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

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PREFACE

The experimental work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg and at the Cedara Weeds Unit (ARC – Plant Protection Research Institute) from January 2006 to January 2008, under the supervision of Doctor Terence Olckers and co-supervision of Doctor Andrew McConnachie and Professor Colleen Downs.

This dissertation, submitted for the degree of Master of Science in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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ABSTRACT

Parthenium hysterophorus (Asteraceae), classified as an emerging weed in South Africa, has become abundant throughout large parts of southern and eastern Africa. In South Africa it has invaded areas in KwaZulu-Natal, Mpumalanga, the North West Province and Limpopo. A biological control programme against parthenium weed was launched in South Africa in 2003, based on the success achieved in Australia. *Zygogramma bicolorata,* a leaf-feeding beetle native to Mexico, was imported into South Africa via Central Queensland, Australia where it was released in the 1980s. This thesis examines aspects of the thermal physiology of *Z. bicolorata* which, in conjunction with its native and exotic geographical distribution, was used to predict the potential distribution of the agent in South Africa, in relation to climate. To determine *Z. bicolorata*'s physiological capability, several physiological parameters were examined for mechanistic modelling purposes. These parameters included the beetle's lethal thermal limits, critical thermal limits, lethal humidities (Chapter 2) and developmental rate at constant temperatures (Chapter 3). In Chapter 4, these physiological parameters were entered into the dynamic modelling program CLIMEX (CLIMEX programme ver. 2, CSIRO Entomology ©) and a map of the areas that are acceptable for the establishment of *Z. bicolorata* was produced. The CLIMEX model predicted that most of South Africa is favourable for the establishment of the beetle, except in the west of the country and in the north of Lesotho, extending into South Africa. All areas in which parthenium currently occurs were predicted to be very favourable for *Z. bicolorata* establishment and proliferation. Optimal release sites aimed at initial establishment were earmarked at three areas in the northeastern part of South Africa (Jozini, Ndumu Game Reserve and along the road from

Swaziland to Mozambique). It is concluded that *Z. bicolorata* is climatically suited to South Africa, increasing the likelihood that populations will establish and proliferate when released.

Keywords: CLIMEX, distribution, *Parthenium hysterophorus*, South Africa, thermal physiology, *Zygogramma bicolorata*

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CONTENTS

CHAPTER 1

INTRODUCTION

1.1. INVASIVE ALIEN PLANTS

The number and scope of exotic plant invasions has become a global threat to agriculture, commercial productivity, conservation and human health (Vitousek 1990, Mooney and Hobbs 2000), with alien invasions being categorized as the second largest threat to biodiversity (Walker and Steffen 1999, Mooney and Hobbs 2000). Plant species are continually being introduced to new ranges, either accidentally or deliberately. Approximately 1 % of these introduced plant species become invasive (Williamson and Fitter 1996). These biotic invaders are defined as "species that establish a new range in which they proliferate, spread, and persist to the detriment of the environment" (Mack *et al.* 2000). Attempts to classify what makes certain species 'good invaders' have been inconclusive (e.g. see van Kleunen and Richardson 2007). It is, however, agreed that factors that contribute to invasions include the release from specialist natural enemy pressure (Elton 1958), plants obtaining greater size or fecundity due to life history trade-offs (Blossey and Notzold 1995), facilitation through disturbance (Mack *et al*. 2000, van Kleunen and Richardson 2007), broken evolutionary relations (Callaway and Aschehoug 2000) and climate change (van Kleunen and Richardson 2007).

Alien vegetation has detrimental impacts on nutrient cycling (Stock *et al.* 1995, Ehrenfeld and Scott 2001), system functioning, fire regimes (D'Antonio 2000, Brooks *et al.* 2004), species composition and water usage (Dyer and Rice 1999, Zavaleta 2000), with high densities of

invasive species greatly reducing land values and production (Khosola and Sobti 1981, Chippendale and Panetta 1994, Mahadevappa 1997, van Wilgen *et al.* 2004). The Conservation of Agricultural Resources Act (CARA) of South Africa (Act No 43 of 1983) lists 198 plant species as declared weeds and invaders (Henderson 2001). All of these invaders negatively affect the country's ecosystems and / or cause economic loss. In South Africa and Lesotho, alien vegetation is estimated to have an accumulative water usage of 3.3 billion m^3/yr (Le Maitre *et al*. 2000) and to cover approximately 10 million hectares of land (Richardson *et al*. 2004). The economic costs caused by alien invasions, although difficult to assess, can be separated into two categories, namely the loss of economic output and ecosystem services following agricultural and natural system destruction and the cost of combating the invasions (Mack *et al*. 2000). It is estimated that over the past ten years, the South African government has contributed over R3 billion to control programmes (Anonymous 2004 in van Wilgen *et al.* 2004).

The threat posed by invaders to natural ecosystems shows no sign of abating, with a rapid increase in the number of alien plant introductions correlated to increased human movement (Wells *et al*. 1986, di Castri 1989), disturbance and climate change (Le Maitre *et al.* 2004). Successful and sustainable control of invasions is therefore extremely important. In order to control the spread of invasive species, species first have to be identified and categorized according to the threat they pose to the environment and the extent of the control that is needed. In South Africa this task is performed by CARA. Once an invasive has been identified, there are three main approaches that can be used to combat its spread and reduce its density, namely chemical, mechanical and biological control. All three methods have advantages and disadvantages, but the use of biological control to maintain alien weed

populations is considered to be the most affordable, sustainable and eco-friendly approach (Zimmerman *et al.* 2004).

1.2. BIOLOGICAL CONTROL OF WEEDS

Classical biological control is the practice of establishing biotic control of the invasive species by introducing one or more natural enemies from the plant's native range, which will cause a decline in the weed's population density, distribution and rate of spread (Zimmermann *et al*. 2004). Classical biological control was first used in South Africa in 1913 with the release of a cochineal insect (*Dactylopius ceylonicus,* Hemiptera: Dactylopiidae) to control drooping prickly pear (*Opuntia monacantha,* Cactaceae: tribe Opuntieae) (Zimmermann *et al*. 2004). Biocontrol has since been estimated to have reduced the South African budget for the management of problem plants by 19.8% (US$ 276 million) (Zimmermann *et al*. 2004).

Despite the many biological control success stories, there is still much concern about the safety of agent introductions. The risk of biocontrol agents attacking non-target species in their introduced range has been reviewed by many authors (e.g. Howarth 1991, Simberloff 1992, Simberloff and Stiling 1996, Thomas and Reid 2007). Although it is commonly agreed that the current protocols followed in assessing an agent's risks are far safer compared to the procedures followed in the early biological control programmes (Simberloff and Stiling 1996), some critics feel that the detection methods used to determine host range expansion are insufficient (Simberloff 1992). However, no host shifts have been recorded in over 350 biocontrol programmes that have been carried out globally (van Wilgen *et al*. 2004).

Of particular concern is *Parthenium hysterophorus* (Asteraceae), which is considered to be an 'emerging weed' because it is in the initial stages of invasion with the potential to cause considerable national losses if not controlled at an early stage (Strathie *et al*. 2005).

1.3. *PARTHENIUM HYSTEROPHORUS*

Parthenium hysterophorus L. (Asteraceae: tribe Heliantheae, subtribe Ambrosinae), commonly known as parthenium, cotton weed, Demoina weed, feverfew and congress grass, is a short-lived annual (McFadyen 1992) that is native to the West Indies and South and North America (Towers *et al*. 1977, McClay 1985, Kulkarni *et al*. 1997). Parthenium has invaded several countries outside of its native range and has caused problems in Australia, India, China, Israel, Madagascar, Mozambique, Nepal, South America, Vietnam (Towers *et al*. 1977, Joel and Liston 1986), Mauritius, the Seychelles (Nath 1988), Kenya, Ethiopia, Reunion, Somalia (CABI 2004) and South Africa (Strathie *et al*. 2005). In particular, parthenium has become a major weed in Australia and India, having been introduced to both countries in the 1950s (McFadyen 1985, Rao 1956 in Bhan *et al.* 1997). In India alone, it has an estimated distribution of several million hectares (Joshi 1990).

Parthenium was first recorded in South Africa in 1880 (Hilliard 1977) and has since invaded areas in KwaZulu-Natal, Mpumalanga, the North West Province and Limpopo (Strathie *et al*. 2005). Despite parthenium's early introduction into South Africa, it appears to have only become invasive in the 1980s following Cyclone Demoina (Strathie *et al*. 2005). In an effort to control its spread, parthenium has been listed as a Category 1 invasive plant by CARA (Henderson 2001).

Parthenium's invasive ability is attributed to its high flower production, with a single plant producing approximately 15 000 flowers in a life time, each bearing four or five seeds (Dhileepan *et al*. 2000). These seeds are primarily dispersed by water and to a lesser extent by wind (Strathie *et al*. 2005). The prolific seed production and all year round germination capabilities of parthenium have aided its competitive ability and rapid spread (McClay 1985). Allelopathic effects through the release of growth inhibitors by the roots and aerial parts of the plant further hamper competitive exclusion by other plants (Chakravarthy and Bhat 1997, Jayanth *et al*. 1997, Singh 1997, Belz *et al.* 2004).

Parthenium dominates agricultural and horticultural fields, greatly reducing yields (Mahadevappa 1997). Grasslands infested with parthenium have been reported to suffer a 90% decrease in forage production (Vartak 1968) and it is estimated to cause annual losses of A$ 16.5 million to the pasture industry in Australia (Chippendale and Panetta 1994). In South Africa it is rapidly invading wasteland, roadsides, rail sides, watercourses, cultivated fields and overgrazed pastures (Strathie *et al*. 2005).

Apart from its negative environmental and economic impacts, parthenium has numerous health implications. Humans in continual contact with parthenium commonly develop dermatitis (Towers *et al*. 1977), nasobronchial allergy and eczema on exposed skin (Shelmire 1939, 1940 in McFadyen 1995). Severe dermatitis caused by parthenium is believed to result from sesquiterpene lactones present within the plant (Swain and Williams 1977). Cattle grazing or walking through fields containing high densities of parthenium have been reported to display inflamed udders, fevers and rashes (Krishnamurthy *et al*. 1977). In an experiment performed by Rao *et al*. (1976), buffalo (*Bubalus bubalis*) and bull (*Bos taurus*) calves feeding

on parthenium developed ulcers in their mouths and digestive tracts and dissection of the livers and kidneys showed a degeneration and necrosis of the tissues.

1.4. CHEMICAL AND MECHANICAL CONTROL OF PARTHENIUM

Chemical control of *P. hysterophorus* is possible using several chemicals, notably paraquat, monosodium methane arsenate, disodium methane arsenate, atrazine 2.4-D sodium salt, bromacil, glyphosate and sodium chlorate (Sukhada 1975, Krishnamurthy *et al*. 1977, Muniappa 1980). In South Africa, the two active ingredients registered for chemical control of parthenium are diuron / sulcotrion and glyphosate trimesium (Strathie *et al*. 2005). However, control using chemicals is only temporary, with regrowth occurring within a few days after application (Mahadevappa 1997). Another drawback of this approach is the high cost of herbicides which makes chemical control largely uneconomical in low production areas (Hobbs and Humphries 1995) and in areas where repeated application is necessary (Mack *et al*. 2000).

Although far less hazardous than chemical control, mechanical control is expensive (Hobbs and Humphries 1995). Although effective in small areas of infestation, the allergic reactions that result from contact with the plant can make mechanical removal difficult to justify (Mahadevappa 1997, Singh 1997).

1.5. BIOLOGICAL CONTROL OF PARTHENIUM

A biocontrol programme, aimed at controlling the spread of parthenium in South Africa, was launched in 2003 by the Agricultural Research Council's Plant Protection Research Institute (ARC-PPRI) (Strathie *et al*. 2005). Based on results from Australia's biological programme against parthenium (see below), three insect agents, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), initially identified as *Zygogramma* sp. v. nr. *malvae* Stal., *Listronotus setosipennis* (Hustache) (Coleoptera: Curculionidae) and *Epiblema strenuana* Walker (Lepidoptera: Tortricidae), and a rust fungus, *Puccinia melampodii* Dietel & Holw. (Uredinales: Pucciniaceae), were identified for introduction (Strathie *et al.* 2005). Currently, the leaf-feeding beetle *Z. bicolorata* and the stem-boring weevil *L. setosipennis* are undergoing host-specificity testing in quarantine. *Epiblema strenuana* was imported from Central Queensland, Australia but the laboratory culture failed to establish and reintroduction is planned for the future (Strathie *et al.* 2005). While host-specificity testing of *P. melampodii* is currently underway, another rust, *Puccinia abrupta* Diet. & Holw (Uredinales: Pucciniaceae), has also been discovered in populations of parthenium in South Africa, Kenya and Ethiopia (Wood and Scholler 2002).

The biological control programme against parthenium in Australia was initiated in 1977 (Dhileepan *et al*. 2000) with nine arthropod species and two fungal pathogens having been released thus far (Dhileepan and McFadyen 1997, Dhileepan *et al*. 2000). *Zygogramma bicolorata* was released in Queensland in 1980 (McFadyen and McClay 1981) but the population only became evident 10 years later in 1990 (Dhileepan and McFadyen 1997). However, by 1997 the *Z. bicolorata* population was estimated to cover approximately 12 000

km^2 in Queensland (Dhileepan *et al*. 2000). A study performed by Dhileepan *et al.* (2000) on the response of parthenium to *Z. bicolorata* in Australia showed that the beetle was capable of causing 92% defoliation, 83% reduction in flower production and a 73% decrease in the soil seed bank after 90 days exposure.

In Bangalore (India), *Z. bicolorata* was mass reared and released in 1984, with visible population growth occurring four years later in 1988 (Basappa 1997, Jayanth *et al*. 1997). By 1997, the *Z. bicolorata* population had dispersed over 200 000 km^2 (Jayanth *et al*. 1997). The beetle is only active during the rainy season, between May and November, and enters diapause during the dry season (Jayanth and Geetha Bali 1994). In India, *Z. bicolorata* causes a 98% reduction in flower production during the wet season (Jayanth and Geetha Bali 1994) and one adult per plant is capable of causing skelentonization of leaves within four to eight weeks (Jayanth *et al*. 1997). The effectiveness of *Z. bicolorata* is, however, thought to be limited in some cases by the time of its emergence from diapause (Dhileepan *et al.* 2000).

1.5.1. Biology of *Zygogramma bicolorata*

Although the beetle is native to Mexico, the population imported into quarantine in South Africa was collected from established sites in Central Queensland, Australia (Strathie *et al*. 2005). Beetles obtained from Mexico for importation into Australia were collected from sites at Nuevo Leon, Veracruz and San Luis Potosi (McClay 1985). This beetle is currently regarded as one of the more promising agents available for release in South Africa (Strathie *et al.* 2005) and along with *E. strenuana* is the only agent to have had measurable negative impacts on parthenium (Dhileepan 2001).

Zygogramma bicolorata lays its eggs either on the leaves, flower heads and surface of the stem or on the terminal and auxiliary buds of parthenium (Dhileepan *et al*. 2000). Eggs can be laid singly or in groups and take three to four days to hatch (Singh 1997, Dhileepan *et al*. 2000). Newly emerged larvae feed on the young leaves of parthenium and the late instar larvae burrow into the soil to pupate (Singh 1997, Dhileepan *et al*. 2000). The larval stages last between four to six weeks (Dhileepan *et al.* 2000). The pupal stage lasts for approximately two weeks after which the adults emerge from the soil (Singh 1997, Dhileepan *et al*. 2000). Adults live for an average of two years (Dhileepan *et al*. 2000) and have an extremely high fecundity of approximately 836 eggs / female in a life cycle (McClay 1985). In both Australia and India adult beetles diapause in the soil for approximately two to three months, depending on weather conditions (Singh 1997, Dhileepan *et al*. 2000). However, Chakravarthy and Bhat (1997) demonstrated that *Z. bicolorata* may not be an obligate diapausing insect.

The success of *Z. bicolorata* as a biocontrol agent has been attributed to numerous factors. It has an extremely high fecundity and egg viability (85-91% hatching success), enabling populations, once established, to increase rapidly (McClay 1985, Kulkarni *et al.* 1997). Feeding on the plant is not restricted to a single life stage as both the adults and larvae cause large scale defoliation of parthenium (McClay 1985). The beetle reduces flower production due to early stage larval feeding on the terminal and auxillary buds, preventing flower emergence (Jayanth *et al*. 1997). *Zygogramma bicolorata* is also able to survive in a wide range of environments (McClay 1985, Kulkarni *et al*. 1997) and uses flight as a means of dispersal, with wind aiding dispersal distance (Jayanth *et al*. 1997).

1.5.2. Host-specificity of *Zygogramma bicolorata*

Extensive host-specificity testing of *Z. bicolorata* was carried out in Mexico, Australia and India. These tests confirmed that *Z. bicolorata* displayed a low level of feeding on a few non-target plants, including cultivated sunflower (*Helianthus annuus L.,* Asteraceae), but was unable to complete its development on any of these species (McFadyen and McClay 1981, Jayanth and Nagarkatti 1987). Also, a study carried out by Swamiappan *et al*. (1997) showed that feeding rates on eleven sunflower varieties was considerably lower than on parthenium. Although *Z. bicolorata* can oviposit on sunflower, it was shown that neonate larvae are unable to feed on the plant (Jayanth *et al.* 1993, Chakravarthy and Bhat 1994, Swamiappan *et al.* 1997) and thus the life cycle can not be completed. Both parthenium and sunflower are native to Mexico, but no feeding by *Z. bicolorata* on sunflower has ever been observed in Mexico (McFadyen and McClay 1981). *Zygogramma bicolorata* was therefore deemed to be host-specific and was released in both Australia and India. Once released, *Z. bicolorata* showed some feeding on sunflowers in the field in India (Anon. 1991 in Singh 1997). The erratic feeding on sunflower was attributed to pollen from adjacent stands of parthenium blowing onto the sunflower plants and inducing abnormal feeding (Jayanth *et al*. 1993). This was confirmed when sunflowers dusted with parthenium pollen in the laboratory induced feeding by adult *Z. bicolorata* (Jayanth *et al*. 1993). As a result, the beetle is not expected to pose any significant risks to non-target plants in South Africa.

1.6. PROJECT BACKGROUND AND AIMS

1.6.1. Factors limiting establishment and success of biological control agents

An important component of the programme to control the spread of parthenium in South Africa is the release of biological control agents from Mexico and South America. The two agents currently being tested in quarantine are *Z. bicolorata* and *L. setosipennis.* Biological control agents are expected to establish, reproduce, spread and cause excessive damage to the weed population (Barton 2004). However, as many post-release evaluations have shown, agents do not always establish and even when they do establish, they may not have a noticeable impact on the weed population in certain areas (e.g. Dennill and Gordon 1990, McEvoy and Coombs 2001, Byrne *et al*. 2002, Goolsby *et al*. 2005, McClay and Hughes 2007). This is commonly a result of edaphic and climatic factors experienced at the release site that either limit or promote population establishment and survival (Sehnal 1991, Stewart *et al.* 1996, Denlinger and Lee 1998, Bale and Walters 2001, Byrne *et al.* 2002). Climate is a particularly important determinant of geographical distribution for ectothermic organisms such as insects (Krebs 1978) and many biological control programmes have failed because agent releases were carried out in climatically unsuitable areas (e.g. Byrne *et al*. 2002). Predicting the probability of an agent's establishment and effectiveness once released is extremely difficult (Goolsby *et al*. 2005), but by understanding the abiotic factors that play a role in limiting the agent's distribution, the prospects for success of a biological control programme are improved (Hoelmer and Kirk 2005). Climate matching and modelling currently allows practitioners to obtain some idea of the role that climate will play in determining a biological control agent's potential for establishment (Sutherst 2003). To save

time and money and prevent any further spread of the weed, agents should be released in areas where successful establishment is most likely to be achieved (Hoelmer and Kirk 2005, Palmer *et al*. 2007). In order to determine where these areas are, the thermal physiological capabilities of the agent need to be examined.

1.6.2. Objectives of study

This dissertation is concerned with examining the thermal physiology of the proposed biological control agent *Z. bicolorata* and, using this information in conjunction with the agent's native and exotic geographical distribution, with mapping the potential distribution of the agent in South Africa in relation to climate. Areas with high probability for *Z. bicolorata* establishment and extensive parthenium invasion will be identified as potential release sites for the agent. To determine a species' physiological attributes, certain physiological parameters are examined for mechanistic modelling purposes. These parameters include lethal thermal limits, critical thermal limits, lethal humidities and developmental rate (at constant temperatures). These parameters will be examined in Chapter 2 and Chapter 3. In Chapter 4, the physiological parameters will be entered into the dynamic modelling programme CLIMEX (CLIMEX programme ver. 2, CSIRO Entomology ©) and a map predicting the areas that are most suitable for the establishment of *Z. bicolorata* will be produced.

The aim of this dissertation is therefore to determine the potential distribution of *Z. bicolorata* in South Africa in relation to climate, in order to identify release sites where establishment and control are most likely to be successful. This will also permit the identification of areas where parthenium invasions are unlikely to be controlled by *Z. bicolorata*, allowing for other

biological control agents to be chosen accordingly in the future. By doing this, the success of *Z. bicolorata* as a control agent in South Africa and the cost effectiveness of the biological control programme against parthenium is expected to be increased.

