Climatic suitability of *Dichrorampha odorata* Brown and Zachariades *(Lepidoptera: Tortricidae)*, a shoot-boring moth for the biological control of *Chromolaena odorata* (L.) R.M. King and H. Robinson (Asteraceae) in South Africa

THESIS

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

at

Rhodes University

By

Slindile Brightness Nqayi

Department of Zoology and Entomology

Rhodes University

P.O. Box 94

Grahamstown

6140

March

2019

ABSTRACT

Biological control using natural enemies introduced from the native range is an integral component of the management of *Chromolaena odorata*, a serious invader in the eastern regions of South Africa. A number of biological control agents for *C. odorata* have been released in South Africa, and one of them, *Dichrorampha odorata*, has failed to establish. To understand if *D. odorata* failed to establish due to climate incompatibility, its thermal physiology was investigated. Thermal tolerance data were used to determine the developmental thresholds and number of generations that *D. odorata* is capable of going through in South Africa per year. These predictions were generated using CLIMEX temperature data and the degree-day parameters K and t_0. Developmental time decreased with increasing temperatures ranging from 20 °C to 30°C, with immature stages not able to complete development at 18°C and 32°C. The developmental threshold, t_o, was determined as 8.45 °C with 872.4 degree-days required to complete development (K), indicating that *D. odorata* is capable of producing a maximum number of 6.5 generations per year in South Africa. The CLIMEX data indicated that the east coast regions of South Africa, which are the heaviest invaded areas by *C. odorata* in South Africa, were climatically most suitable for *D. odorata* t_o. *D. odorata* lower (LLT_{50}) and upper (ULT_{50}) lethal temperatures were -4.5°C and 39.64°C for larvae and 1.83 and 41.02°C for adults, and *D. odorata* adults were able to maintain locomotory functioning at 4.4 to 43.7°C, respectively. Acclimation at low and high temperatures indicate that when *D. odorata* was kept at a lower temperature of 20°C for 7 days, it became tolerant to warmer and cooler temperatures (1.95 and 44.41°C) when compared to *D. odorata* reared at 25°C (3.36 and 43.67°C) and 30°C (5.92 and 42.93°C). *Dichrorampha odorata* is therefore climatically suitable for release and should establish in South Africa to control *C. odorata*. The establishment and persistence of *D. odorata* will not be limited by climatic conditions but rather the distribution of its host weed, *C. odorata* in South Africa. Also, this study presents a decision-making protocol for the release of *D. odorata* to allow better performance in the field.

Keywords: *Chromolaena odorata*, *Dichrorampha odorata*, thermal physiology, temperature, thermal limits, CLIMEX, climate.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following people who have contributed in various ways to conduct and complete my MSc:

- My co-supervisor Dr Costas Zachariades for believing in my potential, guidance throughout my research, for his time and always available to see progress and advise.
- My supervisor Prof. Julie Coetzee and co-supervisor Prof. Martin Hill for also believing in my potential and guidance to how to design my experiments and financial assistance for my tuition. Dr Osariyekemwen Uyi and Dr Frank Chidawanyika who were my mentors, motivated me and believed in me, also with data analysis and trials designs, and also Dr Andrew McConnachie for assisting with CLIMEX, Dr Candice Owen for assisting with running some trials and Ms Pippa Muskett for always being willing to assist me with mapping.
- The staff of the ARC-PHP at Cedara for their assistance, especially Derrick Nkala (former ARC employee), Samora Mqolombeni for planting, Pearl Mhlongo (former ARC employee) and Sthembiso Dlomo.
- My family for their love, support and patience with me sacrificing family time to focus on writing up. Especially my husband Cyril Nqayi and sisters, Elicia, Nolizwi and Boniswa Sithole who were always there.
- A special thanks to the late Lynette Khumalo who played a big role in my life not just as a colleague but also as a mother.
- Lastly Rhodes University and Agricultural Research Council (ARC) for allowing me to study towards my MSc while I was an ARC full time employee, and DEA: NRMP for funding the *Chromolaena odorata* biocontrol project at ARC-PHP.

Table of Contents

List of Figures

Figure 4.1: Average maximum (dark colour) and minimum (light colour) temperatures for two locations each in South Africa (brown) and Jamaica (green) close to where *D. odorata* was collected (Jamaica) or released (South Africa). Data sources: Uyi *et al*. (2017), South African Weather Service (South Africa); https://en.climate-data.org/north-america/jamaica/gordon-town/gordon-town-987260/#climate-graph (Jamaica).

List of Tables

CHAPTER 1: GENERAL INTRODUCTION

1.1 Invasive alien plants

Invasive alien plants (IAPs) are non-native plant species growing in an undesirable place at an undesirable density, which spread rapidly, increasing their distribution and density in their new range (Daehler, 2003; Culliney, 2005). These plants have become a worldwide problem (Richardson and van Wilgen, 2004; Shabbir and Bajwa, 2006) where they have been introduced into new ranges, sometimes unintentionally (Culliney, 2005) but mostly intentionally (Daehler, 2003). Reasons for deliberate introductions of plants into new ranges include use as ornamentals, agroforestry species, crops, hedge plants and fodder (Mgidi *et al*., 2007). Human disturbances in natural ecosystems such as habitat fragmentation, agricultural or commercial practices, have worsened the problem by creating niches for plant invasions worldwide (Culliney, 2005). Such invasions have been problematic for hundreds of years but the rate at which they are occurring is increasing, mainly due to human population growth (Pimentel *et al*., 2001), and an increase in migration, international travel and trade (Culliney, 2005). In their new range, IAPs use more resources (sunlight, water, nutrients and space) than native plants and become a problem by out-competing native vegetation (Pimentel *et al*., 2001; Culliney, 2005). Some IAPs release allele chemicals, resulting in allelopathic effects in the form of suppressed growth of other plants, including native plants (Pimentel *et al*., 2001; Richardson and van Wilgen, 2004; Culliney, 2005; Chown and Terblanche, 2007). These allelopathic effects alter the environment (Javaid *et al*., 2007), thus affecting organisms and components that are essential for healthy soils.

Following rapid spread beyond the areas of introduction, invasive species pose serious threats to ecosystems (Enright, 2000), human health and the economy of the country (Daehler, 2003; Culliney, 2005). Within the introduced range, the rapid spread of IAPs in their introduced range is largely attributed to the absence of their natural enemies that can suppress their growth (Zimmermann *et al*., 2004). This is according to what is now widely regarded as the Enemy Release Hypothesis (ERH) (Keane and Crawley, 2002; Colautti *et al*., 2004; Liu and Stiling, 2006). Indeed, biogeographical analyses among systems have shown a significant decrease in abundance and diversity of natural enemies in introduced ranges compared to their native range (Colautti *et al*., 2004). However, several studies have also reported that the

attributes of the invading species such as high propagule pressure and net reproductive potential aid the competitiveness of IAPs in their introduced range (Theoharides and Dukes, 2007). The presence of abiotic filters such as rainfall and temperature, matching or even more suitable than the native range, has also been argued as factor aiding plant invasions (Theoharides and Dukes, 2007; Grarock *et al.*, 2013). In nature, these factors likely interact, resulting in various scales of invasion.

The influx of alien plants into South Africa started in the mid-1600s (Moran *et al*., 2005) and has continued since. By 2001, South Africa had at least 200 major IAP species (Henderson, 2001), including several significant shrubs such as *Lantana camara* L. (Verbenaceae), *Chromolaena odorata* L. King and Robinson (Asteraceae), *Opuntia* species (Cactaceae) (Richardson and van Wilgen, 2004) and *Parthenium hysterophorus* L. (Asteraceae) (Olckers, 2004). Since then, this number is reported to have increased to more than 380 species (NEMBA, 2014; Henderson and Wilson, 2017).

Most IAPs invading South Africa are originally from Australia, South America and North America (Zimmermann *et al*., 2004). Rapid above-ground biomass accumulation increases evapotranspiration and thus water loss. Such an increase arises because of the greater height and root depth of many IAPs compared to the native plants that they replace. This results in IAPs decreasing surface water runoff, groundwater and bush fires in South Africa, which is a serious issue as South Africa is a water-scarce country (Chamier *et al*., 2012). The 'Working for Water' Programme (WfW), which is now referred to as the Natural Resource Management Programme (NRMP) of the Department of Environmental Affairs (DEA) was established in 1995, aiming to manage IAPs in South Africa (Zimmermann *et al*., 2004; Moran *et al*., 2005; Mgidi *et al*., 2007). The DEA supports integrated methods to manage IAPs through mechanical and chemical control together with biological control (Zimmermann *et al.*, 2004; Turpie *et al*., 2008).

1.2 Types of IAP control methods

1.2.1 Mechanical control

Mechanical control of IAPs involves slashing, burning, ploughing or hand pulling. This method is labour intensive, may be expensive for dense infestations and is only effective in

the short term, because although it reduces the standing biomass, often profuse regrowth occurs from rootstocks (Rusdy *et al*., 2013). Besides being labour intensive, this method may also be ineffective e.g. uprooting or hoeing of *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) has been considered ineffective because small pieces of the plant are left behind and quickly regenerate (McConnachie *et al*., 2011). For mechanical control to be successful, the method needs to be used in conjunction with other methods - for instance after slashing, herbicides may be used as chemical control (Zachariades *et al*., 2002).

1.2.2 Chemical control

Chemical control involves the use of herbicides to control IAPs. Guidance in selecting a correct herbicide for a targeted plant, and using it in the correct manner, is essential. This is because herbicides have the potential to contaminate ground and surface water (Masters and Sheley, 2001). If inappropriate herbicides are used incorrectly, they may be ineffective, resulting in wasted resources. Furthermore, they are expensive, and the costs of repeated applications may be prohibitive (Masters and Sheley, 2001). Herbicidal control of some IAPs is seen to be partially successful, e.g. in South Africa, *C. macrocephalum* has been controlled using herbicides, especially in KwaZulu-Natal province (McConnachie *et al*., 2011). However, it is still a concern that the herbicides may affect non-target plant species, and they have not been recommended for use in ecologically sensitive areas such as wetlands (McConnachie *et al*., 2011). Moreover, due to the extent of current invasions, herbicide use is impractical and too expensive on its own to control *C. macrocephalum* (McConnachie *et al*., 2011).

1.2.3 Cultural control

Cultural control aims to enhance desirable vegetation to minimise weed invasion. This method includes fire, grazing and revegetation/or reseeding (Masters and Sheley, 2001), and includes manipulating farming practices to suppress invasive plant growth, while promoting the development of the preferred plants (DiTomaso, 2000). Restoration of habitats after clearing of IAPs has been undertaken in South Africa, particularly in the fynbos biome (e.g. Holmes *et al*., 2005).

1.2.4 Biological control

Classical biological control is the practice of controlling invasive species through the introduction of one or more natural enemies (insects, mites, pathogens and/or nematodes) from the plant's native range, to reduce populations of the weeds (McFadyen, 1998; Zimmermann *et al*., 2004). Exotic natural enemies that have potential to be biological control agents are imported from the weed's country of origin into the country where the weed is invading. The potential agents are kept in quarantine where they are screened for diseases and parasites. The natural enemies are then tested for host specificity in quarantine on the targeted weed and plants that are closely related to the targeted weed, to ensure that the agent is safe for release and that it will not negatively affect plant species other than the targeted weed (McFadyen, 1998). Once all laboratory tests are complete and indicate that the agent is adequately host specific, an application to release the agent is submitted to the designated government agency. If permission to release is granted, the agent is mass reared in the laboratory and released onto selected field infestations of the weed. Post-release evaluation is critical to determine if the agent has established in the field and whether it is having an impact on the weed and not on non-targeted plant species (Carson *et al*., 2008).

Classical biological control provides a viable solution to plant invasion, because it is self-sustaining and more cost-effective than conventional methods, since many established agents do not need re-application (Barratt *et al.,* 2010). Furthermore, biological control is environmentally friendly because it does not pollute natural resources or harm wildlife. However, it is often a slow process and requires supplementary control methods (mechanical and chemical). Weed biological control was first used in South Africa in 1913 with the release of a cochineal insect (*Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae) to control drooping prickly pear (*Opuntia monacantha* (Wildenow) Haworth (Cactaceae: Opuntioideae) (Zimmermann *et al*., 2004). Since then, biological control programmes have been very successful in South Africa (Moran *et al.,* 2005). Three hundred and fifty one (351) agents have been studied in South Africa since 1913, with the majority being insects and fewer being mites and pathogens (Klein, 2011 updated 2018 (http://www.arc.agric.za/arc-ppri/Documents/Table1-NaturalEnemiesAll.pdf). These agents were studied as potential biological control agents against 84 IAP species in South Africa (Zachariades *et al*. (2017). From the 351 potential agents, 93 species of biological control agents been established on 59

IAP, with 25 plant species still under investigation and establishment of biological control agents not yet confirmed on them (Klein, 2011; Zachariades *et al*., 2017).

1.2.5 Integrated control methods

Using two or more control methods in conjunction with one another can improve overall control and thus have a significant economic impact (e.g. Charudattan, 1986; Zachariades *et al*., 2009). Arguably, the most economical and sustainable control method is biological control, which uses host-specific natural enemies from the native range of the weed to suppress growth of the weed in an introduced country. However, it is often used as a longer-term, landscape-level control method, with mechanical and chemical control methods being used initially.

1.3 *Chromolaena odorata*

Chromolaena odorata, commonly known as chromolaena or triffid weed in South Africa, is a weedy scrambling perennial plant of the tribe Eupatorieae. This sparsely hairy shrub grows up to 4 metres or higher, forming dense thickets (Henderson, 2001; Foxcroft and Martin, 2002). *Chromolaena odorata* originated in the Americas, with a large range stretching from the southern USA to northern Argentina, and is now invasive and widespread in the humid tropics and subtropics of Africa, Asia and Oceania (Figure 1.1) (Zachariades *et al*., 2009). In South Africa, *C. odorata* was first observed to be naturalised in KwaZulu-Natal province in the 1940s (Foxcroft and Martin, 2002). Since then, the weed's distribution has increased to include the provinces of Mpumalanga, Limpopo and Eastern Cape (Foxcroft and Martin, 2002; Zachariades *et al*., 2011) (Figure 1.2).

The *C. odorata* biotype invasive in southern Africa (the "SA biotype") differs in appearance to the more widespread invasive biotype found in Asia, Oceania and other parts of Africa (the "AWA biotype"); it also appears to be more cold-tolerant and susceptible to fire (Zachariades *et al*., 2009, 2011). For many years, the origin of the SA biotype was not clear, but from 1997, increasing evidence pointed to islands in the northern Caribbean region (Zachariades *et al*., 2011). This was confirmed by molecular work which strongly supports a Cuban or Jamaican (two of the islands in the northern Caribbean) origin of the SA biotype (Paterson and Zachariades, 2013; Shao *et al*. 2018).

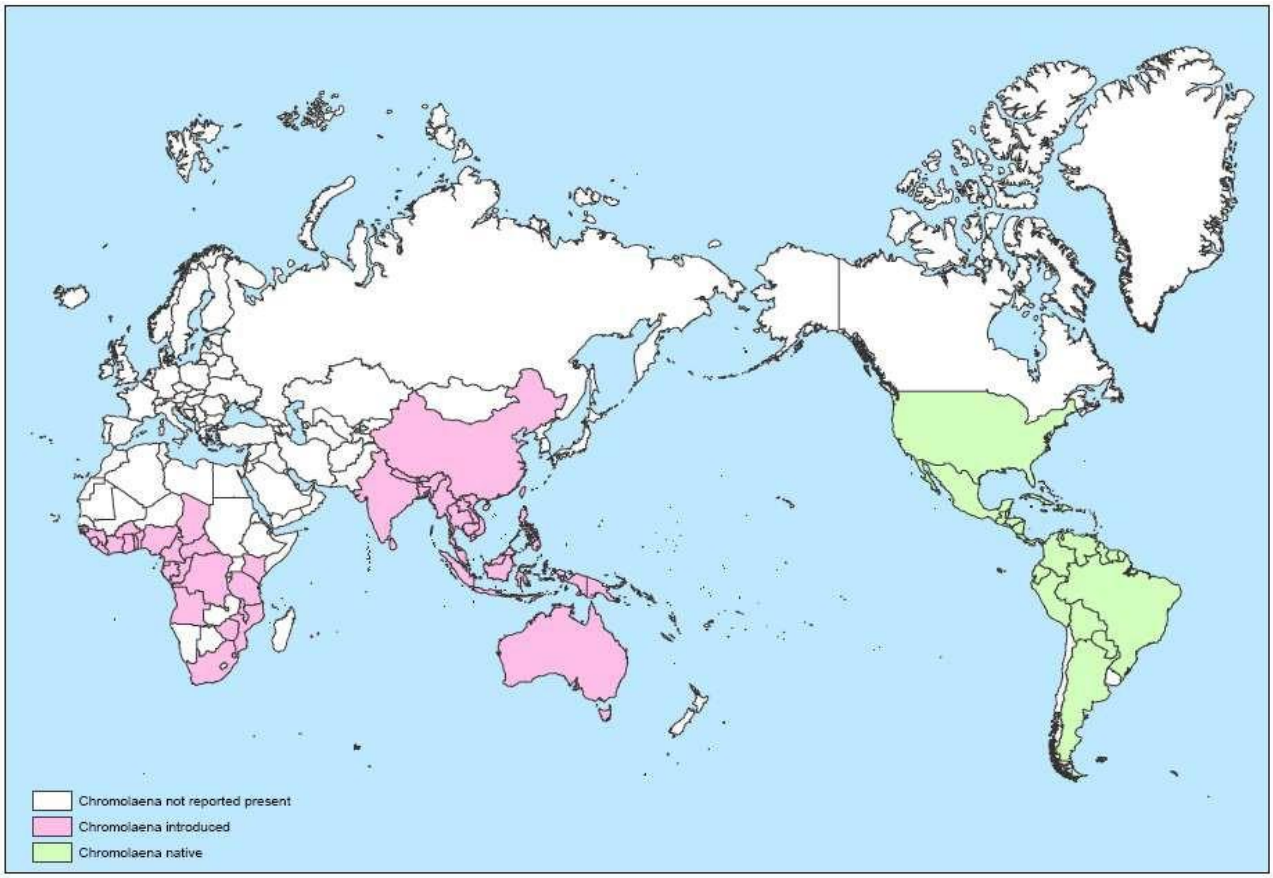

Figure 1.1: Worldwide *Chromolaena odorata* distribution – a version of this map was published in Zachariades *et al*. (2009) and was generated by Jimaima Le Grand (Queensland Department of Primary Industries and Fisheries).

