

**Aspects of the biology, thermal physiology and nutritional ecology of
Pareuchaetes insulata (Walker) (Lepidoptera: Erebidæ: Arctiinae), a
specialist herbivore introduced into South Africa for the biological control
of *Chromolaena odorata* (L.) King and Robinson (Asteraceae)**

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ABSTRACT

Chromolaena odorata (L.) King and Robinson (Asteraceae) is an invasive weedy shrub native to the Americas that has proven to be a significant economic and ecological burden to many tropical and sub-tropical regions of the world where it impacts negatively on agriculture, biodiversity and livelihoods. A distinct biotype of *C. odorata* was first recognised as naturalized in KwaZulu-Natal (KZN) province, South Africa, in the 1940s and has since spread to other climatically suitable provinces. *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebiidae: Arctiinae) was released in KZN, South Africa, as a biological control agent against the weed between 2001 and 2009. Although the moth did establish at one out of some 30 release sites, its population level is generally low in the field. This thesis attempts to unravel the reasons for the poor performance of *P. insulata* in South Africa.

Studies of life history traits of *P. insulata* in the laboratory indicated that the moth possess good biological attributes such as low mortality, high fecundity, egg hatchability and high female mating success. Overall, adult female moths eclosed before their male counterparts suggesting the presence of protogyny. Beyond the contribution of this study to our understanding of the life history traits of erebid moths, it hypothesized that the absence of protandry might have contributed to the low population levels of the moth in the field.

To determine if a degree of agent-host plant incompatibility is culpable for the poor performance of *P. insulata*, insect performance metrics were compared on two distinct *C. odorata* plants (one from Florida and another from South Africa) in laboratory experiments. *Pareuchaetes insulata* performance metrics were similar on both plant forms; there were no significant differences in total leaf area consumed, egg and larval development, immature survival rates, feeding index (FI), host suitability index (HSI), growth index (GI), and fecundity between the Floridian and southern African *C. odorata* plants. In sum, there was no evidence to demonstrate that differences in plant forms in *C. odorata* are culpable for the poor performance of *P. insulata* in South Africa.

The effects of temperature on the developmental and reproductive life history traits, locomotion performance and thermal tolerance range of *P. insulata* were studied in order to elucidate the possible role of temperature on the poor performance of the moth. The results showed that at temperatures below 25 °C, mortality increased and development time was

prolonged. Fecundity and egg hatchability were negatively affected at a constant temperature of 15 °C. Results further showed that third instar larvae were unable to initiate movement at 6 °C and locomotor abilities were significantly reduced at 11 °C. In sum, it is hypothesized that both direct and indirect negative impacts of low temperature may partly explain the poor performance of *P. insulata* in South Africa.

The effects of seasonal and spatial variations in the leaf characteristics of *C. odorata* on the performance of *P. insulata* were investigated. Foliar nitrogen and magnesium concentrations were higher in shaded plants during winter due to low temperatures. Leaves of *C. odorata* plants growing in the shaded habitat (relative to full sun) and leaves of plants during autumn (relative to winter) were more nutritionally balanced and suitable for herbivore performance. Consequently, *P. insulata* developed faster, had heavier pupal mass and increased fecundity when reared on shaded leaves (relative to full sun) or when reared on autumn leaves compared to leaves growing in winter. This study demonstrates that low winter temperatures can indirectly affect insect herbivore performance by changing the phytochemistry of host plant and hypothesized that excess nitrogen and possibly magnesium may have detrimental effects on the insect herbivore performance.

A cross-feeding experiment was conducted to determine *P. insulata* response to a change in the diet of offspring due to a shift in plant quality in shaded versus full sun habitats. The results showed that a 'negative switch' in herbivore diet (i.e. when progeny from parents reared on shaded leaves were fed on full sun leaves) resulted in high (40%) mortality, prolonged development time and reduced fecundity. Thus full sun foliage is an inferior diet for *P. insulata* offspring. In laboratory experiments, foliar nitrogen was positively correlated with the performance of *P. insulata*.

From this study, it is demonstrably evident that the poor performance of *P. insulata* on *C. odorata* in South Africa is caused by multiple factors such as low temperatures as well as spatio-temporal variations in the leaf characteristic of *C. odorata* leaves. This study shows the complexity of determining the causes of low populations and apparent low impact of biological control agents and herbivorous insects generally, in the field. The implications of this research to the biological control programme against *C. odorata* and the direction of future research for the control of *C. odorata* are discussed.

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Publications arising from this thesis

Uyi OO, Hill MP, Zachariades C (2014) Variation in host plant has no effect on the performance and fitness-related traits of the specialist herbivore, *Pareuchaetes insulata*. *Entomologia Experimentalis et Applicata* 153: 64-75.

Uyi OO, Zachariades C, Hill MP (2014) The life history traits of the arctiine moth *Pareuchaetes insulata*, a biological control agent of *Chromolaena odorata* in South Africa. *African Entomology* 22 (3): 611-624.

Uyi OO, Hill MP, Zachariades C (2014) Disentangling the impacts of temperature on the performance of *Pareuchaetes insulata*, a biological control agent of *Chromolaena odorata*. Proceedings of the XIV International Symposium on Biological Control of Weeds, pp 96. FAC. Impson, CA Kleinjan and JH Hoffmann (eds). 2-7 March 2014, Kruger National Park, South Africa.

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DEDICATION

THIS THESIS IS DEDICATED TO MY MOTHER

PATIENCE UYI- ORUMWENSE

WHO WILL NEVER READ IT

SHE POINTED ME THE WAY TO THE TOP, BUT WAS NOT PRIVILEGED TO SEE

ME CLIMB

CHAPTER 1

1.1 INTRODUCTION

The increase in human population and urbanization which is often accompanied by unprecedented alteration and modification of ecosystems, and the promotion and expansion of global trade has led to the widespread distribution of a large number of species outside of their native ranges (Perrings *et al.*, 2010; Bigirimana *et al.*, 2011). Those alien species that become established in a new environment, and then proliferate and spread are considered “invasive alien species”. Invasive alien plant species impact negatively on agriculture, livelihoods and the conservation of biodiversity (Mack *et al.*, 2000; Rejmánek *et al.*, 2005; Pejchar and Mooney, 2009) worldwide, causing significant economic losses (Pimentel, 2002; Perrings *et al.*, 2010). South Africa is host to 117 major invaders and 84 emerging invaders of different categories of alien plants (Nel *et al.*, 2004).

Among the invasive alien plants present in South Africa, *Chromolaena odorata* (L.) King and Robinson (Asteraceae: Eupatorieae) (= *Eupatorium odoratum* L.) (Figure 1.1) is one of the most problematic. *Chromolaena odorata* is an invasive weedy shrub native to the Americas (McFadyen, 1989), that has proven to be a significant economic and ecological burden to many tropical and sub-tropical regions of the world where it impacts negatively on agriculture, biodiversity and livelihoods (Zachariades *et al.*, 2009; Uyi and Igbinosa, 2013). This Chapter consist of a brief review on *C. odorata* and *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebiidae: Arctiinae), a biological control agent that established at one site in South Africa following the release of over 1.9 million individuals at some 30 sites in KwaZulu-Natal (KZN) province, South Africa.

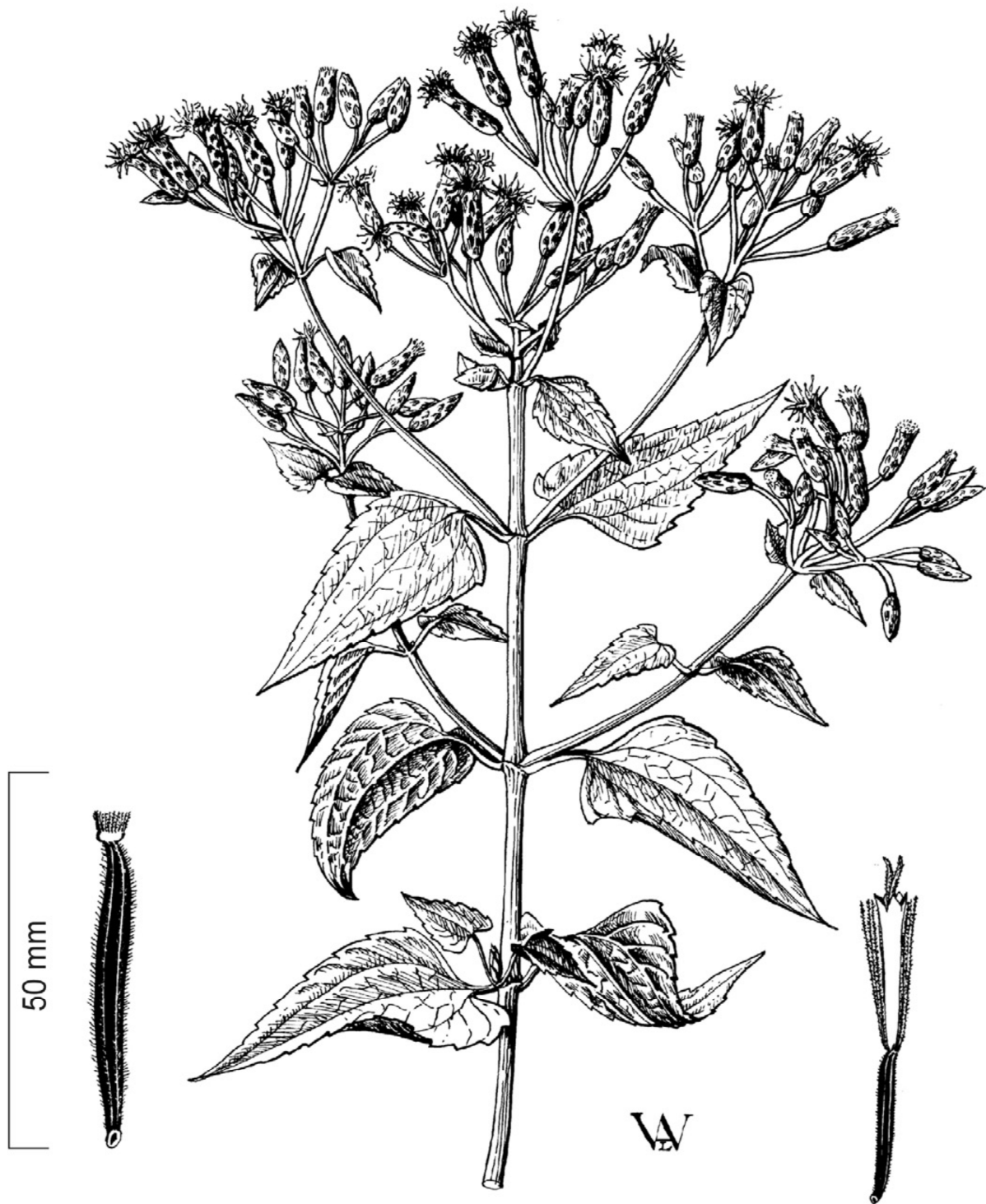


Figure 1.1 *Chromolaena odorata*. (Drawn by A.Walters, first published in Henderson and Anderson (1966), South African National Biodiversity Institute, Pretoria).

A biotype of *C. odorata*, distinct from the more widespread Asian/West African biotype, was first recognised as naturalized in Durban, KZN Province, South Africa, in the 1940s. From

there it has spread both southwards and northwards along the subtropical coastal belt. By the 1970s, it was present throughout the subtropical areas of KZN province and since the 1980s it has also rapidly expanded its range through the Eastern Cape, Mpumalanga and Limpopo provinces (Goodall and Erasmus, 1996; Zachariades *et al.*, 2011a) (Figure 1.2).

The shrub has since been declared a 'Category 1' weed under the Conservation of Agricultural Resources Act (Nel *et al.*, 2004) because of its invasiveness in the north-eastern parts of the country. The invasive success of *C. odorata* is thought to depend upon the combination of several factors such as: (i) high reproductive capacity; (ii) high growth and net assimilation rates; (iii) its capacity to suppress native vegetation through competition for light and allelopathic properties and; (iv) its ability to grow on many soil types and in many climate zones (see reviews in Zachariades *et al.*, 2009; Uyi and Igbinsosa, 2013). The weedy status of the shrub remains a grave concern to conservationists, ecologists and biological control practitioners (e.g. Leslie and Spotila, 2001; Mgobozi *et al.*, 2008; Zachariades *et al.*, 2011a), partly because of the limited success of biological control and other conventional control efforts against the weed in South Africa.

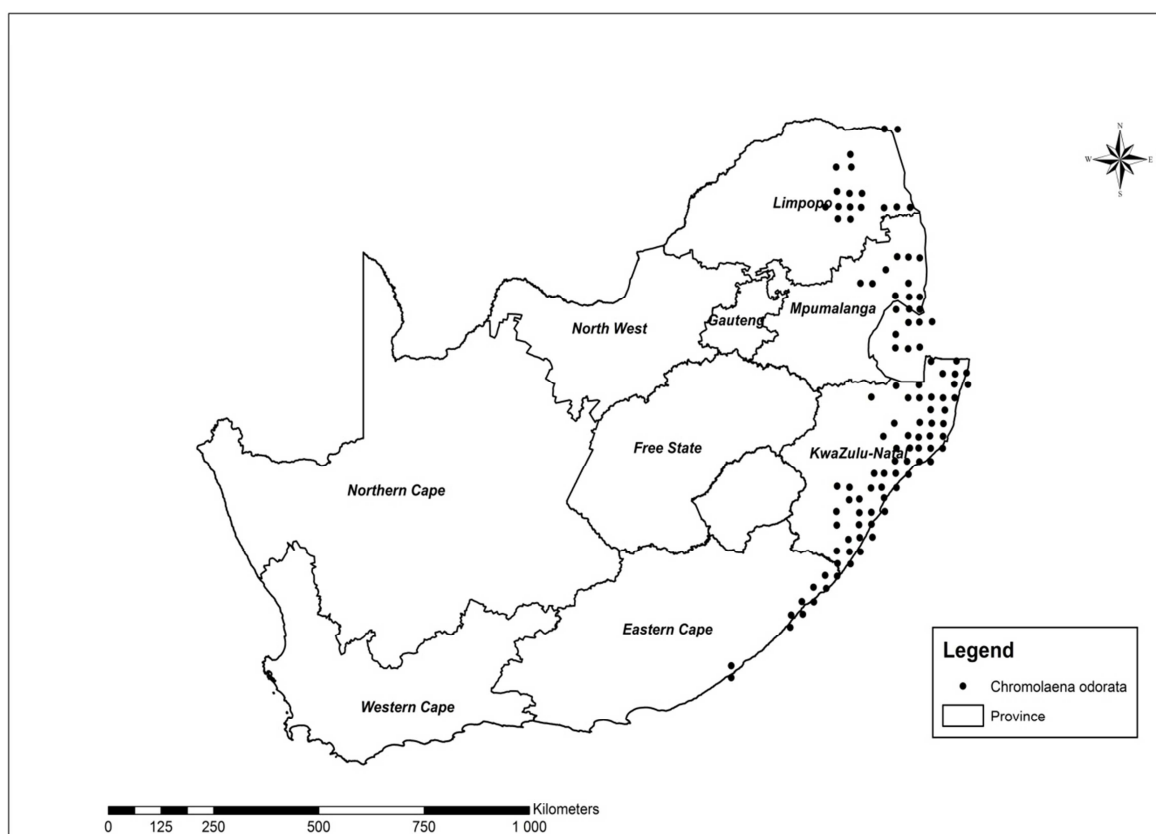


Figure 1.2 Distribution of *Chromolaena odorata* in South Africa and Swaziland. (Originally drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria).

1.2 Descriptive biology and ecology of *Chromolaena odorata*

The biology of *C. odorata* and aspects of its ecology have been documented by several authors. It is a weedy scrambling perennial plant of the tribe Eupatorieae with straight, pithy, brittle stems which branch readily, with three-veined, opposite, ovate triangular leaves and a shallow fibrous root system (Holm *et al.*, 1977; Henderson, 2001). Capitula are borne in panicles at the end of the branches and are devoid of ray florets. The corollas of the florets vary between plants from white to pale blue or lilac and achenes are black with a pale pappus (Holm *et al.*, 1977; McFadyen, 1989). In open (sunny) habitats, *C. odorata* grows up to 3 m in height, but it can reach up to 5-10 m when supported by other vegetation. The plant grows vigorously and profusely throughout the wet season, forming a dense and impenetrable

thicket, but growth ceases as flowering begins with decreasing rainfall and daylength. Flowering peaks in the southern hemisphere during the months of June and July and in the northern hemisphere from December to January. The species can reproduce apomictically (Gautier, 1992; Rambuda and Johnson, 2004) and is a prolific producer of light, wind-dispersed seeds. A single shrub can produce as many as 80 000 seeds in one season (Witkowski and Wilson, 2001). At the start of the wet season, established plants generate new shoots from the crown or from higher, undamaged axillary buds, while seeds in the soil, produced during the previous dry season, germinate (McFadyen, 1989). Stems branch freely and a large plant may have up to 15 or more branches of varying size from a single rootstock. The weed can grow on many soil types, but prefers well-drained soils (Zachariades *et al.*, 2009).

In its native and introduced ranges, the distribution of the weed is limited to between latitudes 30°N and 30°S and altitude of up to 1000 m (McFadyen, 1988; Muniappan and Marutani, 1988). It is also generally limited to areas receiving over 1000 mm rainfall per annum (McFadyen, 1988), but in southern Africa, this limit declines to 500 mm per annum (Goodall and Erasmus, 1996). This is probably because of the biotype difference between the southern African and Asian/West African *C. odorata*. It grows best in sunny, open areas such as roadsides, abandoned fields, pastures, and disturbed forests, but tolerates semi-shade conditions. It does not thrive under the shaded conditions of undisturbed forest or in closely planted, well-established plantations (Zachariades *et al.*, 2009).

1.3 Genetic and morphological variability in *Chromolaena odorata*

Two main biotypes of *C. odorata* are known in its invasive range of distribution viz. the Asian/West African biotype (hereafter referred to as AWAB) and the southern African

biotype (hereafter referred to as SAB). The AWAB, which is the most widely spread form found in Asia, India, West, East and Central Africa and the Oceania, is thought to have originated from Trinidad, Tobago or the adjacent areas (Yu *et al.*, 2014), while the SAB found only in southern Africa is thought to have originated from Jamaica or Cuba (Paterson and Zachariades, 2013). The two biotypes are known to differ in morphology, genetics and aspects of their ecology (Paterson and Zachariades, 2013). The differences between AWAB and SAB plants have been elucidated (C. Zachariades, ARC-PPRI, South Africa, unpubl. data). The SAB plants are substantially different from the widespread invasive biotype found in Asia, West and Central Africa in the following ways;

- (i) the leaves of the AWAB are usually large with fine hairs giving a soft texture particularly to younger leaves, with grey-green to dark-green colour, but often purple at the young stage especially when growing in the sun, while the leaves of the SAB are distinctly small and smooth, with dark-green colour when growing in a semi-shade but yellow-green in the sun and red when young,
- (ii) the stems of the AWAB are hairy, with a grey-green to dark green colour, while those of the SAB are largely smooth with yellow-green colour,
- (iii) the AWAB have broader individual capitula containing disc florets with a pale lilac colour, involucral bracts having sharp tips and which are lax around the capitulum; while the SAB capitula are narrower, containing disc florets with a white colour, involucral bracts having round tips and which are tight around flower-head,
- (iv) the branches of AWAB are not rigid, while SAB has upright growth especially young growth in dense stands and

- (v) the AWAB is more adapted to tropical conditions, and may be more fire resistant, having a tendency to re-grow from the crown, while the SAB may be more cold tolerant and more susceptible to fire.

1.4 History and distribution of *Chromolaena odorata* in Africa

Chromolaena odorata has a wide native range distribution from the southern USA to Argentina, Central America and the Caribbean islands (Gautier, 1992; Kriticos *et al.*, 2005). *Chromolaena odorata* is increasingly becoming widespread in its introduced range with the species being present in Central, East, South and West Africa, India, China, Southeast Asia and parts of Oceania (a region including the islands of the tropical Pacific Ocean) (see Zachariades *et al.*, 2009; 2013).

1.4.1 West Africa

The presence of *C. odorata* in West Africa was first recorded in a forestry plantation near Enugu, in south-eastern Nigeria in 1942 and is thought to have resulted from contaminated seeds of the forest tree *Gmelina arborea* Roxb. (Verbenaceae), imported from southeast Asia in 1937 (Ivens, 1974). Following the introduction of the shrub, it quickly spread across many parts of Nigeria and to neighbouring countries, probably due to human and vehicular movement, road constructions and regional trades (Uyi *et al.*, 2014a). Out of the 36 states in Nigeria, 23 states have already been colonised by *C. odorata* (Uyi *et al.*, 2014a).

Chromolaena odorata is thought to have been first introduced from Nigeria to Ghana, though some other possible modes of introduction have also been identified (Timbilla and Braimah, 1996; Hoevers and M'boob, 1996). The weed has occupied the high forest, semi-deciduous forest, coastal and forest savannah zones, taking over two-thirds of the country's total land

area (Timbilla *et al.*, 2003). Yehouenou (1996), reported the spread of *C. odorata* into southern Benin Republic and Togo around the 1970s and 1980s. The southern parts of Côte d'Ivoire have also been severely infested by *C. odorata* (Zebeyou, 1991). The spread of *C. odorata* to The Gambia, Liberia, Burkina Faso, Guinea and Sierra Leone has also been reported (Timbilla *et al.*, 2003).

1.4.2 East and Central Africa

Chromolaena odorata appeared in East and Central Africa much later than in West and southern Africa. The spread and status of *C. odorata* in the region was recently reviewed in Zachariades *et al.* (2013). It was first confirmed present in Kenya in 2006, while it was recorded in the eastern part of Rwanda in 2003. The west of Busia where Uganda borders Kenya has also been infested by *C. odorata*. The presence of *C. odorata* in Tanzania was recorded between 2009 and 2010 near the eastern shores of Lake Victoria by these authors.

In the mid-1970s *C. odorata* was recorded in the central parts of the Democratic Republic of Congo (Gautier, 1992; Hoevers and M'boob, 1996), and is now present in the western parts of the country, and also the eastern parts close to the border with Burundi and Uganda (Zachariades *et al.*, 2013). *Chromolaena odorata* spread from the south-eastern states of Nigeria to Cameroon and has also been reported in Chad (Zebeyou 1991; Hoevers and M'boob, 1996; Timbilla, 1998). The spread of the weed in this region has been made possible through human and vehicular movements and dispersal of the seeds by wind and water (Zachariades *et al.*, 2013).

1.4.3 Southern Africa

As stated earlier, the *C. odorata* biotype in South Africa is believed to have originated from Jamaica or Cuba (Paterson and Zachariades, 2013) and was first recorded as naturalised in South Africa in 1947 (Zachariades *et al.*, 2011a) at a site east of Ndwedwe (29° 30° S, 30° 56° E) near Durban (Hilliard, 1977). Its spread is restricted to the warmer subtropical eastern and north-eastern parts of the country where it is present in KZN, Mpumalanga, Limpopo and Eastern Cape provinces (Goodall and Erasmus, 1996; Kriticos *et al.*, 2005). *Chromolaena odorata* spread has reached its southern ecological limit around the Port St Johns region of the Eastern Cape Province (Zachariades *et al.*, 2011a). The plant was probably introduced from Asia into Mauritius before 1949 (Zachariades *et al.*, 2009), while it was discovered in Zimbabwe in the late 1960s and in northern Angola in the late 1970s (Gautier, 1992; Hoevers and M'boob, 1996). *Chromolaena odorata* invades the forest and savannah biomes of Swaziland (Zachariades *et al.*, 2011a, 2013), and some parts of Mozambique and possibly Malawi have also been infested (Zachariades *et al.*, 2013).

1.5 Economic and ecological importance

In most of its introduced range, *C. odorata* impacts on cropping and pastoral agriculture and on biological diversity. The weed affects both subsistence and commercial agriculture, including crops and plantations (e.g. oil palm, rubber, coffee, cacao, coconuts, cashew, sugarcane, banana and plantain), grazing lands and silviculture (Zachariades *et al.*, 2009). The weed thus decreases agricultural productivity and increases management cost at both subsistence and commercial scale (Prasad *et al.*, 1996).

Although the status of *C. odorata* in West Africa remains a subject of debates (see Uyi *et al.*, 2014a and references therein), its impacts on agriculture, livelihood and biodiversity have

been documented (Lucas, 1989; Yeboah, 1998; Uyi and Igbinosa, 2013). It is a major weed in crops such as cocoa, coffee, oil palm, cotton, rubber, cassava, banana, plantain, yam as well as vegetables in Nigeria (Uyi *et al.*, 2014a). In Ghana, Yeboah (1998) reported a reduction in the diversity of small mammals in vegetation that was predominantly colonised by *C. odorata*.

In South Africa, its impact on ecotourism and biodiversity (Witkowski and Wilson, 2001; Zachariades and Goodall, 2002; Goodall and Zacharias, 2002; Mgobozi *et al.*, 2008; te Beest *et al.*, 2009) has been documented. For example *C. odorata* stands interfered with the egg-laying potential of the Nile crocodile (Leslie and Spotila, 2001). Also, te Beest *et al.* (2012) showed how fire interacted with the conventional clearing of *C. odorata* in South Africa and induced an intense canopy fire that caused a shift from woodland to grassland. Though not yet perceptibly noticed in East and Central Africa, *C. odorata* is expected to have similar impacts on agriculture, biodiversity and human livelihoods, as has been reported in other regions where the plant is currently invasive.

Despite the weed status of *C. odorata*, it is claimed to have some beneficial attributes by locals in West Africa (see discussions in Uyi *et al.*, 2014a). In South Africa, there is no conflict of interest surrounding the weed's status.

1.6 Control methods

Recent studies have revealed that the impact of invasive plants changes over time (Dostál *et al.* 2013; Yelenik and D'Antonio 2013), but this may not necessarily benefit native species recovery (Yelenik and D'Antonio 2013). However, it is still important to manage plant invasions because of the threat they may pose to natural and semi-natural ecosystems.

Following the invasive nature of *C. odorata* and its presence in large areas, including uncultivated and other public areas, chemical and mechanical measures such as slashing and burning are not practical to check the spread of, or adequately control, this noxious weed. Hence biological control methods using insects which feed on this plant have been advocated as an important strategy for the long-term management of *C. odorata* (Seibert, 1989; Goodall and Erasmus, 1996).

1.6.1 Biological control of *Chromolaena odorata*

The first biocontrol programme for *C. odorata* worldwide originated in West Africa when in 1968, the Nigerian Institute for Oil Palm Research (NIFOR) funded research by the Commonwealth Institute of Biological Control in Trinidad. This led to the release of two biological control agents *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Erebiidae: Arctiinae) (initially misidentified as *Ammalo insulata* Walk.) and *Apion brunneonigrum* Beguin- Billecoq (Coleoptera: Curculionidae) in both Nigeria and Ghana in the early 1970s (Ivens 1974; Cock and Holloway 1982). This initial effort was perceived to have failed because at the time, establishment was not recorded, possibly due to ant predation (Cock and Holloway, 1982). Uyi *et al.* (2011) argued that these initial biological control efforts may have failed because they were not sustained. Following successes of *P. pseudoinsulata* elsewhere (Seibert 1989), a renewed biocontrol effort began in Ghana in 1989, when a culture of *P. pseudoinsulata* was imported from Guam and released in the Ashanti and Central Regions from 1991 to 1997 (Timbilla *et al.*, 2003). Timbilla *et al.* (2003) confirmed the establishment of the moth at Fumesua near Kumasi in 1994, making Ghana the first country in Africa to establish *P. pseudoinsulata*.

The first biological control attempt against *C. odorata* in South Africa started in 1988, when disease-free eggs and early-instar larvae of the leaf feeding moth *P. pseudoinsulata* were imported from the Pacific island of Guam, USA, and mass-reared before several releases in ten localities in KZN without establishment (Kluge, 1991). Kluge (1994) attributed *P. pseudoinsulata* establishment failure to predation of eggs by ants and other invertebrates. In an attempt to minimize the reported predation, *Pareuchaetes aurata aurata* (Butler) (Lepidoptera: Erebidae: Arctiinae), which lays its eggs singly, scattered on the ground, was introduced between 1990 and 1993. However, it failed to establish after the release of 150,000 larvae, pupae and adults at some 18 sites around KZN in the eastern part of South Africa (Zachariades *et al.*, 1999). Following further successes with *P. pseudoinsulata* elsewhere, a new culture was imported from Indonesia and released mainly in Limpopo province in the northern region of South Africa from 1998 to 1999 with about 367,000 larvae and 3,000 adults released at three sites. The reason for the large numbers during these releases was to overcome the possible effects of predation, parasitism, dispersal and Allée effects, but again the insect failed to establish (Strathie and Zachariades, 2002; Zachariades *et al.*, 2011a).

In furtherance of the efforts to biologically control and manage *C. odorata*, a third *Pareuchaetes* species, *P. insulata* (Walker) (Lepidoptera: Erebidae: Arctiinae) with a similar biology to *P. pseudoinsulata* was considered. A culture of *P. insulata* was collected from Florida, USA and tested for host specificity in the early 1990s (Kluge and Caldwell, 1993a). Florida exhibits a similar seasonal climate comparable to that in north-eastern parts of South Africa where the weed is invasive. This moth lays its eggs on *C. odorata* leaves and the caterpillar feeds on the leaves until pupation. A further culture was collected in late 2000 and was mass reared under rigorous hygiene standards at the South African Sugarcane Research

Institute (SASRI), Mt Edgecombe, South Africa. More than 700,000 larvae and 10,000 pupae and adults were released at 17 sites in KZN between January 2001 and April 2003 (Zachariades *et al.*, 2011a). Some of the release areas were climatically similar to Fort Lauderdale, Florida where the insect culture was collected (Parasram, 2003) but the insect did not appear to have established (Strathie and Zachariades, 2004). In an attempt to overcome possible incompatibility of the biological control agent with the southern African *C. odorata* as a factor preventing establishment, *P. insulata* cultures from Cuba and Jamaica were imported, mass-reared and released. 590,000 and 327,000 larvae of the Jamaica and Cuba populations of *P. insulata* respectively were released between 2002 and 2008, apparently without establishment (Zachariades *et al.*, 2011a). In total, approximately 1,920,000 individuals of the Floridian, Jamaican and Cuban strain of *P. insulata* were released at 30 sites. Subsequently however, Zachariades and Strathie (2006) confirmed the establishment of *P. insulata* in 2004 at one site (Sappi Cannonbrae Plantation, Umkomaas, KZN, South Africa). The Cannonbrae site, which lies close to the coast south of Durban, recorded the greatest number of larvae released (Strathie *et al.*, 2007; Zachariades *et al.*, 2011a) and is thought to be the only established site to date.

1.7 Descriptive biology and behaviour of *Pareuchaetes insulata* and other arctiine moths

Pareuchaetes insulata is a moth in the sub-family Arctiinae whose foliage-feeding larvae can cause extensive defoliation damage to *C. odorata*. While the description of the immature stages and adults of *P. insulata* is well documented (Cock and Holloway 1982; Kluge and Caldwell, 1993a; Dube, 2008), little information is available on its biology and behaviour. Adult *P. insulata* are medium sized, golden yellow moths with longitudinal rows of black abdominal spots both dorsally and laterally. Adults are nocturnal and mating occurs on the

first night after eclosion. Newly laid eggs (on the underside of *C. odorata* leaves) are pale yellow and spherical in shape and turn grey before hatching.

The biology of *P. insulata* (Kluge and Caldwell, 1993a) is similar to that of *P. pseudoinsulata* (Bennett and Cruttwell, 1973) and that of *P. aurata aurata* (Kluge and Caldwell, 1993b). Newly hatched first instar larvae are grey with a single black seta on each tubercule. After feeding, they become pale grey with orange and maroon blotches. The second instar larva differs from the first instar in that there are 1-5 seta per tubercule and that the abdominal segment one, seven and nine are maroon. The third, fourth, fifth and sixth instar larvae are similar. Two broken narrow white lines dorso-laterally and ventro-laterally run along the length of the body. On each segment, there are 2-6 verrucae with short black setae. Slightly longer white setae are often visible on the meso- and metathorax and on the 8th abdominal segments. The dorso-lateral and lateral verrucae on the metathorax and abdominal segments 2, 4, 8 and 10 are reddish-orange; although these colourings are sometimes absent from segments 6 and 8.

P. insulata is distinguishable from *P. pseudoinsulata* by the pattern of the red colouring on the abdomen of the 5th instar larvae which are found on the verrucae of segments 3, 5-9 and 11 in *P. pseudoinsulata* (Cock and Holloway, 1982) and 2, 6 and 8 in *P. aurata aurata* (Kluge and Caldwell, 1993b). The two broken white lines running down the length of the body of *P. insulata* are noticeably whiter than those of *P. pseudoinsulata* and *P. aurata aurata* (Kluge and Caldwell, 1993a). In *P. insulata* and *P. psuedoinsulata*, larvae feed on foliage of *C. odorata* during the night and quit the plant foliage at or soon after sunrise and spend the day hidden in leaf litter at the base of the plant.

Late fifth and sixth instars of *P. insulata* migrate down the base of the *C. odorata* plants and pupate in a flimsy cocoon in leaf litters. The pupa of *P. insulata* is dark brown and measures approximately 12 mm in length and 4 mm in width. The larvae of these moths are reported to pass through six instars before pupation, while its life cycle has been reported to be between 40 and 60 days (Kluge and Caldwell, 1993a; Dube 2008).

Arctiine moths are relatively distasteful to predators in all life stages and this allows them to occupy behavioural and ecological contexts unavailable to their more palatable counterparts (Conner, 2009). Larvae of these moths are known to sequester pyrrolizidine alkaloids (PAs) from their host plants which serve as host-finding cues (see reviews in Macel, 2011; Conner, 2009), a feeding stimulant (Kelley *et al.*, 2002) and play an integral role in their mating behaviour (Conner, 2009). For example, in *Utetheisa ornatrix* (Lepidoptera: Erebidae) (L.) and in other arctiines, females choose males based on a courtship pheromone, hydroxydanaidal (HD), derived from defensive PAs (Schneider *et al.*, 1992; Conner, 2009). Females make choices based on the ability of virgin males to transfer spermatophores whose contents are proportional to their HD titre and body size; as a result, selective females receive both phenotypic benefits (more nutrients and PAs) and genotypic benefits (genes for larger body size inherited by the offspring) (Conner *et al.*, 1990; LaMunyon and Eisner, 1994; Conner, 2009; Kelly *et al.*, 2012). The females pass this gift, together with PAs that they themselves procured as larvae, to the eggs for defence against predators and parasitoids (Eisner *et al.*, 2000; Bezzerides *et al.*, 2004).

1.7.1 Spread and impact of *Pareuchaetes insulata*

To date, only a few basic quantitative studies on the populations and impact of *P. insulata* have been undertaken (see Zachariades *et al.*, 2011a). Following establishment at Sappi

Cannonbrae Plantation, the initial feeding damage and insect population levels increased in 2005 and 2006 when there was a dramatic increase in larval numbers within a 5 km radius of the release site, and the distribution of, and damage caused by the moth was predicted to increase annually (Strathie *et al.*, 2007). By April 2006, evidence of spread of the moth was observed up to 25 km to the north-east, 20 km in a south-westerly direction and 10 km inland, to the north-west (Strathie *et al.*, 2007). However, these population and damage levels were not sustained. Much of the weed's infestation in KZN lies inland in areas that become dry in winter and the current pattern of spread observed does not suggest that the moth will establish in these areas. By 2010, evidence of spread of the moth was observed up to 100 km along the coast and 10 km inland (Zachariades *et al.*, 2011a). Although recent outbreaks (Strathie and Zachariades, 2014) have been observed at a number of locations in northern KZN, its overall population in the field remained generally low and consequently its impact on *C. odorata* has been variable, but probably low overall.

In many other countries where a related species, *P. pseudoinsulata* has established, it seems to be an “outbreak species” (Zachariades *et al.*, 2009). The situation in South Africa in terms of establishment and impact is worse compared to many of the areas where *P. pseudoinsulata* has been introduced in terms of establishment and impact. A study of the impact of *P. insulata* in the laboratory and planted field plots (T.D. Rambuda, unpubl. data) was discontinued in South Africa. However, in 2006 large areas of defoliated *C. odorata* thickets were observed within a radius of 1 km of the release point, particularly in shadier parts after establishment was confirmed (Strathie *et al.*, 2007). In a recent review, Zachariades *et al.* (2011a) suggested that “although *P. insulata* may be sporadically very damaging in the coastal belt, overall it is unlikely to provide consistent or adequate suppression of *C. odorata*”. Hence *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae) was released

and readily established. Also, other biological control candidates such as *Lixus aemulus* Petri (Coleoptera: Curculionidae) and *Dichrorampha odorata* Brown and Zachariades (Lepidoptera: Tortricidae) are currently being released against the weed after rigorous host-range tests.

1.8 Possible factors affecting the success of *Pareuchaetes insulata*

The success of biological control agents is sometimes constrained by a multiplicity of biotic and abiotic factors that may limit their efficacy and even result in the failure of some agents to establish. For example, climate (McClay, 1996; Byrne *et al.*, 2002, 2003), top-down factors (e.g., predators and parasitoids) (Goeden and Louda, 1976; Kluge, 1994; Manrique *et al.*, 2011), and plant factors such as plant quality, genetic dissimilarity, plant morphological variations, or biotype incompatibility (Dray *et al.*, 2004; Simelane, 2006; Wheeler, 2006; Baars *et al.*, 2007) have been suggested to deleteriously influence the survival, development, population dynamics, as well as the establishment of biological control agents. It is hypothesized that one or a combination of these factors may be responsible for the poor performance of *P. insulata* in South Africa.

A common practice in weed biological control programmes is to prioritize host range and laboratory impact studies to demonstrate that the candidate agent does not pose an unacceptable risk to non-target plant species (Briese, 2005) and to also show that the agent can decrease the growth and hamper the reproductive ability of the target plants (McClay and Balciunas, 2005; van Klinken and Raghu, 2006; Carson *et al.*, 2008). However, studies on possible factors that could constrain the establishment and performance of biological control agents are often not prioritized pre-release, because they are difficult to perform, and infrequently conducted post-release to provide possible explanations for the establishment

failure of biological control agents, or the limited success of biological control programmes when agent establishment occurs (see McClay, 1996; Byrne *et al.*, 2002, 2003; for exceptions, see Simelane, 2006; May and Coetzee, 2013). This study attempts to explain the poor performance of *P. insulata* by studying the contribution of potential factors that could be responsible for its variable impact on *C. odorata* in the bitrophic relationship between the insect and its host plant.

1.9 Aims and rationale

Studies on *P. insulata* in South Africa have been limited to host-range testing (Kluge and Caldwell, 1993a) and to reporting the establishment and spread of, and basic data on damage caused by the moth on *C. odorata* (Strathie *et al.*, 2007; Zacharides *et al.*, 2011a). Therefore, the principle aim of this thesis was to determine the possible factors that are responsible for the poor performance (low population levels) of *P. insulata* in South Africa. This was carried out by characterising and interpreting both biotic and abiotic factors responsible for the low abundance and population fluctuations of moth.

Aspects of the life history traits of biological control agents may be implicated in the poor establishment and performance of biological control agents (e.g. Boughton and Pemberton, 2012), therefore the life history traits of *P. insulata* were investigated in Chapter 2, to determine whether several aspects of its developmental and reproductive biology can help explain its poor performance in South Africa.

Due to the differential performance of insect herbivores on host plant varieties, genotypes or chemotypes (Dray *et al.*, 2004; Goolsby *et al.*, 2006; Simelane, 2006; Wheeler, 2006; Baars *et al.*, 2007) and the implications such differences could have on biological control agents,

Chapter 3 compared several measurements of performance of *P. insulata* reared as larvae on leaves of two distinct morphological forms (possibly biotypes) of *C. odorata*, one from southern Florida, USA (from which this *P. insulata* population originates) and the other from South Africa where it has established as a biological control agent, albeit poorly. The experiments in this chapter were conducted to test if a degree of agent-host plant incompatibility is culpable for the poor performance of the moth.

