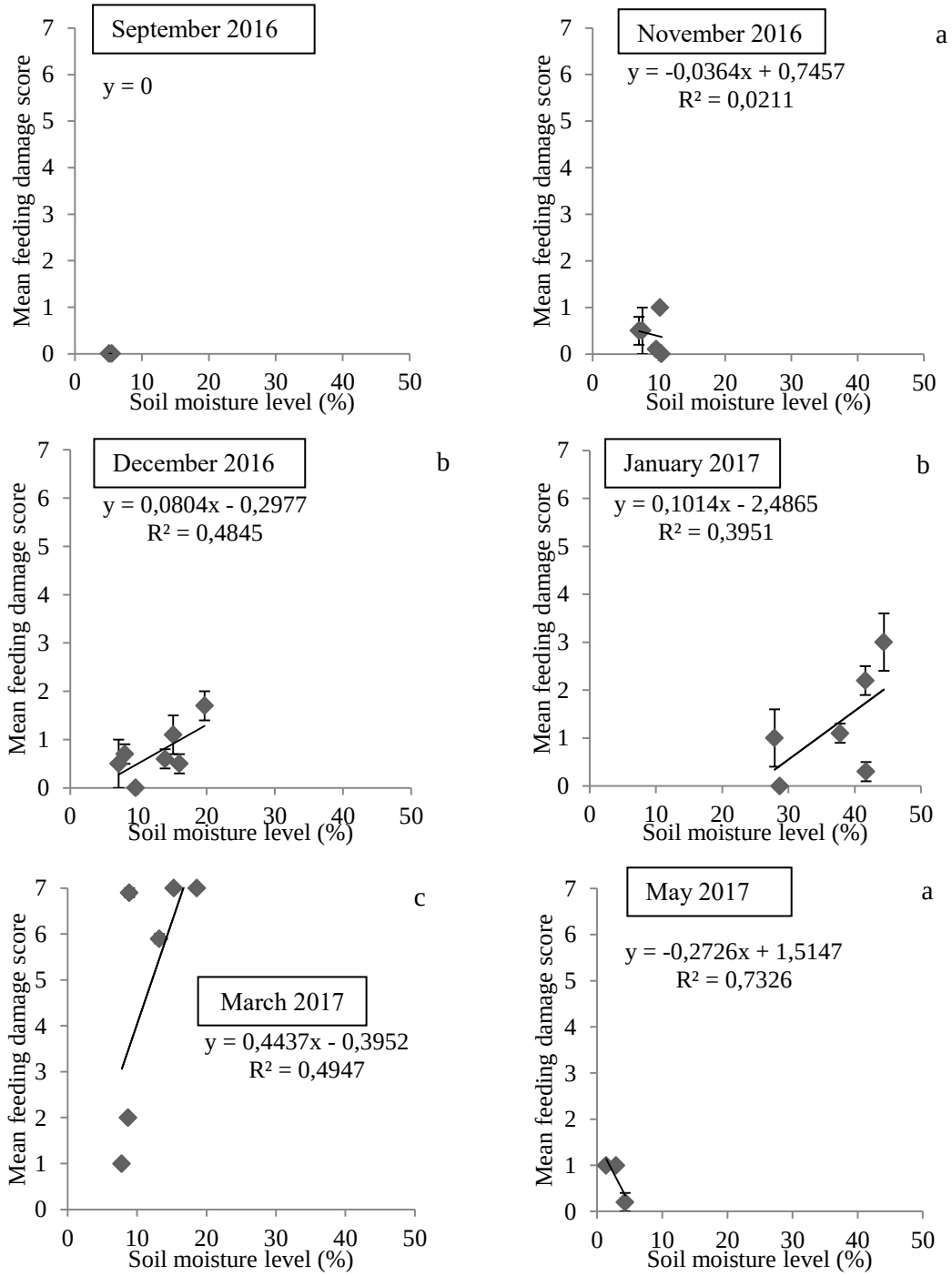


Figure 2.15. The effect of soil moisture upon *Zygogramma bicolorata* feeding damage (mean ±S.E.) on *Parthenium hysterophorus* along a soil moisture gradient during September 2016, November 2016, December 2016, January 2017, March 2017, and May 2017 at the abandoned cane field (Season 2) ($F_{5, 27}$=1.87; P<0.05). Different letters on each graph indicate significant differences between months (P<0.05, Tukey).

2.5.4. Vulnerability of *Z. bicolorata* eggs to predation and parasitoids

No parasitoids were recorded emerging from *Z. bicolorata* eggs. No significant difference ($F_{17, 53}$= 0.33; P>0.05) in egg predation was found between treatments, regardless of the season (Fig. 2.16). Predation of the eggs was high in all cases, with a mean predation of 37.23% at best and 100% at worst. The standard error of the experimental treatment data is consistently higher than that of the control treatment, indicating that crawling insects add a higher degree of variability to the data.

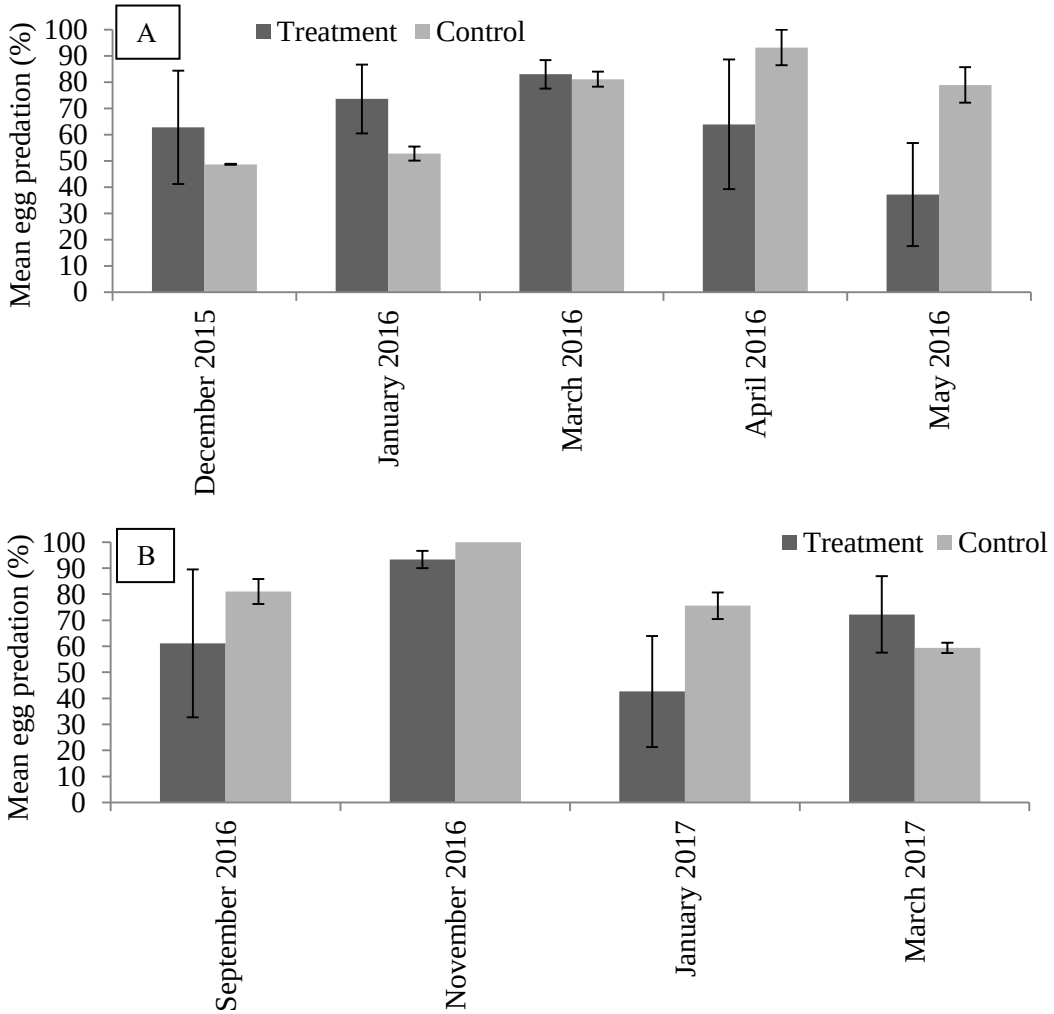


Figure 2.16. Mean percentage mortality of *Zygogramma bicolorata* eggs (mean ± S.E.) that were exposed to both flying and crawling insects (control) and flying predators only (treatment) at the Ivaura field site for five days during season 1 (A) and season 2 (B) ($F_{17, 53}$= 0.33; P>0.05).

2.6. Discussion

2.6.1. The effect of *Z. bicolorata* on *P. hysterophorus* in the field and the implications for the biological control of the weed

Zygogramma bicolorata feeding resulted in a significant decrease of *P. hysterophorus* density, resulting in no *P. hysterophorus* at the later stages of sampling of the second season. It is important to note that the drop in density at both field sites only occurred for prolonged periods during the second season, when the *Z. bicolorata* population had established and been stable in the "beetles present" treatments and the rainfall had been high. This is similar to the biological control program in Australia, where the beetles took ten years to establish over a wide area due to the low rainfall of the first ten years (Dhileepan & McFadyen 2012). However, once the beetle did successfully establish, the density of *P. hysterophorus* was reduced significantly (Dhileepan *et al.* 2000; Dhileepan 2003).