1.3.1. Description of *Chromolaena odorata*

In South Africa, *C. odorata* can reach up to 5-10 m when supported by other vegetation, but 2-3 m when growing in the open. The leaves are light green to yellow, ovate to triangular, three-nerved from the base and glandular-dotted below (Henderson, 2001). When crushed, the leaves exude a strong smell of paraffin or turpentine. The stems are photosynthetic and branches grow opposite to each other (Figure 1.3). In southern Africa and the rest of the southern hemisphere, the flower development is in June-July. The flowers are white (those in southern Africa) to pale blue. The seeds are lightweight and dispersed by wind, animals and vehicles. With its photosynthetic stems, the plant can form new shoots from axillary buds during the start of the rainy season and dispersed seed germinates to give rise to a new

generation (McFadyen, 1989). The weed can grow on different soil types, but preferably on well-drained soil (Zachariades *et al.*, 2009).

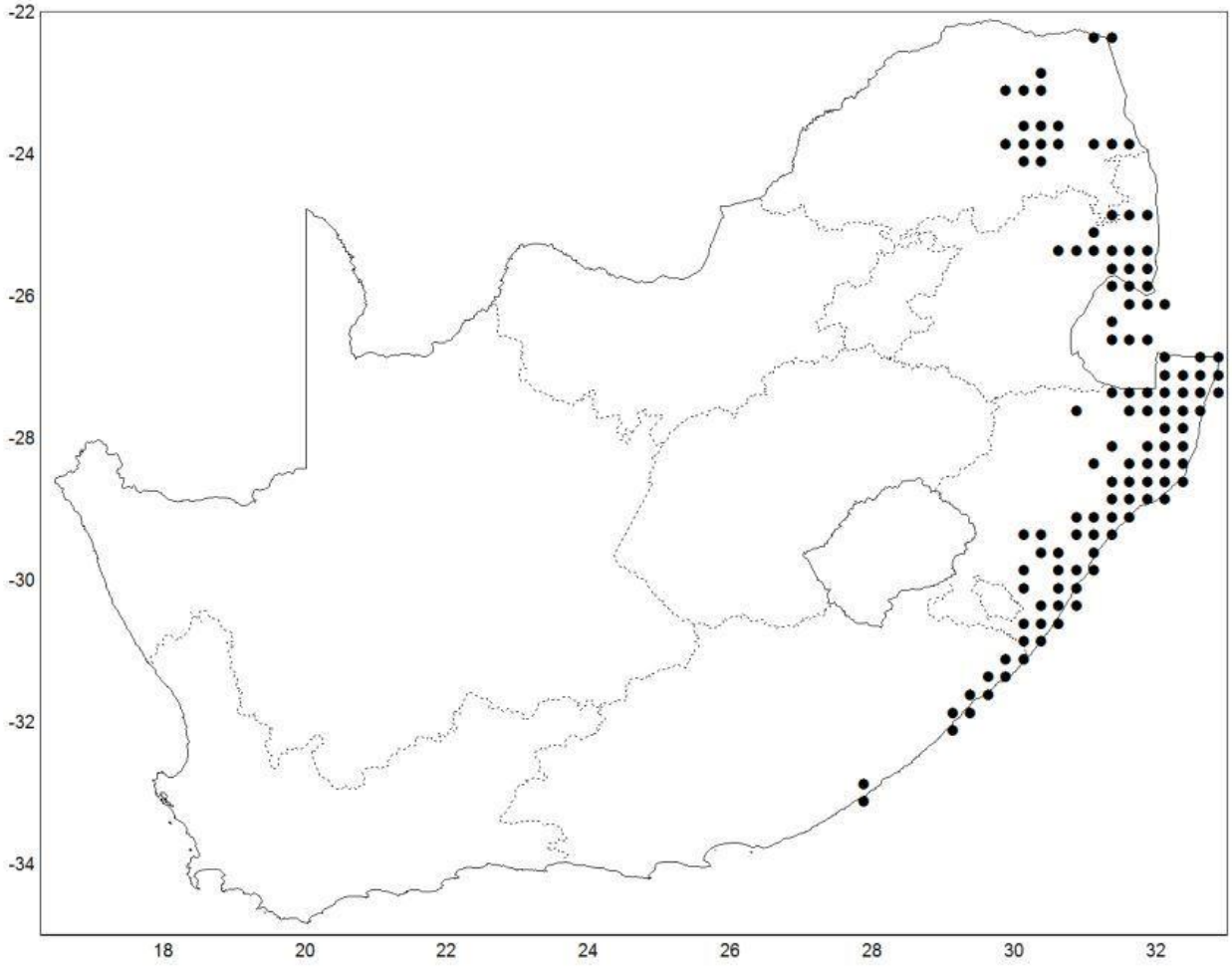

Figure 1.2: Areas invaded by *Chromolaena odorata* in southern Africa (Henderson, 2007).

1.3.2 Ecological and economic impacts

Chromolaena odorata has negative economic impacts when it invades commercial and subsistence agriculture such as crops and plantations, grazing lands and silviculture (Zachariades, *et al*., 2009). Since *C. odorata* requires high light levels for growth, natural forests are not usually invaded by the weed, but during forest degradation the weed invades, resulting in the suppression of native forest tree regrowth (Norbu, 2004; Zachariades, *et al*., 2009).

The weed forms thickets, which smothers native vegetation and prevents the free movement of wildlife and livestock, thus having impacts on biodiversity, conservation and animal husbandry in the areas where it is invasive (Goodman, 2003; Zachariades, *et al*., 2009). Human lives are also affected by *C. odorata*, not only by its effects on agriculture but also because it is a fire hazard, especially during the dry season (Macdonald, 1983; Goodall and Erasmus, 1996).

1.4 Biological control of *Chromolaena odorata*

Initial surveys and host-range testing of natural enemies for potential use as biocontrol agents on *C. odorata* were conducted in Trinidad (Cruttwell, 1974), when *C. odorata* was referred to as *Eupatorium odoratum* L. Species such as *Pareuchaetes pseudoinsulata* Rego Barros (mistakenly identified as *Ammalo insulata* (Walk.) (Lepidoptera: Erebidae: Arctiinae)) and *Apion brunneonigrum* Beguin-Billecoq (Coleoptera: Curculionidae) were tested for host specificity. More investigations were done and completed in 1973 on *Mescinia* sp. nr. *parvula* (Zeller) (Lepidoptera: Pyralidae) (subsequently re-identified as *Phestinia costella* Hampson (Solis *et al*., 2008), *Melanagromyza* sp. (Diptera: Agromyzidae) and *Acalitus adoratus* Kiefer (Acarina: Eriophyidae) (Cruttwell, 1974). Cruttwell (1974) showed that there was significant damage on *E. odoratum* caused by *Perasphondylia reticulata* Mohn (Diptera: Cecidomyiidae) and *Cecidochares* sp. (Diptera: Tephritidae) and suggested that these species may have potential as biological control agents. As could be expected, some of the insect species investigated during surveys were host–specific and some were not (McFadyen, 1988). Three of the species that were host-specific (*P. pseudoinsulata*, *A. brunneonigrum* and *P. costella*) were subsequently released in West Africa, Asia and Oceania, but only *P. pseudoinsulata* established, and even then, establishment was patchy (Zachariades *et al*., 2009) (Table 1.1).

From the late 1980s on, the Agricultural Research Council's Plant Protection Research Institute (ARC–PPRI) (now ARC-Plant Health and Protection) in South Africa imported insects and pathogens from the Americas that were damaging to *C. odorata* into quarantine for host-range testing (Kluge, 1991; Zachariades *et al*., 2011). This resulted in the release of four insect species (*P. pseudoinsulata*, *Pareuchaetes aurata aurata* (Butler), *Pareuchaetes insulata* (Walker) and *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae)) between

1988 and 2009, of which the last two established. Several other species were investigated but not released (Klein, 2011).

Before the origin of the SA biotype was found, the difference between this and the AWA biotype was a problem for biocontrol of *C. odorata* in South Africa, because agents were collected from forms of *C. odorata* different to the SA biotype, which caused compatibility problems between agents and this biotype. (Zachariades *et al*., 2009)

By the late 2000s, seven species of biological control agents had established in the Old World either intentionally or by accident (Zachariades *et al*., 2009; Zachariades, 2011; Winston *et al*., 2014, updated at https://www.ibiocontrol.org/catalog/agents.cfm?weed=55) (Table 1.1):

- *Pareuchaetes pseudoinsulata*, native to eastern Venezuela and Trinidad, is one of the *C. odorata* agents released worldwide and established in countries such as Sri Lanka, Malaysia, Guam (USA), Ghana and Indonesia. However, the moth failed to establish in Vietnam, Thailand and South Africa, and also in Côte d'Ivoire and several other countries where it was released (Zachariades *et al*., 2009).
- *Pareuchaetes insulata*, native to Colombia though Central America to the southern USA (Texas and Florida) and the Caribbean islands, it was subsequently released in South Africa in 2001 and establishment was confirmed (Zachariades *et al*., 2009).
- *Cecidochares connexa* Macquart (Diptera: Tephritidae), a fly with a native range including Trinidad, Mexico and Bolivia, established in a number of countries including Indonesia, Papua New Guinea, Ghana and Côte d'Ivoire, where it has proved extremely damaging. However, it failed to sustain a population in quarantine on the southern African *C. odorata* biotype (Zachariades *et al*., 2009).
- *Actinote thalia pyrrha* Fabr. and *A. thalia thalia* L. (Lepidoptera: Nymphalidae) from Central and South America were not released in South Africa because the host-specificity tests showed the insects feeding on *Mikania capensis* DC. (Asteraceae: Eupatorieae) which is indigenous to South Africa. However, they established in Indonesia.
- *Calycomyza eupatorivora* is a fly whose larvae mine *C. odorata* leaves, with a native range including Jamaica, Hispaniola, Argentina, Venezuela and Guadeloupe. The agent was released and established in South Africa (Zachariades *et al*., 2009).

- *Acalitus adoratus*, native to countries including Brazil, Bolivia and Trinidad was accidentally released in the Philippines and now occurs in Asia and Oceania (Zachariades *et al*., 2009).

In warmer, more humid parts of West Africa, Asia and Oceania, the combination of *P. pseudoinsulata* and *C. connexa* has proved sufficient to bring the AWA *C. odorata* biotype under an excellent level of biocontrol. However, in South Africa, because of the failure of some of the *C. odorata* agents to breed in quarantine or establish in the field, probably due to biotype preference or climate incompatibility, and the limited effectiveness of the two species that did establish, there was still a need to release more agents. Agents released since 2011 include the stem-boring weevil *Lixus aemulus* Petri (Coleoptera: Curculionidae), the longhorn beetle, *Recchia parvula* (Lane) (Coleoptera: Cerambycidae), and *Dichrorampha odorata* Brown & Zachariades (Lepidoptera: Tortricidae), a stem-tip boring moth from Jamaica and Cuba (Strathie and Zachariades, 2004) which is the subject of this thesis.

Lixus aemulus was collected in north-western Brazil. Adults feed on young leaves and females insert eggs singly into green stems containing pith (parenchyma cells forming the medulla). Larvae bore inside the stem for 20-30cm and pupate inside the stem, boring out as adults. Larval boring decreases stem growth and flowering (Kluge & Zachariades, 2006). It was first released in 2011 and although it has persisted in the field, numbers are low. *Recchia parvula*, a univoltine longhorn beetle from Argentina, has adults that feed on shoot tips of *C. odorata*, leaving the shoot tips wilted. After mating, the adult female creates holes below the damaged shoot tips, into which they oviposit. Eggs hatch and larvae feed inside the stems, leaving frass and small holes where the larvae have passed, which eventual destroying the stem. Mature larvae overwinter and later pupate in the root crown, and adults eclose in the springtime. The insect can be extremely damaging to young plants, leaving only a stump in the soil. It was released in South Africa in 2016. Another agent, the stem-galling *Polymorphomyia basilica* Snow (Diptera: Tephritidae) from Jamaica, is still in quarantine, host-specificity tests have been conducted and a release application submitted to the authorities.

1.5 *Dichrorampha odorata*

Dichrorampha odorata is a shoot-boring moth that was collected in Jamaica, and imported into South Africa's ARC-PHP quarantine laboratory at Cedara, KwaZulu-Natal (29°.32'45.5" S, 30°.16'17.7" E) in 2005. The moth was an undescribed species with no literature records until its description in Brown and Zachariades (2007). *Dichrorampha odorata* was considered to be a potentially good agent for control of *C. odorata* because the biotype invading South Africa has been conclusively demonstrated to originate from one of the Greater Antilles islands of the northern Caribbean, particularly Jamaica or Cuba (Paterson and Zachariades, 2013); the moth appears damaging to the plant; and, it is likely to increase and spread in the field because of its short life cycle and high fecundity (Brown & Zachariades, 2007). *Dichrorampha odorata* is easy to rear in the laboratory, and was consequently quite rapidly tested for host specificity. It was considered as a substitute for *P. costella*, long targeted as a biocontrol agent for South Africa and elsewhere, but which could not be reared in the laboratory.

1.5.1 Description

The *Dichrorampha* genus is characterized by a male forewing with a well-developed costal fold and dark dots along the termen of the forewing. In the female genitalia, the sterigma, seventh abdominal sternite and sclerotized posterior portion of the ductus bursa are fused (Brown and Zachariades, 2007). Adults are about 5 mm long, have cream and pale tan scales, a brown dorsum and grey-brown forewing (Figure 1.4f). As in most insects, the females are usually bigger than males (Dube, 2008).

1.5.2 Biology and host range

During surveys of potential biocontrol agents on *C. odorata* in Jamaica in the late 1990s, *D. odorata* was discovered feeding in the shoot tips of *C. odorata* (Brown and Zachariades, 2007). During oviposition, the female moths firmly attach eggs on the upper surface of *C. odorata* leaves (Figure 1.4a) close to a growing vegetative shoot tip. Eggs hatch after ~10 days at 22-28°C and 40-80% RH. Once the egg hatches (Figure 1.4b), the larva bores into the

Table 1.1: List of biocontrol agents released on *Chromolaena odorata* around the world. Adapted from Zachariades (2011), Winston *et al*. (2014) updated at https://www.ibiocontrol.org/catalog/agents.cfm?weed=55.

Species	Released	Established[2]
LEPIDOPTERA		
Actinote anteas (=*thalia thalia*) (Nymphalidae)	Asia: ID	Asia: ID
Actinote thalia pyrrha (Nymphalidae)	Asia: ID	Asia: ID
Dichrorampha odorata (Tortricidae)	Africa: ZA	Africa: ZA – too early to tell
Pareuchaetes aurata aurata (Erebidae)	Africa: ZA	-
Pareuchaetes insulata (Erebidae)	Africa: ZA	Africa: MZ, SZ, ZA
Pareuchaetes pseudoinsulata (Erebidae)	Africa: CI, GH, NG, ZA Asia: ID, IN, LK, MY, TH, VN Oceania: GU, MP, FM, PG, PW	Africa: BJ, GH, NG, TG? Asia: BN, ID, IN, LK, MY, PH Oceania: GU, MP, FM, PG, PH
Phestinia costella (Pyralidae) (=*Mescinia* nr. *parvula*)	Oceania: GU	-
COLEOPTERA		
Apion brunneonigrum (Brentidae)	Africa: GH, NG Asia: IN, LK, MY Oceania: GU	-
Lixus aemulus (Curculionidae)	Africa: ZA	Africa: ZA - too early to tell
Recchia parvula (Cerambycidae)	Africa: ZA	Africa: ZA – too early to tell
DIPTERA		
Calycomyza eupatorivora (Agromyzidae)	Africa: ZA Asia/Oceania: PG	Africa: SZ, ZA
Cecidochares connexa (Tephritidae)	Africa: CI Asia: ID, IN, TP, TH Oceania: GU, FM, MP, PG, PW	Africa: BJ, CI, GH, GN, LR, TG Asia: ID, IN, TP Oceania: GU, FM, MP, PG, PH, PW
ACARINA		
Acalitus adoratus (Eriophyidae)	-[1]	Africa: MU? Asia: BD, BT?, KH?, CN, ID, IN, LA, LK, MM, MP, MY, NP?, PH, SG, TH, TP, TW, VN Oceania: GU, FM, MH?, MP, PG, PW

[1]Released accidentally, possibly in Malaysia with *A. brunneonigrum*.