Temperature has been shown to influence the development, survival, fecundity, locomotion (or flight) performance, distribution as well as establishment and population dynamics of biological control agents and other insects (Stewart *et al.*, 1996; Visalakshy, 1998; Byrne *et al.*, 2002; Chidawanyika and Terblanche, 2011; Li *et al.*, 2011; Tamiru *et al.*, 2012; May and Coetzee, 2013; Ferrer *et al.*, 2014). To date, no quantitative data exist on the effects of temperature on the survival, development, fecundity, locomotion performance and temperature tolerance range of *P. insulata* in South Africa or elsewhere. Hence, Chapter 4 attempts to address this lacuna.

Bottom-up effects of plant nutritional quality can influence the survival, development, fecundity and distribution of insects including those used as biocontrol agents (Preszler and Price, 1988; van Hezewijk *et al.*, 2008; Sarfraz *et al.*, 2009; Moran and Goolsby, 2014). Leaf nutrients and physical characteristics of host plants may also vary in space (e.g. Bryant *et al.*, 1987; Diaz *et al.*, 2011) and time (e.g. Muller *et al.*, 2005, 2011). Understanding how such bottom-up factors may shape the population density of *P. insulata* requires knowledge of how a change in *C. odorata* plant nutritional quality affects the development, survival and fecundity of the herbivore. Therefore, the objective of Chapter 5 was to investigate the effects

of seasonal and spatial variations in the leaf characteristics of *C. odorata* on the performance of *P. insulata*.

Chapter 6 investigated larval feeding history in *P. insulata* by examining the effect of a switch in herbivore diet on larval offspring. This study was conducted to determine *P. insulata* response to a change in diet of neonate offspring due to a shift in plant quality occasioned by mechanical clearing of the weed and/or removal of surrounding vegetation within the vicinity of *C. odorata*, thereby exposing the plants to higher light levels.

Recent studies suggest that the relationships between nitrogen levels in host plants and phytophagous insect performance are not simple (Boesma and Elser, 2006; Clissold *et al.*, 2006; Zehnder and Hunter, 2009). Therefore, Chapter 7 investigated the effect of nitrogen fertilization on the performance of *C. odorata* as well as on the performance of *P. insulata*.

Chapter 8 is a general discussion of the results. The implications of the findings of this thesis on biological control of *C. odorata* and on the population dynamics of *P. insulata* are discussed. The chapter also identified possible areas for future research.

CHAPTER 2

The life history traits of the arctiine moth *Pareuchaetes insulata*, a biological control agent of *Chromolaena odorata* in South Africa

2.1 INTRODUCTION

Pareuchaetes insulata is a moth in the sub-family Arctiinae whose foliage-feeding larvae can cause extensive defoliation damage to *C. odorata*. The description of the immature stages and adults of *P. insulata* is well documented (Cock and Holloway, 1982; Kluge and Caldwell, 1993a).

In a few places, such as Guam, there has been a significant long-term reduction in the population of *C. odorata* using *P. pseudoinsulata*. However, in many of the countries or regions where *P. pseudoinsulata* established, including Sri Lanka, the Marianas, India, Ghana, and Sumatra, initial establishment was followed by a spectacular population build-up and widespread, complete defoliation of *C. odorata* thickets, and high mortality rates of these plants (see review in Zachariades *et al.*, 2009), but, the populations of the moth declined dramatically (after one or two years) and the weed recovered. Subsequent sporadic outbreaks resulting in defoliation occurred, but these were often less spectacular and were unpredictable over time and space (Zachariades *et al.*, 2009). Although occasional outbreaks of *P. insulata* have been reported in South Africa (Zachariades *et al.*, 2011, Strathie and Zachariades, 2014), *P. insulata* is still considered to be a less effective biological control agent (compared with *P. pseudoinsulata*) probably because of the effect of climate (low winter temperatures) and other environmental or intrinsic factors affecting the moth in South Africa.

Although the biology of *P. insulata* appears to be similar to that of *P. pseudoinsulata* (Bennett and Cruttwell, 1973) and *P. aurata aurata* (Kluge and Caldwell, 1993b), a further and comprehensive understanding of its developmental and reproductive biology is needed. Information on several aspects of the life history traits of *P. insulata* might assist in unravelling the reasons for the low population levels in the field (e.g. as in Boughton and Pemberton, 2012) and could enable us to gain a thorough knowledge of the behaviour of the erebid moth in its new environment. Furthermore, previous research (Kluge and Caldwell, 1993a; Walton and Conlong, 2003; Dube, 2008) undertaken to document the biology of *P. insulata* did not consider differences between female and male traits; hence, this study addresses this lacuna because the development and growth of moths sometimes vary as a function of gender (Thiéry and Moreau, 2005; Stillwell and Davidowitz, 2010). Experiments reported here were conducted to investigate several aspects of the biology of *P. insulata* to understand the poor performance of moth in the field and to allow comparison with earlier studies on *P. pseudoinsulata* (Cruttwell, 1972; Muniappan *et al.*, 1989), *P. insulata* (Kluge and Caldwell, 1993a) and *P. aurata aurata* (Kluge and Caldwell, 1993b).

This study compares metrics of the moth's performance such as leaf consumption, development duration, pupal mass, growth rate and longevity between males and females which was not considered by Kluge and Caldwell (1993a) and Dube (2008). A further objective of this study was to investigate survival, realised fecundity (i.e. total number of eggs laid), sex ratio, egg hatchability, mating success, duration of egg laying and pre-oviposition period in *P. insulata*.

2.2 MATERIALS AND METHODS

2.2.1 Origin and maintenance of plant and insect cultures

Pareuchaetes insulata individuals were collected in the Sappi Cannonbrae plantation, Umkomaas (south coast of KZN province), South Africa (30° 13' S, 30° 46' E), where the insect established following large releases made between 2001 and 2003 (Zachariades *et al.* 2011a). The individuals of the moth were maintained for about six generations in the laboratory at the Cedara Weeds Research Unit, ARC-Plant Protection Research Institute (ARC-PPRI) KZN, South Africa on *C. odorata* bouquets from the field (in and around Durban) at 25 ± 2 °C, $65 \pm 10\%$ relative humidity (RH), with a photoperiod of L12:D12. Newly hatched larvae were fed with cut leaves inside 700 ml (1 egg batch per container) aerated plastic containers, while older larvae were reared inside 2 L “Freezette” rectangular plastic trays (32 x 22 x 6 cm) (30 larvae per container) with *C. odorata* bouquets. Fresh leaves were added as needed. Pupae were placed in 2 L Freezette trays containing vermiculite and monitored for eclosion.

Chromolaena odorata plants used in this study were grown from stem-tip cuttings collected from Durban, South Africa. All cuttings were initially planted in a mist bed in vermiculite with rooting hormone (Seradix™ No. 1) before they were later planted in nursery pots (25 cm diameter). All plants benefited from same potting medium (Umgeni sand: Gromor Potting Medium 1:1), fertilizer (Plantacote, AGLUKON Spezialduenger GmbH & Co. KG, Germany) and watering regimes. These plants were maintained in a glasshouse at temperatures between 20 and 30 °C and plants were watered daily using an automatic drip irrigation system. All trials were performed in a growth chamber (Labcon, South Africa) set at 25 °C and a 12L:12D photoperiod. Hygrochron iButtons (model DS 1923, Maxim Integrated Products, San José, CA, USA, 0.5 °C accuracy) were used to measure temperature

(hourly readings: range, 24.10 to 25.71 °C; mean $\pm$ SE, 24.53 $\pm$ 0.01 °C) and RH (range, 66.4 to 79.2%; mean $\pm$ SE, 70.7 $\pm$ 3.3%) inside the chamber.

2.2.2 Survival, growth and developmental biology

To assess survival, growth and development duration of *P. insulata*, larvae were raised individually from day of hatching to pupation in 100 ml aerated plastic containers with a circular net screen window (2.5 cm diameter on the top for ventilation), lined at the bottom with moistened filter paper to maintain RH, and were fed on *C. odorata* leaves for the duration of their development. This protocol presented at least two main advantages: (i) feeding larvae in isolation prevented biases due to competition and consequent food deprivation and; (ii) prevention of variations due to microhabitat effects. Larvae (n = 280) were fed with fresh fully expanded leaf tissues (taken from the upper half of the plants) every 24 hours and their frass was removed at same interval for hygienic reasons (Uyi *et al.*, 2011). The daily use of new leaf tissues probably increases survival, and is consistent with field observation of the *Pareuchaetes* species larva, which, in the presence of an abundant food supply, feeds each night on a leaf which has not previously been fed on, and shelters further down on or underneath the plant during the day. The purpose of using excised leaves was to limit uncontrolled variations among whole plants so that the insect parameters of interest, in this case development time and other fitness-related traits, could be stringently assessed. Although the use of excised leaves in the determination of insect survival and performance has been a subject of debate (Olckers and Hulley, 1994; Blossey and Notzöld, 1995; Palmer, 1999), this practice is seen as an acceptable method for providing uniform materials in laboratory feeding studies of this kind (see Blossey and Notzöld, 1995; Hull-Sanders *et al.*, 2007; Beaton *et al.*, 2011; Oleiro *et al.*, 2011; Boughton and Pemberton, 2012). Moreover, all other previous work (Muniappan *et al.*, 1989; Kluge and Caldwell, 1993b; Dube *et al.*, 2014)

on aspects of the biology of *Pareuchaetes* species have been conducted using excised leaves of *C. odorata*.

All containers were placed inside a ZiplocTM bag (600 x 450 mm) to prevent desiccation. Observations on mortality were made daily until adult eclosion in order to follow the duration of each instar and other immature stages. The stage-specific and overall survival of *P. insulata* was calculated as numbers of larvae (or pre-pupae or pupae) that developed to the next stage, divided by the initial number of that particular stage. The loss of the cephalic capsule was the criterion used to determine the moult. Pupae were extracted from the food source and weighed ($n = 72$) as an index of the adult body size. Sex was determined at the pupal stage, based on the position of the genital orifice (see Dube 2008). Sex ratio and performance metrics were calculated and recorded respectively based on the numbers of surviving pupae ($n = 250$). Specifically, the following variables were measured: (i) duration of each larval instar, (ii) total larval development duration (defined here as the number of days from hatching until pupation), (iii) pre-pupal duration, (iv) pupal duration, (v) total pupal duration (pre-pupal and pupal combined), (vi) total immature development time (defined here as the number of days from hatching until adult eclosion), (vii) growth rate (pupal mass in mg/development time) (for rationale, see Thiéry and Moreau, 2005) and (viii) head-capsule width. The head-capsule growth ratio was calculated following Dyar's (1890) study, i.e. post-moult head-capsule width was divided by pre-moult width. All parameters except head-capsule width were measured according to sex in order to enable comparisons between males and females. Upon adult eclosion, adult longevity was measured as a function of sex.

2.2.3 Leaf area consumption

The influence of gender on total (i.e. lifetime) leaf consumption by *P. insulata* was tested on individual larvae with equivalent amounts of food using similar methods described in the previous section. This protocol enabled accurate quantification of the leaf area consumed by a single individual from first instar larval stage to pupation (for rationale, see van Hezewik *et al.*, 2008; Prasifka *et al.*, 2009). The area of leaf tissue consumed per individual larva per day was assessed by scanning images of the leaf tissues before and after feeding, with a digital scanner (Konica Minolta C360 Series PCL, Langenhagen, Germany). Leaf areas were thereafter measured using the Compu Eye Leaf and Symptom Area program developed by Bakr (2005) (available at <http://www.ehabsoft.com/CompuEye/LeafSArea/>). The above procedure was continued each day for each larva (n = 53) until feeding ceased in the prepupal stage. Pupae were extracted from the food source and weighed as an index of the adult body size. The growth rate was calculated as described previously.

2.2.4 Reproductive life history traits

To enable correlation of life history traits, all adults from individual larvae that were used for the leaf consumption trial were used to determine reproductive life history traits, and these were augmented with adults from the survival trials. Two newly eclosed males and one virgin female were placed in an aerated 700 ml plastic container (with a 5 cm diameter mesh window at the top) with *C. odorata* stem cuttings plugged into a 5 x 5 x 3 cm moistened OasisTM floral foam block wrapped with aluminium foil. Two 90 mm discs of filter paper (moistened with 0.4 ml of water) were placed inside the containers to maintain RH. Adults were supplied with cotton-wool balls soaked with 50% honey solution for feeding. Fifty-three replicates were used for this study. Males and females were held captive in these containers until the death of both sexes. Females could oviposit on the wall of the containers and/or on

the leaves of the plants. Containers were examined daily, until all adults had died, to record: (i) realised fecundity (i.e. total number of eggs laid), (ii) female mating success (i.e. the number of matings that resulted in fertile eggs) assessed by production of fertile eggs (egg fertility was checked by observing hatching larvae), (iii) number of egg batches, (iv) egg hatchability (number of eggs that hatched), (v) pre-oviposition period and (vi) duration of egg laying.

2.2.5 Statistical analysis

All data sets (development time, pupal mass, growth rate, leaf consumption, longevity) satisfied the assumption of parametric tests (i.e. they were normally distributed - after Shapiro-Wilk's test). Only data from individuals that successfully eclosed were considered when analysing growth and developmental biology, while only data from females that had mated were considered when analysing reproductive parameters. To determine differences in development times and other performance metrics between males and females, the independent sample t-test procedure of Genstat statistical software, version 9.0 (VSN International, Hemel Hempstead, UK) was used. Regression analyses were carried out using Microsoft Excel and Genstat.

2.3 RESULTS

2.3.1 Survival, leaf consumption, growth and development

Although one seventh-instar larva was recorded out of 250 larvae, overall, *P. insulata* developed through five or six larval instars with an average growth rate of 1.48 (Tables 2.1 and 2.2). The survival of each development stage was high, with over 88% overall survival and 97% eclosion success (i.e. pupal survival, 250 eclosed out of 256 pupae), when neonates were monitored until adulthood (Figure 2.1).

Table 2.1 Mean head-capsule width (mm $\pm$ SE) and its increase rate in *P. insulata* reared on *Chromolaena odorata* leaves.

Instars	n	Mean (mm $\pm$ SE)	Growth ratio
I	70	0.38 $\pm$ 0.002	
II	70	0.56 $\pm$ 0.004	1.474
III	70	0.89 $\pm$ 0.005	1.589
IV	70	1.43 $\pm$ 0.009	1.607
V	70	2.05 $\pm$ 0.320	1.433
VI	19	2.48 $\pm$ 0.026	1.317
Mean			1.484

Table 2.2 Development duration (days) (mean $\pm$ SE) of *Pareuchaetes insulata* males and females reared on leaves of *Chromolaena odorata*.

Stage	Gender ^a		Statistics	
	Female	Male	Test	P value
Egg	4.80 $\pm$ 0.05 (4581) ⁿ			
First instar	3.15 $\pm$ 0.03 (136)	3.22 $\pm$ 0.03 (114)	$t = -1.28$	0.200
Second instar	3.10 $\pm$ 0.03 (136)	3.04 $\pm$ 0.03 (114)	$t = 1.19$	0.234
Third instar	3.18 $\pm$ 0.05 (136)	3.18 $\pm$ 0.04 (114)	$t = 0.12$	0.901
Fourth instar	3.75 $\pm$ 0.06 (136)	3.68 $\pm$ 0.06 (114)	$t = 0.68$	0.494
Fifth instar	4.94 $\pm$ 0.10 (136)	4.90 $\pm$ 0.07 (114)	$t = 0.29$	0.773
Sixth instar	5.07 $\pm$ 0.16 (28) [#]	5.18 $\pm$ 0.62 (11) [#]	$t = -0.17$	0.868
Seventh instar	4.00 (1) [#]	5.00 (1) [#]		
Total larval dev. Time	19.20 $\pm$ 0.16 (136)	18.56 $\pm$ 0.19 (114)	$t = 2.51$	0.013
Pre-pupal dev. Time	1.92 $\pm$ 0.02 (136)	1.93 $\pm$ 0.02 (114)	$t = -0.38$	0.706
Pupal dev. Time	9.96 $\pm$ 0.07 (136)	11.27 $\pm$ 0.06 (114)	$t = -13.7$	0.001
Total pupal dev. Time	11.88 $\pm$ 0.07 (136)	13.21 $\pm$ 0.06 (114)	$t = -13.74$	0.001
TDT	31.08 $\pm$ 0.19 (136)	31.77 $\pm$ 0.20 (114)	$t = -2.41$	0.017
Range	(26 – 40)	(28 – 42)		

TDT: Total immature development time from neonate larva to adult eclosion.

^a Numbers within parentheses indicate numbers of immature stages (eggs and larvae) that successfully hatched to first instar larvae (for eggs) and/or were reared to adulthood (for larvae).

ⁿ Eggs (gender indeterminate) obtained from 62 female adults of *P. insulata*.

[#] Note that at sixth instar, sample sizes were low; 28 and 11 for female and male, respectively, because most larvae pupated at 5th instar (same applies to seventh instar).

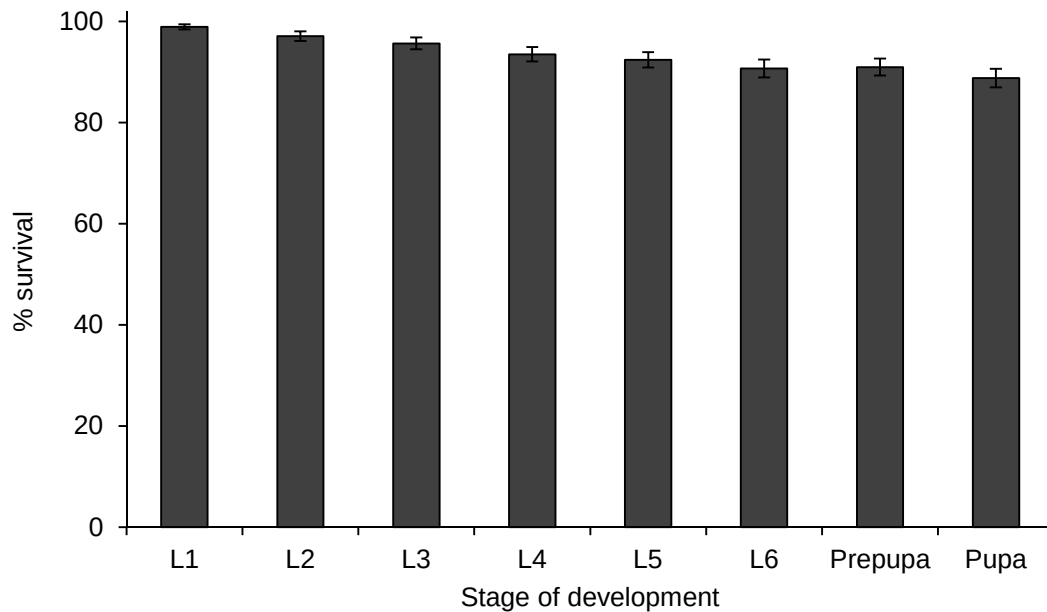


Figure 2.1 Stage-specific percentage survival (mean $\pm$ SE) of *Pareuchaetes insulata* (n = 280) reared on *Chromolaena odorata*. Pupal survival signifies eclosion success.

Apart from total larval development time, pupal development time and total development duration, which were all significantly influenced by gender, other immature development times (eggs, and first instars – sixth instars) were not statistically different between gender (Table 2.2). At the larval stage, females needed more time to develop than males while at the pupal stage, males needed more time to develop before their eventual eclosion. Overall, females emerged earlier than their male counterparts (Figure 2.2, Table 2.2). While over 40% of females emerged within the first five days following the commencement of adult eclosion, only 16% of males emerged within the same time interval. Similarly, over a six-day period, about 73% of females emerged in contrast with the 63% emergence recorded for their male counterparts. Pupal mass and growth rate varied significantly between sex (females had higher growth rates and were always heavier than males) (Table 2.3). Also, females consumed more food than their male counterparts (Table 2.3).

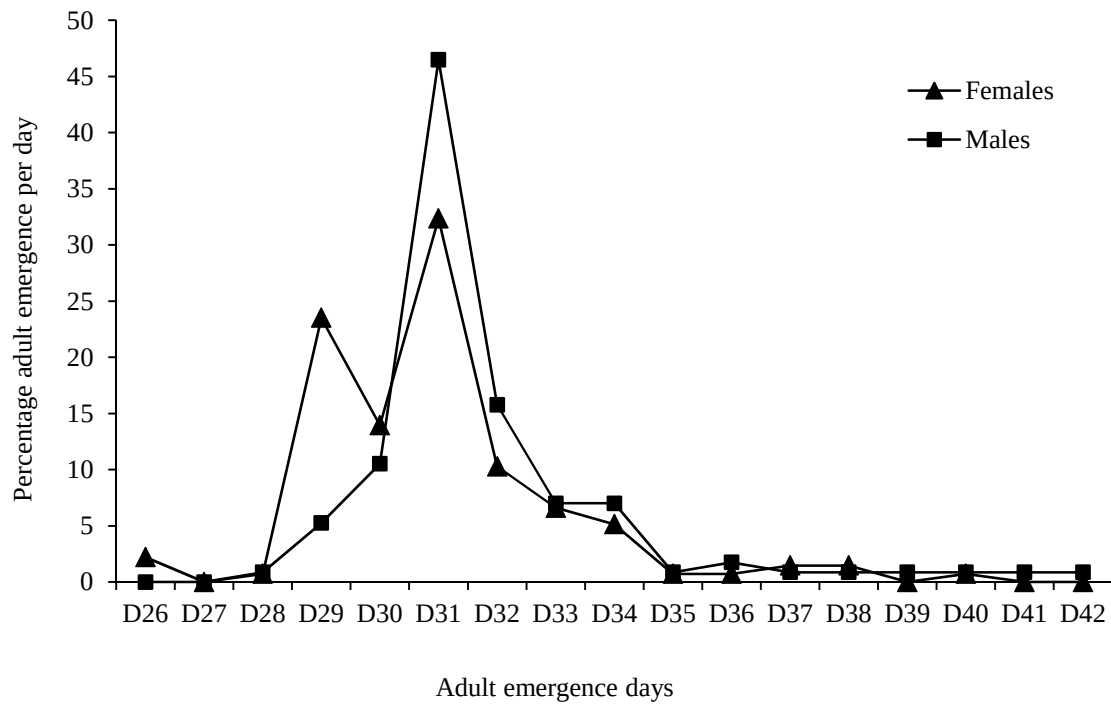


Figure 2.2 Daily mean percentage emergence of *Pareuchaetes insulata* adult (females and males) over a 17-day period. D26 represents the first day of emergence, while D42 represents the last day of emergence.

Table 2.3 Longevity, leaf consumption and growth metrics (mean $\pm$ SE) of *Pareuchaetes insulata* reared on leaves of *Chromolaena odorata*. The numbers in parentheses represent the sample size.

Parameter	Female	Male	Test	P – value
Longevity (days)	5.92 $\pm$ 0.17 (136)	5.50 $\pm$ 0.16 (114)	$t = 1.89$	0.060
Range	3 -11	3 -11		
Pupal mass (mg)	213.10 $\pm$ 5.15 (39)	157.00 $\pm$ 3.31 (33)	$t = 9.16$	<0.001
Range	147.51 - 295.20	126.00 - 198.11		
Growth rate (mg/days)	6.46 $\pm$ 0.14 (38)	4.72 $\pm$ 0.08 (33)	$t = 9.16$	<0.001
Range	4.430 - 8.62	3.90 - 6.19		
Leaf consumption (mm ²)	8566.10 $\pm$ 272.92 (25)	6578.11 $\pm$ 256.40 (28)	$t = 5.31$	<0.001
Range	5505.31 - 11058.43	3255.42 – 9527.22		

2.3.2 Reproductive life history traits

Female mating success was 94.3% (Table 2.4). Egg hatchability ranged from 82.7 to 100.0% while sex ratio was fairly balanced with females having the larger share; out of 250 individuals that were successfully reared to adulthood, 54.4% were females (Table 2.4). Oviposition usually started on the second night after female emergence. Eggs (45 – 661 per female) were laid in batches (1-16 batches per female) over a duration of between 2 and 8 days (Table 2.4). Although females appeared to live longer than males, the difference was not significant (Table 2.3).

Table 2.4 Reproductive performance (mean $\pm$ SE) of *Pareuchaetes insulata* reared on leaves of *Chromolaena odorata*. The numbers in parentheses represent the sample size.

Parameter	Range	Mean
Mating success (%)	-	94.33 (53)
Egg hatchability (%)	87.7-100	97.56 $\pm$ 0.49 (4775) ⁿ
Sex ratio (% of females)	-	54.41 (250)
Duration of egg laying (days)	2 - 8	4.72 $\pm$ 0.25 (50)
Pre-oviposition period (days)	1 - 4	1.70 $\pm$ 0.1 (50)
No. of egg batches	1 - 16	8.96 $\pm$ 0.50 (50)
^a Fecundity	45 - 661	387.62 $\pm$ 19.50 (50)

ⁿ Eggs were from 62 mated adult females of *P. insulata*.

^a Fecundity indicates total number of eggs per female.

2.3.3 Correlations of life history traits

Linear regression analysis showed a significant positive relationship between head-capsule width and larval instars (Figure 2.3). There was a significant positive correlation between the amount of leaf tissue consumed and the resulting female pupal mass (Figure 2.4a), between amount of leaf tissue consumed and female growth rate (Figure 2.4b) and between amount of leaf tissue consumed and fecundity (Figure 2.4c).

The significant positive relationship between pupal mass and the fecundity of the resultant female indicates that females produced 5.25 eggs per mg, with the individuals of the lowest mass in the analysis being just 145.8 mg and carrying 45 eggs, while the heaviest was a 230.9 mg individual that carried 661 eggs (Figure 2.5). The percentage of eggs laid per night decreased with female age after a peak on the second night (Figure 2.6). Over 61% of the total eggs were laid within the first three nights of eclosion, while over 74% of the eggs were laid over a four-night period.

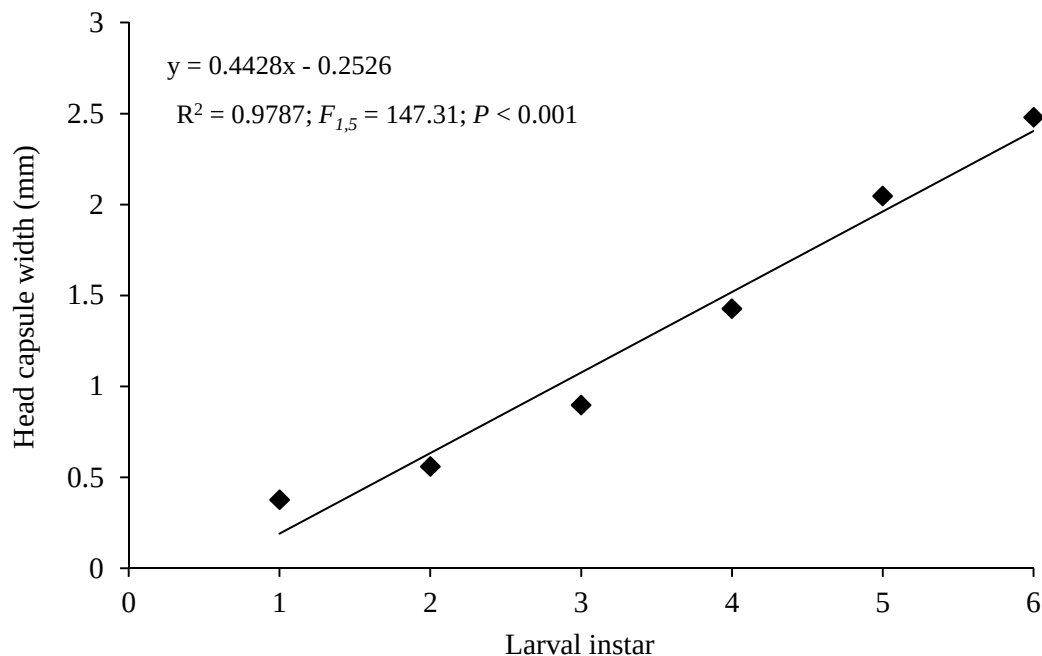


Figure 2.3 Relationship between mean head-capsule width and larval instars of *Pareuchaetes insulata*.

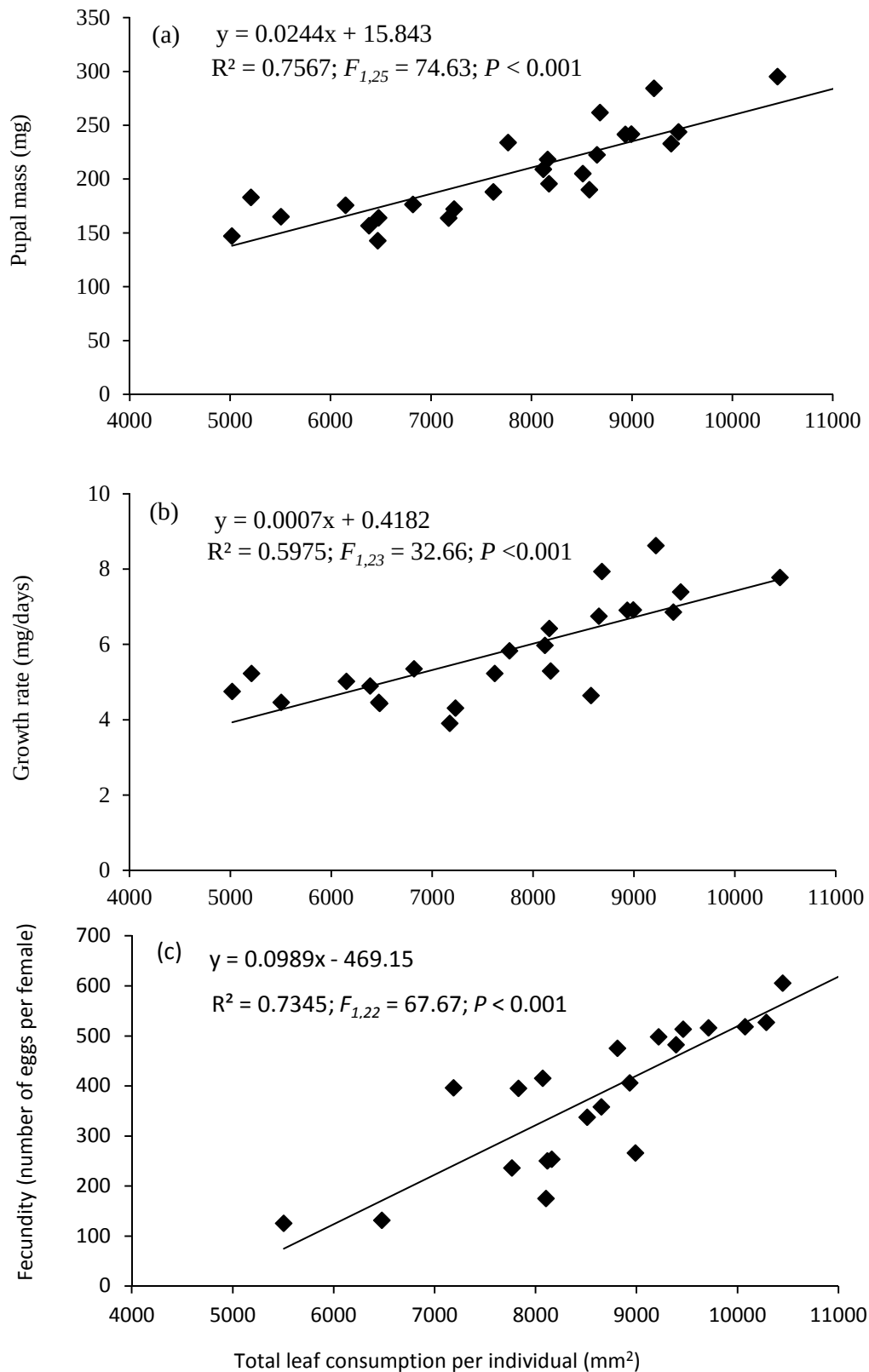


Figure 2.4 Relationship between female pupal mass (a), growth rate (b), fecundity (c) and leaf consumption by the larvae of *Pareuchaetes insulata* (feeding was from first instar until pupation).

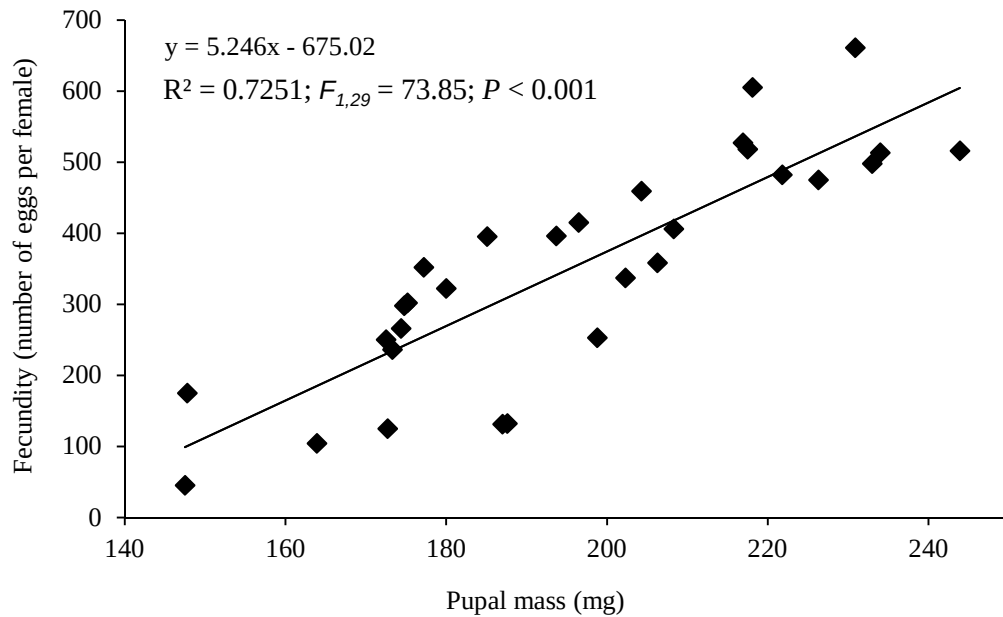


Figure 2.5 Relationship between adult fecundity and pupal mass of female *Pareuchaetes insulata* reared on *Chromolaena odorata*.

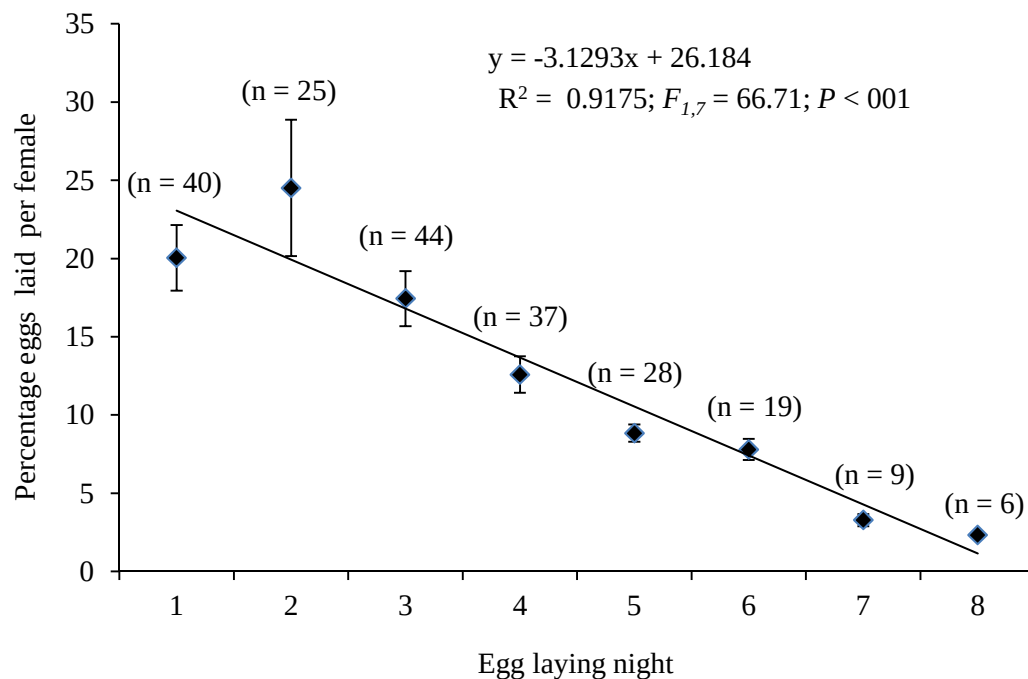


Figure 2.6 The relationship between percentages of eggs laid (per day by females) and egg-laying duration. The number of females that laid on a particular day is given in parentheses. All females except one survived before day 8. The model only considered females that laid eggs on a particular day.

2.4 DISCUSSION

Using a culture of *P. insulata* reared on excised leaves of *C. odorata* plants at constant temperature, the life history traits of the moth and differences in performance between males and females were measured. Mortality was low and females performed better than males in terms of development time and other performance metrics such as leaf consumption, pupal mass and growth rate. Significant positive correlations of life history traits in *P. insulata* were detected between insect performance and leaf consumption, between head-capsule width and larval instars and between pupal mass and fecundity.

The head-capsule width shows a geometric progression pattern of growth consistent with Dyars' rule (Dyar, 1890). Dyar (1890) measured the head-capsule width of 26 species of Lepidoptera and showed that the width of the head capsule increased geometrically and by a relatively constant ratio ranging from 1.3 to 1.7. The over 97% eclosion success recorded in this study is similar to the 98.6% recently found by Uyi *et al.* (2014c, Chapter 3) who investigated the effect of *C. odorata* genotype on the performance of *P. insulata*, but contrasts the findings of Dube *et al.* (2014) who reported 61% eclosion success. The reasons for such discrepancies are unclear, but are likely caused by such factors as experimental conditions, foliage quality and/or smaller sample size used by the latter authors.

No previous study has compared the immature development duration between males and females in *P. insulata*, *P. aurata aurata* or *P. pseudoinsulata*; hence the results of this study cannot be directly compared with the reports of other workers. Nevertheless, the total development time (neonate - adult eclosion of male and female combined) of 31 days in this study is comparable to the findings of Dube *et al.* (2014) who recorded a mean of 35 days from oviposition to adult eclosion in *P. insulata*. The range of 26 to 42 days that we recorded

for development from neonate to adult eclosion is similar to the 32 to 47 days reported by Syed (1979), but shorter than the range (39 to 54 days) reported by Muniappan *et al.* (1989) for *P. pseudoinsulata*. Although the reason why males spent a longer time in the pupal stage in this study is unclear, it is not uncommon in erebid moths (Betzholtz, 2003; Rodríguez-Loeches and Barro, 2008). However, in many insects including erebid moths, females prolong their growth (larval) period in order to have heavier pupae (Betzholtz, 2003; Esperk *et al.*, 2007; Stillwell and Davidowitz, 2010) and the same might also be true for *P. insulata*. Although the overall difference in development times between males and females might seem short (16.6 hours), similar differences have been documented in other erebid moths. For example, the development time in females of *Parasemia plantaginis* (L.) (Lepidoptera: Erebidae: Arctiinae) reared on *Taraxacum* sp. (Cichoriaceae) was on average 0.71 days (17.0 hours) shorter than that of the males (Ojala *et al.*, 2005). The small statistical difference in development time between males and females of *P. insulata* is probably without any ecological significance, but this needs to be explored further to either validate or invalidate this finding.

The emergence of adult females before males suggests the absence of protandry, in which males are reproductively active before females, and the presence of protogyny, in which females emerge and are reproductively active first. Protandry appears common in many insects including Lepidoptera (Honěk, 1997; Morbey and Ydenberg, 2001), but examples of protogyny are also known (Honěk, 1997; Buck 2001). Protandry presents two advantages in that it: (i) maximises copulation opportunities for males (Wiklund and Fagerström, 1977; Bulmer, 1983) and; (ii) minimises the pre-reproductive period of females because they emerge when most males are available for mating (Fagerstrom and Wiklund, 1982; Carvalho *et al.*, 1998; Larsen *et al.*, 2013). In small populations (environments where mate location is

difficult) and in short-lived insects such as *P. insulata*, reproductive asynchrony, expressed as excess protandry, its absence, or its reverse (protogyny), can lead to ‘matelessness’ and can be a mechanism for Allée effects which can negatively affect population growth or result in the extinction of species (Calabrese and Fegan, 2004; Calabrese *et al.*, 2008; Larsen *et al.*, 2013; Fauvergue, 2013). In this context, the absence of protandry or presence of protogyny in *P. insulata* could be a clear disadvantage for adults and could impair their mating success to the extent that their long-term persistence may be jeopardised, as females may have dispersed before the emergence of males. In *P. pseudoinsulata*, gender imbalance has been implicated with infertility of eggs due to matelessness (Torres *et al.*, 1991). It is possible that, with constant and asynchronous eclosion of *P. insulata* in the field, protogyny might not be problematic.

