Aspects influencing the efficacy of *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Phlaeothripidae), a biological control agent for the invasive weed *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) in South Africa

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PREFACE

The research described in this thesis was carried out at the facilities of the Agricultural Research Council- Plant Protection Research Institute (ARC-PPRI) at Cedara and the School of Life Sciences, University of KwaZulu-Natal (Pietermaritzburg), from March 2013 to December 2014, under the supervision of Dr T. Olckers and the co-supervision of Dr A.J. McConnachie.

The work presented in this thesis represents the original work of the author and has not been otherwise submitted in any other form for any degree or diploma to any other University. Where use has been made of the work of others, this has been duly acknowledged in the text.

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ABSTRACT

Pompom weed, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), an unpalatable, perennial, erect invasive herb from South America has become naturalized in South Africa, invading grasslands, savannas and wetlands, where it has a significant impact on biodiversity. In order to sustainably curb the spread and negative impact of the weed, *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripidae) was imported from South America (Argentina) as a candidate biological control agent. Quarantine tests demonstrated that the thrips was suitably host specific and damaging to the target weed and permission for its release in South Africa was granted in 2013. However, numerous biocontrol agents worldwide have displayed exceptional potential while in quarantine but have had little to no success following their release in the field.

This study incorporated both laboratory and field trials to determine the likelihood of success with the thrips. *Liothrips tractabilis* developmental threshold trials were conducted at seven constant temperatures (15, 17.5, 20, 25, 27.5, 30, 32°C) and the data, excluding the uppermost and lowermost temperatures (as the trips did not survive at these temperatures), were ultimately used to develop a degree-day model. The findings of the model were then validated under outdoor conditions. Furthermore, the impact of the thrips was assessed on seedlings and root crown regrowth shoots under outdoor conditions, and the results were compared to those of the laboratory impact trials that were conducted while the agent was still under investigation in quarantine.

The thrips completed development at all five temperatures, with the number of days taken to develop from egg to adult decreasing with increasing temperature. Lethal temperatures were recorded at 15°C and 32.5°C where no development beyond the egg stage was observed. The lower developmental threshold (t) was estimated at 9.6°C with 546.9 degree-days (°D) required by the thrips to complete its development. The degree-day model predicted that in Gauteng, parts of Limpopo, North West and Mpumalanga provinces, where *C. macrocephalum* is invasive, the thrips is likely to complete 3-9 generations per year. The outdoor developmental trials did validate the model and although temperatures recorded in the laboratory and field trials were not equal, the field data largely supported the predictions of the laboratory trials. Furthermore, the thrips developed significantly faster at the Pietermaritzburg site in comparison to Cedara, which was largely a consequence of low

altitude and higher ambient temperatures. A significant difference was also obtained across the three seasons, where the thrips developed fastest during summer, and slowest during winter at Pietermaritzburg. The same was true at Cedara, although no development occurred during the winter trials. The impact trials showed that the thrips significantly reduced the height, number of leaves and both wet and dry masses of *C. macrocephalum* seedlings, which was largely in agreement with the original laboratory study. However, this was not the case with the regrowth trials, where only relative growth rates in terms of wet tuber mass were significantly reduced by thrips feeding. These results were largely a consequence of varying tuber wet masses used at the start of the trials.

Liothrips tractabilis appears to be climatically compatible with conditions in South Africa, since this study has shown that the establishment and persistence of *L. tractabilis* is unlikely to be limited by climatic conditions in areas that are currently invaded by the target weed. Furthermore, the agent should be able to inflict appreciable damage and hence have an impact on *C. macrocephalum* populations in the field. Thus, prospects for the biological control of *C. macrocephalum* in South Africa appear promising.

Key words: Agent impacts, climatic compatibility, developmental rates/threshold, degree-day model, weed biocontrol.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INVASIVE ALIEN PLANTS

Invasive alien plants (IAPs) are non-native species that easily surmount geographic and environmental barriers (often via human assistance), thereby establishing themselves quickly, and then expand their numbers and ranges rapidly within the new habitat, often displacing or extirpating populations of native species in the process (Daehler 2003; Culliney 2005). Such plants are now a worldwide problem and are regularly introduced into new ranges; often unintentionally, but mostly intentionally (Daehler 2003). Human disturbances within natural ecosystems, which include habitat fragmentation, habitat conversion and agricultural or commercial practices, have escalated 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 alarming. This is largely a consequence of human population growth, as well as increasing emigration, international air travel and the intentional movement of species outside their native range (Culliney 2005). Reasons for deliberate introductions of these plants into new ranges include their use as ornamentals, agroforestry species, crops, hedge plants and fodder (Mgidi *et al*. 2007).

The introduction of IAPs into South Africa started in the mid-1600s (Moran *et al*. 2005, 2013) and has persisted for several centuries. South Africa has been invaded by at least 200 major IAP species (Henderson 2001) which includes several noxious plants such as *Lantana camara* L. (Verbenaceae), *Chromolaena odorata* L. King and Robinson (Asteraceae), *Opuntia* species (Cactaceae), *Parthenium hysterophorus* L. (Asteraceae) and, more recently, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) (Olckers 2004).

Invasive alien plants rapidly colonise disturbed areas with the additional ability to encroach upon undisturbed, pristine areas; thereby posing serious threats to ecosystems, human health and the economy of countries (Daehler 2003; Culliney 2005). This is in most part caused by the fact that IAPs, in their introduced ranges, lack the natural enemies with which they have co-evolved in their native range to maintain them at acceptable levels (Zimmermann *et al*. 2004). The enemy release hypothesis (ERH), which stems from the lack of natural enemies in the introduced range, constitutes one of several hypotheses explaining why IAPs become problematic in a novel range (Keane & Crawley 2002). Other hypotheses

that explain plant invasion include biodiversity resistance (Kennedy *et al*. 2002), evolution of improved competitive ability (Blossey & Notzold 1995) and niche opportunity (Shea & Chesson 2002).

Problems arising from IAPs are a major ecological concern as they negatively affect natural habitats by means of a number of processes. These include: reducing biodiversity via competition or hybridization; simplifying food webs; altering fire regimes and hydrological cycles; altering soil chemistry and biology through changes in pH and nutrient cycling, salt accumulation, nitrogen fixation, or changing the composition of soil fauna and flora; affecting usual geomorphological processes through siltation or erosion of stream banks and sand dunes; and altering pollinator activity (Daehler 2003; Culliney 2005). Plant invasions have been recognized as a major factor driving global environmental change, thus rivalling habitat destruction as a contributor to species extinction (Richardson & van Wilgen 2004). In South Africa, IAPs also utilize large volumes of scarce water supplies resulting in reduced river flows and consequently impacting on the economy (Moran *et al*. 2005). It has been acknowledged that IAPs within South Africa currently reduce river-flow by 6-22% and if left uncontrolled, this could increase to 22-95% over a 26-30 year period (Le Maitre *et al*. 2001).

Economic losses caused by IAPs are greater than those caused by any other pest categories (Culliney 2005). This is largely the consequence of the high costs involved in managing plant invasions, loss of agricultural products due to weed seed contamination, reduced quality and yield of valued crops and livestock poisoning (Richardson & van Wilgen 2004). Moreover, the causes and consequences of global climate change may create further avenues for plant invasions, thereby increasing their frequency and severity (Hellmann *et al*. 2008; Verlinden & Nijis 2010).

Many of the IAPs present in South Africa originate from Australia, South and Central America, and North America (Zimmermann *et al*. 2004). The Working for Water (WFW) Programme, established in 1995, is aimed at managing IAPs within South Africa, and since 2003 has invested substantial funds in pursuit of this (Moran *et al*. 2013). The WFW programme utilizes chemical and mechanical control methods and supports the integration of these methods with biological control in the management of IAPs (Zimmermann *et al*. 2004).

1.2 BIOLOGICAL CONTROL OF INVASIVE ALIEN PLANTS

Biological weed control utilizes natural enemies (biological control agents), that are either insects or pathogens, to reduce either the vigour or reproductive potential of an invasive alien plant (McFadyen 1998). The principle underpinning this approach is that IAPs become invasive in their new ranges as there are no natural enemies to regulate their populations. Thus, alien plants acquire a competitive advantage over indigenous vegetation, as indigenous plants have their own natural enemies that either feed on them or result in them developing diseases (Daehler 2003). Biological control aims to introduce the alien plant's natural enemies into its new habitat, assuming that they will remove the plant's competitive advantage until its vigour has declined to a level comparable to that of the natural vegetation (McFadyen 1998).

Biological control programmes involve several procedures (as outlined by Culliney 2005) that start by surveying the target weed in its native range (country of origin) in order to identify candidate biological control agents, as well as surveying the target weed in its introduced range to determine if there are damaging agents already present on the weed. The next step involves importing candidate agents into quarantine where they are selected and screened for diseases and parasites. Selected agents then undergo rigorous host-specificity testing to determine their potential impact on both native and economically important non-target species. This is done to evaluate the risks associated with each agent and to destroy ineffective agents with low host specificity. Once permission for release is obtained, effective agents are mass-reared, released and established to reduce and maintain the target weed populations at non-damaging levels. Biological control provides a viable solution to plant invasion, as it is self-sustaining and cost-effective when compared with conventional methods, since many established agents do not require re-application (Barratt *et al*. 2010). Moreover, it is considered to be an environmentally friendly approach, as it does not result in pollution of natural resources or pose any threats to wildlife (Barratt *et al*. 2010). Furthermore, because agents are tested for host specificity prior to release, the possibility of non-target effects is reduced substantially.

Biological control in South Africa was originally initiated in 1913, based on research conducted in countries such as Australia and the United States of America (Moran *et al*. 2005, 2013). South Africa has since advanced over the years in terms of biological control research and is now recognized as one of the world leaders in the field. The first biological

control programme against an invasive alien plant in South Africa was the introduction of a sap-sucking cochineal insect, *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae), to control drooping prickly pear, *Opuntia monacantha* Haw. (Cactaceae) (Zimmermann *et al*. 2004). Following on from this, several weed biological control programmes were launched in South Africa and some 61% of these have demonstrated varying degrees of success (Moran *et al*. 2005, 2013). Some of these programmes are recent and unique to South Africa and one of these is the programme against *C. macrocephalum* (Asteraceae).

1.3 INFLUENCE OF TEMPERATURE ON ESTABLISHMENT AND SUCCESS OF BIOLOGICAL CONTROL AGENTS

Effective biological control agents should be safe for release, damaging to the target weed and have the ability to persist under variable climatic conditions in their new range (Kluge 2000). However, only 10-20% of weed biological control agents become established in their new range (McFadyen 2003). Successful establishment of agents is limited by factors that put small populations at risk of extinction; among these are demographic stochasticity, environmental variability and Allee effects (Grevstad 1999). According to Byrne *et al*. (2003), 44% of weed biological control agents are unable to establish due to climatic incompatibility of the agent, usually an insect, within its introduced range.

Concerns revolving around climate change and its impact on biodiversity have highlighted studies investigating the effect of temperature on living organisms (Lachenicht *et al*. 2010), including insects. Abiotic factors, in particular temperatures in the introduced range, are some of the causes resulting in unsuccessful establishment of weed biocontrol agents (McClay & Hughes 2007). As a result, the negative effects of environmental conditions may limit the effectiveness and persistence of biological control agents in their new range (Hill & Olckers 2001). This is evident from the study conducted by McClay & Hughes (2007) which revealed that the performance of a stem-mining weevil *Mecinus janthinus* Germar (Coleoptera: Curculionidae), a biological control agent of *Linaria vulgaris* P. Mill. and *Linaria dalmatica* (L.) P. Mill (Scrophulariaceae) from Europe, was limited by climatic factors in Alberta, Canada. Therefore, in addition to quarantine host-specificity testing and impact studies on candidate agents, studies pertaining to aspects of their thermal

physiology and climatic adaptability should be explored to enhance our understanding of their climatic suitability to conditions encountered in the introduced range.

Outputs generated from climate matching studies may enhance the effectiveness of biological control programmes by reducing the failure of agents to establish, or to have impact, as a result of environmental conditions not matching their thermal physiology (McClay 1996). Modelling approaches, such as insect development (degree-day) models, provide a means by which practical and meaningful interpretation can be achieved (Byrne *et al*. 2003). Such models, that use only empirical data, utilize temperature and time to predict the number of generations that an insect can be expected to complete at a given locality and may thus be successful at predicting the likelihood of an agent establishing at a particular locality (Byrne *et al*. 2003). Predictions from such studies can then be verified in the field by undertaking releases at sites that incorporate a range of climatic conditions. For example, a degree-day model predicted 4-20 generations of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) per year at different localities infested by the aquatic weed *Azolla filiculoides* around South Africa (Byrne *et al*. 2003). This was confirmed by widespread establishment of the beetle, with field sampling suggesting that the estimations may have been marginally low (Byrne *et al*. 2003), thus proving that such modelling can play a pivotal role in the field of weed biological control.

1.4 *CAMPULOCLINIUM MACROCEPHALUM*

1.4.1 Description and biology

Campuloclinium macrocephalum (Less.) DC. (Asteraceae), commonly known as pompom weed (Fig. 1), constitutes one of the more recent IAPs to be targeted with biological control in South Africa (McConnachie *et al*. 2011). The plant is an unpalatable perennial, erect herb that grows up to 1.5m high (Henderson 2001; McConnachie *et al*. 2011). Descriptions of the plant are provided by Henderson (2001) and McConnachie *et al*. (2011) and are summarized below. Both stems and leaves (Fig. 1A) are covered by coarse, bristly hairs and the leaves are light green with serrated margins and are scattered along the length of the stem, but become clustered at the base forming a rosette. The plant includes a short woody rootstock ending in thick tuber-like roots; in spring, shoots arise from the rootstock while in autumn, they die back to the rootstock. The attractive and distinctive pink

inflorescences (flower heads) (Fig. 1B) are produced in thick clusters at the ends of the aerial stems (situated terminally). Every flower head, measuring 15mm long x 25mm wide, comprises hundreds of small, star-shaped florets (Fig. 1C) which are surrounded by purple/pink bracts. Each mature floret results in a single-seeded dry fruit (achene) (Fig. 1D) that has a tuft of hairs (pappus) which promotes wind dispersal. Long distance seed dispersal occurs via people who pick the flowers and by attachment to vehicles or machinery (Trethowan *et al*. 2011). *Campuloclinium macrocephalum* typically flowers from December through to March (Henderson 2001).

The plant can establish itself and survive in a wide range of habitats at altitudes of 0-1900m or more (ARC 2007a; McConnachie *et al*. 2011; Trethowan *et al*. 2011). It is tolerant to most soil types with considerable effort invested into its perennial underground structures, the tuber-like roots (McConnachie *et al*. 2011; Trethowan *et al*. 2011). The annual shoots and leaves are clearly visible in summer and account for approximately 30% of the plant's total biomass (ARC 2007a). The plant thus has the ability to survive fires and frost during winter as all its living components remain underground in a dormant state (ARC 2007a; McConnachie *et al*. 2011). When faced with drought conditions during summer, the plant is also able to revert back to a dormant state by withdrawing its nutrients from the shoots back into the roots (ARC 2007a). Therefore, the plant has evolved strategies which facilitate its survival and proliferation in both grassland and savanna ecosystems in South Africa (ARC 2007a). *Campuloclinium macrocephalum* is usually found together with another closely related invasive plant, purple top (*Verbena bonariensis* L.; Asteraceae), which may thus serve as an indicator of areas that are suitable for its establishment (ARC 2007a). The plant has been shown to reach densities of up to 27 mature (flowering) plants/m², and 249 seedlings/m² (McConnachie *et al*. 2011). The viable component of its seed bank has also been found to be as high as 6864 seeds/m² (McConnachie *et al*. 2011). Therefore, efficient wind dispersal of the seeds, combined with the plant's enormous reproductive potential, enables it to rapidly invade large areas.