[2]Country abbreviations: BD = Bangladesh, BJ = Benin, BN = Brunei Darussalam, BT = Bhutan, CI = Côte d'Ivoire, CN = China, FM = Federated States of Micronesia, GH = Ghana, GN = Guinea, GU = Guam, ID = Indonesia, IN = India, KH = Cambodia, LA = Laos, LK = Sri Lanka, LR = Liberia, MH = Marshall Islands, MM = Myanmar, MP = Northern Mariana Islands, MU = Mauritius, MY = Malaysia, NG = Nigeria, NP = Nepal, PG = Papua New Guinea, PH = Philippines, PW = Palau, SG = Singapore, TG = Togo, TH = Thailand, TP = East Timor, TW = Taiwan, VN = Viet Nam, ZA = South Africa.

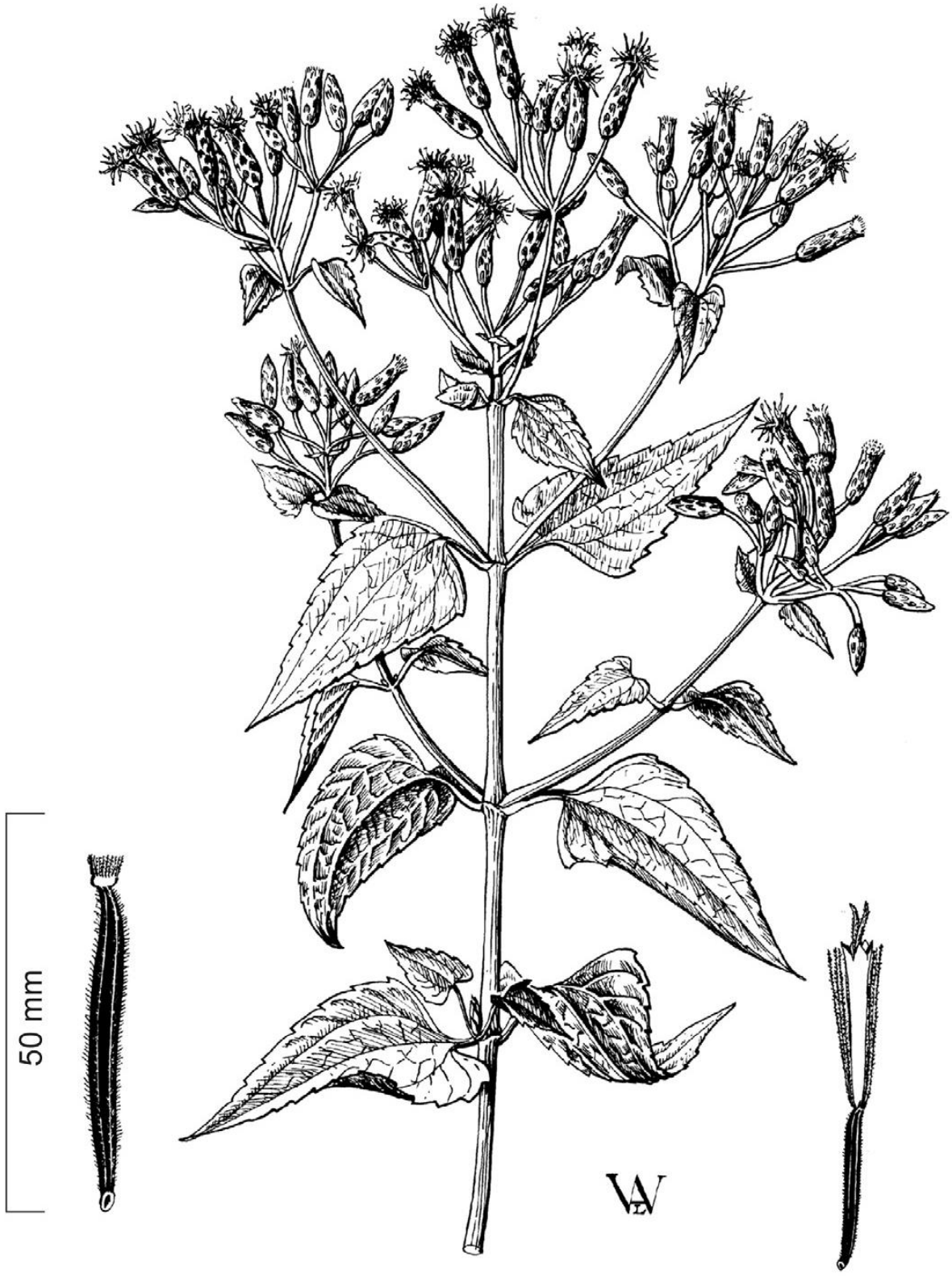

Figure 1.3: *Chromolaena odorata*. (Published in Henderson (2001), South African National Biodiversity Institute, Pretoria).

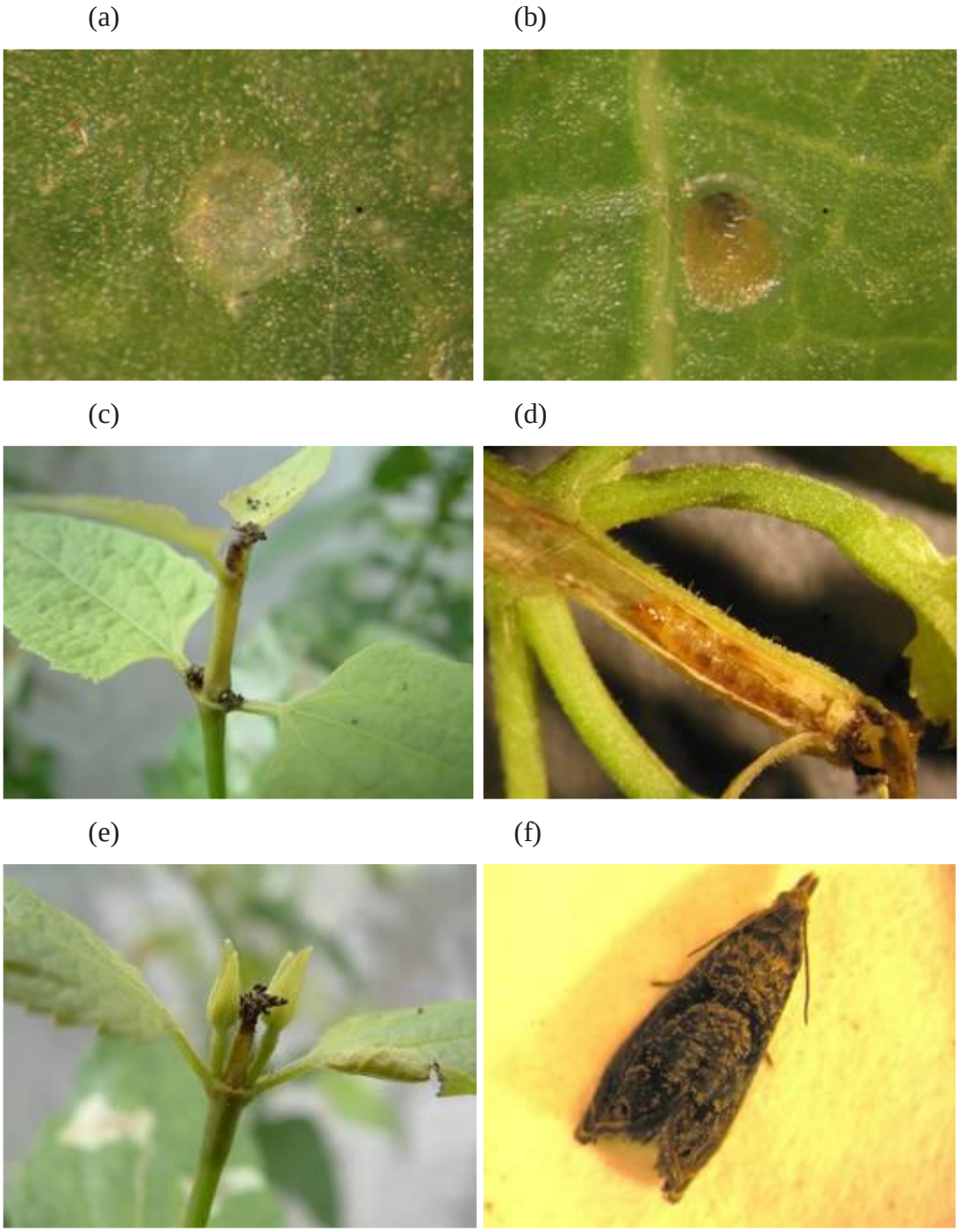

Figure 1.4: *Dichrorampha odorata* stages; (a) newly laid, clear egg; (b) mature egg, orange in colour with brown head capsule, about to hatch; (c) *D. odorata* larval damage on *C. odorata* shoot tip causing small galls/swelling in shoots; (d) *D. odorata* larva inside *C. odorata* shoot tip; (e) *D. odorata* pupa; mature larva exits plant and pupates inside leaf roll; (f) *D. odorata* adult.

shoot tip (one larva per tip), causing a slight swelling and discolouration (Figure 1.4c). Feeding by the larva kills the growth point of the shoot tip. The mature larva (Figure 1.4d) exits the gall to pupate in a tight leaf-roll (Figure 1.4e) (Zachariades *et al*., 2011; Dube *et al*., 2017). The *D. odorata* larval stage has six instars, pupae take 10 days to eclose and the adults live for 3 – 4 days. The complete lifecycle from egg laying to adult eclosion is about 41-45 days in the laboratory.

Preliminary host-specificity trials for *D. odorata* were conducted in 2006 (Brown and Zachariades, 2007), and full testing was subsequently conducted in the laboratory from 2009 to 2011 (Dube *et al*., 2017). Most of these trials consisted of larval no-choice tests, in which first instar larvae of *D. odorata* were inoculated onto test plants. Complete larval development was limited to *C. odorata*. Adult no-choice trials were subsequently conducted on a few test species selected because they were closely related to *C. odorata* or because some larval development was recorded on them; *D. odorata* mostly laid eggs on *C. odorata*, no larval damage was recorded on any test plants. These trials indicated that *D. odorata* was highly specific to *C. odorata*.

The first release of *D. odorata* in South Africa was in 2013, and almost 20,000 individuals of the moth have subsequently been released in KwaZulu-Natal, Mpumalanga and Limpopo provinces, but no establishment has been confirmed. Because it is unlikely that there is a biotype incompatibility problem, non-establishment is most likely either due to climatic incompatibility, predation/parasitism, or the biology of the insect which makes it susceptible to Allée effects and other stochastic events. The current study addresses the climatic incompatibility hypothesis.

1.6 Climatic conditions as one of the keys to insect establishment

Weed biological control agents are faced with challenges associated with climatic conditions (McClay, 1996; Day and McAndrew, 2002) when introduced into a new range, and this can affect their establishment (Byrne *et al*., 2002; de Guzman and Frake, 2007), dispersal (Sutherst and Maywald, 1985; Lekovic *et al*., 2001) and persistence in the field. Studying climatic suitability and thermal physiology of an agent enables one to understand how the insect will reproduce and respond at different temperatures experienced in the field. This helps to predict how an insect will perform when experiencing known climatic conditions and

also enables prediction of the species' eventual geographical range (Keena, 2006) because the insect laboratory thermal responses give an indication of their likely responses in the field under natural environments (Abdullah, 1961; Ramanand *et al*., 2017).

Environmental temperature appears to be one of the most important abiotic factors that affects the performance of insects. It has an effect on reproduction, locomotion, development time, persistence and establishment of biological control agents (Angilletta *et al.,* 2002). It is important for biological control agents to have thermal tolerances that match environmental conditions in the region of the targeted weed into which the agents are released. Thus understanding thermal tolerance of biological control agents may reduce cases where these agents fail to establish due to climatic incompatibility. The effect of climate and thermal tolerance on *D. odorata* is explained in detail in the next chapters.

1.7 Determination of insect thermal biology

1.7.1 Effects of temperature on developmental rate of *Dichrorampha odorata*

Abiotic factors such as temperature, rainfall and humidity determine persistence and establishment of biological control agents in their new range (Van Lenteren *et al*., 2006). Of these abiotic factors, temperature is one of the most important in determining the development of the immature stages of insects and has therefore become the subject of many studies (Terblanche *et al*., 2005). Generally, metabolic activities in ectotherms are limited by upper and lower temperature thresholds (Sutherst and Maywald, 1985). In weed biological control, when biological control agents are introduced into a new range, they may face challenges associated with climatic conditions, resulting in failure to establish (Day and McAndrew, 2002; Byrne *et al*., 2003; May and Coetzee, 2013). According to Byrne *et al*., (2003), Manrique *et al*., (2014) and Ramanand, (2017), climate incompatibility studies can be conducted pre-release to assess agent tolerance to thermal extremes, but can also be conducted post-release to provide explanations for failure to establish (May and Coetzee, 2013).

1.7.1.1 Developmental rate and threshold

According to McClay and Hughes (1995) and Bryant *et al*. (2002), insects that are native to warmer climatic regions have higher temperature thresholds compared to insects that are native to colder climatic regions. In cool temperate climates, the insect's ability to tolerate winter low temperatures and to continue important processes, such as reproduction and development, determines whether establishment of populations is possible (Hughes *et al.* 2011). Similarly, insects from colder regions may be less tolerant of high temperatures. Studying the thermal physiology of an insect in the laboratory may allow a determination to be made of its metabolic response to variation in temperature (Sutherst and Maywald, 1985). Campbell *et al*. (1974) state that studying an insect's developmental rate is a standardized laboratory measure of its thermal physiology because this rate is dependent on ambient temperature. The metabolic activities of insects are limited by upper and lower temperature thresholds.

Developmental threshold is defined as the temperature or developmental base where an insect's development stops due to metabolic activities being limited (Wilson and Barnett, 1983; Sutherst and Maywald, 1985; Kontodimas *et al*., 2003; Garcia & Morrell, 2009; Jalali *et al*., 2010). The developmental rate of an insect increases, often in a linear fashion, from the lower temperature threshold (t_o) (Wagner *et al*., 1991). However, beyond an optimum temperature, the insect becomes thermally stressed and although it continues to develop at a faster rate, its physiological processes start to break down, and beyond an upper threshold, development decreases or stops (Wagner *et al*., 1991), before lethal temperatures are reached.

The developmental threshold (t), and in particular the lower developmental threshold (t_o) can be determined in the laboratory by measuring the period taken for an insect to develop from egg to adult at several constant temperatures (Campbell *et al*., 1974; Wagner *et al*., 1991). The development rate of each immature life stage (egg, larva, pupa) needs to be measured separately, at the designated constant temperatures, and can then also be combined. In insect development, the thermal constant (K) is important as it is the measure of accumulated heat required to complete development (Campbell *et al*., 1974). The parameters t and K are used in various climatic models to predict the distributions of biological control agents in their new range and to predict how many generations an agent is capable of producing in a new range per year using degree-days (Byrne *et al*., 2003; May and Coetzee, 2013).

1.7.1.2 Degree-day model

The degree-day model is used to determine the number of generations an insect can complete in a location, and it relies on the insect's developmental rate and historical temperatures of the new range region. This model measures development of insects using accumulated temperature (thermal constant – K) (Allen, 1976; Nietscke *et al*., 2007) and is measured in degree-days (°D), sometimes referred to as physiological time (Zalom *et al*., 1983). By using historical weather data and available °D above any given lower temperature threshold (t_o), one can estimate which locations will provide adequate physiological time for an insect to complete its development (McClay 1996; Garcia and Morrell 2009). If physiological time allows only one or fewer generations to be completed at a site in a year, then that area/location is not suitable for the insect's establishment; if more than one generation can be completed, then establishment is likely in that area/location (McClay 1996; McClay and Hughes 2007). A good example of what has been discussed above would be of a wasp used as biological control of aphids, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) which was reported to have a lower developmental threshold of 5.8°C (Hughes et al. 2011). With such low developmental threshold, the wasp is able to develop during the cold season. The wasp was also predicted to produce 9.8 generations a year in northern Europe, which was enough for it to establish (Hughes *et al*. 2011).