CHAPTER 2

PHYSIOLOGICAL LIMITS OF *ZYGOGRAMMA BICOLORATA*

2.1. INTRODUCTION

2.1.1. Parthenium in South Africa

Although still in the initial stages of invasion, the spread of *Parthenium hysterophorus* in South Africa has been rapid, with a number of important ecological regions already displaying large areas of infestation (Strathie *et al.* 2005, Strathie and McConnachie 2006). A recent survey, funded by the United States Agency for International Development (USAID) and the Integrated Pest Management Collaborative Research Support Program (IPM CRSP), conducted in South Africa, Swaziland, Ethiopia, Uganda and Botswana revealed parthenium to be abundant throughout large parts of southern and eastern Africa. In South Africa and Swaziland combined, parthenium was recorded in 70 quarter degree squares, over 25 quarter degrees more than was known prior to the survey in 2006 (Figure 1) (Strathie and McConnachie 2006). A follow up survey at the beginning of 2007 led to the discovery of a further four quarter degree squares infested with parthenium (Strathie & McConnachie, pers. comm.).

In South Africa, parthenium occurs primarily along the east coast of KwaZulu-Natal province and extends into the Mpumalanga and North West provinces (Strathie and McConnachie 2006, Figure 1). Important ecological and economic areas invaded by the weed include the Kruger National Park (Mpumalanga Province) and Ndumo, Tembe and Hluhluwe-iMfolozi

Game Reserves (KwaZulu-Natal Province) (Strathie and McConnachie 2006). In Swaziland the weed is present throughout the country (Strathie and McConnachie 2006).

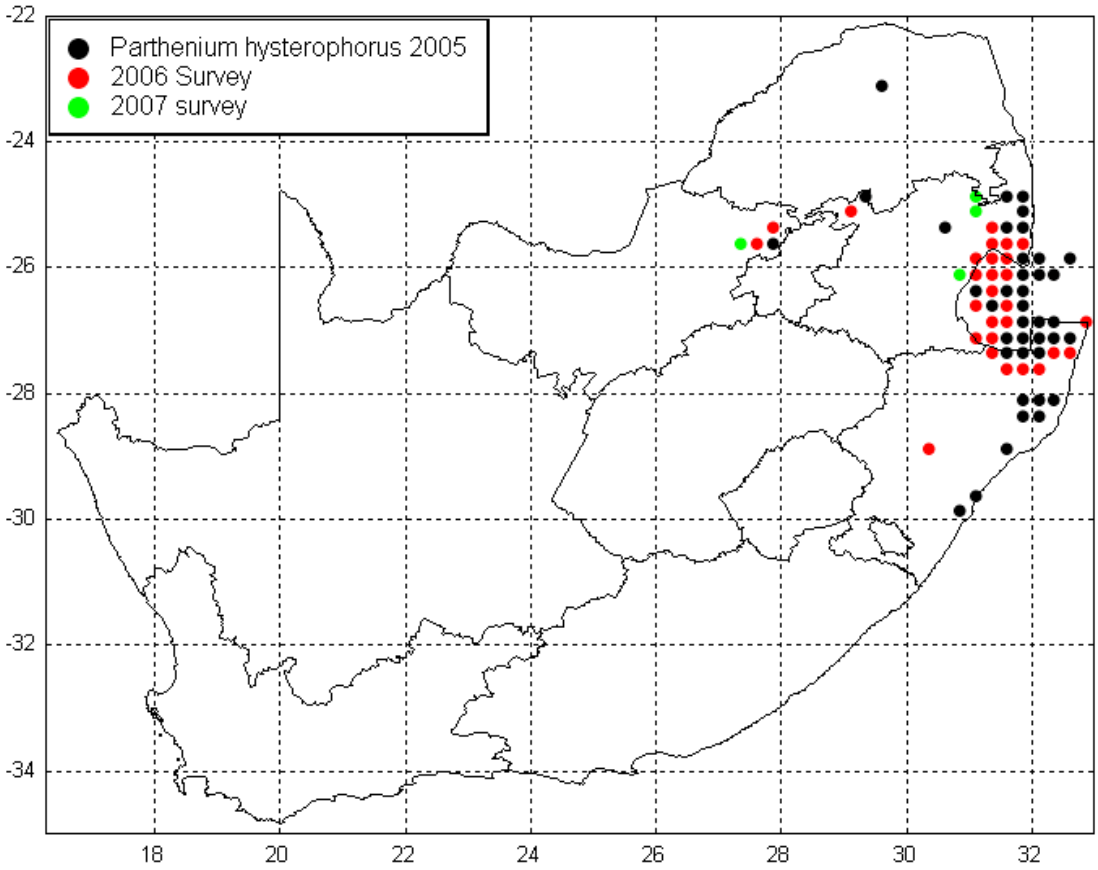
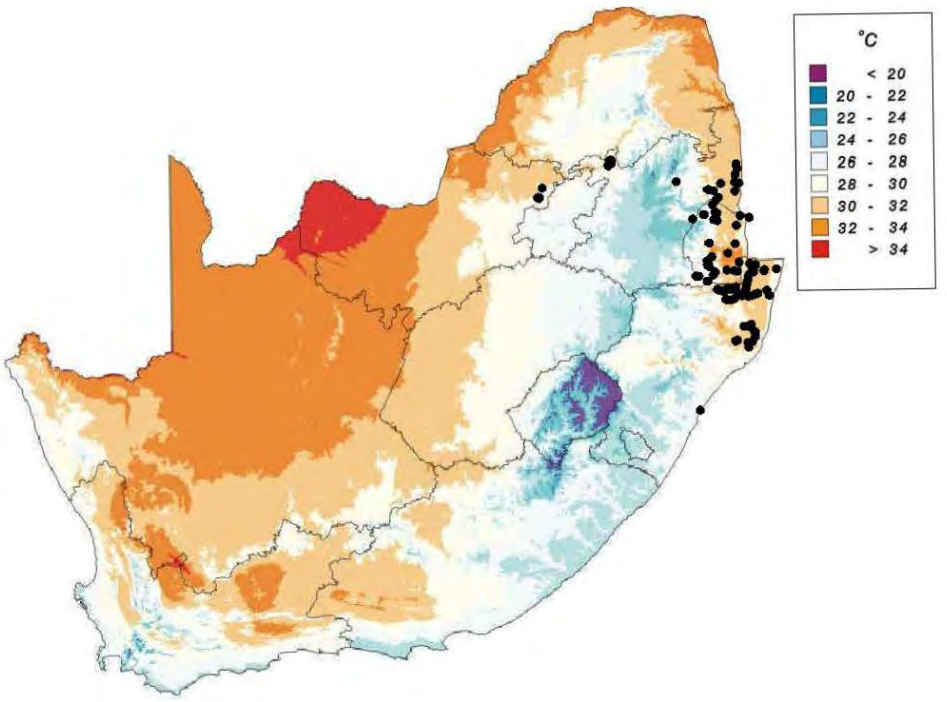


Figure 1: Known distribution of *Parthenium hysterophorus* in South Africa based on surveys carried out in 2005, 2006 and 2007 (Henderson 2001, Strathie and McConnachie 2006).

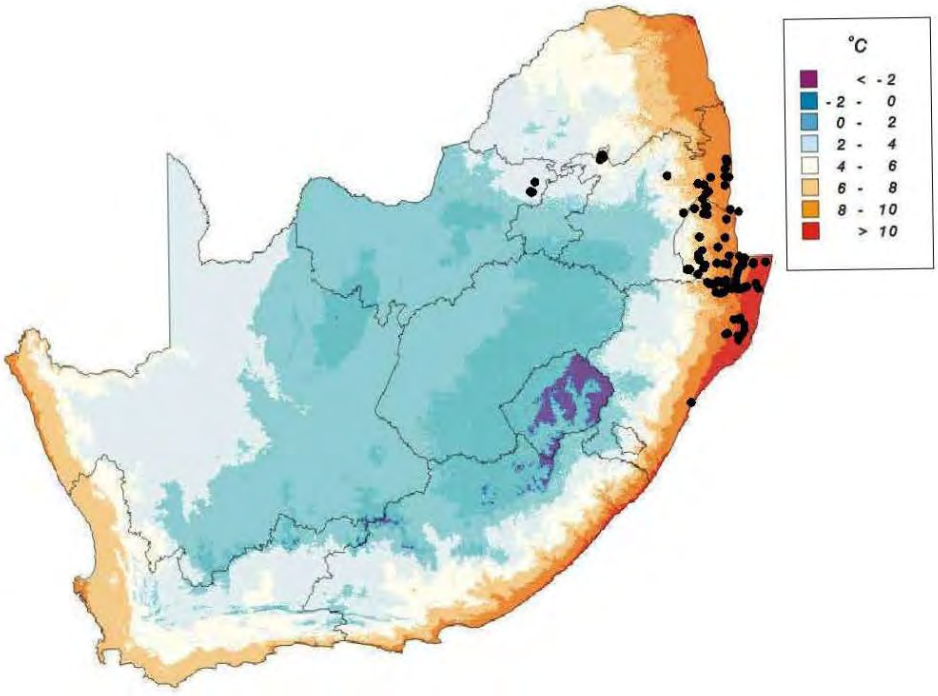
2.1.2. Climatic limitations of ectothermic agents

The wide range of temperature zones which *P. hysterophorus* has invaded in South Africa (Figure 2a & b) could potentially limit the establishment and impact of the proposed biological control agent *Zygogramma bicolorata*. Insect body temperature and metabolic rate is

determined primarily by external environmental factors (Cloudsley-Thompson 1970, Neven 2000) and temperature and humidity can greatly affect population establishment and survival as well as the reproductive ability and development of individuals (Sehnal 1991, Stewart *et al.* 1996, Denlinger and Lee 1998, Bale and Walters 2001, Byrne *et al.* 2002). Although ectothermic organisms are capable of some behavioural and physiological thermoregulation, the extent is limited (Reynolds 1979) and the survival of ectotherms has been shown to be significantly reduced at high and low temperature extremes (Denlinger and Lee 1998). Therefore, the limits of the distribution of most ectothermic species are determined by climate (Krebs 1978). If the climate is too extreme for a biological control agent, it will either fail to establish or will have little or no effect on the weed population (Dennhill and Gordon 1990). Forty-four percent of biocontrol agents released worldwide have failed to establish (McEvoy and Coombs 2001) and others have had limited success due to climatic incompatibility of the agent within the region of establishment (Dennill and Gordon 1990, Byrne *et al*. 2002, Goolsby *et al*. 2005, McClay and Hughes 2007). The ability of *Z. bicolorata* to establish and control parthenium may, therefore, differ throughout the weed's range in South Africa.



(a)



(b)

Figure 2: Distribution of *Parthenium hysterophorus* in South Africa (Henderson 2007) overlayed on (a) mean daily maximum temperatures for January and (b) mean daily minimum temperatures for July (Schulze 1997).

To increase the success and cost-effectiveness of a biological control programme, the agent's potential distribution in the new country should be modelled before agent introduction, and release sites prioritised (Byrne *et al.* 2002, Byrne *et al.* 2003, Rouget *et al.* 2004, Palmer *et al.* 2007). There are two common methods whereby an insect's potential geographical range can be modelled; one is through climate matching (reverse modelling) where the climate from the country of origin is compared to that of the country of introduction, thus inferring the new geographical range from the native range (Sutherst *et al.* 2001, Byrne *et al.* 2003). However, this method is only successful when extensive locality records from the native range are available (Byrne *et al.* 2003). The second method models the potential geographical distribution of the species by examining the native and / or exotic geographical distribution of the species in conjunction with its physiological attributes and limits (mechanistic modelling) (Sutherst *et al.* 2001).

Physiological studies, for mechanistic modelling purposes, rely on determining certain physiological parameters. In this chapter these parameters, as well as other responses of the insect to temperature extremes, were examined to form a basis for modelling (Chapter 4) and to gain insight into the insect's physiological limits. The parameters examined were the beetle's critical thermal limits (CT), lethal temperature limits (LT), lethal humidities and developmental rates. In this chapter, the first three factors, as outlined below, were considered.

2.1.2.1. Critical Thermal Maxima and Minima

When exposed to extreme temperatures, insects enter either chill coma or heat stupor (Renault *et al.* 2005). The thermal limits at which these responses occur are known as the critical

thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}) (Wedemeyer and McLeay 1981). Beyond these limits, insects can no longer react to any additional temperature change in the same direction and locomotory function is lost (Xu and Robertson 1994, Byrne *et al.* 2003). These limits are important ecologically as insects lose their ability to forage and escape from hostile environments and are thus more susceptible to mortality (Kay and Whitford 1978, Layne *et al*. 1985, Mitchell *et al*. 1993, Kelty and Lee 1999). Both CT_{max} and CT_{min} are not constant and can vary with acclimation as well as experimental cooling and heating rates (Kelty and Lee 1999).

2.1.2.2. Lethal Thermal Limits

The physiological temperature-tolerance limits, known as the lower lethal (LLT_{50}) and upper lethal (ULT_{50}) temperatures, define the temperature limits below or above which a species cannot survive after prolonged exposure (Mitchell *et al*. 1993). Thermal limits are examined by exposing insects to specified low or high temperatures for a designated time and then calculating percentage mortality. Insects are usually exposed to the temperature for two hours as this represents an overnight cold period (Byrne *et al.* 2003) or daily heat wave which could result in the death of an insect population. Insects are exposed to high or low temperatures until a temperature is reached at which 100 % mortality occurs.

Death, as a result of exposure to temperature extremes, may not occur during temperature exposure, or directly afterwards, but may instead result in thermal wounding leading to death at a later stage (Denlinger and Yocum 1999). For this reason, survival was recorded after a 24 hour recuperation period and again after five days. Insects with locomotory loss were recorded

as dead as they would be unable to survive to the next developmental stage (Denlinger and Lee 1998).

Thermophysiological studies frequently focus on determining the thermal limits for the adult stage only (Renault *et al*. 2005). However, immature stages are generally less mobile and therefore more likely to experience more extreme temperatures in the field (Coyne *et al*. 1983). The temperature at which death occurs has also been shown to be dependent on the stage of development (Denlinger and Yocum 1999) and it was thus deemed important in this study to explore the thermal limits of both the juvenile stages and adults. The production of viable offspring is another important criterion for survival (Denlinger and Lee 1998). An insect may survive exposure to extreme temperatures, but its ability to develop to the next developmental stage or produce viable young, after exposure, may be negatively affected (Sinclair and Chown 2005, Chown and Terblanche 2007).

2.1.2.3. Lethal Humidities

Although the effect of relative humidity (RH) on insect survival varies between species, it is widely accepted that insects require a sufficient quantity of body water for physiological system functioning (Pelletier 1995). One of the greatest challenges facing insects is the avoidance of dehydration (Danks 2000) through exposure to high temperatures and low RH (Edney 1979, Denlinger and Lee 1998). For most insect species, the lowest RH at which absorption of water into the body can occur (the critical equilibrium humidity) ranges between 70 – 95 % RH (Knulle 1984, Kahl and Alidoust 1997) and high mortality is commonly experienced at humidities lower than 60 % RH (e.g. Weissling and Giblin-Davis 1993).

The lower lethal humidity (LH_{50}) is calculated to determine at what level RH will become a limiting factor for a particular species (Byrne *et al.* 2002). Death may not occur at humidities below the $LH_{50,}$ but moisture stress will start to accumulate, leading to decreased fecundity and / or survival (Byrne *et al.* 2002). The extent of damage caused by moisture stress on population survival, establishment and growth is dependent on the number of times a year the RH drops below the LH_{50}. However, desiccation resistance can vary between populations and individuals of the same species (Phillips *et al.* 1996).

The two developmental stages of *Z. bicolorata* that were hypothesized to be the most vulnerable to dehydration are the egg and pupal stages. These stages are unable to feed or drink, a major means whereby water is obtained by most insects, and are unable to move to more climatically sheltered microclimates (Edney 1977, Danks 2000). Godfrey and Holtzer (1991) concluded that the moisture stress on eggs is additive, independent of whether the stress is constant or irregular. Therefore, months in which the RH drops below the egg LH_{50} on a number of occasions may have detrimental effects on egg hatch regardless of whether the low RH's are consecutive or not. This could result in eggs being extremely vulnerable to low RH.

The RH requirements for successful pupation were examined as it has been noted that, in South Africa, parthenium frequently occurs in very sandy, low moisture soil (McConnachie pers. comm.) and that pupae can be extremely sensitive to dry conditions (Hargrove 2004). Pupae will therefore be reliant on RH for survival. Pupae may not be as vulnerable to RH as the egg stage, however, as *Z. bicolorata* larvae burrow into the soil for pupation and form

pupation chambers within the soil (Dhileepan *et al.* 2000). Pupation chambers and cocoons limit transpiration and, in doing so, protect the pupae from desiccation (Tagawa 1996).

2.1.3. Aims

In this chapter, the physiological limits (temperature and humidity) of *Z. bicolorata* were investigated and examined in conjunction with climatic records to infer which localities in South Africa are suitable for population establishment and growth. For more accurate modelling, the physiological limits and developmental rates of *Z. bicolorata* were later used in conjunction with meteorological data to model the potential distribution of *Z. bicolorata* in South Africa using CLIMEX (CLIMEX programme ver. 2, CSIRO Entomology ©) (Chapter 4).

2.2. METHODS

2.2.1. Insect cultures

The beetles were collected in 2005 from Central Queensland, Australia and reared on parthenium plants in a quarantine facility at ARC-PPRI Weeds Laboratory, Cedara, South Africa. The culture was maintained at a temperature range of 20-30°C, humidity of 40-70% and a 12 hour photoperiod. All beetles used in experimentation were between the ages of six to eight weeks. To avoid pseudo-replication, individuals were excluded from further experimentation after being subjected to a trial.

2.2.2. Critical thermal limits

To determine the CT_{min} and CT_{max} of *Z. bicolorata,* adults were placed individually into sealed glass vials (8cm x 2 cm). Adults were acclimatized at quarantine temperature, as it was from this temperature range that insects were reared and would be released into the field. The CT_{min} vials were sealed with plastic lids and the CT_{max} vials were sealed with moist cotton wool plugs and parafilm, to prevent evaporative cooling by the insects. Insects were left to equilibrate for 15 minutes at room temperature (23^{o}C). Vials were then placed into a programmable water bath, set at 23^{o}C, (Haake Phoenix II, Haake C25P) which contained either absolute alcohol, after which the temperature was decreased (CT_{min}), or water, after which the temperature was increased (CT_{max}), at 0.25^{o}C min^{-1} until a temperature was reached where individuals lost the ability to right themselves. Traditionally in ecophysiological studies, a cooling and heating rate of 1^{o}C min^{-1} is used, however, this is considered too rapid and unrepresentative of temperatures experienced by an insect in the field (Sinclair *et al.* 2003). As the rate of cooling has been shown to affect survival (Sinclair *et al.* 2003) a rate of 0.25^{o}C min^{-1} was selected as it is ecologically more representative of environmental temperature change (Kelty and Lee 1999, 2001, Terblanche *et al.* 2006). Beetles were observed every minute until locomotory function became impaired. Vial temperatures were monitored with a thermocouple (YFE YF-160A Type-K; range of -50^{o}C to 1300^{o}C; accuracy of 0.3^{o}C), the tip of which was inserted into the abdomen of a freshly euthanazed adult, thus acting as an operative thermometer. The body temperature of this individual was taken to represent the body temperature of the experimental insects. Due to limited space in the water bath, only 10 individuals (five males and five females) were tested at a time. A fresh operative thermometer was used in each run.

Mean critical thermal limits and standard errors were determined for the adults (n = 60) and then separately for males and females. A Mann-Whitney U test was carried out to determine whether there was a significant difference between male and female critical thermal limits.

2.2.3. Lethal temperatures

Lethal temperature experiments were conducted on first and fourth instar and adult *Z. bicolorata*. Beetles were placed individually into glass vials (8 cm x 2 cm), sealed with plastic vial lids (LLT_{50}) or cotton wool plugs (ULT_{50}) and parafilm, and left to equilibrate at room temperature (23^{o}C) for 15 minutes. They were then placed into a programmable water bath (Haake Phoenix II, Haake C25P). The water bath contained water in the ULT_{50} trials and absolute alcohol in the LLT_{50} trials and was set at 23^{o}C at the start of each trial. Each temperature treatment group contained 20 individuals (1:1 adult sex ratios; larvae were not sexed). Temperature was increased (ULT_{50}) / decreased (LLT_{50}) at 0.25^{o}C min^{-1} until the exposure temperature was reached. Exposure temperatures were adjusted by one degree increments, between runs, until a temperature was reached at which 100% mortality was experienced. Temperature was then increased / decreased from this temperature at 0.5^{o}C increments, to improve the resolution of the trial. Exposure temperatures ranged from 10^{o}C to -15^{o}C in the LLT_{50} trials and 40^{o}C to 50^{o}C in the ULT_{50} trials. Individuals were exposed to the exposure temperatures for two hours before being removed and allowed to recuperate for 24 h in a Petri dish containing moist filter paper and a fresh parthenium leaf. After the 24 h recuperation period, the number of dead individuals was recorded for each temperature treatment. Insects that showed only limited movement of antennae and legs were recorded as dead (Klok and Chown 1997). As survival assessments made soon after exposure to

temperature extremes have been shown to overestimate survival, insects were left in the laboratory for a further 7 days in order to assess survival (Klok and Chown, 1997). Unimpaired reproductive performance of the surviving adult females was monitored (Coulson and Bale 1992) by exposing females to untested males in plastic tubs containing moist filter paper and fresh parthenium leaves, which were replaced daily. The number of eggs laid within two weeks after exposure, was recorded.