There was a lack of any significant difference in *P. hysterophorus* canopy volume between the "beetles present" and "beetles absent" treatments during either season at the Ivaura field site. There was only one incidence of *P. hysterophorus* canopy volume in the "beetles present" treatment being significantly lower than that of the "beetles absent" treatment at the Mauricedale site, which occurred in the second season, when the feeding damage of the beetle was significantly higher. This result does indicate that the beetles' feeding can reduce the plants' canopy volume, although this does not appear to be a common occurrence. It is important to note that the drop in canopy volume only occurred during the second season, when the *Z. bicolorata* population had established and been stable in the "beetles present" treatments and the rainfall had been high. This mirrors the difficulty of the beetle's establishment in Australia, it is suspected that the long establishment time was due to a dry habitat (Dhileepan & McFadyen 2012).This study did not produce as consistently strong a result as that found in Australia, where *P. hysterophorus* height was found to be significantly reduced (Dhileepan *et al.* 2000; Dhileepan 2003). However, as this study recorded *P. hysterophorus* canopy volume, and not only height, it may be a more accurate example of the effect of the beetles in the field.

There was no significant difference in flower production of *P. hysterophorus* between undamaged and beetle-damaged plants at either field site during both seasons, although this may be a result of the high variation in the data set. The flower production was lowered to nil during the April 2016 field trip at the Ivaura site, when defoliation was recorded at 100%. In Australia, the beetles were found to reduce the reproductive output of *P. hysterophorus* after their introduction (Dhileepan *et al.* 2000; Dhileepan 2003). The lower means in the "beetles present" treatments indicate that the beetle does reduce the plant's reproductive output, but the high variation in the data does not make this a clear-cut trend. As the beetles have only been in South Africa since 2013 (McConnachie 2015), the impact on flower production may increase as the beetle populations increase over time, which may not have been achieved in South Africa as of yet. Additionally, a review of the programme in Australia by Dhileepan and McFadyen (2012) found that there is a high variation in *P. hysterophorus* reproductive output. However, *P. hysterophorus* reproductive output is directly correlated to plant size (Dhileepan & McFadyen 2012). Therefore, the beetle's reduction of *P. hysterophorus* canopy volume in the field in South Africa should indirectly reduce the plant's flower production.

There is no indication that feeding damage from *Z. bicolorata* affects the chlorophyll content of the leaves of *P. hysterophorus*. There are few studies on the effect of arthropod herbivory on a plants' photosynthetic rate; however, the majority concluded that feeding damage results in a reduced rate of photosynthesis (Gonda-King *et al.* 2014; Nabity *et al.* 2009; Nagaraj *et al.* 2002). As chlorophyll content affects photosynthetic rate (Eaten 1931; Emerson 1929), this study's results are opposite to those of the aforementioned studies. However, a study by Retuerto *et al.* (2004) on the effect of the scale insect, *Coccus* sp. (Hemiptera: Coccoidea), feeding damage on European Holly, *Ilex aquifolium* L. (Aquifoliaceae), found that the photosynthetic rate of the plant increased with an increase in herbivory, presumably due to a compensatory response by the plant. The lack of any effect of herbivory on the chlorophyll content of the leaves of *P. hysterophorus* is not cause for concern, as the beetle's feeding does reduce the

plant's leaf surface area, which in turn would reduce the overall levels of photosynthesis that the plant is capable of.

2.6.2. The effect of soil moisture on *Z. bicolorata* and the implications for the selection of release sites

Ivaura and Mauricedale did not display the same temporal pattern of feeding damage scores by the beetle, despite the fact that both field sites occur in the same climate. This indicates that another factor may be affecting the beetle in the field. The primary difference between the field sites is the soil type. As the beetles pupate and diapause in the soil (Dhileepan & McFadyen 2012), soil texture may need to be studied further in order to understand the beetle's biological needs and possible limits to establishment in South Africa. Soil is considered a safe haven for many arthropods which generally prefer soils that are stable and have high levels of moisture (Barbercheck 1992). This is true for *Z. bicolorata*, as feeding damage by *Z. bicolorata* has a strong positive correlation to soil moisture above a VSWC of 10%, which results in >10% defoliation, below which there is little to no feeding damage. As the VSWC remained below this critical level during the first season, when there was low rainfall, it indicates that release sites with higher soil moisture should be selected in periods of low rainfall in order to give the beetle the maximum chance to establish. In periods of heavy rainfall it appears that the beetle would not be as limited in the number of field sites at which it could potentially establish. This has been found to be true for other beetles, such as *P. japonica* which did not establish in a wide area in seasons with low rainfall as it was restricted by its soil-inhabiting stage, but had a wider distribution in seasons with high rainfall (Hawley 1949).

2.6.3. The effect of predators and parasitoids on *Z. bicolorata* and the implications for successful release of the agent in South Africa

The lack of any parasitoids is unexpected, as many parasitoids can attack many different insect species (Holt & Lawton 1993). However, the possibility of parasitoids attacking the beetle larvae and pupae needs to be explored. The high egg predation indicates this life stage has the potential to be highly limited in the

field in South Africa and may be one of the reasons for the limited establishment of the beetle in the field. However, as the eggs have a high viability of 85-91% (McClay 1985) it is likely that most of the surviving eggs would hatch in the field if abiotic factors do not limit the beetle's survival. It is not known which predators target the beetle's eggs, although it is likely that at least some of the predators can fly in order to access the eggs. Generalist predators of *Z. bicolorata* adults and larvae have been found in India (Gupta *et al*. 2004), but predators of the *Z. bicolorata* eggs have yet to be identified in South Africa. The study by Gupta *et al.* (2004) also found that the beetles were attacked once established, but weren't attacked while establishing in the field. However, in this study it is likely that the predators were generalists, which naturally occur in the field and happened upon the eggs. The high variation in the experimental treatment indicate that crawling insects may miss some of the plants containing eggs, but when they do find plants which contain eggs the eggs are almost all preyed upon. This, as well as the fact the eggs were absent, indicates that ants may be the primary crawling predators of the eggs (Stickland *et al.* 1995).

2.7. Conclusion

The beetles have a negative effect on *P. hysterophorus* density and canopy volume, and there is an indication that they also reduce the plant's reproductive output in the field in South Africa, which is suggestive of a successful biological control program. However, there is no indication that the beetles have any effect on the plant's chlorophyll content, but the beetle likely reduces total photosynthetic rate by reducing the plants total leaf surface area. The high levels of predation of the *Z. bicolorata* eggs and low mobility of the larvae (Dhileepan & McFadyen 2012), lead to the conclusion that the best life stage to release into the field in order to maximise the potential for establishment is the adult beetles.

2.8. References

BARBERCHECK, M.E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. *The Florida Entomologist* **75**: 539-548.

BRIESE, D.T. 2004. Weed biological control: applying science to solve seemingly intractable problems. *Australian Journal of Entomology* **43**: 304-317.

BYRNE, M.J., CURRIN, S. & HILL, M.P. 2002. The influence of climate on the establishment and success of the biological control agent *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biological Control* **24**: 128-134.

CARSON, W.P., HOVICK, S.M., BAUMERT, A.J., BUNKER, D.E. & PANDERGEIST, T.H. 2008. Evaluating the post-release efficacy of invasive plant biocontrol by insects: a comprehensive approach. *Arthropod-Plant Interactions* **2**: 77-86.

CLEWLEY, G.D., ESCHEN, R., SHAW, R.H., WRIGHT, D.J. 2012. The effectiveness of classical biological control of invasive plants. *Journal of Applied Ecology* **49**: 1287-1295.

CLIMATE-DATA.ORG. 2017, November. Retrieved from https://en.climate-data.org/location/26824/

DHILEEPAN, K. 2003. Seasonal variation in the effectiveness of leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Curculionidae) and stem-galling moth *Epiblema strenuata* (Lepidoptera: Tortricidae) as biocontrol agents on the weed *Parthenium hysterophorus* (Asteraceae). *Bulletin of Entomological Research* **93**: 393-401.

DHILEEPAN, K & MCFADYEN R.C. 2012. *Parthenium hysterophorus* L. - parthenium. In: JULIEN, M., MCFADYEN, R. & CULLEN, J. (eds) Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne. Pp. 448-462.

DHILEEPAN, K. SETTER, S. & MCFADYEN, R.E. 2000. Impact of defoliation by the introduced biocontrol agent *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on parthenium weed in Australia. *Biocontrol* **45**: 501-512.

EATON, S.V. 1931. Chlorophyll content and photosynthesis. *Botanical Gazette* **91**: 223-224.

EMERSON, R. 1929. Chlorophyll content and rate of photosynthesis. *Proceedings of the National Academy of Sciences of the United State of America* **15**: 281-284.

FRECKLETON, R.P. 2000. Biological control as a learning process. *Trends in Ecology and Evolution* **15**: 263-264.

GONDA-KING, L., GÓMEZ, S., MARTIN, J.L., ORIANS, C.M. & PREISSER, E.L. 2014. Tree responses to an invasive sap-feeding insect. *Plant Ecology* **215**: 297-304.

GUPTA, R.K., KHAN, M.S., BALI, K., MONOBRULLAH, M. & BHAGAT, R.M. 2004. Predatory bugs of *Zygogramma bicolorata* Pallister: an exotic beetle for biological suppression of *Parthenium hysterophorus* L. *Current Science* **87**: 1005-1010.

HAWLEY, I.M. 1949. The effect of summer rainfall on Japanese beetle populations. *Journal of the New York Entomological Society* **57**: 167-176.