1.7.2 Effect of temperature on the thermal limits of *Dichrorampha odorata*

Temperature influences insect behaviour (Neven, 2000; Bradshaw *et al*., 2004; Reznik *et al*., 2009; Saska *et al*., 2010), movement, physiology, survival, fecundity (Hamilton and Zalucki, 1991; Keena, 2006; Booth and Kiddel, 2007; Reznik *et al*., 2009; Saska *et al*., 2010) and fitness (Willott and Hasall, 1998; Chown and Terblanche, 2007; Lachenicht *et al*., 2010). Effects of temperature on these characteristics can negatively affect establishment and persistence of insects used as biological control agents (see above). Insects are highly affected by environmental conditions because they have no internal mechanism for regulating internal body temperature (Jones and Brunner, 1993). Changes in environmental conditions can be stressful to them; therefore, environmental extremes are important (Chown and Terblanche, 2007). If insects experience temperatures outside their optimum temperature range, their performance will be reduced and this may lead to mortality (Lachenicht *et al*., 2010). However, the severity of such effects is based on their rate, intensity, duration and

frequency of occurrence (Chown and Terblanche, 2007). Therefore, in order for insects to survive in their thermal environment, they have to be adapted and tolerant to a wider range of temperatures or by being physiologically adapted to a specific range of temperatures (Willott and Hasall, 1998).

According to Terblanche *et al.* (2007), factors such as photoperiod, acclimation, day temperature and rate of temperature change have an effect on assessment of thermal limits of insects. These limits are measured using static or dynamic methods (Terblanche *et al.*, 2007), where static method refers to lethal temperature limits (LT) and dynamic method refers to critical thermal limits (CT).

1.7.2.1 Static method (lethal temperature limits – LT)

Lethal temperatures (LT_{50}) are temperature limits where death of 50% of the population occurs (Sinclair *et al*., 2006) and these include the lower lethal temperature (LLT_{50}) and the upper lethal temperature (ULT_{50}). Measuring lethal temperatures for an organism makes it easy to identify which extreme temperatures will cause a reduction in the population resulting in no establishment. These limits are examined by exposing insects to low or high temperatures for a designated time and then calculating mortality. According to Byrne *et al*. (2003), it is appropriate to expose insects to the temperature for two hours, as this is the length of time that represents a heat wave during the day and the coldest period at night and can result in death of an insect population.

When conducting LT assays, insects are exposed to high or low temperatures until a temperature is reached where there will be 100 % mortality. It is important to examine survival several hours after running assays because temperature extremes may not cause immediate death but mortality may occur at a later stage. Loss of locomotion in insects is also recorded as death when conducting LT experiments, because once insects lose locomotory function, there is a high probability that they would be unable to survive to the next developmental stage, and would therefore have a lower chance of establishment and dispersal (Denlinger and Lee 1998).

1.7.2.2 Dynamic method (critical thermal limits – CT)

Critical thermal (CT) limits describe behavioural and ecological temperature tolerance limits of the species (Mitchell *et al.*, 1993). Determination of these involves cooling and heating an organism from a starting temperature until physiological failure (Terblanche *et al.*, 2007). Critical thermal minima (CT_{min}) and critical thermal maxima (CT_{max}) are used to indicate chill coma and heat stupor of different species (Klok and Chown, 1997; Klok and Chown, 1998). At these temperatures, normal functioning or activity is lost (Mitchell *et al.*, 1993) but recovery is still possible. The inability of an insect to move prevents it from locating food and it can become defenceless to predators (Layne *et al.*, 1985; Mitchell *et al.*, 1993; Kelty and Lee, 1999). Kelty and Lee (1999) state that, when recording CT_{max} and CT_{min} it is important to note that these can vary with experimental cooling and heating rates as well as with acclimation.

1.7.3 Thermal acclimation

Long-term pre-exposure of insects to heat shock or cold shock temperatures prior to the insects being exposed to extreme temperatures induces plastic responses and is referred to as acclimation (Chidawanyika *et al.*, 2017). Depending on the species, thermal acclimation can be induced within hours or days (Chidawanyika *et al.*, 2017), in the immature stages (developmental acclimation) and/or the adult stage (Denlinger and Yocum, 1999). According to Lagerspetz (2006), acclimation benefits a species in that it can subsequently survive similar environmental conditions in the field. Some studies on acclimation have shown cross-tolerance in insects, such that insects pre-exposed to cold temperatures can improve their heat tolerance (Chidawanyika and Terblanche, 2011; Stotter and Terblanche, 2009).

It is argued that insects used in biological control, reared at controlled environments, may struggle to adapt to more extreme temperatures when they are released into the field. Therefore, it is important to study physiological responses of biological control insects to thermal variation, as this can assist in developing release protocols for insects to perform well in the field (Terblanche, 2014).

1.8 Research aims and objectives

The failure of *Dichrorampha odorata* to establish in South Africa thus far could be due to climate incompatibility. Robertson et al. (2008) reported that FloraMap and CLIMEX identified Jamaica (where *D. odorata* was collected) as not having a climate closely matched to those areas of South Africa in which *C. odorata* is invasive, which are generally cooler and drier than Jamaica. Studying the effects of temperature on the development of *D. odorata* may allow us to understand why the moth has failed to establish and will help to predict climate suitability for *D. odorata* in areas infested by *C. odorata* in South Africa.

The main questions for the study were: (1) is *D. odorata* suitable to be released in South Africa, in terms of climatic compatibility? (2) which areas of South Africa invaded by *C. odorata*, are climatically best suited for the release and establishment of the moth? To answer the questions, the study's objectives were: (1) to elucidate the thermal physiology of *D. odorata* by determination of the critical thermal minima (CT_{min}) and maxima (CT_{max}), lower (LLT), upper (ULT) lethal limits, and developmental thresholds under manipulated laboratory conditions; (2) to determine the potential area of distribution of *D. odorata* in South Africa, by climatic matching using laboratory-determined thermal response traits of *D. odorata*, predicting the number of generations that *D. odorata* is capable of producing; and (3) to determine the thermal plasticity of *D. odorata* by measuring critical thermal limits at high and low temperatures after acclimation at different temperatures.

CHAPTER 2: METHODOLOGY

2.1 Effects of temperature on developmental rate of *Dichrorampha odorata*

2.1.1 Study area, insects and plants

The study was conducted in the quarantine facility of the Plant Health and Protection (ARC-PHP) unit at Cedara (29° 32' 29.5" S, 30° 16' 03.5" E) in KwaZulu-Natal Province, South Africa. Insects used were from the *D. odorata* culture that is maintained in the laboratory at temperatures and humidity (RH) of 22-27°C and 35-80%. This culture was collected from Jamaica in 2005 and supplemented with fresh material from the same source in 2012.

Fresh cuttings of *C. odorata* were collected from the field (Peter Brown Drive, Pietermaritzburg) in KwaZulu-Natal and planted in a seedling tray in vermiculite using Seradix (Seradix^{TM} No. 1) as rooting hormone. The seedling tray was placed in the glasshouse at Cedara, in a heated mist-bed with sprinkler irrigation every 20 minutes. After about two weeks, once roots were visible under the seedling tray, cuttings were transplanted into pots (25 cm diameter) with sand (Umngeni River sand) and Gromor Potting Medium^{TM} (Gromor, Cato Ridge, South Africa) at a 1:1 ratio growth medium. The plants were grown in the greenhouse tunnel and hand-watered with a hosepipe daily. Two weeks after being potted out, 5 ml of Multicote 8^{TM} (Haifa Group RSA (Pty) Ltd) fertilizer was added to each pot. Shoot tips of the plants were nipped off to stimulate growth of side shoots.

2.1.2 Effect of temperature on development of *D. odorata*

Development time, survival, and adult longevity of *D. odorata* was studied at seven constant temperatures: 15, 18, 20, 25, 27, 30 and 32 °C. All trials were performed in growth chambers (Labcon, South Africa) set at one of the above temperature treatments, with photoperiod set at 12L: 12D. iButton dataloggers (model DS 1923, Maxim Integrated Products, San José, USA, 0.5 °C accuracy) were used to log microclimates (temperature and relative humidity) inside each temperature chamber, every hour, to ensure experimental temperatures were maintained.

2.1.3 Egg development

In order to obtain eggs, cages of 0.9 x 0.4 x 0.4m, comprising a steel frame and fine gauze panelling, were placed in a quarantine glasshouse maintained at a temperature of 20-30°C. One *C. odorata* plant (~25cm tall) was placed inside the cage. Two newly eclosed adults, consisting of one putative male (smaller in body size) and one putative female (bigger in body size), were placed inside the cage with the plant for mating and oviposition. After 24 hours of exposing adults to the plant, the adults were removed and placed back in the culture. The number of eggs laid (attached on upper surfaces of leaves, visible as white dots) was recorded for each plant and plants with eggs were placed into one of the seven treatment temperatures (15, 18, 20, 25, 27, 30 and 32°C). Plants in chambers were irrigated using Blumat^{TM} (Tropf-Blumat, Germany) drippers. Eggs were monitored daily and hatching date was recorded. For lower temperatures (15, 18 and 20°C), it was initially noticed that there was high egg mortality, which was thought to be due to lower humidity due to the cooling system in the chambers (RH ranging at average of 26.90% at low temperatures and 58.01% at higher temperature (Table 3.1)). Plants with eggs in these chambers were subsequently covered with plastic bags with pores (280mm x 400mm x 30 micron (Range Plastic cc)) to increase humidity and airflow around the plant, to prevent eggs from drying out. Duration of egg development (number of days to hatching) was calculated for each egg at each temperature treatment.

2.1.4 Larval and pupal development

The newly hatched larvae crawled from their natal eggs on leaves to the closest shoot tips and tunneled in. After the 10th day of shoot damage, damaged *C. odorata* shoots were dissected to determine how many larvae were inside the shoots. Fresh shoot tips collected from stock plants were used to create a bouquet, by cutting the terminal 70-80mm of a shoot tip off the plant using secateurs and individually wrapping the base of each shoot tip with a piece of damp tissue paper and tin foil. Each bouquet was placed into glass Petri dish (9cm in diameter x 1cm high) lined with moistened (2% sodium hypochlorite solution) filter paper. The larvae that had been dissected from the shoot tips were placed onto their own *C. odorata* shoot tip bouquets, to make sure that there was only one larva per shoot tip. Petri dishes were placed inside plastic containers lined with moistened tissue paper and the container was closed with a lid to prevent leaf material from drying out from the airflow inside the chamber.

Plastic containers with Petri dishes were placed in growth chambers and larval development at each temperature was monitored.

The bouquets were changed once a week to provide fresh plant material for larvae – larvae were dissected out of the old shoot tip and placed onto the new shoot tip, into which they readily bored. The larval development period (number of days from hatching to pupation) was calculated, as was pupal period (number of days from pupation to adult eclosion) and survival rate. TIBCO Statistica^{TM} version 13.2 (Tibco Software Inc.) and SPSS Statistical software was used to analyse the data.

A linear relationship between temperature and developmental rate day^{-1} was developed using the method from Campbell *et al*. (1974). To determine geographical areas likely to be suitable for establishment, parameters (K and t_o) were used to calculate the number of generations *D. odorata* is capable of producing in South Africa and across Africa using CLIMEX (v.4, Hearne Scientific Software) weather data and the 'Compare Locations' function (this function predicts potential species geographical distribution of a or a pair of based on its climatic preferences (Sutherst *et al*., 2007)) and ARC-GIS to map.

2.2 Effect of temperature on the thermal limits of *Dichrorampha odorata*

2.2.1 Study area and insects

The study was conducted in the quarantine facility of the Plant Health and Protection (ARC-PHP) laboratory, at Cedara in KwaZulu-Natal, South Africa. Adult insects used were from the *D. odorata* culture that was maintained in a laboratory at temperatures and humidity (RH) of 22-27°C and 35-80%, respectively. Larvae and pupae were from the *D. odorata* culture that was maintained in a glasshouse at temperatures of 19-29°C, as above.

2.2.2 Static method (lethal temperature limits – LT)

Lower lethal temperature (LLT) and upper lethal temperature (ULT) for adults and 1-month-old larvae were assessed using a standard "plunge" protocol from Sinclair *et al.* (2006) and Terblanche *et al.* (2008). The experiment was conducted using a programmable water bath (Haake C25P, Thermo Electro Corporation, Karlsruhe, Germany). Newly eclosed *D. odorata*

adults and 1-month-old larvae were placed in groups of 5 per sealed (with cotton wool) plastic vial, and were exposed to a set constant temperature treatment for two hours. A thermocouple (YFE YF-160A Type K) was used to monitor the temperature inside the water bath to ensure that the desired temperature during treatment was achieved and maintained.

To determine ULT, five vials with five adults or larvae in each vial (sealed with moistened cotton wool for sufficient humidity) were placed inside a zip-lock bag and tightly closed. The water bath was set at 25°C for 15 minutes to acclimate insects; thereafter, temperature was increased to the desired value and was at that constant temperature for two hours. To determine LLT, the water bath was filled with 70% ethanol to allow for sub-zero temperature use without freezing. Five vials with five adults or larvae in each vial were placed inside a zip-lock bag and tightly closed to prevent them from drowning. The water bath was set at 25°C for 15 minutes to acclimate the insects; thereafter the temperature was decreased to the desired value and was at that constant temperature for two hours.

Following treatments, the adults or larvae were placed in petri dishes with filter paper and a *C. odorata* bouquet comprising a shoot tip, to allow the surviving larvae to bore into the shoots. The petri dishes were then placed in a growth chamber set at constant 25°C. Survival was scored after 24 hours by recording indicators of normal behaviour. For adults, a behaviour such as walking or flying was considered normal, and for larvae, a behaviour such as boring inside *C. odorata* shoot tips was considered normal. The effect of temperature on larval and adult LT at exposure time was analysed using a Generalized Linear Model (GLM) and LT_{50} (the temperature causing 50% of tested individuals to die in a given period (Li *et al*., 2011)), using SPSS Statistical software.

2.2.3 Dynamic method (critical thermal limits – CT)

A programmable water bath (Haake C25P, Thermo Electro Corporation, Karlsruhe, Germany) connected to a series of chambers (‘organ pipes’) was used to determine critical thermal limits (CTL) following methods outlined in Terblanche *et al.* (2008). Ten newly eclosed *D. odorata* adults were individually placed in holding vials into chambers of the organ pipes; vials were plugged with cotton wool to prevent the insects from escaping before the onset of each trial. The cotton wool was moistened with water to prevent dehydration. A

thermocouple (YFE YF-160A Type K) connected to a digital thermometer was inserted into the control chamber to record the actual temperature faced by adults in the chamber.

The water bath was pre-set at 25°C for 15 minutes, to allow adults to equilibrate with chamber temperatures. After 15 minutes, temperature was decreased or increased at a ramping rate of 0.25°C per minute to determine the CT_{min} and CT_{max}. This rate was chosen as it is a relatively ecologically relevant rate (Nyamukondiwa and Terblanche, 2010) resembling temperature changes in the natural environment. For the start and duration of the experiment, adults were repeatedly turned on their backs by agitating them at one-minute intervals using a small paint brush until the temperature at which they lose the ability to self-right was reached. The insects were carefully observed to assess at what temperature they could no longer self-right, and the temperature at which locomotory function was deemed to have been lost was recorded as the critical thermal limit. Following treatments, the adults were placed at 25°C and checked after 1 hour to determine whether they had recovered.

2.2.4 Thermal acclimation

To determine the effects of thermal acclimation and hardening on CT, developmental acclimation was conducted. *Dichrorampha odorata* pupae were harvested from the culture in the glasshouse. The pupae were placed into thermal treatments for 7 days (acclimation) inside Labcon growth chambers before assessing CT_{min} or CT_{max}. For acclimation, three growth chambers were set at 20, 25 (control) and 30°C.

Harvested pupae were placed in petri dishes (20 pupae per petri dish) lined with moistened filter paper. Petri dishes were then placed inside a plastic container lined with damp paper towel and closed with a lid to prevent pupae from dehydrating due to airflow inside the growth chamber. The containers with pupae were then placed in growth chambers set at 20, 25 and 30°C for 7 days. After 7 days the containers from 20 and 30°C were removed and placed at 25°C. The pupae were monitored until adult eclosion.

Once adults emerged, CT_{min} and CT_{max} was scored using a programmable water bath (LAUDA ECO GOLD RE1050, Lauda DR. R. Wobser GMBA & CO. KG, Germany) connected to a series of chambers (‘organ pipes’) following methods outlined in Terblanche *et al.* (2008). The water bath was pre-set at 25°C for 15 minutes, to allow adults to equilibrate

with chamber temperatures. After 15 minutes, temperature was reduced or increased at a ramping rate of 0.25°C per minute to determine the CT_{min} and CT_{max} respectively. Self-righting response was assessed in the same manner as above. Data were analysed using One-way ANOVA, in the Statistica package.

CHAPTER 3: RESULTS

3.1 Effects of temperature on developmental rate of *Dichrorampha odorata*

Temperature and RH data were recorded using iButtons for five of the growth chambers (Table 3.1). These indicated that RH was lower in the chambers set at lower temperatures. The temperatures maintained in the three warmest chambers was slightly under the set points, and in the cooler two, slightly over. For the purposes of the results, however, the temperatures indicated are the set temperatures.