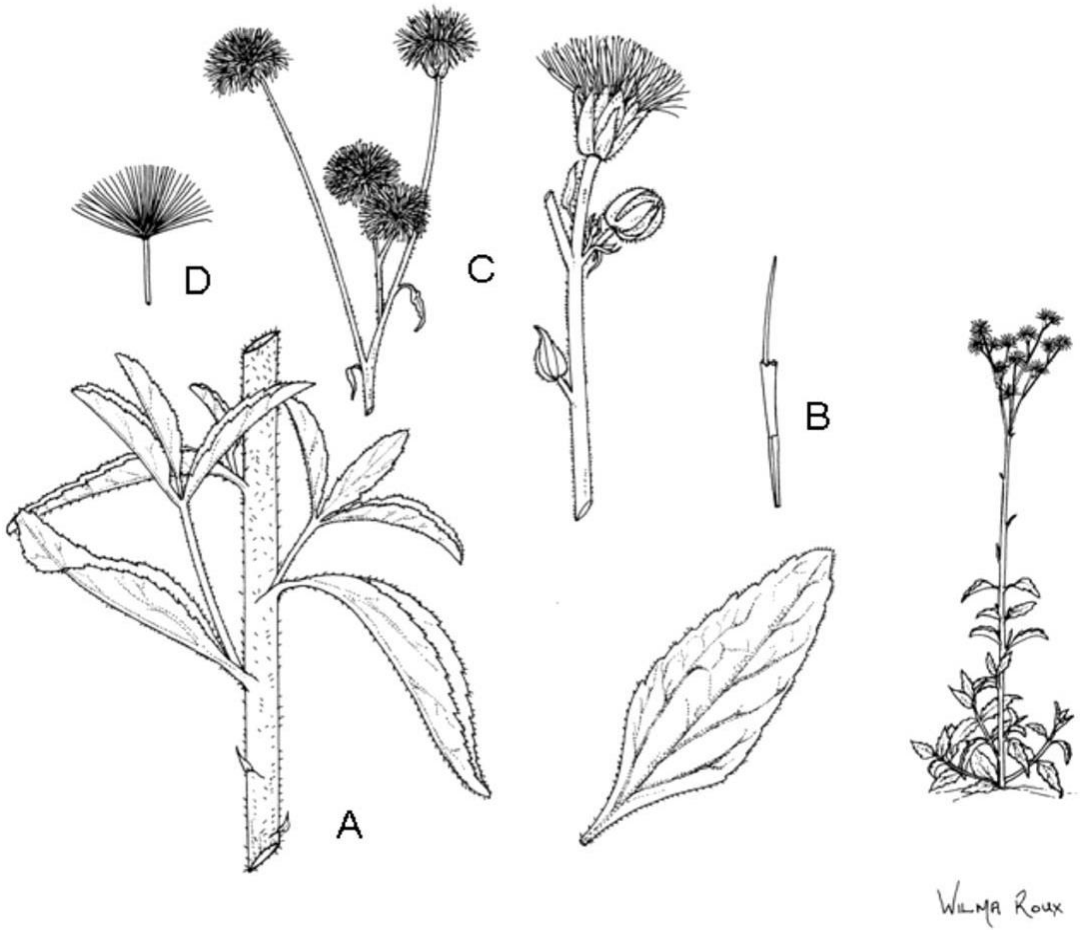

Fig. 1. *Campuloclinium macrocephalum* stems and leaves (A), inflorescences (B), florets (C) and achenes (D) (reproduced from McConnachie *et al*., 2011).

1.4.2 Distribution and negative effects

Campuloclinium macrocephalum has a wide natural distribution extending from Argentina in South America, to Costa Rica and Honduras in Central America, and to Mexico in North America (Henderson 2001; McConnachie *et al*. 2011; Trethowan *et al*. 2011). This plant has become naturalized in South Africa and questions still persist as to how and when it was introduced (McConnachie *et al*. 2011). A specimen collected in 1962 (Pretoria National Herbarium), apparently from the Johannesburg area, is the earliest record of the plant in South Africa (McConnachie *et al*. 2011). During the early 1960s, *C. macrocephalum* was initially recorded as an escapee from cultivation within the Fountains Valley area of Pretoria (25°46'52"S 28°11'37"E), as well as at Westville (29°49’43"S 30°55’58"E), not far from

Durban, during 1972 (McConnachie *et al*. 2011). The spread of *C. macrocephalum* has been documented via roadside surveys and the Southern African Plant Invaders Atlas (SAPIA) project since the 1980s (McConnachie *et al*. 2011). *Campuloclinium macrocephalum* gradually increased within the Pretoria (Gauteng) area, spreading to the Limpopo Province in the 1980s (McConnachie *et al*. 2011). During the 1990s and 2000s, there was an alarming, exponential expansion phase, resulting in vast tracts of land being invaded in Gauteng (Highveld grasslands), parts of Limpopo, and the North West and Mpumalanga provinces (McConnachie *et al*. 2011). The SAPIA project revealed a near-doubling in the number of quarter-degree squares (48 in March 2005 to 93 in March 2010) (Fig. 2) in which the weed was recorded over a 5-year period (McConnachie *et al*. 2011). *Campuloclinium macrocephalum* has also been recorded in the KwaZulu-Natal and Free State provinces (McConnachie *et al*. 2011; Trethowan *et al*. 2011). A confirmed occurrence was also reported near George in the Western Cape (Trethowan *et al*. 2011). The plant has also been found in Swaziland (McConnachie *et al*. 2011). According to the Conservation of Agricultural Resources Act, 1983 (Act No 43 of 1983) (CARA) in South Africa, this plant is designated as a Category 1 plant (declared weed), meaning that it must be controlled wherever present and may not be propagated or spread (Henderson 2001).

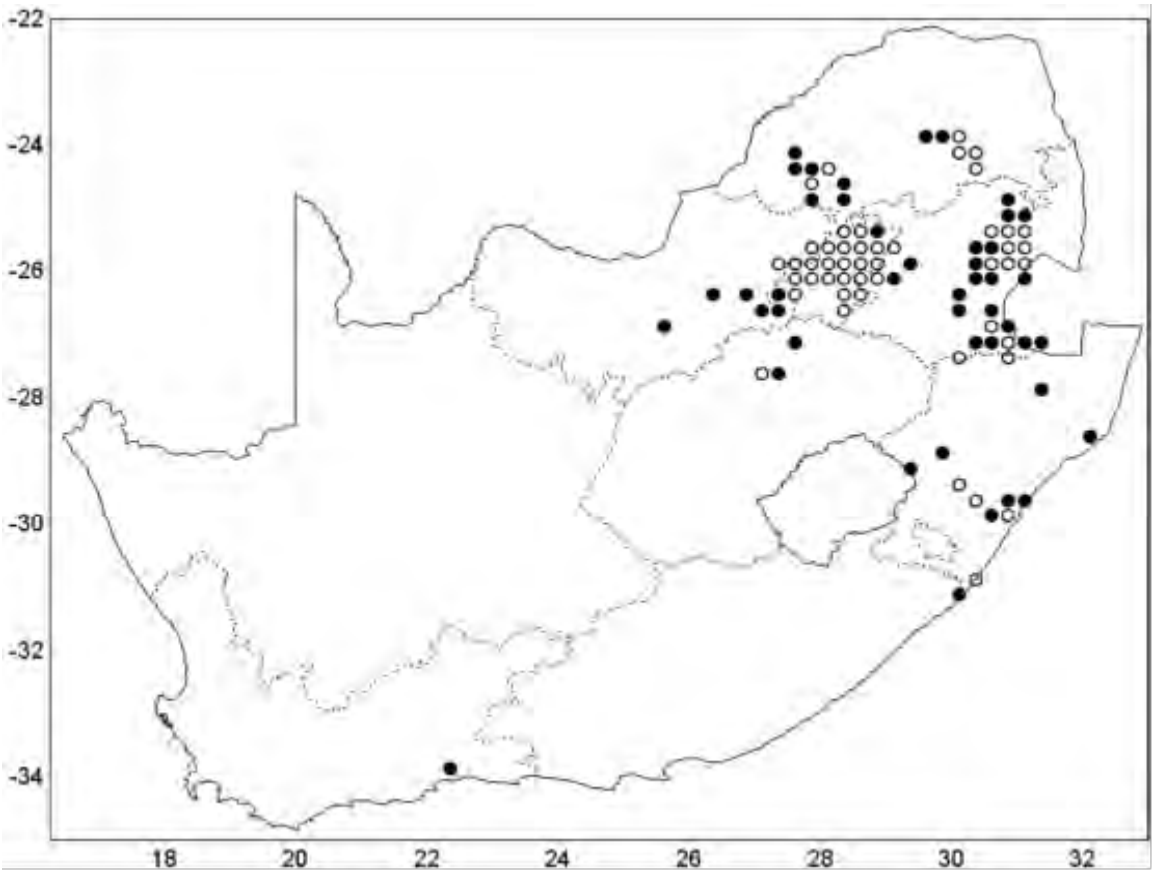

Fig. 2. Distribution of *Campuloclinium macrocephalum* in South Africa as at March 2010. Closed circles represent quarter degree squares occupied from April 2005 to March 2010 (reproduced from McConnachie *et al*. 2011).

Campuloclinium macrocephalum is negatively affecting the conservation of grasslands in South Africa and if no action is taken against it, the plant may invade the entire grassland biome (McConnachie *et al*. 2011; Trethowan *et al*. 2011). The fleshy, tuber-like roots deprive the soil of water and nutrients, inhibiting the growth of indigenous wild flowers and veld grasses in their vicinity (Moremi 2010). Several models on the predicted distribution of *C. macrocephalum* have been provided by Trethowan *et al*. (2011), indicating that the weed may indeed spread across a greater region than it currently occupies (Fig. 3). It was also predicted that the savanna and grassland biomes are most vulnerable to invasion (Trethowan *et al*. 2011).

The plant first establishes itself in disturbed sites such as roadsides, but then invades natural grasslands, open savanna and wetlands (McConnachie *et al*. 2011; Trethowan *et al*.

2011). The ability of *C. macrocephalum* to invade is better explained by the absence of natural enemies than by allelopathy which is not deemed to be a causal mechanism for the invasiveness of the plant, as indicated by preliminary studies (Goodall *et al*. 2011). A significant decline in plant diversity has been shown to be caused by *C. macrocephalum*; however, there has been no effect on insect diversity (McConnachie *et al*. 2011; Trethowan *et al*. 2011). *Campuloclinium macrocephalum* is recognized as being unpalatable to livestock, as well as grazing wildlife, and will thus result in a reduction of the carrying capacity of farms and game reserves (McConnachie *et al*. 2011; Trethowan *et al*. 2011). Some of the most threatened vegetation types in South Africa are situated within the grassland biome and a considerable portion of this biome has already been transformed by *C. macrocephalum* (Trethowan *et al*. 2011). The seriousness of the negative effects and problems associated with *C. macrocephalum* requires immediate intervention in order to manage the plant and prevent it from spreading further.

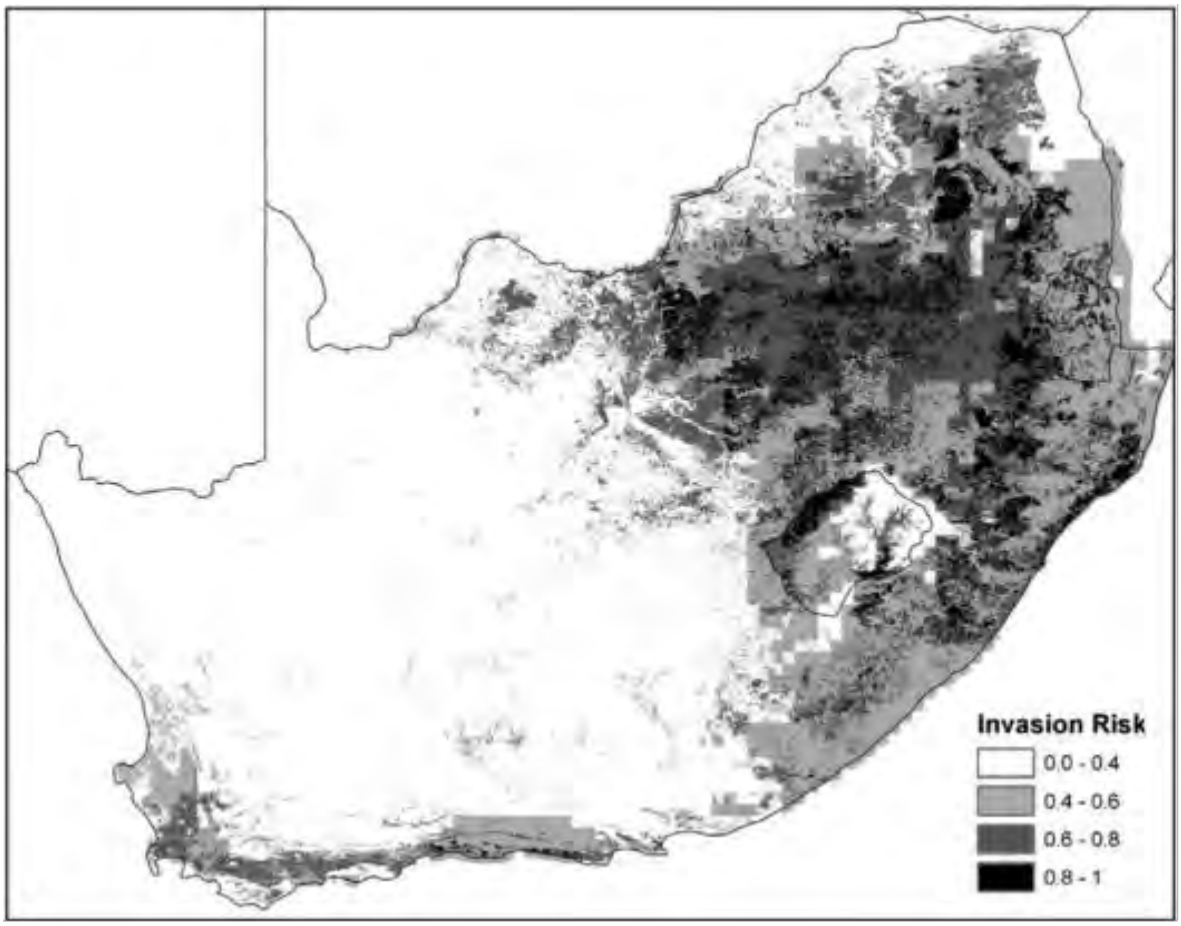

Fig. 3. The invasion risk posed by *C. macrocephalum* in South Africa, Lesotho and Swaziland (reproduced from Trethowan *et al*. 2011).

1.5 METHODS OF CONTROLLING *CAMPULOCLINIUM MACROCEPHALUM*

1.5.1 Mechanical control

Mechanical control is widely practiced in countries where problematic IAPs are present but is labour intensive and requires repeated implementation (Zimmermann *et al*. 2004). In general, physical methods utilized for controlling *C. macrocephalum*, such as uprooting or hoeing, have been deemed ineffective and further exacerbate the problem through disturbance (McConnachie *et al*. 2011). Ploughing lands where *C. macrocephalum* is present is also not advised as this merely results in damage to the rootstock, thereby stimulating further vegetative growth and denser stands (McConnachie *et al*. 2011).

1.5.2 Chemical control

Chemical control has always been considered as the quickest method to manage weeds in the short term, although it encompasses a number of side effects. The major disadvantage is that it requires re-application, with additional costs, and weeds often develop resistance to herbicides over time (Labrada 1994). Several herbicides have been utilized against *C. macrocephalum* in South Africa, especially for roadside applications (Goodall *et al*. 2011). These include metsulfuron methyl (600g kg^{-1}) (Brushoff®, made by DuPont), which provided 80% control of pompom weed in field trials, and picloram (240g l^{-1}) (Access 240®, made by Dow Agro-Sciences), at concentrations of 0.25g and 3.5 ml l^{-1} water, respectively (ARC 2007b). Both herbicides have to be applied with a mineral oil adjuvant and should ideally be applied to actively growing plants in early summer when flowering begins (ARC 2007b).

All known infestations of *C. macrocephalum* in KwaZulu-Natal are treated with herbicides by the Invasive Alien Species Programme of the Department of Agriculture, Environment and Rural Development (McConnachie *et al*. 2011). Considerable progress has also been realized with chemical control programmes initiated in the North West, Limpopo, Mpumalanga and Free State provinces during the summer of 2009/2010 (McConnachie *et al*. 2011).