Although up to seven instars were recorded, most larvae pupated at the fifth instar. Kluge and Caldwell (1993a) did not mention variations in the number of larval instars in *P. insulata*, but other authors (Cruttwell, 1972; Dube, 2008) documented between five and six instars for *P. pseudoinsulata* and *P. insulata*, respectively. The number of larval instars for erebid moths has been recorded to be as low as five and as high as seven for different species (Betzholtz, 2003; Rodríguez-Loeches and Barro, 2008). Variations in the number of larval instars is common in Lepidoptera but is not considered as an important life-history trait (Pöykkö, 2005). As for other Lepidoptera, the variation in the number of *P. insulata* larval instars could be influenced by biotic and abiotic factors such as temperature, day length and foliage quality (Calvo and Molina, 2005).

The greater female pupal mass compared to males recorded in this study is not uncommon in Lepidoptera and in other insects (Singer *et al.*, 2004; Thiéry and Moreau, 2005; Stillwell *et*

al., 2010; for an exception see LaMunyon and Eisner, 1993) and the reasons or the underlying mechanisms involved have been disentangled (see Awmack and Leather, 2002; Davidowitz and Nijhout, 2004; Stillwell and Davidowitz, 2010). Stillwell and Davidowitz (2010) studied sexual size dimorphism in *Manduca sexta* L. (Lepidoptera: Sphingidae) and reported that the timing of hormonal events that determines when growth ends and when to initiate pupation, occurs slightly later in female larvae, thus explaining the higher female pupal mass. As is evident from this study, pupal mass provides a good estimate of individual fecundity in many insects including Lepidoptera (e.g. Doak, 2000; Zhang *et al.*, 2012). Czypionka and Hill (2007) suggested that this relationship could be used as an index to judge the suitability of an insect (in terms of its reproductive potential) for the biological control of an invasive alien weed. The fact that *P. insulata* female growth rate is higher than that of males is typical of capital breeders (i.e. species with adults that do not feed or ingest little food) since male body weight is not directly coupled with fitness as it is for females (Haukioja and Neuvonen, 1985). Food quality/quantity is considered a good determinant of fecundity in herbivorous insects. As observed for *P. insulata*, the quantity and/or quality of resources consumed by herbivores during ontogenesis are directly related to their final body weight and other life history traits (Stillwell *et al.*, 2007; 2010). Those insects that do not acquire proteins as adults must obtain all these reserves during their larval stage. This may explain the significantly longer larval development period of females and larger female pupal mass in this study, because females needed more time to eat and/or acquire sufficient food (either in terms of quality and/or quantity) in order to cope with reproductive responsibilities such as egg production.

Mean lifetime fecundity (387 ± 19.62 eggs) per female was 23% higher than that recorded for a closely related species, *P. aurata aurata*, where lifetime fecundity averaged 241.9 eggs per

female (Kluge and Caldwell, 1993b). The findings of this study also indicate that over 74% of the eggs were laid during the first four nights (after mating) with peak egg laying on the 2nd night. This concurs with the findings of Kluge and Caldwell (1993b) who reported that 70% of oviposition occurred within the first four nights for *P. aurata aurata*. This relatively short window exemplifies why protogyny could lead to matelessness. The ranges of adult longevity, duration of egg laying and pre-oviposition periods are comparable to that reported for *P. aurata aurata* (Kluge and Caldwell, 1993b). The fact that longevity was similar for both sexes corroborates the findings of Jiménez-Pérez and Villa-Ayala (2009), who reported that being larger does not necessarily confer greater longevity in insects. Female longevity is known to influence fecundity in long-lived insects or in income breeders (i.e. species using both current nutritional inputs as adults and resources obtained during earlier life stages for reproduction), where long-lived individuals lay more eggs (e.g. Herrera *et al.* 2011). The negative relationship between the percentage of eggs laid on a night in relation to the time since eclosion probably occurs because *P. insulata* is a capital breeder.

The high egg hatchability (97%) reported in this study is similar to the mean of 95% recorded by Visalakshy (1998), but higher than the 79% recorded by Dube *et al.* (2014) and the 74% by Kluge and Caldwell (1993b) for *P. pseudoinsulata*, *P. insulata* and *P. aurata aurata*, respectively. The high mating success recorded in this study could be attributed to the fact that two virgin males and one female were always paired in one container. The finding of this study likely reflects the field situation where females might have a choice of a number of males. In *Pareuchaetes* species, the females call (using tubular pheromone glands) and males respond by locating them (Schneider *et al.*, 1992). In *Utetheisa ornatatrix* (L.) (Lepidoptera: Erebiidae: Arctiinae) and in other arctiines, females choose males based on a courtship pheromone, hydroxydanaidal (HD), derived from defensive pyrrolizidine alkaloids (PAs).

Females make choices based on the ability of virgin males to transfer spermatophores whose contents are proportional to their HD titre and body size; as a result, selective females receive both phenotypic benefits (more nutrients and PAs) and genotypic benefits (genes for larger body size inherited by the offspring) (Conner *et al.*, 1990; LaMunyon and Eisner, 1994; Conner, 2009; Kelly *et al.*, 2012). Similar to the findings of the above reports, it is possible that females may have chosen or mated with large males in this experiment and this might be a true reflection of the actual field situation.

This study is the first report of the growth and reproductive performance of, and life history correlations in, *P. insulata*, thus contributing to our understanding of the life-history traits of erebid moths in the subfamily Arctiinae.

CHAPTER 3

The effect of variation in host plant on the performance and fitness-related traits of the specialist herbivore, *Pareuchaetes insulata*

3.1 INTRODUCTION

Differential growth, fecundity, feeding, and oviposition preference of insect populations on host plant varieties, genotypes, or chemotypes have been suggested to have important implications for biological control of weed species (Dray *et al.*, 2004; Goolsby *et al.*, 2006; Simelane, 2006; Wheeler, 2006; Baars *et al.*, 2007). Therefore many recent biological control programmes have been careful to locate the exact origin, within its broader range, of the target weed in order to collect biological control agents that are optimally compatible with the weed (e.g., Pemberton, 1998; Goolsby *et al.*, 2003; Ramadan *et al.*, 2011; Center *et al.*, 2012).

However, for some biocontrol programmes, agents have been collected from an area of the native range other than that from which the invasive population originated, from plants which were morphologically distinct from those in the invasive range [e.g., *Chromolaena odorata* in South Africa]; often this was because the exact origin of the invasive population of the target weed could not be found. These agents may have established, but appear to perform poorly in the field. In such cases, partial incompatibility between the agent and its host plant may provide at least some explanation for this poor performance. In other cases, such as *Lantana camara* L. (Verbenaceae) (Day and McAndrew, 2003) and *Prosopis* spp. (Fabaceae) (Zachariades *et al.*, 2011b), the invasive plants are hybrids of several native populations or species, and thus agents collected from one of these native populations or species may not perform well on the invasive population. It has thus been considered worthwhile to conduct

pre- [e.g., Simelane (2006) on *L. camara*] or post-release evaluation studies [e.g., Paterson *et al.*, 2012, on *Pereskia aculeata* Mill. (Cactaceae)] to determine whether the performance of biocontrol agents differs as a function of the origin or form of host-plants on which they are fed. Such studies, when conducted prior to the release of an agent, may be useful in predicting on which varieties or genotypes of the host plant, if any, the agent is likely to perform well. Conducted after the agent has been released, they can explain performance and prevent the release of further, perhaps sub-optimal agents.

Here, several insect performance metrics of *P. insulata* on two morphological forms of *C. odorata*, which are probably also genetically distinct (Paterson and Zachariades, 2013) were compared. One form came from within the native range (southern Florida, USA) where *P. insulata* was collected, and the other was the invasive form (from South Africa), onto which it was released as a biological control agent.

In order to test if a degree of agent-host plant incompatibility may be culpable for the poor performance of *P. insulata*, it was hypothesized that *P. insulata* larvae are more attracted to Floridian *C. odorata* (which is morphologically similar to AWAB *C. odorata*) than to the SAB and that larvae reared on Floridian *C. odorata* have higher fitness and performance than those reared on SAB. This study specifically compared insect preference and metrics of herbivore performance such as leaf consumption, feeding index, survival, development duration, pupal mass, growth index, host suitability index, realised fecundity (total number of eggs laid), sex ratio, egg hatchability, mating success, duration of egg laying, pre-oviposition period, and female longevity between the Floridian and SAB *C. odorata* in this plant - herbivore system.

3.2 MATERIALS AND METHODS

3.2.1 Study system: origin and maintenance of plants and moths

For this study, *P. insulata* individuals were collected in the Sappi Cannonbrae plantation, Umkomaas (south coast of KZN), South Africa (30°13'S, 30°46'E), where the insect established following large releases made between 2001 and 2003 after it was imported from Florida (Zachariades *et al.*, 2011a). Moth individuals were maintained for about five generations on *C. odorata* bouquets from the field at 25 ± 2 °C, $60 \pm 10\%$ relative humidity (RH), with a photoperiod of L12:D12. All tests were performed in a growth chamber (Labcon, South Africa). Hygrochron iButtons (model DS 1923; Maxim Integrated Products, San José, CA, USA, 0.5 °C accuracy) were used to measure temperature (hourly readings: range = 23.12–25.62 °C; mean $\pm$ SE; 24.62 ± 0.01 °C) and RH (range = 65.3–78.1%; mean $\pm$ SE, $70.51 \pm 2.13\%$) inside the chamber.

The southern African *C. odorata* plants were grown from stem-tip cuttings collected from several plants growing along a 50-m stretch of road near Durban, South Africa, whereas the Floridian *C. odorata* plants were grown from stem-tip cuttings taken from four clones of a single plant in the 'international' *C. odorata* collection of the ARC-Plant Protection Research Institute, Cedara, South Africa. The parent (AcCe 108) of the Floridian plants (which is morphologically similar, but not identical, to the AWAB plants as described in Chapter 1) was originally collected from Fort Lauderdale, Florida (26°05'42"N, 80°18'45"W) on 28 October 2002; from the same vicinity as the *P. insulata* culture was collected. All cuttings were initially planted in the mist bed in vermiculite with rooting hormone (Seradix™ No. 1) before they were later planted in nursery pots (25 cm diameter) for establishment. All treatment plants benefited from the same potting medium (Umgeni coarse sand: Gromor Potting Medium 1:1, Gromor, Cato Ridge, South Africa), fertilizer (9g of Plantacote,

AGLUKON Spezialduenger GmbH & Co. KG, Germany: 14% nitrogen, 8% phosphorus pentaoxide, and 15% potassium oxide – all soluble in water) and watering regimes. Plants were grown outside between November 2011 and April 2012 and hand-watered with a hose. Fifty SAB and 49 Floridian *C. odorata* plants were grown and used for this study.

A single plant from Florida is not necessarily representative of that population, and even the SAB plants used were not selected to be representative of the invasive population as a whole. However, I believe that comparisons using this small selection of plant material still has validity for several reasons, even though it limits the strength of the conclusions that can be drawn: (i) Although sample sizes were small, it appears that *C. odorata* growing in Florida is genetically distinct from that invading South Africa, which is strongly related with plants from Jamaica and Cuba (Paterson and Zachariades, 2013). However, because it cannot be proved that the plant from Florida that was used differs genetically from the SAB plants I used, I refer to them as different plant forms rather than different genotypes; (ii) Plants from Florida (including the parent plant used here) can be clearly distinguished from those from South Africa based on their morphological characteristics (e.g., leaf pubescence, odour, flower colour, and growth form); (iii) Both SAB and Floridian *C. odorata* display a high degree of within-population morphological homogeneity in their respective ranges in South Africa and Florida (C. Zachariades, unpubl.). The genetic and morphological similarities among SAB suggest that they may have come from a single plant introduced into Durban. In addition, SAB *C. odorata* is at least facultatively apomictic (Rambuda and Johnson, 2004), and could thus be largely clonal. Paterson *et al.* (2012) followed a similar procedure, using cuttings from single *P. aculeata* plants from several parts of the native range of the species.

3.2.2 Larval preference

To test the attractiveness of SAB and Floridian *C. odorata* to *P. insulata*, freshly collected young, fully expanded leaves of each of these two forms were used. Rectangular pieces of leaf tissue (20 × 40 mm) were placed in 140-mm diameter Petri dishes containing a 90-mm-diameter disc of filter paper moistened with 0.4 ml of water. Two rectangles of leaf tissue from the Floridian *C. odorata* were placed alternately with those of SAB (for rationale, see Prasifka *et al.*, 2009) and 25 unfed neonate *P. insulata* were placed haphazardly (but not on the leaves) in each Petri dish. Forty replicates and 1 000 neonates were used and the leaf tissues used were taken from 80 different plants (40 per plant form). The Petri dishes were placed in a tray wrapped with a ZiplocTM bag (45 × 65 cm) to minimize desiccation. After 24 hours in the growth chamber, each dish was opened and the number of larvae on each leaf piece was counted. The presence of larvae on a piece of leaf was used to determine preference or attractiveness. A similar experiment was conducted (with the same number of larvae and replicates) concurrently with the above, but Petri dishes were only opened for larval counts after 48 hours. There were no mortalities in all cases.

3.2.3 Leaf area consumption

The effect of Floridian and SAB *C. odorata* on total (i.e. lifetime) leaf consumption by *P. insulata* was tested on individual larvae with equivalent amounts of food. Larvae were raised individually from day of hatching to pupation in 100 ml aerated plastic containers with a circular net screen window on top for ventilation (2.5 cm diameter), lined at the bottom with moistened filter paper to maintain RH. The larvae were fed on one of the two *C. odorata* forms for the duration of their development. This protocol presented at least three main advantages; (a) feeding larvae in isolation prevented biases due to competition and consequent food deprivation, (b) accurate quantification of the leaf area consumed by a single

individual from first instar to pupation could be made and (c) variations due to microhabitat effects could be prevented. Larvae ($n = 30$ per diet) were fed with fresh, fully expanded leaf tissues (taken from the upper half of the plants) every 24 hours and their frass was removed at the same time for hygienic reasons (Uyi *et al.*, 2011). The daily use of new leaf tissues probably increases survival, and is also consistent with field observations of *Pareuchaetes* species preferably feeding on undamaged leaves in the presence of an abundant food supply. The purpose of using excised leaves was to limit uncontrolled variations among whole plants within the two plant forms so that the insect parameters of interest, in this case, leaf consumption, attractability, development rates, and other fitness-related traits could be stringently assessed. A second reason was to control environmental conditions such as temperature. The use of excised leaves is a standard method for providing uniform materials in the laboratory feeding studies of this kind (see Blossey and Nötzold, 1995; Greenberg *et al.*, 2001; Hull-Sanders *et al.*, 2007; Beaton *et al.*, 2011). All containers were placed inside a ZiplocTM bag (600×450 mm to prevent desiccation). The area of leaf tissue consumed per individual larva per day was assessed by scanning images of the leaf tissues before and after feeding, with a digital scanner (Konica Minolta C360 Series PCL, Langenhagen, Germany) and were thereafter measured using Compu Eye Leaf and Symptom Area program developed by Bakr (2005) (available at <http://www.ehabsoft.com/CompuEye/LeafSArea/>). The above procedure was continued each day for each larva until feeding ceased in the prepupal stage. Pupae were removed from the food source and weighed as an index of the adult body size, and their sex was determined. Greenberg's feeding index (FI) was calculated by dividing pupal mass (fresh biomass) by mean leaf area consumed for each host plant (modified after Greenberg *et al.*, 2001). Greenberg and colleagues used this index to determine the convertibility of plant biomass into insect body weight. The assumption of this index is that insects feeding on a more suitable host plant are likely to have greater mass (as pupae or

adults) and a higher feeding index than if the plant is less suitable. Although Greenberg *et al.* (2001) used mean weight of leaf tissue consumed to calculate the feeding index, mean leaf area consumed was used here because that is what was measured.

3.2.4 Survival and development

To assess survival, growth, and duration of development of *P. insulata*, 80 newly hatched larvae per plant form were used following the methods described in leaf area consumption section, and observations on mortality were made daily until adult eclosion. The stage-specific and overall survival of *P. insulata* on the two different plant forms were calculated as numbers of larvae (or pre-pupae or pupae) that developed to the next stage divided by the initial number (of that particular stage). The larvae were monitored daily until pupation and/or adult eclosion in order to follow the duration of each instar and other immature stages. The loss of the cephalic capsule was the criterion used to determine the moult. Pupae were extracted from the food source and weighed ($n = 73$ for those reared on Floridian *C. odorata*, $n = 72$ for SAB). Sex ratio and performance indexes were calculated and recorded based on the numbers of surviving pupae ($n = 70$ for those reared on Floridian *C. odorata*, $n = 71$ for SAB). Additionally, the following variables were measured: (i) duration of each immature stage (first instar to adult eclosion) and (ii) growth rate (pupal mass in mg/development time). Sétamou's growth index (GI) was calculated by dividing the percentage survival of immatures by development time (Sétamou *et al.*, 1999). This index emphasizes the importance of both survival and development time in measuring food quality. We also calculated Maw's host suitability index (HSI) (for rationale, see Maw, 1976) using the equation: $HSI = (\text{Female pupal mass}) (\% \text{ pupation}) / \text{Immature development time}$.

Maw (1976) used the HSI to determine which plant species were superior hosts for *Cassida hemisphaerica* Hbst (Chrysomelidae). The assumptions of the index are that insects feeding on more suitable host plants are likely to have greater mass, higher survival rates and shorter developmental times than those feeding on inferior host plants.

3.2.5 Reproductive life history traits

Two newly eclosed males and one virgin female were placed in aerated 700 ml plastic containers (with 5-cm-diameter mesh window at the top) with Floridian *C. odorata* stem cuttings plugged in 5 × 5 × 3 cm moistened OasisTM floral foam block wrapped with aluminium foil. Two 90 mm discs of filter paper (moistened with 0.4 ml of water) were placed inside the containers to maintain RH. Adults were supplied with cotton-wool balls soaked with 50% (wt/vol) honey solution for feeding. The same procedure was repeated using the SAB form. Males and females were held captive in these containers until they all died. Females could oviposit on the wall of the containers and/or on the leaves of the plants.

Containers were examined daily to record (i) adult mortality, (ii) realised fecundity (total number of eggs laid), (iii) female mating success assessed by production of fertile eggs (egg fertility was checked by observing hatching larvae), (iv) egg hatchability (number of eggs that hatched), (v) duration of egg laying and (vi) pre-oviposition period.

3.2.6 Statistical analysis

Normality of frequency distribution in all data sets was tested using Shapiro-Wilk's test and Levene's test for homoscedasticity of variance. Data that violated the assumption of parametric tests were analysed using appropriate statistical methods. Due to the generally normal form of frequency distribution, the percentage preference for host plant by neonate

larvae was evaluated using Generalized Linear Model (GLM) assuming a normal distribution with an identity link function. The effect of plant form and sex on total leaf consumption, pupal mass, growth rate, and longevity were evaluated using univariate General Linear Model analysis of variance (GLM ANOVA). When the overall results were significant in a two-way analysis, the differences among the treatments were compared using the Sequential Bonferroni test. This was done in order to (i) reduce the chance of making a Type I error, (ii) increase statistical power and (iii) to ensure a Type II error no greater than α (see Rice, 1989). To compare the effect of diet on stage-specific survival, sex ratio, and on mating success, Pearson's χ^2 test was employed. Because the assumptions of normality of data and homoscedasticity of variance were often violated, a non-parametric test (Mann-Whitney U-test) was used to evaluate the effect of plant form on development durations of each immature stage and on total developmental time (larval to adult). Greenberg's feeding index (FI) and Maw's host suitability index (HSI) were analysed using Mann-Whitney U-test and Student's t-test, respectively. However, it was impossible to perform a statistical analysis on Sétamou's growth index because only one value was obtained for each host plant. Duration of egg laying, realised fecundity, and pre-oviposition period were analysed using a t-test, and egg hatchability was evaluated using Mann-Whitney U-test. With the exception of the GLM and GLM ANOVA that were performed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA), all other analysis were performed using Genstat 9.0 (VSN International, Hemel Hempstead, UK).

3.3 RESULTS

3.3.1 Larval preference

Neonate larvae (newly emerged first instars) of *P. insulata* preferred to feed on Floridian *C. odorata* leaves (GLM Wald $\chi^2_{1,159} = 16.322$; $P < 0.001$) following 24 and 48 hours' exposure

to leaves from both plant forms (Figure 3.1). Exposure time did not significantly influence neonate larval preference (GLM Wald $\chi^2_{1,159} = 0.01$; $P = 0.980$).

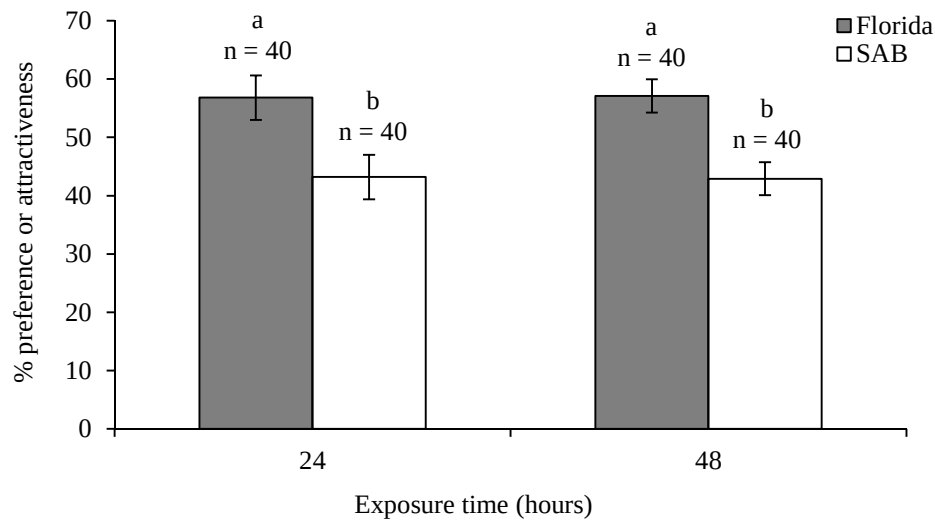


Figure 3.1 Percentage (mean $\pm$ SE) larval preference or attractiveness of neonate larvae of *Pareuchaetes insulata* exposed simultaneously to two distinct *Chromolaena odorata* morphological forms [Floridian vs. southern African (SAB) *C. odorata*] for 24 and 48 hours. Means (following GLM) with different letters are significantly different (Sequential Bonferroni test: $P < 0.05$). Figures above each bar represent sample sizes.

3.3.2 Effect of host plant form on leaf area consumption, survival and development

Total leaf area consumed by individuals of *P. insulata* did not differ between SAB and Floridian *C. odorata* (GLM ANOVA: $F_{1,52} = 0.444$, $P = 0.508$) (Figure 3.2). However, females fed more than their male counterparts (GLM ANOVA: $F_{1,52} = 24.79$, $P < 0.001$) and there was no interaction between plant form and sex on the amount of leaf tissue consumed. The feeding index was not statistically significant between diets ($U = 342.0$, $P = 0.881$) (Table 3.1).

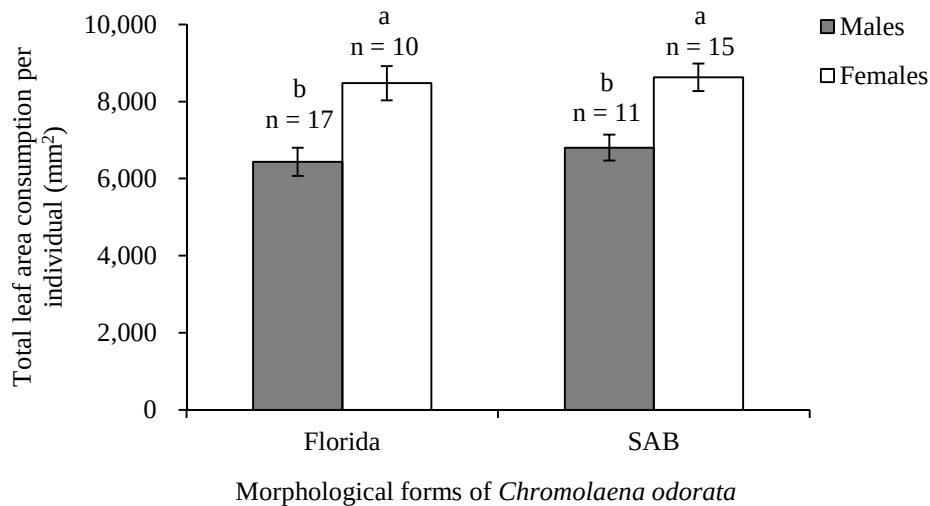


Figure 3.2 Leaf consumption per larva (mean $\pm$ SE) from first instar stage to pupation in individuals of *Pareuchaetes insulata* reared on two distinct *Chromolaena odorata* morphological forms. Means (following GLM ANOVA) with the same letters, both within and among morphological forms, are not significantly different (Sequential Bonferroni test: $P > 0.05$). Figures above each bar represent sample sizes.

Table 3.1 Total leaf area consumption and performance indexes $\pm$ SE (sample sizes are in parentheses) of *Pareuchaetes insulata* on two distinct *Chromolaena odorata* morphological forms (Floridian and southern African *Chromolaena odorata*).

Index	Florida	SAB	Test	P
Total leaf consumption	7190.3 $\pm$ 339 (27)	7854.5 $\pm$ 304 (26)	$F_{1,52} = 0.444$	0.508
Feeding index (FI)	5.82 $\pm$ 0.02 (27)	5.61 $\pm$ 0.01 (26)	$U = 342.0$	0.881
Sétamou's growth index	2.650 (70)	2.678 (71)	1	1
Host suitability index (HSI)	5.38 $\pm$ 0.16 (31)	5.78 $\pm$ 0.14 (40)	$t_{1,69} = -1.87$	0.066

¹Unable to perform a statistical analysis because only one value was obtained for each morphological form.

The survival rates for each development stage and total immature survival (from first instar to adult eclosion) were not significantly affected by the *C. odorata* form on which they fed (Table 3.2). Mortality rates were less than 5% at each stage with 87.5 and 88.8% overall survival for larvae fed on Floridian and SAB *C. odorata*, respectively.

Table 3.2 Stage-specific and overall survival (mean % $\pm$ SE) of *Pareuchaetes insulata* reared on two distinct *Chromolaena odorata* morphological forms (Floridian and southern African).

Morphological form	Larval instar ^{1,2}						Pre-pupa ^{1,2}	Pupa ^{1,2,3}	Total immature (larva-adult) ^{1,2}
	First	Second	Third	Fourth	Fifth	Sixth			
Florida	100.0 $\pm$ 0.0 (80)	98.8 $\pm$ 1.25 (80)	97.5 $\pm$ 1.78 (79)	98.7 $\pm$ 1.30 (77)	98.7 $\pm$ 1.32 (76)	96.8 $\pm$ 3.23 (31)	98.7 $\pm$ 1.35 (74)	95.9 $\pm$ 2.30 (73)	87.5 $\pm$ 3.71 (80)
SAB	98.8 $\pm$ 1.25 (80) $\chi^2_1 = 1.74$	96.2 $\pm$ 2.2 (79) $\chi^2_1 = 1.38$	100.0 $\pm$ 0.0 (76) $\chi^2_1 = 0.003$	98.7 $\pm$ 1.32 (76) $\chi^2_1 = 0.005$	100.0 $\pm$ 0.0 (75) $\chi^2_1 = 1.81$	100.0 $\pm$ 0.0 (20) $\chi^2_1 = 3.20$	96.0 $\pm$ 2.3 (75) $\chi^2_1 = 1.35$	98.6 $\pm$ 1.39 (72) $\chi^2_1 = 1.36$	88.8 $\pm$ 3.63 (80) $\chi^2_1 = 0.826$

χ^2 = Pearson Chi-Square.

¹Numbers within parentheses indicate numbers of larvae/pupae reared in each stage.

²Survival rates were not significantly affected by plant morphological form (χ^2 : P>0.05).

³Pupal survival signifies eclosion success.

Table 3.3 Development duration (days) (mean $\pm$ SE) of *Pareuchaetes insulata* on two *Chromolaena odorata* morphological forms.

Stage	Morphological form ¹		Statistics	
	Florida	SAB	Test	P
Egg	4.75 $\pm$ 0.07 (3635) ²	4.90 $\pm$ 0.04 (3841) ²	t _{1,38} = -1.64	0.106
First instar	3.20 $\pm$ 0.06 (70)	3.46 $\pm$ 0.06 (71)	U = 1876.0	0.003
Second instar	3.21 $\pm$ 0.05 (70)	3.27 $\pm$ 0.06 (71)	U = 2400.0	0.661
Third instar	3.41 $\pm$ 0.07 (70)	3.34 $\pm$ 0.09 (71)	U = 2280.0	0.404
Fourth instar	3.75 $\pm$ 0.07 (70)	3.70 $\pm$ 0.10 (71)	U = 2207.5	0.196
Fifth instar	4.64 $\pm$ 0.18 (70)	5.01 $\pm$ 0.13 (71)	U = 1855.0	0.007
Sixth instar	5.12 $\pm$ 0.28 (28) ³	5.11 $\pm$ 0.27 (19) ³	U = 262.0	0.935
Seventh instar	5.00 (1) ³	4.50 (2) ³		
Total larval dev. time	20.34 $\pm$ 0.34 (70)	20.24 $\pm$ 0.28 (71)	U = 2420.5	0.787
Pre-pupal dev. time	1.84 $\pm$ 0.04 (70)	1.94 $\pm$ 0.02 (71)	U = 2234.5	0.053
Pupal dev. time	10.83 $\pm$ 0.12 (70)	10.94 $\pm$ 0.11 (71)	U = 2341.5	0.535
Total pupal dev. time	12.67 $\pm$ 0.12 (70)	12.88 $\pm$ 0.11 (71)	U = 2206.0	0.228
TDT	33.01 $\pm$ 0.30 (70)	33.13 $\pm$ 0.30 (71)	U = 0.06	0.831

TDT: Total immature development time from neonate larva to adult eclosion.

¹Numbers within parenthesis indicates numbers of immature stages (eggs and larvae) that successfully hatched to first instar (for eggs) and/or reared to adulthood (for larvae).

²Number of eggs obtained from 40 female adults of *Pareuchaetes insulata*.

³Note that at sixth instar, sample sizes were 28 and 19 for Florida and SAB, respectively, because most larvae pupated at fifth instar (same applies to seventh instar).

Apart from the development duration of the first and fifth instars of *P. insulata* that were significantly affected, plant form did not have a significant influence on other larval development times (second, third, fourth, sixth and seventh instars), total larval development time (all larval instars combined), pre-pupal development time, pupal development time, total pupal development time, and total immature development time (larva-adult eclosion) (Table 3.3). Similarly, egg development duration was not affected by the plant form on which adults had fed during larval development (Table 3.3). Although one and two seventh instars were recorded for Florida and SAB, respectively, overall *P. insulata* developed through five or six

instars before pupating (Table 3.3). More females (Florida: 60.7%; SAB: 73.6%) developed through six instars than males for both plant forms (Florida: Pearson $\chi^2 = 8.60$, d.f. = 1, $P = 0.003$, $n = 28$; SAB: $\chi^2 = 48.44$, d.f. = 1, $P < 0.001$, $n = 19$) before pupating. There were 19% more sixth instars for Floridian *C. odorata* than for SAB (Pearson $\chi^2 = 7.33$, d.f. = 1, $P = 0.007$; Table 3.3).

Some variation in pupal mass was recorded as a function of plant form (GLM ANOVA: $F_{1,144} = 4.371$, $P = 0.038$) and sex (GLM ANOVA: $F_{1,144} = 101.46$, $P < 0.001$; Figure 3.3a). However, there was no significant interaction between plant form and sex (GLM ANOVA: $F_{1,144} = 2.57$, $P = 0.111$). Female pupae were always heavier than their male counterparts on both plant forms. Female larvae that fed on SAB had higher pupal mass compared to female larvae that fed on Floridian plants. The growth rate did not differ significantly between plant forms (GLM ANOVA: $F_{1,140} = 3.560$, $P = 0.061$) but varied significantly between sexes (GLM ANOVA: $F_{1,140} = 111.134$, $P < 0.001$) (Figure 3.3b). The growth rate of females was always higher than that of males. Maw's host suitability index (HSI) shows that SAB *C. odorata* approaches significance in being superior to that from Florida ($t_{1,69} = -1.87$, $P = 0.066$) and Sétamou's growth index was similar for both Floridian and SAB *C. odorata* (Table 3.1).

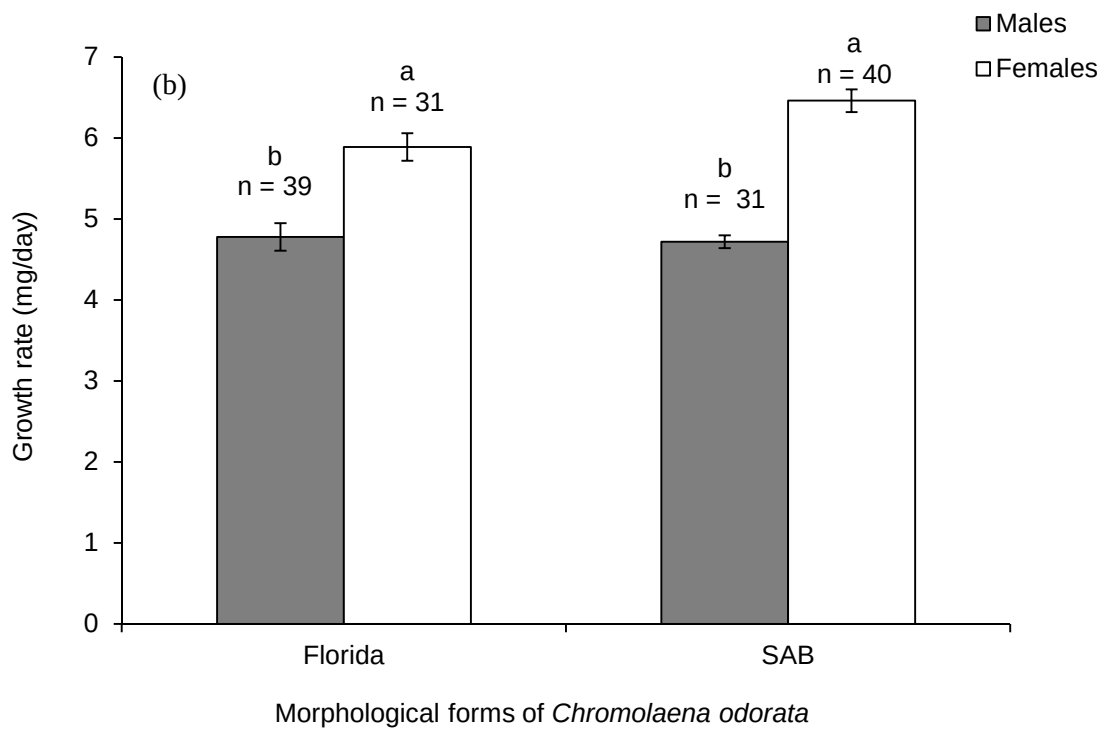
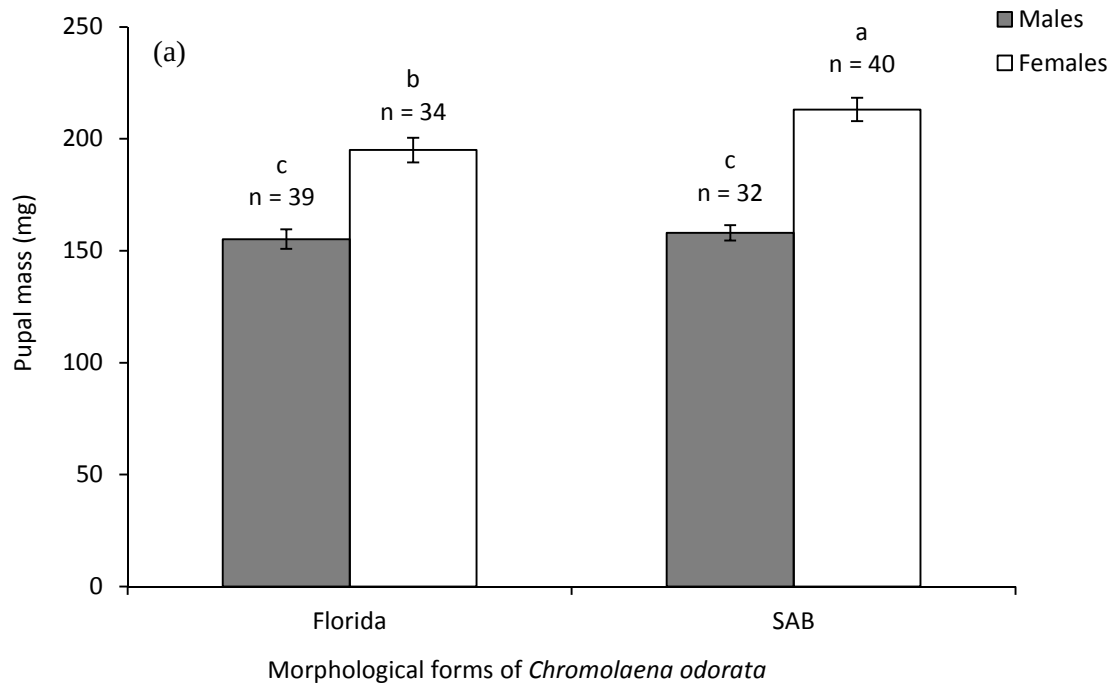


Figure 3.3 (a) Mean ($\pm$ SE) pupal mass and (b) growth rate (pupal mass (mg)/total development time (days) of *Pareuchaetes insulata* females and males reared on two distinct *Chromolaena odorata* morphological forms. Means (after GLM ANOVA) with the same letters are not significantly different (Sequential Bonferroni test: $P > 0.05$). Figures above each bar represent sample sizes.

3.3.3 Effect of host-plant form on reproductive life history traits

Female mating success (number of females mated) did not differ significantly as a function of the plant form they were fed as larvae, nor did plant form significantly affect F_1 egg hatchability (Table 3.4). Duration of egg laying ranged from 2 to 8 days for females reared on Floridian plants and 2 to 7 days for females reared on SAB plants. Thus plant form did not appear to have influence on the duration of egg laying. Realised fecundity did not differ significantly between Floridian *C. odorata* and SAB. Oviposition commencement time (egg-laying start date, after adult emergence) ranged from 1 to 3 days and from 2 to 3 days for females reared as larvae on Floridian and SAB *C. odorata*, respectively, but differences were not significant. The numbers of emerged females did not differ between plant forms. Finally, female longevity varied slightly between plant forms (Table 3.4).

Table 3.4 Performance and fitness-related traits of *Pareuchaetes insulata* adults according to the *Chromolaena odorata* morphological form on which they developed as larvae.

Parameter	Florida	SAB	Test	P
Mating success (%)	95.2 (20)	92.9 (20)	$\chi^2_1 = 0.507$	0.992
Egg hatchability (%)	98.9 $\pm$ 2.31 (3676) ¹	97.6 $\pm$ 3.10 (3914) ¹	U = 629.5	0.08
Duration of egg laying (days)	4.53 $\pm$ 0.42 (15)	3.73 $\pm$ 0.43 (15)	t _{1,28} = 1.42	0.167
Achieved fecundity (total number of eggs laid)	395.10 $\pm$ 35.60 (15)	308 $\pm$ 43.94 (15)	t _{1,28} = 1.53	0.136
Pre-oviposition period (days)	2.07 $\pm$ 0.18 (15)	2.20 $\pm$ 0.10 (15)	t _{1,28} = -0.63	0.532
Sex ratio (% of females)	44.28 (70)	56.39 (71)	$\chi^2_1 = 0.205$	0.153
Longevity (days)	6.30 $\pm$ 0.26 (70)	5.23 $\pm$ 0.27 (71)	F _{1,140} = 4.804	0.030
Pupal mass (mg)	175.09 $\pm$ 4.31 (70)	185.05 $\pm$ 3.5 (71)	F _{1,140} = 4.371	0.038

The numbers in parentheses represent the sample size.