Dichrorampha odorata did not complete development at constant rearing temperatures of 15ºC, where no eggs hatched; 18ºC, where only one egg hatched but the resultant larva died after 2 days; or 32°C where 21 eggs hatched but larvae died before pupation, after developing for 16.0 ± 1.8 (mean ± SEM) days. Complete development occurred at constant rearing temperatures of 20, 25, 27 and 30°C (Table 3.2). Developmental time increased as temperatures decreased; for example, eggs placed in the 20°C chamber took 75.8 ± 1.71 (mean ± SEM) days to develop to adulthood whereas eggs in the 30°C chamber took 40.6 ± 1.34 (mean ± SEM) days. Adult progeny from each temperature were kept inside growth chambers at that rearing temperature and adults from the 20°C trial lived longer with average number of days 8.6 ± 0.97 (mean ± SE) ($F_{1,62}$ = 9.83; P = 0.001) than those from higher temperatures (Table 3.2).

Table 3.1: Microclimate data inside growth chambers recorded with iButtons.

Set Temperature (°C)	Actual Temperature (°C)	RH (%)
20	20.04	26.90
25	25.36	46.09
27	26.25	48.37
30	29.53	48.43
32	31.33	58.01

Table 3.2: Eggs laid, hatched and total development time from egg to adult for *Dichrorampha odorata* at all tested constant temperatures. Different letters after each value within a row denote significant differences across temperature. Tukey post-hoc test performed on data.

Period	**Mean duration (days) ± SEM (N)**							**F statistic**	***P* value**
	15°C	**18°C**	**20°C**	**25°C**	**27°C**	**30°C**	**32°C**		
No. eggs laid	67	57	50	94	52	58	40	-	-
Oviposition to egg hatch	-	15 (1)	19.3 ± 0.39 (27) a	10.5 ± 0.25 (36) c	11.8 ± 0.15 (30) b	10.4 ± 0.15 (37) c	10.2 ± 0.10 (21) c	$F_{4,149} = 251.40$	$P < 0.001$
Egg hatch to pupation	-	-	35.8 ± 1.93 (22) a	26.8 ± 0.51 (26) b	23.6 ± 0.75 (26) c	21.9 ± 1.45 (15) c	-*	$F_{3,84} = 23.95$	$P < 0.001$
Pupation to adult eclosion	-	-	22.5 ± 0.9 (17) a	14.4 ± 0.25 (22) b	12.4 ± 0.4 (20) b	7.4 ± 2.0 (7) c	-	$F_{3,65} = 78.80$	$P < 0.001$
Oviposition to adult eclosion	-	-	75.8 ± 1.71 (17) a	51.6 ± 0.79 (22) b	47.7 ± 0.85 (20) b	40.6 ± 1.34 (7) c	-	$F_{3,65} = 149.75$	$P < 0.001$
Adult longevity	-	-	8.6 ± 0.97 (17) a	4.9 ± 0.27 (22) b	5.7 ± 0.27 (20) b	4.3 ± 0.19 (7) b	-	$F_{1,62} = 9.83$	$P < 0.001$
Total percentage survival	-	-	62.96	61.11	66.67	19.44	-	-	-

*****No development after early larval instar development

For all three immature stages (eggs, larvae, pupae), there was a significant positive linear relationship between temperature and developmental rate (Figure 3.1a, b, c). For the combined immature stages, the linear regression method shows a linear relationship (R^2 = 0.99; P = 0.001) between temperature and developmental rates (Figure 3.1d) and reduced major axis regression (R^2 = 0.98; P = 0.005) between product of development time x temperature (DT) and developmental time (days) (Figure 3.2) Using the equation, the lower temperature threshold (t_o) for all stages was estimated at 8.45°C and the thermal constant (K) at 872.4°D (y = 8.45x + 872.36) (Table 3.3). Ikemoto and Takai (2000) reduced major axis regression method (Figure 3.2) was done to validates linear regression method and the method gave similar results as with the Campbell (1974) method.

Table 3.3: Thermal parameters K and t_o of *Dichrorampha odorata* from (a) linear regression using the Campbell *et al*. (1974) method; (b) reduced major axis regression using the Ikemoto and Takai (2000) method.

(a)

Stage	**R^2**	**t_O**	**K**
Eggs	0.729	6.37	232.6
Larvae	0.984	5.11	555.6
Pupae	0.995	10.92	177.7
Eggs-Adults	0.997	8.55	909.1

(b)

Stage	**R^2**	**t_O**	**K**
Eggs	0.809	10.75	180.0
Larvae	0.878	5.06	533.2
Pupae	0.955	14.60	134.7
Eggs-Adults	0.989	8.45	872.4

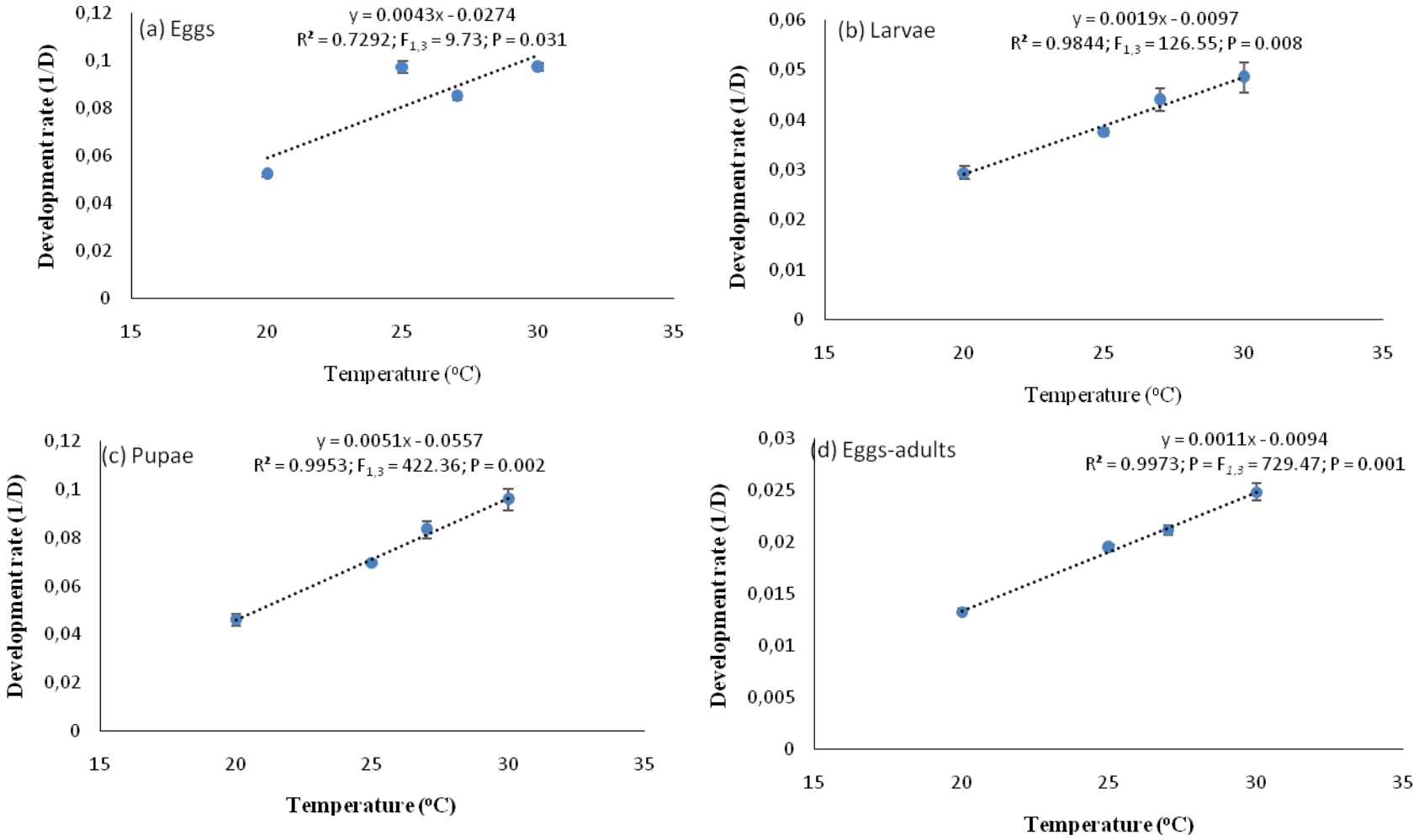

Figure 3.1: Linear relationship between temperature and developmental rates (1/Days) of each *Dichrorampha odorata* stage (a) eggs; (b) larvae; (c) pupae and (d) total development (from eggs to adults) using the method of Campbell *et al*. (1974).

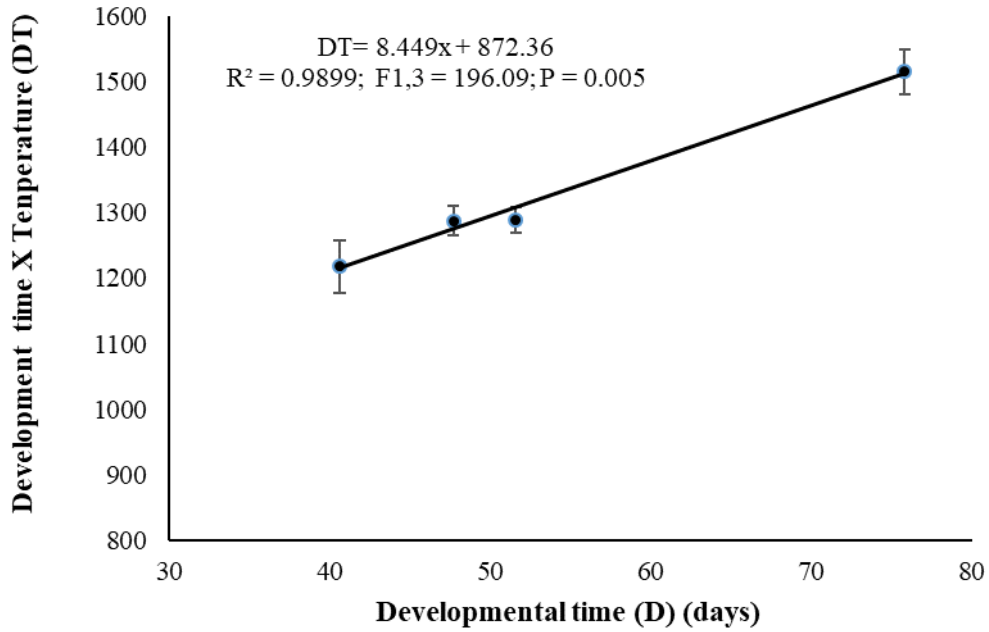

Figure 3.2: Reduced major axis regression (eggs to adults) at four constant temperatures where *Dichrorampha odorata* developed from egg to adult using method of Ikemoto and Takai (2000).

The percentage survival of combined immature stages at 30°C was significantly lower than that at any of the three other temperatures ($F_{3,129} = 7.787$, $P < 0.001$). There was no significant difference in survival of combined immature stages between 20, 25 and 27°C. The highest overall survival (66.67%) was recorded at 27°C, followed by 20, 25 and 30°C (62.96, 61.11, 19.44% overall survival respectively) (Figure 3.3).

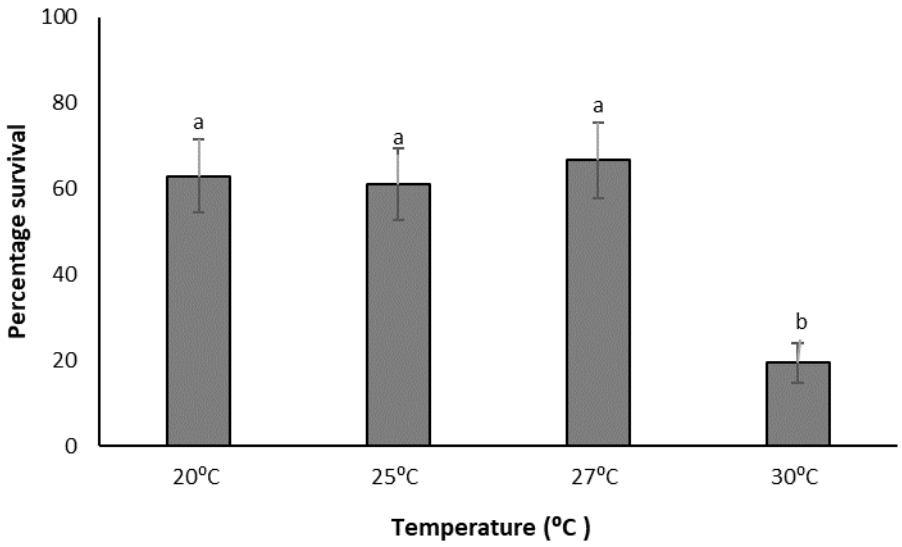

Figure 3.3: Percentage survival (mean ± SE) of combined immature stages (larva to adult) of *Dichrorampha odorata* at four constant temperatures. Means capped with different letters are significantly different [after Tukey's Honest Significant Difference (HSD) Test: $P < 0.05$)].

Using parameters (K = 872.4 days and t_o = 8.45°C) from the linear regression model, *D. odorata* is capable of completing a maximum of 6.3 generations per year in South Africa, especially along the east coast where *C. odorata* is mostly found (Figure 1.2), whereas in other parts of Africa, it is capable of completing a maximum of 9 generations per year (Figure 3.4).

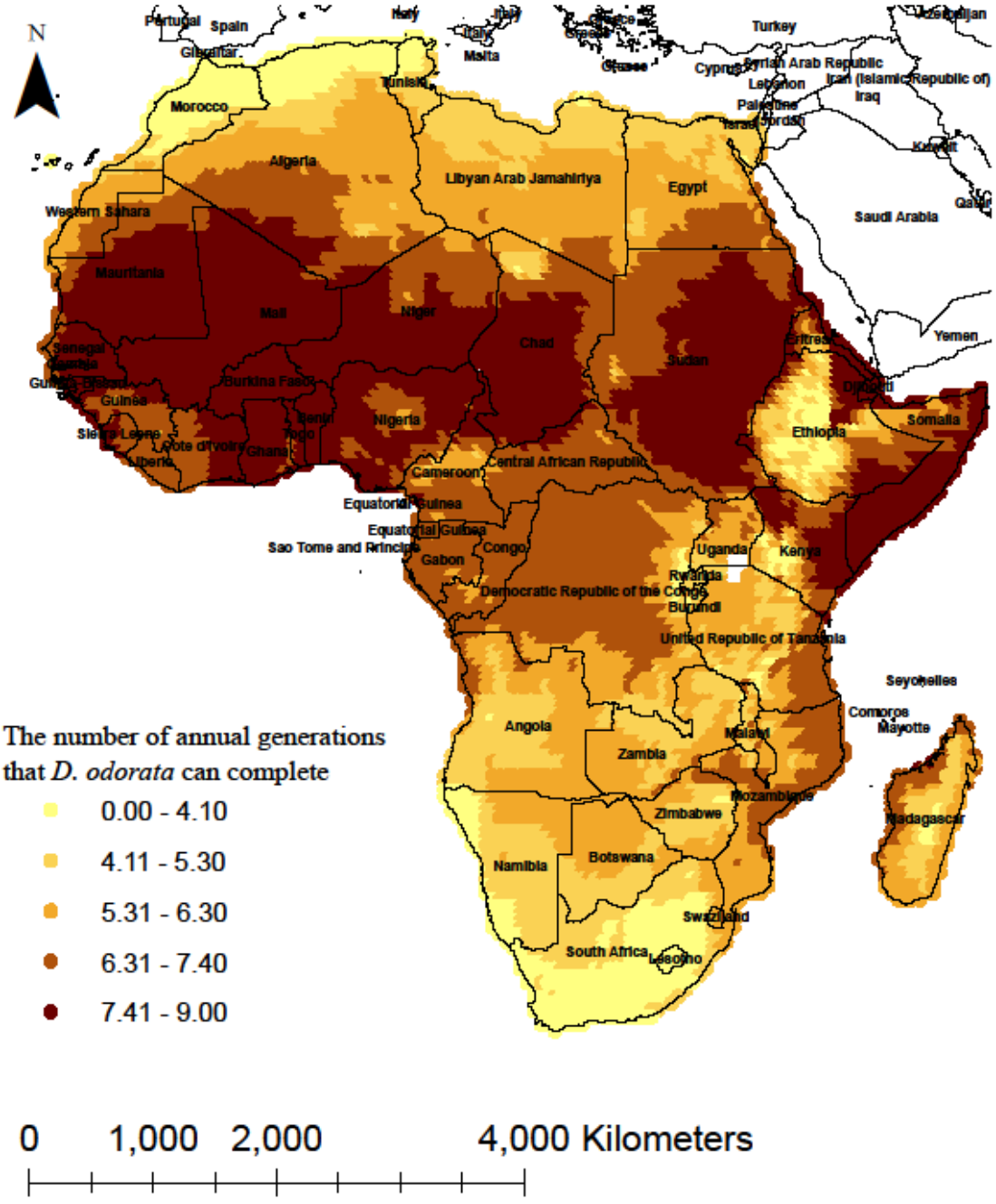

Figure 3.4: Number of generations that *Dichrorampha odorata* is capable of completing in a year throughout Africa, using parameters from the linear regression model.