So far, control of *C. macrocephalum* in South Africa has been based on herbicides (McConnachie *et al*. 2011). However, these herbicides may affect non-target plant species and have also not been recommended for use in ecologically sensitive areas such as wetlands (McConnachie *et al*. 2011). Also, due to the extent of current invasions of *C.*

macrocephalum, chemical control is likely to become impractical and unaffordable over the long term (McConnachie *et al*. 2011).

1.5.3 Biological control

Given the above shortcomings, biological control is likely to become the only sustainable and cost effective method for controlling *C. macrocephalum* in South Africa. In 2003, a biological control programme against *C. macrocephalum* was first initiated in South Africa by the Agricultural Research Council's Plant Protection Research Institute (ARC-PPRI) as part of an initiative to target "emerging weeds" (i.e. plants at an early stage of invasion) for biological control (Olckers 2004).

Surveys for natural enemies were conducted in Argentina during 2003, 2005, 2006, 2008, 2011 and 2013, as well as in Brazil in 2006 (McConnachie *et al*. 2011; McConnachie & McKay 2015; A. McConnachie, pers. comm.). Northern Argentina was found to have the highest diversity of natural enemies associated with *C. macrocephalum* and a total of nine biocontrol candidates were collected from surveys in this region (McConnachie *et al*. 2011). However, only three insect species and one pathogen species were selected for further study on the basis of their impact, distribution or field host-range attributes, namely *Zeale* (= *Adesmus*) *nigromaculatus* Klug (Coleoptera: Cerambycidae), *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Phlaeothripidae), *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae) and *Puccinia eupatorii* Dietel (Pucciniales: Pucciniaceae) (McConnachie *et al*. 2011; McConnachie & McKay 2015).

These agents were imported into South Africa where they were cultured and studied in quarantine. The leaf rust *P. eupatorii* was imported into South Africa in 2003 and was subjected to laboratory trials which included host-specificity testing in quarantine (McConnachie *et al*. 2011). The rust was found to be suitable for release, but in 2006 a rust fungus, whose identity was later confirmed as *P. eupatorii*, was discovered on *C. macrocephalum* in the field near Pretoria, Gauteng Province (25°53'49"S 28°17'38"E) (Goodall *et al*. 2012). Field populations of the inadvertently introduced *P. eupatorii* are believed to have spread widely throughout the invaded range of *C. macrocephalum* in South Africa (McConnachie *et al*. 2011; McConnachie & McKay 2015). Although it is too early to estimate the field impact of the rust on the plant, laboratory studies have suggested that over time it will reduce the weed's underground root stores (McConnachie *et al*. 2011). So far,

there is no evidence to suggest that the quarantine isolate of the rust is any more effective than the field isolate and, as a result, it will not be released in the near future (McConnachie *et al*. 2011; McConnachie & McKay 2015).

Data from the native range and laboratory trials indicated that the host-range of *Z. nigromaculatus* was too broad and that this stem-boring beetle may therefore pose a risk to indigenous South African Asteraceae species. The beetle was thus rejected as a biological control agent for *C. macrocephalum* in South Africa, with all quarantine cultures being destroyed (McConnachie *et al*. 2011). Although quarantine cultures of the flower-feeding moth *C. campuloclinium* have suggested that the immature stages are able to inflict appreciable damage to *C. macrocephalum*, further laboratory testing is still required for confirmation of host range before an application for release can be considered (McConnachie *et al*. 2011). The fourth agent, *L. tractabilis*, which is the subject of this study, is discussed further in the following section.

1.6 *LIOTHRIPS TRACTABILIS*

Liothrips tractabilis is a stem-galling thrips which was first recorded on *C. macrocephalum* in 2004 and was described as a new species by Mound & Pereyra (2008). The thrips was present at 19 of the 66 *C. macrocephalum* sites that were surveyed in Brazil and Argentina (McConnachie *et al*. 2011; McConnachie & McKay 2015). Feeding by the adults and immature stages of *L. tractabilis* causes distortion of the growing parts of *C. macrocephalum*, resulting in a significant reduction in flowering ability (McConnachie *et al*. 2011; McConnachie & McKay 2015). The thrips are believed to survive the dry winter periods, when the above-ground parts of the plant have died back, by retreating underground to feed off the fleshy roots of the plant (A. McConnachie, pers. comm.).

In South Africa, the laboratory host range of *L. tractabilis* was determined by adult no-choice and paired-choice tests under strict quarantine conditions which involved 43 plant species within the family Asteraceae. These included 11 closely related tribes present in the family, as well as tribes containing ornamental and crop species (McConnachie *et al*. 2011; McConnachie & McKay 2015). In the no-choice trials, feeding and/or oviposition was recorded on 13 test species in four tribes, but at lower levels than on the *C. macrocephalum* controls. Paired-choice trials were then undertaken for the 13 species that were utilized in the

no-choice trials. During these trials, there were no traces of feeding or oviposition on any of the test species in comparison to the control plants, which were heavily attacked. Therefore, *L. tractabilis* was considered to be suitably host specific and permission for its release in South Africa was granted in 2013.

The biology and life history of the thrips was described by McConnachie *et al*. (2011) and is summarized below. *Liothrips tractabilis* feeds mainly on new growth (i.e. sepals and stems) of *C. macrocephalum*, which also includes seedlings. The eggs (Fig. 4B) may be laid either singly or in batches on the stems, leaves, as well as sepals, often in regions that have been heavily fed upon by the thrips. The eggs are oval in shape, orange-yellow in colour, 0.45 ± 0.02mm long and 0.19 ± 0.01mm wide, with bumps evenly spaced over the surface. Hatching occurs after approximately 10 days at 25°C. Since *L. tractabilis* is typical of species in the Phlaeothripidae, as with most members of the genus it includes two actively feeding larval stages (Fig. 4C, 5) followed by three "pupal" stages (Palmer *et al*. 1989). The first pupal stage (prepupa) has short antennae-like horns and displays no wing buds (Fig. 5C). The second pupal stage has the antennae turned back over the head, but the wing buds are short (Fig 5D), while the third has similar antennae, but long wing buds (Fig. 4D, 5E). The larval period lasts for about 11 days, with the pupal stages lasting around seven days (McConnachie *et al*. 2011). Development of the thrips from egg to adult (Fig. 4, 5) took about 28 days at 25°C.

Fig. 4. The biological control agent, *Liothrips tractabilis*: (A) adult, (B) eggs, (C) larva, (D) pupa (reproduced from McConnachie 2012).

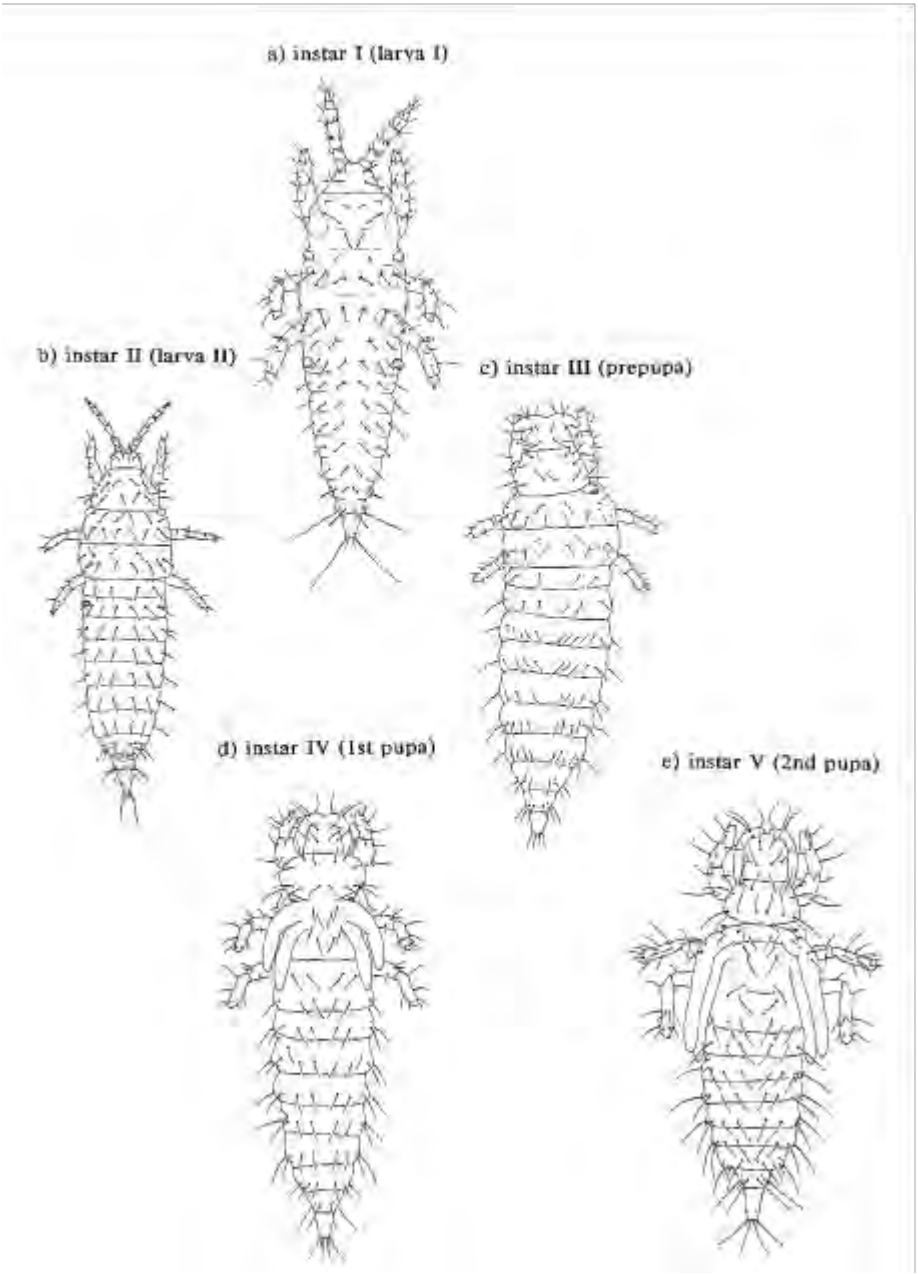

Fig. 5. The immature stages of the Phlaeothripidae (suborder Tubulifera) (reproduced from Palmer *et al*. 1989).

Laboratory impact studies have indicated that *L. tractabilis* significantly affects the growth of *C. macrocephalum* (McConnachie *et al*. 2011). Seedlings at the 8-12 leaf stage that had been inoculated with two pairs of adult thrips, suffered a significant reduction in plant height, number of leaves and wet mass (McConnachie *et al*. 2011). The same was true for root crown regrowth shoots, which also showed a significant reduction in plant height, number of leaves, as well as, wet and dry masses (McConnachie & McKay 2015). When selected initially, the thrips was considered to have potential to survive under South African

climatic conditions, most of which should be favourable for it (McConnachie *et al*. 2011). However, in order to highlight areas where this agent may be limited by climatic or environmental conditions, aspects of its thermal physiology still require investigation.

1.7 RESEARCH AIMS AND OBJECTIVES

This study will incorporate both laboratory and field components. The key question pertaining to this study is whether the candidate biological control agent *L. tractabilis* has the necessary physiological and ecological attributes to become established and proliferate in all areas in South Africa that have become (or may become) invaded by *Campuloclinium macrocephalum*. The study thus aims to predict, and confirm in the field, the suitability of the introduced thrips as a biological control agent of *C. macrocephalum* in South Africa. The objectives are simply threefold: (1) to study the thermal physiology of *L. tractabilis* in order to determine its developmental threshold and develop a degree-day model that can predict the likelihood of its survival in the field; (2) to test the model under actual climatic conditions in the field and; (3) to set up a garden experiment and assess the thrips' impact on seedlings and root crown re-growth for comparison with the results of the laboratory impact study. The outcomes of this study should make an important contribution towards elucidating the potential efficacy of *L. tractabilis* as a biological control agent.

CHAPTER 2: CLIMATIC SUITABILITY OF SOUTH AFRICA FOR *LIOTHRIPS TRACTABILIS*

2.1 INTRODUCTION

Climate influences various population parameters (e.g. size and distribution) of insects, as well as other living organisms, which has attracted increasing interest from entomologists. Consequently, climatic conditions such as rainfall, humidity, light, wind, and temperature all play a role in determining the distribution of biological control agents (van Lenteren *et al*. 2006). As a result of variable climatic conditions, insects thus experience both favourable and unfavourable growing seasons in a particular region (Sutherst 2003). Temperature is one of the most important components of climate that affect insect development (Sutherst & Maywald 1985). This is because the biological activities of poikilothermic organisms depend on energy from chemical reactions that are limited by upper and lower temperature thresholds, thereby affecting insect development (Sutherst & Maywald 1985). As a result of the effects of temperature on insect physiology, climate has a substantial effect on the distribution, abundance (Ulrichs & Hopper 2008) and establishment (Byrne *et al*. 2002; de Guzman & Frake 2007) of insect biological control agents.

Numerous biological control agents have had limited establishment success due to climate incompatibility (Byrne *et al*. 2003; May & Coetzee 2013; Manrique *et al*. 2014) and this could have been predicted by determining their thermal physiological requirements and climatic compatibility prior to release. Indeed, studies on the thermal requirements of biocontrol agents are generally conducted post-release, in order to provide possible explanations for failed establishment (May & Coetzee 2013). However, at this stage, the failure of agents to establish represents a waste of research efforts and funding, particularly in resource-limited countries (Byrne *et al*. 2003). Worldwide, biocontrol practitioners generally do not undertake thermal physiology studies before an agent's release, as they are considered to be time consuming (and therefore costly). Instead, it is generally perceived to be of greater importance to release numerous host-specific agents, in the hope that at least a single agent will establish and bring about appreciable control (Byrne *et al*. 2003; Dhileepan *et al*. 2013). Nonetheless, although thermal physiology studies take time and effort to conduct, the procedures are not tedious and don't require much funding. Moreover, 'pre-release' thermal

physiology studies enable the selection of appropriate and well-adapted candidate agents, which in turn reduces expenditure (e.g. mass-rearing and release efforts) in achieving agent establishment (Byrne *et al*. 2003).

In South Africa, such studies have mostly been neglected in weed biocontrol programmes. One such example is the biological control programme against water hyacinth, *Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae), South Africa's most problematic aquatic weed (Hill & Olckers 2001). Despite the release of seven agent species (six arthropods and one pathogen), more than anywhere else in the world, control has been successful in some areas but not in others (May & Coetzee 2013). The majority of infestations occur in the Highveld, the high-lying interior plateau of South Africa, which is characterized by extreme winter temperatures. Due to the lack of pre-release thermal requirement studies, most of the agents released against this weed have since proven to be adapted to low-altitude warm climates, with none specifically selected for high altitude climates where they were ultimately unsuccessful (May & Coetzee 2013).

To understand how different insect species respond to temperature variation, it is thus crucial to determine their thermal physiology, since exposure to different temperatures in the laboratory indicates the optimal range of temperatures in a natural system (Sutherst & Maywald 1985). This helps to predict how that species will perform when experiencing known climatic conditions and also enables predictions of its potential geographical range (Keena 2006) because optimal temperatures in the laboratory are similar to those in their natural environments (Abdullah 1961). Therefore, experimentally determining an insect's thermal physiology provides insight into its performance when experiencing varying thermal conditions in the field.

2.1.1 Developmental rates

A standard measure of insect thermal physiology is the determination of developmental rate (Campbell *et al*. 1974). Developmental rates of most insects are largely dependent on the temperatures to which they are exposed; however, this relationship is typically non-linear (Ikemoto & Takai 2000; Jalali *et al*. 2010). Insect development takes place within a definite temperature range, and its rate increases from zero at a low temperature threshold (t), reaches a maximum at an optimal temperature, and then decreases rapidly to zero beyond an upper lethal temperature (Wagner *et al*. 1991). The lower

developmental threshold for a species is the temperature at and below which development stops, whereas the upper developmental threshold is taken as the temperature at and above which growth or development starts to decrease (Wagner *et al*. 1991; May & Coetzee 2013). The heat accumulation that is required to complete development is known as the thermal constant (K) (Campbell *et al*. 1974). This measure of accumulated heat is termed 'physiological time', which provides a common reference for the development of poikilothermic organisms (Wagner *et al*. 1991).