¹Eggs were from 20 adult females of *Pareuchaetes insulata*.

3.4 DISCUSSION

This study compared several measurements of performance of *P. insulata* reared as larvae on excised leaves of two distinct forms of *C. odorata*, one from southern Florida, USA, from which this *P. insulata* population originates, and the other from South Africa, where it has established as a biological control agent, albeit poorly. Larval preference for excised leaves of the two plant forms was also examined. The study was undertaken as part of an effort to understand why the insect has not performed well as a biological control agent in South Africa. Neonate larvae of *P. insulata* preferred Floridian over SAB *C. odorata*, even after being established on the latter in South Africa for over a decade, although the biological significance of this remains to be seen. However, the insect performed equally well on SAB as on Floridian plants, suggesting that insect fitness and reproductive performance when developing on the two plants is similar. The only differences detected in *P. insulata* life history parameters were in pupal mass and adult longevity, with pupal mass being slightly higher on SAB plants while increased female longevity was evident in individuals that fed on Floridian plants.

Larvae consumed similar amounts of leaves of SAB and Florida plants, so that leaf consumption rate in a no-choice situation did not reflect preference. Total leaf consumption by *P. insulata* has been shown to be positively correlated with female pupal mass and adult fecundity (Uyi *et al.*, 2014d). Food quality is considered a good determinant of fecundity in herbivorous insects (Awmack and Leather, 2002). Those insects that do not acquire proteins as adults must obtain all the reserves during their larval stage. This may explain the significantly higher leaf consumption by females in this study because females require more food to produce eggs.

The overall survival rate for insects feeding on SAB (88.8%) and on *C. odorata* from Florida (87.5%) is similar to the 88% recorded in an earlier study on SAB by Uyi *et al.* (2014d). The pupal survival rate for insects feeding on SAB (98.6%) and on *C. odorata* from Florida (95.9%) is higher than that recorded by Walton and Conlong (2003) and Dube *et al.* (2014), who recorded 78.4% and 61.0% pupal survival, respectively, for *P. insulata* feeding on SAB. All other survival rates for the immature stages were also high and similar for both plant forms. The similarity in larval, pupal, and total immature development durations on both plant forms showed that the insect responded equally well to the two diets. The mean development time of eggs, larvae, and pupae for both diets is marginally within the range of values (5.0, 27.6, and 9.6 days for eggs, larvae, and pupae, respectively) reported for *P. insulata* by Walton and Conlong (2003). Development time is potentially an important component of fitness and survival in the field because it determines how long larvae are exposed to predators and parasitoids (Benrey and Denno, 1997; Williams, 1999) and unfavourable environmental conditions.

The slightly higher mass of female pupae recorded on the SAB diet did not influence reproductive metrics such as mating success, duration of egg laying, pre-oviposition period, female sex ratio and fecundity of the resulting adults. Although leaf characteristics (physical and chemical features) were not measured, it can be hypothesized that the heavier pupal mass for SAB females might be a direct response to host plant characteristics (Wheeler and Center, 1997; Diaz *et al.*, 2011) - as insect preference and performance can be driven by plant traits which are occasionally difficult to disentangle (Jermy, 1984; Bernays and Chapman, 1994; Hull-Sanders *et al.*, 2007; Clissold *et al.*, 2006) because of the multiplicity of indirect biotic and abiotic effects that may explain a correlation (Hunter and Price, 1998). Although growth rate (pupal mass/development time) was similar on both plant forms, female growth rate was

always higher than that of males. This is classic in capital breeders (species with non- or less-feeding adults), as male body weight is not as directly coupled with fitness as it is for females (Haukioja and Neuvonen, 1985).

In the assessment of the effect of the two plant forms on *P. insulata*, three key statistics: feeding index (FI), growth index (GI), and host suitability index (HSI) were compared. Greenberg *et al.* (2001) used the FI score to reflect the convertibility of plant biomass into insect body weight in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and they concluded that better food quality usually led to a higher feeding-index score. The GI emphasizes the importance of survival and development time when assessing food quality (Sétamou *et al.*, 1999). Higher survival rates and faster development times will yield higher GI values and these can be used as a measure of host plant quality. The FI, GI, and HSI did not differ between the two *C. odorata* plant forms. Due to the similarity of all the three calculated indexes between Floridian and SAB plants, it was safe to hypothesize that both plants contain equal amounts of energy/nutrient source per unit to support the development of *P. insulata*, and therefore neither one of the plant forms can be considered as a superior host. Dube *et al.* (2014) have shown that *P. insulata* from Jamaica and Florida do not exhibit reproductive or genetic barriers between them. Therefore it may not be surprising that *P. insulata* from Florida develops equally well on SAB, which has its origin in Jamaica, as it does on Floridian *C. odorata*.

The equal performance of *P. insulata* on *C. odorata* plants from South Africa and Florida provides substantial evidence that incompatibility between the insect and plant does not play a role in the generally poor performance of the insect as a biological control agent in South Africa. However, the neonate larval preference recorded for Floridian *C. odorata* indicates

that performance may not be the sole criterion needed to definitively show that plant form has no effect on the insect's field population dynamics in South Africa. Trials on comparative acceptability as mates of adult males fed on SAB and Floridian *C. odorata* may inform us on whether low field populations of *P. insulata* in South Africa may be due to poor mating success as a result of a partial incompatibility with the host-plant form, most likely mediated through qualitative or quantitative differences in pyrrolizidine alkaloids (PAs) between Floridian and SAB plants. The high mating success recorded in this study could be attributed to the fact that two virgin males and one virgin female were always paired in one 700-ml container with a single plant form. This might contrast to the field situation, where adults may need to travel a considerable distance before finding and accepting a mate, especially at low population levels. In *Utetheisa ornatrix* (L.) (Lepidoptera: Erebidæ: Arctiinae) and in other arctiines, females choose males based on a courtship pheromone, hydroxydanaidal (HD), derived from defensive pyrrolizidine alkaloids (PAs) (Schneider *et al.*, 1992; Conner, 2009). Females make choices based on the ability of virgin males to transfer spermatophores whose contents are proportional to their HD titre and body size; as a result, selective females receive both phenotypic benefits (more nutrients and PAs) and genotypic benefits (genes for larger body size inherited by the offspring) (Conner *et al.*, 1990; LaMunyon and Eisner, 1994; Conner, 2009; Kelly *et al.*, 2012). The females pass this gift, together with PAs that they themselves procured as larvae, to the eggs for defence against predators and parasitoids (Eisner *et al.*, 2000; Bezzerides *et al.*, 2004). Thus the assessment of male dietary background by the adult female is a sine qua non for choosing mates and is a key to the reproductive success in species such as *U. ornatrix*. Investigations on the PA concentrations in Florida vs. SAB plants, PA concentrations of various life stages of *P. insulata* which fed on the two plant forms and the role of PAs in mating biology would not only help to explain mate finding, but mate acceptance by females in the field.

There are several other possibilities regarding poor performance of *P. insulata* in South Africa, including poor climatic matching and top-down factors such as predation, parasitism, and disease. Uyi *et al.* (2014b) investigated the role of temperature and reported that both direct and indirect negative impacts of low temperature may partly explain the poor performance of *P. insulata* in South Africa (also see Chapter 4). Although a preliminary study (O. Uyi, unpubl. data) did not record any egg parasitoids or predators, the role of these top-down factors on the different life stages of this insect needs further investigation across a spatio-temporal spectrum. Exotic herbivores are likely to have fewer parasitoids in their new range than in their country of origin, and their parasitoid fauna is likely to be chiefly generalists with few specialist species (Cornell and Hawkins, 1993). McFadyen (1997) reported extremely low levels of parasitism in a related species (*P. pseudoinsulata*), which was introduced into many countries in Asia and Africa. In that paper, she concurred with previously published papers by concluding that “herbivorous insects from families with exposed larvae (such as *P. insulata*), are unlikely to be heavily parasitized in a new geographical range”. While it is possible that the PAs sequestered by *Pareuchaetes* species may also act as defences against predators and parasitoids as has been reported for other erebid moths (e.g., Eisner *et al.*, 2000; Bezzerides *et al.*, 2004), a test of this hypothesis is needed to either validate or invalidate this conjecture.

3.5 CONCLUSION

In general, herbivore-host plant incompatibility is considered a less likely reason for poor performance of *Pareuchaetes* species, as they are believed not to have a highly restricted host range; for example, both *P. pseudoinsulata* and *P. insulata* feed on *Ageratum conyzoides* L. (Asteraceae: Eupatorieae) in the field in native and introduced ranges (Cruttwell, 1972;

Zachariades *et al.*, 2011a; C. Zachariades, unpubl. data) although probably only as a secondary host in the presence of *C. odorata*. However, several studies (Karban, 1992; Hanks and Denno, 1994; Dray *et al.*, 2004; Wheeler, 2006) have reported variations in insect fitness and survival within a single plant species (but with different genotypes, chemotypes, varieties or from different geographic ranges) (also see Simelane, 2006).

The equal performance of *P. insulata* from Florida on SAB and Floridian *C. odorata* plants is not a criterion to argue that the ancestral origin of invasive alien weed populations should not be prioritized when collecting biological control agents in the native range. Because the *C. odorata* biotype invasive in South Africa is genetically similar to the Jamaican *C. odorata* (Paterson and Zachariades, 2013), pre-release studies should be conducted on potential agents collected in areas other than the origin of SAB in the Americas to compare insect preference for, and performance on, the genotype of *C. odorata* from which it was collected and the SAB genotype. This will help to eliminate scepticism about biotype incompatibility, should the agent fail to establish, or perform poorly. The findings of this Chapter are important because they have helped to eliminate uncertainties about biotype incompatibility as a possible factor responsible for the poor performance of the moth, hence redirecting research focus on the poor performance of the insect to other aspects such as climate (e.g. the role of temperature, as in Chapter 4), bottom-up effects of plant quality (Chapters 5, 6 and 7) or top-down factors (e.g. predators and parasitoids).

CHAPTER 4

The effects of temperature on developmental and reproductive life history traits, locomotory performance and thermal tolerance range of *Pareuchaetes insulata*

4.1 INTRODUCTION

Ambient temperature is a key environmental factor influencing a variety of aspects of animal ecology and evolution, especially for ectotherms. The effects of temperature on the performance and behaviour of insects are well known (Bale *et al.*, 2002; Hughes *et al.*, 2004; Deere and Chown, 2006; Tamiru *et al.*, 2012; Chidawanyika and Terblanche, 2011; Li *et al.*, 2011; Ferrer *et al.*, 2014). Insect responses to temperature extremes over short periods may be an important driver of population dynamics and consequently species abundance and geographic distribution over longer timescales (Bale, 2002; Chown and Terblanche, 2007; Denlinger and Lee, 2010). Over longer periods, temperature influences the seasonal and evolutionary responses of insects (Bale, 2002; Chown and Nicholson, 2004; Denlinger and Lee, 2010). Likewise, the ability of temperature fluctuation to affect activity (e.g. locomotion, feeding) and survival of insects at short time-scales is also of critical importance (Lachenicht *et al.*, 2010; Chidawanyika and Terblanche, 2011; Li *et al.*, 2011).

For insects, temperature is an important abiotic factor, with each species having its optimal temperature range and, outside this optimal range, development is slowed and damage to physiological and metabolic processes occurs, leading to either high mortality or reduced performance (Anguilletta *et al.*, 2002; Bale, 2002; Chown and Nicholson, 2004; Martin and Huey, 2008; Anguilletta, 2009). When exposed to temperature extremes, insects employ a range of mechanisms to adjust their body temperature to the extremes they can withstand, using either physiological or behavioural mechanisms or some combination of both. For

example, an insect experiencing adverse high ambient temperature can lower its body temperature by avoidance of sunny hot spots (or seeking a shaded microsite) or vice versa (e.g. Kührt *et al.*, 2006; Huey and Pascual, 2009). However, behavioural adjustments only act as the first line of defence against sub-optimal ambient temperatures and depend to a large extent on microhabitat opportunities in their habitat (Kührt *et al.*, 2006). If unfavourable environmental conditions persist, physiological mechanisms may become critical to ensure survival. Examples of such physiological adjustments include alteration of thermal tolerance at daily (Overgaard and Sørensen, 2008) or seasonal (Khani and Moharramipour, 2010) time-scales. Temperatures lethal to insects are a function of both the magnitude of the temperature variation and the duration of exposure (Chown and Nicholson, 2004; Angilletta, 2009; Denlinger and Lee, 2010). Alternatively, they may rapidly develop biochemical protection, e.g., heat shock proteins, which maintain cell function during or after experiencing potentially damaging conditions (Denlinger and Lee, 2010; Terblanche, 2013).

Understanding the effects of temperature on the development, tolerance and locomotion performance of *P. insulata* is not inconsequential because numerous biological control agents have either failed to establish or performed poorly in their introduced ranges due to low temperatures (=climate incompatibility) (McClay and Hughes, 1995; McClay, 1996; Stewart *et al.*, 1996; Good *et al.*, 1997; Byrne *et al.*, 2002, 2003; Bale, 2011).

Therefore, the reasons for the low populations of *P. insulata* could include temperature incompatibility or sub-optimal temperatures over long periods or at short time-scales. There are no published studies on the effects of temperature on the performance of *P. insulata* in South Africa and elsewhere in the world – as a previous attempt to do this was unsuccessful (C. Zachariades, pers. comm.). Fecundity, fertility, developmental period and longevity are

the best measures of the biotic potential of an insect (see Southwood and Henderson, 2000). Knowledge of the effects of temperature on the development of *P. insulata* will help to establish the development threshold (or optimal temperature range) and lethal limits of temperature and this can be helpful in terms of predicting and understanding the distribution and performance of the moth in relation to the South African climate. The aims of this study was several-fold; (i) To determine the role of constant temperatures (15, 20, 25, 27, 30 and 35 °C) on development, survival, fecundity and longevity of *P. insulata* and to establish day degree requirements for each stage of development of the moth (Tamiru *et al.*, 2012; May and Coetzee, 2013) (ii) To determine the range of time-temperature combinations (thermal tolerance) which may be lethal at short time-scales (Li *et al.*, 2011; Chidawanyika and Terblanche, 2011) in *P. insulata* and (iii) to determine the effect of temperature and acclimation on locomotion performance (Lachenicht *et al.*, 2010; Boiteau and Mackinley, 2012) of *P. insulata* larvae.

4.2 MATERIALS AND METHODS

4.2.1 Origin and maintenance of plant and insect cultures

Pareuchaetes insulata individuals were collected in the Sappi Cannonbrae plantation, Umkomaas (south coast of KZN province), South Africa (30° 13' S, 30° 46' E), where the insect established following large releases made between 2001 and 2003 (Zachariades *et al.*, 2011). The individuals of the moth were maintained for about two generations on *C. odorata* bouquets from the field (in and around Durban) at 25 ± 2 °C, $65 \pm 10\%$ relative humidity (RH), with a photoperiod of L12:D12. Newly hatched larvae were fed with cut leaves inside 700 ml (1 egg batch per container) aerated plastic containers, while older larvae were reared inside 2 L “Freezette” rectangular plastic trays (32 x 22 x 6 cm) (30 larvae per container)

with *C. odorata* bouquets. Fresh leaves were added as needed. Pupae were placed in 2 L Freezette trays containing vermiculite and monitored for eclosion.

Chromolaena odorata plants used in this study were grown from stem-tip cuttings collected from Durban, South Africa. All cuttings were initially planted in a mist bed in vermiculite with rooting hormone (SeradixTM No. 1) before they were later planted in nursery pots (25 cm diameter). All plants benefited from same potting medium (Umgeni sand: Gromor Potting Medium 1:1, Gromor, Cato Ridge, South Africa), fertilizer (Plantacote AGLUKON Spezialduenger GmbH & Co. KG, German) and watering regimes. Plants were grown outside between September 2011 and April 2012 and hand-watered with a hose.

4.2.2 Effect of temperature on development and other life history traits

Development time, survival, adult longevity and fecundity of *P. insulata* were studied at six constant temperatures of 15, 20, 25, 27, 30 and 35 °C at 70±10% relative humidity. These temperature ranges were chosen because daily mean temperatures in the *P. insulata* established sites in KZN province never fall below 14 °C. All trials were performed in growth chambers (Labcon, South Africa) set at either of the above temperature treatments. Hygrochron iButtons (model DS 1923, Maxim Integrated Products, San José, USA, 0.5 °C accuracy) were used to measure hourly temperature readings inside the chambers to make sure that the desired temperature was achieved. In all growth chambers, photoperiod was set at 12L:12D (h).

4.2.2.1 Effect of temperature on egg development

To investigate the effect of constant temperature on egg development, two newly eclosed males and one virgin female were placed in an aerated 700 ml plastic container (with a 5 cm

diameter mesh window at the top) with *C. odorata* stem cuttings plugged into a 5 x 5 x 3 cm moistened OasisTM floral foam block wrapped with aluminium foil. Two 90 mm discs of filter paper (moistened with 0.4 ml of water) were placed inside the containers to maintain RH. Adults were supplied with cotton-wool balls soaked with 50% honey solution for feeding. Ten replicates were used for this study. Eggs laid on the leaf of *C. odorata* were detached and placed inside a 100 ml aerated plastic containers with a circular net screen window (2.5 cm diameter on the top and lined at the bottom with moistened filter paper to maintain RH). All containers (with eggs) were placed inside a ZiplocTM bag (600 x 450 mm) to prevent desiccation. The eggs were then exposed to one of six treatment temperatures (15, 20, 25, 27, 30, and 35 °C). Eggs from 10 females were observed at each temperature treatment. Observations were carried out once a day and egg development period (number of days to hatching) and percentage egg hatch was calculated. Egg development duration was analysed using a Generalised Linear Model (GLM) while egg hatchability (number of eggs hatching) was analysed using a Pearson χ^2 test. All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA)

4.2.2.2 Effect of temperature on larval development

To assess the effects of different constant temperatures on immature (larval and pupal) survival, growth and development duration of *P. insulata*, larvae were raised individually from day of hatching to pupation in 100 ml aerated plastic containers with a circular net screen window (2.5 cm diameter on the top for ventilation), lined at the bottom with moistened filter paper to maintain RH, and were fed on *C. odorata* leaves for the duration of their development. All containers (larvae) were placed inside a ZiplocTM bag (600 x 450 mm) to prevent desiccation. The larvae were then exposed to one of six treatment temperatures (15, 20, 25, 27, 30, and 35 °C). Fifty larvae (n = 50) per temperature treatment were fed with

fresh fully expanded leaf tissues (taken from the upper half of the plants) every 24 hours and their frass was removed at same interval for hygienic reasons. The purpose of using excised leaves was to limit uncontrolled variations among whole plants so that the insect parameters of interest, in this case development time and other fitness-related traits, could be stringently assessed. Observations on mortality were made daily until adult eclosion in order to follow the duration of each instar and other immature stages. The stage-specific and overall survival of *P. insulata* was calculated as numbers of larvae (or pre-pupae or pupae) that developed to the next stage, divided by the initial numbers at the start of the experiment. The loss of the cephalic capsule was the criterion used to determine the moult. Pupae were extracted from the food source and sex was determined at the pupal stage, based on the position of the genital orifice. Sex ratio and development duration were calculated and recorded respectively based on the numbers of surviving pupae. Specifically, the following variables were measured: (i) duration of each larval instar, (ii) total larval development duration (defined here as the number of days from hatching until pupation), (iii) pre-pupal duration, (iv) pupal duration, (v) total pupal duration (pre-pupal and pupal combined) and (vi) total immature development time (defined here as the number of days from hatching until adult eclosion). All parameters were measured according to sex in order to enable comparisons between males and females. Upon adult eclosion, adult longevity was observed. The effects of constant temperature on immature development durations (from first instar to adult eclosion) and adult longevity were analysed using a Generalised Linear Model (GLM) while sex ratio (% females) was analysed using a Pearson χ^2 test. All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA).

4.2.2.3 Effect of temperature on reproductive life history traits

To determine the influence of different constant temperatures on fecundity, two newly eclosed males and one virgin female were placed in aerated 700 ml plastic containers (with 5 cm diameter mesh window at the top) with *C. odorata* stem cuttings plugged in 5 × 5 × 3 cm moistened OasisTM floral foam block wrapped with aluminium foil. Two 90 mm discs of filter paper (moistened with 0.4 ml of water) were placed inside the containers to maintain RH. Adults were supplied with cotton-wool balls soaked with 50% (wt/vol) honey solution for feeding. All containers (20 containers per treatment) were placed inside a ZiplocTM bag (600 x 450 mm) to prevent desiccation and they were thereafter exposed to one of six treatment temperatures (15, 20, 25, 27, 30, and 35 °C). Males and females were held captive in these containers until they all died. Females could oviposit on the wall of the containers and/or on the leaves of the plants. Containers were examined daily to record (i) adult mortality, (ii) realised fecundity (total number of eggs laid), (iii) duration of egg laying and (iv) pre-oviposition period. The effect of constant temperature on longevity, realised fecundity, duration of egg laying and pre-oviposition period were analysed using a Generalised Linear Model (GLM). All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA).

4.2.3 Lower developmental threshold (t_0) and the rate of development (K)

The developmental rate relationship [$R(T) = a + bT$] for each insect stage and all immature stages combined was calculated using the linear regression method (Campbell *et al.*, 1974). T refers to temperature, and a and b are the intercept and slope estimates respectively. The lower temperature threshold was estimated by the intersection of the regression line at $R(T) = 0$, $t_0 = -a/b$. Degree-day requirements for each stage and all immature stages combined were calculated using the inverse slope ($1/b$) of the fitted linear regression line (Campbell *et al.*,

1974).

Because the relationship between temperature and development rate is not linear (especially at lower and upper temperature thresholds), Ikemoto and Takai (2000) proposed the reduced major axis regression method. They suggested that this method produces more accurate thermal parameters than the linear regression model proposed by Campbell *et al.* (1974). The number of days to complete immature development (D) was plotted against the temperature multiplied by the numbers of days to complete immature development (DT). The lower development threshold (t_0) and the thermal constant K were calculated using the equation obtained from the reduced major axis regression method, where t_0 is determined from t and k (y-intercept) represents K: $DT = k - tD$. The thermal constant K is the number of degree-days of development above the developmental threshold t_0 (i.e. the temperature at which zero development occurs). There is no major error associated with the reduced major axis regression method, as the parameters K and t_0 are drawn straight from the line parameters (Ikemoto and Takai, 2000), therefore this method was used.

Daily maximum and minimum temperature records were obtained from the CLIMEX model database for 129 locations throughout South Africa. The thermal parameters obtained from the reduced major axis regression method and the climate data were used to develop degree-day models for *P. insulata* for each year and location according to the equation: $\Sigma[(T_{\max} + T_{\min})/2 - t]$, where $T_{\max}$ and $T_{\min}$ represent maximum and minimum temperature experienced by *P. insulata*, and t represents the lower developmental threshold for the insect. The reduced major axis regression method was used because it has been reported to produce more accurate thermal parameters than the linear regression model (see discussion in Ikemoto and Takai, 2000, also see May and Coetzee, 2013). The number of generations per year was predicted by

dividing the cumulative degree-days per station by K, the degree-day requirement for egg to adult. Maps were created using ArcGis version 10.2 (ESRI, Redlands, CA) indicating the numbers of generations that *P. insulata* potentially completes in a year within the weed's distribution range (Eastern Cape, KZN, Limpopo and Mpumalanga provinces) in South Africa.

4.2.4 Thermal tolerance (effects of lethal temperature and exposure time on survival)

The lower lethal temperature (LLT) and the upper lethal temperature (ULT) for adults and third instar larvae were assessed using a standard “plunge” protocol (e.g. Sinclair *et al.*, 2006; Terblanche *et al.*, 2008). Adults (males and females combined) and larvae of moths used were reared under controlled constant conditions (25 ± 2 °C, $70 \pm 10\%$ RH and 12L: 12D photoperiod). Thermal tolerance was measured as a proportion of survival after exposure to a constant temperature for a fixed period of time over a range of experimental temperatures using a circulating programmable water bath (Haake C25P, Thermo Electro Corporation, Karlsruhe, Germany). The water bath was filled with 90% ethanol to allow for sub-zero temperature use without freezing.

For ULT and LLT trials, empty 60 ml glass vials (sealed at the top with cotton wool) containing either individuals of 2-day old moths (for the adult trials) or third instar larvae (for larval trials) (4 in each vial x 10 vials = 40 individuals per temperature treatment) were placed inside a plastic bag placed inside the programmable water bath and were subjected to temperature treatments for a fixed time period (0 hour = control group, 0.5, 1, 2 and 4 hours). The range of conditions tested always encompassed the full range of moth survival from 0 to 100% covering a temperature range of -10 to 0 and 25 to 43 °C for LLT and ULT trials respectively. A fine type-T thermocouple connected to a digital thermometer (TECPEL 305,

Taiwan, ± 0.1 °C accuracy) was regularly used to monitor the temperature inside the vials and the water bath to ensure that the desired temperature during treatments was achieved and maintained. Following treatments, the moths (adults or larvae) were removed from the water bath and placed inside a 100 ml plastic container with a circular net screen window (2.5 cm diameter on the top for ventilation) and lined at the bottom with filter paper. The moths were either provided with 50% honey solution or excised leaves depending on their life stage and with moistened cotton wool to maintain humidity. The containers were then placed inside a growth chamber set at normal rearing temperature (25 °C) and survival was scored after 24 hours. Survival was considered as a coordinated response to gentle stimuli (e.g. normal behaviour such as walking, feeding or flying for adults upon gentle prodding).

Following arcsine square root transformation, the effects of temperature and time was analysed using a Generalized Linear Model (GLM) (assuming normal distribution with an identity link function). Probit regression was used to estimate LT_{50} , the temperature causing 50% of tested individuals to die in a given period (Li *et al.*, 2011). All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA)

4.2.5 Locomotion performance trials

Day-old third instar larvae of *P. insulata* were obtained from a colony maintained at 25 °C as described above. Some of these larvae were placed in 2 L “Freezette” rectangular plastic trays (32 x 22 x 6 cm) (about 100 larvae per tray) with *C. odorata* bouquets and placed inside a growth chamber set at the rearing temperature (25°C) for the warm acclimation treatment, while for the cool acclimation treatment, day-old third instar larvae inside 2 L “Freezette” trays were placed in a growth chamber set at 10 °C. The actual temperature and humidity experienced by the larvae in each chamber was recorded at hourly intervals using data

loggers (Hygrochron iButtons, model DS 1923, Maxim Integrated Products, San José, CA, USA, 0.5 °C accuracy) attached to the inside wall of each tray (hourly readings for warm acclimation treatment: range, 24.53 to 25.47 °C; mean $\pm$ SE, 24.71 ± 0.01 °C; RH: range, 68.3 to 77.6%; mean $\pm$ SE, $71.2 \pm 2.1\%$; hourly readings for cool acclimation treatment: range, 9.68 to 10.58 °C; mean $\pm$ SE, 10.43 ± 0.01 °C; RH: range, 66.5 to 75.8%; mean $\pm$ SE, $70.1 \pm 1.9.1\%$). The chosen acclimation temperatures represent the range of conditions likely to be encountered by the larvae in the various release sites in KZN province. The two sub-colonies were exposed to their new environment for 48 hours (2 days) before the start of the experiment to ensure acclimation (based on preliminary observations). Previous work on insects has indicated that a 1 to 7-day acclimation period is sufficient for the full change of phenotype to be realized (Hoffman and Watson, 1993; Terblanche *et al.*, 2006; Weldon *et al.*, 2011).

The influence of temperature and acclimation temperature on locomotion performance of *P. insulata* larvae was investigated by recording the proportion of warm-acclimated and cold-acclimated third instar larvae capable of walking when exposed to a range of temperatures (four test temperatures; 6, 11, 15 and 20 °C) and the time spent moving during each 30-second exposure (for rationale, see Boiteau and Mackinley, 2012). To ensure that the test temperature was kept constant, the experiments were done on a 0.60 m² temperature-controlled stage connected to a programmable water bath (Haake C25P, Thermo Electro Corporation, Karlsruhe, Germany). Larvae were allowed to equilibrate for 2 mins prior to estimating the locomotion parameters of interest. A fine type-T thermocouple connected to a digital thermometer (TECPEL 305, Taiwan, ± 0.1 °C accuracy) was placed on the floor of the stage to ensure that the desired test temperature was achieved and maintained. Five larvae were individually exposed to a particular test temperature on the stage for 30 seconds and the

number of insects mobile at each temperature, and the time spent walking during each exposure, was recorded with an electronic stop watch. This observation constituted one replicate and a total of five replicates were used for each treatment temperature (or exposure) (i.e. 25 larvae were used for each treatment temperature). Relative humidity was at 100% at the surface of the stage because of condensation at the cold surface of the aluminium plate. A Pearson χ^2 test was applied to comparisons of walking frequency and a student's t-test to mean duration of movement between warm-acclimated and cold-acclimated larvae. The analyses were performed using Genstat 9.0 (VSN International, Hemel Hempstead, UK).

4.2.6 Microclimate data

Hygrochron iButtons (Hygrochron iButtons, model DS 1923, Maxim Integrated Products, San José, USA, 0.5 °C accuracy) were used to record microclimate temperatures and relative humidity at 1-hour sampling frequencies at three locations in Sappi Cannonbrae Plantation (*P. insulata* established site), near the coastal town of Umkomaas, South Africa over a period of 12 months. Two iButtons were used to record climate data per site; one was placed at near-ground level (3.5 cm above the ground) while the other was suspended (60 cm above ground level) within *C. odorata* thickets. The iButtons were placed inside 100 ml screw-top plastic containers with circular screen windows and holes around the container. This was done to prevent biased temperature readings and to easily allow air flow. The iButtons were never placed in direct sunlight and were protected from direct rainfall. The iButtons placed at the near-ground level were to determine climate conditions at supposed pupation sites of the moths, while the suspended ones were to record climate data at the feeding microsite because larvae feed on the leaves of the plant. Data from three sites at near-ground level and those from the suspended microsite were compared using a Mann-Whitney U-test because their distribution violated the assumption of a parametric test.

4.3 RESULTS

4.3.1 Survival, development and reproductive life history traits

Survival of eggs and neonate larvae to adult stage of *P. insulata* was obtained at 15, 20, 25, 27 and 30 °C, but all larvae and eggs either died or failed to hatch at 35 °C (Figure 4.1; Table 4.1a).

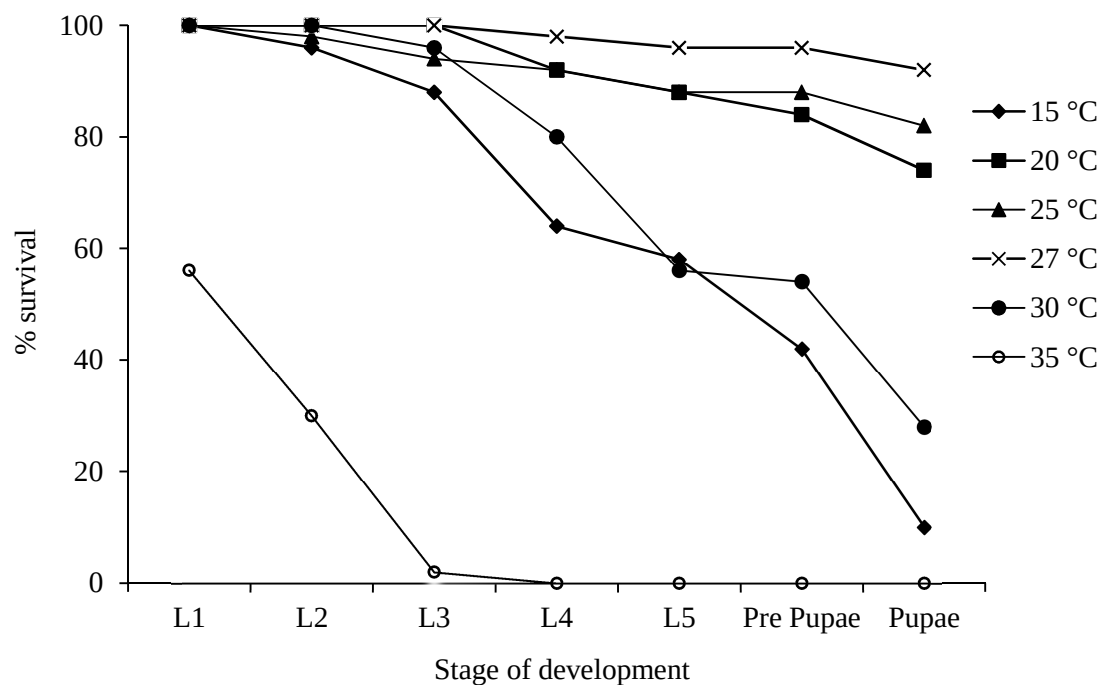


Figure 4.1 Mean percentage survival of immature stages of *Pareuchaetes insulata* at six constant temperatures. Survival at each stage and overall survival were significantly affected by rearing temperatures (Pearson χ^2 , $P < 0.001$, d.f. = 5). Fifty individuals were reared under each temperature regime.

Table 4.1a Mean developmental durations ($\pm$ SE) for different life stages (eggs, first – fifth instars) of *Pareuchaetes insulata* at five constant temperatures.

Sex	Temp (°C)	N ¹	Eggs	Larva				
				First	Second	Third	Fourth	Fifth
Male	15	1	18.65 $\pm$ 0.30 (559) ²	6.00 $\pm$ 0.00a	10.00 $\pm$ 0.00a	7.00 $\pm$ 0.00a	10.00 $\pm$ 0.00a	9.00 $\pm$ 0.00a
	20	20	9.55 $\pm$ 0.13 (652) ²	4.62 $\pm$ 0.11b	4.52 $\pm$ 0.14b	4.74 $\pm$ 0.10b	5.45 $\pm$ 0.15b	6.40 $\pm$ 0.22b
	25	18	4.60 $\pm$ 0.12 (811) ²	3.11 $\pm$ 0.07c	2.92 $\pm$ 0.09c	3.11 $\pm$ 0.11c	3.67 $\pm$ 0.22c	5.05 $\pm$ 0.09c
	27	21	4.00 $\pm$ 0.00 (912) ²	2.95 $\pm$ 0.04c	2.11 $\pm$ 0.07d	2.90 $\pm$ 0.11c	3.00 $\pm$ 0.00d	4.00 $\pm$ 0.00d
	30	7	4.00 $\pm$ 0.08 (508) ²	2.94 $\pm$ 0.00c	2.00 $\pm$ 0.00d	2.10 $\pm$ 0.21d	3.28 $\pm$ 0.18cd	4.00 $\pm$ 0.00d
Female	15	4		6.00 $\pm$ 0.00a	10.51 $\pm$ 0.05a	7.75 $\pm$ 0.25a	10.13 $\pm$ 0.07a	10.10 $\pm$ 0.50a
	20	17		4.81 $\pm$ 0.10b	4.00 $\pm$ 0.17b	4.90 $\pm$ 0.16b	5.60 $\pm$ 0.25b	6.82 $\pm$ 0.39b
	25	23		3.25 $\pm$ 0.08c	3.00 $\pm$ 0.07c	3.22 $\pm$ 0.08c	4.04 $\pm$ 0.14c	5.47 $\pm$ 0.21c
	27	25		2.73 $\pm$ 0.09c	2.40 $\pm$ 0.10d	3.04 $\pm$ 0.07c	2.98 $\pm$ 0.04d	4.40 $\pm$ 0.11d
	30	7		3.00 $\pm$ 0.00c	2.00 $\pm$ 0.00d	2.00 $\pm$ 0.00d	3.14 $\pm$ 0.14d	4.28 $\pm$ 0.18d
GLM analysis	Intercept		$\chi^2_1 = 670.1^{***}$	$\chi^2_1 = 448.37^{***}$	$\chi^2_1 = 1030.44^{***}$	$\chi^2_1 = 430.08^{***}$	$\chi^2_1 = 804.89^{***}$	$\chi^2_1 = 1157.14^{***}$
	Temp		$\chi^2_4 = 3160.54^{***}$	$\chi^2_4 = 27.91^{***}$	$\chi^2_4 = 261.13^{***}$	$\chi^2_4 = 41.93^{***}$	$\chi^2_4 = 60.04^{***}$	$\chi^2_4 = 36.53^{***}$
	Sex			$\chi^2_1 = 0.002^{NS}$	$\chi^2_1 = 0.032^{NS}$	$\chi^2_1 = 0.057^{NS}$	$\chi^2_1 = 0.020^{NS}$	$\chi^2_1 = 0.820^{NS}$
	Interaction			$\chi^2_4 = 0.403^{NS}$	$\chi^2_4 = 3.39^{NS}$	$\chi^2_4 = 0.108^{NS}$	$\chi^2_4 = 0.312^{NS}$	$\chi^2_4 = 3.069^{NS}$

¹Larval sample size, note that 50 replicates were used for larval development trial at each temperature, only individuals that survived to eclosion were considered for these analyses.

²Numbers of eggs investigated are indicated in parentheses.

Mean developmental times at each life stage were significantly affected by rearing temperature (Generalized Linear Model, either assuming a normal distribution with an identity link function or Poisson distribution with a log link function). Means within a column followed by the different letters are significantly different within each sex (Sequential Bonferoni test, $P < 0.05$).

^{NS} $P > 0.05$; ^{***} $P < 0.0001$

Table 4.1b Mean developmental durations ($\pm$ SE) for total larval, total pupal and total immature (1st instar – adult eclosion) stages of *Pareuchaetes insulata* at five constant temperatures.

Sex	Temp (°C)	N ¹	Total larva ²	Total pupa ²	Total immature (1 st instar – adult eclosion) ²
Male	15	1	59.00 $\pm$ 0.00a	59.00 $\pm$ 0.00a	118.00 $\pm$ 0.00a
	20	20	26.05 $\pm$ 0.52b	22.45 $\pm$ 0.18b ²	48.45 $\pm$ 0.57b ²
	25	18	17.89 $\pm$ 0.31c ²	12.94 $\pm$ 0.15c ²	30.83 $\pm$ 0.31c
	27	21	15.00 $\pm$ 0.01d	11.04 $\pm$ 0.08d ²	26.05 $\pm$ 0.11d
	30	7	14.42 $\pm$ 0.20d	9.57 $\pm$ 0.22e	24.00 $\pm$ 0.00e
Female	15	4	60.75 $\pm$ 0.63a	52.04 $\pm$ 0.13a	112.75 $\pm$ 0.14a
	20	17	26.88 $\pm$ 0.36b	19.94 $\pm$ 0.40b	46.82 $\pm$ 0.57b
	25	23	18.86 $\pm$ 0.23c	11.78 $\pm$ 0.13c	30.50 $\pm$ 0.19c
	27	25	15.48 $\pm$ 0.19d	10.00 $\pm$ 0.00d	25.48 $\pm$ 0.19d
	30	7	14.42 $\pm$ 0.20d	9.03 $\pm$ 0.02e	23.42 $\pm$ 0.30e
GLM analysis	Intercept		$\chi^2_1 = 49942.63^{**}$	$\chi^2_1 = 25889.99^{**}$	$\chi^2_1 = 12925.55^{**}$
	Temp		$\chi^2_4 = 8426.91^{**}$	$\chi^2_4 = 8304.29^{**}$	$\chi^2_4 = 3296.93^{**}$
	Sex		$\chi^2_1 = 7.89^*$	$\chi^2_1 = 82.48^{**}$	$\chi^2_1 = 37.12^*$
	Interaction		$\chi^2_4 = 17.46^*$	$\chi^2_4 = 39.96^{**}$	$\chi^2_4 = 27.46^*$

¹Larval sample size, note that 50 replicates each was used for larval development at each temperature, only individuals that survived to eclosion were considered for these analyses.