The development of exposed larvae to the subsequent life stage was monitored. First instar larvae were placed separately into rectangular plastic tubs (10 cm x 5 cm) containing moist filter paper and a fresh parthenium leaf. Old leaves were removed daily and fresh leaves added. Survival and development of the larvae to the next instar was monitored on a daily basis. Larvae that showed only limited locomotion were considered dead. Fourth instar larvae received the same treatment except that the tubs were filled with soil for the larvae to pupate in. The number of adults eclosing was recorded daily.

Lethal temperatures for all three developmental stages were determined using Probit Analysis (Finney 1962) of survival data collected after 24 hrs and one week after experimentation. A Wilcoxon matched pairs test was conducted to determine whether there were significant differences between survival data collected after 24 hrs and one week after experimentation. The numbers of eggs laid within two weeks, by adult females in the different temperature treatments, and the number of eggs that hatched were analyzed using a simple linear regression to test for correlation with temperature. A simple linear regression was used to test whether the number of fourth instar larvae that emerged as adults was correlated with test temperature.

2.2.4. Lethal Humidities

2.2.4.1. Effect on egg hatch

To determine lethal humidities the methods of Byrne *et al.* (2002) were followed. Eggs laid within a 12 hr period were collected from the laboratory culture of *Z. bicolorata*. These were then placed into Petri dishes (5 cm diameter) covered with a fine gauze, and spaced on a wire-mesh shelf in 1*l* plastic humidity chambers (20 cm x 10 cm). Thirty eggs were placed in a humidity chamber for each of the six experimental treatments (given below). Eggs were collected in the batches in which they were laid to avoid unnecessary dehydration (Clark and Faeth 1998). Humidity within the chambers was controlled by saturated salt solutions of silica gel, NaOH, $CaCl_2 \cdot 6H_2O$, NaCl, water, and KH_2PO_4 (Winston and Bates 1960). Relative humidity and temperature within these chambers was monitored using a thermohygrometer (Hobo Data-logging Units, Onset Computer Corporation, Bourne, USA). Humidity chambers were placed in a constant-temperature incubator set at 27.5^{o}C (optimal temperature for development derived in chapter 3) with a 12 hour photoperiod. Egg hatch was recorded every 12 hrs. Eggs were monitored for twice the mean egg developmental period at 27.5^{o}C (see chapter 3) to allow for slower development resulting from humidity stress (Cook and Spain 1982).

2.2.4.2. Effect on pupation and adult emergence

Fourth instar larvae were collected from the laboratory culture and exposed to the same humidity treatments as described above. In place of Petri dishes, larvae were placed in plastic dishes (10 cm diameter x 2 cm high), 15 in each, containing standard dry culturing soil,

comprised of 1:1 mix of uMgeni River sand (coarse) and mushroom compost. Thirty fourth instar larvae were used in each humidity treatment. The dishes were monitored every 24 hrs and time to adult emergence was recorded. The lower lethal humidity for egg hatch and eclosion was calculated using Probit Analysis (Finney 1960).

2.3. RESULTS

2.3.1. Critical thermal limits

The mean CT_{min} for adult *Z. bicolorata* is given in Table 1. There was a significant difference (Mann-Whitney U, $P = 0.004$) between the CT_{min} recorded for males and females. The loss of locomotory function was recorded between 45.5°C and 51.0°C and the mean CT_{max} for adult *Z. bicolorata* was 47.8°C ± 0.2 (mean ± SE, n = 40) (Table 1). Males had a mean CT_{max} of 47.6°C ± 0.3 (mean ± SE, n = 20) and females a mean of 48.1°C ± 0.3 (mean ± SE, n = 20), but these differences were not significant (Mann-Whitney U; $P = 0.27$) (Table 1).

Table 1: Mean Critical Thermal Minimum (CT_{min}) and Critical Thermal Maximum (CT_{max}) (± standard error) of *Zygogramma bicolorata* adults. BT = body temperature of the operative thermometer.

	Both Sexes (BT°C)	n	Male (BT°C)	n	Female (BT°C)	n
CTmin	11.8 (± 0.3)	60	11.0 (± 0.3)	30	12.5 (± 0.3)	30
CTmax	47.8 (± 0.2)	60	47.6 (± 0.3)	30	48.1 (± 0.3)	30

2.3.2. Lethal limits

The LLT_{50} of the adult beetles was -6.7°C after 24 hours, but increased to -5.9°C after one week (Table 2). There were significant differences (Wilcoxon MPT; $P = 0.04$) in survival at the different test temperatures after 24 hrs and one week from experimentation. The number of eggs laid per female, within two weeks, was not significantly correlated with test temperature (linear regression; $r^2 = 0.06$, $P = 0.21$). The proportion of eggs which hatched was positively correlated, albeit weakly, to treatment temperature, but this correlation was not statistically significant (linear regression; $r^2 = 0.3$, $P = 0.05$).

Table 2: Lower and Upper Lethal Temperature of adults and first and fourth instar larvae of *Zygogramma bicolorata* calculated from survival data collected after 24 hours and 1 week after experimentation.

Stage	Lethal Thermal Limit (°C)	24 hrs	1 week	n
Adult	LLT_{50}	-6.7	-5.9	260
	ULT_{50}	45.9	45.9	180
1st instar	LLT_{50}	-7.3	-5.6	140
	ULT_{50}	45.3	44.4	160
4th instar	LLT_{50}	-11.9	-7.7	140
	ULT_{50}	44.6	44.3	160

First instar larvae had a LLT_{50} of –7.3°C after 24 hours recovery, but this increased to –5.6°C one week after experimentation (Table 2). There was a significant difference (Wilcoxon MPT; $P = 0.05$) between survival at the different test temperatures after 24 hrs and one week after experimentation. Of the individuals that were alive 24 hrs after testing, 68.7 % moulted to the next developmental stage. Development to the next stage was significantly positively correlated to treatment temperature (linear regression; $r^2 = 0.82$, $P = 0.01$). The LLT_{50} of fourth instar larvae was -11.9°C after 24 hours recovery, but increased to -7.7°C a week after the trial was run. There was a significant difference (Wilcoxon MPT, $P = 0.04$) between these survival data at the different test temperatures. The proportion of surviving fourth instars that emerged as adults was positively correlated to an increase in treatment temperature but this correlation was not statistically significant (linear regression; $r^2 = 0.6$, $P = 0.07$).

The ULT_{50} for adult beetles was 45.9°C (Table 2) after 24 hours recovery, and did not change after one week of recovery. There was no significant difference (Wilcoxon MPT, $P = 0.1$) when survival data after 24 hrs and one week were compared. The number of eggs laid, within two weeks by females from the different temperature treatments, showed a slight, but not statistically significant, negative correlation to the treatment temperature (linera regression; $r^2 = 0.3$, $P = 0.08$). The number of eggs that hatched was negatively and significantly correlated to an increase in treatment temperature (linear regression; $r^2 = 0.7$, $P = 0.003$).

The ULT_{50} calculated for first instar larvae was 45.3°C after 24 hours recovery and 44.4°C one week after experimentation (Table 2). There was no significant difference (Wilcoxon MPT, $P = 0.07$) in survival recorded after 24 hrs versus one week after experimentation. Of the

individuals that were alive 24 hrs after testing, 81.6 % moulted to the next developmental stage. Development to the next stage (measured as survival to the next moult) was significantly negatively correlated to treatment temperature (linear regression; $r^2 = 0.6$, $P = 0.03$).

Fourth instar larvae had similar ULT_{50}'s, 44.6°C and 44.3°C, when survival was monitored 24 hours and one week respectively after experimentation (Table 2). There was no significant difference (Wilcoxon MPT, $P = 0.58$) in survival between these recording periods. Forty percent of the individuals emerged as adults in the 41°C treatment and 55 % of the 4th instars developed into adults in the 42°C treatments. Survivors from the 44°C, 45°C, 46°C and 48°C treatments did not develop further.

2.3.3. Lethal Humidities

Saturated salt solutions were used to control humidity within the chambers. The mean (± SD) humidities obtained were 1.79 % ± 1.33 (silica gel), 14.12 % ± 1.82 (NaOH), 39.36 % ± 1.44 ($CaCl_2 \cdot 6H_2O$), 74.69 % ± 0.8 (NaCl), 100.36 % ± 1.45 (water), and 103.7 % ± 0.01 (KH_2PO_4). The LH_{50} for egg hatch was calculated by Probit Analysis as 59.92 % RH (n = 30 egg cases, 95 % confidence limit = 52.28 to 67.29) (Figure 3) and the LH_{50} for adult eclosion was 23.85 % RH (n = 30 fourth instar, 95 % confidence limit = 19.73 to 27.89) (Figure 4).

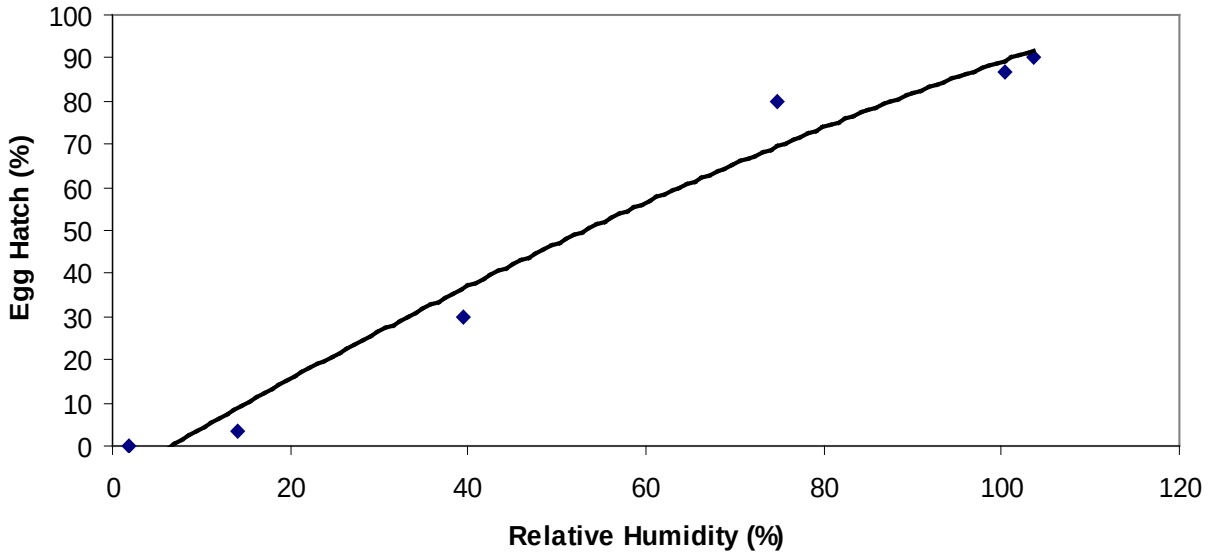


Figure 3: Linear regression of percentage egg hatch against relative humidity of *Zygogramma bicolorata* at six constant humidities.
Equation of line: $y = -0.0026x^2 + 1.2303x - 8.0823$; $r^2 = 0.98$ ($P < 0.01$).

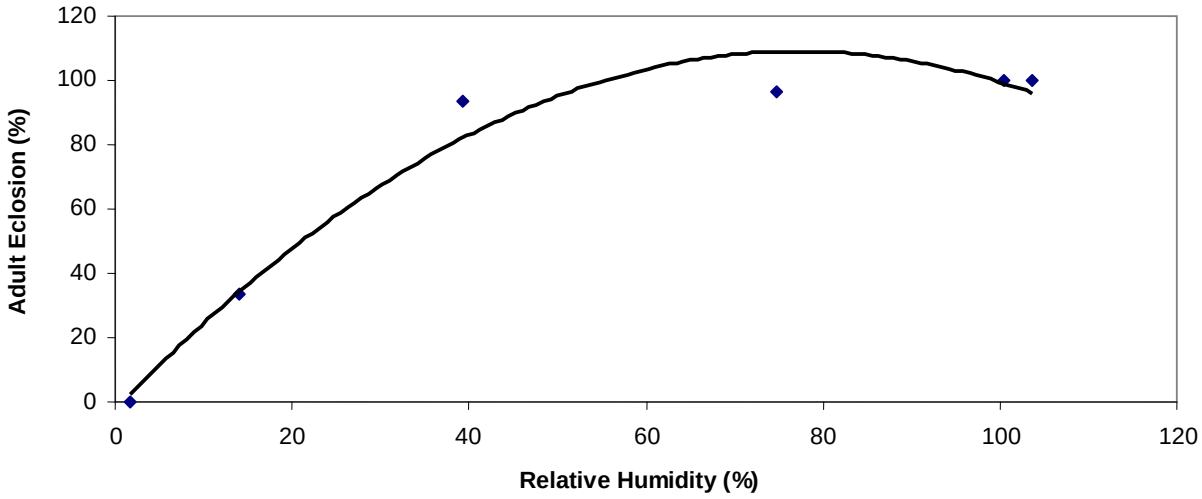


Figure 4: Linear regression of percentage adult eclosion against relative humidity of *Zygogramma bicolorata* at six constant humidities.
Equation of line: $y = -0.0187x^2 + 2.8884x - 2.56$; $r^2 = 0.97$ ($P < 0.01$).

2.4. DISCUSSION

In comparison with other summer-active Coleoptera species, the temperature at which *Z. bicolorata* enters chill coma is relatively high (CT_{min} = 11.8°C ± 0.3). *Stenopelmus rufinasus* (Curculionidae), a weevil released in South Africa for the biological control of *Azolla filiculiodes*, has a CT_{min} of 1.3°C (McConnachie 2004). Also, *Gratiana spadicea* (Chrysomelidae), the agent for *Solanum sisymbriifolium* in South Africa, has a CT_{min} of 4.9°C (Byrne *et al.* 2002) while darkling beetles (Tenebrionidae) collected from the Namib Desert have a CT_{min} of 10°C (Roberts *et al.* 1991). The reason for the higher CT_{min} of *Z. bicolorata* is unclear and cannot be fully explained by past evolutionary adaptations to the local environment, as *G. spadicea* originated from subtropical South America (Byrne *et al.* 2002), *S. rufinasus* from tropical Florida (McConnachie 2004) and *Z. bicolorata* from warm Mexico (Strathie *et al.* 2005).

One explanation for the higher CT_{min} is that the confinement of the *Z. bicolorata* population examined, to a temperature-regulated quarantine facility for a number of generations, has led to genetic changes resulting from both inbreeding depression and selective breeding for traits imposed by quarantine conditions (Center *et al.* 1997, Grodowitz *et al.* 1997, Harshman and Hoffmann 2000, Matos *et al.* 2000, Sgro and Partridge 2000, Terblanche *et al.* 2006). This selection can lead to a decrease in resistance to thermal stress, further promoted by the absence of dispersal between metapopulations (Chown and Terblanche 2007). The *Z. bicolorata* population used in this experiment has gone through two periods of quarantine confinement, once in Australia and once in South Africa, whereas field-collected *G. spadicea*, *S. rufinasus* and tenebrionids were used to determine CT_{min} in the other studies (Roberts *et al.* 1991, Byrne

et al. 2002, McConnachie 2004). Negative effects resulting from confinement in Australian quarantine, in all probability, are now negligible, as the *Z. bicolorata* population established in the field for some 20 years (McFadyen and McClay 1981) before being exported to South Africa. However, the population in South Africa has been confined to quarantine for approximately two and a half years with no field releases. This population could have adapted to quarantine conditions and may be suffering from inbreeding depression resulting from inevitable drops in population numbers. The adaptation of insect populations to laboratory conditions has been noted in a number of studies (e.g. Huey *et al.* 1991, Gibbs 1999, Kingsolver *et al.* 2001, Zatsepina *et al.* 2001) and is not easily rectified in pre-release biological control studies, as truly representative genetic stocks are difficult to obtain. As phenotypic variation in thermo-tolerance is heritable (Wang and Kang 2005) and small quarantine-adapted populations tend to have low genetic variability (Hoffmann and Blows 1994, Gaston 2003, Blows and Hoffmann 2005), *Z. bicolorata's* ability to establish in the range of environments that it will be released in, could have been diminished by extended quarantine confinement.

A second explanation, for the high CT_{min} of *Z. bicolorata,* could be phenotypic plasticity resulting from acclimation at quarantine temperatures. In an experiment performed by Terblanche *et al.* (2006), quarantine acclimatized populations of *Glossina pallidipes* (Diptera: Glossinidae) had a mean CT_{min} that was 3.3^{o}C higher than field fresh populations. Variations in the CT_{min} resulting from phenotypic plasticity can be rapid, with individual *G. pallidipes,* that were acclimatized in the laboratory for five days having substantially higher CT_{min}'s compared to field-reared individuals (Terblanche *et al.* 2006). Terblanche *et al.* (2006) hypothesized that the variation in CT_{min} was most likely caused by a mixture of phenotypic

plasticity and genetic evolution. Therefore, to increase establishment success, releases of *Z. bicolorata* should be carried out using new genetic stocks that have recently been collected in the field (Center *et al.* 1997).

Even though *Parthenium hysterophorus* currently occurs predominately along the warmer eastern coast of South Africa (Strathie *et al.* 2005, Strathie and McConnachie 2006) the majority of potential release sites may experience temperatures below *Z. bicolorata*'s CT_{min} (Figure 2b). Insects will accumulate cold stress when temperatures drop below *Z. bicolorata's* CT_{min} (Byrne *et al.* 2002). Populations of parthenium that occur further inland, e.g. around Brits which experiences daily mean winter (June/July) temperatures between 2 – 4^{o}C (Schulze 1997), will be less suitable for *Z. bicolorata* establishment. Although not an obligate diapausing insect (Chakravarthy and Bhat 1997), *Z. bicolorata* adults do overwinter in the soil, in Australia and India, during the climatically unfavourable months of the year (Singh 1997, Dhileepan *et al.* 2000) and are thus expected to diapause in the colder regions of South Africa to escape low winter temperatures. Establishment of *Z. bicolorata* populations may be negatively affected by the substantially higher mean CT_{min} of the females (12.5^{o}C ± 0.3) compared to that of the males (11^{o}C ± 0.3). Females will recover from chill coma but the chilling injury that results from repeated short exposure to sub-lethal temperatures could have lasting negative effects on reproduction and fitness (Denlinger and Lee 1998, Rinehart *et al.* 2000, Shreve *et al.* 2004, Sinclair and Chown 2005, Chown and Terblanche 2007). Temperature effects on female fitness were found to be minimal in this experiment but these results may change if short exposure to sub-lethal temperatures were repetitive. This could lead to a depressed growth rate of the population (Sinclair and Chown 2005) and, therefore, variability in the agent's effectiveness between sites.

The mean CT_{max} measurements of subtropical insect species commonly fall in the range of 47 – 50°C, irrespective of family (Roberts *et al.* 1991, Mitchell *et al.* 1993, Klok and Chown 1997). *Zygogramma bicolorata* follows this trend with a mean CT_{max} of 47.8°C (± 0.2). The beetle is unlikely to be exposed to temperatures this extreme, once released (Figure 2a). Parthenium seed germination is also negatively affected at temperatures greater than 35°C (Dhawan and Dhawan 1994), and the weed is thus not likely to spread to areas where temperature frequently exceeds *Z. bicolorata*'s CT_{max}.

Adult *Z. bicolorata* had the highest LLT_{50} (-6.7°C) when compared with first (-7.3°C) and fourth (-11.9°C) instar larvae. *Zygogramma bicolorata's* adult LLT_{50} is high in comparison to *S. rufinasus* (-12.1°C) (McConnachie 2004) and similar to that of the similar-sized chrysomelid *G. spadicea* (-7.1°C) (Byrne *et al.* 2002), and the trend of higher thermal tolerance shown by the late instar larvae (-11.9°C) was similar to that of *G. spadicea* (-10°C) (Byrne *et al.* 2002). However, the adults' ability to move to climatically buffered microenvironments is likely to increase its thermal tolerance (May 1979, Reynolds 1979, Dreisig 1980, Denlinger and Lee 1998). During experimentation, insects were restricted from carrying out behavioural thermal regulation. However, once released, exposure of adults to temperatures close to those of their lower lethal limits could be avoided by both behavioural and physiological responses, thereby increasing the beetle's chances of establishing in marginal environments. *Zygogramma bicolorata*'s establishment will be aided further by mean daily minimum temperatures of the areas where parthenium occurs being substantially higher than the lower lethal temperature limits of *Z. bicolorata* (Figure 2b). These limits are, therefore, only likely to become a problem if the spread of parthenium continues inland, which

is improbable as parthenium seed germination is also negatively affected at temperatures below 10^{o}C (McFadyen 1992, Dhawan and Dhawan 1994). However, any unseasonable low temperature weather events (Chown and Terblanche 2007) in areas where *Z. bicolorata* establishes, can influence survival and areas that experience variable climates will have fluctuating mortality and fecundity rates (Roy *et al.* 2001, Hargrove 2004).