The upper and lower developmental thresholds can be determined directly in the laboratory by measuring the period taken by an insect to develop through a series of developmental stages at different constant temperatures (Campbell *et al*. 1974; Wagner *et al*. 1991).

2.1.2 Degree-day models

The value of determining the parameters t and K is that they can be used to predict potential biocontrol agent distributions in climate matching models (Byrne *et al*. 2003; May & Coetzee 2013). Physiological time for developing insects is usually measured in degree-days (°D), where one degree-day is equal to the amount of development that will take place for a given insect, which is maintained at one degree above its lower developmental threshold over 24 hours (Jones & Brunner 1993).

Using historical weather records from specific geographical locations, available °D above any given threshold can be calculated and used to estimate whether these locations will provide sufficient physiological time for a particular insect species to complete its development (Garcia & Morrell 2009). Mapping these data will depict areas where the insect will be able to establish (> 1 generation per year) and areas where establishment will not be achieved (< 1 generation per year).

2.1.3 Study aims

As discussed in Chapter 1, *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Phlaeothripidae) is a promising biocontrol agent for *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) in South Africa. However, aspects of its biology have not been documented in the literature, particularly its thermal physiology, since the species was only fairly recently described (Mound & Pereyra 2008). Therefore, the aim of this chapter was to conduct

laboratory trials to determine the agent's developmental threshold and produce a degree-day model to highlight areas in South Africa that are most suited for its establishment. Furthermore, the development of *L. tractabilis* under controlled field (i.e. outdoor) conditions was investigated during summer, winter and spring. The results from these trials were used to validate the strength/relevance of the degree-day model under more natural climatic conditions.

2.2 MATERIAL AND METHODS

2.2.1 Experimental subjects

The *L. tractabilis* culture (originally established from material collected in Argentina in 2005) was maintained in the quarantine facility of the Plant Protection Research Institute (ARC-PPRI), Cedara (29°32'45.5"S, 30°16'17.7"E). At the beginning of this study, the agent had not yet been cleared for release; however, permission for its release was later granted in mid-2013 which permitted the movement of the cultures out of quarantine. Adults that were used in both the laboratory and controlled field trails were selected from the culture that was reared under laboratory conditions at Cedara. *Campuloclinium macrocephalum* plants that were used in the controlled field trials were obtained from stock plants that were maintained under drip fertigation with a 2% solution of Gromor™ 3:1:3 (37 w.s.) plus Gromor™ Calmag N + microelements, twice a day in the greenhouse at Cedara.

2.2.2 Lower developmental threshold (t) and rate of development (K)

To obtain eggs for these trials, 10 adult mating pairs were collected from the laboratory culture and confined overnight within a growth chamber set at 27°C. The thrips were placed in glass Petri dishes with filter paper, moistened with dilute (2%) sodium hypochlorite solution (to ensure a stable humidity level and prevent fungal infection) and were provided with young shoots of *C. macrocephalum* for oviposition. After 24 hours, 280 eggs were harvested from the Petri dishes and placed individually, each with a fresh *C. macrocephalum* leaf (replaced daily) close to it, in glass Petri dishes with moist filter paper. A total of 40 (20 and 20) eggs were placed into two sealed plastic containers with moistened paper towel (to ensure a stable humidity due to the drying effect of the chambers). These were placed in Labcon LTGC 40 growth chambers that had been pre-set at seven constant

temperatures (15°C, 17.5°C, 20°C, 25°C, 27.5°C, 30°C and 32.5°C). Photoperiod was set at 16 hours light: 8 hours dark. Temperatures were logged at 15 minute intervals using iButtons (DS 1921G-F5#MAXIM, Thermochron (-40°C to +85°C), Fairbridge Technologies, Sandton, South Africa, Acc 1°C) that were placed with the Petri dishes in each of the two containers used for each experimental temperature. New adult mating pairs were used to obtain additional eggs when necessary, to replace individuals that were lost (i.e. escaped from the dishes or were damaged during handling). Development was monitored every 24 hours using a dissecting microscope and the time (number of days) to complete each developmental stage (see Table 1) at the different test temperatures was recorded. The immature thrips life stages were fed on leaf material (dipped in a dilute (2%) sodium hypochlorite solution to prevent pathogen infection) that was replaced daily, along with the filter paper. The filter paper in each Petri dish and the paper towel in the plastic containers were also moistened daily with the dilute sodium hypochlorite solution.

The average number of days taken to develop from egg to adult was calculated for each surviving individual thrips at each of the experimental temperatures. Two linear methods were utilized in determining the developmental zero. The linear regression method was used to plot the inverse of the developmental duration (developmental rate) against temperature, for complete development, where $y = a + bx$ (Campbell *et al*. 1974). The lower developmental threshold was calculated by the intersection of the regression line at $R(T) = 0$, $t = - a/b$. The thermal constant, K, was estimated by calculating the inverse of the gradient of the slope (1/b) of the fitted linear regression line (Campbell *et al*. 1974).

Since the relationship between temperature and developmental rate is not linear, particularly at the lower and upper temperature thresholds, the reduced major axis regression method as proposed by Ikemoto & Takai (2000) was also utilised. This method has been reported to produce a better fit than the linear regression method proposed by Campbell *et al*. (1974). The Ikemoto & Takai method plots the product of development time and temperature (DT) against development time (D). The method follows the equation for a straight line, $y = a + bx$, where $y = DT$, $a = K$ and $b = t$. This method does not require an estimation of standard error because its line parameters are the direct parameters, K and t (Ikemoto & Takai 2000).

2.2.3 Degree-day calculations

Daily maximum and minimum temperature records were obtained from the CLIMEX model database for 128 locations throughout South Africa. The parameters K and t were used to calculate the accumulated degree-days for each year and location according to the equation below, where T_{max} and T_{min} represent the maximum and minimum temperatures experienced, and t represents the lower developmental threshold for *L. tractabilis*.

$$K = \sum \{ \frac{(T\max + T\min)}{2} - t \}$$

(if $T_{min} < t$, t was used)

The available degree-days (°D) were then calculated for each of the 128 locations in South Africa. This facilitated the calculation of the number of generations that *L. tractabilis* is likely to complete in different localities throughout South Africa. The CLIMEX programme was used to generate maps using these data, to determine the likely suitability of these areas for the establishment and persistence of the thrips.

2.2.4 Controlled field trials

2.2.4.1 Study sites

These trials were set up in a shade house at the Botanical Garden of the University of KwaZulu-Natal (UKZN) (29°37.5'40"S, 30°24.2'60"E) and in a shade house (22 x 11m, 40% shade cloth on the roof and 30% around the sides) at Cedara. The Botanical Garden is situated in Pietermaritzburg and is 750 m above sea level, while Cedara is 1000 m above sea level. Pietermaritzburg falls within the Coast Hinterland bioclimatic zone (Le Roux 1993), with a steep and broken topography, altitudes ranging from 450 to 900 m and annual rainfall varying between 850 and 1300 mm. Average annual temperatures vary from 17.5 to 20°C with relatively high humidity. Short term droughts occur occasionally, with little to no frost in winter. In contrast, Cedara falls within the Mist-belt bioclimatic zone (Le Roux 1993), with altitudes ranging from 900 to 1400 m and annual rainfall varying between 800 and 1600 mm. Mist is common and average annual temperatures are cooler, ranging between 16 and 18°C. Climatic extremes in the Mist-belt include occasional dry spells of short duration in

summer, excessive cloudiness in early summer, slight to sometimes severe frosts (particularly in winter), occasional severe hail and hot berg winds in early spring. Altitude, in particular, was expected to have a major influence on the results from the two sites since atmospheric temperature typically decreases with increasing altitude, with frost becoming more prominent (Le Roux 1993). The trials were conducted during the peak of the southern hemisphere spring (28 September to 4 December), summer (25 January to 9 March) and winter (14 June to 30 August) of 2014.

2.2.4.2 Experimental procedure

Eggs for the trials were obtained by exposing 10 mating pairs of *L. tractabilis* to young *C. macrocephalum* shoots (± 120 mm long) for 24 hours, in each of 12 Petri dishes lined with moistened (2% sodium hypochlorite solution) filter paper. Excess eggs were removed from the shoots to ensure that each shoot contained 20 eggs. The shoots were then placed into glass vials containing water and sealed with parafilm® (stems were left sticking out through the parafilm where the eggs were situated) to ensure that they remained fresh until the eggs hatched. Each vial was secured with parafilm® onto a potted *C. macrocephalum* plant, which was held erect using a wooden stake. This setup was replicated six times in each shade house (UKZN and Cedara), with the plants spaced at 1m intervals. To record mean exposure temperatures, two iButtons were secured with Presstick™ onto the stems of two randomly selected *C. macrocephalum* plants at each site. Eggs were monitored daily and the duration from egg to adult was recorded for each surviving individual on each plant at each site. This procedure was repeated for each of the three seasons (see above). Sample sizes were supplemented (by adding eggs) to ensure that at least 20 individuals completed development to adulthood at each site for each season. This was because eggs, especially during winter, often failed to hatch (collapsed) or the thrips succumbed to extreme temperatures or burrowed into the soil for refuge on the roots of *C. macrocephalum*.

2.2.4.3 Statistical analysis

Since the data were not normally distributed, Mann-Whitney U tests were used to determine if there were significant differences in the number of days taken to complete development (egg-adult) between the two sites for each season. The number of days taken to complete development at the Pietermaritzburg site, across the three seasons, was compared using a Kruskal-Wallis test. Since development to adulthood at the Cedara site occurred in

only two of the three seasons, the data were compared using a Mann-Whitney U test. Analyses were conducted using SPSS Statistics 22.0, and Microsoft Excel 2010.

2.3 RESULTS

2.3.1 Lower developmental threshold (t) and rate of development (K)

Liothrips tractabilis successfully completed development from egg to adult emergence at mean temperatures of 17.01°C, 19.96°C, 24.78°C, 27.03°C and 29.65°C (Table 1). These temperatures were slightly different to those at which the growth chambers were set (see Methods) and were caused by the microclimatic conditions of the Petri dishes. The duration of development of each of the six life stages recorded, as well as the overall time taken from egg-hatch to adult emergence, decreased linearly (Fig. 6) as temperature increased (Table 1). The time taken to develop to adulthood was quickest (25.60 ± 0.82 days) at 29.65ºC and slowest (74.23 ± 2.02 days) at 17.01ºC (Table 1). Development of *L. tractabilis* was not supported at the lowest and highest temperatures of 14.8°C and 32°C, respectively, as these proved to be lethal. At 14.8°C, the eggs took around one month to develop, but collapsed before any thrips had hatched. Conversely, at 32ºC, eggs took only three days to develop, but also collapsed and produced no larvae.

Table 1: Mean (± SD) developmental time from egg-hatch to adult emergence for *Liothrips tractabilis* at five constant temperatures. Temperatures represent the means of those recorded for the duration of the trials.

	Duration of development (days) at actual temperatures				
Stage*	**17.01ºC**	**19.96ºC**	**24.78ºC**	**27.03ºC**	**29.65°C**
Egg	20.16 ± 0.37	14.16 ± 0.37	9.30 ± 0.46	8.13 ± 0.34	5.93 ± 0.35
L1	11.16 ± 0.37	6.16 ± 0.37	4.16 ± 0.37	3.83 ± 0.37	3.13 ± 0.34
L2	21.16 ± 0.37	18.16 ± 0.37	15.73 ± 0.44	13.83 ± 0.37	10.13 ± 0.34
Pre-pupa	7.16 ± 0.37	5.16 ± 0.37	2.70 ± 0.46	2.86 ± 0.34	2.16 ± 0.37
2nd pupal	7.43 ± 0.50	4.16 ± 0.37	2.73 ± 0.44	2.23 ± 0.42	2.16 ± 0.37
3rd pupal	7.14 ± 0.34	4.16 ± 0.37	2.93 ± 0.25	2.26 ± 0.44	2.13 ± 0.34
Total	**74.23 ± 2.02**	**51.97 ± 2.23**	**37.56 ± 1.40**	**33.16 ± 1.09**	**25.60 ± 0.82**
(n)	**(30)**	**(31)**	**(33)**	**(31)**	**(30)**

*****L 1-2 indicates the two larval feeding stages. Total (n) indicates the number of days from egg to adult emergence, with n representing the number of individuals that survived to adulthood.

There was a very strong linear relationship ($y = 0.0019x - 0.0193$; $r^2 = 0.97$) between developmental rate (i.e. inverse of the developmental duration) and temperature (Fig. 6). Using this linear regression approach, the lower developmental threshold (t) was estimated at 10.2ºC and the thermal constant (K) at 526.3ºD (Fig. 6).

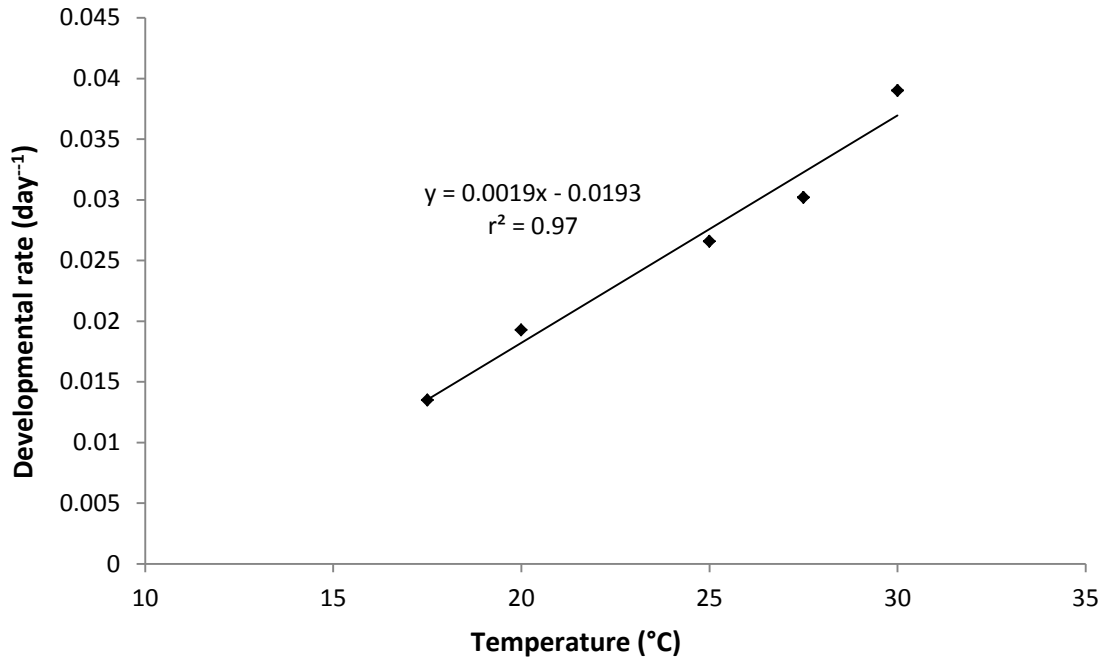

Fig. 6. Developmental rate from egg-adult of *Liothrips tractabilis* at five constant temperature treatments, using the linear regression method.

When the reduced major axis regression approach was adopted, there was also a very strong linear relationship ($y = 9.6x + 546.9$; $r^2 = 0.97$) between the product of developmental time and temperature (DT) and developmental time (D) (Fig. 7). Using this approach, the low temperature threshold (t) was estimated at 9.6ºC and the thermal constant (K) at 546.9ºD (i.e. from the equation presented in Fig. 7).