HILL, M.P. & HULLEY, P.E. 1995. Host-range extension by native parasitoids to weed biological control agents introduced to South Africa. *Biological Control* **5**: 297-302.

HOLT, R.D. & LAWTON, J.H. 1993. Apparent competition and enemy-free space in insect host-parasitoid communities. *American Naturalist* **142**: 623-645.

JULIEN, M.H., KERR, J.D. & CHAN, R.R. 1984. Biological control of weeds: an evaluation. *Protection Ecology* **7**: 3-25.

KING, H. 2008. Thermal physiology and predicted distribution of *Zygogramma bicolorata* (Chrysomelidae), a promising agent for the biological control of the invasive weed *Parthenium hysterophorus* in South Africa. Unpublished. Masters diss., Faculty of Science and Agriculture, University of KwaZulu-Natal, 2008.

KING, A.M. 2011. The effect of temperature on biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae) in South Africa. MSc dissertation. University of the Witwatersrand, Johannesburg, South Africa.

LAWTON, J.H. 1985. Ecological theory and choice of biological control agents. *Proceedings of the VI International Symposium on Biological Control of Weeds*: 13-26.

MCCLAY, A.S. 1985. Biocontrol agents for *Parthenium hysterophorus* from Mexico. In: DEL FOSSE, E.S. (ed) Proceedings of the Sixth International Symposium on Biological Control of Weeds. Ottawa, Canada. Pp. 19-25.

MCCONNACHIE, A.J. 2015. Host range and risk assessment of *Zygogramma bicolorata,* a defoliating agent released in South Africa for the biological control of *Parthenium hysterophorus. Biocontrol Science and Technology* **25**: 975-991.

MCEVOY, P.B. & COOMBS, E.M. 1999. Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecological Applications* **9**: 387-401.

MORIN, L., REID, A.M., SIMS-CHILTON,N.M., BUCKLEY, Y.M., DHILEEPAN, K., HASTWELL, G.T., NORDBLOM, T.L. & RAGHU, S. 2009. Review of approaches to evaluate the effectiveness of weed biological control-agents. *Biological Control* **51**: 1-15.

NABITY, P.D., ZAVALA, J.A. & DELUCIA, E.H. 2009. Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. *Annals of Botany* **103**: 655-663.

NAGARAJ, N., REESE, J.C., KIRKHAM, M.B., KOFOID, K., CAMPBELL, L.R. & LOUGHIN, T.M. 2002. Relationship between chlorophyll loss and photosynthetic rate in greenbug (Homoptera: Aphididae) damaged sorghum. *Journal of the Kansas Entomological Society* **75**: 101-109.

NORRIS, R.J., MEMMOTT, J. & LOVELL, D.J. 2002. The effect of rainfall on the survivorship and establishment of a biocontrol agent. *Journal of Applied Ecology* **39**: 226-234.

PAYNTER, Q., FOWLER, S.V., GOURLAY, A.H., GROENTEMAN, R., PETERSON, P.G., SMITH, L. & WINKS, C.J. 2010. Predicting parasitoids accumulation on biological control agents of weeds. *Journal of Applied Ecology* **47**: 575-582.

RETUERTO, R., FERNANEZ-LEMA, B., RODRJGUEZ-ROILOA & OBESO, J.R. 2004. Increased photosynthetic performance in holly trees infested with scale insects. *Functional Ecology* **18**: 664-669.

ROBERTSON, M.P., KRITICOS, D.J. & ZACHARIADES, C. 2008. Climate matching techniques to narrow the search for biological control agents. *Biological Control* **46**: 442-452.

SASRI WEATHERWEB. 2017, November. Retrieved from https://sasri.sasa.org.za/weatherweb/weatherweb.ww_menus.menu_frame?menuid=1

STICKLAND, T.R., BRITTON, N.F. & FRANKS, N.R. 1995. Complex trails and simple algorithms in ant foraging. *Proceedings: Biological Sciences* **260**: 53-58.

THOMAS, M.B. & WILLIS, A.J. 1998. Biocontrol- risky but necessary? *TREE* **13**: 325-329.

WALKER, B.H. 1976. An approach to the monitoring of changes in the composition and utilization of woodland and savanna vegetation. S*outh African Journal of Wildlife Research* **6**:1-32.

CHAPTER THREE

Zygogramma bicolorata (Pallister) (Coleoptera: Chrysomelidae) Diapause and Pupation Success in Relation to Soil Depth and Texture: Is Soil Moisture Limiting the Beetle's Ability to Establish on *Parthenium hysterophorus* (L.) (Asteraceae: Heliantheae) in South Africa?

3.1. Abstract

Parthenium hysterophorus is one of the worst invasive weeds in the world today. *Zygogramma bicolorata* is one of the agents to have been released in order to control the weed. However, the beetle displayed limited establishment and persistence at release sites around South Africa, resulting in a low impact on the weed in the field. *Zygogramma bicolorata* pupates and diapauses in the soil, therefore, soil type and soil field capacity may be a possible factor constraining its establishment. Diapause depth was not affected by soil type or soil field capacity, but remained at 0.3 cm or shallower. However, pupation survival was found to be limited in dark soils (<37% survival) which indicate high amounts of organic matter in the soil, and pupation depth was found to be reduced at 100% soil field capacity (<0.37 cm). The beetles did not survive diapause at 100% soil field capacity. Therefore, soil type and soil moisture should be considered when choosing field sites for release.

Keywords: Biological control; diapause; field capacity; parthenium; pupation; soil moisture; soil texture.

3.2. Introduction

3.2.1. *Zygogramma bicolorata* soil requirements

Soil texture and soil moisture, defined as the quantity of water contained in the soil, are important factors which determine the likelihood of survival in insects that pupate and diapause in the soil (Ellis *et al*. 2004). Soils with large particle sizes, such as sandy soils, have more air spaces and are less compacted, and drain more easily than clay soils (Dimou *et al*. 2003). This promotes movement and penetration of insect larvae to greater depths (Dimou *et al.* 2003; Hennessey

1994). Therefore, beetles and beetle larvae burrowing into sandy soils would be expected to burrow deeper into the soil than in clay soils, which are more compacted than sandy soils. Soil that is dry can cause pupal death by dessication as demonstrated in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae) (Jackson *et al.* 1998; Vargas *et al.* 1987). However, studies by Ellis *et al.* (2004) on *Aethina tumida* (Murry) (Coleoptera: Nitidulidae) and Hulthen and Clarke (2006) on *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) found that soil type alone did not significantly influence the pupation success or duration of the respective insects. Hulthen and Clarke (2006) also found that soil saturation, defined as all pores between soil particles being occupied by water, of 0% or 100% significantly increased the mortality rate of *B. tryoni*. However, between 10% and 75% soil saturation, mortality of *B. tryoni* was low (Hulthen & Clarke 2006). Cheng *et al*. (2017) found that the diapause period of the wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) was significantly lengthened when the soil moisture deviated from the fly's ideal range of 20-60% of the soils dry weight. However, the diapause of the blister beetle, *Mylabris phalerata* (Pallas) (Coleoptera: Meloidae) was not affected by soil moisture (Zhu *et al*. 2006).

Zygogramma bicolorata (Pallister) (Coleoptera: Chrysomelidae) is a beetle native to South America that has been introduced to South Africa in order to control the invasive alien weed *Parthenium hysterophorus* (L.) (Asteraceae: Heliantheae). Lack of knowledge regarding the agent's biological requirements, has resulted in some beetles being released in unsuitable environments, leading to limited establishment. A thorough investigation of *Zygogramma bicolorata*'s thermal and humidity level limits was conducted in South Africa (King 2008). The study concluded that *Z. bicolorata* was highly likely to establish widely in South Africa as most of the country had favourable temperature and humidity conditions for the beetle (King 2008). The beetle has, however, failed to establish in high numbers at most release sites in South Africa, indicating that additional factors other than temperature and humidity are affecting *Z. bicolorata* populations in the field. The beetle pupates in the soil during Mpumalanga's wet season during spring and

summer, emerging as an adult. Soil is, therefore, a potentially important factor in the beetle's lifecycle, and one that has been overlooked so far. This is the knowledge gap that this study will be filling. It is also important to document whether the beetle's ability to establish in the field could be limited by the moisture of the soil in which it is diapausing. It has also been suggested that an integrated control program using a burning regime in the winter to destroy *P. hysterophorus* seeds, whilst *Z. bicolorata* adults are diapausing in the soil, be implemented.