In the lower lethal thermal limit experiments, survival data collected 24hrs and one week after experimentation were significantly different for both sexes of adults (Wilcoxon MPT; P = 0.04) and first instar larvae (Wilcoxon MPT; P = 0.05), with the calculated LLT_{50} being higher for all three life stages when survival data were collected one week after experimentation. Usually, in lethal limit experiments, survival is only monitored once after 24hrs (e.g. Byrne *et al.* 2002). As thermal wounding, resulting from exposure to sub-lethal temperatures (Chown and Terblanche 2007), was most likely the cause of lower survival levels one week after exposure, these survival data should be considered instead of the 24 hr data. Insects that die within a week of exposure to sub-lethal low temperatures will have suffered from chilling injury which negatively affects reproduction (Chown and Terblanche 2007) and therefore population growth rates.

The range of ULT_{50}'s calculated for adults and first and fourth instar larvae of *Z. bicolorata* was narrow (44.6 – 45.9^{o}C) with these limits being higher than two other Coleoptera species (Staphylinidae) from the subtropics, (namely *Philonthus sanamus*: 42.8^{o}C and *Philonthus labdanus*: 40.5^{o}C) (Byrne *et al.* 2003). However, as in the case of the LLT_{50}, no areas in South Africa frequently experience such high temperature extremes and the distribution of *Z. bicolorata* is thus unlikely to be limited by its ULT_{50} (Figure 2a).

The LH_{50} for *Z. bicolorata* egg hatch (59.92 % RH) was similar to that calculated for *Gratiana spadicea* (56.6 % RH) (Byrne *et al.* 2002). However, pupation of *G. spadicea* was not significantly affected by RH (Byrne *et al.* 2002), whereas *Z. bicolorata* eclosion was negatively affected by low RH (LH_{50} = 23.85 % RH). *Zygogramma bicolorata* establishment and population growth is therefore likely to be affected by high egg mortality in areas were RH drops below the LH_{50} on a number of occasions in a month (Figure 5). However, this mortality is likely to be avoided due to the microclimate experienced by the eggs on the leaf being higher than the ambient RH. Byrne *et al.* (2002) argued that the microclimate RH experienced by *G. spadicea* on the leaves of *S. sisymbriifolium* is unlikely to be that different from the ambient RH, as plants close their stomata during periods of low humidity. However, *Z. bicolorata* lays its eggs predominately on the under surface of *P. hysterophorous* leaves (pers. obs.) and plant stomatal aperture is less affected by low humidities on the lower leaf surface compared to the upper surface (Yasutake *et al.* 2001). This is likely to have the effect of increasing the microclimate RH beneath the under surface of the leaf. *Zygogramma bicolorata* is unlikely to be negatively affected by its low LH_{50} for pupation as RH below 30 % is extremely rare in South Africa (Figure 5) (Schulze 1997).

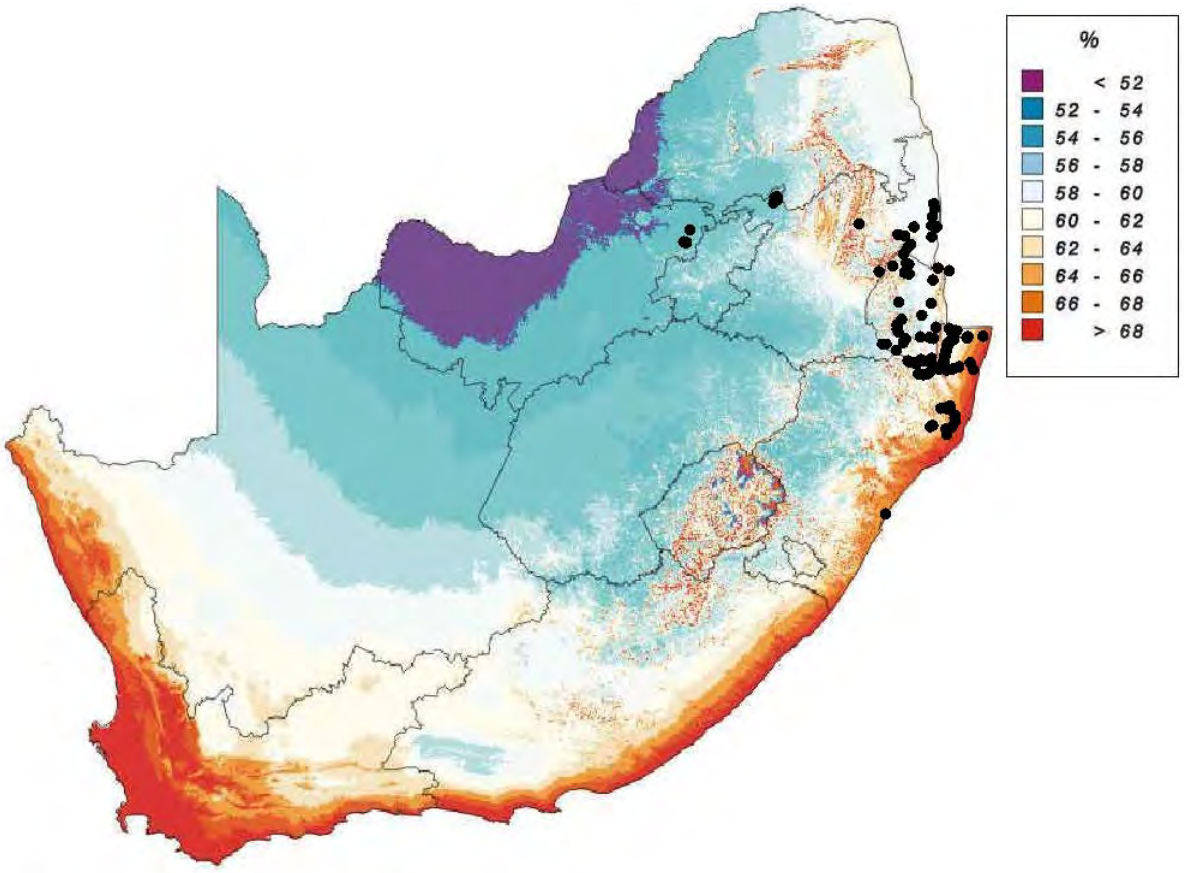


Figure 5: Distribution of *Parthenium hysterophorus* in South Africa (Henderson 2007) overlayed on mean daily relative humidity for July (Schulze 1997).

The value of the different thermal parameters in predicting the climatic distribution of insects has been debated (e.g. Byrne *et al.* 2003). The use of upper thermal limits has been criticized since these are regularly above the average environmental temperatures (Byrne *et al.* 2003), as is the case for *Z. bicolorata*, and therefore do not identify localities where high temperatures will limit establishment. In South Africa the average maximum summer temperatures do not reach either the ULT_{50} or the CT_{max} of *Z. bicolorata,* and therefore, these limits are limited as predictive tools. In contrast, the lower thermal limits are often considered more useful when predicting insect distribution and give a good indication of areas where survival will be limited

in winter (Byrne *et al.* 2003). Although the winter temperature frequently drops below the beetle's LLT_{50} in many regions of South Africa, parthenium occurs predominantly in the warmers areas of South Africa, where excessively low temperatures are infrequent. Therefore it is predicted that low temperature extremes are unlikely to limit survival of *Z. bicolorata*. One criticism of the lower lethal thermal limit is that cold stress is accumulative and over 50% of the population may thus die as a result of thermal wounding before the LLT_{50} is approached (Byrne *et al.* 2002). In this study, the CT_{min} is considered to be the best indicator of *Z. bicolorata* population establishment and success in South Africa. However, thermal tolerance within an insect species is not constant and can vary considerably within a population and also throughout the geographical range of the species (Denlinger and Lee 1998). Terblanche *et al.* (2006) showed that in the case of the Tsetse fly (*Glossina pallidipes*, Diptera: Glossinidae), the CT_{min} was highly variable among populations, with acclimation to laboratory conditions for as little as 10 days altering the CT_{min} between 4°C and 10°C. By collecting fresh genetic stocks from the field, selecting for increased cold tolerance in the laboratory (Tucic 1979) or selecting individuals from colder regions (Denlinger and Lee 1998), the CT_{min} of *Z. bicolorata* could be decreased. This would facilitate the successful establishment of the agent in the field.

From this discussion, it is clear that other predictive tools are required to define the climatic limitations of a species' distribution. One way to achieve this is by determining the developmental rate of a species at a range of constant temperatures and, from this, calculate the amount of thermal energy (degree-days) that the species requires to complete a generation. This information can then be used to map areas which have suitable climates to support populations of the species. This will be discussed further in Chapter 3.

CHAPTER 3

DEVELOPMENTAL RATE & DEGREE-DAY MODELLING OF *ZYGOGRAMMA BICOLORATA*

3.1. INTRODUCTION

3.1.1. Developmental limitations of ectotherms

External environmental factors are a primary determinant of an ectotherm's body temperature and metabolic rate (Cloudsley-Thompson 1970, Neven 2000) and temperature and humidity can greatly affect population establishment and survival as well as the reproductive ability and development of individuals (Sehnal 1991, Stewart *et al.* 1996, Denlinger and Lee 1998, Bale and Walters 2001, Byrne *et al*. 2002). In Chapter 2 the role of thermal limits in limiting *Z. bicolorata*'s survival and distribution were explored. However, these limits are not the only way in which a species' survival can be limited at a specific location. Some areas will not receive adequate accumulated heat for insect development, thereby decreasing survival. If an area does not receive adequate accumulated heat for the species to complete one or more generations per year, it is predicted that the area will not support the species (McClay 1996). When determining the potential geographic range of *Z. bicolorata* in South Africa, it is therefore important to determine which areas will have sufficient accumulated heat for population survival and growth.

3.1.2. Developmental rate

The rate of development of ectothermic organisms is dependent on ambient temperature (Prect *et al.* 1973, Cossins and Bowler 1987, Nylin *et al.* 1989, Nylin 1992, Blanckenhorn 1997). As a general rule, cooler temperatures slow development, increasing the period spent at each immature life stage, and warmer temperatures accelerate development, decreasing the period spent at each immature stage (Briere *et al*. 1999, Russo *et al.* 2004, Logan *et al.* 2006, Manush *et al.* 2006). This temperature-development relationship is commonly described as curvilinear (McClay 1996), as although the rate of development increases with an increase in temperature (roughly linearly) (Briere *et al*. 1999), temperature thresholds are reached at low and high temperatures, beyond which development ceases (Del Fosse 1977, Briere *et al.* 1999, Daane *et al*. 2004). The predicted curvilinear relationship is extremely difficult to express mathematically (Briere *et al.* 1999), and the linear approximation method is therefore frequently used to describe the temperature-development relationship and determine the lower thermal threshold (t) for a species. Studies in which this method has recently been used include McConnachie (2004), Bernardo *et al.* (2006) and Kheradmand *et al.* (2007).

Using the linear approximation method, t is determined by extending the linear section of the curve until it intercepts with the x-axis. Although it is accepted that this method overestimates t, minimal development occurs in this low temperature region and the overestimation is assumed to not significantly affect inferences made from the parameter (McConnachie 2004).

3.1.3. Degree-day model

The degree-day model uses developmental rate and temperature, to determine the number of generations an insect can complete at a given location. From developmental threshold experiments, the amount of accumulated heat that an insect requires to develop (i.e. thermal constant K) is determined. This accumulated heat is measured in degree-days (^{o}D) and is referred to as 'physiological time' (Zalom *et al.* 1983). A degree-day is defined as the number of degrees above the lower thermal threshold, over a 24 hour period, which an insect requires for development (Zalom *et al.* 1983).

Using historical weather data, the number of available degree-days above a specified threshold (t), can be determined for a defined location (McClay 1996). The number of degree-days that *Z. bicolorata* requires to complete development will be compared throughout South Africa to predict which areas will have sufficient physiological time for population establishment and growth. If there is only enough physiological time for one or less generations to be completed at a site in a year, then that particular locality is unsuitable for establishment (McClay 1996, McClay and Hughes 2007).

3.1.4. Aims

This chapter compares the development and survival of *Z. bicolorata* at eight constant temperature regimes. The lower thermal threshold and the required number of degree-days for development of *Z. bicolorata* were calculated and CLIMEX was used to model the number of generations that will be completed at different localities throughout South Africa.

3.2. METHODS

3.2.1. Insect Culture

Beetles were cultured as reported in section 2.2.1.

3.2.2. Developmental rate

Pairs of male and female *Z. bicolorata* were placed on fresh host plants for 12h at quarantine temperature (20 – 30^{o}C). After 12h, eggs were removed and placed singly into glass Petri dishes (9 cm in diameter x 1 cm high) to ensure that they were 12hrs old or younger. Each Petri dish contained moist filter paper and a fresh parthenium leaf and was placed into a constant temperature growth chamber (Labcon, LTGC 40). Temperature data for each treatment were recorded using a data logger (Onset, Hobo U12), with a J-type thermocouple probe (iron-constantan) placed in a Petri dish. Treatment temperatures (mean ± SD) included 15.1^{o}C ± 0.58, 17.1^{o}C ± 0.31, 20^{o}C ± 0.46, 25.4^{o}C ± 0.39, 27.2^{o}C ± 0.40, 29.1^{o}C ± 0.95, 31.8^{o}C ± 0.26 and 34.1^{o}C ± 0.82. Eggs were continuously added to the chambers to ensure that as many adults developed from each temperature treatment as was possible (Table 1). Incubators were set on a 12h photoperiod throughout. Dishes were inspected every 24h for evidence of hatching, moulting for each instar, pupation and adult eclosion and new leaves were added as required. Egg hatch success was determined from the number of unhatched eggs. The larval instars were identified using head capsule dimensions and evidence of moulting. Head capsules were measured using an ocular micrometer, calibrated with an ocular graticule. Developmental time was recorded as the mean number of days from egg to

adult eclosion for each temperature treatment. Developmental rate was calculated as the inverse of developmental time.

Table 1: Number of *Zygogramma bicolorata* eggs placed in each temperature treatment chamber.

Temperature (^{o}C)	15.1	17.1	20	25.4	27.2	29.1	31.8	34.1
Eggs	131	133	121	133	63	131	71	96

Linear approximation was used to plot developmental rate against temperature, where y = a + bx. The lower thermal threshold (t) was calculated by –a / b and degree-days of developmental time (K) was computed as 1 / b (where a is the x-intercept and b is the slope of the graph) (Kheradmand *et al.* 2007). Population viability at the different temperature regimes was calculated from the number of adult beetles obtained from the initial number of eggs exposed (Petavy *et al.* 2001). Data, for larval survival and population viability, were analyzed using One-way Analysis of Variance (ANOVA) and Tukey's multiple range test was used to compare differences between means of parameters at the different temperatures.

3.2.3. Degree-day model

From the thermal constant (K), the lower thermal threshold (t) and maximum and minimum CLIMEX temperature records, the accumulated ^{o}D for each year was calculated for 134 weather station locations in South Africa using the equation:

$$K = \sum \left\{ \frac{(T_{max} + T_{min})}{2} - t \right\} \quad (\text{if } T_{min} < t,\ t \text{ was used})$$

From the mean annual ^{o}D total, the number of *Z. bicolorata* generations that each location would be able to support per year was determined and mapped using CLIMEX.

3.3. RESULTS

3.3.1. Developmental rate

Zygogramma bicolorata did not complete development at constant rearing temperatures of 15.1^{o}C, 31.8^{o}C and 34.1^{o}C. Complete development occurred at constant rearing temperatures of 17.1^{o}C, 20^{o}C, 25.4^{o}C, 27.2^{o}C and 29.1^{o}C (Table 2). Developmental time increased as temperatures decreased, with eggs placed in the 17.1^{o}C incubator taking 59.1 ± 2.6 days (mean ± SD) to develop to adulthood and eggs in the 29.1^{o}C incubator taking 22.4 ± 1.2 days (mean ± SD). The standard deviation of the means of developmental time was highest for the low temperature treatments and decreased with increasing temperature (range = ± 3.0 to ± 1.2 days) (Table 2).

Table 2: Developmental time (days) from egg to adult for *Zygogramma bicolorata* at five constant temperatures

	Mean ± SD duration (days), (N), and % total development time at indicated temperature				
Stage	**17.1°C**	**20°C**	**25.4°C**	**27.2°C**	**29.1°C**
Egg	10.9 ± 0.7	7.4 ± 1.3	5.2 ± 0.9	4.9 ± 0.6	4.3 ± 0.6
	(80) 18.5	(82) 16.2	(85) 18.6	(53) 22.3	(101) 19.11
L1	7.1 ± 1.8	8.7 ± 2.0	4.1 ± 1.1	3.2 ± 0.8	3.1 ± 1.2
	(33) 12	(24) 18.3	(37) 14.7	(33) 14.4	(61) 13.89
L2	6.6 ± 1.4	4.1 ± 1.5	2.5 ± 0.8	1.7 ± 0.8	2.0 ± 0.6
	(29) 11.2	(22) 8.9	(30) 8.9	(27) 7.7	(49) 8.92
L3	4.3 ± 1.1	3.8 ± 0.8	2.3 ± 0.8	1.5 ± 0.7	1.9 ± 0.8
	(24) 7.3	(21) 8.1	(28) 8.2	(26) 6.8	(40) 8.47
L4	4.3 ± 1.2	4.2 ± 1.3	3.4 ± 1.5	2.1 ± 0.7	2.5 ± 1.6
	(19) 7.3	(20) 9.2	(22) 12.4	(18) 9.5	(20) 11.28
Pupa	25.8 ± 1.7	18.3 ± 1.3	10.3 ± 1.7	8.7 ± 0.9	8.6 ± 0.8
	(19) 43.7	(20) 39.3	(22) 37.2	(18) 39.4	(20) 38.34
Total	59.1 ± 2.6	46.5 ± 3.0	27.8 ± 2.5	22.2 ± 1.2	22.4 ± 1.2
	(19) 100	(20) 100	(22) 100	(18) 100	(20) 100

A linear regression analysis was applied to the rearing temperatures that produced adults (Figure 1). The lower thermal threshold (t) for all life stages combined was calculated as 11°C (linear regression; $r^2 = 0.99$; $P < 0.01$) and physiological time (K) at 384.6°D (Table 3). The linear regression equation fitted the data very accurately, as indicated by the high r^2 (0.99). A linear regression equation was applied to all six of the life stages separately, and the lower thermal threshold (t) and physiological time (K) calculated (Table 3). The life stage with the highest lower thermal threshold was the second larval instar (t = 12.5°C; $r^2 = 0.95$; $P < 0.01$) but it was also this stage that required the least amount of degree-days to complete development (29.6°D). The pupal stage had the highest degree-day requirements to complete development (142.9°D) and the fourth instar had the lowest developmental threshold (t = 5.1°C; $r^2 = 0.90$; $P < 0.01$).

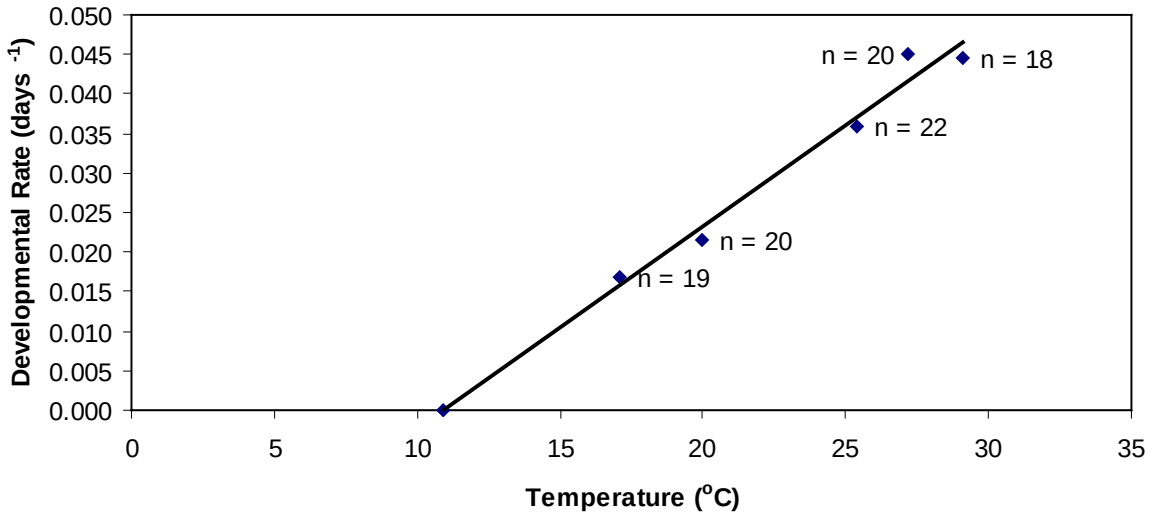


Figure 1: Linear regression of developmental rate against rearing temperature of *Zygogramma bicolorata* from egg to adult emergence at five constant temperatures.
Equation of line: y = 0.0026x – 0.0277, $r^2 = 0.99$ ($P < 0.01$).