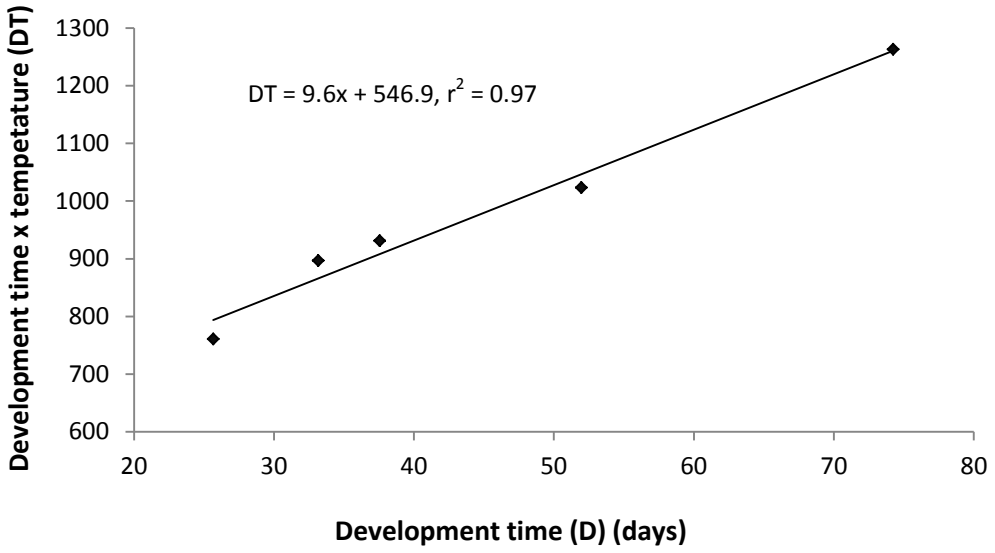

Fig. 7. Reduced major axis regression for *Liothrips tractabilis* in which the product of developmental time and temperature (DT) is plotted against developmental time (D).

Although the differences in the two thermal parameters between the two models were relatively small, the parameters derived by the reduced major axis regression were used to develop the degree-day model, as this method is considered to be more accurate because it reduces error in the estimation of the parameters K and t. Hence, only these results will be considered in the discussion.

2.3.2 Degree-day model

The map generated by the degree-day model revealed that the ecoclimatic suitability of South Africa for *L. tractabilis* varied throughout the different regions (Fig. 8). The model predicted that there are sufficient degree-days for *L. tractabilis* to complete at least two and up to nine generations per annum throughout most of South Africa (Fig. 8). Warmer regions across South Africa and neighbouring countries (e.g. Mozambique) were found to be most suitable, potentially supporting 6 -10 generations per annum (Fig. 8). In Gauteng, parts of Limpopo, North West and Mpumalanga provinces, where *C. macrocephalum* is most abundant (see Fig. 2 in Chapter 1), the thrips are predicted to complete 3-9 generations per year (Fig. 8).

It should be noted that the model "assumes" that plants are always available to the thrips in the field. This is unlikely, particularly since the plants die back in late autumn, so the thrips will probably complete fewer annual generations than predicted. Even though the thrips may feed on the underground tissues during winter, the temperatures experienced will presumably be very different to the above-ground temperatures (i.e. weather station data) that were used to calculate the number of generations above ground.

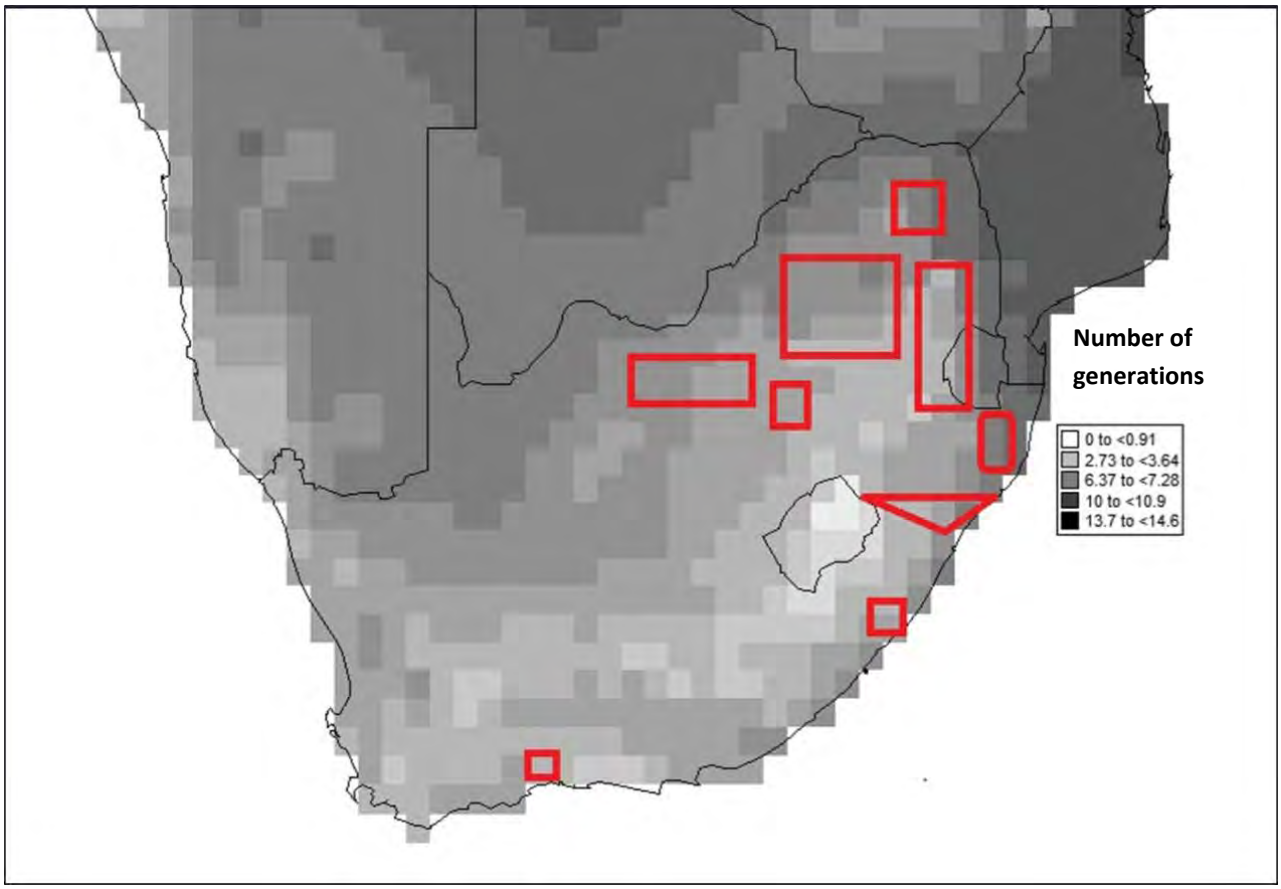

Fig. 8: Potential number of generations of *Liothrips tractabilis* per year in southern Africa based on the degree-day model that was developed from laboratory-derived data (spatial resolution of the grid: 15 minutes). Shaded areas represent the potential number of annual generations of *L. tractabilis*; the darker the shading, the higher the number of generations that could be supported in a particular area. The areas demarcated in red indicate the current distribution of *Campuloclinium macrocephalum*.

2.3.3 Controlled field trials

During the spring trials, the development of *L. tractabilis* from egg-adult was significantly faster (U = 0.0, d.f. =1, $P < 0.0005$) at the Pietermaritzburg site (Mean ± SD =

60.08 ± 0.81 days; n = 25) than at the Cedara site (65.3 ± 0.77 days; n = 26), where the mean recorded temperature was 0.9°C lower (Fig. 9a). The same trend was recorded in summer, with significantly faster development (U = 0.0, d.f. = 1, $P < 0.0005$) at Pietermaritzburg (39.1 ± 0.51 days; n = 32) than at Cedara (43.1 ± 0.57 days; n = 36), where the mean temperature was 1.3°C lower (Fig. 9b). Although average spring and summer temperatures differed slightly between the two sites (by ± 1°C), this was sufficient to cause significant differences in the developmental time of *L. tractabilis*. There was a substantial difference in average winter temperatures between the two sites, with temperatures at Cedara lower by some 7.9°C. Consequently, no eggs survived to adulthood at the Cedara site (n =20), while the thrips took 77.1 ± 0.79 days (n = 21) to complete their development at the Pietermaritzburg site (Fig. 9c).

There were significant differences in developmental times across the three seasons (H = 70.036, d.f. = 2, $P < 0.0005$) at Pietermaritzburg. The thrips developed significantly faster (U = 28.5, d.f. = 1, $P < 0.0005$) during summer (around 39 days) than in spring (60 days), as a result of a 5°C increase in temperature. Following a 7.4°C decrease in temperature between summer and winter, there was significantly slower (U = -23.0. d.f. = 1, $P = 0.001$) development (77 days) during winter (Fig. 9). Despite no winter development, there was a similar pattern at the Cedara site, where the thrips also developed significantly faster (U = 0.0, d.f. =1, $P < 0.0005$) during summer (around 43 days) than in spring (65 days), following a 5.1°C increase in temperature (Fig. 9).

The outdoor data were also compared to those of the laboratory trials (see discussion section), but no statistical analyses were performed given the large temperature fluctuations that typically occur under field conditions. In contrast, temperature fluctuations were prevented by the controlled conditions in the laboratory trials, so the average temperatures recorded during the laboratory and field trials may not necessarily be comparable (see below).

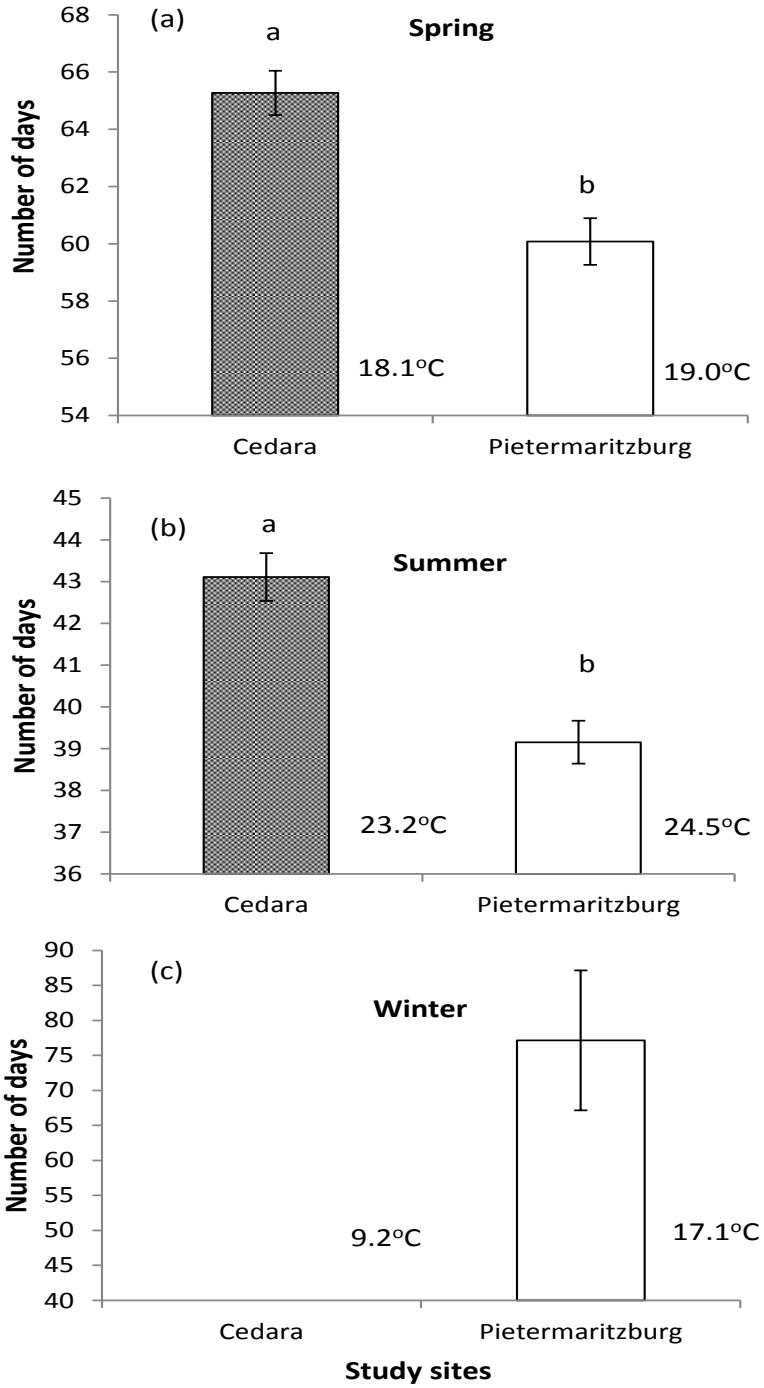

Fig. 9. The number of days (mean ±SD) taken for *Liothrips tractabilis* to complete development from egg-adult during spring (a), summer (b) and winter (c) at Pietermaritzburg and Cedara. Means followed by different letters between the two sites within a season are significantly different ($P < 0.05$). Temperatures next to the bars are the mean temperatures recorded by the iButtons at each site, within each season, for the duration of the trials.

2.4 DISCUSSION

The developmental rate of *L. tractabilis* increased with increasing temperature between 17ºC and 30ºC (Table 1, Fig. 6), which was consistent with many similar studies conducted on various insect species (e.g. Ulmer *et al*. 2006; Zhou *et al*. 2010; May & Coetzee 2013). This is because at higher temperatures, physiological changes during insect development occur at faster rates (Dingha *et al*. 2009) resulting in quicker growth rates (Matsuki *et al*. 1994). The failure of *L. tractabilis* to develop at constant temperatures at or above 32ºC was consistent with species such as the southern pine beetle, *Dendroctonus frontalis* Zimm. (Coleoptera: Curculionidae), where development ceased at temperatures above 32ºC (Friedenberg *et al*. 2008). A decrease in the survival rate of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) was similarly recorded at temperatures of below 14ºC and above 32ºC (Huang *et al*. 2008). Moreover, the hatch rate of eggs of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae) was very low at 32.5ºC compared to eggs that were kept at temperatures between 15ºC and 30ºC (McAvoy & Kok 1999). In a study by Dhileepan *et al*. (2013), temperatures between 20ºC and 30ºC proved most favourable for adult survival, oviposition, egg hatching, and both larval and pupal development for the leaf-tying moth *Hypocosmia pyrochroma* Jones (Lepidoptera: Pyralidae), a biocontrol agent for cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohman (Bignoniaceae), in Australia and South Africa. This moth was also negatively affected by both higher (>30ºC) and lower (<20ºC) temperatures.

High temperatures can hinder the synthesis and release of neurosecretory materials (Lekovic *et al*. 2001) and stop the production of moulting hormone in larvae (Okasha 1970). Thus, temperatures greater than 30ºC generally appear to be the point at which physiological processes are affected and therefore lead to reduced development and mortality. On the other hand, at low temperatures, the development of insect immature stages takes longer (Angilletta *et al*. 2004) because physiological reactions take place at a slower rate, sometimes resulting in reduced survival and fitness of the progeny (Ernst & Isaaks 2000). Failure of *L. tractabilis* to develop at constant temperatures at or below 14.8°C is consistent with observations on other insect species. For example, development of *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) was inhibited at temperatures between 15°C and 17°C (Rueda & Axtel 1996). Moreover, cold stress can result in abnormalities and defects in certain insects, thereby reducing food consumption (De Guzman & Frake 2007). For example, *A. diaperinus*

adults did not feed when exposed to temperatures of 6°C and 10°C and starved to death (Renault *et al*. 1999). The effects of reduced feeding at low temperatures was also demonstrated in two weevil species, *Exapion ulicis* Forster and *E. lemovicinum* Hoffmann (Curculionidae: Apioninae) (Barat *et al*. 2010).

It should be noted that constant temperature is the key criterion in this context (i.e. accumulated heat stress without reprieve). In other words, if the day time temperature drops below 14.8°C or exceeds 32°C for a few hours, this is unlikely to prove lethal for *L. tractabilis*. Furthermore, certain life stages of the thrips may well be more susceptible to temperature than others. This study found that the egg stage was particularly susceptible where, although development occurred at the abovementioned lethal temperatures, the eggs all collapsed before any hatching could occur. While the egg stage appears unable to tolerate these temperatures, the larval, pupal and adult stages may be able to better withstand them. Therefore, it should be emphasised that these results for *L. tractabilis* are consistent with a standard methodology that has been adopted by biological control practitioners, and may not be an absolute reflection of the insect's ability to survive in the field when exposed to the lethal temperatures mentioned above.