3.2.2. Integrated control using fire and biocontrol agents as a way to control *Parthenium hysterophorus*.

Fire has been recommended as a low cost and effective way to control alien weeds (Briese 1996; Dew *et al*. 2017; Goodall & Erasmus 1996). However, management of alien weeds that have biological control agents released against them may not be compatible with fire as it may promote the spread and density of the weed and may slow the agents' impact against the weed or possibly eliminate the agent (Briese 1996). When considering fire as a way to control an alien plant it is important to consider the fire intensity, fire frequency, the season that the fire burns, the scale of the fire, and how the weed and biological control agents respond to the fire (Briese 1996). It is possible to destroy sub-surface seeds of invasive alien weeds, such as *Chromoleana odorata* (L.) King & Robinson (= *Eupatorium odoratum* L.), by heating up the soil with fire (te Beest *et al.* 2012; Witkowski & Wilson 2001). Intense fires in South Africa, West Africa, and north eastern India have resulted in the death of *C. odorata* plants and it has been suggested that a burning regime is a way to destroy the plants and seeds of *C. odorata* (Goodall & Erasmus 1996; Witkowski & Wilson 2001). Experiments conducted by te Beest *et al.* (2012) found that mechanical and chemical clearing of the weed combined with a burning regime in savannah invaded by *C. odorata* resulted in a reduced reinvasion of the weed and an increase in natural grasses. However, a managed burning regime may result in a change from the rate of natural fire occurring in the field, which may itself threaten natural ecosystems (te Beest *et al.* 2012)

Once-off burns of fields of parthenium in Australia resulted in increased parthenium densities, but repeated burns in early autumn and late summer over a two year period resulted in a rapid decline of *P. hysterophorus* density (Vogler *et al*. 2002). However the burning of parthenium fields had no effect on parthenium germinable soil seed banks (Vogler *et al.* 2002). *Zygogramma bicolorata* adults diapause in the soil from autumn to winter (McFadyen & McClay 1981), when parthenium plants are scarce and the soil is dry, in order to survive through the harsher climatic conditions (Dhileepan & McFadyen 2012; Hahn & Denliger 2007). It appears that winter would be the best time to burn parthenium in order to reduce the sub-surface soil seed bank. However the beetle's diapause depth in different soils needs to be measured in order to establish whether diapausing beetles would be able to survive the high soil temperatures caused by the fire.

3.3. Aims

The aim of this study is to determine whether soil habituating life stages of *Z. bicolorata* are constrained to certain soil types in the field and to determine the likelihood of success of an integrated control programme by using a burning regime. The study objectives were as follows:

Objective 1: Measure *Zygogramma bicolorata* diapause and pupation success and pupation depths in differing soil types and soil moisture levels in order to establish if these soil factors affect the agent's ability to establish in the field.

Objective 2: Measure *Z. bicolorata* diapause depth in different soil types and soil moisture levels in order to establish whether integrated control of parthenium with the use of fire in winter is a viable option.

3.4. Materials and methods

Twenty kilograms of soil at a depth range of 0- 10cm was collected from each of the following field sites at which *Z. bicolorata* had established during January of 2016: Mauricedale (25°31'46"S; 31°36'38"E); Ivaura (25°32' 46"S; 31°38' 51"E); and an abandoned cane field (25°29'21"S; 31°31'38"E). The soil particle

size from each site was measured by using the Bouyoucos Hydrometer Method (Bouyoucos 1962), the percentages of each particle size was then compared to a soil texture triangle in order to determine the soil texture of each field site.

Each site's soil was sterilized by autoclaving (Sturdy SA-300VL) at 120°C for 30 minutes at 1 atm. Each field site's soil was watered 100%, 75%, 50%, or 25% of its field capacity, which is defined as the maximum amount of water that soil can hold, before the water is drawn away by gravity, resulting in twelve soil and moisture treatments for each of the two experiments. Field capacity was calculated for each field site's soil by slowly wetting 2 kg of compacted soil from the relevant field site until it was fully saturated, at which point run-off of water occurred from the surface (100% field capacity). The 100% field capacity soil was then weighed. The soil was then allowed to dry in an oven at 50 °C for two weeks; at this point 0% field capacity was achieved. The dry soil was then weighed. The weight of the dry soil was then subtracted from the weight of the soil when it was at 100% field capacity in order to calculate the weight of the water that was needed to achieve 100% field capacity for that soil weight. The correct percentage of field capacity was then prepared by adding the amount of dry soil to the percentage of the relative total water volume for that soil, for example 25% saturation of Ivaura soil was achieved by adding Ivaura soil to 25% of the relative water volume that would be needed to achieve 100% field capacity for that weight of Ivaura soil. Final instar beetle larvae were raised in laboratory conditions where the temperature was kept at 25 °C with a 12:12 hour day:night cycle. Thirty were used for each pupation treatment, and 30 adult beetles were used for each diapause treatment, resulting in 360 individuals being used for each of the two experiments.

3.4.1. *Zygogramma bicolorata* pupation depth and success rate

Thirty larvae were placed in a 15cm deep cylindrical container with a diameter of 15cm for each soil and moisture treatment combination. The containers were then sealed to prevent moisture loss. The larvae were allowed to pupate. Fresh leaves were provided to the larvae daily until all individuals had pupated, or had died.

Dead individuals were counted and removed daily. Once all living individuals had burrowed into the soil, they were left alone and allowed to pupate for 7 days (Dhileepan & McFadyen 2012), at which point the experiment was considered complete. The number of individuals that pupated was counted, their pupation depth into the soil, and the weight of each of individual was measured. Pupation depth was measured by covering the container with a tray before carefully turning each container upside-down on the tray, so that the soil remained in the shape of the container and no soil from the top of the container was removed. The soil was then scraped away in 1 cm increments from the front to the back of the soil mound. When an individual was found, the distance was measured from the tray (soil surface) to the midpoint of the beetle's pupation chamber. The pupa was then removed from the soil mound and individuals which had pupated were weighed on a scale (ADAM CBK 16). The number of individuals that had pupated of the initial thirty from each treatment was also recorded in order to determine the pupation success.

3.4.2. *Zygogramma bicolorata* diapause depth and success rate

Thirty beetles were placed in a 15 cm deep cylindrical container with a diameter of 15 cm for each soil and moisture treatment combination. The containers were sealed to prevent moisture loss and the day length was reduced from >12 hours to 8 hours per 24 hour cycle in order to simulate a shortened day associated with the induction of diapause by the beetles (Dhileepan & McFadyen 2012). Diapause was induced by lowering the ambient temperature of the experimental chamber from 25 °C to 10 °C at which point the population began to diapause. Once all individuals had either burrowed into the soil in order to diapause or were dead, they were left alone and allowed to diapause for 7 days (Dhileepan *et al.* 2000), at which point the experiment was considered complete. The number of individuals that diapaused was counted and their diapause depth from the soil surface was measured. Diapause depth was determined in the same manner as pupation depth. The beetle was then removed from the soil mound. The number of individuals that had diapaused of the initial thirty from each treatment was also recorded in order to determine the diapause success.

3.4.3. Statistical Analysis

The difference in *Z. bicolorata* diapause and pupation survival between soil types was calculated using a χ^2 test on R 3.4.3. The differences between different levels of soil field capacity in *Z. bicolorata* diapause depth, as well as pupation depth, was calculated using an ANOVA test in Microsoft Excel 2010, with a post-hoc Tukey test when needed in R 3.4.3. The relationship between *Z. bicolorata* pupal weight and pupation depth below the soil was calculated using a correlation analysis with the use of Microsoft Excel 2010.

3.5. Results

Mauricedale's soil is red in colour due to the presence of rusted iron, indicating that it has often been hydrated, and is a silt soil type (Table 3.1); Ivaura's soil is dark in colour, indicating the presence of organic materials and is a silt soil type. The abandoned cane field's soil is red in colour and is a loamy sand.

Table 3.1 Percentages of clay, silt and sand (mean ± S.E.) contents of the soil from each field site (n=5 per soil site).

Soil location	% silt mean ± S.E.	% clay mean ± S.E.	% sand mean ± S.E.	Description of soil texture
Mauricedale	93 ± 2	3 ± 2	4 ± 2.45	silt
Ivaura	96 ± 1.87	1 ± 1	3 ± 1.22	silt
Abandoned cane field	2 ± 1.22	13 ± 1.22	85 ± 2.25	loamy sand

The number of *Z. bicolorata* which survived attempting diapause was low for all treatments of the experiment, ranging from 0% to 57% survival across all treatments, and from 40-57% for the 25%, 50%, and 75% moisture treatments across all soil types (Fig. 3.1). No beetles entered diapause at 100% soil moisture in any soil source. Only 139 of the 360 beetles used in the experiment diapaused successfully. The mean diapause success across all treatments was 38.6%, but was 51.5% when the 100% soil moisture treatment was excluded. Soil type did not have a significant effect on the survival of the beetles (χ^2_4= 1.76, P>0.05). Chi-

squared tests were conducted without the 100% treatments as no beetles survived that treatment.

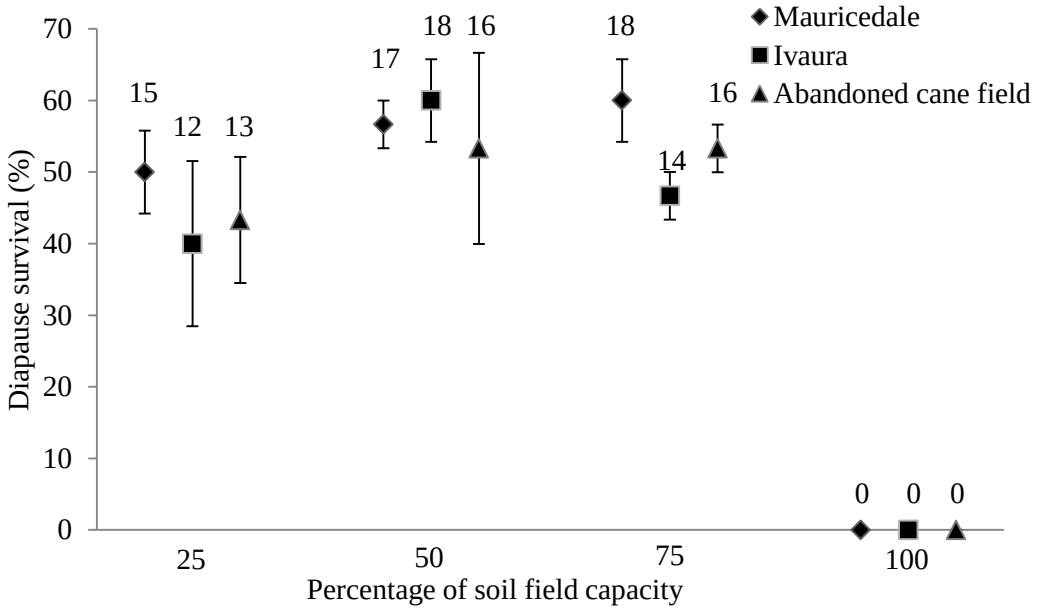


Figure 3.1. The effect of soil source and percentage of soil field capacity on *Zygogramma bicolorata* diapause survival (mean ± S.E.) with the number of survivors of the initial 30 displayed (χ^2_4= 1.76, P> 0.05).