Table 3: Lower thermal thresholds, degree-days and linear regression of developmental rates (y) on temperature (x) for the immature stages of *Zygogramma bicolorata.*

Stage	n	Lower thermal threshold (t)^{o}C	Degree-Days (K)^{o}D	Regression	r^2	p
Egg	401	8.6	89.3	y = 0.0113x - 0.0966	0.997	$P < 0.01$
L1	188	11.1	55.6	y = 0.0180x - 0.1996	0.95	$P < 0.01$
L2	157	12.5	29.6	y = 0.0338x - 0.4240	0.95	$P < 0.01$
L3	139	10.7	30.8	y = 0.0325x - 0.3476	0.91	$P < 0.01$
L4	99	5.1	57.1	y = 0.0175x - 0.0891	0.90	$P < 0.01$
Pupa	99	11.7	142.9	y = 0.0070x - 0.0818	0.99	$P < 0.01$
Total	99	11	384.6	y = 0.0026x - 0.0277	0.99	$P < 0.01$

3.3.2. Life stage development

At all temperatures, the longest life stage was the pupal stage (range: 8.6 - 25.8 days) and the shortest was the third instar (range: 1.5 - 4.3 days) (Table 2). Developmental rate of all life stages showed a significant positive correlation with temperature [r^2 range = 0.997 ($P < 0.01$) to 0.90 ($P = < 0.01$)] (Table 2).

3.3.3. Egg hatch

Egg hatch success was lowest at 15.1^{o}C (8.4 %) and highest at 31.8^{o}C (86 %). The overall trend was an increase in egg hatch with increasing temperature; however, there was a decrease in egg hatch at the highest test temperature treatment (34.1^{o}C, egg hatch = 54.2 %) (Figure 2). A polynomial regression gave the best fit for percentage egg hatch regressed against temperature ($r^2 = 0.73$, $P = 0.04$) (Figure 2).

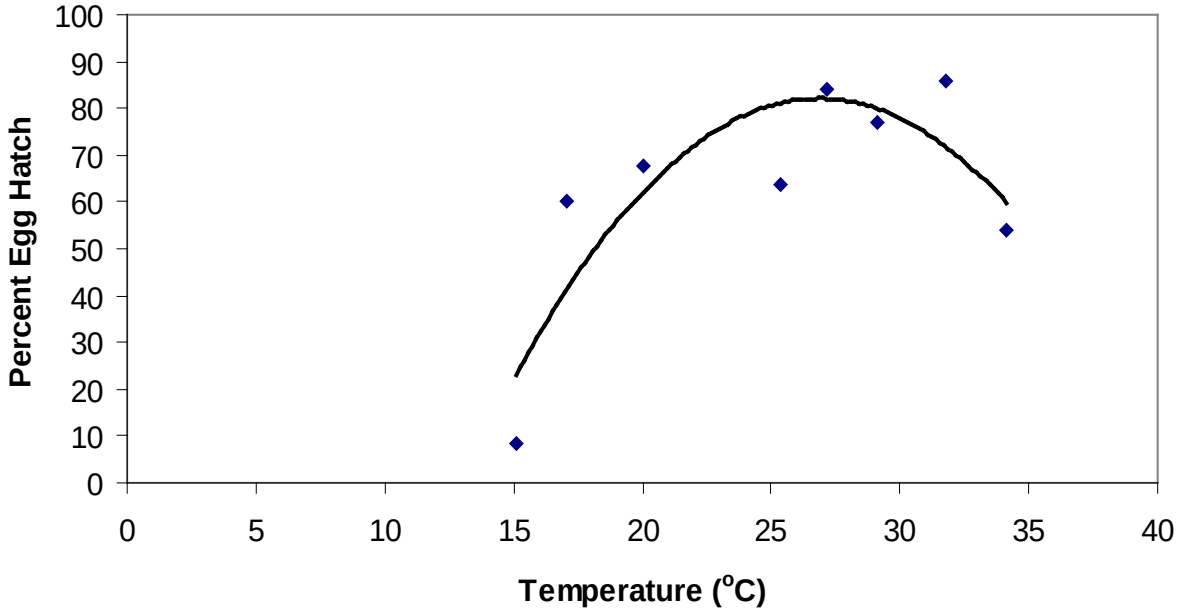


Figure 2: Polynomial regression of percentage egg hatch against rearing temperature of *Zygogramma bicolorata* at eight constant temperatures.
Equation of line: $y = -0.4252x^2 + 22.851x - 224.94$, $r^2 = 0.73$ ($P = 0.04$).

3.3.4. Larval survival

Larval survival was lowest at the two highest temperature treatments (31.8°C and 34.1°C) with only 11.48 % of 1st instars in the 31.8°C temperature treatment surviving to the second instar and 0 % of 1st instars in the 34.1°C temperature treatment developing to the second instar. The stage with the lowest survival, when lethal temperatures (15.1°C, 31.8°C and 34.1°C) were excluded, was the 1st instar. Survival at this stage ranged between 29.27 % (20°C) to 62.26 % (27.2°C), which was significantly lower than all other stages ($P < 0.001$; Tukey's post hoc, survival arcsine transformed).

The response to temperature, in terms of survival, varied significantly between the stages (P = 0.006; ANOVA, survivorship arcsine transformed). Survival increased or stayed constant with temperature for most life stages, except for the second and fourth instar stages. Survival decreased, but not significantly, in the second instar stage and decreased significantly in the fourth instar stage (P = 0.07 and $P < 0.001$ respectively).

3.3.5. Population viability

Development to the adult stage was highest at 27.2°C with 28.57 % of the eggs placed into the temperature chamber developing to adulthood. Besides the three lethal temperatures (15.1°C, 31.8°C and 34.1 °C), population viability was lowest at 17.1°C, with only 14.29 % of the eggs developing to adulthood (Figure 3). Population viability was constant at all non-lethal temperatures (range = 14.3 % to 16.5 %), except at 27.2°C where it was significantly greater (28.6 %) (ANOVA; $P < 0.01$) (Figure 3).

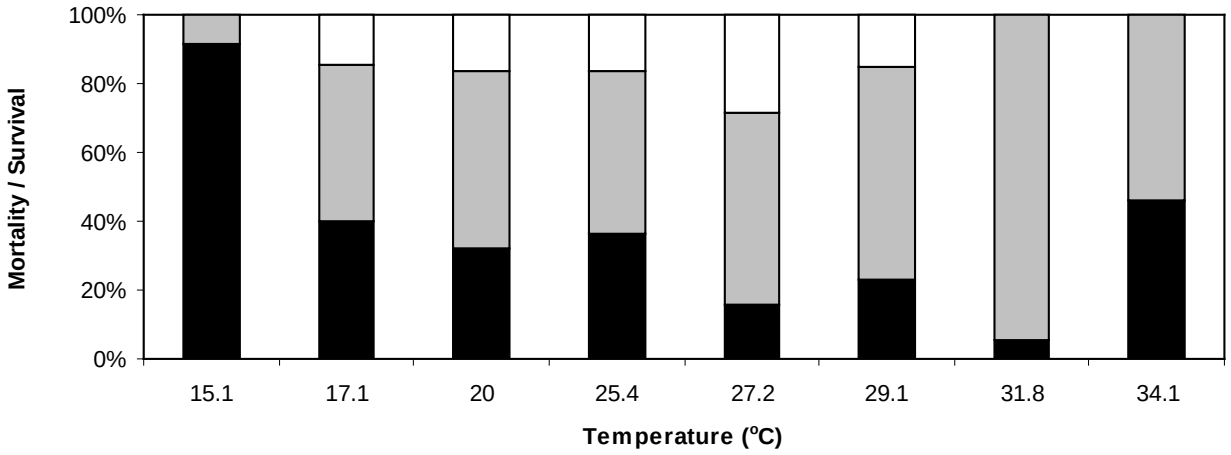


Figure 3: Stacked column chart comparing percentage egg mortality (black), larval mortality (grey) and adult emergence (white) of *Zygogramma bicolorata* at eight constant temperature treatments.

3.3.6. Degree-days

A colour-graded map of the predicted number of generations that *Z. bicolorata* will be able to complete annually throughout South Africa was generated using CLIMEX. *Parthenium hysterophorus'* current distribution was overlayed onto this map using MapViewer 7 (Golden Software, Inc.) (Figure 4). At all locations in South Africa, *Z. bicolorata* was predicted to be able to complete at least one annual generation. The location within South Africa with the lowest predicted number of completed generations was the region north of Lesotho (28°48'E; 28°48'S), with only 1.5 generations a year. The north-eastern region of South Africa, and along the Mozambique border, was predicted to support the highest number of generations (± 11 generations / year). Throughout parthenium's current distribution in South Africa, the range of predicted generations varies between 6.22 (25°18'E; 29°48'S) and 11.22 generations / year (25°18'E; 32°18'S).

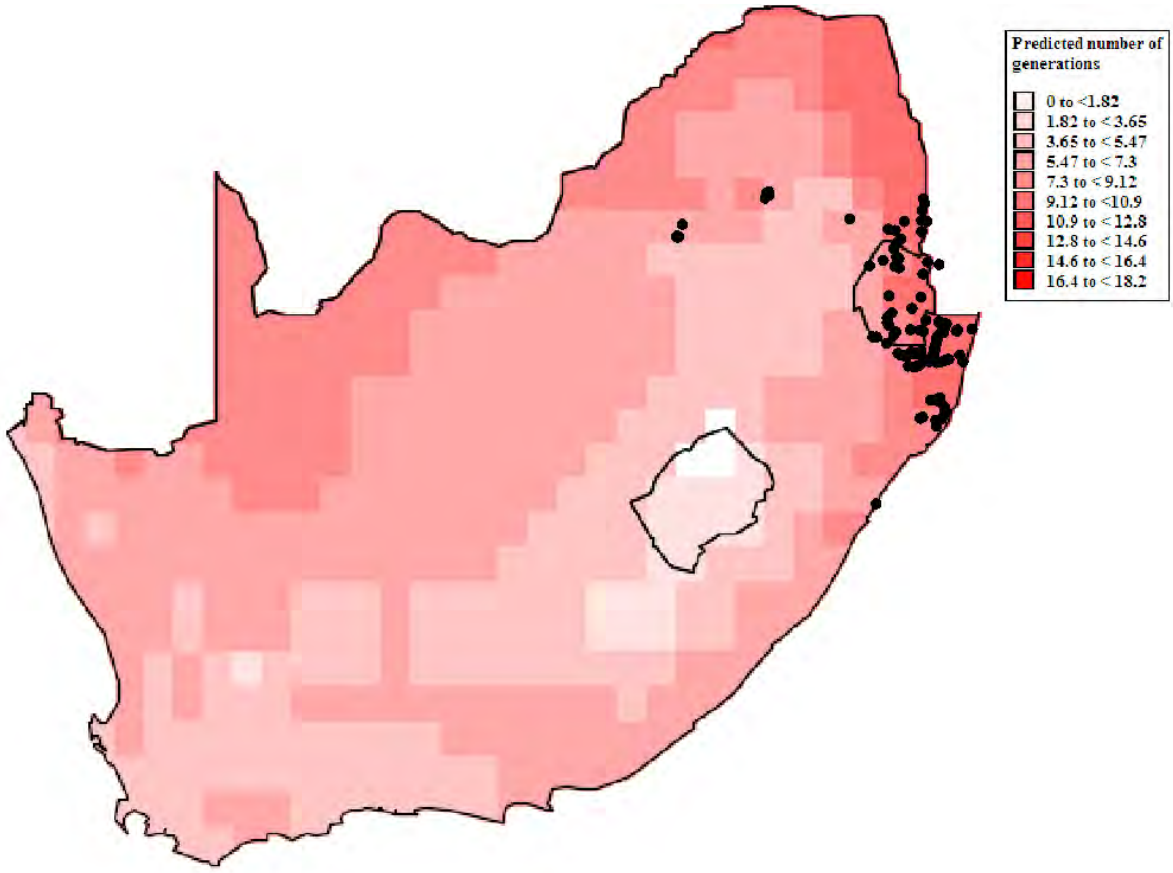


Figure 4: CLIMEX generated colour-graded map of South Africa depicting the number of annual generations that *Zygogramma bicolorata* will have sufficient physiological time to complete throughout South Africa. *Parthenium hysterophorus*'s current distribution in South Africa (black dots) has been overlayed (SAPIA database 2007)

3.4. DISCUSSION

Of the constant temperatures tested, *Z. bicolorata* was only able to develop to adulthood within the range of 17.1°C and 29.1°C, with high mortality experienced at temperature extremes (15.1°C, 31.8°C and 34.1°C). Optimum development at constant temperatures occurred at 27.2^{o}C (22.2 days ± 1.2) and 29.1^{o}C (22.4 days ± 1.2). The capacity for cold tolerance has been noted to differ between insect stages (Chen *et al.* 1991) and is commonly

greatest during the pupal stage, followed by the adult stage, with the larval stages being the least cold tolerant (Denlinger and Lee 1998). The pupal stage of *Z. bicolorata* showed constant survival across the temperature range tested. All other stages, with the exception of the second and fourth instars, showed low survivorship at low temperatures with an increase in survival as temperature increased.

The developmental rate of *Z. bicolorata* at constant temperature regimes is similar to that of *Zygogramma suturalis* (Coleoptera: Chrysomelidae) (Igrc 1990, Igrc *et al.* 1995) (Table 4). *Zygogramma suturalis*, native to the United States, was introduced to Russia and Croatia in the 1980s as part of a biological control programme to control the spread of common ragweed (*Ambrosia artemisiifolia* L.*,* Asteraceae) (Igrc 1990, Igrc *et al.* 1995). *Zygogramma bicolorata* has been documented as feeding on common ragweed in Australia (McFadyen pers. comm.) and is very similar in appearance to *Z. suturalis*. In developmental rate experiments using constant temperature regimes, *Z. suturalis* took 52.2 ± 0.4 (mean ± SD) days to develop from egg to adult at 19^{o}C and 27.1 ± 0.3 days at 27^{o}C (Table 4). This is comparable to *Z. bicolorata* which took 46.1 ± 3.0 days at 20^{o}C and 22.3 ± 0.3 at 27.2^{o}C (Table 4). The release of *Z. suturalis* in Croatia during the late 1980s and early 1990s was reported, after subsequent monitoring, as having a low success rate with only small populations establishing (Igrc *et al.* 1995). At one release site, near Bjelovar, temperature dropped to 2^{o}C two days after release, killing larvae and adults (Igrc *et al.* 1995). Only small *Z. suturalis* populations established at the other two release sites, both around Zagreb (Igrc *et al.* 1995). This is contrary to reports that *Z. suturalis* is believed to have established large populations within a short time after release in Russia (Igrc *et al.* 1995).

Table 4: Comparison of mean (± SD) developmental time (days) of life stages between *Zygogramma suturalis* (Z.s) and *Zygogramma bicolorata* (Z.b) for the range of temperatures tested by Irgc *et al.* (1995) and the similar temperatures tested in this study.

	Z.s	Z.b	Z.s	Z.b	Z.s	Z.b
	16°C	**17.1°C**	**19°C**	**20°C**	**27°C**	**27.2°C**
Egg	15.6 ± 1.2	10.9 ± 0.7				
Larvae			24.3 ± 3.0	20.8 ± 2.3	10.3 ± 0.6	8.5 ± 0.8
Pupae			24.5 ± 8.0	18.3 ± 1.3	12.5 ± 0.6	8.7 ± 0.9
Adult			52.2 ± 0.4	46.1 ± 3.0	27.1 ± 0.3	22.3 ± 1.2

The similarity in physiology between the two species is a good indication of the establishment success that *Z. bicolorata* is likely to experience in South Africa. *Zygogramma suturalis* established successfully in Russia (Irgc *et al.* 1995), a country which experiences far lower temperature extremes, in comparison to South Africa. However, low temperature weather events in areas with adequate physiological time could have a substantial effect on this insect population establishment in South Africa, as they did on *Z. suturalis* establishment in Croatia (Irgc *et al.* 1995). The negative effects of extreme weather events are a known cause of population establishment failure (Chown and Terblanche 2007) and are very difficult to predict.

From developmental rate experiments, the physiological time (^{o}D) that *Z. bicolorata* requires for development was calculated at 384.6^{o}D. Compared with other Coleopteran species, *Z. bicolorata* degree-day requirements are substantially higher. The number of degree-days required by *Diomus austrinus* (Coleoptera: Coccinellidae) to complete development varies

from 240.4 ^{o}D to 261.8^{o}D, depending on which prey species it is reared on (Chong *et al.* 2005). Using data from McClay and Hughes (2007), the degree-day requirement of *Mecinus janthinus* (Coleoptera: Curculionidae) was calculated at 270.3^{o}D. The far higher number of degree-days that is required by *Z. bicolorata* results from the extended period that *Z. bicolorata* requires for pupal development. At 20^{o}C, *D. austrinus* requires 9.5 ± 0.1 or 9.2 ± 0.1 days for pupal development, depending on the prey species used to rear the beetle (Chong *et al.* 2005), whereas *Z. bicolorata* requires 18.3 ± 1.3 days for pupal development. Although this is far greater than a number of other insect species (McConnachie 2004, Chong *et al.* 2005), it is similar to that recorded for *Z. suturalis,* which took 24.5 ± 8.0 days to complete pupal development at 19^{o}C (Irgc *et al.* 2005) (Table 4). Despite the high degree-day requirement of pupae, *Z. bicolorata* is not limited by physiological time requirements as there are a sufficient number of available degree-days throughout South Africa for establishment.

The number of generations that *Z. bicolorata* was able to complete was greater than one throughout the entire country (range: 1.5 to 11.2 generations / year). Any number of generations above one is considered adequate for population establishment and growth (McClay 1996). All areas within South Africa should therefore be able to support *Z. bicolorata*, in terms of physiological time, if parthenium was to spread as predicted. The highest predicted number of generations is 11.2 per year on South Africa's eastern border with Mozambique. In this region, the rapid development of the species will further promote population growth as the effect of any possible larval stage-based predation will be decreased, as a result of the decrease in the length of these stages, and the period available for reproduction will be increased (Logan *et al.* 2006).

Throughout Mexico, *Z. bicolorata*'s country of origin, the number of predicted generations does not drop below 3.5 (Figure 5). Using known collection localities of *Z. bicolorata* (Pallister 1953, McClay pers. comm.) the range of generations that *Z. bicolorata* is predicted to be able to complete in Mexico varies from 3.5 to 13.2 generations / year (Figure 5). In Australia, where *Z. bicolorata* has been introduced as an agent for *P. hysterophorus*, the range of predicted generations is similar to that in South Africa. The number of generations ranges from 1.75 to 10.1/ year at known establishment sites in Australia (McFadyen pers. comm.) (Figure 6). The fact that *Z. bicolorata* originates from Mexico, has been successfully established in Australia for over 20 years, and that both countries have similar numbers of predicted generations as South Africa, is a good indication that there are a sufficient number of available degree-days in South Africa to support *Z. bicolorata* establishment.

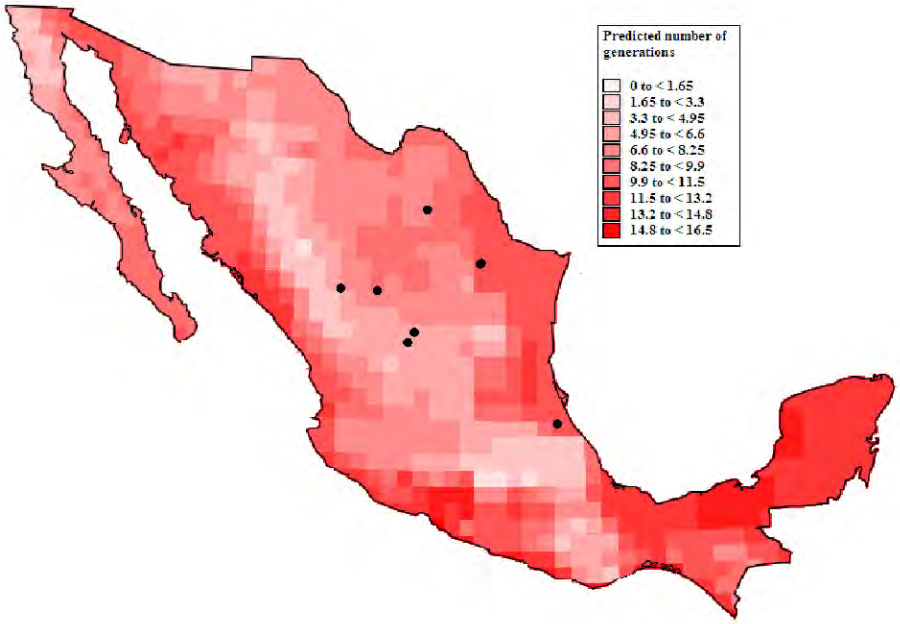


Figure 5: CLIMEX generated colour-graded map of Mexico, depicting the number of generations that *Zygogramma bicolorata* will have sufficient physiological time to complete. Known occurrence sites of *Zygogramma bicolorata* have been overlayed (McClay, pers. comm.).