Mean developmental times at each life stage were significantly affected by rearing temperature (and occasionally sex) (Generalized Linear Model, either assuming a normal distribution with an identity link function or Poisson distribution with a log link function). Means within a column followed by the different letters are significantly different within each sex (Sequential Bonferroni test, $P < 0.05$).

* $P < 0.01$; ** $P < 0.0001$.

²Significant differences in development durations between males and females at each temperature and development stage (student's t -test, $P < 0.05$).

Temperature significantly affected overall survival (first instar to adult eclosion; Pearson $\chi^2 = 317.30$, d.f. = 5, $P < 0.001$). The highest overall survival (92.0%) was recorded at 27 °C, followed by 25, 20 and 15 °C (82.2, 74.0, 10.0% respectively) and the lowest was obtained at 35 °C (0.0%) (Figure 4.1). Egg hatchability was high and similar at 20, 25 and 27 °C with 15 °C experiencing the lowest (70.8%) (Table 4.2). Development time of each insect stage significantly decreased with increasing temperatures from 15 to 30 °C and total immature development duration was longest (118.0 and 112.7 days for male and female respectively) at 15 °C and shortest (24.0 and 23.4 days for male and female respectively) at 30 °C (Tables 4.1 a and b). The longest egg development period was at 15 °C (18.6 days), while the shortest (4.0 days) was at 27 and 30 °C (Table 4.1a). At all temperatures, larval instars did not differ between sexes, but a difference was detected in the total larval development time between sexes at 25 °C with males developing faster than females (Tables 4.1 a and b). However, female pupae developed faster than their male counterparts at 20, 25 and 27 °C. Finally total immature development time at 20 °C was shorter in females compared to their male counterparts.

Although temperature did not significantly affect sex ratio (Table 4.2), pre-oviposition period, duration of egg laying, adult longevity and realised fecundity varied among temperatures (Table 4.2; Figure 4.2). Pre-oviposition period was shortest (1.0 days) at 25 °C and longest (3.2 days) at 15 °C. Duration of egg laying was longest (7.0 days) at 15 °C and shortest (1.6 days) at 30 °C. Individuals reared at the lowest temperature (15 °C) lived longer compared to those reared at 20, 25, 27 and 30 °C. Finally, realised fecundity was higher at 20, 25 and 27 °C compared to 15 and 30 °C.

Table 4.2 Egg hatchability and reproductive performance of *Pareuchaetes insulata* at different constant temperatures.

Temperature	Hatchability (%) ¹	Sex ratio (% females)	Pre-oviposition period (days)	Duration of egg laying (days)	Longevity (days)
15 °C	70.82 ± 1.17 (771)c	80.00 (5)	3.25 ± 0.34 (20) a	7.00 ± 0.58 (20)a	9.98 ± 0.30 (60)a
20 °C	96.39 ± 1.09 (652)a	45.90 (37)	2.05 ± 0.05(20)b	6.10 ± 0.24 (20)a	7.43 ± 0.19 (60)b
25 °C	97.45 ± 1.14 (811)a	36.09 (41)	1.05 ± 0.05 (20)c	5.60 ± 0.41 (20)ab	6.88 ± 0.22 (60)b
27 °C	99.39 ± 0.26 (2133)a	54.35 (46)	1.70 ± 0.21 (20)b	4.55 ± 0.25 (20)b	4.38 ± 0.14 (60)c
30 °C	92.27 ± 1.87 (557)b	50.00 (14)	2.00 ± 0.22 (20)b	1.60 ± 0.21 (20)c	3.25 ± 0.08 (60)d
35 °C	0 (878)	-	-	-	-
Intercept	$\chi^2_1 = 83299.58$		$\chi^2_1 = 404.01$	$\chi^2_1 = 3470.09$	$\chi^2_1 = 11560.10$
Temperature	$\chi^2_4 = 1099.034$	$\chi^2_4 = 2.44$	$\chi^2_4 = 51.40$	$\chi^2_4 = 346.56$	$\chi^2_4 = 2620.99$
P	< 0.001**	= 0.601*	< 0.001**	< 0.001**	< 0.001**

The numbers in brackets represent sample size.

¹Eggs were from twenty different females.

*Pearson χ^2

** GLM Wald χ^2

Means (±SE) within a column followed by the different letters are significantly different (Sequential Bonferroni test, $P < 0.05$).

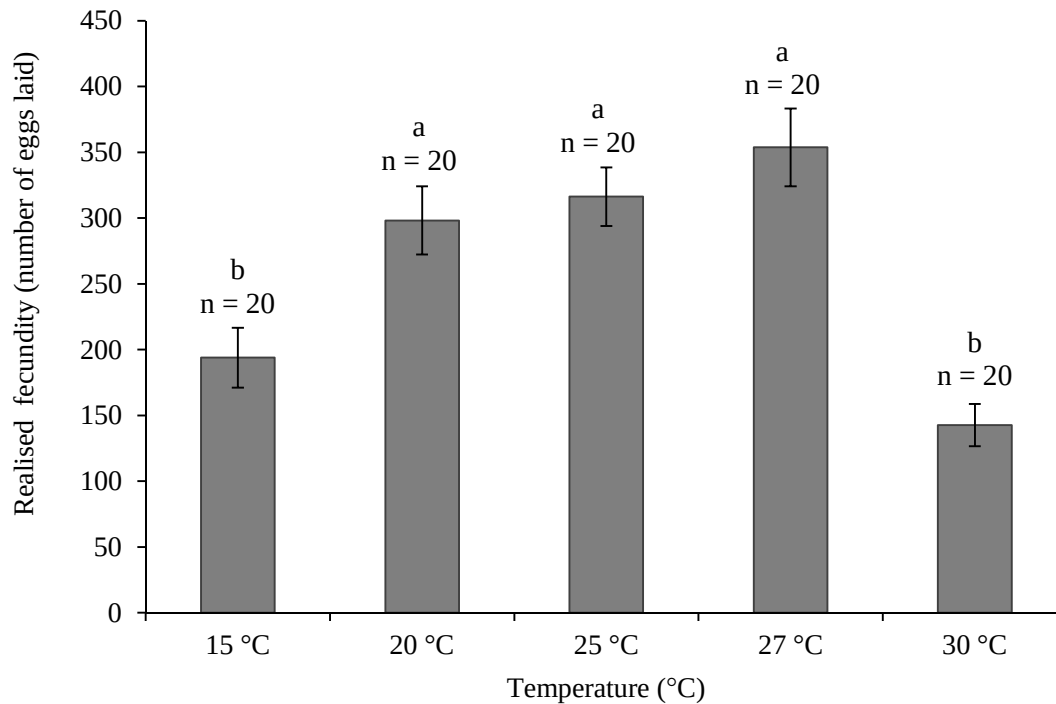


Figure 4.2 Realised fecundity as the number of eggs laid (mean $\pm$ SEM per female) by *Pareuchaetes insulata* reared under different temperature conditions. Means (following GLM Analysis, $\chi^2 = 2312.45$, $P < 0.001$) with different letters are significantly different after Tukey's honestly significant difference (HSD) test, ($P < 0.05$). The figures above the bars represent sample sizes.

4.3.2 Lower developmental threshold (t_0) and the rate of development (K)

The linear regression model and the reduced major axis regression model parameters for each insect stage and all immature stages combined for *P. insulata* are shown in Tables 4.3 a and b and Figure 4.3. There was not much difference in thermal parameters between the two models. For the linear regression model, the lower temperature or developmental thresholds (t_0) varied from 11.58 °C for eggs to 12.08 °C for pupae and for all stages combined (egg-adult) was 11.05 °C, while for reduced major axis model t_0 was 11.29 °C for all stages combined (egg-adult). While the degree-day requirement (K) from egg to adult was 500 days for the linear method, the reduced major axis regression method calculated 491 days.

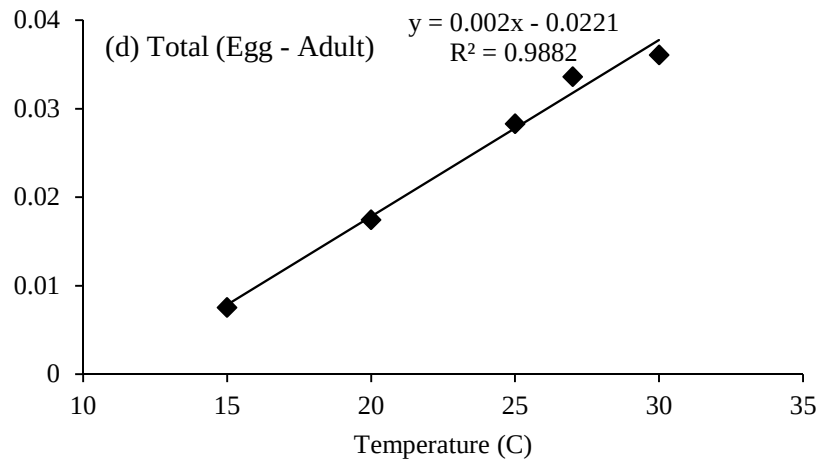
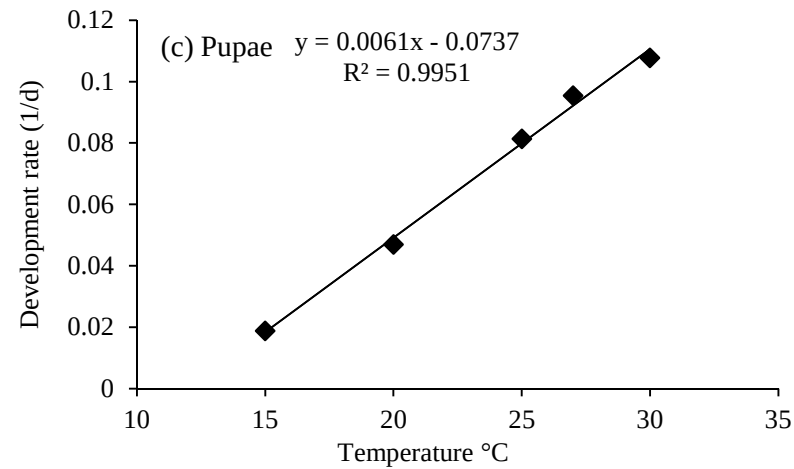
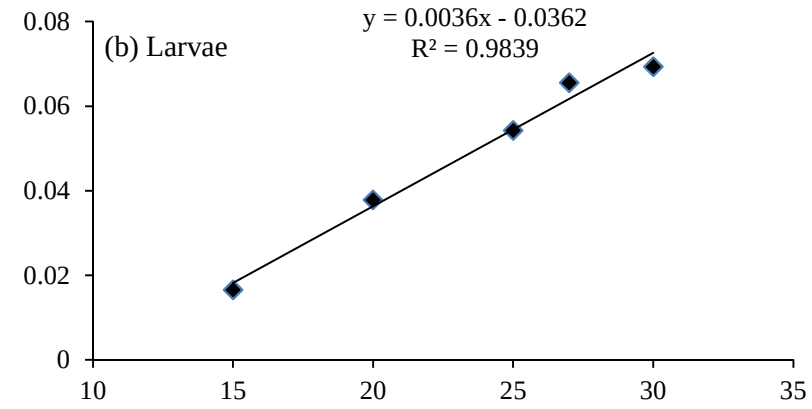
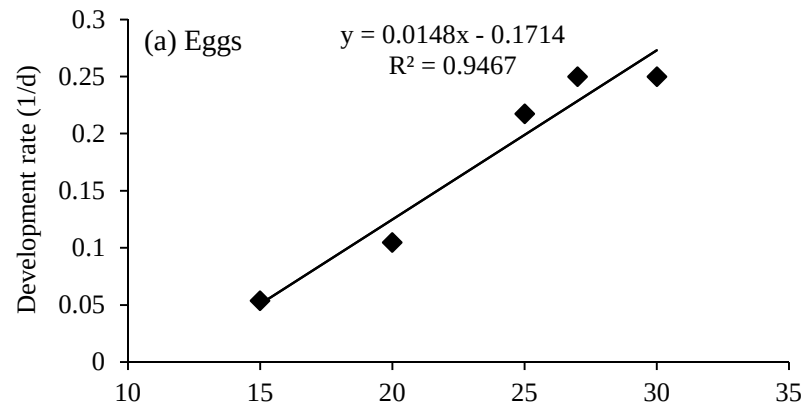


Figure 4.3 Linear relationship between temperature and developmental rate (1/days) of each immature stage (a: eggs; b: larvae; c: pupae and d: total immature stages) of *Pareuchaetes insulata* using the method of Campbell *et al.* (1974).

Table 4.3 (a) Linear regression parameter estimates describing the relationship between temperature and developmental rate (1/d) of *Pareuchaetes insulata* using the method of Campbell *et al.* (1974) and (b) the thermal parameters K and t_0 for *Pareuchaetes insulata* obtained from the reduced major axis method developed by Ikemoto and Takai (2000).

(a)

Stage	Intercept	Slope	R ²	N	t_0	K
Eggs	-0.1714	0.0148	0.947	4144	11.58	67.56
Larvae	-0.0362	0.0036	0.984	143	10.05	277.77
Pupae	-0.0737	0.0061	0.995	143	12.08	163.93
Total (egg – adult)	-0.0221	0.0020	0.988	143	11.05	500.48

(b)

Stage	Equation	t_0	K	N	Regression coefficient
Eggs	DT = 11.567x + 68.361	11.56	68.36	4144	F ₁ = 225.93, R ² = 0.987, P < 0.001
Larvae	DT = 10.621x + 261.710	10.62	261.71	143	F ₁ = 750.95, R ² = 0.996, P < 0.001
Pupae	DT = 11.965x + 163.681	11.96	163.68	143	F ₁ = 4837.03, R ² = 0.999, P < 0.001
Total (egg – adult)	DT = 11.293x + 491.943	11.29	491.94	143	F ₁ = 2084.09, R ² = 0.998, P < 0.001

D = Duration of development

T = Temperature (°C)

t_0 = Lower temperature threshold

K = Thermal constant

4.3.2.1 Degree-day accumulation and number of generations of *Pareuchaetes insulata*

The number of generations that *P. insulata* is capable of producing in one year in areas where *C. odorata* is invasive in South Africa was estimated from the thermal parameters obtained from the reduced major axis regression method and the climate data obtained from the CLIMEX model database. In KZN province where *P. insulata* is present, the moth is capable of producing a minimum of 2.5 and a maximum of 7 generations per year, whereas in other regions such as Mpumalanga and Limpopo provinces, the number of generations varied between 1 and 9 per year (Figure 4.4).

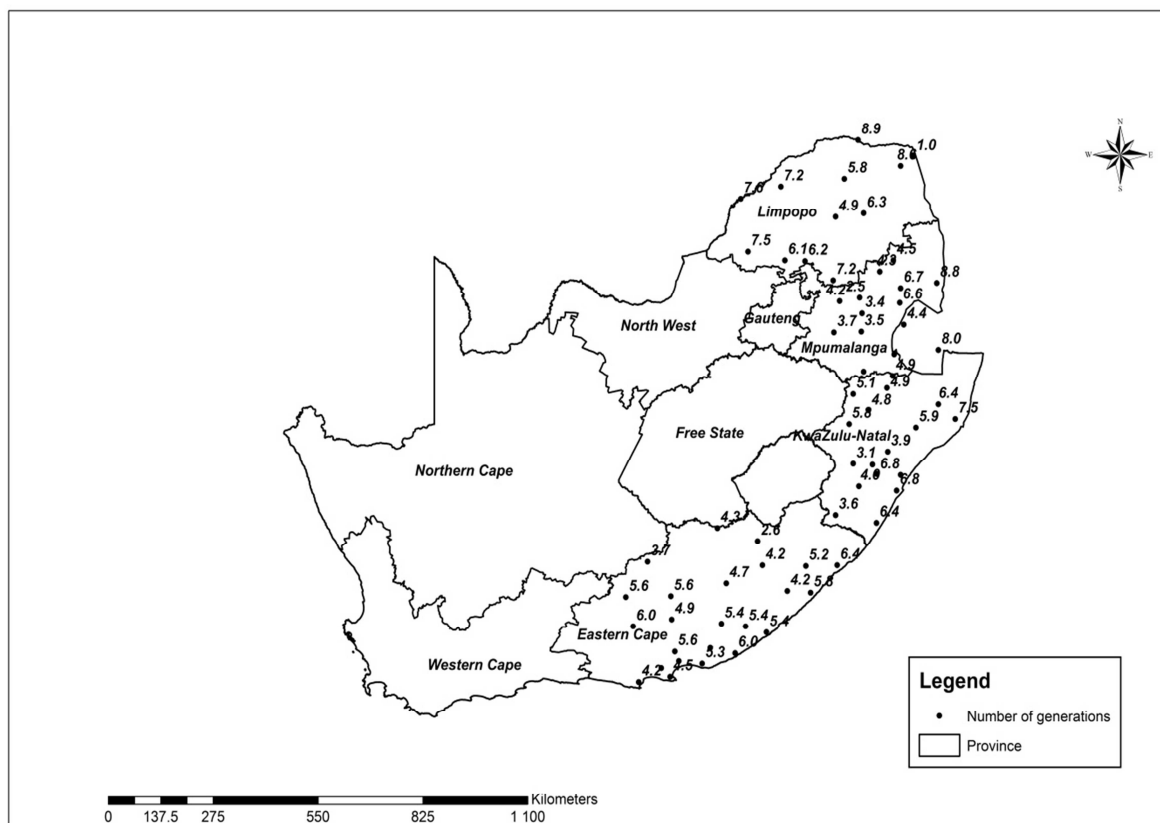


Figure 4.4 Number of generations that *Pareuchaetes insulata* is capable of producing per year in *Chromolaena odorata*-infested provinces in South Africa, estimated from the recorded reduced major axis regression model and meteorological data from 129 South African weather stations.

4.3.3 Thermal tolerance (effects of lethal temperature and exposure time on survival)

The temperature, and the time period that *P. insulata* was exposed to it, significantly affected their survival at either high (ULT) or low (LLT) temperatures (Table 4.4). An increase in severity of exposure (or longest exposure time) at low or high temperatures resulted in increased mortality (Figures 4.5 and 4.6).

Table 4.4 Generalized linear model (GLM) results for effects of temperature, time, life stage and all interactions on higher and lower lethal limits in *Pareuchaetes insulata*. Following arcsine square root transformation, normal distributions with an identity link function were assumed.

Effect	d.f.	Wald χ^2	P
Upper lethal temperature (ULT)			
Intercept	1	2140.14	0.0001
Temperature	8	412.31	0.0001
Time	3	94.31	0.0001
Life stage	1	0.08	0.771
Temperature x time	24	122.26	0.0001
Temperature x life stage	8	0.21	0.996
Time x life stage	3	0.27	0.964
Temperature x time x life stage	16	1.07	0.998
Lower lethal temperature (LLT)			
Intercept	1	1608.76	0.0001
Temperature	8	335.24	0.0001
Time	3	47.55	0.0001
Life stage	1	5.64	0.018
Temperature x time	24	48.84	0.002
Temperature x life stage	8	6.14	0.632
Time x life stage	1	0.18	0.894
Temperature x time x life stage	8	1.03	0.998

Statistically significant values are indicated in bold.

Likewise, an increase in the duration of exposure at any given temperature resulted in a reduction in *P. insulata* survival (Figures 4.5 and 4.6). Life stage significantly affected survival in the LLT trials, but did not in the ULT trials (Table 4.4). The interaction of temperature and the duration of exposure was highly significant resulting in shorter periods of time required to inflict 100% mortality at extremely severe low or high temperatures, suggesting limited plasticity of survival in these trials (Table 4.4; Figures 4.5 and 4.6).

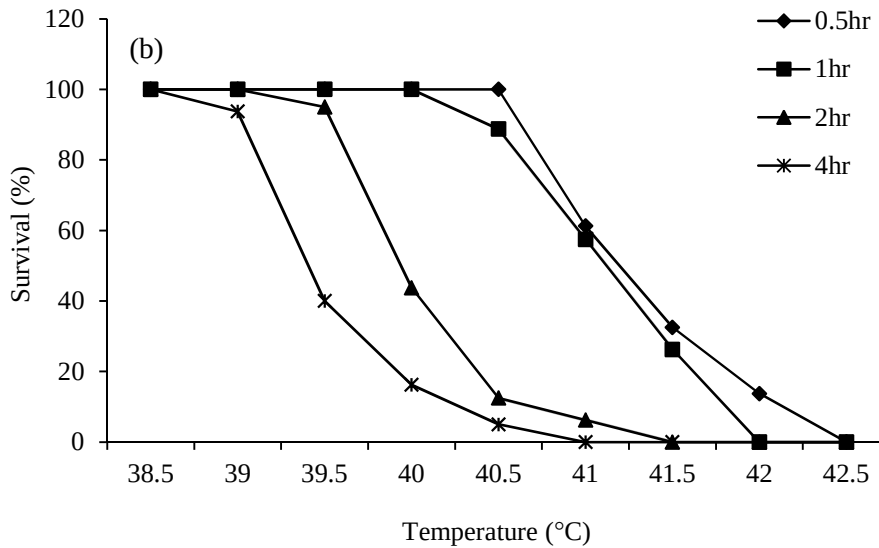
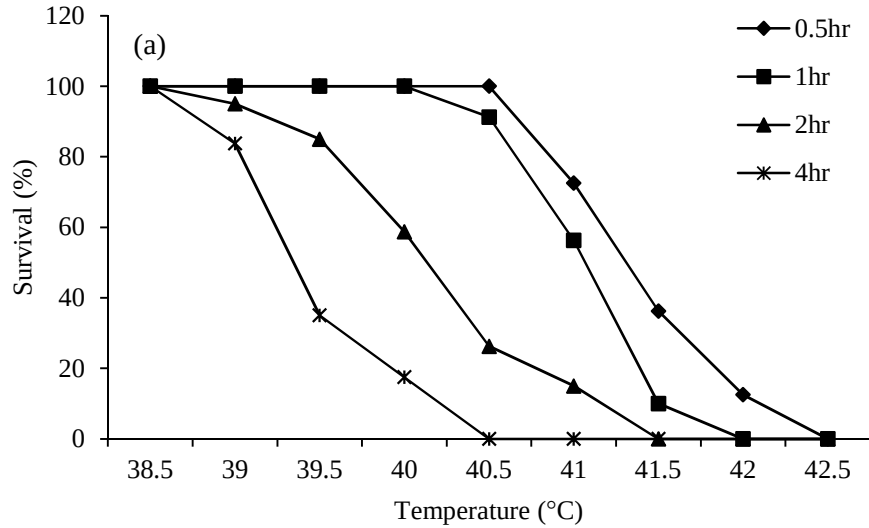


Figure 4.5 Mean survival of *Pareuchaetes insulata* (adults and larvae [a and b]) exposed to different high temperatures for four exposure durations. Each symbol is a mean of ten replicates of 4 individuals per replicate ($n = 40$ per symbol). Note that 0.0 hour treatments (handling controls) experienced no mortality during these experiments (data not shown).

Based on the results of the LLT and ULT trials, the temperatures estimated to cause 50% mortality (LT_{50}) were calculated (Table 4.5). In the ULT trials, LT_{50} for each life stage decreased with extended exposure time (Table 4.5). Exposure to 40.1 °C and 40.0 °C for 2 hours respectively caused 50% mortality of adults and larvae. LT_{50} for each life stage in the LLT trials fell as the exposure time increased. When third instar larvae were exposed for 0.5,

1, 2 and 4 hours, the LT_{50} values were -8.1 , -6.5 , -5.9 and -4.3 °C respectively. When adults were exposed for 2 hours, LT_{50} value was -4.7 °C.

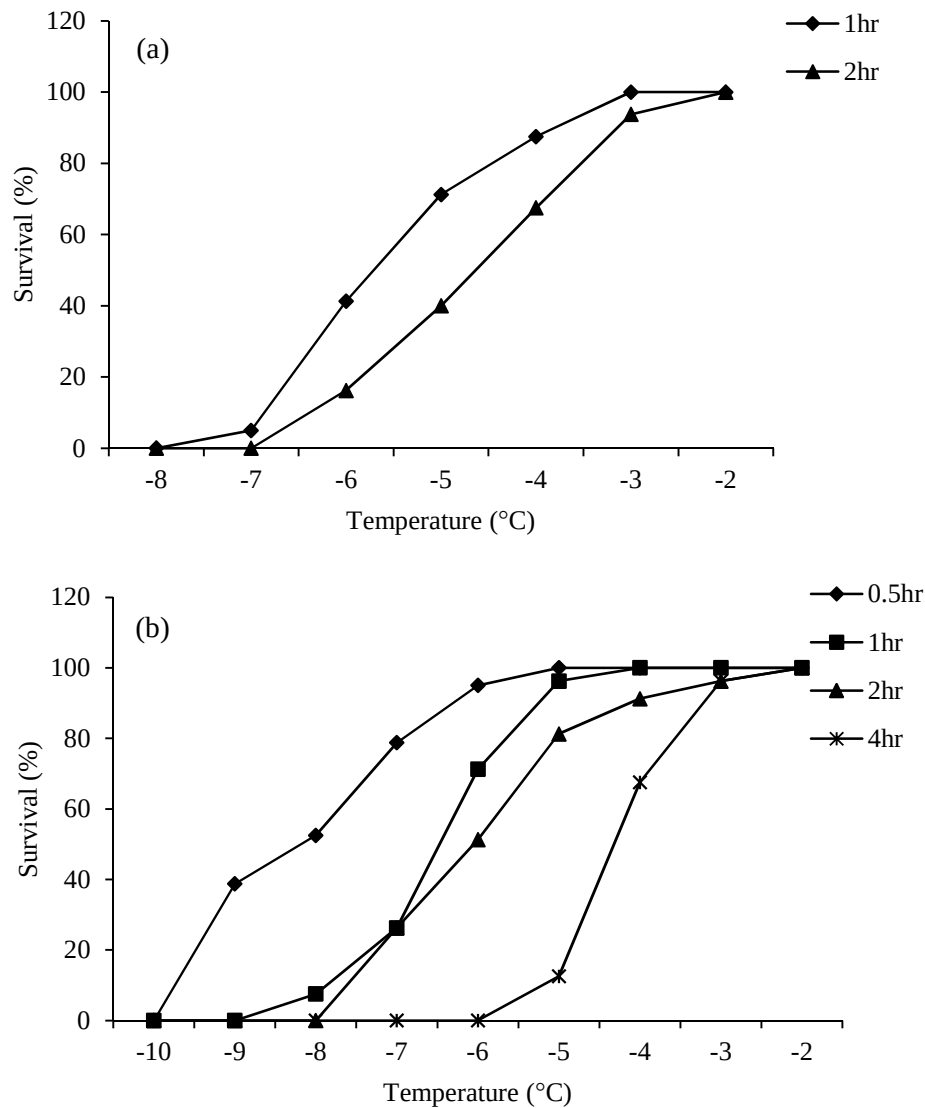


Figure 4.6 Mean survival of *Pareuchaetes insulata* (adults and larvae [a and b]) exposed to different low temperatures for four exposure durations. Each symbol is a mean of ten replicates of 4 individuals per replicate ($n = 40$ per symbol). Note that 0.0 hour treatments (handling controls) experienced no mortality during these experiments (data not shown). Data are not available for adult exposure at 0.5 and 4 hours because of the huge numbers of adults needed for these experiments.

Table 4.5 Higher lethal temperatures (LT₅₀) and lower lethal temperatures (LT₅₀) of *Pareuchaetes insulata* when exposed to a range of temperatures for various durations.

Exposure (hours)	Life stage	LT ₅₀ (°C)	95% confidence interval
Higher lethal temperatures (LT ₅₀)			
0.5	Adult	41.4	41.1 – 41.7
	larva	41.2	40.9 – 41.6
1	Adult	41.3	40.9 – 41.8
	Larva	41.4	41.0 – 41.8
2	Adult	40.1	39.7 – 40.4
	Larva	40.0	39.8 – 40.6
4	Adult	39.2	39.0 – 39.5
	Larva	39.5	39.2 – 39.8
Lower lethal temperatures (LT ₅₀)			
0.5	Adult	¹	¹
	Larva	– 8.1	– 8.8 to – 7.5
1	Adult	– 5.5	– 6.1 to – 4.9
	Larva	– 6.5	– 7.1 to – 5.9
2	Adult	– 4.7	– 5.3 to – 4.1
	Larva	– 5.9	– 6.7 to – 5.3
4	Adult	¹	¹
	Larva	– 4.2	– 4.8 to – 3.8

¹Data are not available for adult exposure at 0.5 and 4 hours.

Note that data presented here are analyses from upper lethal temperature (ULT) and lower lethal temperature (LLT) trials. For both ULT and LLT trials, insects were exposed to a range of temperatures (38.5 to 42.5 and -10 to -2 for ULT and LLT respectively).

4.3.4 Locomotion performance

Cold-acclimated third instar larvae of *P. insulata* had a higher level of locomotor activity than warm-acclimated larvae when exposed to low temperatures. When both groups of acclimated larvae were placed on the locomotion stage set at 11 °C, only 68.2 ± 4.7% of warm-acclimated larvae dispersed at the set temperature compared with 100.0 ± 0.0% for cold acclimated larvae (Pearson $\chi^2 = 38.10$, d.f. = 1, $P < 0.001$; Figure 4.7a). The greater mobility of cold acclimated larvae was evident not only in the frequency of individuals capable of movement but even more clearly in the duration of walking activity for cold-acclimated larvae (Figure 4.7b). When both groups of acclimated larvae were exposed to 11 °C, cold-acclimated larvae spent 70.8% (21.24 ± 1.15 seconds) of their time moving compared with 43.8% (13.16 ± 1.03 seconds) for warm-acclimated larvae ($t = 5.30$, d.f. = 8,

$P < 0.001$; Figure 4.7b). Similarly, cold-acclimated larvae spent more time walking when both group of acclimated larvae were exposed to 15 °C; cold-acclimated larvae spent more (95.2%) time moving compared with the warm-acclimated ones (84.6%) ($t = 4.06$, d.f. = 8, $P < 0.004$; Figure 4.7b).

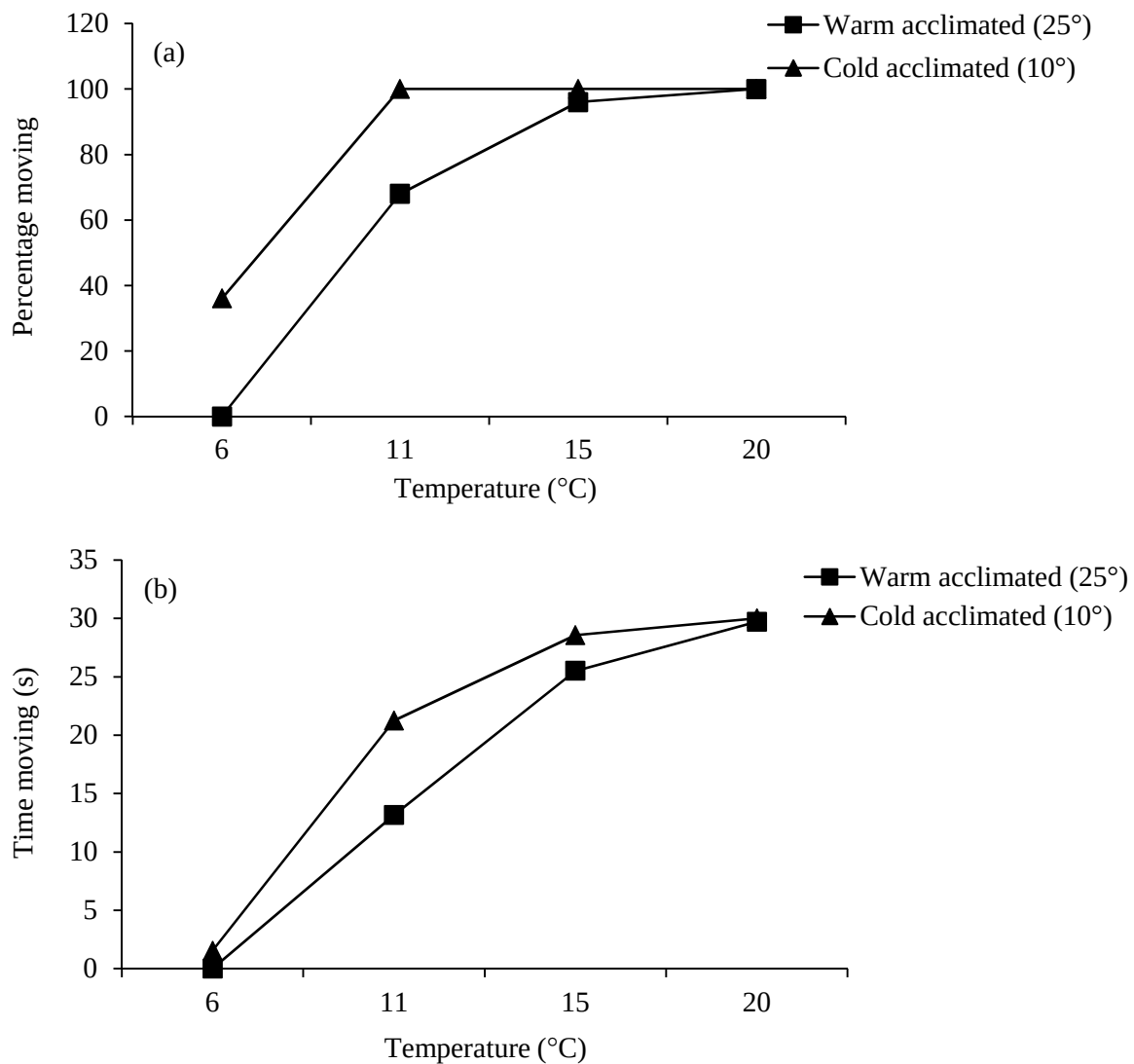


Figure 4.7 Comparative mobility of third instar larvae of *Pareuchaetes insulata* acclimated to warm (reared at 25 °C) or cold (at 10 °C) for 2 days after consecutive 30-second exposure periods to 6, 11, 15 and 20 °C on a temperature-controlled locomotion stage. Five individuals were exposed to each temperature and the test was replicated five times. The mean total number (%) of individuals moving (a) and the mean total time spent moving (b) for each treatment at each temperature are presented.

4.3.5 Microclimate data

Temperatures significantly differed between microsites (Table 4.6). In February, September and in winter months (June, July and August), the suspended iButtons recorded significantly higher temperatures compared to those placed at near ground levels (Table 4.6).

4.4 DISCUSSION

Of a suite of climate factors, temperature is perhaps the single most important environmental factor affecting insect distribution, behaviour, survival, reproduction (Gilbert and Raworth, 1996; Bale *et al.*, 2002; Fantinou *et al.*, 2004; Chidawanyika and Terblanche, 2011; Tamiru *et al.*, 2012; Ferrer *et al.*, 2014) as well as the establishment and performance of biological control agents in their introduced ranges (e.g. McClay and Hughes, 1995; Byrne *et al.*, 2002; Bale, 2011; May and Coetzee, 2013). This study documents the effect of constant temperatures on the life history traits of *P. insulata* and predicts the numbers of generations the moth is capable of producing in the eastern parts of South Africa. Additionally, the effects of temperature and acclimation on locomotion performance as well as the response of the moth to extreme high and low temperatures were investigated. The study was undertaken as part of an effort to understand the low population abundance and poor performance of *P. insulata* in South Africa.

4.4.1 Survival, developmental and reproductive life history traits

Survival and development durations of *P. insulata* were negatively impacted at temperatures below 25 °C, which has potentially important implications for larval populations of this insect during the cool sub-tropical winter season where absolute minimum and maximum

temperatures range between 4.6 and 7.6 °C and 23.6 and 38.1 respectively (mean $\pm$ SE; 15.75 $\pm$ 0.04 °C) (see Table 4.6) at the only known established site in South Africa.

Table 4.6 Summary results for temperatures recorded using hygrochron iButtons (0.5 °C accuracy; 1 hour sampling frequency) between June 2013 and May 2014 at three locations within *Chromolaena odorata*-infested areas in Sappi Cannonbrae Plantation, Umkomaas, South Africa where *Pareuchaetes insulata* established following its release (between 2001 and 2003). At each location, two iButtons were placed in two microsites; one was placed at near ground level (3.5 cm above ground level), while the other was suspended within *Chromolaena odorata* thickets (60 cm above ground level). The absolute minimum (Min), absolute maximum (Max) and mean ($\pm$ SE) temperatures are given in °C per microsite. Means are average values from all three sites, all days, all records.

Months (2013-2014)	Ground level			Suspended			Statistics	
	Min	Max	Mean	Min	Max	Mean	Test	P
2013								
June	6.1	23.6	14.7 $\pm$ 0.1	4.6	37.1	15.1 $\pm$ 0.1	U = 2159101.0	<0.001
July	7.6	24.4	15.6 $\pm$ 0.1	6.6	28.1	15.9 $\pm$ 0.1	U = 2213610.0	<0.001
August	6.6	28.6	16.5 $\pm$ 0.1	5.1	38.1	17.0 $\pm$ 0.1	U = 2275364.0	<0.001
September	5.1	32.1	18.0 $\pm$ 0.1	3.6	35.1	18.2 $\pm$ 0.1	U = 2214153.0	0.004
October	10.0	33.6	19.0 $\pm$ 0.1	9.1	36.6	19.1 $\pm$ 0.1	U = 1208503.0	0.074
November	15.1	36.6	21.8 $\pm$ 0.1	14.1	33.1	21.5 $\pm$ 0.1	U = 257886.0	0.868
December	17.1	35.6	22.3 $\pm$ 0.1	16.1	32.6	22.0 $\pm$ 0.1	U = 272588.0	0.614
2014								
January	20.1	38.6	24.9 $\pm$ 0.1	18.6	33.6	24.9 $\pm$ 0.1	U = 266102.0	0.198
February	18.6	40.1*	24.8 $\pm$ 0.1	17.6	34.1	24.9 $\pm$ 0.1	U = 208501.0	0.015
March	16.6	38.1	23.6 $\pm$ 0.1	16.1	35.1	23.5 $\pm$ 0.1	U = 269325.0	0.369
April	9.6	42.1*	19.8 $\pm$ 0.2	11.5	30.1	19.6 $\pm$ 0.1	U = 244201.0	0.564
May	9.1	30.2	17.5 $\pm$ 0.2	11.0	23.0	17.3 $\pm$ 0.1	U = 276060.0	0.457

Asterisks indicate temperatures above, or that approached the higher lethal temperatures (LT₅₀) for both larvae and adults.

Over longer periods (5 years of data obtained from South Africa Weather Service), monthly average minima were 10.2, 9.4 and 11.1 °C in June, July and August respectively, across *C. odorata*'s South African range of distribution (Figure 4.8). This further substantiates the view that low temperatures may affect the survival and development of *P. insulata* in the field at certain times of the year. In laboratory rearing studies, survival of *P. insulata* larvae to adults at 15 °C was only 10%, which was eight-fold lower than at 27 °C, and development took almost four times longer. These findings support the predictions that low temperatures are likely to restrict the establishment and distribution of some biological control agents (e.g. May and Coetzee, 2013).