3.2 Effect of temperature on the thermal limits of *Dichrorampha odorata*

3.2.1 Static method (Lethal temperature limits – LT)

The LLT_{50} and ULT_{50} at which 50% of the larval population died were determined to be -4.50°C and 39.64°C respectively, and of the adult population were 1.83°C and 41.02°C. In the larval population, 100% mortality was recorded at -9°C (LLT) and 44°C (ULT), and for the adult population at 0°C (LLT) and 45°C (ULT) (Table 3.4).

Table 3.4: Lower and Upper Lethal Temperatures of adults and larvae of *Dichrorampha odorata* calculated from survival data collected 24 hours after lethal temperature exposure.

Stage	Lethal thermal limits (°C)	24 hours after exposure	95% confidence interval	*P*
Larvae	LLT_{50}	-4.5	-5.20 – (-4.10)	0.0001
	ULT_{50}	39.64	38.51 - 39.87	0.0001
Adults	LLT_{50}	1.83	0.85 - 3.42	0.0001
	ULT_{50}	41.02	40.20 - 42.70	0.0001

Using Generalized Linear Model (GLM) the statistical analysis indicates that temperature significantly affected ($F_{1,7}$ =343.3, P<0.001) survival of *D. odorata* larvae and adults when exposed to lethal temperatures (ULT or LLT) for 2 hours (Table 3.4).

3.2.2 Dynamic method (Critical thermal limits – CT)

The mean CT_{min} and CT_{max} of *D. odorata* were determined to be 4.4 ± 0.22°C and 43.73 ± 0.12°C (mean ± se, n = 30).

3.2.3 Thermal acclimation

Dichrorampha odorata pupae kept at 20°C for seven days before CT assays, had a significantly lower CT_{min} (1.95 ± 0.06°C) than insects kept at 30°C (5.92 ± 0.08°C)

($F_{1,3}$=818.87, P<0.001). Pupae kept at 20°C had higher CT_{max} of 44.41 ± 0.07°C, compared to 42.92 ± 0.09°C for insects kept at 30°C (Table 3.5).

Acclimation significantly affected CT_{min} and CT_{max} of *D. odorata* in all temperature treatments, with CT_{min} being more responsive to cold acclimation ($F_{1,2}$ = 818.87, P < 0.001).

Table 3.5: Effects of acclimation on CT_{min} and CT_{max} of *Dichrorampha odorata* at three acclimation temperatures (mean ± SE (n)).

	Acclimation Temperature (°C)		
	20°C	25°C	30°C
CT_{min}	1.95 ± 0.06 (30)	3.36 ± 0.07 (30)	5.92 ± 0.08 (30)
CT_{max}	44.41 ± 0.07 (30)	43.67 ± 0.12 (30)	42.92 ± 0.09 (30)

CHAPTER 4: DISCUSSION

Modelling the potential distribution of biological control agents has become important in predicting distribution prior to release, and in explaining their failure to establish and/or reducing weed populations, post-release. One of the programmes commonly used in biological control is CLIMEX, which allows predictions of climatic suitability for an agent in its introduced range (Kriticos *et al*., 2003), but, to determine areas climatically suitable for biological control agents using CLIMEX, thermal physiology data on that agent are required. In the current study, the thermal physiology of *D. odorata*, a biological control agent of *C. odorata*, was determined, in order to predict areas that are climatically suitable for *D. odorata*, if any, in South Africa.

Thermal parameters such as lower development threshold and degree-days are important for predicting insect establishment and distribution (Manrique *et al*., 2008; May and Coetzee, 2013). Fluctuating temperatures from natural environments may result in variable insect development (Brakefield and Mazzotta, 1995), generally, higher temperatures result in faster growth and development (Matsuki *et al*., 1994; Dingha *et al*., 2009). However, above a certain optimal temperature, physiological processes become compromised and mortality increases. When temperatures become cooler, insect development slows, and below a certain temperature, it stops. Slow development can lead to poor feeding and higher risk of predation. Each species of insect has a different thermal profile, which can best be elucidated through a fairly standard experimental setup.

4.1 Lower developmental threshold (t_o) and the rate of development (K)

Although development rate increased with temperature, survival of *D. odorata* decreased at the two highest temperatures (30ºC and 32ºC). The optimal development temperature, at which both development rate and survival were high, was 27°C. Only one egg hatched at 18ºC, and none at 15ºC. The lack of egg hatch at these lower temperatures does not exclude the possibility that larvae could develop through to pupation successfully at these temperatures, albeit at a slow rate. Furthermore, if *D. odorata* eggs were exposed to the lower experimental temperatures (15ºC and 18ºC) for only short periods, as will happen at night in the field, they may well hatched. In the trial in the growth chambers, the long accumulation of

cold stress at constant 15ºC and 18ºC may be the reason why no eggs hatched at 15ºC and very poor hatching was recorded at 18ºC. It is nevertheless surprising that this is the case, given that t_o for eggs was calculated to be 6.37ºC. Perhaps low RH, caused by the cooling mechanism in the growth chambers (which dries air as it cools) was a greater factor here.

Padmavathi *et al.* (2013), working on the rice leaf folder *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae), showed a similar trend as the current study: as temperature increased from 18ºC to 34ºC, the developmental time of stages was reduced although *C. medinalis* is more heat and cold intolerant than *D. odorata*. Lower threshold temperatures (t_o) and thermal constants (K) were estimated by linear regression analysis and are indicated in Table 4.1. The lower threshold temperature of the rice leaf folder was estimated to be 11.2ºC (higher than that of *D. odorata* which was 8.54ºC) (Padmavathi *et al*., 2013). The t_o and K values for *P. insulata*, which has successfully established as a biological control agent on *C. odorata* in South Africa, were determined by Uyi *et al*. (2016) (Table 4.1), although they used the reduced major axis method developed by Ikemoto and Takai (2000), rather than Campbell's (1974) linear regression method. Ramanand *et al*. (2017), also using the Ikemoto and Takai (2000) method, found a t_o of 9.6°C and a K value of 546.9°D for *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Phlaeothripidae) successfully established as a biological control agent of *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), which grows in cooler areas of South Africa than *C. odorata*. It is interesting to note that for both lepidopteran species (Table 4.1), the K value for egg development was much lower than that for *D. odorata* (Table 3.4), and that this results in *D. odorata* having a far higher K value for total development than do the other two species. Perhaps this helps explain the poor establishment record of *D. odorata* in South Africa.

Table 4.1: Lower developmental threshold (t_o) and thermal constant (K) values for two species of Lepidoptera from tropical and subtropical regions and *Dichrorampha odorata*.

Stage	*C. medinalis*		*P. insulata*		*D. odorata*	
	t_o (ºC)	K (°D)	t_o (ºC)	K (°D)	to (ºC)	K (°D)
Egg	11.0	69	11.56	68.36	10.75	180.0
Larva	10.4	270	10.62	261.71	5.06	533.2
Pupa	12.8	106	11.96	163.68	14.60	134.7
Total	11.1	455	11.29	491.94	8.45	872.36

The number of generations that *D. odorata* is capable of producing in one year in areas where *C. odorata* is invasive in South Africa was estimated from the thermal parameters obtained from the linear regression method and the climate data obtained from the CLIMEX model weather database. Results indicate that the number of generations that *D. odorata* can produce in South Africa (maximum of 6.5 generations per year) is lower than other parts of Africa (maximum of 9 generations per year). According to McClay (1996) and McClay and Hughes (2007), if more than one generation can be completed, then establishment is likely in that area. Thus, looking at only temperature as the only factor, *D. odorata* is likely to establish in some parts of South Africa invaded by *C. odorata*, especially along the east coast (Figure 3.4). However, when comparing the number of generations of *D. odorata* per year in South Africa to other moth species, particularly *P. insulata* which is also a biological control agent of *C. odorata*, has a lifecycle of similar length with that of *D. odorata*, and is predicted to have 10 generations per year in South Africa (Uyi *et al.*, 2016), this may imply that temperature may be an important limiting factor affecting *D. odorata* establishment in South Africa. When temperatures for Jamaica are compared with those for South Africa (Figure 4.1), it is apparent that winter temperatures in South Africa, even at the most optimal release sites, are considerably lower than those in Jamaica.

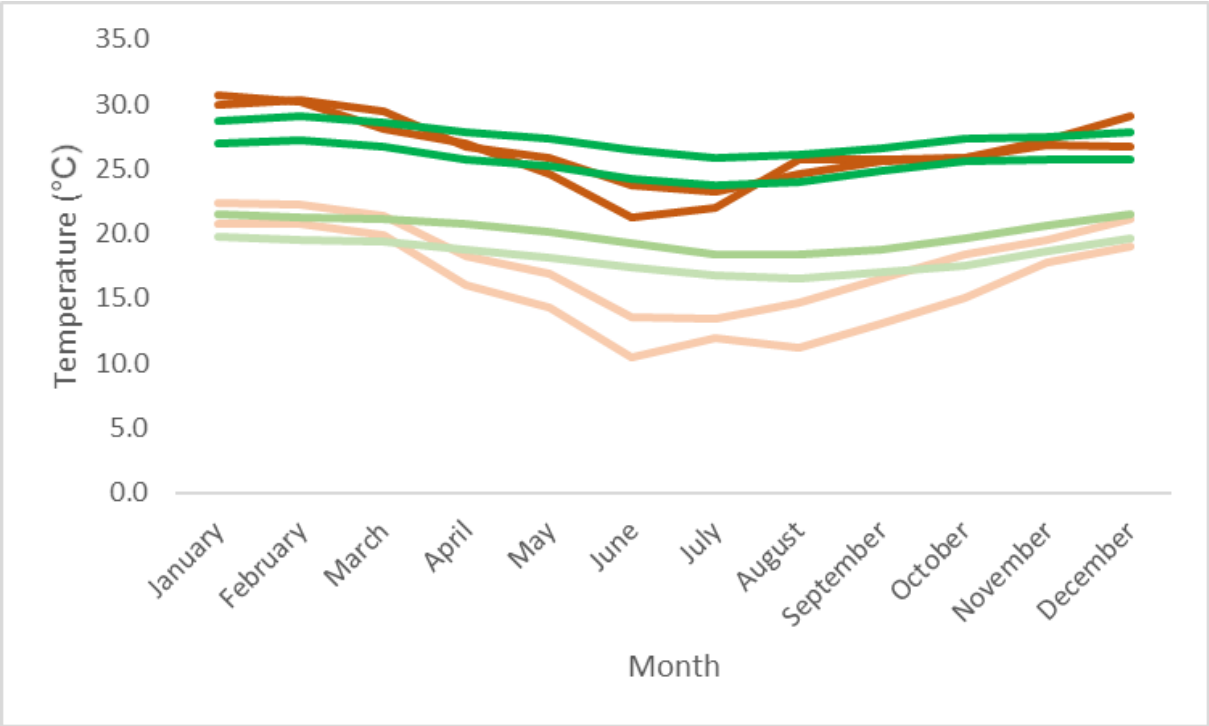

Figure 4.1: Average maximum (dark colour) and minimum (light colour) temperatures for two locations each in South Africa (brown) and Jamaica (green) close to where *D. odorata* was collected (Jamaica) or released (South Africa). The data for Jamaica were shifted by 6 months in order to compare seasonal variation across northern and southern hemispheres. Sites in South Africa: Richard's Bay (28.7376 S, 32.0942 E), Cannonbrae (30.2167 S, 30.7667 E); sites in Jamaica: Gordon Town (18.0355 N, 76.7196 W), Mavis Bank (18.0271 N, 76.6625 E). Data sources: Uyi *et al*. (2017), South African Weather Service (South Africa); https://en.climate-data.org/north-america/jamaica/gordon-town/gordon-town-987260/#climate-graph (Jamaica).

4.2 Thermal limits

According to Coulson and Bale (1992) and Bale (2002), low temperatures are the most important abiotic factor that governs insect abundance and distribution in all seasons; also, because insects have small bodies, their sensitivity to high temperatures is another important factor to note (Somero, 2010). The negative impacts of extremely high temperatures on insects include water loss, disruption of the structure of membranes (Yoder *et al*., 2009;

Hochachka and Somero, 2002) and restricted enzyme-catalysed reactions due to denaturation of protein (Chown and Nicholson, 2004). When looking at survival of D. odorata adults and larvae under lethal temperatures (LLT_{50} and ULT_{50}) for a 2-hour exposure period, both LLT (-4.5°C for larvae, 1.83°C for adults) and ULT (39.64°C for larvae, 41.02°C for adults) differed between life stages. This indicates that larvae are more tolerant to cold than adults, and that adults are more tolerant to heat than larvae. Other studies have reported that thermal tolerance in insects varies significantly among life stages (Jensen *et al*., 2007; Marais *et al*., 2009). When considering a similar study on *P. insulata* (Uyi *et al.*, 2016), the LLT_{50} and ULT_{50} of larvae was –5.9°C and 40.0°C respectively, which is almost equal to larvae lethal temperatures of *D. odorata*; and –4.7 and 40.1 °C respectively for adults, where LLT is more than 5°C lower and ULT is slightly lower, than that of *D. odorata*.

Given that *C. odorata* is intolerant of frost (Goodall and Erasmus, 1996), it does not grow in areas which reach close to freezing point, and none of the sites at which *D. odorata* has been released experience those at which LLT_{50} would be experienced by either the larvae or adults. There is a possibility that some sites may reach temperatures at which ULT_{50} for adults or larvae may occur, so this may be a limiting factor. However, the plant tissues in which larvae live for most of their development, and microhabitats for adults, may allow them to escape these temperatures.

The CT_{min} and CT_{max} values for *D. odorata* adults (4.4°C and 43.7°C respectively) indicate that adults should in general remain active across the range of temperatures experienced in the field. Cowie *et al*. (2016) showed that the adults of *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a bud-feeding weevil which was released as a biological control agent on *Solanum mauritianum* Scop. (Solanaceae), in South Africa but only established at warmer, more coastal areas (it now has a similar distribution to *C. odorata*), have a CT_{min} of 4.1°C, which is similar to that of *D. odorata* adults. Cowie *et al*. (2016) demonstrated that this CT_{min} should only prevent *A. santacruzi* from establishing at sites in the colder Highveld region of South Africa, not in any of the warmer areas where *C. odorata* is present.

4.3 Effects of temperature and acclimation

Experimental protocol has a major effect when measuring parameters such as critical thermal limits (CT). Terblanche *et al*. (2006) reported that there was a variation in CT_{min} and CT_{max} in

the same insect species when thermal acclimation had been induced over a 10-day period, compared to when it was not. *Dichrorampha odorata* reared at normal rearing conditions or at 25°C had a CT_{min} that is higher than CT_{min} from cold acclimation and CT_{max} that is lower than CT_{max} from cold acclimation treatments. The results indicate that when *D. odorata* is reared at lower temperatures, the moth becomes more tolerant to both cold and heat compared to when it is reared at optimal or higher temperatures (Table 3.5). In addition, adults lived significantly longer at lower temperatures (Table 3.2).

It is important to bear in mind that laboratory acclimation may not replicate field conditions experienced by insects, but it does provide an indication of the extent of plasticity (Sinclair *et al*., 2015). Sinclair *et al*. (2015) reported that survival of rusty grain beetle, *Cryptolestes ferrugineus* Stephens (Coleoptera: Laemophloeidae) increased when the beetle was cold-acclimated for a six-week period. This is supported by Terblanche (2014), who showed that where cold-acclimated insects are released into a hotter field environment, the insects suffered performance costs but still performed better than insects reared at the optimum rearing temperature (control). This is still to be verified on *D. odorata* since acclimation before release is still on going.

4.4 Further research

4.4.1 Climate-related issues

The study indicates that *D. odorata* adults can survive in areas that have temperatures below 40°C and above 1°C in South Africa, and be active between 4°C and 44°C. However, the temperature range over which adults are able to fly to find mates, and to mate, was not examined in the study; this might be another factor that affects reproduction and fitness of the moth at varying temperatures.

The eggs of *D. odorata* appear to be extremely fragile and vulnerable to desiccation. Anecdotally, if the leaf on which an egg has been laid is removed from the plant and kept in a Petri dish, the egg dies, even if the larva was at an advanced stage of development (N. Dube, pers. comm.). During periods of low RH in the glasshouse, it was noticed that a large proportion of eggs on plants used for culturing the insect had died (pers. obs.). It is thus possible that some of the egg mortality recorded at low temperatures in the growth chambers

was due to desiccation rather than low temperatures *per se*. It would be valuable to conduct trials to determine the vulnerability of eggs to desiccation (cf. Cowie *et al*., 2016). If the suspected desiccation problem could be overcome in growth chambers at low temperatures, it would also be valuable to rerun the trials at 15°C and 18°C to determine larval development time.

Further climatic modelling could be done using CLIMEX to compare the number of generations of *D. odorata* that can be expected in areas of origin such as Jamaica and Cuba. If humidity trials were conducted, the results could also be extrapolated to a field situation in both South Africa and the areas of origin (cf. Cowie *et al.*, 2016), which would also give an indication of likelihood of establishment.