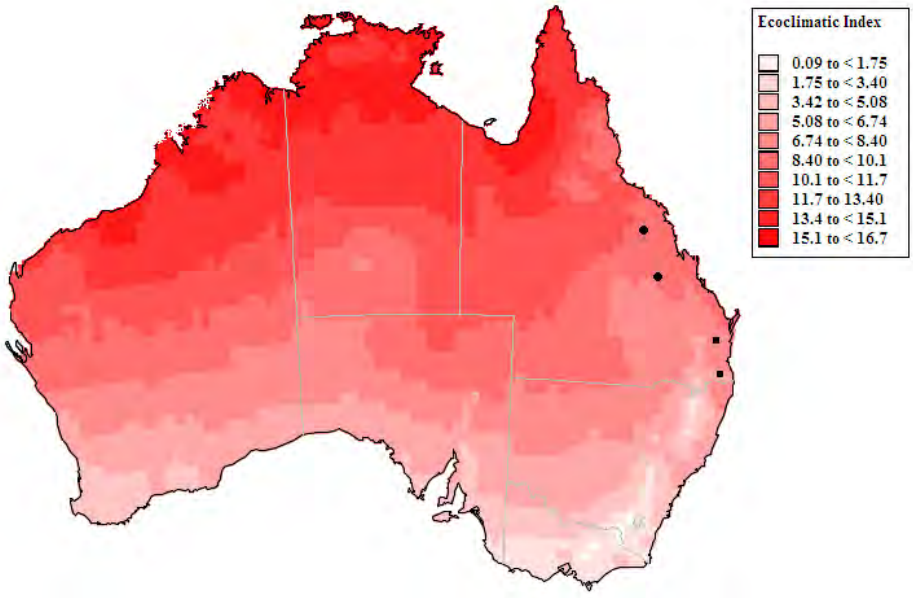


Figure 6: CLIMEX generated colour-graded map of Australia, depicting the number of generations that *Zygogramma bicolorata* will have sufficient physiological time to complete. *Zygogramma bicolorata* occurs on *Parthenium hysterophorus* between Injune and Clermont (circles) and on *Ambrosia artemisiifolia* between Grafton North and Brisbane (squares) (McFadyen pers. comm.).

Although the degree-day model can be a good predictor of site suitability for a particular species in relation to climate, the model does have limitations. Degree-day models have been found to work best when dealing with univoltine species which have defined overwintering strategies, in extreme climates (Stewart *et al.* 1996, Byrne *et al.* 2003). *Zygogramma bicolorata* is a multivoltine species which is known to diapause in unfavourable climates (Singh 1997, Dhileepan *et al*. 2000) but is not an obligate diapausing species (Chakravarthy and Bhat 1997). In such cases, degree-day model results are often considered to be inconclusive (Stewart *et al.* 1996). For multivolitine species', the number of generations predicted by CLIMEX should be interpreted as indicating whether a location receives enough accumulated heat for the production of multiple generations, and not that the exact number of generations will occur (Kriticos *et al.* 2007). Factors such as plasticity in the number of degree-days required to complete a generation, soil moisture, day length and rainfall will affect the number of generations produced at a particular location (Kriticos *et al.* 2007). Another criticism of the degree day model is that the required accumulated heat for development of the average individual is determined (Kriticos *et al.* 2007). However, it is argued that in the cool range margins, it will be the fastest developing individuals that survive and for these regions the accumulated heat requirements for these fast developing individuals should be determined (Kriticos *et al.* 2007). It was suggested that this could be done by determining the accumulated heat requirements for each stage, less two standard deviations (Kriticos *et al.* 2007). As all areas in South Africa have been predicted as being able to sustain at least one generation per year, this criticism will not affect the predicted distribution of *Z. bicolorata* in South Africa.

In order to include more physiological limits and climatic variables for predicting the likely distribution of *Z. bicolorata* in South Africa, thereby increasing model reliability, a CLIMEX model was generated (Chapter 4). CLIMEX incorporates the degree-day requirements, along with a number of other physiological requirements of the specific species, to generate a more realistic predicted distribution. The CLIMEX model also includes a number of other climatic variables, such as humidity and soil moisture, and not just temperature as in the degree-day model, when determining the suitability of a site for agent establishment. This model will be considered in the next chapter.

CHAPTER 4

MODELLING THE POTENTIAL DISTRIBUTION OF *ZYGOGRAMMA BICOLORATA* IN SOUTH AFRICA USING CLIMEX

4.1. INTRODUCTION

4.1.1. Ecological modelling systems

A number of systems, varying in their predictive scale and sophistication, have been developed to aid in the prediction of plant and insect persistence in a new range. These include computer-based systems such as BIOCLIM (recently renamed ANUCLIM) (Busby 1991, Houlder 2004), CLIMATE (Pheloung 1996), Climate Envelopes, CLIMEX (Sutherst and Maywald 1985, Sutherst *et al.* 2004), DOMAIN (Carpenter *et al.* 1993), GARP (Stockwell and Peters 1999), HABITAT (Walker and Cocks 1991) and STASH (Sykes *et al.* 1996). In a review by Kriticos and Randall (2001) the applicability of these systems to model the potential range of a species in an exotic environment was examined, and a number of negative attributes were identified. For example, ANUCLIM identifies the likely range of indigenous species in their native range only, and is therefore not useful when comparing international climates. Also, Climate Envelopes do not allow for any user intervention in the modelling process besides the inputting of locations, DOMAIN does not determine which sites are most suitable for species persistence and HABITAT is known to reject erroneously some occurrence sites from the predicted distribution (Kriticos and Randall 2001). Of all the modelling systems, only CLIMATE and CLIMEX included global meteorological databases (Kriticos and Randall 2001). Useful functions also included in CLIMEX are a degree-day

variable, the ability to map stress and growth functions and to graph their seasonal fluctuations, and the option to create climate change scenarios (Sutherst *et al.* 1999). The predictions produced by CLIMEX are therefore thought to be more 'biologically meaningful and predictive' compared to other modelling systems (Zalucki and van Klinken 2006). CLIMEX has proven valuable in a number of recent biological control and pest risk assessment programmes (e.g. Julien *et al.* 1995, Hoddle 2004, McConnachie 2004, Kriticos *et al.* 2005, Kriticos *et al.* 2007, Palmer *et al.* 2007) and was thus chosen as the most suitable simulation to model the potential distribution of *Z. bicolorata* in South Africa in relation to climate.

4.1.2. CLIMEX

CLIMEX (CLIMEX Programme Ver. 2, CSIRO Entomology©) is a multi-parameter, dynamic, eco-climatic modelling package that estimates the potential geographical distribution and seasonal abundance of a species using long term, average meteorological data (Julien *et al.* 1995, Sutherst *et al*. 1999, Sutherst *et al.* 2004, Kriticos *et al.* 2005). The model assumes that the climatic requirements of a species can be inferred from its native and / or exotic geographical distribution, with the option of fine tuning the model with species specific physiological data. The species' ecological niche and not its fundamental niche is therefore determined. CLIMEX was originally designed for reverse modelling, i.e. using only the native range for predicting a species' distribution, but the model can also be used for mechanistic modelling to determine possible distributions. Mechanistic modelling requires some knowledge of the native range of the species, but relies more heavily on physiological parameters to predict distribution in the new range. Mechanistic (and not reverse) modelling

was the primary method used in this study as reverse modelling requires accurate data on the species' distribution in its native range (Sutherst *et al.* 2004) and extensive distribution data are not available for *Z. bicolorata* in its native range (Mexico).

CLIMEX contains three functions, 'Match Climates', 'Compare Years' and 'Compare Locations'. For this study only the 'Compare Locations' function was required.

4.1.2.1. Compare Locations

The Compare Locations function determines the potential geographical distribution of a species in relation to climate, based on the species' climatic preferences (Sutherst 2003). Climatic preferences of a species are obtained from the native and / or exotic distributions and empirical data (Kriticos *et al.* 2005). Once the climatic preference has been determined, CLIMEX uses a meteorological database of monthly climatic averages to calculate a Growth Index and several stress indices (Sutherst *et al.* 2004, Kriticos *et al.* 2005). These indices are based on the assumption that a species will experience one season that is favourable for growth and one that is unfavourable for growth, in a single year (Sutherst *et al.* 2004). The Growth Index describes the potential growth of the population during the favourable season and the four stress indices (see below) describe the probability of population survival during the unfavourable season (Southwood 1977). The growth and stress indices are combined into an ecoclimatic index (EI), scaled between 0 (unsuitable) and 100 (optimum), which describes the overall probability of a population surviving at a particular site (Julien *et al.* 1995).

4.1.2.2. Stress Indices

There are four stress indices that were used to model the distribution of *Z. bicolorata* in South Africa, namely Cold Stress (CS), Heat Stress (HS), Dry Stress (DS) and Wet Stress (WS). It is a CLIMEX stipulation that the threshold values for the stress indices (e.g. cold stress temperature threshold and heat stress temperature threshold) do not fall within the range of the growth parameters, as a population can not be growing and simultaneously accumulating stress (Kriticos *et al.* 2005).

4.1.2.3. The Weekly Growth Index

CLIMEX uses two growth indices, the Weekly Growth Index (GI_w) and the Annual Growth Index (GI_A), to determine the final Growth Index. The GI_w (0 – 1) describes the weekly suitability of the climate for growth through four indices; temperature (TI_w), moisture (MI_w), light (LI_w) and diapause (DI_w) (see Equation 1 below). The species-specific values for the indices are inferred from the species' geographical distribution and fine-tuned using ecophysiological data (Sutherst 2003, Kriticos *et al.* 2005).

Equation 1: $\mathbf{GI_w = TI_w \times MI_w \times LI_w \times DI_w}$

Included in the TI_w is the function to calculate the number of degree-days above the developmental threshold (DVO) that a species requires to complete a generation (PDD). This parameter is important because if the number of potential generations in a year is less than one, the EI becomes zero, even if stresses are not excluding the species from the locality (Kriticos *et al.* 2005).

4.1.2.4. The Annual Growth Index

The Annual Growth Index (0 – 100) is an average of the GI_w (see Equation 2 below) and describes the general potential of the population for growth and its potential relative abundance over the population's entire range, relative to climate.

Equation 2:

$$GI_A = 100 \sum_{i=1}^{52} GI_{Wi} /52$$

4.1.3. Aims

In Chapters 2 and 3, the thermal limits and developmental temperature requirements of *Z. bicolorata* were examined separately and broad potential areas in South Africa, climatically suitable for *Z. bicolorata's* persistence, were identified. In this chapter, these parameters were used in conjunction with native and exotic distribution data of *Z. bicolorata* to produce a more accurate and detailed ecological model of the beetle's potential range and abundance in South

Africa, as limited by climate. Using this model, suitable release sites and the appropriate timing of releases at these sites were identified.

4.2. METHODS

4.2.1. Parameter derivation

Through an iterative process, of comparing the predicted distribution to the known distribution, the CLIMEX Semi-arid Template was shown to describe *Z. bicolorata's* distribution in Mexico (native distribution), India and Australia (exotic distributions) most accurately. Both the native and exotic distributions were used to fit the model parameters as the native range represented the species' realized niche and the exotic ranges its fundamental niche (Crutwell McFadyen 1991, Kriticos *et al.* 2005). The decision was therefore made that the starting point for the CLIMEX model for both *P. hysterophorus* and *Z. bicolorata* would be the Semi-arid Template. For the Moisture Index (used to determine the Growth Index) the Colorado Beetle Template (see below) was used for estimations of the upper optimal soil moisture (SM2) and upper soil moisture threshold (SM3). The values for these parameters were found to be too low in the Semi-arid Template and unrepresentative of *Z. bicolorata*'s native distribution. *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), commonly known as the Colorado Beetle, is a member of the same family as *Z. bicolorata* and also originates from central Mexico (Cappaert *et al.* 2006). The two beetles have a similar biology as both have four larval instars that are leaf-feeders and burrow into the soil to pupate (Cappaert *et al.* 2006). The Semi-arid Template and Colorado Beetle Template parameters were refined using the thermal limits of *Z. bicolorata,* collected in Chapters 2 and 3. In determining the predicted distribution of *Z. bicolorata,* the light and growth indices were not

used. The LI_w is only applicable when predicting the potential range of a plant species (Sutherst *et al.* 2004), and the DI_w was not used as *Z. bicolorata* is not an obligate diapausing insect (Chakravarthy and Bhat 1997). Explanations for the parameter values used in the model are given in Table 1.

Table 1: Growth and stress parameters of the CLIMEX model used to assess the potential distribution of *Zygogramma bicolorata* in South Africa

Parameter	Value	Definition	Explanation
Temperature:			
DVO	10.9	Lower temperature threshold	t of *Z. bicolorata*
DV1	20	Lower optimum temperature	obtained from developmental rate graph
DV2	30	Upper optimum temperature	obtained from developmental rate graph
DV3	45.4	Upper temperature threshold	ULT_{50} of *Z. bicolorata*
PDD	384.6	Annual minimum degree-days (^oD)	K of *Z. bicolorata*
Moisture:			
SMO	0.05	Lower soil moisture threshold	obtained from LH_{50} of *Z. bicolorata*
SM1	0.08	Lower optimal soil moisture	obtained from LH_{50} of *Z. bicolorata*
SM2	0.8	Upper optimal soil moisture	Colorado Beetle Template (CBT)
SM3	1.5	Upper soil moisture threshold	CBT
Cold Stress:			
DTCS	-5.6	Cold stress temperature threshold	LLT_{50} of *Z. bicolorata*
DHCS	-0.002	Cold stress accumulation rate	Semi-arid Template (SAT)
Heat Stress:			
TTHS	45.5	Heat stress temperature threshold	obtained from ULT_{50} of *Z. bicolorata*
THHS	0.002	Heat stress accumulation rate	SAT
Dry Stress:			
SMDS	0.04	Dry stress threshold	obtained from SM0
HDS	-0.005	Dry stress accumulation rate	SAT
Wet Stress:			
SMWS	1.51	Wet stress threshold	obtained from SM3
HWS	0.01	Wet stress accumulation rate	SAT

4.2.2. Model validation

To ground truth the distribution model of *Z. bicolorata* in South Africa, the CLIMEX predicted distribution of *Z. bicolorata* in Mexico, Australia and India were overlayed with the known distribution of *Z. bicolorata* in these countries. This technique was used as a means of model validation and not parameter fitting as there were insufficient data available on the known distribution of *Z. bicolorata* in these countries.

4.2.3. Mapping

Zygogramma bicolorata's CLIMEX model was run and a map of the predicted EI's in South Africa was produced and overlayed with the current parthenium distribution in South Africa (Henderson 2007, Strathie and McConnachie 2007). The predicted distribution of *Z. bicolorata* and the known distribution of parthenium in South Africa were compared.

4.2.4. EI interpretation

The EI interpretations of Vera *et al.* (2002), Hoddle (2004) and Venette and Cohen (2006) were followed: EI values of 0 were considered unsuitable, 1- 10 marginal, 11 – 25 favourable, and ≥ 26 very favourable for establishment.

4.2.6. Release site identification

Four criteria were decided on for selecting release sites for *Z. bicolorata*: 1) the location had to have a 'very favourable' EI (> 26); 2) the level of parthenium infestation had to be significant; 3) the surrounding locations had to have a positive EI; and; 4) the Growth Index had to be high throughout the year. Locations with 'very favourable' EI's were chosen as the chances of establishment and population long term survival are highest in these areas, decreasing the amount of time and money spent on agent release efforts and increasing project success. Also, by releasing agents at locations where surrounding areas have positive EI's, the chances of the population spreading outwards from the release site, once established, is increased. Areas of high parthenium infestation are required as the *Z. bicolorata* population requires a sufficient food source for population growth. The Weekly Growth Index was graphed to help determine both the timing of agent releases at particular sites and the identification of sites where agent abundance is expected to be high throughout the year (Zalucki and van Klinken 2006). The predicted best release time was estimated from when the Weekly Growth Index was high enough for substantial population growth, with sufficient time for the population to increase in numbers before the Weekly Growth Index started to decrease again.

4.3. RESULTS

4.3.1. Model validation

4.3.1.1. Mexico

The predicted distribution of *Z. bicolorata* in Mexico according to the CLIMEX model indicated that all known collection localities of *Z. bicolorata* have an EI greater than 26 (very favourable for establishment and survival, range = 29 to 57), validating the model (Figure 1). According to the model it can be predicted that *Z. bicolorata's* range is likely to extend across most areas in Mexico with abundant populations occurring in the east of the country.

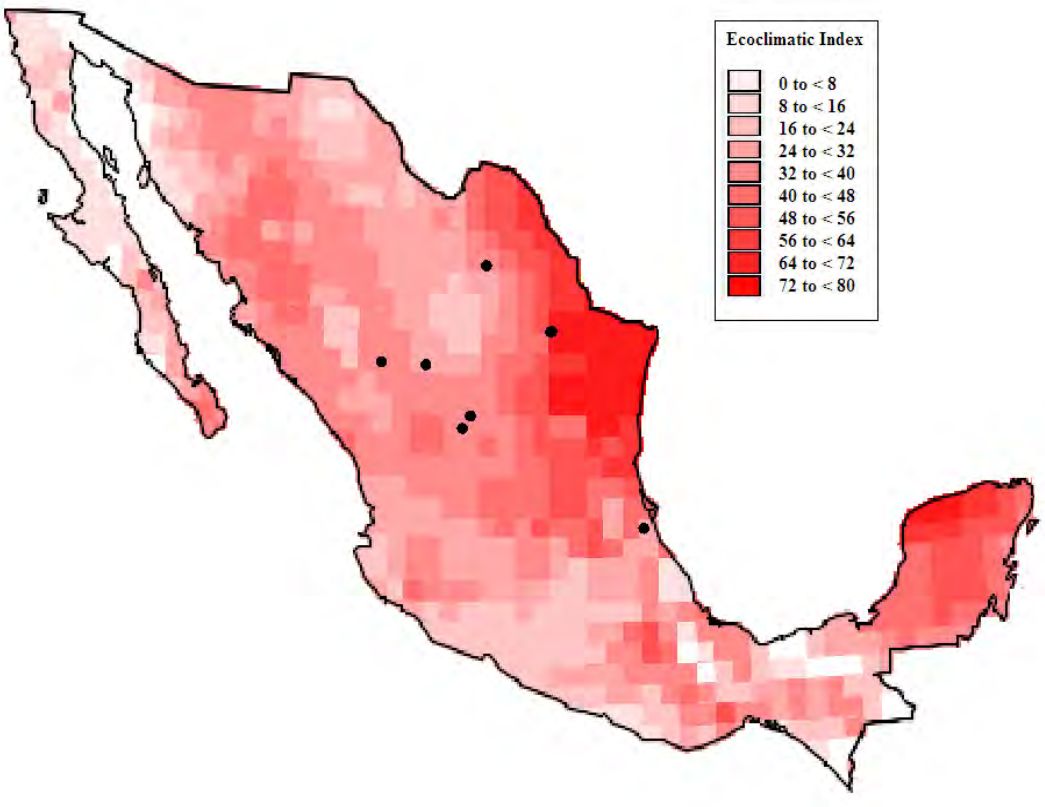


Figure 1: CLIMEX generated map of the ecoclimatic suitability of Mexico for *Zygogramma bicolorata* persistence. Solid circles indicated known occurrence sites of *Zygogramma bicolorata* (Pallister 1953, McClay pers comm.).

4.3.1.2. Australia

The predicted distribution of *Z. bicolorata* in Australia covers a far larger area than the current known distribution of *Z. bicolorata* in Australia (Figure 2). Currently *Z. bicolorata* is known to occur on *P. hysterophorus* between Injune North and Clermont, and on *Ambrosia artemisiifolia* between Grafton North and Brisbane (McFadyen pers. comm.). *Zygogramma bicolorata* does not have a larger range in Central Queensland due to the absence of both host plants (McFadyen pers. comm.). The EI values in the areas where *Z. bicolorata* does occur are all very favourable and range between 45 and 56 (between Brisbane and Grafton North) and 71 and 74 (between Injune North and Clermont), further validating the model.