No significant difference was found in the *Z. bicolorata* diapause depth between any of the soil types ($F_{39, 78}$= 1.16, P>0.05) or soil moisture levels within each of the soil types ($F_{2, 78}$= 0.46, P>0.05) (Fig. 3.2). The beetles all diapaused at a mean depth less than 0.3 cm below the soil surface in all treatments. The majority of beetles only burrowed down into the soil deep enough to create a chamber for diapause, and no deeper. The deepest individuals were found 0.4 cm below the surface of the soil, though this occurrence was rare (6.47% (n=9) of the individuals which diapaused) and not limited to a specific soil type or moisture level.

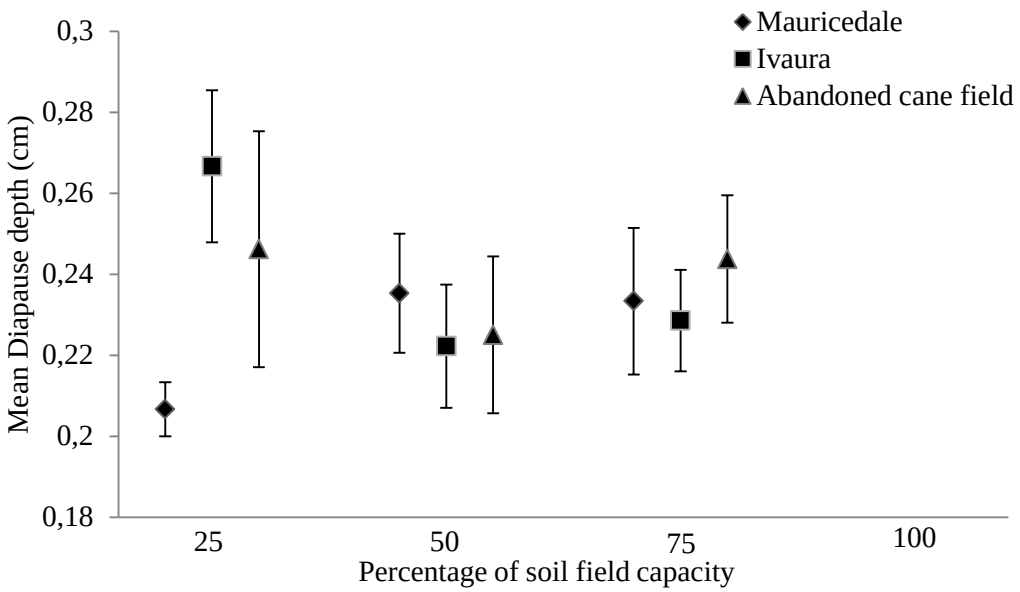


Figure 3.2. The effect of soil source ($F_{39, 78}$= 1.16, P>0.05) and percentage of soil field capacity ($F_{2, 78}$= 0.46, P>0.05) on *Zygogramma bicolorata* average diapause depth (mean ± S.E.).

Although the number of beetles which pupated was lower in the Ivaura soil than the Mauricedale or abandoned cane field soil, there was no significant difference between any of the soils in the number of beetles that pupated (χ^2_6= 10.94, P> 0.05) (Fig. 3.3). Only 142 of the 360 beetles (39.44%) pupated. Even though there was no significant difference, the data indicates that the beetles do not pupate as successfully in the Ivaura soil (dark soil) compared to the Mauricedale and the abandoned cane field soils (red soils), with a maximum survival of 36.7% in the Ivaura treatment, which occurred at the 100% field capacity treatment.

Beetle pupation depth was not affected by soil type ($F_{25, 75}$= 1.37, P>0.05) (Fig. 3.4). There was significant variation in pupal depth between the different soil moisture treatments ($F_{3, 75}$= 8.04, P<0.05) and the 100% field capacity treatments differed significantly from the remaining soil field capacities at P<0.05. The 25%, 50% and 75% soil field capacities were not significantly different from each other in relation to the depth of pupation. The majority of individuals pupated less than 1 cm below the surface of the soil. Two pupating beetles were found at the maximum depth of 1.7 cm below the soil surface, which occurred in both Ivaura and the abandoned cane field soil at 50% of the carrying capacity of each soil.

Some individuals pupated on the soil surface, although this did not happen consistently within any soil type or soil moisture carrying capacity percentage.

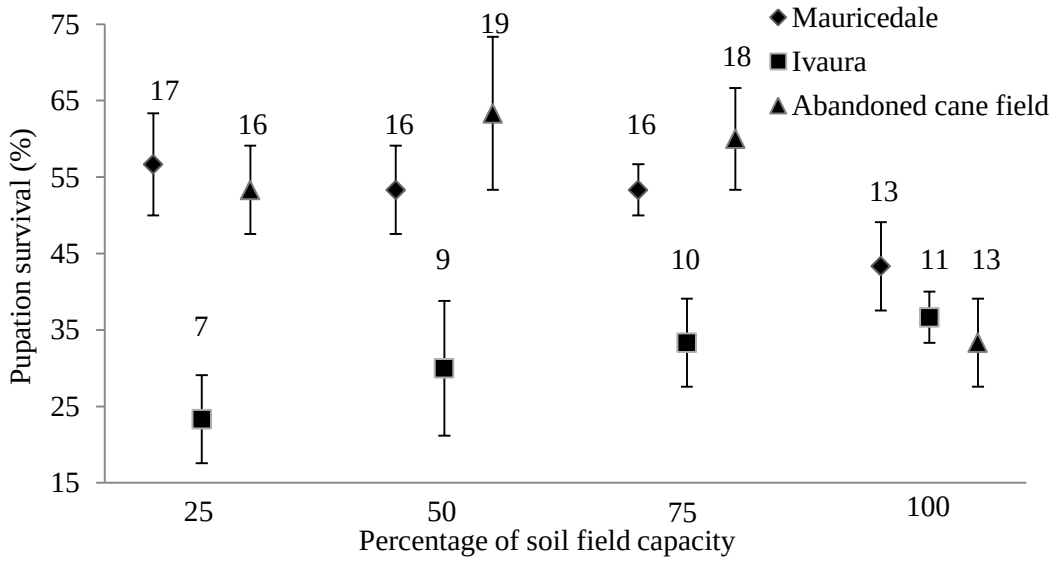


Figure 3.3 The effect of soil source and percentage of soil field capacity on *Zygogramma bicolorata* pupation survival percentage (mean ± S.E.) with the number of survivors of the initial 30 displayed (χ^2_6= 10.94, P>0.05).

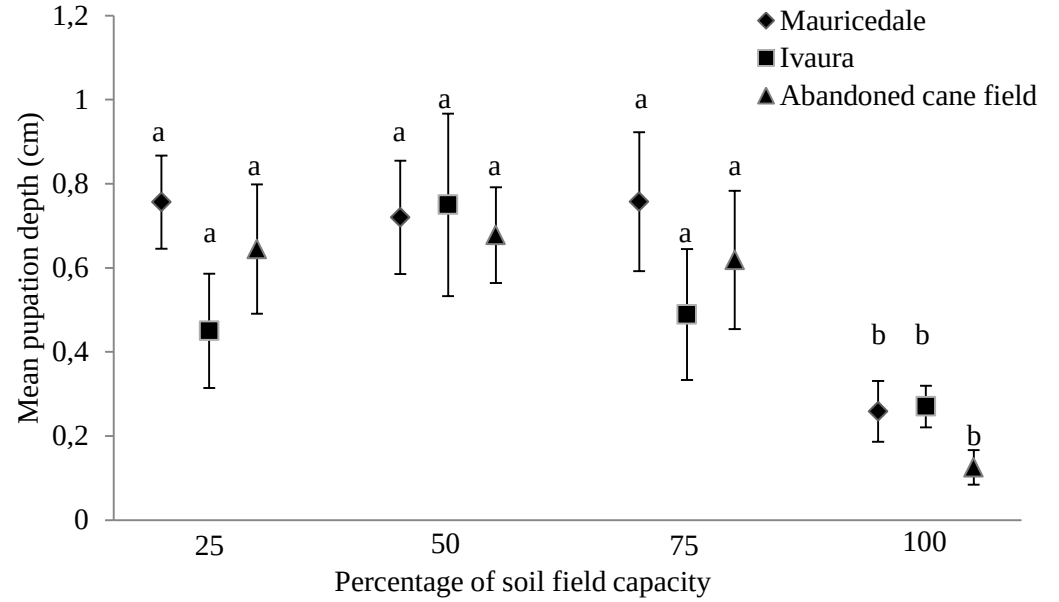


Figure 3.4. The effect of soil source ($F_{25, 75}$= 1.37, P>0.05) and percentage of soil field capacity ($F_{3, 75}$= 8.04, P <0.05; differences displayed) on *Zygogramma bicolorata* average pupation depth (mean ± S.E.). Different letters above bars indicate significant differences between treatments (P<0.05, Tukey).