In order to generate the CLIMEX model, temperature data from weather stations were used. However, the temperatures and humidities within the sites (microclimate) may be somewhat different, and it would be useful to measure these using ibuttons or similar techniques, in order to determine more accurately the field conditions to which *D. odorata* is being exposed, and then to compare it with the insect's thermal tolerances determined in the laboratory trials in this thesis.

4.4.2 Other reasons for non-establishment

High mortality of both larvae and pupae in the field has been recorded (Zachariades *et al*., in press). Predation played a role here, with a high proportion of the vegetative shoot tips and pupal leaf rolls having been bored into and the insect inside eaten. Predators were not seen as high proportion of the larvae and/or pupae brought into quarantine from Jamaica were parasitized (ARC-PHP, unpubl. data), and braconid parasitoids have also emerged from pupae in the laboratory culture in South Africa, indicating the parasitism may be a factor here. Formal, quantitative studies examining mortality rates of all life stages in the field, and the causes of mortality, would be valuable.

The induction of a laboratory-adapted culture may occur quickly (Hoffmann and Ross, 2018). The *D. odorata* laboratory culture has been held at ARC-PHP, Cedara since 2005, at temperatures and humidities which do not reflect field conditions. Although fresh material was imported in 2012, this was not fully integrated into the laboratory culture.

4.5 Conclusions and recommendations

The practice of weed biological control using live organisms (insects and pathogens) needs continued and patient scientific effort in order to improve its application and effectiveness (Julien *et al*., 2007). There is considerable criticism of biological control of weeds especially regarding lack of post-release assessments (McEvoy and Coombs, 2000; Louda *et al*., 2003; Julien *et al*., 2007). A number of pre-release techniques and tools have been developed and improved to ensure the safety and efficacy of weed biological control (Rector, 2008; Mills and Kean, 2010). Together with all the screening and tests conducted on potential agents against targeted weeds, the establishment potential of a biological control agent in a new range should be studied and determined through species distribution modelling programmes to predict whether or not that agent will be able to establish or not (Byrne *et al*., 2003). Once biological control agents are released, post-release evaluations need to be conducted to determine whether the released agent has an impact on the targeted weed and, if there is no impact, to determine factors affecting the dispersal and effectiveness of agents (Thomas and Willis, 1998; Delfosse, 2005; Morin *et al*., 2009).

Biological control of *C. odorata* in South Africa started in the late 1980s (Zachariades *et al*., 2011). In South Africa, biological control of *C. odorata* has faced difficulties because of the failure of some of the *C. odorata* agents to breed in quarantine or establish in the field, probably due mainly to incompatibility with the SA biotype or with the climate in South Africa. The origin of the SA *C. odorata* biotype has since been shown to be Jamaica or Cuba, and therefore a number of insect species from these islands, which have many *C. odorata* herbivores in common (Strathie and Zachariades, 2004), have been considered as candidate biocontrol agents, including *D. odorata*. However, the climates of these islands are dissimilar to many parts of southern Africa invaded by *C. odorata*, and te Beest *et al*. (2013) showed that in South Africa, many of the areas invaded by *C. odorata* are climatically only marginally suitable for it. Because of this, biological control agents which can be expected to be more drought tolerant than insects from Jamaica continue to be considered, despite coming from areas of the neotropics where the SA *C. odorata* biotype does not occur (e.g. *R. parvula* from north-western Argentina, which is climatically similar to southern Africa (Robertson *et al*., 2008)).

Apart from *D. odorata*, there are currently three other species which either are being currently released (*L. aemulus* and *R. parvula*), or for which an application to release has been submitted (*P. basilica*). Although *L. aemulus* has persisted in the field since 2011 (ARC-PHP, unpubl. data), it has not performed well and may ultimately not establish. Releases of *R. parvula* were initiated only in 2016, and being a univoltine species which cannot easily be reared in large numbers, it may take many years before sufficient insects have been released in the field to effect establishment. Tephritidae, and Diptera in general have a good establishment record, and thus *P. basilica* is expected to establish quite readily, should permission for release be granted. Its potential efficacy as a biological control agent in the field is unknown, however.

Funding permitting, research and implementation will continue on the six *C. odorata* biocontrol agents *P. insulata*, *C. eupatorivora*, *L. aemulus*, *D. odorata*, *R. parvula* and *P. basilica*. Should the combination of established agents be deemed insufficient in the future for biocontrol of *C. odorata*, the stem-tip boring fly *Melanagromyza eupatoriella* Spencer (Diptera: Agromyzidae) would be a good option as the next agent: it is widespread, occurring in Jamaica; it has long been targeted as a possible biocontrol agent (Zachariades *et al*., 1999) [it was replaced by *D. odorata* due to the similar damage caused by this species, and the relative ease of laboratory rearing of the moth]; and field host range surveys and trials indicated that it is host specific (Robinson *et al*., 2013). Dipteran biocontrol agents often establish and disperse well.

REFERENCES

Abdullah, M. 1961. Behavioural effects of temperature on insects. The Ohio Journal of Science, **61**: 212-219.

Allen, J. C. 1976. A modified sine wave method for calculating degree days. Environmental Entomology, **5**: 388-396.

Angilletta J. R. M. J., Niewiarowski, P. H. & Navas, C. A. 2002. The evolution of thermal physiology in ectotherms. Journal of Thermal Biology**, 27**: 249- 268.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D. & Whittaker, J. B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology, **8**: 1-16.

Barratt, B. I. P., Howarth, F. G., Withers, T. M., Kean, J. M. & Ridley, G. S. 2010. Progress in risk assessment for classical biological control. Biological Control, **25**: 245-254.

Booth, D. T. & Kiddell, K. 2007. Temperature and the energetics of development in the house cricket (*Acheta domesticus*). Journal of Insect Physiology, **53**: 950-953.

Bradshaw, W. E., Zani, P. A. & Holzapfel, C. M. 2004. Adaptation to temperate climates. Evolution, **58**: 1748-1762.

Brakefield, P. M. & Mazzotta, D. 1995. Matching field and laboratory environments: effect of neglecting daily temperature variation on insect reaction norms. Journal of Evolutionary Biology, **8**: 559-573.

Brown, W. J. & Zachariades, C. 2007. A new species of *Dichrorampha* (Lepidoptera: Tortricidae: Grapholitini) from Jamaica: a potential biocontrol agent against *Chromolaena odorata* (Asteraceae). Proceedings of Entomological Society of Washington, **109**: 938-947.

Bryant, S. R., Thomas, C. D & Bale, J. S. 2002. The influence of thermal ecology on the distribution of three nymphalid butterflies. Journal of Applied Ecology**, 39**: 43-55.

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

Byrne, M. J., Coetzee, J., McConnachie, A. J., Parasram, W. & Hill, M. P. 2003. Predicting climate compatibility of biological control agents in their region of introduction. In: Proceedings of the XI International Symposium on Biological Control of Weeds (Eds. Cullen, Briese J. M., , Kriticos D. T., , Lonsdale D. J., , Morin W. M., & Scott, J. K.), 28- 35. CSIRO Entomology, Canberra, Australia.

Campbell, A., Frazer, B. D., Gilbert, N., Gutierrez, A. P. & Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. Journal of Applied Ecology, **11**: 431- 438.

Carson, W. P., Hovick, S. M., Baumert, A. J., Bunker, D. E. & Pendergast IV, T. H. 2008. Evaluating the post-release efficacy of invasive plant biocontrol by insects: a comprehensive approach. Arthropod – Plant Interactions, **2**: 77- 86.

Chamier, J., Schachtschneider K., le Maitre, D. C., Ashton P. J., & van Wilgen B. W. 2012 Impacts of invasive alien plants on water quantity with particular emphasis on South Africa. Water SA, **38**: 345-356.

Charudattan, R. 1986. Integrated control of waterhyacinth (*Eichhornia crassipes*) with a pathogen, insects, and herbicides. Weed Science, **34** (Suppl. 1): 26-30.

Chidawanyika, F. & Terblanche, J. S. 2011. Rapid thermal response and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). Journal of Insect Physiology, **57**: 108-117.

Chidawanyika, F. Nyamukondiwa, C. Strathie, L & Fischer, K. 2007. Effects of thermal regimes, starvation and age on heat tolerance of the parthenium beetle *Zygogramma*

bicolorata (Coleoptera: Chrysomelidae) following dynamic and static protocols. PLoS ONE **12**: 1-17.

Chown, S. L. & Nicholson, S. W. 2004. Insect Physiological Ecology: Mechanisms and Patterns. Oxford Press, New York.

Chown, S. L. & Terblanche, J. S. 2007. Physiological diversity in insects: ecological and evolutionary context. Advances in Insect Physiology, **33**: 50-152.

Colautti, R. I., Ricciardi, A., Grigorovich, I. A. & MacIsaac, H. J. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters, **7**: 721–733.

Coulson, S. J & Bale, J. S. 1992. Effects of rapid cold hardening on reproduction and survival of offspring in the housefly *Musca domestica*. Journal of Insect Physiology, **38**:421 – 424.

Cowie, B. W., Venturi, G., Witkowski, E. T. F & Byrne, M. J. 2016. Does climate constrain the spread of *Anthonomus santacruzi*, a biological control agent of *Solanum mauritianum*, in South Africa? Biological Control, **101**: 1–7.

Cruttwell, R. E. 1974. Insects and mites attacking *Eupatorium odoratum* in the Neotropics: An annotated list of the insects and mites recorded from *Eupatorium odoratum* L., with a key to the types of damage found in Trinidad. Commonwealth Institute of Biological Control, Technical Bulletin, **17**: 87-125.

Culliney, T. W. 2005. Benefits of classical biological control for managing invasive plants. Critical Reviews in Plant Sciences, **24**: 131-150.

Daehler, C. C. 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annual Review of Ecology, Evolution, and Systematics, **34**: 183-211.

Day, M. D. & McAndrew, T. D. 2002. Status of *Charidotis pygmaea* (Coleoptera: Chrysomelidae) as a biological control agent of *Lantana montevidensis* (Verbenaceae) in Australia. Biological Control, **23**: 27–34.

De Guzman, L. I. & Frake, A. M. 2007. Temperature affects *Aethina tumida* (Coleoptera: Nitidulidae) development. Journal of Apicultural Research, **46**: 88-93.

Delfosse, E. S. 2005. Risk and ethics in biocontrol. Biological Control, **35**: 319-329.

Denlinger, D. L. & Lee, R. E. 1998. Physiology of cold sensitivity. In: Hallman, G. J. & Denlinger, D. L. (eds). Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press, Boulder, Colorado, pp: 55 – 95.

Denlinger, D. L & Yocum, G. D. 1999. Physiology of heat sensitivity. In: Hallman, G. J. & Denlinger, D. L. (eds). Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press, Boulder, Colorado, pp. 7 – 53.

Dingha, B. N., Appel, A. G. & Vogt, J. T. 2009. Effects of temperature on the metabolic rates of insecticide resistant and susceptible German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Midsouth Entomologist, **2**: 17-27.

DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. Weed Science, **48**: 255-265.

Dube, N. 2008. Investigation of the biology and cross-breeding of populations of *Pareuchaetes insulata* (Lepidoptera: Arctiidae) and the implications for the biological control of *Chromolaena odorata* (Asteraceae) in South Africa. M.Sc. thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Dube, N., Zachariades, C., Munyai, T. C. & Uyi, O. O. 2017. Laboratory studies on the biology and host range of *Dichrorampha odorata* (Lepidoptera: Tortricidae), a biological control agent for *Chromolaena odorata* (Asteraceae). Biocontrol Science and Technology, **27**: 222–236.

Enright, W. D. 2000. The effect of terrestrial invasive alien plants on water scarcity in South Africa. Physics and Chemistry of the Earth, **25**: 237-242.

Foxcroft, L. C. & Martin, B. W. 2002. The distribution and current status of *Chromolaena odorata* in Kruger National Park. Scientific Services Section, Kruger National Park, Skukuza.

Garcia, C. M. & Morrell, J. J. 2009. Development of the powderpost beetle (Coleoptera: Bostrichidae) at constant temperatures. Environmental Entomology, **38**: 478–483.

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

Goodman, P. S. 2003. Assessing management effectiveness and setting priorities in protected areas in KwaZulu-Natal. BioScience, **53**: 843-850.

Grarock, K., Lindenmayer, D. B., Wood, J. T. & Tindemann, C. R. 2013. Using invasion process theory to enhance the understanding and management of introduced species: a case study reconstructing the invasion sequence of the common myna (*Acridotheres tristis*). Journal of Environmental Management, 15: 129-398.

Hamilton, J. G. & Zalucki, M. P. 1991. Effect of temperature on development rate, survival and fecundity of cotton tipworm, *Crocidosema plebejama* Zeller (Lepidoptera: Tortricidae). Australian Journal of Zoology, **39**: 191-200.

Henderson, L. 2001. Alien weeds and invasive plants: A complete guide to declared weeds and invaders in South Africa. Plant Protection Research Institute Handbook No.12. Agricultural Research Council, Pretoria, pp. 300.

Henderson, L. 2007. Invasive, naturalised and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia **37**: 215–248.

Henderson, L. & Wilson, J. R. U. 2017. Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. Bothalia, 42(2), a2172. https://doi. org/10.4102/abc.v47i2.2172.

Hochachka, P. W. & Somero, G. N. 2002. Mechanisms and Processes in Physiological Evolution. Oxford University Press, New York.

Hoffmann, A. A. & Ross, P. A. 2018. Rates and patterns of laboratory adaptation in (mostly) insects. Journal of Economic Entomology, **111**: 501-509.

Holmes, P. M., Richardson, D. M., Esler, K. J., Witkowski, E.T.F. & Fourie, S. (2005). A decision-making framework for restoring riparian zones degraded by invasive alien plants in South Africa. South African Journal of Science, **101**: 553-564.

Ikemoto, T & Takai, K. 2000. A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. Environmental Entomology, **29**: 671-682.

Jalali, M. A., Tirry, L., Arbab, A. & De Clercq, P. 2010. Temperature-dependent development of the two-spotted ladybeetle, *Adalia bipunctata*, on the green peach aphid, *Myzus persicae*, and a factitious food under constant temperatures. Journal of Insect Science, **10**: 1–14.

Javaid, A., Shafique, S. & Shafique, S. 2007. Causes of rapid spread of *Parthenium hysterophorus* L. in Pakistan and possible control measures - A review. Pakistan Journal of Botany, **39**: 2611-2618.

Jensen, D., Overgaard, J. & Sørensen, J. G. 2007. The influence of developmental stage on cold shock resistance and ability to cold-hardening in *Drosophila melanogaster*.
Journal of Insect Physiology, **53**: 179-186.

Jones, V. P. & Brunner, J. F. 1993. Degree day models. Orchard pest management, Washington State University.

Julien, M. H., Scott, J. K., Orapa, W. & Paynter, Q. 2007. History, opportunities and challenges for biological control in Australia, New Zealand and the Pacific islands. Crop Protection, **26**: 255-265.

Keane, R. M. & Crawley, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution, **17**: 164-170.

Keena, M. A. 2006. Effects of temperature on *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Environmental Entomology, **35**: 912-921.

Kelty, J. D. & Lee, R. E. 1999. Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. Journal of Insect Physiology, **45**: 719-726.

Klein, H. 2011. A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in South Africa. African Entomology, **19**: 515–549. Updated at http://www.arc.agric.za/arc-ppri/Documents/Table1-NaturalEnemiesAll.pdf.

Klok, C. J. & Chown, S. L. 1997 Critical thermal limits, temperature tolerance and water balance of a Sub-Antartic caterpillar, *Pringleophaga marioni*. Journal of Insect Physiology, **43**: 685 – 694.

Klok, C. J. & Chown, S. L. 1998. Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). Journal of Insect Physiology, **44**: 615-628.

Klok, C. J. & Chown, S. L. 2000. Lack of cold tolerance in a small, brachypterous sub-Antarctic fly, *Apetaenus litoralis* Eaton (Diptera: Tethinidae), from Marion Island. African Entomology, **8**: 305-308.

Kluge, R. L. 1991. Biological control of triffid weed, *Chromolaena odorata* (Asteraceae), in South Africa. Agriculture, Ecosystems and Environment**, 37**: 193-198.

Kontodimas, D. C., Eliopoulos, P. A., Stathas, G. J. & Economou, L. P. 2003. Comparative temperature-dependent development of *Nephus includens* (Kirsch) and *N. bisignatus* (Boheman) (Coleoptera: Coccinellidae) preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): evaluation of linear and various nonlinear models using specific criteria. Environmental Entomology, **33**: 1-11.

Kriticos, D. J., Sutherst, R. W., Brown, J. R., Adkins, S. W. & Maywald, G. F. 2003. Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp. *indica* in Australia. Journal of Applied Ecology, **40**: 111-124.

Lachenicht, W., Clusella-Trullas, S., Boardman, L., Le Roux, C. & Terbranche, J. S. 2010. Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). Journal of Insect Physiology, **56**: 822-830.

Lagerspetz, K. Y. H. 2006. What is thermal acclimation? Journal of Thermal Biology, **85**: 332-336.

Layne, J. R, Manis, M. L & Claussen, D. L. 1985. Seasonal variation in the time course of thermal acclimation in the crayfish *Orocoectes rusticus*. Freshwater Invertebrate Biology, **4**: 98 – 104.