The degree-day model (Fig. 8) predicted that *L. tractabilis* should complete at least two generations per year throughout South Africa, with three or more generations in most of the areas that are infested with *C. macrocephalum*. In some parts of the Mpumalanga and North West provinces, where warmer conditions prevail, up to seven generations per year are possible. In the Gauteng, Limpopo, Mpumalanga and North West provinces, where the weed is currently a major problem, the thrips should be able to complete more than four generations each year. Thus, optimal release site selection, involving climatically suitable sites in the main regions that are invaded by *C. macrocephalum* will be important. Similarly, an appropriate release strategy that involves large numbers of thrips (preferably thrips-infested whole plants or shoot tips that include all life stages of the insect) should increase the likelihood of establishment and impact on the target plant.

In the field in South Africa, *C. macrocephalum* plants typically die back in winter, leaving no above-ground foliar material for the thrips. However, it is believed that the thrips are likely to persist during this period, by moving underground to feed on the fleshy roots of the plant (A. McConnachie, pers. comm.). Since this has not been verified in the field, it provides an opportunity for future research. If the thrips is able to persist underground, either

by diapausing or by feeding on the roots, cold winter temperatures are unlikely to limit its success, particularly in areas in Gauteng where cold winters are typical. The lower developmental threshold of 9.6ºC also becomes less of a concern, as the plant reaches its peak during the spring and summer months, where temperatures will rarely drop below this threshold for a sustained period of time.

Byrne *et al*. (2003) considered the degree-day model to be satisfying from the perspective that the results are sensible, and useful for various geographical areas. Such models have proven to be informative for a variety of pest management applications. These include scheduling of pest management actions, monitoring of pest or biocontrol agent activity, avoiding wastage of efforts in trying to establish climatically incompatible agents, optimising release strategies and promoting the release of agents whose potential was previously not known (Byrne *et al*. 2003). For example, May & Coetzee (2013) found that *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a candidate agent for water hyacinth in South Africa, had high thermal requirements, was poorly adapted to Highveld temperatures and would thus not fare any better than other agents already released against this weed.

The controlled field trials (Fig. 9) revealed significant differences in developmental time to adulthood for *L. tractabilis* between the two sites, during both spring and summer. During both seasons, development was faster at the Pietermaritzburg site where the mean temperatures were ca. 1°C higher than at Cedara. Even though the sites are only some 20 km apart, Cedara is situated some 250 m higher than Pietermaritzburg and thus displays cooler average temperatures (see section 2.2.4.1). Therefore, it is not surprising that the thrips developed faster at the warmer site. However, humidity, which is known to affect egg hatch and pupation in insects (e.g. Bell 1975; Howe 1956), may also have played a role as Cedara and Pietermaritzburg have very different humidity profiles. This is because Pietermaritzburg is generally drier due to the region being warmer, whereas Cedara is cooler in comparison with the air having a higher moisture content (D. Chapman, pers. comm.).

A key question in this study was whether the laboratory-derived data were an accurate indicator of the situation in the field. During spring, development of *L. tractabilis* to adulthood took an average of 60.08 days at a mean temperature of 19.0°C at the Pietermaritzburg site, compared to 65.3 days at a mean temperature of 18.1°C at Cedara. This was comparable to what was predicted by the laboratory trials (e.g. 51.97 days at 19.96°C). Although the average temperatures recorded during the laboratory and field trial were not

identical (see Table 1 and Fig. 9), the field developmental data largely support the predictions of the laboratory trials (see Table 1). Similarly, during summer, development to adulthood took 39.15 days at 24.54°C at Pietermaritzburg and 43.11 days at 23.18°C at Cedara, which was also in agreement with the laboratory predictions (e.g. 37.56 days at 24.78°C). During winter, no development to adulthood was recorded at Cedara where a low mean temperature of 9.2°C prevailed. This was also in agreement with the laboratory data which determined a lower developmental threshold of 9.6°C, where development will cease. In contrast, the mean winter temperature at Pietermaritzburg was 17.1°C, which explains why successful development to adulthood was accomplished. Moreover, development to adulthood at Pietermaritzburg took 77.1 days at an average temperature of 17.1°C, which was also in line with the laboratory predictions (74.23 days at 17.01°C). Similar trends were recorded by Goebel (2006), who examined the effect of temperature on the development and reproduction of the sugarcane stalk borer, *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae) in the laboratory and was able to validate these results in the field. Developmental times measured in sugarcane fields were similar to those in artificial laboratory conditions, without any large inconsistencies.

The controlled field trials also demonstrated significant differences in developmental times across seasons, at both sites. Developmental times decreased from winter to spring to summer as the average temperatures at the study sites increased. Therefore, as average temperatures change between the seasons, so will the times taken for *L. tractabilis* to develop to adulthood. Thrips populations can thus be expected to thrive during summer and probably to a lesser extent during spring and autumn. However, populations will be inhibited during the winter months, particularly at higher altitudes. How thrips populations will be able to cope with high altitude winters will depend on their overwintering strategies given that *C. macrocephalum* populations die back during this time (see above).

In conclusion, the laboratory data which determined the temperature tolerances of *L. tractabilis* were verified by the field data, ensuring their suitability for degree-day modelling. The number of generations predicted by the degree-day model across the different regions of South Africa suggests that *L. tractabilis* should be able to establish and proliferate, to varying degrees, throughout the range invaded by *C. macrocephalum*.

CHAPTER 3: ASSESSING THE IMPACT OF *LIOTHRIPS TRACTABILIS* ON *CAMPULOCLINIUM MACROCEPHALUM* UNDER NATURAL CLIMATIC CONDITIONS

3.1 INTRODUCTION

Impact assessment is an integral part of any classical biological control programme. Such assessment is used to determine whether an agent is inflicting appreciable damage on the target weed (Morin *et al*. 2009). This can be conducted either before or after an agent has been released into the introduced range of the target weed (McClay & Balciunas 2005). However, biological control practitioners have come under the spotlight for their lack of rigorous evaluations on the ultimate outcomes of deliberate introductions of exotic organisms (Carson *et al*. 2008). This has resulted in increased pressure to conduct research to identify, prior to their release, which agents are most likely to be effective (van Klinken & Raghu 2006).

Pre-release impact evaluation is either conducted in the field in the native range of the target weed or on individual plants under controlled conditions in laboratories or glasshouses, in conjunction with host-specificity testing (Morin *et al*. 2009). Such studies enable researchers to assess the effectiveness of prospective agents, thus providing an indication of their potential to negatively affect key growth parameters of the target weed and assisting in the prioritisation of agents (Sheppard 2003). Data collected during such studies may be crucial in convincing reviewers/decision makers that a particular agent inflicts significant damage on the target weed, and that permission for its release should be granted. However, one can never fully predict how a candidate agent will perform on weed populations in the introduced range, particularly when faced with a new set of environmental conditions (Broughton & Pemberton 2008).

Post-release impact evaluation measures how effective released agents are at reducing target weed populations within the introduced range and thereby quantifies the benefits for associated plant communities, ecosystems and the economy and society in general (Morin *et al*. 2009). However, this is quite a challenging task as not only do researchers need to determine whether the agent has adversely affected the weed, but they

also need to demonstrate that the observed suppression is greater than would be anticipated given the underlying spatio-temporal variability of the biological system and abiotic conditions (McClay 1995). It is for this reason that, to date, the majority of biological control programmes have focused on subjective assessments of agent establishment and impact at the individual plant level (Morin *et al*. 2009).

The impact of *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Phlaeothripidae) on individual *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) seedlings and root crown regrowth shoots was initially assessed in a quarantine glasshouse as these stages were considered, from field observations, to be the most vulnerable to thrips attack (McConnachie & McKay 2015). These authors found that *L. tractabilis* significantly reduced the growth of *C. macrocephalum*, even under low inoculation densities (test plants inoculated with two pairs of thrips). Seedlings suffered significantly reduced heights, numbers of leaves and wet masses when compared to the control plants. Root crown regrowth shoots also displayed significantly reduced heights, numbers of leaves, wet masses and dry masses, while bud and flower production was also significantly reduced (McConnachie & McKay 2015). However, as mentioned above, results obtained from laboratory-based studies are not necessarily a true reflection of what can be expected under natural conditions.

Therefore, following approval for the release of *L. tractabilis*, a decision was made to repeat the laboratory impact study under more natural conditions, using a garden-type experimental set-up. The aim of the study was thus to confirm that the thrips would be just as damaging to *C. macrocephalum* seedlings and root crown regrowth shoots under natural conditions.

3.2 MATERIAL AND METHODS

3.2.1 Study site

The trials were carried out in a vacant plot of land belonging to the Agricultural Research Council at the Cedara Weeds Research Unit (29°32'45.5"S, 30°16'17.7"E), Hilton, South Africa, between October and December 2014. Plants remained in the ground for 10-12 weeks before harvesting.

3.2.2 Test plants and thrips

Campuloclinium macrocephalum plants that were used in the regrowth trials were obtained from stock plants maintained under drip fertigation with a 2% solution of Gromor™ 3:1:3 (37 w.s.) plus Gromor™ Calmag N + microelements, twice a day in the greenhouse at Cedara. Mature plants that had recently flowered were cut back and their tubers were washed, weighed and then transplanted at the experimental site where they were allowed to re-sprout. Seedlings were obtained by germinating *C. macrocephalum* seeds in a glasshouse in trays containing Gromor™ (potting medium). Once these had reached the 4-6 leaf stage, the tubers were also washed, weighed and then transplanted at the study site. *Liothrips tractabilis* adults that were used in the study were obtained from the culture that was maintained in the quarantine facility of the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI) at Cedara.

3.2.3 Experimental design

The seedlings and root crowns (tubers) of the cut back plants were transplanted into pits (25 x 25 x 25 cm) that were dug at the study site in late spring (October 2014). The pits were 2m apart from each other, allowing the plants sufficient space. Impact assessment was initiated on *C. macrocephalum* seedlings at the 8–12 leaf stage and on root crown regrowth shoots with 10-12 leaves. Ten replicates of each growth form (i.e. seedlings and regrowth) were inoculated with five pairs of (unsexed) thrips (treatment), with a further 10 that remained free of thrips (controls). The plants were monitored daily to ensure that no thrips had moved onto the control plants or between inoculated plants, as well as to prevent feeding damage from other generalist herbivores such as grasshoppers. The trials were terminated after 10-12 weeks.

3.2.4 Data collection

The growth parameters measured in this study included plant height, number of leaves produced and biomass of the above-ground and below-ground material. Measurements for plant height (cm) and number of leaves were taken at the start (prior to thrips inoculation for each growth form, at the respective growth stage as stipulated in 3.2.3.) and at the end of the study. Plants were then harvested and separated into above-ground and below-ground material. The biomass of the above- and below-ground plant material was measured before (final wet mass) and after drying (dry mass) in an oven set at 55ºC for 72 hours.

3.2.5 Statistical analysis

Since the starting tuber masses were not equal in the individual plants for both growth forms, the initial measurements for plant height, numbers of leaves and tuber wet masses were compared statistically to confirm that the differences between the control and experimental plants were not significant ($P > 0.05$). Since the data did not meet the assumptions of normality, Mann-Whitney U tests were used for these comparisons. Where the initial measurements were not significantly different ($P > 0.05$) between the treated and control plants, the final measurements were similarly compared using Mann-Whitney U tests. Where there were significant differences in the initial measurements, the relative growth rates (= growth increment (initial – final measurement) / initial measurement) were calculated and compared using Mann-Whitney U tests. With regard to plant biomass, where the initial tuber wet masses were similar (i.e. not statistically different), the dry masses of the above-ground, below-ground and total plant material at termination were then compared using Mann-Whitney U tests. However, where the initial tuber wet masses were dissimilar (i.e. statistically different), the relative growth rates of treated and control plants were compared in relation to their final wet masses. All analyses were conducted using SPSS Statistics 22.0 and Microsoft Excel 2010.

3.3 RESULTS

3.3.1 Seedlings

Under natural conditions, the thrips had a significant negative impact on the growth of *C. macrocephalum* seedlings. Seedlings infested with thrips displayed significantly reduced heights ($U = 0.0$, d.f. = 1, $P < 0.0005$) (Fig. 10A), numbers of leaves ($U = 0.0$, d.f. = 1, $P < 0.0005$) (Fig. 10B) and wet tuber masses ($U = 1.0$, d.f. = 1, $P < 0.0005$) (Fig. 10C) in relation to the uninfested controls. There were no significant differences in the initial measurements (i.e. height, numbers of leaves and tuber wet mass) of the control and experimental (i.e. thrips infested) plants, indicating that the plants were all of similar size at the start of the trials (Fig. 10).

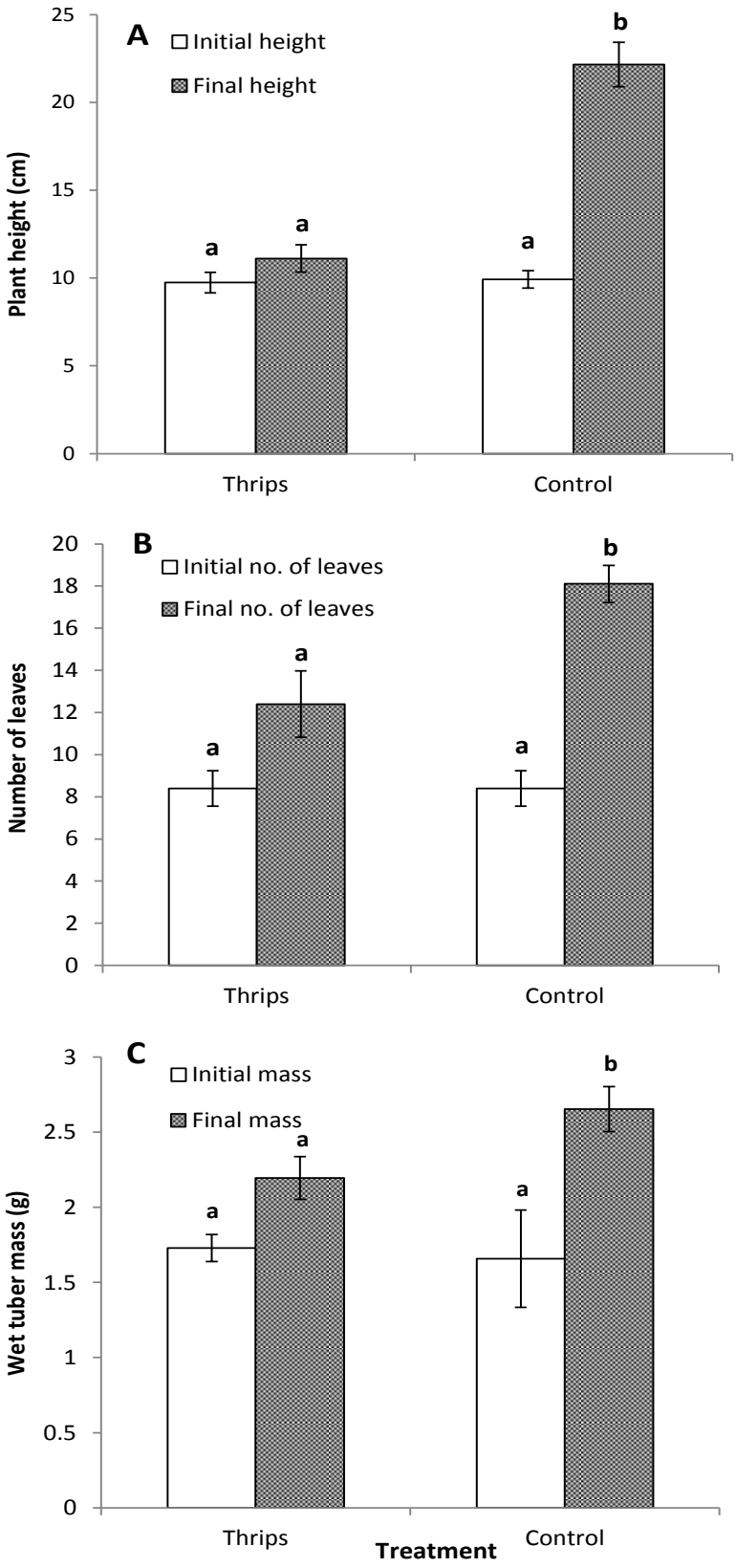

Fig. 10. Impact of feeding by *Liothrips tractabilis* on *Campuloclinium macrocephalum* seedlings as determined by plant height (A), leaf production (B) and wet tuber mass (C).