There was no relationship between pupal weight and pupation depth (r = 0.162; df = 140; P>0.05) (Fig. 3.5). Most of the beetles (80.99%), including the heaviest and lightest individuals ($\bar{x}$ = 47.34 ± 1.43 mg, n = 115) were found to have pupated at shallow depths of 1 cm or less. Of these individuals, three (2.11% of the total number of beetles which pupated) pupated on the soil surface. The average weight of these individuals did not stand out from the rest of the data and the weight range was large (range = 39-49 mg; $\bar{x}$ = 43 ± 3.06 mg) indicating that no particular beetle weight is vulnerable to this behaviour. Comparatively few individuals pupated between 1 cm and 2 cm below the soil surface (16.90%), but these individuals were marginally, but not significantly, lighter than the individuals which pupated below 1 cm ($\bar{x}$ = 44.0 ± 2.85 mg, n= 24). Therefore, individuals of the same weight can be found within the entire range of burrowing depths.

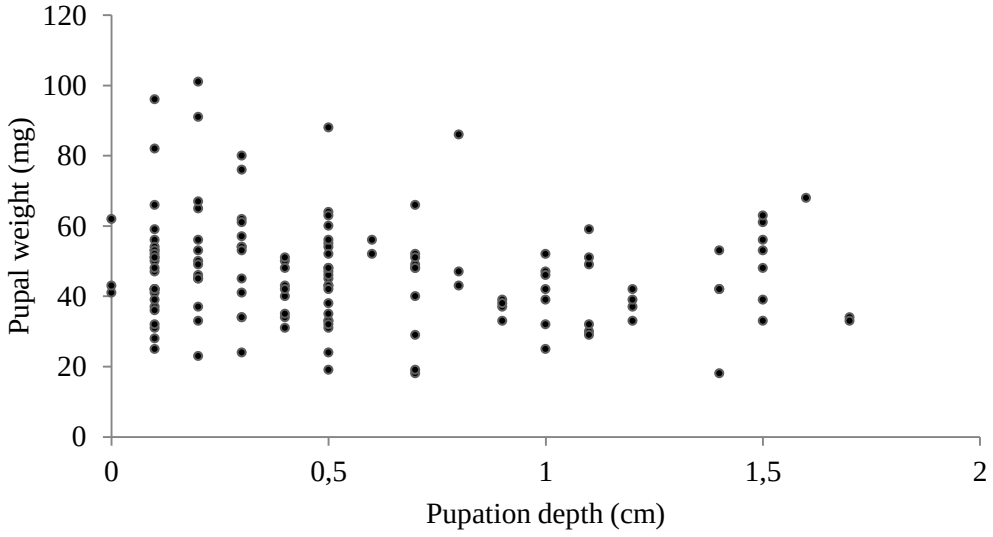


Figure 3.5. The relationship between *Zygogramma bicolorata* pupal weight and pupation depth (r = -0.162; df = 140; P>0.05).

3.6. Discussion

3.6.1. *Zygogramma bicolorata* diapause depth and the implications for integrated pest management using fire

Soil type and soil moisture didn't have any effect on the depth at which *Z. bicolorata* beetles diapaused. Of the beetles that did diapause, the vast majority diapaused at a depth less than 1 cm below the surface of the soil. This depth does not appear particularly unusual as *Contarinia nasturnii* (Kieffer) (Diptera:

Cecidomyiidae), a common pest in Europe and invasive pest in North America also typically burrows only into the top 1 cm of the soil (Chen & Shelton 2007). The average across all soil types and soil moisture treatments was between 0.2 cm and 0.3 cm below the surface of the soil, with many individuals digging down just deep enough for them to create a diapause chamber in the soil. A diapausing individual was also found in the abandoned cane field site in the winter of 2017 at a depth of 0.4 cm below the surface of the soil.

The shallow diapause depth indicates that the beetles are unlikely to survive a burning regime in the field. This combined with the lack of an impact of a burning regime on *P. hysterophorus* germinable seed banks in Australia (Vogler *et al.* 2002) suggest that integrated control is unlikely to be successful. The lack of any correlation relating to diapause depth and soil type indicates that using a burning regime would have the same effect on the beetle population in any soil type. However, further study is required in order to determine whether fire is likely to result in *Z. bicolorata*'s lethal thermal limit being reached at 1 cm below the surface of the soil.

3.6.2. *Zygogramma bicolorata* pupation and diapause success and the implications for field site selection for beetle release and the beetle's ability to establish in the field

Previous studies have shown that soil type and moisture can have a significant effect on the life cycle of an insect (Ellis *et al*. 2004; Pacchioli & Hower 2004; Shililu *et al.* 2004). Insects typically tolerate a wide range of soil conditions, but do not tolerate extremely dry or extremely wet soils (Lapointe & Shapiro 1999). Hulthen and Clarke (2006) found that the mortality of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) increased at the extreme points of soil moisture (85% mortality at 10% soil moisture and 30% mortality at 100% soil moisture), whilst Lapointe & Shapiro (1999) found that the ideal soil moisture content for Diapausing *Diaprepes abbreviates* (L.) (Coleoptera: Curculionidae) adults was 60 ± 10% of the soil by weight. Therefore, the 100% soil moisture treatments resulting in no individuals diapausing and a drop in successful pupation is not

surprising as the water likely resulted in anoxic conditions in the soil, preventing the beetles from burrowing into it (Skopp *et al.* 1990).

The lack of a significant relationship between diapause survival and soil type is not surprising as the beetles have established and persisted at all three sites in the field, meaning that the beetles must be able to diapause in each of the soils over the winter. This result also indicates that soil type is not a determining factor for the three sites that were tested in the likelihood of *Z. bicolorata*'s ability to complete its lifecycle, as has been found with other insects (Hulthen & Clarke 2006). Whilst there is no correlation between beetle diapause depth at 25%, 50%, and 75% field capacity; it does show that the beetles are capable of diapausing and surviving in a wide range of soil conditions, and none of these soil moisture levels are low enough to significantly affect the beetles' ability to diapause. The lack of any significant drop off of diapause success between the 25-75% soil moisture treatments indicate that *Z. bicolorata* is able to tolerate a wider variety of soil moisture conditions than *D. abbreviates* (Lapointe & Shappiro 1999) and therefore, *Z. bicolorata* is likely a good agent.

The low percentage (38.61%) of beetles diapausing is a cause for concern, however, there is no indication that either soil moisture below 100% field capacity or soil type is the reason for the low diapause success. The low diapause rate may be a result of possible suboptimal conditions during the laboratory trial, although the cause of such conditions is not known. The low rate of diapause could equate to heavy losses to the established beetle population in the winter, especially when considering the shallow diapause depth of the survivors. Any sudden fluctuations in temperature above ground could result in a significant temperature fluctuation at the low depth that the beetles diapause, which may result in more individuals dying off.

A lower pupation rate in the Ivaura soil treatment compared to other soil types is surprising, as the soil texture is the same as that of Mauricedale soil. Ivaura soil is the only soil used in these experiments with a dark colour, indicating that perhaps

other factors, such as soil content or pH, which are factors that can determine soil colour, could limit the beetle's ability to successfully pupate in the soil, and therefore establish in the field. However, as the beetles have established at all three sites, these factors clearly do not prevent the beetle from establishing, though organic content and pH should be further explored as they may be a good indication of the beetles' ability to establish in the field.

The mean pupation depth of between 0.1 cm and 0.75 cm below the soil surface across all treatments is comparable to other insects (Chen & Shelton 2007). It is very rare to find individuals deeper than 1 cm below the surface of the soil, and there was no correlation between maximum depth of an individual and soil type or soil moisture. The significant drop-off between beetle pupation success and a 100% soil field capacity is not surprising as one would expect the beetle's pupation rate to drop off at the 100% soil moisture treatment due to the anoxic conditions created in the soil (Skopp *et al*. 1990). However, as *Z. bicolorata* diapauses in winter, when the soil moisture is typically lower than in summer, it is unlikely that soil moisture would restrict the beetle in the field. The shallower average pupation depth may be an indication that the individuals are exposed to conditions which are less than ideal for them, perhaps demonstrating that these conditions could have an effect on the beetle population's ability to persist in the field when exposed to these circumstances. The experiment does indicate that the beetles are capable of establishing in the field where soils can reach a carrying capacity of 100%. Therefore there does not appear to an upper limit to the beetle's ability to establish in wet soil. However, with factors such as predators and fluctuating temperatures that would be included in real world field conditions, it is unlikely that these individuals would survive whilst pupating in an extremely high soil moisture content. Pupal weight does not appear to affect soil pupation depth in any way. The few individuals pupating on the surface of the soil is cause for concern as these individuals would likely be preyed upon or trampled (Holomuzki 1985; Holomuzki 1988).

3.7. Conclusion

It appears that soil moisture conditions have an effect on *Z. bicolorata*'s lifecycle, particularly diapause success, as has been shown with other insects (Ellis *et al.* 2004, Hulthen & Clarke 2006, Pacchioli & Hower 2004, Shililu *et al.* 2004). Therefore, soil moisture conditions should be considered when selecting sites for *Z. bicolorata* release and establishment. Soils that have summer soil moistures between 25% and 75% of the soil field capacity should not have an effect on the beetle's ability to establish in the field in South Africa and sites that fit these soil moisture conditions should be selected for *Z. bicolorata* release in order to maximise the likelihood of establishment. However, sites with high soil moisture levels (>75% of the soil field capacity) should be disregarded as *Z. bicolorata* populations are unlikely to be able to complete their lifecycle under these conditions.