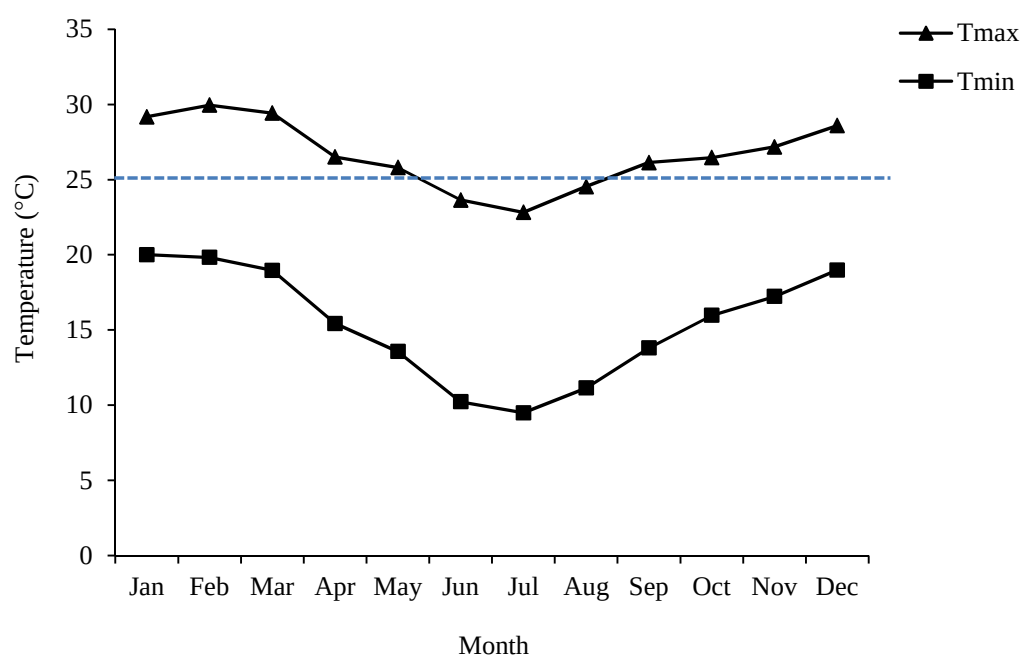


Figure 4.8 Summary of five years (2008 to 2012) of weather data per month averaged over eight locations across *Chromolaena odorata*-infested areas in Limpopo, Mpumalanga and KwaZulu-Natal provinces in South Africa. Data were obtained from the South African Weather Service. Tmin: monthly average minimum temperature; Tmax: monthly average maximum temperature. The broken line in the middle of the graph indicates that constant temperatures below 25 °C negatively affected survival and development of *Pareuchaetes insulata* in laboratory experiments.

In addition to a direct negative impact of low winter temperatures on survival of *P. insulata* larvae and eggs, it is not impossible that larvae will also suffer an indirect negative impact in the form of increased mortality resulting from slowed development and increased exposure time to predators and parasitoids. The eggs of, *P. pseudoinsulata* were recorded to be attacked by predatory ants in South Africa (Kluge, 1994). Other studies have documented that predation can be the major source of mortality for lepidopteran larvae (Feeny *et al.*, 1985) and that larvae are at risk of predation during movement (Marston *et al.*, 1978; Bergelson and Lawton, 1988) and feeding (Bernays, 1997). Also, Cruttwell (1972) found that daytime predation and parasite attack was the sole reason for *P. pseudoinsulata* larvae ceasing feeding, leaving the leaves and hiding at ground level during the daytime. *Pareuchaetes insulata* larvae show the same behaviour, probably for similar reasons. Furthermore, low winter temperatures can increase the nitrogen content in some plants as is demonstrably evident in *C. odorata* (see Chapter 5). This has been suggested to have negative consequences for the populations of the weed's herbivore, *P. insulata* (Chapter 5), as there might be a fitness cost associated with excess nitrogen in the diet of insect herbivores (Lee *et al.*, 2002; Boersma and Elser, 2006; Clissold *et al.*, 2006). The longer larval development time for females, longer pupal development time for males and the longer total development time for males recorded in this study has been previously documented (Uyi *et al.*, 2014d; Chapter 2)

Although the reduced fecundity at 15 °C (82% less than at 27 °C) has not been previously documented for this insect, temperature has been shown to influence fecundity in lepidopterans (e.g. Tamiru *et al.*, 2012) including *P. pseudoinsulata* (Visalakshy, 1998). The increased longevity at 15 °C did not amount to increased fecundity.

4.4.2 Lower developmental threshold (t_0), the rate of development (K) and number of generations

Insect development may vary at fluctuating temperatures found in natural environments (Brakefield and Mazzotta, 1995). However, biological interpretations obtained from linear and non-linear models using constant temperatures have been shown to approximate fluctuating temperature conditions (e.g. Worner, 1992). The reduced major axis regression model estimated a lower development threshold of 11.3 °C and 492 degree-days (K) for *P. insulata* to complete development (from egg to adult). Based on the above thermal parameters, the number of generations *P. insulata* is capable of producing per year range between 2.5 and 7 in different locations in KZN while in other provinces (Eastern Cape, Limpopo and Mpumalanga), the number of generations varied between 1 and 9 per year, indicating the existence of favourable conditions for both the establishment and spread of this agent in South Africa. However the moth failed to establish at a number of release sites in KZN province (see Zachariades *et al.*, 2011a). The reasons for this might be due to the direct or indirect effects of temperature, food quality and/or top-down forces (e.g. predation and parasitism) experienced by this insect in the field. Although thermal parameters such as lower development threshold and degree-days are important for predicting insect establishment and distribution (Manrique *et al.*, 2008; May and Coetzee, 2013), models incorporating other thermal parameters such as the locomotion performance and feeding rates of insects at different temperatures may provide us with more information on the actual thermal requirements for insects used as biological control agents. Bottom-up effects (through host plant quality) might also be responsible for the poor performance of a biological control agent in its introduced range, although this is not often appreciated by biological control practitioners (Price, 2000).

4.4.3 Thermal tolerance (effects of lethal temperature and exposure time on survival)

This study investigated the survival of adults and larvae of *P. insulata* under varying low or high temperatures of different short durations and found that both the magnitude of temperature variation (from 25 to – 10 °C and from 25 to 43 °C) and duration of exposure were important in determining the survival of the moth, as might be expected for insects in general (Chown and Terblanche, 2007; Chidawanyika and Terblanche, 2011; Li *et al.*, 2011). Over a 2-hour period for example, 50% of adult populations would be killed by temperatures of 40.1 °C or – 4.7 °C, while 50% of larval populations would be killed by temperatures of 40.0 °C or – 5.9 °C. By contrast, the high temperatures which would be lethal for 50% of adults and larvae were respectively 41.4 and 41.2 °C for 4-hour exposure. While low temperature is considered the most important abiotic factor that governs the year-to-year abundance and geographic distribution of insects (Coulson and Bale, 1991; Bale, 2002), insects are sensitive to high temperatures because of their variable body temperature and their small bodies. High extreme temperatures have been linked with water loss, disruption of structure of membranes (Yoder *et al.*, 2009; Hochachka and Somero, 2002) and denaturation of protein, which restrict enzyme-catalysed reactions (Chown and Nicholson, 2004). Meanwhile, thermal stress is known to cause neuronal damage (Chown and Terblanche, 2007).

One important question of ecological significance that arises from the findings of this research is whether adults or larvae of *P. insulata* are likely to die from thermal stress in the field. This can be addressed by combining the microclimate temperature data with thermal tolerance estimates recorded in the laboratory experiments. The one-year high frequency microclimate temperature data recorded at two microsites (suspended and ground level) in Sappi Cannonbrae Plantation, Umkomaas, South Africa ranged from 3.6 to 38.1 °C and 5.1 to

42.1 °C for the suspended and ground level microsites respectively. This microclimate temperature data suggests that temperatures potentially causing low temperature mortality never really occurred; neither did they even approach lethal levels at any duration. On the contrary, the high temperatures recorded at near-ground levels fell within the range of lethal high temperatures (Figure 4.6). For example, absolute temperatures recorded in the months of February and April 2014 were 40.1 and 42.1 respectively for a period of not more than 3 hours, suggesting that higher temperatures are more likely to cause direct larvae mortality as compared to low temperatures during winter months particularly for this South African location. Although the thermal tolerance of pupae of this insect was not studied, it is hypothesized that they would suffer significant mortality at temperatures higher than 39.2 °C or 39.5 °C, the temperature at which 50% adult or larval mortality would occur when exposed for four hours. From the five years of temperature data obtained from the South African Weather Service, absolute minima and maxima recorded in eight locations respectively averaged 2.9 (in winter) and 34.5 °C (in summer) further corroborating the hypothesis that minimum temperatures are not likely to be lethal to *P. insulata*, although these temperatures (below 6 °C) can seriously affect the locomotion abilities of larvae (see discussion below) especially as they are nocturnal feeders (adults are also only active at night).

The larvae of this moth appeared to be cold-tolerant compared to adults, suggesting larvae can survive more extreme cold conditions. Although the ecological significance of this remains to be seen, several other studies have reported the thermal tolerance in insects to vary significantly among life stages (Marhoof *et al.*, 2003; Jensen *et al.*, 2007; Marais *et al.*, 2009). However, exceptions do exist, such as in the sub-Antarctic *Apetaenus littoralis* Eaton (Diptera: Canacidae) (Klok and Chown, 2000), where no differences among life stages have been recorded.

4.4.4 Locomotion performance

Beyond behavioural adjustments, insects use physiological and biochemical mechanisms to cope with temperature variations in their environments (Cossins and Bowler, 1987; Hochachka and Somero, 2002) and such responses are considered as ‘acclimation’ in the laboratory or ‘acclimatization’ in the field (Lachenicht *et al.*, 2010). Weldon *et al.* (2011) define acclimation as the rapid and reversible change in phenotype (be it physiological, biochemical or anatomical) in response to chronic exposure to a new environmental condition, and these plastic changes are often beneficial for the survival of insects in a changing environment (e.g. Terblanche *et al.*, 2006).

When larvae were placed on the locomotion stage set 6 °C, none of the warm-acclimated individuals were able to initiate movement, while only 36% of the cold-acclimated individuals dispersed, walking for only 2 seconds of the 30 seconds’ exposure time. Similarly, only 68% of warm-acclimated larvae dispersed when exposed to 11 °C compared to the 100% dispersal for cold-acclimated individuals. Furthermore, warm-acclimated and cold-acclimated larvae respectively spent 43% and 71% of their time moving at 11 °C. These results support the beneficial acclimation hypothesis and other studies that reported improvement in locomotion (or flight) abilities in mites and insects due to acclimation (e.g. Deere and Chown, 2006; Marais and Chown, 2008; Lachenicht *et al.*, 2010). Across insect taxa, acclimation to temperature is most pronounced at the lower temperature threshold, while there is little flexibility at the upper thermal limit (Klok and Chown, 2003; Slabber and Chown, 2005; Chown and Terblanche, 2006; Terblanche and Chown, 2006), although exceptions to this trend have been found in several species including moths (Klok and Chown, 1998; Sinclair and Chown, 2003).

Low temperature significantly affected the locomotor abilities of *P. insulata* larvae and this is likely to be of serious ecological significance in the field during winter months – as the absolute minimum temperature in Sappi Cannonbrae Plantation (one year of microclimate temperature data) Umkomaas during winter ranged between 3.6 and 4.6 for the suspended microsite (Table 4.6). This suggests that *P. insulata* larvae would be unable to move or feed in winter when the temperature falls below 6 °C, thereby preventing escape from indigenous natural enemies (that are more cold-tolerant) or promoting larval starvation. When larvae are dislodged from the foliage of *C. odorata* plants (either by wind or other factors), the situation may exacerbate further – as attempts to locate its host plant (to either initiate feeding or seek shelter) might prove unsuccessful. This might lead to an increase in indirect mortality and consequently affect the populations of this biological control agent in field situations. Although the effect of temperature on flight performance was not investigated, low temperature is likely to affect the flight capacity of this insect because adults are more sensitive to low temperatures compared to larvae (see results and discussion in thermal tolerance section). In order to fully elucidate the effect of temperature on the performance of *P. insulata*, further studies on the flight and mating abilities of the insects under variable low and high temperatures are clearly needed.

4.5 CONCLUSION

As is usual with insects which are ectothermic, this study showed that temperature significantly affects development time, mortality and reproductive life history traits in *P. insulata*. The survival and development time were negatively impacted at constant temperatures below 25 °C. It has been shown that *P. insulata* is predicted to be capable of producing between 1 and 9 generations per year in the weed's distribution range in South

Africa. One of the significant observations from these experiments was that thermal fluctuations are likely to play a role in the survival of both adults and larvae of *P. insulata*, especially at high temperatures. Therefore, the high temperatures potentially experienced by *P. insulata* during summer or autumn are likely to negatively impact on the population of this insect in the field, although such a speculation requires further information regarding the use of microclimates and behavioural thermoregulation. Furthermore, third instar larvae of the moths were unable to initiate movement at 6 °C and locomotion abilities were reduced at 11 °C. Although low temperature seem unlikely to be a direct cause of mortality in this species, low environmental temperature may still contribute to reducing population abundance due to reduction in development rates, fecundity, locomotion and other activities. For example, larvae may suffer indirect negative effects in the form of increased mortality resulting from slowed development, increased exposure to natural enemies, and inability to move or feed. In sum, it is hypothesized that both direct and indirect negative impacts of low temperature may partly explain the poor performance of *P. insulata* in parts of South Africa.

CHAPTER 5

The effects of seasonal and spatial variation in the leaf characteristics of an invasive alien shrub, *Chromolaena odorata* (L.) on the performance of *Pareuchaetes insulata*

5.1 INTRODUCTION

The physiology, anatomy and phytochemistry of plant species often vary according to microhabitat, prevailing environmental conditions or seasonality (Bryant *et al.*, 1983; 1987; Herms and Mattson, 1992; Wang *et al.*, 2003; Migita *et al.*, 2007; Zhang and Wen, 2009; Zhang *et al.*, 2009; Diaz *et al.*, 2011; Muller *et al.*, 2011) and the understanding of how phytophagous insects respond to these changes is central to our knowledge of insect life-history evolution (Wolda, 1978; Janzen, 1987; Wolda, 1988; Jansen and Stamp, 1997; Sipura and Tahvanainen, 2000; Gripenberg *et al.*, 2010; Münzbergová and Skuhrovec, 2013).

In a field situation, individuals within plant populations may be exposed to different levels of resources such as sunlight, nutrients and water. For example, light intensity varies between open and shaded habitats, resulting in changes in plant characteristics (chemical or physical) and/or physiology. Light intensity directly influences growth, development and biochemical activities in plants due to the crucial role of sunlight and carbon during photosynthesis (Wang *et al.*, 2003; Roberts and Paul, 2006; Zhang and Wen, 2009). The theory of resource allocation of plants in relation to balance between carbon and mineral nutrients predicts that the leaves of plants growing under sub-optimal levels of photosynthetically active radiation (e.g. shade habitats) should contain relatively more mineral nutrients (especially nitrogen) and relatively less carbon-based secondary (defence) compounds compared to plants growing in direct sunlight (Bryant *et al.*, 1983; 1987; Herms and Mattson, 1992).

Studies that tested the carbon nutrient balance hypothesis in terms of the preference and performance of herbivores in shaded and open habitats, either as a main aim or a sideline, suggests that herbivore responses are not always straightforward and appear to be species specific. For example, some laboratory and field studies have documented improved immature survival, rapid development time, increased pupal mass and high fecundity in insects that fed on shaded foliage (Bultman and Faeth, 1988; Trumbule and Denno, 1995; Jansen and Stamp, 1997; Sipura and Tahvanainen, 2000; Diaz *et al.*, 2011), while others show some of these variables to be greater in insects fed on leaves of plants growing in direct sunlight (Bultman and Faeth, 1988; Moore *et al.*, 1988; Sipura and Tahvanainen, 2000; Moran and Showler, 2005; Osier and Jennings, 2007). Still others have reported no differences in herbivore performance on shade versus full sun foliage (Moore *et al.*, 1988; Horner and Abrahamson, 1992; Potter, 1992; Franca and Tingey, 1994).

Plant traits and leaf characteristics (including foliar nutrients) typically exhibit temporal variation over time (Migita *et al.*, 2007; Zhang *et al.*, 2009; Muller *et al.*, 2011; Flaherty *et al.*, 2013) and this may in turn affect the performance of associated herbivores (Scriber and Slansky, 1981; Mattson and Scriber, 1987; Slansky, 1993; Alonso and Herrera, 2000; Osier *et al.*, 2000; Ishihara and Ohgushi, 2006). Despite reports that spatial and temporal variations in abiotic factors or conditions have been found to affect the abundance and performance of herbivores both directly (e.g. Shreeve, 1986; Byrne *et al.*, 2002, 2003; Sipura and Tahvanainen, 2000; Chidawanyika and Terblanche, 2011) and indirectly through host plant quality (e.g. Jansen and Stamp, 1997; Ishihara and Ohgushi, 2006; Diaz *et al.*, 2011), studies that have explicitly and simultaneously assessed the relative roles of indirect influences of abiotic factors on host plant and on herbivore performance in space and time are still scarce (but see Flaherty *et al.*, 2013). Therefore understanding how a specialist herbivore such as *P.*

insulata responds to seasonal and spatial variations in the quality of its host plant, would not only advance our knowledge of the nutritional ecology of this erebid moth but may also help to explain its low abundance or variable population levels in the field following its establishment at one site after large populations of the insect were released at some 30 sites in KZN province, South Africa between 2001 and 2008.

One of the important plant characteristics that may vary seasonally or spatially is foliar nitrogen. Although the role of nitrogen and water in insect survival, development, reproduction and abundance have been extensively studied (Mattson, 1980; Myers and Post, 1981; van der Meijden *et al.*, 1984, 1989; Minkenberg and Ottenheim, 1990, Heard and Winterton, 2000; Hinz and Müller-Schärer, 2000; Henriksson *et al.*, 2003; Wheeler, 2003; Ricklefs, 2008; van Hezewijk *et al.*, 2008; Münzbergová and Skuhrovec, 2013), the relative importance of other nutrients such as phosphorus, magnesium, potassium and calcium are only beginning to be explored (e.g. Huberty and Denno, 2006; Joern *et al.*, 2012; Ge *et al.*, 2013) partly because a combination of nutrients or a balanced diet may be more important for the development and reproduction of insects.

Understanding how habitat conditions (e.g. light conditions) and season influence the chemical and physical traits in *C. odorata* and how its biological control agent *P. insulata* responds to these traits is particularly important for the biological control of weeds because insect response to, or performance on, the target weed will determine the level of control in the country of introduction.

Anecdotal reports and field observations suggest that leaf-feeding moths of the genus *Pareuchaetes* are more abundant and prefer to feed on shaded leaves of *C. odorata* plants in

shaded habitats or wetland environments (Cock and Holloway, 1982; D. Conlong, pers. comm.; pers. obs.) probably because of the higher nutrient (e.g. nitrogen) levels of plants in such environments (Bryant *et al.*, 1983, 1987), although no empirical data exist to validate this conjecture. Most studies on the indirect effects of microhabitat conditions (through host plant quality) on insect performance have examined such effects at only one point in time (e.g. Sipura and Tahvanainen, 2000; Diaz *et al.*, 2011). However, insect herbivores may exhibit differential performance when fed on food from different habitats (e.g. Diaz *et al.*, 2011) and this performance may vary during the course of the year, with a particular habitat providing a better food resource at one time, and another habitat providing a better food resource at a later time. Therefore, this study examined the effects of habitat conditions (shade versus full sun) on the leaf characteristics of *C. odorata* plants during winter versus autumn months in order to determine the season or microhabitat that offers the best food nutrient for *P. insulata*. A further objective of this study was to evaluate the performance of *P. insulata* on leaves obtained from plants growing in both shaded and full sun habitats during winter and autumn because no previous study exists on the effects of seasonality and habitat type on the performance of this insect.

5.2 MATERIALS AND METHODS

5.2.1 Measurement of physical and chemical characteristics of leaves

Plant traits and leaf characteristics of *C. odorata* plants growing in fields within the vicinity of the South African Sugarcane Research Institute (SASRI), Mount Edgecombe (29° 70' S, 31° 05' E), near Durban, South Africa, were studied during winter of 2013 and Autumn of 2014. The field chosen consisted of full sun (or open) and shaded habitats and measured 0.7 hectares. The full sun habitat was fully exposed to sunlight and was predominantly dominated by *C. odorata* with a sparse population of *L. camara*, while the shaded habitat was partially

exposed to sunlight and consisted trees of *Syzygium guineense* (Wild.) DC. (Myrtaceae) and bugweed, *Solanum mauritianum* Scop. (Solanaceae). Light intensity (measured by a light meter, LX – 101, Taiwan) differed significantly between the two habitats (mean $\pm$ SE: 1882.100 ± 34.251 and 358.233 ± 14.873 lux for open and shaded environment respectively: GLM ANOVA: $F_{1,19} = 921.243$, $P < 0.0001$). The field was initially used for growing sugarcane and was last mowed on 22 February 2012. *Pareuchaetes insulata* was absent from this site at the time.

The study was conducted in winter/early spring 2013 (from July 15th to October 6th, 2013) and late summer/autumn 2014 (from March 11th to June 4th, 2014). For purposes of convenience, the late summer/autumn trial will be called the ‘autumn trial’ and the winter/ early spring trial will be called the ‘winter trial’ from here on. All plants used for this study in the winter trials were at the flowering stage whereas the ones used in the autumn trials had not started flowering. In *C. odorata*, the initiation of flowering in winter causes all further production of new leaves to cease.

For the winter study, in August 2013, leaf toughness of 100 fully expanded leaves (taken from the upper half of the plants) obtained from 20 plants per habitat (5 leaves per plant) was estimated in each habitat following the methods described in Steinbauer (2001). A hole punch (diameter 5.54 mm) was used to take a leaf disc from the middle of the leaf. Fresh discs were weighed before being wrapped in individual pieces of aluminium foil, and were oven dried at 64 °C for 48 hours before being re-weighed. Specific leaf weight (SLW), which is an indication of leaf toughness (Steinbauer, 2001), was calculated for each habitat using the formula: $SLW = \text{dry weight of leaf disc in mg} / \text{area of hole punch in mm}^2$. On August 22nd, leaf materials were collected from 10 randomly selected *C. odorata* plants along a 20 m

transect in each habitat and subjected to analyses at the laboratories of the Fertilizer Advisory Service, SASRI and the provincial KZN Department of Agriculture and Rural Development, South Africa. The leaves were weighed before drying for 72 hours at 65 °C and water content was calculated using the formula: (%) = (leaf fresh weight – leaf dry weight) / leaf fresh weight × 100. Nitrogen and carbon contents were determined as a percentage of dry weight using a CN analyser (TruSpec[®]CN, LECO, Michigan, USA). Phosphorus was analysed using the colorimetric method in a spectrophotometer. Potassium, calcium and magnesium concentrations were analysed using Atomic Absorption Spectrophotometry (SpectrAA 220 Fast Sequential AAS, Varian Inc. California, USA). The amount of the total non-structural carbohydrate (NSC) in leaves was analysed using the acid hydrolysis procedure (Marais, 1979). Finally, the acid detergent lignin content was analysed using the methods described in van Soest (1963). For the autumn study, leaf characteristics were determined in both shaded and full sun habitats in April 2014 following the methods and protocols described above.

5.2.2 Development and reproductive performance of *Pareuchaetes insulata*

For the winter 2013 study, the larvae used in the insect performance experiments were obtained from eggs laid by F_1 adult females whose original parents were collected in May 2013 on light traps at the Sappi Cannonbrae plantation, Umkomaas (30° 13' S, 30° 46' E) (south coast of KZN province), South Africa, where the insect established following large releases made between 2001 and 2003 (Zachariades *et al.*, 2011a). For the late summer/autumn 2014 study, the larvae used in the insect performance experiments were obtained from eggs laid by F_1 adult females whose original parents were collected in February 2014 on light traps at the same location as above.

The parents (males and females) were placed in aerated 700 ml plastic containers, each with a 5 cm diameter mesh window at the top, with *C. odorata* stem cuttings plugged into a 5 × 5 × 3 cm moistened OasisTM floral foam block wrapped with aluminium foil for egg laying. They were provided with a cotton-wool ball soaked with a 50% honey solution and kept in the laboratory (25 ± 2 °C, 65 ± 10% relative humidity (RH), L12:D12) at the Cedara Weeds Research Unit, ARC-Plant Protection Research Institute (ARC-PPRI), KZN, South Africa (29° 32' S, 30° 16' E). Hatched larvae from these adults (from eggs laid) were fed on cuttings with fully expanded leaves obtained from plants in 25 cm-diameter pots (see Uyi *et al.*, 2014d for details of the potting medium). The resulting adults (1 virgin female and 2 newly eclosed males) were placed in 700 ml containers as described above. The eggs laid by these females were used for this study.

The performance experiments were conducted in a temperature-controlled room maintained at 25 °C, 68% RH and 12D:12L photoperiod at SASRI. Hygrochron iButtons (model DS 1923, Maxim Integrated Products, San José, USA, 0.5 °C accuracy) were used to measure temperature and RH at hourly intervals during winter (temperature range, 23.69 to 26.19 °C; mean ± SE, 24.84 ± 0.01 °C; RH range, 66.5 to 75.3%; mean ± SE, 70.51 ± 2.13%) and autumn (temperature range, 24.45 to 26.74 °C; mean ± SE, 24.95 ± 0.01 °C; RH range, 68.4 to 78.1%; mean ± SE, 71.34 ± 2.16%). For both the winter and autumn study, newly hatched *F*₁ larvae (= “parental generation” in this experiment) were placed individually, using a fine brush, into transparent 100 ml aerated plastic containers (one larva per container), each with a circular screen window of 2.5 cm diameter at the top, lined at the bottom with moistened filter paper to maintain RH, and fed on *C. odorata* foliage (fully expanded leaves taken from the upper half of plants in the field) obtained from either shaded or full sun habitat. One hundred replicates (= 100 larvae) were used for each habitat and generation. Leaf materials

were replaced every 48 hours and frass were removed at same interval for hygienic reasons (Uyi *et al.*, 2011). All leaf materials were obtained fresh from over 8 plants per habitat on each visit or collection date at the field site. The larvae were monitored daily until pupation and/or adult eclosion in order to record mortality and follow the duration of larval instars and the pupal stage. Based on the number of surviving pupae, the following variables were measured or recorded for both shaded and full sun foliage-fed individuals during winter and autumn: (i) total immature development time (duration from egg hatch to adult eclosion), (ii) pupal mass and (iii) growth rate (pupal mass (mg) / development time). Maw's host suitability index was calculated (HSI; for rationale, see Maw, 1976) (and made comparisons between habitats and between seasons) using the following equation: $HSI = (\text{female pupal mass} \times \% \text{ pupation}) / \text{immature development time}$. The assumptions of the index are documented in Chapter 3.

Newly hatched larvae (F_2 or their progeny) resulting from F_1 adults in this experiment were also subjected to similar treatment as described above (for the larval performance trial). Two generations of the insect were studied, because only two generations of this insect can be obtained within a particular season – as the average total development time of this insect (at 25 °C) is 31 days (Uyi *et al.*, 2014d; Chapter 2). When the adults of both the “parental generation” (F_1) and progeny (F_2) eclosed, 1 virgin female and 2 newly eclosed males that had fed on either shaded or full sun foliage as larvae were placed in 700 ml containers as described above (as was done for oviposition of field-collected adults) but they were provided with stem cuttings (with leaves) of the plant type (shaded and full sun) they had fed on as larvae. During the winter trials, 43 replicates (86 in total) each were used for full sun and shaded habitat trials, while during the autumn trials, 34 and 35 replicates (69 in total) were respectively used for full sun and shaded habitat trials. The containers and leaves were

examined daily to record the following: (i) adult longevity, (ii) numbers of eggs laid, (iii) female mating success (the number of matings that resulted in fertile eggs), assessed by production of fertile eggs and (iv) egg hatchability (number of eggs that hatched).

In this way, 800 individuals of *P. insulata* were studied for two successive generations (i.e. parents and progeny) on shaded versus full sun foliage in winter/early spring 2013 (from July 15th to October 6th, 2013) and late summer/autumn 2014 (from March 11th to June 4th, 2014) under constant laboratory conditions. While the rearing conditions were relatively homogenous, the quality of the foliage offered to the larvae as food could not be regarded as uniform because it was obtained fresh from field sites. Therefore, any noticeable change in herbivore fitness could be attributed to habitat (shaded vs. full sun) or seasonal differences (winter vs. autumn).

5.2.3 Statistical analysis

The effect of season and habitat on leaf characteristics (nitrogen, carbon, phosphorus, calcium, magnesium, potassium, lignin, non-structural carbohydrate, leaf water content and leaf toughness) were evaluated using univariate General Linear Model analysis of variance (GLM ANOVA). When the overall results were significant in a two-way analysis, the differences among the treatments were compared using the Tukey's Honestly Significant Difference (HSD) test because of the equality of sample sizes. Data from two successive generations per habitat for both seasons were combined and used for the analysis of insect performance. Pearson's χ^2 test was used to compare the effect of season and habitat on total immature survival, egg hatchability and mating success. The effect of season and habitat on total development duration, pupal mass, growth rate, host suitability index, realised fecundity (total number of eggs laid) and adult longevity were evaluated using GLM ANOVA. When

the overall results were significant, the differences among the treatments were compared using Tukey-Kramer's test. With the exception of the GLM ANOVA that was performed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA), all other analysis were performed using Genstat 14.0 (VSN International, Hemel Hempstead, UK).

5.3 RESULTS

5.3.1 Leaf characteristics

Most of the leaf characteristics exhibited seasonal changes and varied between shaded and full sun habitats (Table 5.1; Figure 5.1 a-h). Foliar nitrogen concentration was 18.61% higher in winter compared to autumn in the shaded habitat, but its concentration in the full sun habitat did not differ between winter and autumn. In winter, foliar nitrogen concentration was 69.07% higher in shaded leaves compared to leaves in the full sun habitat, while it was 52.68% higher in autumn (Figure 5.1a). Although carbon concentration did not differ between seasons, it was slightly higher in the shaded habitat in both winter and autumn (Figure 5.1b). Phosphorus concentration was slightly higher in the full sun habitat in both autumn and winter (Figure 5.1c).

Table 5.1 Statistical details (GLM ANOVA) for the analyses of the effects of season and habitat condition on physical and chemical characteristics of *Chromolaena odorata* leaves.

Analysis	Source of variation	DF	MS	F-value	P- value
Nitrogen	Season	1	2.162	33.58	< 0.0001
	Habitat	1	28.190	437.86	< 0.0001
	Season $\times$ Habitat	1	0.761	16.25	< 0.0001
	Total	39			
Carbon	Season	1	0.484	0.93	0.342
	Habitat	1	10.012	19.15	< 0.0001
	Season $\times$ Habitat	1	0.009	0.02	0.896
	Total	39			
Phosphorus	Season	1	0.032	5.35	0.027
	Habitat	1	0.046	7.61	0.009
	Season $\times$ Habitat	1	0.001	0.01	0.904
	Total	39			
Calcium	Season	1	0.091	0.47	0.499
	Habitat	1	0.150	0.77	0.387
	Season $\times$ Habitat	1	1.315	7.13	0.011
	Total	39			
Magnesium	Season	1	0.998	17.78	< 0.0001
	Habitat	1	0.392	6.98	< 0.0001
	Season $\times$ Habitat	1	0.101	1.78	0.19
	Total	39			
Potassium	Season	1	3.733	24.66	< 0.0001
	Habitat	1	6.609	43.67	< 0.0001
	Season $\times$ Habitat	1	0.334	2.21	0.146
	Total	39			
Non-structural Carbohydrate	Season	1	0.000	0.00	0.982
	Habitat	1	36.243	36.88	< 0.0001
	Season $\times$ Habitat	1	0.052	0.05	0.819
	Total	39			
Lignin	Season	1	1.600	0.10	0.757
	Habitat	1	138.681	8.43	0.006
	Season $\times$ Habitat	1	10.283	0.63	0.434
	Total	39			
Leaf water content	Season	1	47.524	25.68	< 0.0001
	Habitat	1	165.345	89.34	< 0.0001
	Season $\times$ Habitat	1	1.024	0.55	0.462
	Total	39			
SLW(Leaf toughness)	Season	1	0.012	43.51	< 0.0001
	Habitat	1	0.079	273.99	< 0.0001
	Season $\times$ Habitat	1	0.008	28.07	< 0.0001
	Total	399			

DF: degrees of freedom; MS: mean squares.

Statistically significant values are indicated in bold.

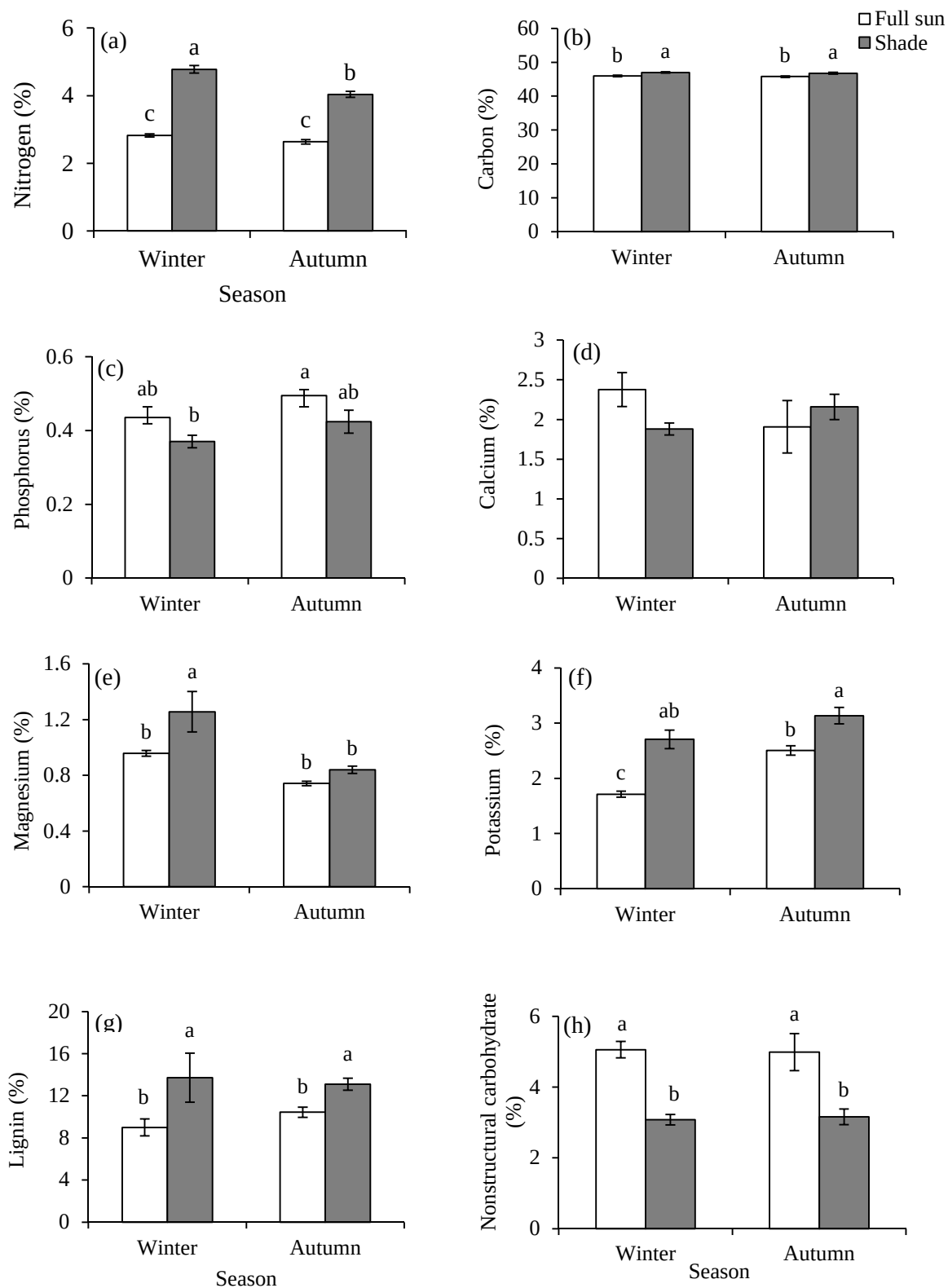


Figure 5.1 Seasonal variation in the phytochemistry (including nutrients, % dry mass) of the leaves of *Chromolaena odorata* plants in two habitats (shade vs. full sun). Data represent means $\pm$ SE. Bars within each graph not sharing a common letter differ significantly ($P < 0.05$) after Tukey's Honestly Significant Difference (HSD) test.

The calcium concentration did not differ between seasons or between habitats (Figure 5.1d). Although magnesium concentration was higher in shaded habitat in winter, this variable did not differ between habitats in autumn (Figure 5.1e). The concentration of potassium was higher in autumn than winter and the shaded habitat had significantly higher concentration compared to full sun habitat (Figure 5.1f). Lignin content did not differ between seasons, but was statistically higher in shaded habitats in both autumn and winter (Figure 5.1g). Although the concentration of total non-structural carbohydrate did not vary between autumn and winter, it was 64.28 and 49.97% higher in the full sun habitat in winter and autumn respectively (Figure 5.1h) compared to the shaded habitat. Leaf water content was higher for plants growing in the shaded habitat in both autumn and winter and was higher in autumn compared to winter (Figure 5.2). Leaf toughness (indicated as SLW) in the full sun habitat was 97.35% greater than that in the shaded habitat in winter and 54.28% greater than shaded habitat in autumn (Figure 5.3).

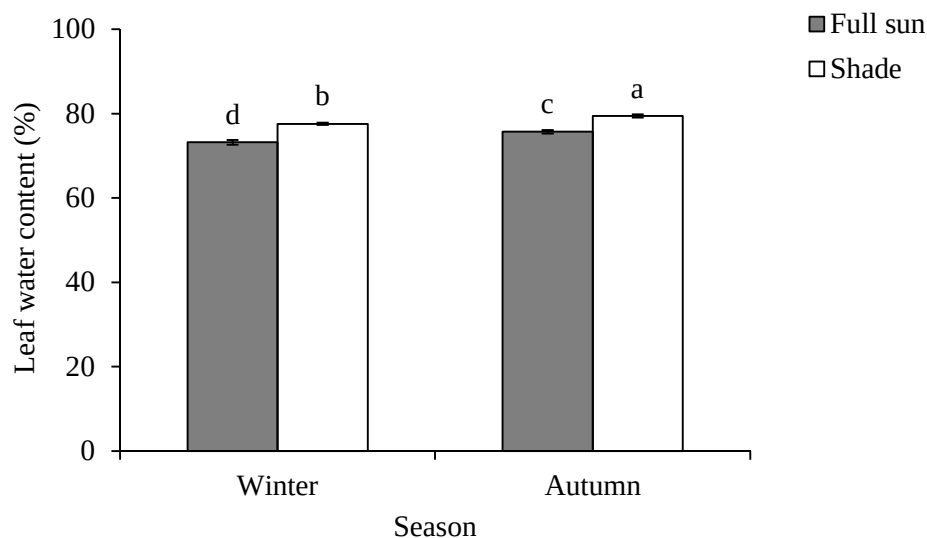


Figure 5.2 Seasonal variation in the leaf water content (mean % $\pm$ SE) of *Chromolaena odorata* plants in two habitats (shade vs. full sun). Bars within each graph not sharing a common letter differ significantly ($P < 0.05$) after Tukey's Honestly Significant Difference (HSD) test.

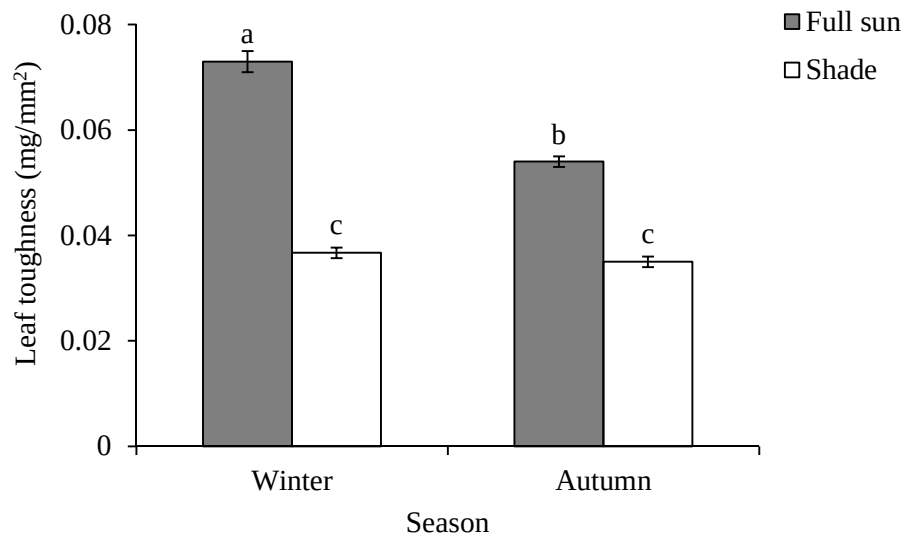


Figure 5.3 Seasonal variation in leaf toughness [as indicated by specific leaf weight (SLW)] of *Chromolaena odorata* plants in two habitats (shade vs. full sun). Data represent means $\pm$ SE. Bars within each graph not sharing a common letter differ significantly ($P < 0.05$) after Tukey's Honestly Significant Difference (HSD) test.