Comparisons are made between the control and thrips-infested plants at the start of the trials (white bars) and then at their termination (shaded bars). Means (± SD) followed by different letters are significantly different from each other ($P < 0.05$).

Since there were no significant differences in the initial wet tuber masses between the control and thrips-treated plants (Fig. 10C), indicating similar-sized seedlings, comparisons of biomass increments were made using the dry masses of the above- and below-ground material at the termination of the trials. Seedlings infested with thrips displayed significantly reduced dry masses for the below-ground (U = 0.0, d.f. = 1, $P < 0.0005$), above-ground (U = 0.0, d.f. = 1, $P < 0.0005$) and total plant material (U = 0.0, d.f. = 1, $P < 0.0005$) (Fig. 11).

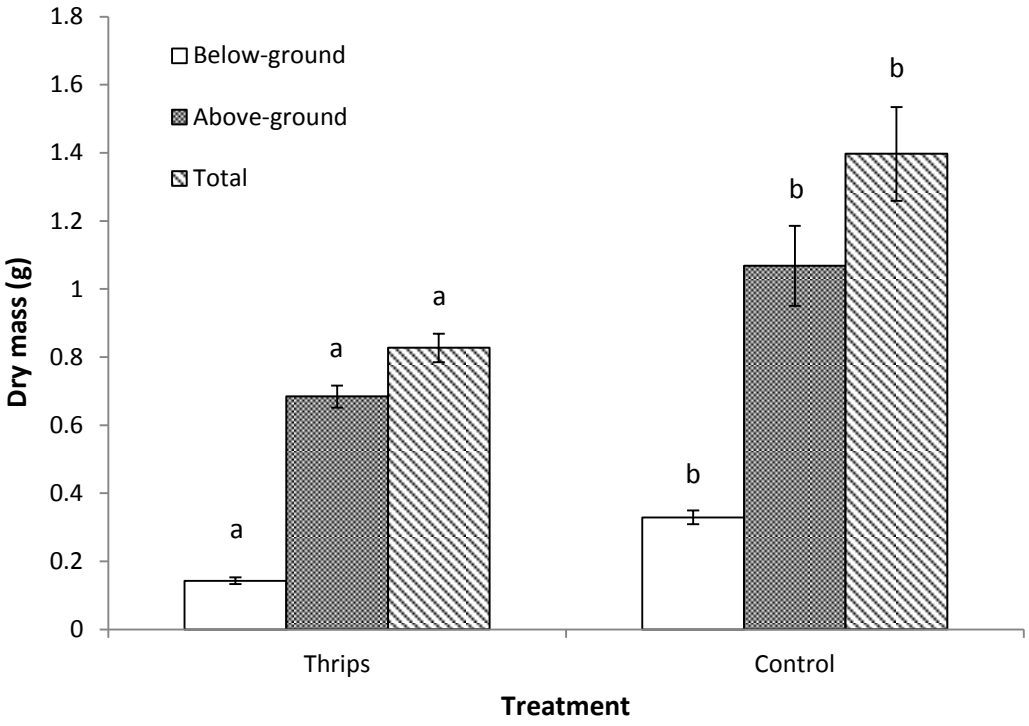

Fig. 11. Comparisons of the dry masses (mean ± SD) of the below-ground, above-ground and all plant tissues between the thrips-treated and control seedlings of *Campuloclinium macrocephalum* at the termination of the trials (i.e. pairwise comparisons were made between the thrips-infested and the control plants for the below-ground, above-ground and finally the total biomass). Means followed by different letters for each biomass comparison are significantly different ($P < 0.05$).

3.3.2 Root crown regrowth

In contrast to the seedlings, thrips feeding did not appear to negatively affect the regrowth of larger *C. macrocephalum* plants. There were no significant differences in plant height (U = 33.0, d.f. = 1, $P = 0.218$) (Fig. 12A) and the numbers of leaves produced (U = 62.5, d.f. = 1, $P = 0.353$) (Fig. 12B) between the thrips-infested and control plants. There were no significant differences in the initial measurements of plant height and numbers of leaves of the control and experimental (i.e. thrips infested) plants, suggesting that the plants were all of similar size at the start of the trials (Fig. 12). However, the differences in the initial measurements of wet tuber mass between the experimental and control plants were significantly different (U = 84.0, d.f. = 1. $P = 0.009$), with the thrips-infested plants displaying significantly higher tuber masses prior to exposure (Fig. 12C). Consequently, the significant differences in final tuber mass (U = 85.0, d.f. = 1. $P = 0.007$) (Fig. 12C) cannot be accurately interpreted and the relative growth rates of the tubers (see below) were analysed instead.

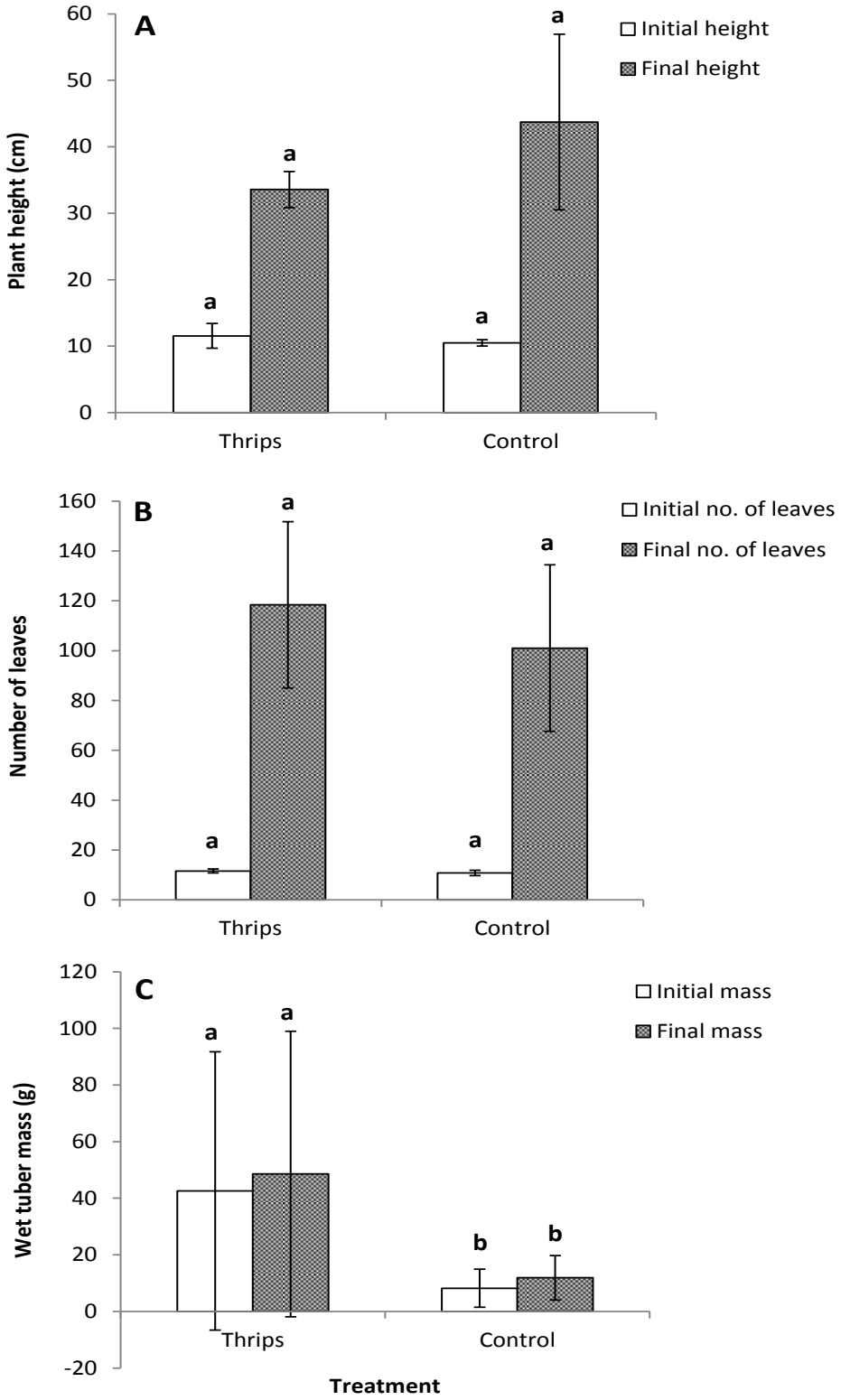

Fig 12. Impact of feeding by *Liothrips tractabilis* on *Campuloclinium macrocephalum* root crown regrowth shoots as determined by plant height (A), leaf production (B) and wet tuber mass (C). Comparisons are made between the control and thrips-infested plants at the start of

the trials (white bars) and then at their termination (shaded bars). Means (± SD) followed by the same letter are not significantly different from each other ($P > 0.05$).

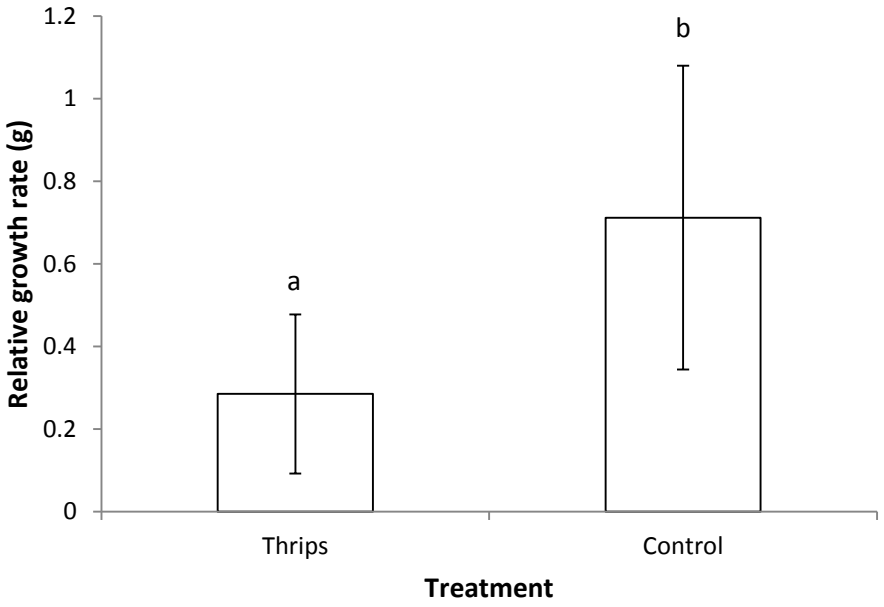

Fig. 13. The effect of *Liothrips tractabilis* on the relative root biomass accumulation (i.e. growth increments (initial – final values) / initial values) of *Campuloclinium macrocephalum*, as indicated by the mean (± SD) relative increments in tuber wet mass. Means followed by different letters are significantly different ($P < 0.05$).

Despite the inconsistent and variable initial wet tuber masses between the experimental and control plants, those inoculated with *Liothrips tractabilis* displayed a significantly lower relative growth rate in terms of wet tuber mass ($U = 15.0$, d.f. = 1, $P = 0.007$) (Fig. 13).

3.4 DISCUSSION

The outdoor trials clearly revealed that feeding by *L. tractabilis* caused significant reductions in plant height, number of leaves and tuber wet mass in seedlings of *C. macrocephalum*, which was consistent with the results of the initial laboratory trials

(McConnachie & McKay 2015). In contrast, while the laboratory study showed no significant reductions in dry mass as a result of thrips feeding, this outdoor study clearly revealed significant reductions in below-ground, above-ground and total plant biomass in *C. macrocephalum* seedlings. These findings have thus provided further, and somewhat stronger, evidence of the damage that *L. tractabilis* inflicts on *C. macrocephalum* seedlings.

However, the results of the trials involving root crown regrowth were not consistent with those involving seedlings and did not provide consistent evidence of impact by *L. tractabilis.* Although there was a reduction in plant height in the thrips-damaged plants, the differences were not significant; presumably because of high variation in the data from the control plants (Fig. 12A). Surprisingly, the control plants displayed lower numbers of leaves but the differences were also not significant. In particular, there were significant differences in initial wet tuber mass between the thrips-infested and control plants as well as high variation in initial tuber mass in the thrips-infested plants, which could have influenced the results of the trials. Consequently, comparisons of final wet tuber masses were made using relative growth rates in tuber mass. The thrips-infested plants displayed a significantly lower relative growth rate in tuber mass in relation to the control plants, indicating some effect of thrips feeding.

Substantial variation in the data sets of recorded plant variables can mask the effects of insect herbivory during impact studies such as these. Ziganira & Olckers (2012) investigated the response of the invasive cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohman (= *Macfadyena unguis-cati* (L.) Gentry) (Bignoniaceae), to simulated and actual defoliation by *Charidotis auroguttata* (Boheman) (Coleoptera: Chrysomelidae), and also recorded a lack of consistent significant responses, presumably because of considerable variation in the data. Ziganira & Olckers (2012) suggested that differences in the size and age of the tubers that were used to propagate the *D. unguis-cati* test plants may have masked any trends, as plants of varying size and age may respond differently to herbivory. Since the same may have occurred in the regrowth trials, any future studies on the effects of insect herbivory on *C. macrocephalum* should limit variability in the response variables by ensuring that similar-sized tubers are used at the outset when propagating the test plants.

Unlike the laboratory impact study, this study regrettably did not quantify bud formation or flowering. In retrospect, this was an oversight. However, the main concern was that with such variation in tuber masses between the control and treated plants during the

regrowth trials, flowering of the control plants would not have occurred at the same time, with considerable delays in some plants, thus confounding the results. During the initial laboratory trials (McConnachie & McKay 2015), plants of a similar age and size were used which allowed flowering to occur at the same time. It is, however, well known that carbohydrate reserves in roots, as well as storage organs, are generally used to sustain cellular respiration and facilitate plant recovery, at the expense of reproductive output, when photosynthesis becomes reduced due to herbivory (Meyer 2000). Continuous herbivory by the leaf beetle *Diorhabda elongata* Brulle (Chrysomelidae) significantly lowered carbohydrate reserves and regrowth of invasive *Tamarix* L. species (Tamaricaceae) in the field in the USA (Hudgeons *et al*. 2007). During the present study, both growth forms of *C. macrocephalum* displayed significantly reduced wet tuber masses as a result of thrips feeding, suggesting that plant resources were reallocated from the tubers to the areas where the damage was inflicted and that flowering would probably have been affected. Numerous other studies have also demonstrated significant reductions in below-ground biomass with increasing levels of insect herbivory/defoliation (e.g. Dhileepan *et al*. 2000; Kleinjan *et al*. 2004).

In conclusion, this study has provided evidence that feeding by *L. tractabilis* has the potential to reduce the growth and biomass accumulation of *C. macrocephalum* seedlings and root crown regrowth shoots under natural conditions. More clear-cut trends in the regrowth trials were presumably masked by high variability in the starting wet masses of the tubers used. Flowering and bud formation still needs to be quantified under field conditions, as a significant reduction in flowering will in turn result in a reduction in the number of seeds produced, and ultimately the spread of the weed. Based on the data presented in this study, the use of *L. tractabilis* for the biological control of *C. macrocephalum* looks promising. However, while this study was conducted under more natural conditions, these were not actual field conditions within the invaded range of *C. macrocephalum*. Therefore, the original laboratory study, together with this study, should be extrapolated to the field with caution, as plant responses to herbivory are strongly influenced by a variety of factors which affect their growth, notably competition, but also drought, disturbance and nutrients (e.g. Cottam *et al*. 1986).

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSION

4.1 INTRODUCTION

The primary purpose of this study was to examine the potential efficacy of *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Phlaeothripidae) as a biological control agent of the invasive weed, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), in South Africa. This chapter, therefore, summarizes the major findings of this study and discusses the compatibility of *L. tractabilis* with South African climatic conditions as well as its impact on the growth and biomass accumulation of *C. macrocephalum*.