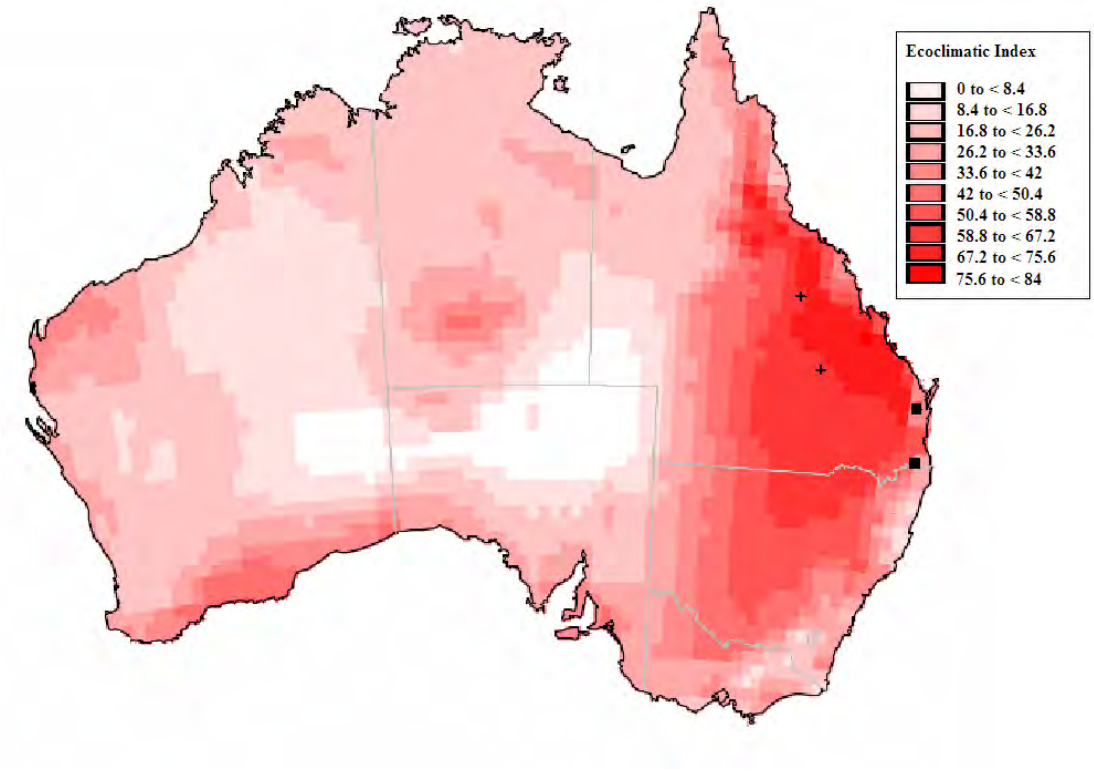


Figure 2: CLIMEX generated map of the ecoclimatic suitability of Australia for *Zygogramma bicolorata* persistence. *Zygogramma bicolorata* occurs on *Parthenium hysterophorus* between Injune North and Clermont (crosses) and on *Ambrosia artemisiifolia* between Grafton North and Brisbane (squares) (McFadyen pers. com.).

4.3.1.3. India

Zygogramma bicolorata has been recorded as established in Tamil Nadu, Karnataka, Andhra Pradesh, Jammu, Punjab and Madhya Pradesh States in India (Basappa 1997). When CLIMEX was used to model the potential distribution of *Z. bicolorata* in India, the EI values for the established areas ranged between 26 and 53 (Figure 3). There were no areas in India where the beetle has established that CLIMEX predicted as being unsuitable.

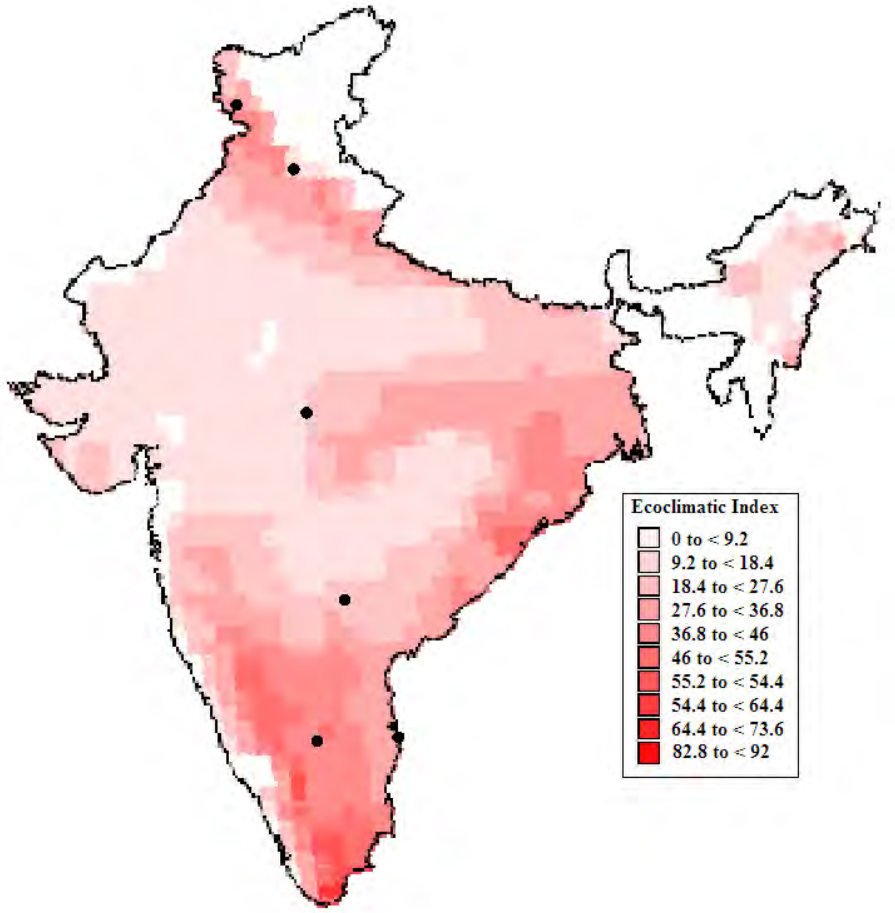


Figure 3: CLIMEX generated map of the ecoclimatic suitability of India for *Zygogramma bicolorata* persistence. Solid circles indicate known occurrence sites of *Zygogramma bicolorata* (Basappa 1997).

4.3.2. Predicted South African distribution

The CLIMEX model prediction for *Z. bicolorata* distribution in South Africa depicts the country as being favourable for the establishment of the beetle throughout all regions, except in the west of the country and in the north of Lesotho, extending into South Africa (Figure 4). All areas in which parthenium is known to occur (Figure 4) have 'very favourable' EI values (> 26) for *Z. bicolorata* persistence in the area (range = 35 to 91) with the highest EI occurring in the north-eastern region of South Africa (27°18'E; 32°18'S) extending into the south east of Swaziland, and the lowest in areas west of this (25°18E'; 30°48'S).

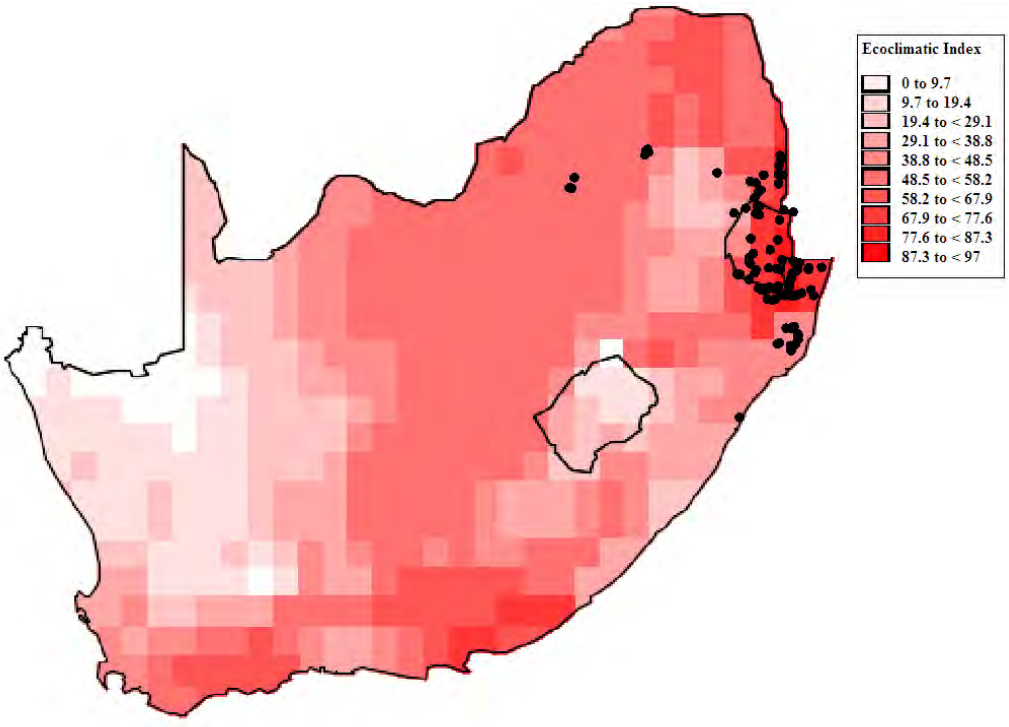
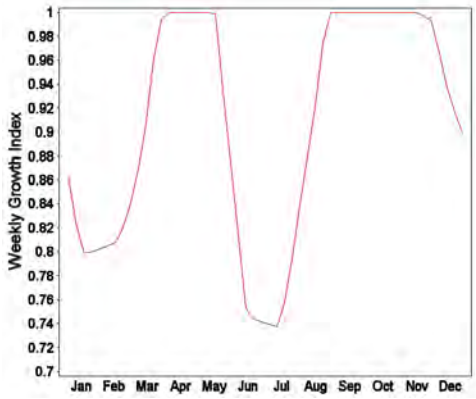


Figure 4: CLIMEX generated map of the ecoclimatic suitability of South Africa for *Zygogramma bicolorata* establishment and long term survival. The current distribution of *Parthenium hysterophorus* (black dots) (Henderson 2007) in South Africa has been overlayed.

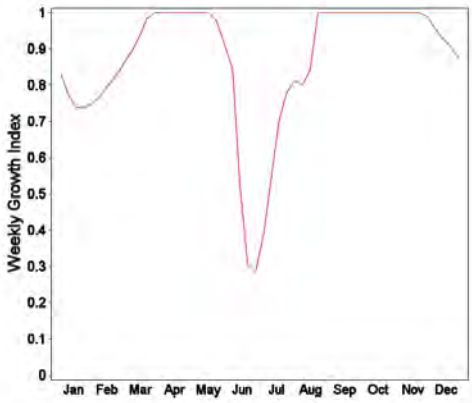
4.3.2. Release sites

For initial releases aimed at establishing the agent, three optimal release sites were identified as being highly suitable for the establishment of *Z. bicolorata* in South Africa (Figure 4). These were inland along the road from Swaziland to Mozambique (2532CC) (although releases should not be performed directly on the road verge), Ndumu Game Reserve (2632CC) and Jozini (2732AC). All three sites have very high EI values (87 to 91), abundant to very abundant parthenium densities (Henderson 2007), are surrounded by areas with high EI's and have a high Weekly Growth Index throughout the year (Figure 5). The Weekly Growth Index does not drop below 0.74 within the year at Jozini and does not drop below 0.29 and 0.34 along the road from Swaziland to Mozambique and in Ndumu Game Reserve respectively (Figure 5). All Stress Indices are zero at the three locations. The beetle populations will therefore continue to grow throughout the entire year at the three chosen locations. At all these localities, the predicted best release time is from late August to early September, when climate is at an optimum for growth.

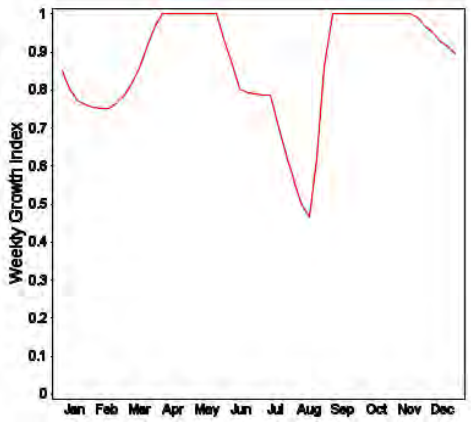
Populations of parthenium occurring further west in South Africa, at Brits (2527BD) and Siyabuswa (2529AA), were not chosen as release sites even though they displayed 'very favourable' EI values (range = 62 to 63). These sites were not categorised as optimal release sites, as from May to September, the Weekly Growth Indices dropped to zero, inhibiting population establishment and growth (Figure 6). Furthermore, the density of parthenium at Siyabuswa is categorised as 'frequent' and only 'occasional' at Brits (Henderson 2007).



(a)

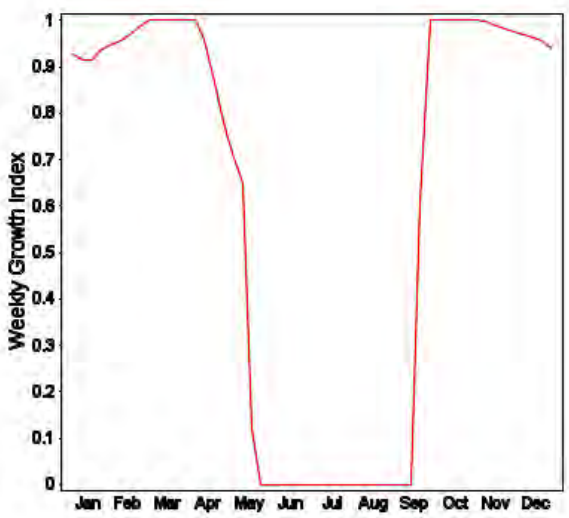


(b)

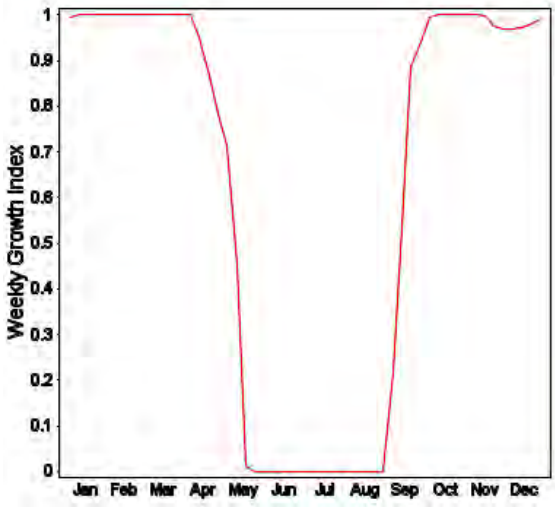


(c)

Figure 5: Weekly Growth Indices of *Zygogramma bicolorata* at (a) Jozini (b) Ndumu Game Reserve and (c) along the road from Swaziland to Mozambique



(a)



(b)

Figure 6: Weekly Growth Indices of *Zygogramma bicolorata* at (a) Brits and (b) Siyabuswa

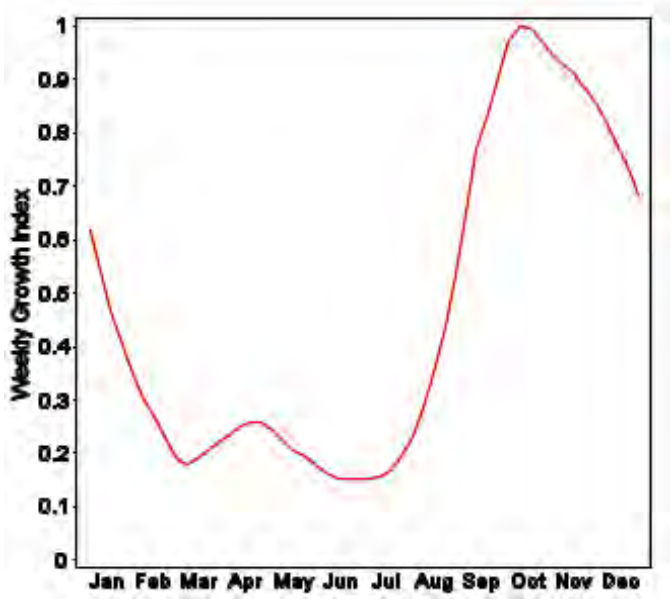
4.4. DISCUSSION

The wide range of EI values (range = 26 to 74) of the sites where *Z. bicolorata* is known to occur in Mexico (native range), Australia and India (introduced ranges) gives an indication of the range of EI values that will be suitable for the beetle in South Africa. From this, it is predicted that the potential distribution of *Z. bicolorata* will not only incorporate parthenium's current range in South Africa, but is also wide enough to spread into new areas when parthenium expands its distribution. *Zygogramma bicolorata* is therefore considered to be a suitable agent for the control of parthenium throughout South Africa, in terms of the potential climatic range it can inhabit. However, the extent of the impact that *Z. bicolorata* will have on the parthenium population in South Africa is also dependent on several additional factors, including how *Z. bicolorata* feeding will affect parthenium growth and reproduction (Raghu *et al.* 2006).

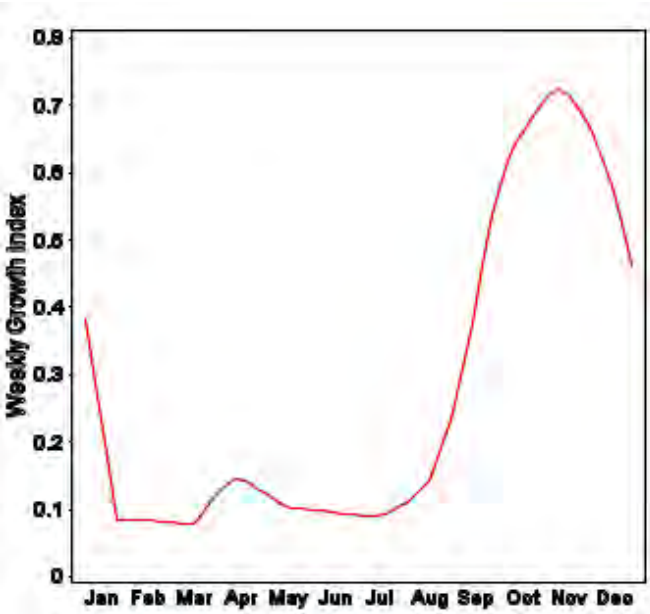
Three optimal release sites were chosen for the initial release of *Z. bicolorata* in South Africa; namely along the road from Swaziland to Mozambique (2532CC), Ndumu Game Reserve (2632CC) and Jozini (2732AC). These three release sites were chosen over other potential sites as although other sites fulfilled most of the release site criteria, they did not have a positive Weekly Growth Index throughout the year. *Zygogramma bicolorata* will most likely be able to persist at these sub-optimal sites, but rapid population growth will be limited, thereby increasing the time needed for population establishment and spread. The mean (± SD) Weekly Growth Indices for the three chosen release sites were 0.88 ± 0.14 (2532CC), 0.87 ± 0.18 (2632CC) and 0.91 ± 0.10 (2732AC). The two most westerly sites of parthenium

distribution in South Africa, although having high EI values for *Z. bicolorata* persistence, had mean Weekly Growth Indices of 0.62 ± 0.46 (Brits) and 0.65 ± 0.44 (Siyabuswa).

In Australia, *Z. bicolorata* was released in Central Queensland in the 1980s (McFadyen and McClay 1981). Although *Z. bicolorata* is present in Australia today, it took 10 years after the final release before the population was considered to have become established (Dhileepan and McFadyen 1997). The low average Weekly Growth rates predicted by the CLIMEX model for these areas could be a possible explanation for the slow population growth rates. In Grafton North, the average Weekly Growth Index predicted for a year was 0.28 (± 0.24) and in Brisbane it was 0.46 (± 0.3) (Figure 7). These average Weekly Growth Indices for the year were extremely low when compared to the proposed release sites in South Africa. The rate of population growth, and therefore abundance, is likely to much higher at the proposed release sites in South Africa compared to the sites / areas where the beetle is established in Australia. The chances of *Z. bicolorata* establishing at the release sites in South Africa are therefore considered high.



(a)



(b)

Figure 7: Weekly Growth Indices of *Zygogramma bicolorata* at (a) Brisbane and (b) Grafton North, Australia

The CLIMEX model predicted an extensive potential distribution for *Z. bicolorata* in South Africa. However, this predicted range is determined solely by climatic variables and is likely to be an overestimation of *Z. bicolorata*'s potential range. Other abiotic and biotic variables that will be a factor in further limiting *Z. bicolorata*'s distribution include the frequency of disturbance at a particular site (Hulme 2003), predation, food availability (Hoddle 2004) and synchronicity with its host (Zalucki and van Klinken 2006). There is an ongoing debate over the importance of abiotic versus biotic variables in determining plant and animal realized niches. Some authors state that climate is the primary determinant of a plant's realized distribution but that biotic factors play a more important role in determining an animal's realized distribution (Samways *et al.* 1999, Austin 2002). Conversely, climate is viewed by other authors as the primary determinant of both plant and animal realized ranges (Lawton 2000, Sutherst 2003). The latter theory was supported by a study performed by Huntley *et al.* (2004) which concluded that climatic model performance was not related to trophic level, and that climate was the best variable for modelling species distribution. Although biotic factors play an important role in determining species distribution, they are altered by abiotic factors, which play the primary role in determination (Lawton 2000).

Abiotic factors, principally climate, are the best determinant of a species' potential range, but there are limitations in their predictive power when using most meteorological databases, including the CLIMEX meteorological database. The minimum and maximum temperatures in the database are averaged and at any location, the temperature may drop below or rise above the minimum or maximum temperature a number of times a year (Kriticos *et al.* 2005). Therefore, although the climate of an area (as stipulated in the CLIMEX database) fits within the physiological limits of a species, mortality at a site could be experienced due to

temperature extremes that are outside of the physiological range of the species. Conversely, the potential range of a species could be greater than that predicted by CLIMEX. This happens when the species that is introduced adapts to the new environment (Hufbauer and Roderick 2005).