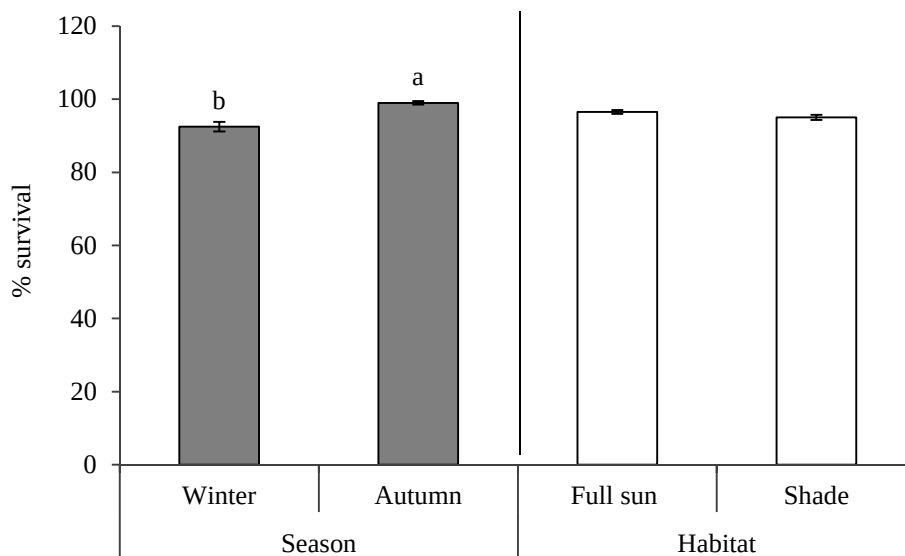


Figure 5.4 Seasonal variation in percentage survival (mean $\pm$ SE) of combined immature stages of *Pareuchaetes insulata* reared on *Chromolaena odorata* leaves from two different habitats (shade vs. full sun) under constant laboratory conditions at 25 °C, 12:12 (L:D)h. Bars with different letters are significantly different (Pearson χ^2 ; $P < 0.05$). Each bar represents percentage survival out of 400 first instar larvae monitored until adult eclosion.

5.3.2 Developmental and reproductive performance of *Pareuchaetes insulata*

Although there was no significant difference in overall survival of *P. insulata* (first instar to adult eclosion) between shaded and full sun foliage (Pearson $\chi^2 = 0.28$, d.f. = 1, $P = 0.599$), this variable was higher for combined immature stages of individuals that fed on autumn compared to winter foliage (Figure 5.4). Total development was significantly faster on shaded leaves in both autumn and winter (Table 5.2; Figure 5.5a).

Table 5.2 Statistical details (GLM ANOVA) for the analyses of the effects of season and habitat condition (larval food source) on total development duration, pupal mass, growth rate, host suitability, female fecundity and adult longevity.

Analysis	Source of variation	DF	MS	F-value	P- value
Total development Duration	Season	1	622.166	199.17	< 0.0001
	Habitat	1	483.049	154.65	< 0.0001
	Season $\times$ Habitat	1	50.760	16.25	< 0.0001
	Total	765			
Pupal mass	Season	1	110934.110	94.43	< 0.0001
	Habitat	1	16938.000	14.42	< 0.0001
	Season $\times$ Habitat	1	6848.231	5.83	0.016
	Total	765			
Growth rate	Season	1	250.196	171.68	< 0.0001
	Habitat	1	73.726	50.59	< 0.0001
	Season $\times$ Habitat	1	11.962	8.21	0.004
	Total	765			
Host suitability index	Season	1	385.161	547.03	< 0.0001
	Habitat	1	84.327	119.77	< 0.0001
	Season $\times$ Habitat	1	5.030	7.14	0.008
	Total	356			
Fecundity	Season	1	226299.110	30.96	< 0.0001
	Habitat	1	152326.400	20.84	< 0.0001
	Season $\times$ Habitat	1	51417.201	7.03	0.009
	Total	154			
Longevity	Season	1	36.459	13.93	< 0.0001
	Habitat	1	17.443	6.69	0.01
	Season $\times$ Habitat	1	3.611	1.38	0.24
	Total	765			

DF: degrees of freedom; MS: mean squares.

Statistically significant values are indicated in bold.

Although faster development time was evident in autumn compared to winter irrespective of habitats, this variable did not differ between individuals that fed on shaded leaves in winter

and those that fed on full sun leaves in autumn. A significant difference in pupal mass was detected between habitats and between seasons with evidently heavier pupal mass on shaded leaves in winter, but this variable did not vary between habitats in autumn (Table 5.2; Figure 5.5b). There was a significant effect of season and habitat on growth rate (Table 5.2; Figure 5.5c). Individuals reared on foliage growing in autumn had a higher growth rate than those that were fed on winter foliage. Irrespective of season, growth rate was always higher on individuals fed on shaded leaves compared to full sun leaves.

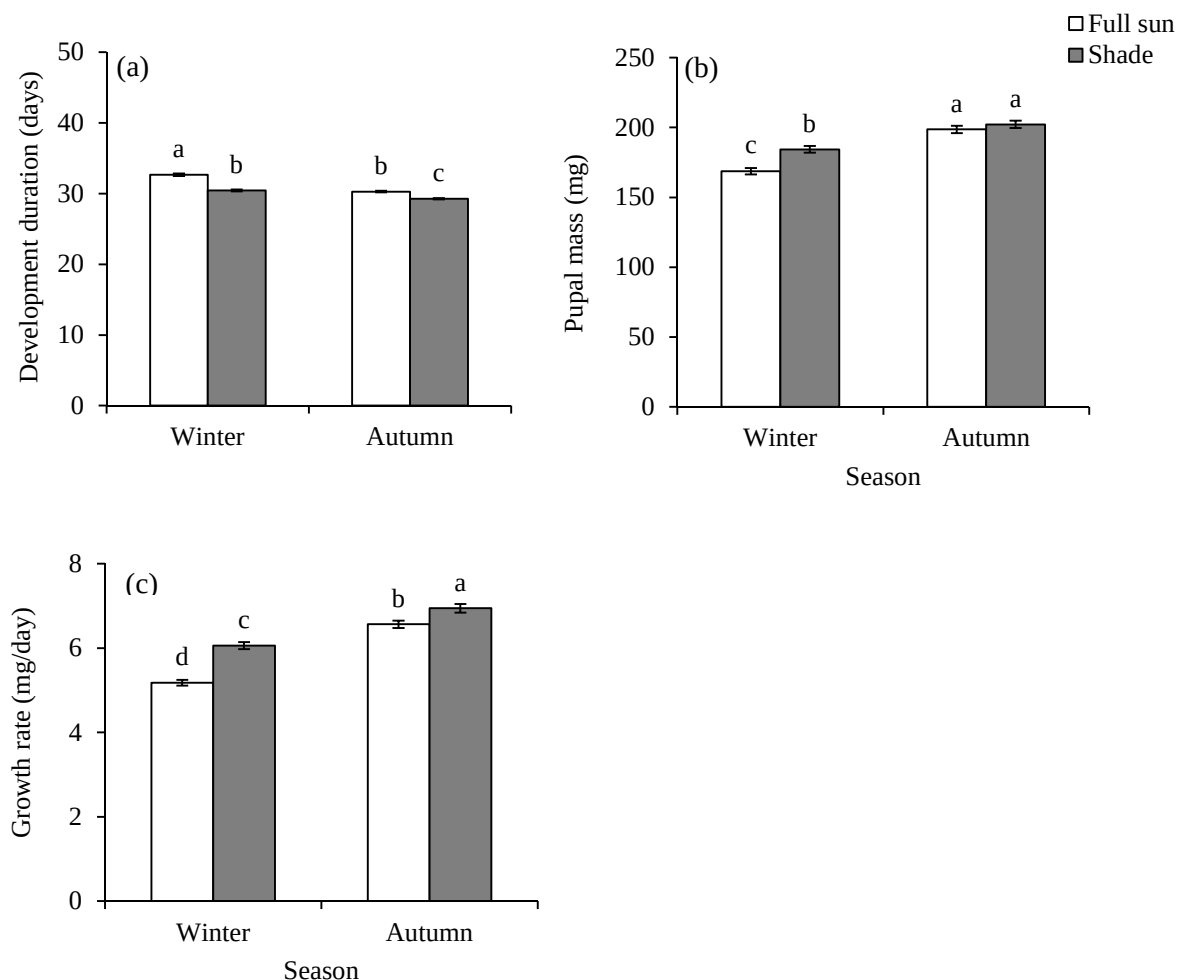


Figure 5.5 Seasonal variation in total development duration (a), pupal mass (b) and growth rate (c) of *Pareuchaetes insulata* reared on *Chromolaena odorata* leaves from two different habitats (shade vs. full sun) under constant laboratory conditions at 25 °C, 12:12 (L:D)h. Data represent means $\pm$ SE. Bars within each graph not sharing a common letter differ significantly ($P < 0.05$) after Tukey-Kramer test.

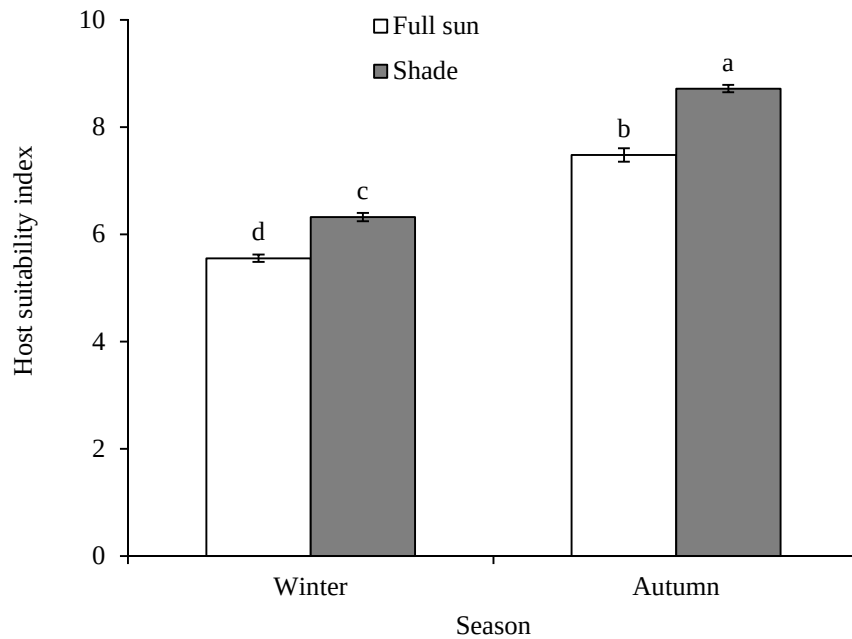


Figure 5.6 Seasonal variation in host suitability index of *Pareuchaetes insulata* reared on *Chromolaena odorata* leaves from two different habitats (shade vs. full sun) under constant laboratory conditions at 25 °C, 12:12 (L:D)h. Data represent means $\pm$ SE. Bars within each graph not sharing a common letter differ significantly ($P < 0.05$) after Tukey-Kramer test.

Maw's HSI indicated that leaves of *C. odorata* plants in the shaded habitat are more suitable for the growth and development of *P. insulata* in both autumn and winter (Table 5.2; Figure 5.6). Regardless of habitat, *C. odorata* leaves growing during autumn provided a more suitable food source for the moth. Realised fecundity only differed significantly between seasons within the sunny site (Figure 5.7a). Similarly, adult longevity did not differ between habitats in autumn, but differed between habitats in winter – with individuals that fed on full sun foliage living slightly longer than their counterparts that were reared on shaded leaves (Table 5.2; Figure 5.7b).

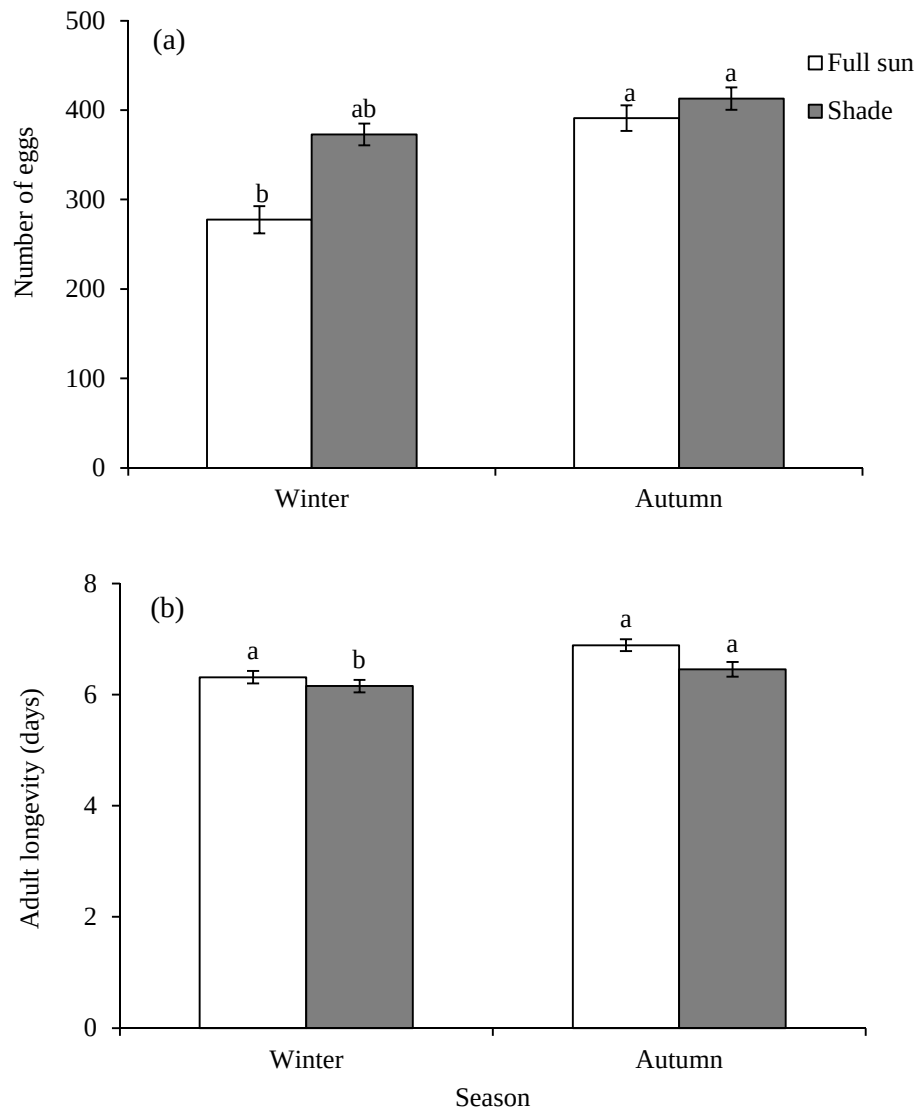


Figure 5.7 Seasonal variation in mean female fecundity (a) and mean adult longevity (b) of *Pareuchaetes insulata* reared on *Chromolaena odorata* leaves from two different habitats (shade vs. full sun) under constant laboratory conditions at 25 °C, 12:12 (L:D)h. Data represent means $\pm$ SE. Bars within each graph not sharing a common letter differ significantly ($P < 0.05$) after Tukey-Kramer test.

Finally, egg hatchability (Season: Pearson $\chi^2 = 2.87$, d.f. = 1, $P = 0.090$; Habitat: Pearson $\chi^2 = 0.08$, d.f. = 1, $P = 0.777$) and mating success (Season: Pearson $\chi^2 = 0.07$, d.f. = 1, $P = 0.786$; Habitat: Pearson $\chi^2 = 0.28$, d.f. = 1, $P = 0.597$) did not statistically differ between habitats and between seasons (Table 5.2; Figure 5.8a and b).

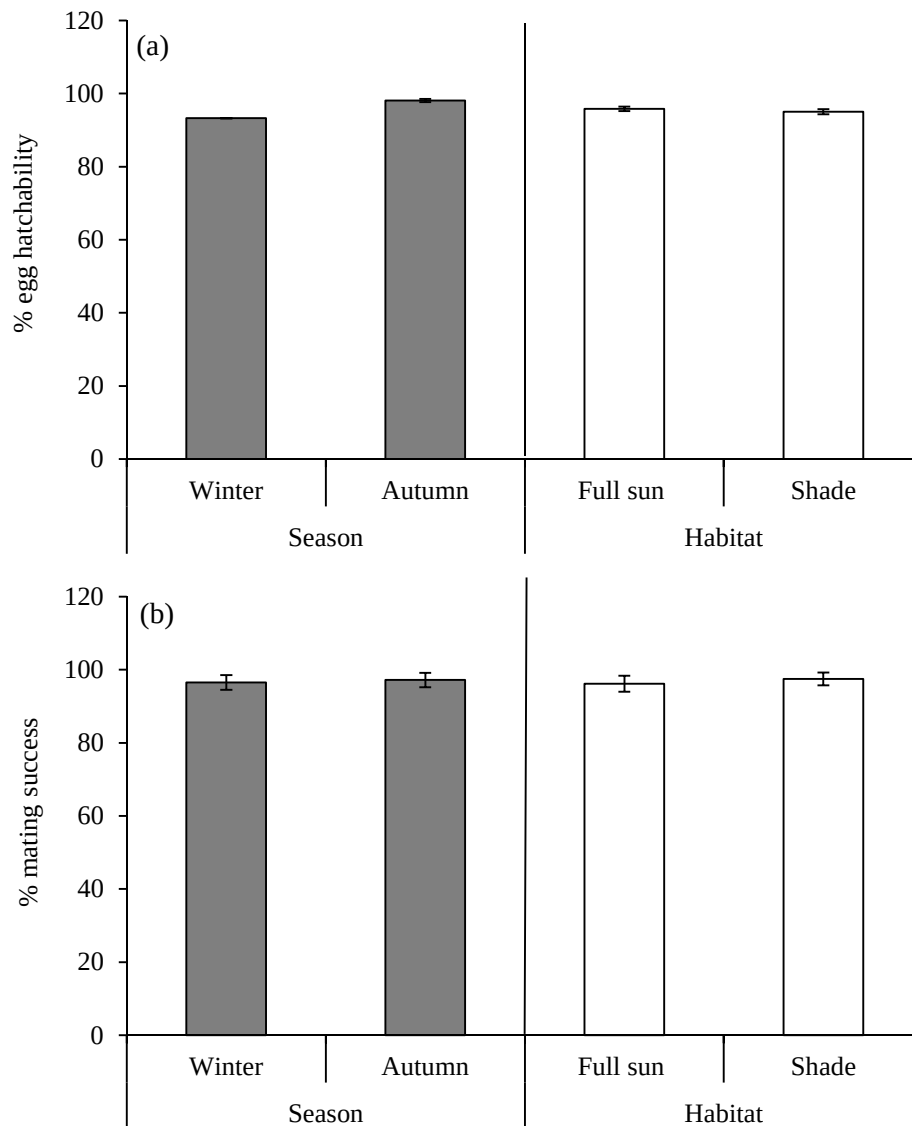


Figure 5.8 Percentage egg hatchability (a) and percentage mating success (b) of *Pareuchaetes insulata* reared on *Chromolaena odorata* leaves from two different habitats (shade vs. full sun) during winter and autumn under constant laboratory conditions at 25 °C, 12:12 (L:D)h. Data represent means $\pm$ SE.

5.4 DISCUSSION

This study documents spatial (habitat: shade vs. full sun) and temporal (seasonal: autumn versus winter) variations in leaf characteristics of *C. odorata* and reports on how the specialist herbivore *P. insulata* responds to these variations. Beyond the ecological significance of this study, it was undertaken as part of an effort to understand why *P. insulata* has not performed well as a biological control agent against *C. odorata* in South Africa.

5.4.1 Variation in leaf characteristics

Climate- and light-mediated changes in plant environment significantly affected several leaf characteristics in *C. odorata*. For instance, leaves from the shaded habitat had reduced toughness, higher water content, increased concentrations of nitrogen, carbon, magnesium, potassium, and lignin as well as reduced non-structural carbohydrate and phosphorus concentrations. The leaf characteristics of *C. odorata* plants also exhibited seasonal variation. While studies simultaneously investigating seasonal variations in leaf characteristics in shaded and full sun habitats are still scarce, studies elucidating spatial variations in leaf characteristics in shaded versus full sun habitats (e.g. Diaz *et al.*, 2011) or seasonal variations (e.g. Alonso and Herrera, 2000; Osier *et al.*, 2000; Migita *et al.*, 2007; Muller *et al.*, 2011) in plants are not uncommon. Several studies report consistent differences in the chemistry of shaded and full sun foliage. For example, water content and nitrogen concentration tends to be higher in shaded foliage, while leaf toughness tends to be higher in full sun foliage (Collinge and Louda, 1988; Potter, 1992; Jasen and Stamp, 1997; Moran and Showler, 2005; Diaz *et al.*, 2011). The high foliar nitrogen concentration in shaded plants supports the prediction of the carbon/nutrient balance hypothesis (Bryant *et al.*, 1983, 1987) that the leaves of plants growing under sub-optimal levels of photosynthetically active radiation (e.g. shaded habitats) should contain relatively more nutrients (especially nitrogen) compared to plants growing in direct sunlight.

Nitrogen is a limiting resource for plant growth in most natural ecosystems (Aerts and Chapin, 2000). Of plant organs, leaves are the largest sink of nitrogen and more than half of leaf nitrogen is invested in photosynthetic apparatus (Evans, 1989; Hikosaka, 2004). The higher concentration of foliar nitrogen in winter has not previously been reported in *C.*

odorata, however it has been shown in several plant species that leaf nitrogen increases in winter compared to summer or autumn (Weih and Karlsson, 2001; Muller *et al.*, 2005, 2011). The seasonal variation in leaf nitrogen suggests that the plants alter their nitrogen concentration to maximize daily nitrogen-use efficiency of carbon gain in response to varying seasonal temperatures, as has been reported by other workers (e.g. Muller *et al.*, 2011). *Chromolaena odorata* growing in shaded environments has broader and longer leaves compared with full sun plants (Uyi *et al.*, submitted). This might be a strategy to maximize the leaf area exposed to sunlight, and consequently photosynthesis and carbon gain (Pearcy *et al.*, 2005). Although phosphorus, magnesium and potassium are important in several processes in plant growth and development, their role in *C. odorata* plants and the reasons why they seasonally and spatially vary in *C. odorata* leaves remains to be established. The lignin content of leaves in the shaded habitat may be higher because the leaves are broader and thinner (Uyi *et al.*, submitted) and thus need more structural support. However, there are currently no data to support this hypothesis in this study. While the increased concentration of non-structural carbohydrate in full sun leaves concurs with previous studies in herbaceous plants (Rowe and Potter, 2000; Moran and Showler, 2005; Diaz *et al.*, 2011), the lower leaf water content in winter and the higher leaf toughness in the full sun habitat during the winter trials (relative to autumn) might be due to the seasonally dry winter (low winter rainfall) in South Africa, as well as because the winter-trial leaves were older than the autumn-trial leaves.

5.4.2 Performance of *Pareuchaetes insulata*

While there was no significant difference in overall immature survival between insects feeding on shaded *C. odorata* foliage and on foliage from the full sun habitat, development time was faster, pupal mass was heavier and increased growth rate was evident in individuals

that were reared on shaded foliage compared to the full sun foliage. The above performance metrics were also better in autumn compared to winter. Reduction of final body weight and increase in development time would both bear fitness costs for larvae and the resulting adults. Prolonging development time may negatively affect larval survival (in field situations) by exposing them to possible predation, parasitism or unfavourable environmental conditions for a longer period (Williams, 1999; Fordyce and Shapiro, 2003), whereas a reduction in larval and pupal weight or adult body size is likely to affect fecundity and/or future reproductive success (Honěk, 1993; Awmack and Leather, 2002). Lower pupal mass has been shown to affect dispersal distances of emerging adults in *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae) (Woodrow *et al.*, 1987). In the winter trials, the heavier pupal mass translated into increased fecundity in females that were fed shaded leaves as larvae compared to those that were fed full sun foliage as larvae. In other species of Arctiinae such as *U. ornatatrix*, smaller adult males have less chance of mating (Conner *et al.*, 1990; LaMunyon and Eisner, 1994; Conner, 2009; Kelly *et al.*, 2012), and if mating does occur, they may produce small-sized offspring resulting in reduced adult fecundity and compromising fitness of immature stages – as smaller-sized adults are likely to produce eggs with reduced pyrrolizidine alkaloids (PAs: a defence compound that protects eggs and larvae from predation and parasitism) (Eisner *et al.*, 2000; Bezzerides *et al.*, 2004; Conner, 2009).

The responses of insects, in terms of performance metrics (such as development time, growth rate and fecundity), to foliage grown in sunny or shaded environments are not always straightforward and appear to be species specific. For example, improved immature survival rate, larval performance (decreased development time and larger pupal mass), adult fecundity and leaf consumption on shaded plants were found on *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae) feeding on Tropical Soda Apple, *Solanum viarum* Dunal

(Solanaceae) (Diaz *et al.*, 2011); and on the tingid *Stephantis pyrioides* (Scott) (Hemiptera: Tingidae) feeding on azalea (*Rhododendron* L. spp.) (Ericaceae) (Trumbule and Denno, 1995). In other plant species, studies have demonstrated neutral (Moore *et al.*, 1988; Franca and Tingey, 1994; Rowe and Potter, 2000) or detrimental performance (Dult and Shure, 1994; Sipura and Tahvanainen, 2000; Moran and Showler, 2005; Osier and Jennings, 2007) of insect folivores feeding on shaded leaves in either laboratory or field conditions. The superior performance of *P. insulata* on autumn (relative to winter) and on shaded foliage (irrespective of season) as indicated by the higher values of Maw's host suitability index (HSI) seems to be in accordance with field observations and reports by other workers (e.g. Cock and Holloway, 1982; Diaz *et al.*, 2011) that larvae of species in this genus and other species generally perform better on shaded foliage or on leaves of plants growing in relatively cool, moist climate conditions. The higher HSI value in autumn suggests that foliage of *C. odorata* plants growing in this period is more suitable for growth, development and reproduction (see Figures 5.5 and 5.6).

So the question arises: why do *P. insulata* perform better on shaded *C. odorata* leaves and on leaves of plants growing in autumn? The spatial and temporal variations in leaf characteristics of *C. odorata* plants can help explain the apparent superiority of leaves of plants growing in autumn and in the shaded habitat. For instance, foliar nitrogen concentration was 69 and 52% higher in shaded (relative to full sun) plants in winter and autumn respectively and its concentration on shaded leaves was 18% higher in winter compared to autumn. Nitrogen is considered to be the most limiting macronutrient for insect herbivores (Mattson, 1980) due to its fundamental role in protein synthesis (Sterner and Elser, 2002). Increased or high leaf nitrogen concentration has been linked to faster development, higher survival rates, increased body mass, higher growth rates, increased

fecundity and population density in insects (Mattson, 1980; Myers and Post, 1981; Scriber and Slansky, 1981; Hinz and Müller-Schärer, 2000; Huberty and Denno, 2006; Moran and Goolsby, 2014, also see Chapter 7). However, the detrimental effects of excess nitrogen have also been documented (e.g. Hartley and Lawson, 1992; Lee *et al.*, 2002; Clissold *et al.*, 2006; Zehnder and Hunter, 2009). The faster development time, increased pupal mass and growth rate as well as the improved adult longevity and fecundity in individuals that fed on shaded leaves in autumn relative to those that fed on same leaves in winter (when foliar nitrogen was 18% higher than autumn) suggests that increased or elevated nitrogen levels in winter negatively affected the above performance metrics in *P. insulata*. This hypothesis can be validated by an inference in Chapter 7 that *P. insulata* larvae reared on the leaves of *C. odorata* with more than 3.84% nitrogen might not get any additional benefits, rather there might be a fitness cost associated with excess leaf nitrogen (e.g. Lee *et al.*, 2002; Clissold *et al.*, 2006) presumably because of elevated metabolic costs related to catabolising protein (for use as an energy source) and/or excreting nitrogen (Schroeder, 1986; Boersma and Elser, 2006; Clissold *et al.*, 2006).

The increased water content in shaded foliage (relative to full sun) and in leaves of plants growing in autumn (relative to winter) may have also contributed to the improved performance of *P. insulata* on this foliage. Elsewhere, increased water content has been demonstrated to positively influence the performance of insect herbivores (Henriksson *et al.*, 2003; Diaz *et al.*, 2011), the attractiveness of foliage to insect herbivores (Ricklefs, 2008) and insect herbivory levels in several Asteraceae species (Münzbergová and Skuhrovec, 2013). The increased toughness of full sun foliage in both autumn and winter may be partly responsible for the prolonged development time in *P. insulata* because larvae needed more time to consume food and assimilate major macronutrients in the required ratio (Clissold *et*

al., 2009). The lower total non-structural carbohydrate level in shaded leaves (in both autumn and winter) suggests that carbon-based defences, such as terpenoids and flavonoids may have been compromised (Wilkens *et al.*, 1996), thus favouring rapid larval growth. The comparatively better larval performance on shaded leaves despite their seemingly higher lignin content, suggests that other carbon compound (secondary chemicals) other than lignin may be more important in larval selection of foliage for consumption or utilization by *P. insulata* and that the insect might have neutralized the threat posed by lignin.

Phosphorus concentration in leaves did not appear to correlate with larval performance, but *P. insulata* performed better in autumn following a slight increase in the concentration of this nutrient. The importance of phosphorus on the growth and development, survival, behaviour and fecundity of insects has been documented (Popp *et al.*, 1989; Janssen, 1994; Perkins *et al.*, 2004; Huberty and Denno, 2006). An increase in potassium and a decrease in magnesium concentrations in autumn coincided with improved herbivore performance in this study. Like nitrogen, magnesium is used for structural purposes, while potassium is involved in electrochemical function, including message transmission in nerves and energy metabolism in insects (Fraústo da Silva and Williams, 1991). Although very little is known about how variations in potassium and magnesium concentrations can affect insect herbivores, the concentrations of both elements are known to be positively associated with insect population density (Joern *et al.*, 2012). Furthermore, increased magnesium concentration in rice, *Oryza sativa* (L.) (Poaceae) has been demonstrated to improve egg production in *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Pyralidae) (Ge *et al.*, 2013). Similar to nitrogen, the increase in magnesium concentration in the shaded habitat in winter might have contributed to the poor performance (prolonged development time and reduced fecundity) of *P. insulata* in the winter trials. The lack of spatio-temporal variation in calcium concentration in *C. odorata*

leaves invites questions as to its function in *P. insulata*. The lack of significant differences in pupal mass and realised fecundity between individuals that fed on shaded and full sun leaves (as larvae) in autumn suggests that leaf nutrients were more balanced during autumn compared to winter. The same reason can also be suggested for the higher HSI value for shaded leaves in autumn relative to winter. The lack of significant difference in survival between habitats suggests that nutrient levels in full sun foliage were not reduced to a level where it could cause direct mortality due to poor food quality.

5.4.3 Implications for the population dynamics of *Pareuchaetes insulata*

The improved performance of *P. insulata* on shaded and full sun leaves in autumn (relative to winter) and the higher HSI in autumn suggests that plants growing in autumn were more nutritionally balanced and satisfied the nutritional requirements of the insect. Although field populations of this insect remained low in the field, this study suggests that the insect should be more abundant during autumn or summer months because *C. odorata* leaves are more suitable during this period. In normal field plants, once flowering has started in winter, all leaves are old – until regrowth starts with the onset of rains at the beginning of the summer and continues until late autumn. However, if plants have been slashed or otherwise damaged, there may be non-flowering regrowth with young leaves present (even in winter) and this will present a very different food source for the larvae of *Pareuchaetes* species. Outbreak populations of *Pareuchaetes* species often occur where there is young regrowth after plants have been slashed or mowed (R.E.C. McFadyen; C. Zachariades pers. comm.).

The occasional localised “outbreaks” of this insect observed in South Africa in 2005-6 (Zachariades *et al.*, 2011a) and 2014 (Strathie and Zachariades, 2014) occurred in summer and autumn when food is much better because prevailing climate conditions (e.g. increased

temperatures and/or rainfall) not only directly improve the performance of the insect (see Chapter 4), but also indirectly improve the leaf quality of the host plant. The higher nitrogen and possibly magnesium concentrations in the shaded leaves during winter (triggered by low winter temperatures) coupled with direct impact of low temperatures (Chapter 4) may be responsible for the extremely low populations of *P. insulata* in winter (relative to autumn) and in colder areas within the weed's distribution range in South Africa. This hypothesis is supported by the prolonged development time, reduced pupal mass, growth rate, longevity and fecundity of *P. insulata* on the shaded foliage in winter compared to autumn.

These results suggest that mass-rearing of *P. insulata* and possibly other species in the genus, could be enhanced by feeding larvae with shaded foliage and that when evaluating the abundance or impact of biological control agents, practitioners should consider habitat heterogeneity because this may affect the leaf characteristics and the subsequent performance of the agent. The improved performance of *P. insulata* on the shaded foliage might have implications for the weed's biological control. First, *C. odorata* plants in shaded habitats are sparse and do not produce many flowers, so if *P. insulata* prefers or performs better in shade, it will be a poor biological control agent except when there are outbreaks. Second, the fact that *C. odorata* plants are known to be shade intolerant (Zachariades *et al.*, 2009; Zhang and Wen, 2009), suggests that female adults might struggle to locate oviposition sites (assuming females would prefer to oviposit on high quality plants, such as those growing in shaded habitats) because of the sparse distribution of the plant in such habitats. Alternatively, when eggs are laid on leaves of plants in full sun habitats, survival might be negatively affected due to their exposure of the resulting early instars to adverse environmental conditions. Also larvae might suffer fitness costs (expressed as prolonged development time and reduced pupal mass and fecundity) on full-sun plants (especially in winter), and any attempts by

larvae to migrate to a more nutritious environment might expose them to predation or other mortality factors. While all these could either lead to reduced fitness or a decline in population levels of this insect in the field, further speculation on the implications of the findings of this study on biological control of *C. odorata* and on the population dynamics of *P. insulata* in the field should be deferred, until field experiments on the effect of light environment (shaded vs. full sun) on oviposition and larval preference of *P. insulata* are conducted, because Sipura and Tahvanainen (2000) clearly showed that in field situations, some insects not only prefer open habitats, but develop faster in such habitats. Also, studies on predator- and non-predator-caused mortality on larval migration between shade and full sun habitats (or *vice versa*) are needed.

5.5 CONCLUSION

This study demonstrates that low winter temperatures can indirectly affect insect herbivore performance through variability in the phytochemistry of host plant and hypothesized that excess nitrogen and possibly magnesium may have detrimental effects on insect herbivore performance. While the effect of excess nitrogen on the performance of insect herbivore have been documented (van der Meijden *et al.*, 1984; Lee *et al.*, 2002; Clissold *et al.*, 2006; Zehnder and Hunter, 2009), the effect of excess magnesium (in herbivore diets) on the performance of insect herbivores needs further experiments and comments before drawing any firm conclusions. In sum, this study shows that the relationships between spatial and temporal variations in leaf characteristics (such as magnesium, nitrogen, phosphorus, potassium and lignin concentrations) and insect herbivore performance are more complex than previously thought.

CHAPTER 6

Larval feeding history in *Pareuchaetes insulata* and its implications for biological control of *Chromolaena odorata*

6.1 INTRODUCTION

Recent studies have demonstrated that the relationships between food quality and phytophagous insect performance are more complex than previously thought (Clissold *et al.*, 2006; Röder *et al.*, 2008; Zehnder and Hunter, 2009; Joern *et al.*, 2012; also see Chapter 5). These relationships remain complex because insect preference and performance can be driven by plant traits which are sometimes difficult to disentangle (Jermy, 1984; Bernays and Chapman, 1994; Hull-Sanders *et al.*, 2007; Clissold *et al.*, 2006) because of the multiplicity of indirect biotic and abiotic effects that may explain a correlation (Hunter and Price, 1998).

Foliar characteristics of the invasive alien plant, *C. odorata* are known to exhibit seasonal and spatial variations [mediated by climate (temperature and rainfall) or sunlight] in the field (Uyi *et al.*, submitted; Chapter 5). When *C. odorata* plants are exposed to direct sunlight, leaf nitrogen and water contents are reduced and leaf toughness is increased and this has been demonstrated to compromise insect fitness because of the nutritional imbalance in full sun leaves (especially during winter) (Uyi *et al.*, submitted; Chapter 5). The response of *P. insulata* to these variations has been studied (Uyi *et al.*, submitted; Chapter 5). Both the parents and offspring of this herbivore perform better (faster development time, increased growth rate, larger pupal mass and improved fecundity) when reared on leaves of plants growing in shaded habitats (relative to full sun habitats). However, the performance of offspring whose parents fed on foliage different from that which is available for offspring

consumption and utilization remains to be established. The knowledge of the impact of feeding history or cross feeding in this insect can have implications for its population dynamics and therefore on the biological control of *C. odorata* in South Africa because of the presupposed shift in plant quality occasioned by the coordinated and uncoordinated rapid clearing of the weed and removal of trees within the vicinity of the weed. Due to the dynamic forces operational in field situations, it is not impossible that *P. insulata* offspring whose parents fed on a superior host plant (e.g. leaves of plants growing in shaded habitats), would be confronted with nutritionally unbalanced foliage of *C. odorata* (such as that growing in full sun).

Several studies have suggested that the nutritional state of parents can influence the performance of their offspring (Slansky and Scriber, 1985; Rossiter, 1991a,b; Fuentealba and Bauce, 2012). For example, Fuentealba and Bauce (2012) found that the nutritional experience of spruce budworm (*Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) parents can influence the development time and pupal mass of the offspring. However, the quality and quantity of food that is consumed by an insect offspring influences its overall fitness and performance, thereby affecting growth rate, development time, final body mass, dispersal ability and probability of survival (Slansky and Scriber, 1985). Whether the nutritional status of the parents of *P. insulata* (fed on suitable foliage) can influence the performance of their progeny that fed on a nutritionally unbalanced diet or the current (suitable) food consumed by larvae (whose parents fed on nutritionally unbalanced diet) can influence the overall fitness and performance of offspring remains to be seen.

If there is a carry-over effect of host nutritional quality on the performance of *P. insulata*, offspring whose parents fed on shaded leaves (nutritionally balanced diet) might perform

equally well or better when fed on full sun foliage (nutritionally unbalanced diet) but not *vice versa*. The objective of this study was to investigate how a continuous shift in leaf nutrients mediated by sunlight in shaded and full sun habitats affects the performance of *P. insulata* progeny when fed on a diet similar to that which the parents were reared and on switched diets. Larval feeding history was grouped into four classes; (i) FSFS: progeny were reared on full sun foliage similar to their parental diet; (ii) SHSH: progeny were reared on shaded foliage similar to their parental diet; (iii) FSSH (cross-feeding): progeny reared on shaded foliage whereas the parents had been reared on full sun foliage and (iv) SHFS: (cross-feeding): progeny reared on full sun foliage whereas the parents had been reared on full shaded foliage. The cross-feeding experiment was performed in order to understand how *P. insulata* progeny will respond to a diet dissimilar to that which their parents fed on as larvae.

6.2 MATERIALS AND METHODS

6.2.1 Study system: site description, origin and maintenance of moths

Plant traits and leaf characteristics of *C. odorata* plants growing in fields within the vicinity of the South African Sugarcane Research Institute (SASRI), Mount Edgecombe (29° 70' S, 31° 05' E), near Durban, South Africa, were studied. The field chosen consisted of full sun (or open) and shaded habitats and measured 0.7 hectares. The full sun habitat was fully exposed to sunlight and was dominated by *C. odorata* with a sparse population of *L. camara*, while the shaded habitat was partially exposed to sunlight and consisted trees of *S. guineense* and bugweed, *S. mauritanum*. Light intensity (measured by a light meter, LX – 101, Taiwan) differed significantly between the two habitats (mean $\pm$ SE: 1882.100 $\pm$ 34.251 and 358.233 $\pm$ 14.873 lux for open and shaded environment respectively; GLM ANOVA: $F_{1,19} = 921.243$, $P < 0.0001$). The field was initially used for growing sugarcane and was last mowed on 22

February 2012. *Pareuchaetes insulata* was absent from this site at the time. Plants used for this study in either habitat were all at the flowering stage (i.e. winter foliage).