Lekovic, S., Lazarevic, J., Nenadovic, V. & Ivanovic, J. 2001. The effect of heat stress of A1 and A2 neurosecretory neurons of *Morimus funereus* (Coleoptera: Cerambycidae) larvae. European Journal of Entomology, **98**: 13-18.

Li, H. B., Shi, L., Lu, M. X., Wang, J. J. & Du, Y. Z. 2011. Thermal tolerance of *Frankliniella occidentalis*: effects of temperature, exposure time, and gender. Journal of Thermal Biology, **36**: 437-442.

Liu, H. & Stiling, P. 2006. Testing the enemy release hypothesis: a review and meta-analysis. Biological Invasions, **8**:1535–1545.

Louda, S. M., Arnett, A. E., Rand, T. A. & Russell, F. L. 2003. Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. Conservation Biology, **17**: 73-82.

MacDonald, I. A. W. 1983. Alien trees, shrubs and creepers invading indigenous vegetation in the Hluhluwe-Umfolozi Game Reserve Complex in Natal. Bothalia, **14**: 949-959.

Manrique, V., Cuda, J. P., Overholt, W. A. & Diaz, R. 2008. Temperature-dependent development and potential distribution of *Episimus utilis* (Lepidoptera: Tortricidae), a candidate biological control agent of Brazilian peppertree in Florida. Environmental Entomology, **37**: 862-870.

Manrique V., Diaz R., Erazo L., Reddi N., Wheeler G. S. 2014 Comparison of two populations of *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) as candidates for biological control of the invasive weed *Schinus terebinthifolius* (Sapindales: Anacardiaceae). Biocontrol Science and Technology, **24**: 518–535.

Marais, E., Terblanche, J. S. & Chown, S. L. 2009. Life stage-related differences in hardening and acclimation of thermal tolerance traits in the kelpfly, *Paractora dreuxi* (Diptera, Helcomyzidae). Journal of Insect Physiology, **55**: 336-343.

Masters, R. A. & Sheley, R. L. 2001. Principles and practices for managing rangeland invasive plants. Journal of Range Management, **54**: 502 – 517.

Matsuki, M., Ayres, M. P. & Maclean, S. F. 1994. Temperature effects on growth and molt of *Nematus calais* (Hymenoptera: Tenthredinidae). Environmental Entomology, **23**: 719- 725.

May, B. & Coetzee, J. 2013. Comparisons of the thermal physiology of water hyacinth biological control agents: predicting establishment and distribution pre- and post-release. Entomologia Experimentalis et Applicata, **147**: 241-250.

McClay, A. S. 1996. Biological control in a cold climate: temperature responses and climatic adaptation of weed biocontrol agents. In: Proceedings of the IX International Symposium on Biological Control of Weeds (Eds. Moran, V. C. & Hoffman, J. H.), 377-383. University of Cape Town, Stellenbosch, South Africa.

McClay, A. S & Hughes, R. B. 1995. Effects of temperature on developmental rate, distribution and establishment of *Calophasia lunula* (Lepidoptera: Noctuidae), a biocontrol agent for toadflax *(Linaria* spp.). Biological Control, **5**: 368–377.

McClay, A. S. & Hughes, R. B. 2007. Temperature and host-plant effects on development and population growth of *Mecinus janthinus* (Coleoptera: Curculionidae), a biological control agent for invasive *Linaria* spp. Biological Control, **40**: 405-410.

McConnachie, A. J., Retief, E., Henderson, L. & McKay, F. 2011. The initiation of a biological control programme against pompom weed, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), in South Africa. African Entomology, **19**: 258-268.

McEvoy, P., Cox, C. & Coombs, E. M. 1991. Successful biological control of ragwort *Senecio jacobaea*, by the introduced insects in Oregon. Ecological Applications, **14**: 430-442.

McFadyen, R. E. C. 1988. Ecology of *Chromolaena odorata* in the neotropics. In Muniappan, R. (Ed.) Proceedings of the First International Workshop on Biological Control of *Chromolaena odorata*. 13 -20. Agricultural Experiment Station, University of Guam, Mangilao, Guam, U.S.A.

McFadyen, R.E.C. 1989. Siam weed: a new threat to Australia's north. *Plant Protection Quarterly*, **4**: 3-7.

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

Mgidi, T. N., Le Maitre, D. C., Schonegevel, L., Nel, J. L., Rouget, M. & Richardson, D. M. 2007. Alien plant invasion- incorporating emerging invaders in regional prioritization: a pragmatic approach for Southern Africa. Journal of Environmental Management, **84**: 173-187.

Mills, N. J. & Kean, J. M. 2010. Review- Behavioral studies, molecular approaches, and modeling: methodological contributions to biological control success. Biological Control, **52**: 255-262.

Mitchell, J. D., Hewitt, P. H. & van Der Linde, T. C. 1993. Critical thermal limits and temperature tolerance in the harvester termite *Hodotermes mossambicus*. Journal of Insect Physiology, **39**: 523-528.

Moran, V. C., Hoffmann, J. H. & Zimmermann, H. G. 2005. Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. Frontiers in Ecology and the Environment, **3**: 77-83.

Morin, L., Reid, A. M., Sims-Chilton, N. M., Buckley, Y. M., Dhileepan, K., Hastwell, G. T., Nordblom, T. L. & Raghu, S. 2009. Review of approaches to evaluate the effectiveness of weed biological control agents. Biological Control, **51**: 1-15.

National Environmental Management: Biodiversity Act 2004 (ACT No, 10 of 2004) – Draft Alien and Invasive Species Lists. 2014. Department of Environmental Affairs. Government Gazette, **584**: 1-96.

Neven, L. G. 2000. Physiological responses of insects to heat. Postharvest Biology and Technology, **21**: 103-111.

Nyamukondiwa, C. & Terblanche, J. S. 2010. Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: thermal history affects short-term responses to temperature. Physiological Entomology, **35**: 255-264.

Nietschke, B. S., R. D. Magarey, D. M. Borchert, D. D. Calvin, & E. Jones. 2007. A developmental database to support insect phenology models. Crop Protection **26**: 1444-1448.

Norbu, N. 2004. Invasive success of *Chromolaena odorata* in the Terai of Nepal. Unpublished MSC.thesis, International Institute for Geo-information Science and Earth Observation, Enschede, Netherlands.

Olckers, T. 2004. Targeting emerging weeds for biological control in South Africa: the benefits of halting the spread of alien plants at an early stage of their invasion. South African Journal of Science, **100**: 64-68.

Padmavathi, C., Katti, G., Sailaja, V., Padmakumari, A. P. Jhansilakshmi, V., Prabhakar, M. & Prasad, Y. G. 2013. Temperature thresholds and thermal requirements for the development of the rice leaf folder, *Cnaphalocrocis medinalis*. Journal of Insect Science, **13**: 1-13.

Paterson I.D & Zachariades C. 2013. ISSRs indicate that *Chromolaena odorata* invading southern Africa originates in Jamaica or Cuba. Biological Control, **66**: 132–139.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. & Tsomondo, T. 2001. Economical and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems and Environment, **84**: 1-20.

Ramanand, H. McConnachie, A. J & Olckers, T. 2017. Thermal tolerance of *Liothrips tractabilis*, a biological control agent of *Campuloclinium macrocephalum* recently established in South Africa. Entomologia Experimentalis et Applicata, **162**: 234–242.

Rector, B. G. 2008. Review- Molecular biology approaches to control of intractable weeds: New strategies and complements to existing biological practices. Plant Science, **175**: 437-448.

Reznik, S. Y., Voinovich, N. D. & Vaghina, N. P. 2009. Effect of temperature on the reproduction and development of *Trichogramma buesi* (Hymenoptera: Trichogrammatidae). European Journal of Entomology, **106**: 535-544.

Richardson, D. M. & van Wilgen, B. W. 2004. Invasive alien plants in South Africa: how well do we understand the ecological impacts? South African Journal of Science, **100**: 45-52.

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

Robinson, M. A., Zachariades, C., Robinson, D. E., Cohen, J. E. & Younger, N. 2013. Field host range of *Melanagromyza eupatoriella* in Jamaica: an insect with potential as a biological control agent on *Chromolaena odorata* in South Africa. In: Zachariades, C., Strathie, L.W., Day, M.D. & Muniappan, R. (eds) Proceedings of the Eighth International Workshop on Biological Control of *Chromolaena odorata* and other Eupatorieae, Nairobi, Kenya, 1-2 November 2010. ARC-PPRI, Pretoria, South Africa, pp. 102-109.

Rusdy, M., Sjahril, R., Riadi, M. & Budiman. 2013. Integration of mechanical and cultural control treatments to manage invasive shrub *Chromolaena odorata* and other weeds under

drought conditions in pasture area. Journal of Indonesian Tropropical Animimal Agriculture, **38**: 65-71.

Saska, P., Martinkova, Z. & Honek, A. 2010. Temperature and rate of seed consumption by ground beetles (Carabidae). Biological Control, **52**: 91-95.

Sinclair, B. J., Alvrado, L. E. C., & Ferguson, L. V. 2015. An invitation to measure insect cold tolerance: Method, approaches, and workflow. Journal of Thermal Biology, **53**: 180-197.

Sinclair, B. J., Terblanche, J. S., Scott, M. B., Blatch, G. L., Klok, C. J. & Chown, S. L. 2006. Environmental physiology of three species of collembolan at Cape Hallet, North Victoria Land, Antarctica. Journal of Insect Physiology, **52**: 29-50.

Solis, M. A., Metz, M. A., & Zachariades, C. 2008. Identity and generic placement of *Phestinia costella* Hampson (Lepidoptera: Pyralidae: Phycitinae) reared on the invasive plant *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae). Proceedings of the Entomological Society of Washington, **110**: 292-301.

Somero, G. N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. Journal of Experimental Biology, **213**: 912-920.

Stotter, R. L. & Terblanche, J. S. 2009. Low-temperature tolerance of false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricicidae) in South Africa. Journal of Thermal Biology, **34**: 320-325.

Strathie, L. W. & Zachariades, C. 2004. Insects for the biological control of *Chromolaena odorata*: surveys in the northern Caribbean and efforts undertaken in South Africa. In: Day, M.D. & McFadyen, R. E. (Eds) Proceedings of the Sixth International Workshop on Biological Control and Management of Chromolaena. ACIAR Technical Reports, **55**: 45–52.

Sutherst, R. W. & Maywald, G. F. 1985. A computerized system for matching climates in ecology. Agriculture, Ecosystems and Environment, **13**: 281-299.

Sutherst, R. W., Maywald, G. F. & Kriticos, D. 2007. CLIMEX V E R S I O N 3: User's Guide. Hearne Scientific Software Pty Ltd.

Tamiru, A., Getu, E. Jembere, B. & Bruce, T. 2012. Effect of temperature and relative humidity of the development and fecundity of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). Bulletin of Entomological Research, **102**: 9-15.

Te Beest, M., Elschot, K., Olf, H., & Etienne, R. S. 2013. Invasion success in a marginal habitat: An experimental test of competitive ability and drought tolerance in *Chromolaena odorata*. Plos One, 8(8): e68274.

Terblanche, J. S. 2014. Physiological performance of field-released insects. Current Opinion in Insect Science, **5**: 1-7.

Terblanche, J. S., Clusella-Trullas, S., Deere, J. A. & Chown, S. L. 2008. Thermal tolerance in a south-east African population of tsetse fly *Glossina pallidipes* (Diptera: Glossinidae): implications for forecasting climate change impacts. Journal of Insect Physiology, **54**: 114-127.

Terblanche, J. S., Klok, C. J., Krafsur, E. S. & Chown, S. L. 2006. Phenotypic flexibility and geographic variation in thermal tolerance and water loss of the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modelling. American Journal of Tropical Medicine and Hygiene, **74**: 786-794.

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C. & Chown, S. L. 2007. Critical thermal limits depend on methodological context. Proceedings of the Royal Society B, **274**: 2935-2942.

Terblanche, J. S., Sinclair, B. J., Klok, C. J., McFarlane, M. L. & Chown, S. L. 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). Journal of Insect Physiology, **51**: 1013-1023.

Theoharides, K. A. & Dukes, J. S. 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. New Phytologist, **176**: 256–273.

Thomas, M. B. & Willis, A. J. 1998. Biocontrol: Risky but necessary? Trends in Ecology and Evolution, **13**: 325-329.

Turpie, J. K., Marais, C. & Blignaut, N. J. 2008. The Working for Water Programme: Evolution of a payments for ecosystem services mechanism that addresses both poverty and ecosystem service delivery in South Africa. Ecological Economics, **65**: 788-798.

Ulmer, B. J., Jacas, J. A., Pen, J. E., Duncan, R. E. & Castillo, J. 2006. Effect of temperature on life history of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae), an egg parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biological Control, **39**: 19-25.

Uyi, O. O., Zachariades, C., Hill, M. P. & McConnachie, A. J. 2016. Temperature-dependent performance and potential distribution of *Pareuchaetes insulata*, a biological control agent of *Chromolaena odorata* in South Africa. Biological Control, **61**:815 – 825.

Uyi, O. O., Zachariades, C., Marais, E. & Hill, M.P. 2017. Reduced mobility but high survival: thermal tolerance and locomotor response of the specialist herbivore, *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebidae), to low temperatures. Bulletin of Entomological Research, **107**: 448–457.

Van Lenteren, J. C., Bale, J., Bigler, F., Hokkanen, H. M. T. & Loomans, A. J. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annual Review of Entomology, **51**: 609-634.

Wagner, T. L., Olson, R. L. & Willers, J. L. 1991. Modeling arthropod development time. Journal of Agricultural Entomology, **8**: 251-279.

Willott, S. J. & Hassall, M. 1998. Life-history responses of British grasshoppers (Orthoptera: Acrididae) to temperature change. Functional Ecology, **12**: 232-241.

Winston, R. L., M. Schwarzländer, H. L. Hinz, M. D. Day, M. J. W. Cock & M. H. Julien, Eds. 2014. Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds, 5th edition. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia. FHTET-2014-04. 838 pp. Updated at: https://www.ibiocontrol.org/catalog/agents.cfm?weed=55.

Yoder, J. A., Chambers, M. J., Tank, J. L. & Keeney, G. D. 2009. High temperature effects on water loss and survival examining the hardiness of female adults of the spider beetles, *Mezium affine* and *Gibbium aequinoctiale*. Journal of Insect Science, **9**:1-8.

Zachariades, C. 2011. Biological control agents released and established on *Chromolaena odorata* worldwide. *Chromolaena odorata* Newsletter, **18**:3-4.

Zachariades, C., Muniappan, R. & Strathie, L. W. (eds) 2002. Proceedings of the Fifth International Workshop of Biological Control and Management of *Chromolaena odorata*. ARC-Plant Protection Research Institute, Pretoria, South Africa.

Zachariades, C., Strathie-Korrûbel, L. W. & Kluge, R. L. 1999. The South African programme on the biological control of *Chromolaena odorata* (L.) King & Robinson (Asteraceae) using insects. African Entomology Memoir, **1**: 89-102.

Zachariades, C., Day, M., Muniappan, R. & Reddy, G. V. P. 2009. *Chromolaena odorata* (L.) King and Robinson (Asteraceae). In: Muniappan, R., Reddy, G.V.P. & Raman, A. (Eds) Biological Control of Tropical Weeds Using Arthropods, 130–160. Cambridge University Press, Cambridge, U.K.

Zachariades, C., Strathie, L. W., Retief, E. & Dube, N. 2011. Progress towards the biological control of *Chromolaena odorata* (L.) R.M. King and H. Rob. (Asteraceae) in South Africa. In: Moran, V.C., Hoffmann, J.H. and Hill, M.P. (eds), Biological Control of Invasive Alien Plants in South Africa (1999-2010). African Entomology, **19**: 282-302.

Zachariades, C., Dube, N., Nqayi, S. B., Dlomo, S. I. & Uyi, O. O. 2019. Attempts to establish *Dichrorampha odorata* on *Chromolaena odorata* in South Africa. Extended

Abstract, in Proceedings of the 15th International Symposium on Biological Control of Weeds, Engelberg, Switzerland, 26-31 August 2018. CABI, in press.

Zachariades, C., Paterson, I. D., Strathie, L. W., Hill, M. P. & van Wilgen, B. W. 2017. Assessing the status of biological control as a management tool for suppression of invasive alien plants in South Africa. Bothalia: **47**, a2142. https://doi.org/10.4102/abc. v47i2.2142.

Zalom, F. G., Goodell, P. B., Wilson, L. T., Barnett, N. W., & Bentley, W. J. 1983. Degree days: the calculation and use of heat units in pest management. Division of Agriculture and Natural Resources, University of California Leaflet 21373, pp. 10.

Zhou, Z. S., Guo, J. Y., Chen, H. S. & Wan, F. H. 2010. Effects of temperature on survival, development, longevity, and fecundity of *Ophraella communa* (Coleoptera: Chrysomelidae), a potential biological control agent against *Ambrosia artemisiifolia* (Asterales: Asteraceae). Environmental Entomology, **39**: 1021-1027.

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