3. 8. References

BOUYOUCOS, G.J. 1962. Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal* **54**: 464- 465.

BRIESE, D.T. 1996. Biological control of weeds and fire management in protected natural areas: are they compatible strategies? *Biological Conservation* **77**: 135-141.

CHEN, M. & SHELTON, A.M. 2007. Impact of soil type, moisture, and depth on Swede Midge (Diptera: Cecidomyiidae) pupation and emergence. *Environmental Entomology* **36**: 1349-1355.

CHENG, W., LONG, Z., ZHANG, Y., LIANG, T. & ZHU-SALZMAN, K. 2017. Effects of temperature, soil moisture and photoperiod on diapause termination and post-diapause development of the wheat blossom midge, *Sitodiplosis mosellana* (Géhin)(Diptera: Cecidomyiidae). *Journal of Insect Physiology* **103**: 78-85.

DEW, L.A., ROZEN-RECHELS, D., LE ROUX, E., CROMSIGT, J.P.G.M. & TE BEEST, M. 2017. Evaluating the efficiency of invasive plant control in response to ecological factors. *South African Journal of Botany* **109**: 203-213.

DHILEEPAN, K. SETTER, S. & MCFADYEN, R.E. 2000. Impact of defoliation by the introduced biocontrol agent *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on parthenium weed in Australia. *Biocontrol* **45**: 501-512.

DHILEEPAN, K & MCFADYEN R.C. 2012. *Parthenium hysterophorus* L. - parthenium. In: JULIEN, M., MCFADYEN, R. & CULLEN, J. (eds) Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne. Pp. 448-462.

DIMOU, I., KOUTSIKOPOULOS, C., ECONOMOPOULUS, A.P. & LYKAKIS, J. 2003. Depth of pupation of the wild olive fruit fly, *Bactrocera (Dacus) oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. *Journal of Applied Entomology* **127**: 12-17.

ELLIS, J.D. JR, HEPBURN, R., LUCKMAN, B. & ELZEN, P.J. 2004. Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). *Environmental Entomology* **33**: 794-798.

GOODALL, J.M. & ERASMUS, D.J. 1996. Review of the status and integrated control of the invasive alien weed, *Chromolaena odorata*, in South Africa. *Agriculture, Ecosystems and Environment* **56**: 151-164.

HAHN, D.A. & DENLIGER, D.L. 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology* **53**: 760-773.

HENNESSEY, M.K. 1994. Depth of pupation of Caribbean fruit-fly (Diptera, Tephritidae) in soils in the laboratory. *Environmental Entomology* **23**: 1119-1123.

HOLOMUZKI, J.R. 1985. Life history aspects of the predaceous diving beetle, *Dytiscus dauricus* (Gebler), in Arizona. *The Southwestern Naturalist* **30**: 485-490.

HOLOMUZKI, J.R. 1988. Pupation site and pupal mortality of the predaceous diving beetle *Dytiscus dauricus. The Southwestern Naturalist* **33**: 485-486.

HULTHEN, A.D. & CLARKE, A.R. 2006. The influence of soil type and moisture on pupal survival of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology* **45**: 16-19.

JACKSON, C.G., LONG, J.P. & KLUNGNESS, L.M. 1998. Depth of pupation in four species of fruit flies (Diptera: Tephritidae) in sand with and without moisture. *Journal of Economic Entomology* **91**: 138-142.

KING, H. 2008. Thermal physiology and predicted distruibution of *Zygogramma bicolorata* (Chrysomelidae), a promising agent for the biological control of the invasive weed *Parthenium hysterophorus* in South Africa. Unpublished. Masters diss., Faculty of Science and Agriculture, University of KwaZulu-Natal, 2008.

LAPOINTE, S.L. & SHAPIRO, J.P. 1999. Effect of soil moisture on development of *Diaprepes abbreviates* (Coleoptera: Curculionidae). *The Florida Entomologist* **82**: 291-299.

MCFADYEN, R.E. & MCCLAY, A.S. 1981. Two new insects for the biological control of parthenium weed in Queensland. *Proceedings of the Sixth Australian Weeds Conference* **1**: 145-149.

PACCHIOLI, M.A. & HOWER, A.A. 2004. Soil and moisture effects on the dynamics of early instar clover root curculio (Coleoptera: Curculionidae) and biomass of alfalfa root nodules. *Environmental Entomology* **33**: 119-127.

SHILILU, J.I., GRUEBER, W.B., MBOGO, C.M., GITHURE, J.I., RIDDIFORD, L.M. & BEIER, J.C. 2004. Development and survival of *Anopheles gambiae* eggs in drying soil: influence of the rate of drying, egg age, and soil type. *Journal of the American Mosquito Control Association* **20**: 243-247.

SKOPP, J., JAWSON, M.D. & DORAN, J.W. 1990. Steady-state aerobic microbial activity as a function of soil water content. *Soil Science Society of America Journal* **54**: 1619-1625.

TE BEEST, M., CROMSIGT, J.P.G.M., NGOBESE, J. & OLFF, H. 2012. Managing invasions at the cost of native habitat? An experimental test of the impact of fire on the invasion of *Chromolaena odorata* in a South African savannah. *Biological Invasions* **14**:607-618.

VARGAS, R.I., CHANG, H.B., KOMURA, M. & KAWAMOTO, D. 1987. Mortality, stadial duration, and weight loss in three species of mass-reared fruit fly pupae (Diptera: Tephritidae) held with and without vermiculite at selected relative humidities. *Journal of Economic Entomology* **80**: 972-974.

VOGLER, W., NAVIE, S., ADKINS, S. & SETTER, C. 2002. Use of fire to control parthenium weed. *A Report for the Rural Industries Research and Development Corporation, Australia* **41**.

WITKOWSKI, E.T.F. & WILSON, M. 2001. Changes in density, biomass, seed production and soil seed banks of the non-native invasive plant, *Chromolaena odorata* along a 15 year chronosequence. *Plant Ecology* **152**: 13-27.

ZHU, F., XUE, F. & LEI, C. 2006. The effect of environmental conditions on diapause in the blister beetle, *Mylabris phalerata* (Coleoptera: Meloidae). *European Journal of Entomology* **103**: 531-535.

CHAPTER FOUR

Synthesis and Conclusions

4.1. General overview

Parthenium hysterophorus (L.) (Asteraceae: Heliantheae) is an annual herbaceous alien invasive weed, which has become prevalent in India, Australia, and South Africa (Adkins & Shabbir 2014). The negative effects of the weed's invasion upon people, livestock and native plant species is well known and has resulted in the initiation of several control methods in the affected countries. Mechanical and chemical control methods were deemed unsuitable as they were not cost effective and posed health concerns for those involved (Hobbs & Humphries 1995; Muniyappa *et al.* 1980; Wakjira *et al.* 2009). The efforts to control the weed have therefore primarily been through biological control.

Based on the Australian biological control program *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) was released into the field in South Africa in 2013 (McConnachie 2015; Strathie *et al*. 2005). However; *Z. bicolorata* has failed to establish at many release sites around South Africa, and has had little apparent impact on the target weed. This study represents a post-release assessment of *Z. bicolorata* in South Africa, with particular emphasis placed on factors which may be limiting the beetle's establishment and impact on the target weed. The purpose of this synthesis is to discuss the conclusions from previous chapters, while drawing attention to the recommendations for the future deployment of *Z. bicolorata* in South Africa.

4.2. Implications of release site selection for *Zygogramma bicolorata*

Often biological control programs are not as successful as expected, with many agents failing to establish, or only establishing in limited ranges- often resulting in a low impact on the target weed (Coombs *et al.* 1999). This has been the case for *Z. bicolorata* in South Africa, as the majority of populations did not establish at release sites and appear to have had limited impacts on the target weed in the field. However, the results of this dissertation suggest that *Z. bicolorata* feeding can facilitate control of the plant in the field by reducing *P. hysterophorus* density

(from 50 plants/m^2 to no plants in some cases) and canopy volume (from 20 cm^3 to 2 cm^3 at some sites), which indicates that a successful biological control program is possible. Previous laboratory-based research by King (2008) suggested that the climate of South Africa is suitable for the beetle, but a major part of the beetles' life history was ignored, namely its pupation and diapause in the soil. An unsuitable climate is the primary reason for the failure of most biological control programs and studies suggest that the successful establishment of an agent occurs when the release site habitat mirrors that of the agent's native habitat (Byrne *et al.* 2002; Stiling 1993). This study found that *Z. bicolorata* is likely to experience soil moisture and soil texture stresses in South Africa. Therefore, failure of *Z. bicolorata* to establish across much of South Africa is likely due to the beetle's poor compatibility with the soils at the release sites, particularly during the seasons with low rainfall, a conclusion which was deemed as the likely reason for the initial difficulties in the beetle's establishment in Australia (Dhileepan & McFadyen 2012).

The ecological requirements of *Z. bicolorata* need to be considered when selecting release sites for the beetle. Sites that have perennially dry soil i.e. below a volumetric soil water content (VSWC) of 10%, or soil that is saturated at 100% field capacity, and soil with high organic content (grey texture) should be deemed unsuitable release sites. Releases should be focused at sites that have a high VSWC (>10%) throughout the year, and soil that has red texture. Studies on the organic and inorganic content of the soils in which the beetle would likely establish would further increase the understanding of the beetle's restrictions. The beetles should also be released during high rainfall periods in order to increase the chances of establishment.