4.2 CLIMATIC SUITABILITY OF *LIOTHRIPS TRACTABILIS* IN SOUTH AFRICA

This aspect of the study typically contributes an important element of a weed biological control programme, namely to assess the potential distribution of a biological control agent prior to its release (Byrne *et al*. 2003; May & Coetzee 2013). This study has also contributed new knowledge on the biology of *L. tractabilis*, which has assisted in understanding how its performance is affected by climatic conditions, notably temperature. It has also enabled the identification of areas that are climatically suitable for *L. tractabilis* so that its establishment in the field can be maximized. The developmental threshold trials showed that with increasing temperature, the number of days from egg to adulthood decreased. The lower developmental threshold of *L. tractabilis* was estimated to be 9.6°C with a relatively short generation time of 546.9 degree-days (see Chapter 2). These data were used to generate a degree-day model using the climate-matching programme CLIMEX. The model predicted that *L. tractabilis* is likely to establish throughout the invaded range of *C. macrocephalum* in South Africa. The optimal areas for release were identified as the warmer regions across South Africa and neighbouring countries. Within the invaded range of *C. macrocephalum* (Gauteng, parts of Limpopo, North West and Mpumalanga provinces) the thrips are predicted to successfully complete 3-9 generations per year.

Degree-day models have successfully predicted the number of generations per year that an agent is likely to complete; for example, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) on the invasive aquatic weed, *Azolla filiculoides*, in South Africa

(Byrne *et al*. 2003). However, predictions have not always been realized in the field. For example, the moth, *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebidae: Arctiinae) released against *Chromolaena odorata* (L.) King and Robinson (Asteraceae) in South Africa, was predicted to have 4-6 generations per year at subtropical release sites in KwaZulu-Natal province (Byrne *et al*. 2003). Initially it was thought that the moth had failed to establish a viable permanent population (Byrne *et al*. 2003). Although establishment has now been confirmed throughout much of KwaZulu-Natal, it is believed that climate did play a role in this agent's poor performance, particularly at low temperatures (C. Zachariades, pers. comm.). Another example is that of *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), a leaf-feeding bug, released in South Africa for the control of water hyacinth (Byrne *et al*. 2003). This species was predicted to complete 3-14 generations per year at various localities in South Africa, as well as five generations at localities around Johannesburg where it failed to overwinter. Therefore, the degree-day model generated for *L. tractabilis* was tested under controlled field conditions to determine whether the laboratory-derived data were representative of field conditions.

The data gathered from the controlled field trials that were conducted during the middle of spring, summer and winter at Pietermaritzburg and Cedara, were largely in agreement with the laboratory-derived data (see Chapter 2). Development to adulthood was fastest during summer, moderate in spring and considerably slower in winter. While development to adulthood was recorded during winter at Pietermaritzburg, this was not the case at Cedara, which was consistent with the thrips' lower developmental threshold and the mean temperatures experienced during that period (Chapter 2). Development was also significantly faster at Pietermaritzburg than at Cedara during the three seasons, which was attributed to the effects of altitude (higher at Cedara) on temperature. Although the differences in temperature between the two sites were only ca. 1°C during spring and summer, it illustrates how relatively small changes in temperature can affect insect development. Furthermore, as discussed in Chapter 2, the above-ground parts of *C. macrocephalum* are not actively growing in the field (or have died) during winter and therefore, when plant populations are proliferating during spring and summer, the thrips should not be hindered thermally. Overall, the results from the controlled field trials have backed up those of the laboratory trials and therefore, the degree-day model for *L. tractabilis* can be relied upon in this context.

A shortcoming of the model is that it does not take into account the effect of other abiotic factors on the distribution of the thrips. To improve predictions on the potential distribution of the thrips, it is suggested that future studies include: (i) determining the effect of other climatic variables such as humidity and soil moisture on the thrips' development and survival, notably the below-ground life stages; (ii) diapause studies, particularly during winter, which will indicate whether or not the thrips is able to persist during that period; and (iii) testing the thermal limits of other developmental stages (in the laboratory) of the thrips which were not included in this study (i.e. larval and pupal stages). One also needs to consider biotic factors such as natural enemies, phenology and availability of the host (Samways *et al*. 1999), dispersal capacity, and interactions between different insect species that utilize the same host plant (Baker *et al*. 2000) as these can also alter a species' response to temperature (Messenger 1959) and its distribution. Therefore, such models are not definitive and they do not replace field-based data that need to be gathered post-release. However, in terms of temperature alone, the degree-day model put forward here can be relied upon.

The advantages of conducting this type of pre-release study is that it provides useful information on the extent to which climate (in this case, temperature) is likely to be a limiting factor for the establishment of a biological control agent and highlights which areas are most suitable for supporting populations of the agent (Byrne et al. 2003; May & Coetzee 2013). This will help to ensure that the implementation of *L. tractabilis* is well planned and to prevent wasted efforts and funds by ensuring that the thrips is not released in climatically unsuitable areas. Besides contributing to the biological control of *C. macrocephalum* in South Africa, the biological data and model can also be applied to other countries which may require *L. tractabilis* in future. Thus, biological control practitioners elsewhere on the continent will also be able to determine the climatic suitability of *L. tractabilis* to their regions prior to release, in order to maximize its establishment success.

Investigating the climatic suitability of an agent should be done concurrently with host-specificity testing. This will enable the suitability of an agent to be determined well in advance and would help to prioritise its release; particularly if it was part of a suite of natural enemies being considered.

4.3 IMPACT OF *LIOTHRIPS TRACTABILIS* ON *CAMPULOCLINIUM MACROCEPHALUM*

As discussed in Chapter 3, laboratory-based impact studies of biological control agents are not necessarily a true reflection of what can be expected in the field. This formed the basis of this aspect of the study, where the impact of *L. tractabilis* was assessed on *C. macrocephalum* seedlings and root crown regrowth shoots under natural conditions in an outdoor experimental set-up. The thrips-infested seedlings displayed significantly reduced heights, numbers of leaves and both wet and dry masses relative to the control plants, which was largely in agreement with the laboratory impact study conducted by McConnachie & McKay (2015) (see Chapter 3). No significant differences between thrips-infested and uninfested regrowth plants were observed in relation to height and numbers of leaves. Since the starting tuber masses were not similar in the latter trials (see Chapter 3), the relative growth rates for wet tuber mass were calculated and compared and these displayed significant differences. These results were dissimilar to those of McConnachie & McKay (2015) (where starting wet masses were more similar) and were presumably largely a consequence of variable starting wet tuber masses for both the test and control plants, which may have masked more clear-cut trends.

Bud and flower formation was not measured as it was beyond the scope of this study. However, based on field observations in Argentina, *L. tractabilis* typically feeds on the actively growing shoots of *C. macrocephalum* (see Chapter 1), substantially reducing flowering and leaf surface area. Should flowers or buds still be produced, albeit in probably lower numbers following herbivory by *L. tractabilis*, the flower-feeding moth *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae) which is still currently under investigation in quarantine (see Chapter 1), should augment this damage, further limiting seed production and ultimately, the spread of the weed.

As mentioned in Chapter 3, the impact of numerous biological control agents has been tested under quarantine conditions in glasshouses, often in conjunction with host-specificity tests, with agents being released directly into the field once approval was obtained from the relevant authorities. Simelane & Phenye (2005) conducted a study on the growth and reproductive response of *Lantana camara* to herbivory by *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) under field cage conditions. Their findings suggested that *O. camarae* would be effective as part of a complementary guild of biological control agents.

Although such studies should be extrapolated into the field with caution (see conclusion in Chapter 3), they have predictive value. Like thermal tolerance studies, they can predict the potential efficacy of an agent under natural conditions, as well as save money and effort that would otherwise be channelled into the mass-rearing and distribution of ineffective agents (Simelane & Phenye 2005, May & Coetzee 2013).

In order to justify continued funding for a biological control project, post-release impact studies are required once agent establishment is confirmed in order to demonstrate that the agent is supressing the weed. Two strategies are often employed (Morin *et al*. 2009) to assess damage to weed populations in the field namely: (i) removal of the control agent(s), largely via chemical exclusion, to determine the extent to which the weed population recovers; or (ii) addition of the control agent(s) to uninfested weed populations to determine how they are negatively affected. As mentioned in Chapter 3, agent damage is often assessed at the individual plant level. However, as shown in this study, trends may be masked if there is substantial variation in pre-exposure plant features (e.g. tuber masses in this case). Since such between-plant variation is typical in field populations, individual plant assessments should consider aspects such as how different sized plants (or tubers) respond to varying numbers of thrips. From this study, it is clear that while low thrips inoculation was sufficient to significantly damage seedlings, this was not the case for the regrowth plants, some of which included individuals with large tubers. This suggests that releases of large numbers of thrips would be required to achieve impact on larger plants in the field.

Although this is currently not a problem in South Africa, there is often limited investment of time and resources in quantifying the effectiveness of agents that have been released (Morin *et al*. 2009) as opposed to investment into the discovery of new agents. Consequently, biocontrol practitioners often have to promote the importance of this component to influence stakeholders and funding bodies. This is crucial to reliably demonstrate the utility of biocontrol as a valuable tool in weed management.

4.4 THRIPS AS WEED BIOCONTROL AGENTS

This section gives a brief insight into the genus *Liothrips* Uzel (Phlaeothripidae) and considers the species that have currently been documented, but focuses primarily on the success that thrips have had as biological control agents. The genus *Liothrips* comprises 260

species worldwide, making it one of the three largest genera in the Thysanoptera (Mound 2005; Mound & Pereyra 2008). Currently, five species of *Liothrips* have been documented in Argentina, the native country of *C. macrocephalum*, namely *Liothrips atricolor* De Santis, *Liothrips tandiliensis* Liebermann & Gemigniani, *Liothrips tractabilis* Mound & Pereyra, *Liothrips vernoniae* Moulton and finally, *Liothrips ludwigi* Zamar (Zamar *et al*. 2013). Species in this genus utilize a range of host plants that include some 28 plant families; however, the vast majority of known species (93%) were recorded from a single host, showing a strong tendency towards monophagy (Cock 1982; Zamar *et al*. 2013). There have been only four instances worldwide, other than the present study, where Thysanoptera have been deployed as classical weed biological control agents (Winston *et al*. 2014). Two of these four agents belong to the genus *Liothrips*. Details on these species are highlighted in Table 2 and discussed below.

Amynothrips andersoni O'Neill (Phlaeothripidae) which was released against the aquatic weed *Alternanthera philoxeroides* (Mart.) Griseb in the USA did not prove effective since damage was usually light and recorded only at a few scattered sites (Buckingham 1996). Predation and the thrips' limited dispersal ability (i.e. most adults are flightless) might have been responsible for its lack of success (Buckingham 1996). The effectiveness of *Sericothrips staphylinus* Haliday (Thripidae) that became established on *Ulex europaeus* L. in Australia, Hawaii and New Zealand, has not been determined (Table 2). However, in Australia, it is believed that its impact may have been restricted by 'bottom up' effects of plant quality limiting its rate of natural increase, as well as its inability to reach large, damaging populations under field conditions (Ireson *et al*. 2008).

To date, the most unsuccessful thrips to have been deployed is *Liothrips mikaniae* Priesner, which failed to establish on *Mikania micrantha* Kunth in Malaysia and the Solomon Islands (Table 2), largely because of predation pressure (Cock *et al*. 2000). In contrast, *Liothrips urichi* Karny has so far been the most successful thrips agent (Table 2), contributing to the successful control of *Clidemia hirta* (L.) D. Don in Fiji (Reimer 1985). However, despite becoming established in Hawaii (Table 2), *L. urichi* inflicted negligible damage on weed populations, largely because of ant predation; in particular, from the alien big-headed ant, *Pheidole megacephala* Fabricius (Hymenoptera: Formicidae) (Reimer 1988).

These limited precedents suggest that while *L. tractabilis* is likely to become established in South Africa (see below), it may be influenced by generalist predators. The

alien ant *P. megacephala* is widespread in the country (McGlynn 1999) although it is unclear as to whether it has negatively affected weed biocontrol programmes. At this stage, the impact of predation on the success of *L. tractabilis* is speculative.

4.5 CONCLUSION

Based on the data gathered in this study, the prospects for *Liothrips tractabilis* as a biological control agent for *Campuloclinium macrocephalum* in South Africa appear promising. The thrips should not be hampered by temperature and should have a negative impact on the weed, provided that any disruption by generalist predators is limited (Reimer 1988). This study has also highlighted two aspects (climate matching and impact assessments) (May & Coetzee 2013; Simelane & Phenye 2005) that should form part of pre-release and post-release evaluations and that are ultimately as important as host-range assessments in weed biocontrol programmes.

Releases of *L. tractabilis* have so far been conducted during the summer months of 2013/2014 and 2014/2015 in all affected provinces in South Africa. Establishment of the thrips has since been confirmed at some sites (Table 2) but, since not all sites have been inspected (L. van der Westhuizen, pers. comm.), it has not been possible at this stage to determine the accuracy of the climate-matching predictions. Confirmation of establishment success and monitoring of population proliferation at all of the release sites is important to illustrate the value of climate-matching studies and should be prioritized. Similarly, field impact studies also need to be initiated in order to verify the predictions of the impact trials reported here.

The impact of the rust *Puccinia eupatorii* Dietel (Pucciniales: Pucciniaceae), which was inadvertently introduced into South Africa and has become widely established on *C. macrocephalum*, is currently being monitored (McConnachie *et al*. 2011, Goodall *et al*. 2012). Data gathered in the weed's native range in Argentina has revealed the coexistence of *P. eupatorii* and *L. tractabilis* on *C. macrocephalum* (McConnachie & McKay 2015). Therefore, the release of *L. tractabilis* should not result in negative interactions between the two agents and should augment the moderate level of control currently being achieved by the rust (A. Den Breeyen, pers. comm.). Furthermore, biocontrol of *C. macrocephalum* will be enhanced once permission is granted for the release of the second insect agent, the flower-

feeding moth *C. campuloclinium*, which is expected later in 2015. Thus, prospects for the biological control of *C. macrocephalum* in South Africa appear promising.

Table 2: Details of thrips species that have been utilized as classical weed biological control agents worldwide.

Plant species Origin *Thrips species*	Country	Establishment	Degree of control[a]	Reference
Amaranthaceae				
***Alternanthera philoxeroides* (Mart.) Griseb** South America				
Amynothrips andersoni O'Neill	United States	Established	Negligible	1
Fabaceae				
***Ulex europaeus* L.** Europe				
Sericothrips staphylinus Haliday	New Zealand	Established	Unknown	2
	Hawaii	Established	Unknown	2
	Australia	Established	Unknown	2
Asteraceae				
***Mikania micrantha* Kunth** Central and South America				
Liothrips mikaniae Priesner	Solomon Islands	Unsuccessful	N/A	3
	Malaysia	Unsuccessful	N/A	3
***Campuloclinium macrocephalum* (Less.) DC.** South America				
Liothrips tractabilis Mound & Pereyra	South Africa	Established (at some sites)	Under assessment	4
Melastomataceae				
***Clidemia hirta* (L.) D. Don** America				
Liothrips urichi Karny	Fiji	Established	Complete	3, 5
	Hawaii	Established	Negligible	6

[a]Definition of terms

Degree of control – The effectiveness of the thrips species in reducing the numbers or spread of the target plant where:

- Complete: Thrips has completely controlled the plant, no other control methods necessary.
- Substantial: Other control methods still required, but most control accomplished by thrips.
- Negligible: Thrips not shown to be effective in controlling plant (still able to spread or no reduction in numbers).
- Unknown: No information available on the effectiveness of the thrips.
- N/A: Thrips either not established or rejected and not released.
- Under assessment: Studies into the effectiveness of the thrips in controlling the plant are currently underway.

References: 1. Buckingham (1996); 2. Ireson *et al*. (2008); 3. Cock *et al*. (2000); 4. L. van der Westhuizen, pers. comm.; 5. Reimer (1985); 6. Reimer (1988).

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