Despite the criticisms levied against the CLIMEX model, it is widely believed that it is one of the most 'biologically meaningful and predictive' models currently available for predicting species distribution in relation to climate (Zalucki and van Klinken 2006). The CLIMEX model of the potential distribution of *Z. bicolorata* in South Africa can, therefore, be used to predict that *Z. bicolorata* establishment is unlikely to be limited by climate in most areas of the country, particularly in those areas where parthenium currently occurs.

CHAPTER 5

THE FUTURE OF WEED BIOLOGICAL CONTROL IN SOUTH AFRICA

5.1. CURRENT ENVIRONMENTAL CONCERNS

In recent decades there has been a social and political awakening as to the importance of, and dangers facing, the environment. This awakening has been driven by the increased pressure placed on the world's natural resources, resulting from dramatic population growth, globalization and technology and associated impacts such as biological invasions and climate change (Carter 2007). This concern towards the environment is fuelled by the anxiety that the world is heading towards a series of devastating ecological crises and safeguards need to be implemented to prevent this (Carter 2007).

The new political regime in South Africa, emerging from the 1994 elections, saw the beginning of a new era of conservation and environmental protection (Moran *et al.* 2005). The Bill of Rights and Biodiversity Bill both set precedents for preserving environmental health and the control of alien invasive plants in particular (Moran *et al.* 2005). South Africa has invested a large amount of money and research into developing effective methods of invasive plant control and is considered to be the third most active country practicing biological control, behind the USA and Australia (van Wilgen *et al.* 2004). However, the increase in environmental awareness that has occurred over the last few decades has increased public pressure on biological control, with the opinion of a number of authors that it is not an

ecologically safe science (Howarth 1991, Simberloff and Stiling 1996, Follett and Duan 1999). Biological control also battles to win support from the public, government and funders as although it preserves ecosystem services, these results are difficult to visualise or quantify (van Wilgen *et al.* 2004) making its necessity difficult to substantiate. If biological control is to be seen as a viable and safe option in this increasingly sceptical environment, it needs to guarantee that the greatest possible effort has been invested to ensure that each biological control agent released is a safe and necessary one.

5.2. CURRENT STATUS OF WEED BIOLOGICAL CONTROL IN SOUTH AFRICA

Biological control has experienced much success in South Africa, since its initiation early in the 20th century (Zimmermann *et al.* 2004) (Table 1). This success has been aided by the initiation of the Working for Water Programme in 1995, by the South African government (Zimmermann *et al.* 2004). Working for Water endeavours to decrease alien vegetation cover and promote the re-emergence of natural vegetation, with the overall intention being to increase the country's water supply (Blanchard and Holmes 2007). The programme is considered to have enhanced biological control in South Africa through the provision of funding, research into emerging weeds, improved international cooperation, well organised implementation programmes and the formation of research teams involving entomologists, plant pathologists, resource economists and plant ecologists (Zimmermann *et al.* 2004). Despite these recent advances and success in limiting the spread of invasive weeds (Zimmermann *et al.* 2004), the practice is still criticised for the perceived risk of direct and indirect non-target effects (e.g. Simberloff and Stiling 1996, McEvoy and Coombs 2001, Denslow and D'Antonio 2005, Louda *et al.* 2005). In order to reduce the potential risk of

negative impacts, the cost of biological control programmes and the time taken to control a weed, a more intensive agent selection process needs to be developed. This selection process should not only be based on an agent's host-specificity, but also on its potential efficacy in establishing and controlling the weed (McClay and Balciunas 2005). Hoelmer and Kirk (2005) quote the rhetorical question posed by van Lenteren (1980): "does art have to become science?". Van Lenteren (1980) concluded that the best approach to biocontrol is presented by trial and error (Hoelmer and Kirk 2005), but in the current environmental and economic climate, this approach is unsatisfactory.

Table 1: Summary of outcomes of biological control programmes targeting 44 weed species in South Africa (Zimmermann *et al.* 2004)

Level of Control	**Percentage**
Complete	25
Substantial	36
Negligible	27
Unknown	11

complete	no other control methods are needed to reduce the weed to acceptable levels, at least in areas where the agent has established
substantial	other management methods are needed to supplement biological control and reduce the weed to acceptable levels, but these efforts are less than they were prior to biological control
negligible	in spite of obvious damage to the weed by the agent, management efforts are entirely reliant on other measures, not biological control
unknown	the impact of the agent is unknown either because no follow-up evaluations were done at the time or because it is too early for the programme to be meaningfully evaluated

5.3. CHALLENGES FACING WEED BIOLOGICAL CONTROL

There are currently many protocols and laws in place to ensure that when an agent is released from quarantine, it has been tested effectively and efficiently for host-specificity (Zimmermann *et al.* 2004). However, the biggest challenges currently facing weed biological control are predicting which agents are going to be effective (Denslow and D'Antonio 2005, Raghu and van Klinken 2006) and quantifying their ecological impacts once released (Denslow and D'Antonio 2005). In South Africa where 86 biological control agents were released before 2004, 23 failed to establish anywhere in the country (Zimmermann *et al.* 2004). Establishment failures should be minimised as they are not only detrimental to the reputation of biocontrol, but also add to the cost and risk of the programme (McClay and Balciunas 2005). An example given by Raghu and van Klinken (2006) demonstrating the waste of resources as a result of poor agent selection is the programme against the prickly pear *Opuntia ficus-indica* in Australia. Natural enemies associated with the genus *Opuntia* totalled 150 insects, 52 of which were imported into Australia and subjected to host-specificity testing, while only 21 were released. Of the agents released, only four (19%) were considered to contribute effectively to the control of the weed (Walton 2005 in Raghu and van Klinken 2006). Although the biological control of *O. ficus-indica* is regarded as highly successful, the project was criticized for its high risk of non-target effects and the time taken to control the weed (Raghu and van Klinken 2006).

Biological control practitioners have identified the need for executing more detailed agent selection procedures, with a number of authors' attitudes being that the population dynamics of the target weed needs to be the primary determinant in agent selection (Briese 1993,

McEvoy and Coombs 2001, Sheppard 2003, Raghu and van Klinken 2006). The biological control project targeting *Onopordum* sp., a hybrid between *O. acanthium* and *O. illyricum* (O'Hanlon *et al.* 1999), in Australia is an example of a recent project in which extensive research was carried out on agent selection before agent importation, to limit the risk of non-target effects (Briese 2006). The agent selection process is described by Briese (2006). The ecology and population dynamics of the weed species guided the decision on which agents should be introduced for effective control. Agents were selected based on their potential to reduce the soil seed bank, biomass and vigour of *Onopordum* sp. The selection of these agents was decided using results from surveys executed throughout the Mediterranean basin, quantitative and experimental field data, experimental impact studies and agent biology observations (Briese *et al.* 2002 in Briese 2006). Of nine potential agents identified, eight were exported to Australia for host range testing, seven of which were released and four of which became established. Despite the research efforts, time and money that were dedicated to this biological control project, three of the seven agents that were released still failed to establish. Briese (2006) concluded that another agent 'filter' needed to be included in biological control programs, namely that of the 'likelihood of establishment'.

The importance of biotic factors (e.g. plant demography, agent-host interactions, agent release density, timing of release and host density) in determining the success of a biological control agent have been extensively discussed and included in biological control programmes (e.g. Cameron *et al.* 1993, Hoelmer and Kirk 2005, Shwab and Raghu 2006). However, the implications of abiotic factors, such as climate and substrate in agent success have, until recently, mostly been unexplored (Raghu *et al.* 2006, Schwab and Raghu 2006). Releasing agents that establish over a large part of the weed's range obviates the release of additional

agents and not only increases the effectiveness of the biological control project but also decreases the risk of non-target effect, as the more agents that are released the greater the chances of unpredictable non-target effects (Schwab and Raghu 2006).

Historically, little emphasis has been placed on climatically matching biological control agents to release sites. This has led to agents not establishing at release sites and projects being abandoned due to perceived agent failure (e.g. Day and McAndrew 2002). Modern biological control research not only places more emphasis on the ecology of the weeds and the evolution of the interactions between the agents and their target weeds (e.g. Blossey and Notzold 1995, Kriticos *et al.* 1999, McEvoy and Coombs 1999, van Klinken and Edwards 2002, Raghu and van Klinken 2006) but also on climatically matching agents to release sites (Hoelmer and Kirk 2005). Protocols need to be developed to ensure that biological control programs are carried out in the most efficient manner, in terms of money, time and effective control.

5.4. CLIMATE MODELLING – WHY IS IT IMPORTANT?

The need for effective agent selection, in terms of its ability to both establish and control the weed, is important for two reasons. First, the environmental crises facing South Africa and the rest of the world have resulted in a rapid decrease in the level of tolerable risk that is placed on the environment (Sheppard *et al.* 2003) making it extremely important for biological control to limit the number of agents released, particularly ineffective ones, so decreasing its 'hit-and-miss' reputation (McClay and Balciunas 2005). Second, the increased time and cost required to carry out stringent host range testing means that as few agents as possible should be selected for these tests (Briese 2006). Climate matching and modelling currently allows

practitioners to obtain some idea of the role that climate will play in determining a biological control agent's potential for establishment (Sutherst 2003). However, a procedure needs to be set out in which the full potential of both climate matching and modelling will be achieved so that ineffective agent releases are reduced. As the primary aim of biological control is 'to obtain effective, permanent control of a pest' (Hoelmer and Kirk 2005), successful agent establishment is vital.

Of the available climate modelling programmes, CLIMEX is deemed to be the most appropriate for biological control situations. In order to develop the most effective climate modelling protocol, the procedures within climate modelling, using CLIMEX, have to be examined and the most effective methods selected. The first choice facing the modeller is whether to perform reverse or mechanistic modelling.

Although CLIMEX was first developed for reverse modelling, there is very rarely enough data on the distribution of the agent in its native range, for this to be performed. In order for reverse modelling to be applicable, Zalucki and van Klinken (2006) have suggested that when agents are surveyed and collected in their native range, records of localities where the agents are present, but also absent, should be compiled. They also argued that systematic sampling of potential agents on the host plant should be carried out across its entire climatic distribution. This is based on the assumption that agent species will be abundant in areas which are climatically suitable and less abundant, or absent, in areas in which the climate is limiting (Zalucki and van Klinken 2006). Although this method of sampling will increase the known distribution of the agent in its native range, it would be extremely time consuming and expensive. The method also requires that extensive data on the native distribution of the weed

needs to be available, as the more information available, the more robust a model becomes (Zalucki and van Klinken 2006). Therefore, except in the rare cases where there are extensive data available on both the weed's and the agents' native distribution, mechanistic modelling remains the most applicable form of modelling for most biological control programmes. Mechanistic modelling was thus used in this thesis in Chapter 4.

To ensure that reliable results are obtained from mechanistic modelling, the most effective parameters for determining a species' distribution need to be identified and a prescriptive set of methods developed for determining these physiological parameters. Although this is not possible for all species, it is possible to develop a set that will be feasible for a large number of species. This will not only improve model reliability and resource usage, but will also make comparisons between species and models possible. The various model parameters are discussed below.

5.5. MODEL PARAMETERS

This thesis has examined a number of physiological parameters to aid in developing a mechanistic model to predict the potential distribution of *Z. bicolorata* in South Africa, in relation to climate. The ease of determination of the various parameters, their applicability and the manner in which they could be determined was varied. A brief conclusion of the most useful parameters for mechanistic modelling is given below.

5.5.1. Thermal Limits

The upper lethal limit (ULT_{50}) and the critical thermal maximum (CT_{max}) of a species are regularly too high to be used as a predictive tool (Byrne *et al.* 2003), as was the case for *Z. bicolorata* (Chapter 2). For most biological control programmes it is therefore not a significant requirement that these be determined.

The lower thermal limits (LLT_{50} and CT_{min}) do commonly have predictive use as they are good indicators of how survival will be limited in winter (Byrne *et al.* 2002). There are, however, pro's and con's to both of these lower thermal limit parameters. Critical thermal minimum experiments are far less costly in time and the number of individuals required for experimentation compared to LLT_{50} (e.g. *Z. bicolorata*: LLT_{50}, n = 260; CT_{min}, n = 60). However, it is easier to determine the LLT_{50} of the immature life stages than it is to determine the CT_{min} as it is easier to monitor death than self-righting ability in these stages. The accuracy of the LLT_{50} data may also be greater than data collected in CT_{min} experiments, for the same reason, especially if there are multiple observers recording the data. The accuracy of CT_{min} experiments is further criticised as CT_{min} results vary greatly with acclimation and cooling rate (Terblanche *et al.* 2007). Even short confinement in temperature-regulated quarantine has been shown to alter considerably the results of CT_{min} experiments (Terblanche *et al.* 2007). The LLT_{50} is criticised because of the accumulative effect of cold stress - when the LLT_{50} is reached, 50 % of the population will already be dead as a result of thermal wounding (Byrne *et al.* 2002), with the end result that population survival is greatly affected.

The applicability of LLT_{50} data may vary depending on species and the climate of the country of introduction. For *Z. bicolorata* in South Africa, the most predictive parameter is currently the CT_{min}, as no areas in which parthenium occurs experience average winter temperatures as low as the LLT_{50} (Chapter 2). No matter which physiological limit is used to obtain predictions of the potential distribution of a species, the accuracy of the limit and chance of establishment will be improved if field-fresh individuals are tested and released. This will decrease the time spent confined in quarantine facilities, thereby decreasing the negative effect on thermal tolerance.

5.5.2. Developmental rates

The accumulated heat required by a species to complete development is a vital indicator of the potential secondary production of a population (Hirst *et al.* 2005). In biological control, the degree-day model is used to predict sites at which a species will have sufficient accumulated heat to complete development and also sites at which population growth will fail (Chapter 3). The model can also identify sites at which population growth has the potential to be rapid, thereby earmarking these sites as 'optimum' release locations. In climate modelling it is important that both the thermal limits and the developmental rates are determined for a species, as although thermal limits give an indication of which locations will be suitable for population survival, they give no indication of the potential population growth at those locations.

A well planned biological control programme would involve determining the thermal limits of potential agents concurrently with host-specificity testing. This would give practitioners an

indication of which agents have the potential to survive in the new range early on in the project. If the agents are found to be thermally unsuitable, then priority can be given to other potential agents and host-specificity testing discontinued. There is also the advantage that the individuals being tested for cold tolerance would be 'fresh' from the field, so reducing the chances of them having become acclimated to artificial quarantine rearing conditions (as discussed in Chapter 2).

5.6. CONCLUSIONS FROM CLIMATIC MODELLING OF *ZYGOGRAMMA BICOLORATA* IN SOUTH AFRICA

From the climatic modelling performed on *Z. bicolorata*, it is predicted that the agent will be able to establish throughout the range of parthenium in South Africa (Chapter 4). The chances of agent establishment will be further increased by choosing sites with high average weekly growth indices. It is hypothesized that at these sites, the beetle should readily establish, undergo population growth and disperse to new areas of infestation. It is hoped that by choosing these 'optimum' release sites, the time required for visible population growth will not be as protracted as it was in Australia and India (Basappa 1997, Dhileepan and McFadyen 1997, Jayanth *et al.* 1997). Given that, despite these time lags, *Z. bicolorata* was effective in curbing the spread and vigour of the weed in Australia and India (Jayanth and Geetha Bali 1994, Jayanth *et al.* 1997, Dhileepan *et al.* 2000), it seems likely that the beetle could make a substantial contribution towards controlling parthenium in South Africa.

Although the CLIMEX model predicts beetle establishment and survival throughout the entire range of parthenium in South Africa, it must be remembered that it is based on climate alone

and is therefore predicting the insect's fundamental niche. The realised niche will only be determined by considering climate and *Z. bicolorata*'s interaction with other abiotic and biotic variables. The distribution based on the realised niche is therefore most likely to be smaller than that based on the fundamental niche. It was also for this reason that 'optimum' release sites were chosen, as the higher the predicted long term survival, the greater the chance of the site falling within the beetle's realised distribution. If there are areas of parthenium invasion in which *Z. bicolorata* does not establish, other control options need to be considered. These control options could either be mechanical, chemical or the introduction of another biological control agent. If another biological control agent is deemed necessary, then its thermal parameters will similarly need to be determined and modelled before it is released, so ascertaining whether it will indeed be able to establish in the areas where *Z. bicolorata* failed.

5.7. CLIMATE CHANGE AND MODELLING

The effect of climate change on the future distribution of both parthenium and *Z. bicolorata* is another important factor that needs to be considered in the management of parthenium. Climate change will very likely affect the distribution and invasive potential of parthenium, thereby affecting the management and control of the weed.

The effects of climate change on the South African climate is expected to vary throughout the country, with a number of future climate scenarios having been developed (e.g. Walker and Schulze 2008). Regardless of how climate change will alter the climate in South Africa, it is agreed that it will have a substantial impact on the distribution of invasive species (Midgley *et al.* 2003, Mgidi *et al.* 2007).

The effect of climate change on alien invasions has become a popular research topic (e.g. Midgley *et al.* 2003, Root *et al.* 2003, Logan *et al.* 2006). Climate change is predicted to have a positive effect on the growth and spread of parthenium in South Africa. It has been shown that plants grown under high CO_2 levels grow more vigorously, generally producing taller and larger individuals (Ackerly 2003). As parthenium is a C3 plant, it is expected to benefit from increased CO_2 levels and compete effectively in fields dominated by C4 grasses (Adkins *et al.* 1997). Increased precipitation is predicted to be beneficial to parthenium population growth as the plant is very abundant in irrigated areas in India (Patil *et al.* 1997). Increased precipitation leading to floods is also expected to aid in the dispersal of seeds (Adkins *et al.* 1997) as is believed to have occurred during Cyclone Demoina in KwaZulu-Natal in the 1980s (Strathie *et al.* 2005).

In areas where climate change leads to warming and altered precipitation patterns, the abundance and distribution of biological control agents and their ability to colonize new habitats is also expected to be affected (Sutherst 2000). Warming, resulting from climate change, is predicted to cause increased agent activity due to an extended growing season of the target weed, shorter generation times, increased growth and developmental rates, altered time of emergence and geographic distribution, increased niche differentiation and evolution and reduced overwintering mortality (Portner *et al.* 1991, Ackerly 2003). However, it is still not entirely certain whether climate change will have positive or negative effects on insect populations. Logan *et al.* (2006) suggest that a variation or decrease in daily temperature amplitude will result in insects not meeting their nutritional needs. However, this may be counteracted by an increase in food quality and digestion rate resulting from increased temperatures and a change in Carbon: Nitrogen ratios (Yang and Joern 1994, Logan *et al.*

2006). All of these factors will have an effect on agent distribution and therefore need to be studied further so that they can be considered when building a more refined climatic model for the potential distribution of an agent in the future.

5.8. FUTURE OF WEED BIOLOGICAL CONTROL

The future of weed biological control relies on the continuation of its good safety record. Also, a higher record of success in controlling the target weeds will do much to improve its public profile. For this to happen, a more stringent *modus operandi* needs to be implemented. Climate modelling needs to be a component of this new protocol in order to decrease the chances of agent releases failing because of climatic constraints.

Although pre-release efficacy assessments have been shown to be beneficial to biological control programmes, a paper by McClay and Balciunas (2005) identifies reasons for practitioners being reluctant to carry out these assessments. First, it is felt that agent impact studies are financially unattractive, with the money better spent on host-range testing (McFadyen 2003). Second, is the worry of a 'false-negative' situation, leading to an agent which could have been effective being rejected due to laboratory efficacy studies wrongly concluding that an agent will be ineffective in the field. However, McClay and Balciunas (2005) argued against these criticisms by demonstrating that pre-release efficacy assessments can make a programme more cost-effective by reducing the number of ineffective field releases.

A number of procedures are recommended for inclusion into biological control programmes to achieve a higher chance of success:

1. Climate matching should be carried out between the native range of an invasive weed and the area of invasion to ensure that candidate agents are surveyed for and collected in appropriate climatic regions. This also improves the chance that the life history of the agent is strongly synchronised with the host (Cameron *et al.* 1993).

2. Lower thermal limit experiments should be carried out on the agents collected in the survey at an early stage. This can be done either in the country of origin or in quarantine directly after importation into the new country. These are quick experiments which will identify which agents should be exposed to host range testing and screen out potentially maladapted ones.

3. Selected agents should then undergo host range testing at the same time as developmental rate experiments. Thereafter, climate modelling can be completed during this time.

4. Following the completion of host-range tests, a field-fresh culture should be imported from the host country for mass rearing and releases at 'optimum' sites as identified by the climate model. This new culture should be re-tested using a shorter list of test plants.

5. Areas of invasion identified by climate modelling as unsuitable for the biological agent should be acknowledged and research carried out into which control method would be most

suitable for the area. Releases should nevertheless be carried out in these ‘sub-optimum’ areas to further check the accuracy of the climatic model.

Although the above procedures would increase the costs spent on a single potential agent, they have the potential to decrease the number of agents undergoing host-range testing as well as the number of agents that are released but fail to establish. This would go a long way towards decreasing the reputation of biological control as a ‘hit-or-miss’ science.

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