Other studies have also found a correlation between soil abiotic factors and beetle population size and spread. The Japanese beetle *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae) has a high population density in when rainfall is high compared to that in years when rainfall is low due to the soil-inhabiting stages of the beetle dying off in low rainfall years due to low soil moisture (Hawley 1949).

Additionally, a study by Ushatinskaya (1977) on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), found that the beetle has a high mortality when diapausing in water-logged soil. This suggests that soil moisture and soil type can have a huge effect on the distribution of Chrysomelid beetles with below-ground life stages, and therefore the success of establishment in its non-native range. As such, abiotic soil factors should not be ignored.

4.3. Biological implications for *Zygogramma bicolorata*

Aside from the climatic unsuitability, biotic factors, such as predation of *Z. bicolorata* eggs, and potentially other life stages such as the larval stage has been suggested for low beetle establishment and persistence, and consequently, the low impact on the target weed in the field (Gupta *et al.* 2004). Predation is listed as the second most common reason for the failure of a biological control program, after climatic unsuitability (Stiling 1993). Agents may be released from the predation pressure of their native predators, but indigenous generalist predators will continually threaten the released population while establishing and once established in the field (Rosenheim *et al*. 1999; Snyder & Ives 2001). Previous studies have found that predators do attack *Z. bicolorata* adults and larvae in their introduced range in India (Gupta *et al.* 2004). This study found that *Z. bicolorata* eggs experience high predation pressure (up to 100% of the eggs can be consumed) in the introduced range, though it is not known which species attack the beetle eggs. This high predation pressure could severely inhibit the chances of the beetle establishing after release. A temporary solution to the problem may be to release only adults. However, other biological control programs suggest that the best way to counteract high predation pressure is to release higher numbers of agents on fewer occasions (Patrick & Olckers 2014), which may be the way forward for *Z. bicolorata*.

4.4. Integrated control with the use of an agent and a burning regime

Fire has been recommended as a low cost and effective way to control alien weeds (Briese 1996; Dew *et al*. 2017; Goodall & Erasmus 1996). However, management

of alien weeds that have biological control agents released against them may not be compatible with fire as it may promote the spread and density of the weed and may slow the agents' impact against the weed or possibly eliminate the agent (Briese 1996). Previous studies have found that it is possible to destroy sub-surface seeds of invasive alien weeds, such as *Chromoleana odorata* (L.) King & Robinson (= *Eupatorium odoratum* L.), by heating up the soil with fire (te Beest *et al.* 2012; Witkowski & Wilson 2001). It was suggested that a burning regime be implemented on *P. hysterophorus* in winter when *Z. bicolorata* is diapausing in the soil in order to reduce the plant's seed bank. However, this study found that the shallow diapause depth (<0.3 cm below the soil surface) of *Z. bicolorata* may render the beetles unlikely to survive a burning regime in the field; therefore integrated control using fire is unlikely to be successful. However, further study is required in order to determine whether fire is likely to result in *Z. bicolorata*'s lethal thermal limit being reached at 1cm below the surface of the soil. A previous study conducted in Australia found that repeated burns of fields of *P. hysterophorus* in early autumn and late summer over a two year period resulted in a rapid decline of *P. hysterophorus* density, but had no effect on parthenium germinable soil seed banks (Vogler *et al*. 2002). Therefore, it is suggested that integrated control with *Z. bicolorata* and a burning regime should not be implemented.

4.5. Conclusion

This research is part of the post-release assessment of the agent *Z. bicolorata* against the invasive weed *P. hysterophorus*, and provides some understanding towards the current biological control program and its limitations. This study shows that *Z. bicolorata* has the potential to be an effective agent against *P. hysterophorus*, and advocates that releases of the agent into the field should be continued. However, release sites should be chosen carefully, as the beetles are particularly sensitive to soil moisture and soil type. The release methods should also be modified in order to lessen the impact of indigenous predators. This research could also improve release efforts of *Z. bicolorata* internationally (e.g. Australia and India). Although the incorporation of these additional factors has

enhanced our understanding of the agent and its impact as a whole, *P. hysterophorus* is an invader which has the potential to directly impact many lives; as such it will require continued biological control efforts in order to successfully regulate the weed.

4.6. References

ADKINS, S.W. & SHABBIR, A. 2014. Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.). *Pest Management Science* **2014**.

BRIESE, D.T. 1996. Biological control of weeds and fire management in protected natural areas: are they compatible strategies? *Biological Conservation* **77**: 135-141.

BYRNE, M.J., CURRIN, S. & HILL, M.P. 2002. The influence of climate on the establishment and success of the biocontrol agent *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biological Control* **24**: 128-134.

COOMBS, E.M., MCEVOY, P.B., PIPER, G.L. & VILLEGAS, B. 1999. Why do weed biocontrol agents fail to establish or to control their hosts? *Proceedings of the X International Symposium on Biological Control of Weeds* p.197.

DEW, L.A., ROZEN-RECHELS, D., LE ROUX, E., CROMSIGT, J.P.G.M. & TE BEEST, M. 2017. Evaluating the efficiency of invasive plant control in response to ecological factors. *South African Journal of Botany* **109**: 203-213.

DHILEEPAN, K & MCFADYEN R.C. 2012. *Parthenium hysterophorus* L. - parthenium. In: JULIEN, M., MCFADYEN, R. & CULLEN, J. (eds) Biological Control of Weeds in Australia. CSIRO Publishing, Melbourne. Pp. 448-462

GOODALL, J.M. & ERASMUS, D.J. 1996. Review of the status and integrated control of the invasive alien weed, *Chromolaena odorata*, in South Africa. *Agriculture, Ecosystem and Environment* **56**: 151-164.

GUPTA, R.K., KHAN, M.S., BALI, K., MONOBRULLAH, M. & BHAGAT, R.M. 2004. Predatory bugs of *Zygogramma bicolorata* Pallister: an exotic beetle for biological suppression of *Parthenium hysterophorus* L. *Current Science* **87**: 1005-1010.

HAWLEY, I.M. 1949. The effect of summer rainfall on Japanese beetle populations. *Journal of the New York Entomological Society* **57**: 167-176.

HOBBS, R.J. & HUMPHRIES, S.E. 1995. An integrated approach to the ecology and management of plant invasions. *Conservation Biology* **9**: 761-770.

KING, H. 2008. Thermal physiology and predicted distribution of *Zygogramma bicolorata* (Chrysomelidae), a promising agent for the biological control of the invasive weed *Parthenium hysterophorus* in South Africa. Unpublished. Masters diss., Faculty of Science and Agriculture, University of KwaZulu-Natal, 2008.

MCCONNACHIE, A.J. 2015. Host range and risk assessment of *Zygogramma bicolorata,* a defoliating agent released in South Africa for the biological control of *Parthenium hysterophorus. Biocontrol Science and Technology* **25**: 975-991.

MUNIYAPPA, T.V., PRASAD, T.V.R. & KRISHNAMURTHY, K. 1980. Comparative efficacy and economics of mechanical and chemical methods of control of *Parthenium hysterophorus* Linn. *Indian Journal of Weed Science* **12**: 137-144.

PATRICK, K. & OLCKERS, T. 2014. Influence of shade on the persistence of *Gargaphia decoris* (Tingidae), a biological control agent of *Solanum mauritianum* (Solanaceae) in South Africa. *African Entomology* 22: 891-895.

ROSENHEIM, J.A., LIMBURG, D.D. & COLFER, R.G. 1999. Impact of generalist predators on a biological control agent, *Chrysoperla carnea*: direct observations. *Ecological Applications* **9**: 409-417.

SNYDER, W.E. & IVES, A.R. 2001. Generalist predators disrupt biological control by a specialist parasitoids. *Ecology* **82**: 705-716.

STILING, P. 1993. Why do natural enemies fail in classical biological control programs? *American Entomologist* **39**: 31-37.

STRATHIE, L.W., WOOD, A.R., VAN ROOI, C. & MCCONNACHIE, A. 2005. *Parthenium hysterophorus* (Asteraceae) in southern Africa, and initiation of biological control against it in South Africa. In: RAMACHANDRA PRASAD, T.V. (ed) Proceedings of the Second International Conference on Parthenium Weed Management. Bangalore, India. Pp. 127-133.

TE BEEST, M., CROMSIGT, J.P.G.M., NGOBESE, J. & OLFF, H. 2012. Managing invasions at the cost of native habitat? An experimental test of the impact of fire on the invasion of *Chromolaena odorata* in a South African savannah. *Biological Invasions* **14**:607-618.

USHATINSKAYA, R.S. 1977. Seasonal migration of the imago of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) in different types of soil and the physiological variations of specimens in hibernating populations. *Ecological Bulletins* **25**: 526-529.

VOGLER, W., NAVIE, S., ADKINS, S. & SETTER, C. 2002. Use of fire to control parthenium weed. *A Report for the Rural Industries Research and Development Corporation, Australia* **41**.

WAKJIRA, M., BERECHA, G. & TULU, S. 2009. Allelopathic effects of an invasive alien weed *Parthenium hysterophorus* L. compost on lettuce germination and growth. *African Journal of Agricultural Research* **4**: 1325-1330.

WITKOWSKI, E.T.F. & WILSON, M. 2001. Changes in density, biomass, seed production and soil seed banks of the non-native invasive plant, *Chromolaena odorata* along a 15 year chronosequence. *Plant Ecology* **152**: 13-27.