The larvae of *P. insulata* used in the performance experiments were obtained from eggs laid by F_1 adult females whose original parents were collected in May 2013 on light traps at the Sappi Cannonbrae plantation, Umkomaas (30° 13' S, 30° 46' E) (south coast of KwaZulu-Natal Province), South Africa, where the insect established following large releases made between 2001 and 2003 (Zachariades *et al.*, 2011a). The parents (males and females) were placed in aerated 700 ml plastic containers, each with a 5 cm diameter mesh window at the top, with *C. odorata* stem cuttings plugged into a 5 × 5 × 3 cm moistened Oasis™ floral foam block wrapped with aluminium foil for egg laying. They were provided with a cotton-wool ball soaked with a 50% honey solution and kept in the laboratory (25 ± 2 °C, 65 ± 10% RH, L12:D12) at the Cedara Weeds Research Unit, ARC-Plant Protection Research Institute (ARC-PPRI), KZN, South Africa (29° 32' S, 30° 16' E). Hatched larvae (from eggs laid) were fed on cuttings with fully expanded leaves obtained from plants in 25 cm-diameter pots (see Uyi *et al.*, 2014d for details of the potting medium). The resulting adults (1 virgin female and 2 newly eclosed males) were placed in 700 ml containers as described above. To avoid differences in egg provisioning (Pöykkö and Mänttari, 2012), the eggs of two females (approximately of the same weight as indicated by pupal mass) laid on the same night were used for this study.

The performance experiments were conducted in a temperature-controlled room maintained at 25 °C, 68% RH and 12D:12L photoperiod at SASRI. Hygrochron iButtons (model DS 1923, Maxim Integrated Products, San José, CA, USA, 0.5 °C accuracy) were used to

measure temperature and RH at hourly intervals (temperature range, 23.69 to 26.19 °C; mean $\pm$ SE, 24.84 ± 0.01 °C; RH range, 66.5 to 75.3%; mean $\pm$ SE, $70.51 \pm 2.13\%$).

6.2.2 Development and reproductive performance of *Pareuchaetes insulata*

These experiments only describe the performance of *P. insulata* progeny while that of the parental generation has been described in Chapter 5 of this thesis. On the day of hatching, larvae (= “progeny” in this experiment) were placed individually, using a fine brush, into transparent 100 ml aerated plastic containers (one larva per container), each with a circular screen window of 2.5 cm diameter at the top, lined at the bottom with moistened filter paper to maintain RH and fed *C. odorata* foliage (fully expanded leaves taken from the upper half of plants in the field) obtained from either shaded or full sun habitat. One hundred replicates (n = 100 larvae) were used for each feeding history group (a total of 400 individuals was used for this study). Leaf materials were replaced every 48 hours and frass were removed at same interval for hygienic reasons (Uyi *et al.*, 2011). All leaf materials were obtained fresh from over 8 plants per habitat on each visit or collection date at the field site and replaced by new materials from different plants every 48 hours. The larvae were monitored daily until pupation and/or adult eclosion in order to record mortality and follow the duration of larval instars and the pupal stage. Based on the number of surviving pupae, the following variables were recorded for both shaded and full sun foliage-fed individuals: (i) total larval development time, (ii) total immature development time (duration from egg hatch to adult eclosion), (iii) pupal mass and (iii) growth rate (pupal mass (mg) / development time).

When the adults eclosed, 1 virgin female and 2 newly eclosed males that had fed on one of four treatments as larvae were placed in 700 ml containers as described above (as was done for oviposition of field-collected adults) but they were provided with stem cuttings (with

leaves) of the plant type (shaded and full sun) they had fed on as larvae. Twenty-one and 19 replicates respectively were used for FSFS and SHSH treatments while 10 replicates each were used for FSSH and SHFS treatments. The containers and leaves were examined daily to record adult mortality and realized fecundity (total number of eggs laid).

In this way, the larval feeding history in *P. insulata* on shaded and full sun foliage as well as on switched foliage between winter and spring (from July 15th to October 6th, 2013) under constant laboratory conditions was studied. While the rearing conditions were relatively homogenous, the quality of the foliage offered to the larvae could not be regarded as uniform because it was obtained fresh from field sites. Therefore, any noticeable change in herbivore fitness could be attributed to larval diet and feeding history.

6.2.3 Statistical analysis

The effect of larval feeding history (as indicated by the feeding history group) and sex on overall immature survival, larval development duration, total development duration, pupal mass, growth rate and adult longevity were evaluated using univariate General Linear Model analysis of variance (GLM ANOVA). When the overall results were significant, the difference among the treatments was compared using the Sequential Bonferroni test (for rationale, see Rice, 1989). The effect of larval feeding history on realized fecundity was evaluated using Generalized Linear Model (GLM) assuming a normal distribution with an identity link function. All analyses were performed using SPSS Statistical software, version 16.0 (SPSS, Chicago, IL, USA).

6.3 RESULTS

Larval feeding history affected the performance of *P. insulata* (Table 6.1; Figures 6.1-6.4). When reared on full sun leaves, immature mortality was higher in offspring from parents reared on shaded leaves (SHFS) than those of other feeding history groups (Figure 6.1). Progeny from parents reared on full sun leaves exhibited the fastest larval and total development times when reared on shaded leaves (FSSH) (Figure 6.2a,b), while prolonged development was evident in the opposite cross-feeding group (SHFS: progeny reared on full sun foliage but whose parents had been reared on shaded foliage).

Table 6.1 General linear model (GLM) analyses on the effects of larval feeding history on survival, development duration, pupal mass, growth rate and adult longevity of *Pareuchaetes insulata* progeny.

Analysis	Source of variation	DF	MS	F-value	P-value
Survival	Feeding history	3	24900.250	19.611	< 0.0001
	Total	399			
Larval development duration	Feeding history	3	369.712	128.109	< 0.0001
	Sex	1	93.745	32.482	< 0.0001
	Feeding history $\times$ sex	3	2.464	0.854	0.465
	Total	329			
Total development duration	Feeding history	3	351.631	112.023	< 0.0001
	Sex	1	0.397	0.126	0.722
	Feeding history $\times$ sex	3	1.807	0.576	0.631
	Total	329			
Pupal mass	Feeding history	3	8059.874	26.200	< 0.0001
	Sex	1	26783.653	737.192	< 0.0001
	Feeding history $\times$ sex	3	65.189	0.212	0.888
	Total	329			
Growth rate	Feeding history	3	36.089	100.810	< 0.0001
	Sex	1	238.328	665.747	< 0.0001
	Feeding history $\times$ sex	3	0.610	1.704	0.166
	Total	329			
Adult longevity	Feeding history	3	44.437	26.270	< 0.0001
	Sex	1	108.696	64.258	< 0.0001
	Feeding history $\times$ sex	3	6.175	3.651	0.013
	Total	329			

DF: degrees of freedom; MS: mean squares.

Statistically significant values are indicated in bold.

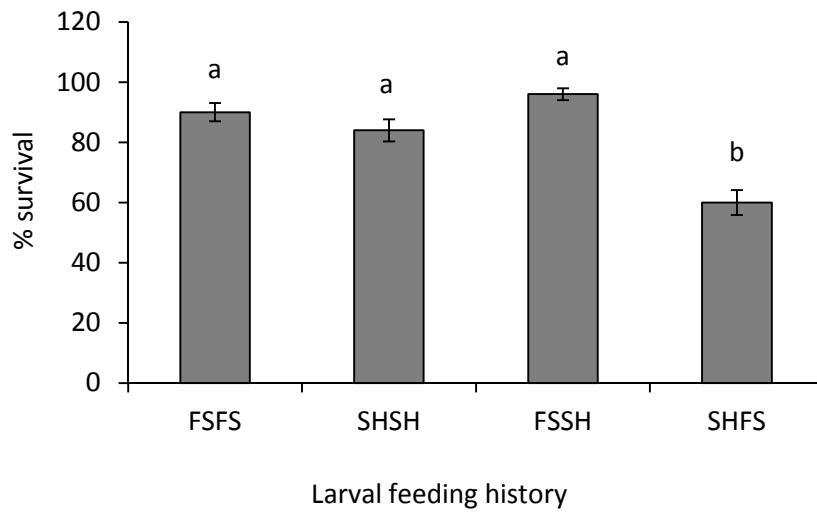


Figure 6.1 The effect of larval feeding history (FSFS, SHSH, FSSH and SHFS) on the percentage survival (mean $\pm$ SE) of combined immature stages (first instar to adult eclosion) of *Pareuchaetes insulata* progeny reared under constant laboratory conditions at 25 °C, 12:12 (L:D) h. Only the quality of host leaves as food could not be regarded as a constant condition because they were collected fresh from two different field sites (shade and full sun habitats). Comparisons with different letters indicate significant differences (Sequential Bonferoni test, $P < 0.05$). FSFS: progeny were reared on full sun foliage similar to their parental diet; SHSH: progeny were reared on shaded foliage similar to their parental diet; FSSH (cross-feeding): progeny reared on shaded foliage whereas the parents had been reared on full sun foliage and SHFS (cross-feeding): progeny reared on full sun foliage whereas the parents had been reared on shaded foliage.

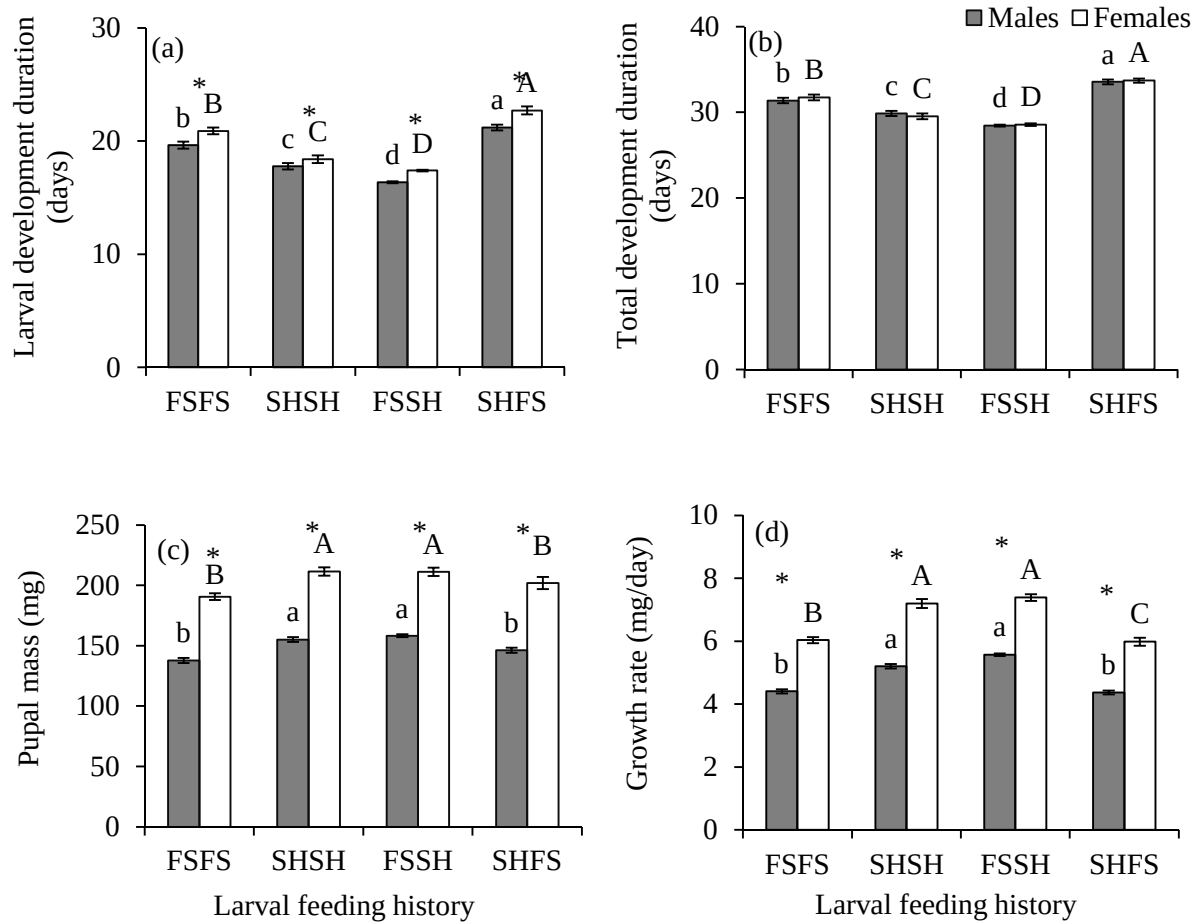


Figure 6.2 The effect of larval feeding history (FSFS, SHSH, FSSH and SHFS) on (a) larval development duration, (b) total development duration, (c) pupal mass and (d) growth rate of *Pareuchaetes insulata* progeny (females and males) reared under constant laboratory conditions at 25 °C, 12:12 (L:D) h. Only the quality of host leaves as food could not be regarded as a constant condition because they were collected fresh from two different field sites (shade and full sun habitats). Data represent mean $\pm$ SE. Among-feeding history comparisons were not significantly different if the same uppercase letter appears above white columns (females) or if the same lowercase letter appears above black columns (males) (Sequential Bonferroni test $P < 0.05$). For each feeding history, differences between males and females are indicated with an asterisk (student's t -test, $P < 0.05$). FSFS: progeny were reared on full sun foliage similar to their parental diet; SHSH: progeny were reared on shaded foliage similar to their parental diet; FSSH (cross-feeding): progeny reared on shaded foliage whereas the parents had been reared on full sun foliage and SHFS (cross-feeding): progeny reared on full sun foliage whereas the parents had been reared on shaded foliage.

Pupal mass was higher in progenies that fed on shaded leaves (SHSH and FSSH) irrespective of parental diets, although there was no significant difference in pupal mass between these two feeding history groups (Figure 6.2c). Likewise, when reared on full sun leaves, pupal mass did not differ between progenies from parents reared on either full sun (FSFS) or

shaded (SHFS) leaves. Growth rate was higher in progeny that fed on shaded leaves (SHSH and FSSH) irrespective of parental diets, although there was no significant difference in this variable between these two feeding history groups (Figure 6.2d).

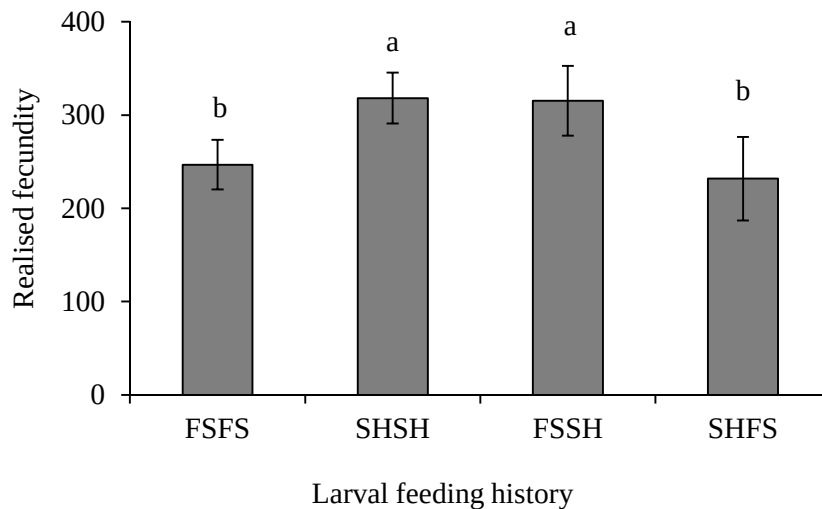


Figure 6.3 The effect of larval feeding history (FSFS, SHSH, FSSH and SHFS) on realised fecundity of of *Pareuchaetes insulata* progeny reared under constant laboratory conditions at 25 °C, 12:12 (L:D) h. Only the quality of host leaves as food could not be regarded as a constant condition because they were collected fresh from two different field sites (shade and full sun habitats). Data represent mean $\pm$ SE. Comparisons with different letters indicate significant differences (Sequential Bonferoni test, $P < 0.05$). FSFS: progeny were reared on full sun foliage similar to their parental diet; SHSH: progeny were reared on shaded foliage similar to their parental diet; FSSH (cross-feeding): progeny reared on shaded foliage whereas the parents had been reared on fun sun foliage and SHFS (cross-feeding): progeny reared on full sun foliage whereas the parents had been reared on shaded foliage.

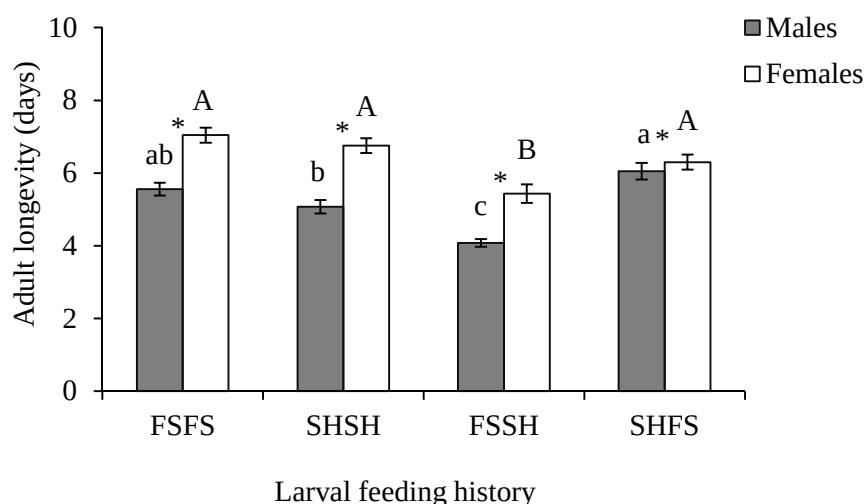


Figure 6.4 The effect of larval feeding history (FSFS, SHSH, FSSH and SHFS) on adult longevity of *Pareuchaetes insulata* progeny (females and males) reared under constant laboratory conditions at 25 °C, 12:12 (L:D) h. Only the quality of host leaves as food could not be regarded as a constant condition because they were collected fresh from two different field sites (shade and full sun habitats). Data represent mean $\pm$ SE. Among-feeding history comparisons were not significantly different if the same uppercase letter appears above white columns (females) or if the same lowercase letter appears above black columns (males) (Sequential Bonferroni test $P < 0.05$). For each feeding history, differences between males and females are indicated with an asterisk (student's t -test, $P < 0.05$). FSFS: progeny were reared on full sun foliage similar to their parental diet; SHSH: progeny were reared on shaded foliage similar to their parental diet; FSSH (cross-feeding): progeny reared on shaded foliage whereas the parents had been reared on full sun foliage and SHFS (cross-feeding): progeny reared on full sun foliage whereas the parents had been reared on shaded foliage.

In all treatments, larval development time was longer for females compared to their male counterparts, but total development time did not differ between sexes. Females had higher growth rate and heavier pupal mass compared to males in all treatments. Realized fecundity was higher in progeny that fed on shaded leaves (SHSH and FSSH) irrespective of parental diets (GLM ANOVA: Wald $\chi^2_{3,59} = 308.437$; $P < 0.0001$; Figure 6.3), although there was no significant difference in this variable between these two feeding history groups. Similarly, when reared on full sun leaves, realized fecundity did not differ between progenies from parents reared on either full sun (FSFS) or shaded (SHFS) leaves. Finally, when reared on shaded leaves, *P. insulata* progeny from parents reared on full sun (FSSH) exhibited reduced adult longevity (Figure 6.4). In all treatments, females lived longer than males.

6.4 DISCUSSION

This study was conducted to understand how *P. insulata* progeny will respond to a diet dissimilar (in nutrition) to that which their parents fed on as larvae. Comparisons of performance parameters were made among feeding history groups and the results showed that progeny of *P. insulata* performed better on shaded leaves (SHSH and FSSH) of *C. odorata* irrespective of parental diets.

The higher mortality (40%) experienced by progeny that fed on full sun leaves but whose parents were originally reared on shade leaves (SHFS) suggest that a “negative switch” (i.e. offspring from parents reared on shaded leaves feeding on full sun leaves) in diet in the field could be deleterious for the population of *P. insulata*. Although leaves of *C. odorata* plants growing in shaded habitats are known to be nutritionally unbalanced during winter relative to autumn (with higher foliar nitrogen and magnesium concentrations in shaded habitat probably causing reduction in pupal mass and growth rate as well as prolonged development time), no difference in survival was detected when two successive generations of the insect were reared exclusively on full sun versus shaded leaves (Chapter 5). The severe effect of the negative switch in diet (SHFS) of offspring, expressed as high mortality, prolonged development time, reduced pupal mass and growth rate reinforces the unsuitability of full sun leaves as source of food for *P. insulata* and suggests that metabolic costs may be associated with a change in diet from suitable quality to a nutritionally unbalanced diet (e.g. Schoonhoven and Meerman, 1978).

The faster larval and total development times, heavier pupal mass and increased growth rate in progeny that fed on shaded leaves, but whose parents fed on full sun (FSSH) leaves, suggests that a “positive switch” (i.e. offspring from parents reared on full sun feeding on

shaded leaves) in diets did not come at a cost, rather that it enhances offspring performance (e.g. Shah and Liu, 2013). Shorter development time may be important to larval survival because the duration of larval exposure to predators, parasitoids, pathogens and other potentially harmful agents is reduced (Slansky, 1990; Fordyce and Shapiro, 2003; Williams, 1999). The prolonged larval development time of female moths (relative to males) and the lack of a significant difference in total development time between sexes in all treatments concur with the findings of Uyi *et al.* (2014c).

The reductions in pupal mass in progeny that fed on full sun leaves irrespective of parental diets (FSFS and SHFS: negative switch) translated into reduced fecundity. Reduced pupal mass is known to affect fecundity in *P. insulata* (Uyi *et al.*, 2014d) and in other insects (Honěk, 1993; Awmack and Leather, 2002). Although *P. insulata* longevity was shorter (with a mean of 4.08 and 5.43 days for males and females respectively) on the positive switch diet compared to others, which lived for between 5 and 7 days, longevity is unlikely to affect fecundity in this insect because the moth is known to lay over 74% of its eggs within the first four nights, with peak egg-laying on the 2nd night (Uyi *et al.*, 2014d). While some studies suggest that the nutritional experience of parents may affect the performance of the progeny (e.g. Rossiter, 1991a,b; Fuentealba and Bause, 2012), others have reported the contrary (e.g. Shah and Liu, 2013). The prolonged development time and the reduced pupal mass as well as fecundity of *P. insulata* on the negative switched diet (SHFS) suggest that parents feeding on a superior diet (e.g. shade foliage) may not convey any advantage to their progeny especially when the progeny are fed on nutritionally unbalanced diets (e.g. full sun foliage).

6.4.1 Implications for biological control

The effects of the negative switch in diet (i.e. offspring from parents reared on shaded leaves feeding on full sun leaves) on *P. insulata* can have important implications for the population dynamics of this insect and on the biological control of *C. odorata* in South Africa. *Chromolaena odorata* grows in an ever-changing environment that is characterized by frequent clearings and the implementation of other management practices such as herbicide applications. While these actions are likely to influence the phytochemical (and nutrient) dynamics of *C. odorata* leaves (e.g. Chapter 5), the response of *P. insulata* to these changes might be negatively affected as has been demonstrated in this study because a negative switch in diet may lead to high mortality (up to 40%), prolonged development time (5 days longer) and reduced fecundity (by 27%). Consequently, this would decrease the populations of *P. insulata* in field situations and reduce the impact of this biological control agent in the field, although if the ovipositing adult females respond by flying to other infestations in search of good quality plants on which to oviposit, such negative effects as demonstrated by the study may be mitigated.

The poor performance exhibited by the offspring that fed on the negative switch diet (SHFS) points to full sun foliage as a poor quality food for *P. insulata* (at least during winter) (Uyi *et al.*, submitted; Chapter 5). These results also show the importance of studying feeding history in insects to fully understand the effects of a switch in the diet of an insect herbivore on its performance. In conclusion, this study demonstrates that a negative switch in diet may be deleterious for *P. insulata*.

CHAPTER 7

Effect of nitrogen fertilization on growth of *Chromolaena odorata* and the performance of a specialist herbivore, *Pareuchaetes insulata*

7.1 INTRODUCTION

The role of foliar nitrogen on the life-history traits and population dynamics of insect herbivores has received a great deal of attention (Mattson, 1980; Myers and Post, 1981; Minkenberg and Ottenheim, 1990; Heard and Winterton, 2000; Hinz and Müller-Schärer, 2000; Wheeler, 2003; Cineros and Godfrey, 2001; Huberty and Denno, 2006; van Hezewijk *et al.*, 2008; Zehnder and Hunter, 2009; Moran and Goolsby, 2014) primarily because nitrogen is considered the most limiting macronutrient for insect herbivores (Mattson, 1980; McNeill and Southwood, 1978) and is the raw material for protein synthesis (Sternner and Elser, 2002). Most plants profit from increased nitrogen availability to a certain threshold by increasing uptake (Herms and Mattson, 1992) and phytophagous insects may respond to variations in foliar nitrogen concentration by increasing (White, 1993) or decreasing (Awmack and Leather, 2002) feeding, depending on plant resource allocation and insect nutritional requirements (Maschinski and Whittham, 1989). Increased nitrogen concentration in plants has been reported to improve the survival and growth rate of insects (Wheeler, 2003; Scriber and Slansky, 1981; Minkenberg and Ottenheim, 1990; Huberty and Denno, 2006). Also, increased body mass and fecundity, together with population growth rate, have been positively linked with nitrogen addition or increased nitrogen in host plants (Myers and Post, 1981; Minkenberg and Ottenheim, 1990; Heard and Winterton, 2000; Hinz and Müller-Schärer, 2000; Awmack and Leather, 2002; van Hezewijk *et al.*, 2008). However, elevated nitrogen levels in plants have also been reported to have no (e.g. Abrahamson and McCrea,

1986; Auerbach and Strong, 1981) or even negative (Simpson *et al.*, 2004; Raubenheimer *et al.*, 2005) effects on herbivorous insects. This complexity in insect response to nitrogen addition underscores the importance of understanding this bitrophic interaction, if nitrogen is to be effectively used in the enhancement of weed biological control.

The usefulness of nitrogen in mass-rearing of weed biological control agents (Wheeler, 2001; Wheeler, 2003), improving the population establishment of biological control agents (Heard and Winterton, 2000; Hinz and Müller-Schärer, 2000; van Hezewijk *et al.*, 2008) and in initiating insect outbreaks in weed biological control (Room and Thomas, 1985) is only beginning to be appreciated by practitioners. For example, increased nitrogen concentrations in *Cynoglossum officinale* L. (Boraginaceae) resulted in a 25% increase in the fecundity of its biological control agent, *Mugulones cruciger* Herbst. (Coleoptera: Curculionidae) and the insect population increased in the field following nitrogen fertilization (van Hezewijk *et al.*, 2008).

An earlier study (Uyi *et al.*, submitted; see Chapter 5) highlighted the importance of *C. odorata* leaf quality for the growth, development and fecundity of *P. insulata*. In the study, when the insect was reared on *C. odorata* leaves obtained from plants growing under shaded conditions, the insect developed faster, had greater pupal mass and increased fecundity. These increases were associated with relatively high nitrogen and water content in the shaded plants compared to plants growing under full-sun conditions. While other inorganic nutrients may be important in the development, growth and population growth rates of phytophagous insects (Perkins *et al.*, 2004; Huberty and Denno, 2006; Behmer, 2009; Joen *et al.*, 2012; Ge *et al.*, 2013; Münzbergova and Skuhrovec, 2013), foliar nitrogen content is ubiquitously considered an appropriate indicator to measure plant quality for herbivorous insects.

Although the mass rearing and release of *P. insulata* is no longer a priority in South Africa, knowledge of the response of this insect to increased nitrogen fertilization of its host plant (*C. odorata*) may be of importance to other countries where the weed is invasive. Most importantly, such studies would help further our understanding of the nutritional ecology of this arctiine moth, a species whose biology and ecology (see review in Conner, 2009) has received limited attention. The objective of this study was to investigate the effect of different nitrogen fertilization levels on the performance of *C. odorata* and to elucidate the response of *P. insulata* (both larvae and adults) to fertilization of *C. odorata*.

7.2 MATERIALS AND METHODS

7.2.1 Study system, test plants and insect cultures

Chromolaena odorata plants were grown from stem-tip cuttings collected from several plants along a stretch of road near Durban, South Africa. Similarly sized stem-tip cuttings were initially planted in vermiculite with rooting hormone (Seradix[®] No 1) and placed in a mist bed before they were later planted in nursery pots (25-cm-diameter) containing a standard potting mixture (Umgeni coarse sand: Gromor Potting Medium[®], 1:1) in September 2013. In October 2013, three hundred plants were assigned to three different nitrogen fertilization levels (high, medium and low) with 100 plants per fertilizer treatment. Plants in high, medium and low fertilization treatments received 13.5, 9.6 and 5.8 g of Plantacote (Plantacote, AGLUKON Spezialduenger GmbH & Co. KG, Germany; 14 – 8 – 15, N-P-K; the various treatments did not exceed the recommendations of the manufacturers) respectively in a slow release formulation. All treatment plants were maintained in a greenhouse tunnel at the ARC-PPRI Weeds Biological Control Unit, Cedara, near Pietermaritzburg, South Africa, between September and October 2013, before they were

transferred outside in November 2013. All treatment plants were hand-watered with a hose pipe and benefited equally from rain water. The medium fertilizer treatment (9.6 g) represents the range (8 – 10 g) used for routine plant maintenance for a variety of experiments with other biological control agents such as *L. aemulus* and *D. odorata* (pers. obs.). Plants were grown at a higher fertilization level (13.5 g) to determine if *P. insulata* larvae would benefit from feeding on higher quality leaves.

For this study, *P. insulata* individuals were collected in the Sappi Cannonbrae Plantation, Umkomaas, KZN province, South Africa (30° 13' S, 30° 46' E) where the insect established following large releases made between 2001 and 2003 (Zachariades *et al.* 2011a). The insect was maintained in the laboratory for about five generations, on *C. odorata* leaf bouquets collected from the field, at 25 ± 2 °C, 60 ± 10 % relative humidity (RH) and at a photoperiod of 12:12 (L:D)h. All insect trials were performed inside a growth chamber (Labcon, South Africa) set at 25 °C and 12:12 (L:D) h photoperiod. Hygrochron iButtons (model DS 1923, Maxim Integrated Products, San José, USA, 0.5 °C accuracy) were used to measure temperature (hourly readings range, 24.1 to 25.54 °C; mean $\pm$ SE, 24.83 ± 0.01 °C) and RH (hourly readings range, 67.4 to 78.9%; mean $\pm$ SE, 71.4 ± 2.35 %) inside the chamber.

7.2.2 Effect of fertilization on plant and leaf characteristics

In December 2013, the longest leaf lengths of 15 *C. odorata* plants (5 leaves per plant) each belonging to one of three treatments were measured. Basal stem diameter and the height of the main shoot were also measured for the respective fertilization treatments (n = 15 per treatment). In February 2014, the above-ground parts of five randomly selected plants from each fertilizer treatment were harvested and separated into leaves and stems, oven dried for 72 hours at 64 °C and weighed to obtain dry biomass. In the same month, leaf materials

(fully expanded leaves taken from the upper half of the plants) were collected from five randomly selected plants in the respective treatments and the nitrogen content of the leaf samples was analysed. The leaves were dried for 72 hours at 65 °C and the nitrogen content was determined as a percentage of dry weight using a CN analyser (TruSpec[®] CN, LECO, MI, USA).

7.2.3 Effect of fertilization on insect survival, development and fecundity

On the day of hatching, *P. insulata* larvae were placed individually (with the aid of a fine brush) into transparent 100 ml aerated plastic containers with circular screen windows (2.5-cm-diameter at the top) lined at the bottom with moistened filter paper (to maintain relative humidity) and fed with *C. odorata* leaves obtained from plants in one of the three treatment levels. The advantages of this protocol have been documented in previous studies (e.g. Uyi *et al.* 2014c,d, Chapters 2 and 3). Larvae (n=100 per fertilizer treatment) were fed with fresh fully expanded leaves (taken from the upper half of the plants) every 48 hours and their frass was removed at the same interval for hygienic reasons (Uyi *et al.*, 2011). The larvae were monitored daily until pupation and/or adult eclosion in order to record mortality and/or to follow the duration of larval instars and pupae. The total development duration (duration from egg hatch to adult eclosion) was recorded only for individuals that survived to adult eclosion, in all three treatments. Pupae were weighed (3 days after pupation), although only those that successfully eclosed were used in the final statistical analysis. Upon adult eclosion, adult longevity was measured.

When the adults emerged, one virgin female and two newly eclosed males that had fed as larvae on the same fertilizer treatment were placed in a 700 ml containers as described in Uyi *et al.* (2014d; Chapter 2), and provided with stem cuttings (with leaves) from this treatment.

Fifteen replicates each were used for high and medium treatments while 19 replicates were used for the low fertilizer treatment. Containers and leaves were examined daily to record the number of eggs laid (i.e. fecundity) and adult longevity.

7.2.4 Statistical analysis

Plant data (nitrogen concentration, basal stem diameter, height of main shoot, length of longest leaf and leaf- and stem-biomass) satisfied the assumption of parametric tests (after Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variances). A one-way analysis of variance was used to evaluate the effect of fertilization on plant and leaf characteristics. When the results were significant, the differences among fertilization treatments were compared using Tukey's Honestly Significant Difference (HSD) test because of the equality of sample sizes. The effect of fertilization treatments on total development duration, pupal mass, female fecundity and adult longevity of *P. insulata* were evaluated using General Linear Model analysis of variance (GLM ANOVA). Due to the unequal sample sizes among treatments in insect performance trials, means were compared using Tukey-Kramer's test. With the exception of the GLM ANOVA that were performed using SPSS statistical software, version 16.0 (SPSS INC., USA), all analyses were performed using GENSTAT statistical software, version 14.0 (VSN International Ltd, UK)

7.3 RESULTS

7.3.1 Effect of fertilization on plant and leaf characteristics

The percentage leaf nitrogen concentration was influenced by fertilization treatments (Table 7.1; Figure 7.1a).

Table 7.1 Results of a one-way ANOVA for effects of three fertilizer treatments on *Chromolaena odorata* plants.

Response variable	Source of variation	DF	MS	F-value	P
Nitrogen (%)	Treatment ^(a)	2	1.503	10.85	0.002
	Error	12	13.861		
	Total	14			
Basal stem diameter (cm)	Treatment ^(a)	2	0.504	44.01	< 0.001
	Error	42	0.012		
	Total	44			
Height of main shoot (cm)	Treatment ^(a)	2	1527.301	12.59	< 0.001
	Error	42			
	Total	44			
Length of longest leaf (cm)	Treatment ^(a)	2	14.174	17.82	< 0.001
	Error	42	0.796		
	Total	44			
Leaf biomass (g dry mass)	Treatment ^(a)	2	47.218	18.53	< 0.001
	Error	12	2.576		
	Total	14			
Stem biomass (g dry mass)	Treatment ^(a)	2	636.727	34.21	< 0.001
	Error	12	18.612		
	Total	14			

DF: degrees of freedom.

MS: mean squares.

^aTreatments: three fertilizer levels, viz. high, medium and low.

Foliar nitrogen concentrations of plants in the medium and high treatments were 23.6 and 34.5% higher than plants that received low fertilization, although foliar nitrogen did not differ between high and medium fertilization treatments. High- and medium-fertilized plants had significantly greater stem diameter relative to low-fertilized plants (Table 7.1; Figure 7.1b). High- and medium-fertilized plants were taller and had leaves that were on average longer (26% and 19.8% for high- and medium-fertilizer treatments respectively) than those of low-fertilized plants (Table 7.1; Figure 7.1 c and d).

Above-ground biomass (leaf- and stem-biomass) was influenced by fertilization treatment (Table 7.1, Figure 7.2 a and b). While high- and medium-treatment plants had higher leaf biomass relative to low-fertilized plants, the three different treatments resulted in different stem biomass with the high-fertilizer treatment having the greatest stem biomass (over 1.5 fold greater than the low-fertilized plants).

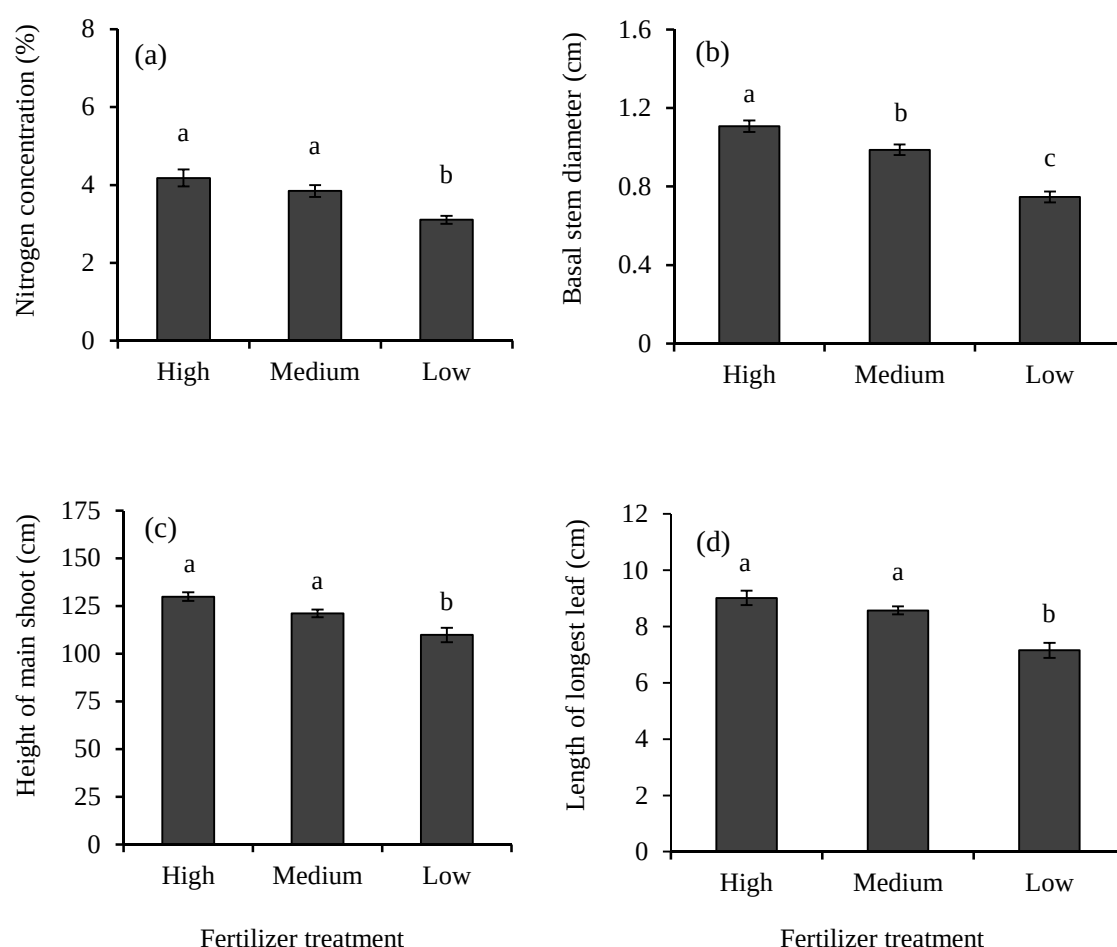


Figure 7.1 Effect of fertilization on foliar nitrogen (a), basal stem diameter (b), shoot height (c) and the length of longest leaf (d) of *Chromolaena odorata* plants. F-ratios of one-way ANOVA with fertilization treatments as factors are given in Table 1. Means ($\pm$ SE) with different letters above bars are significantly different ($P < 0.05$) after Tukey's Honestly Significant Difference (HSD) test.

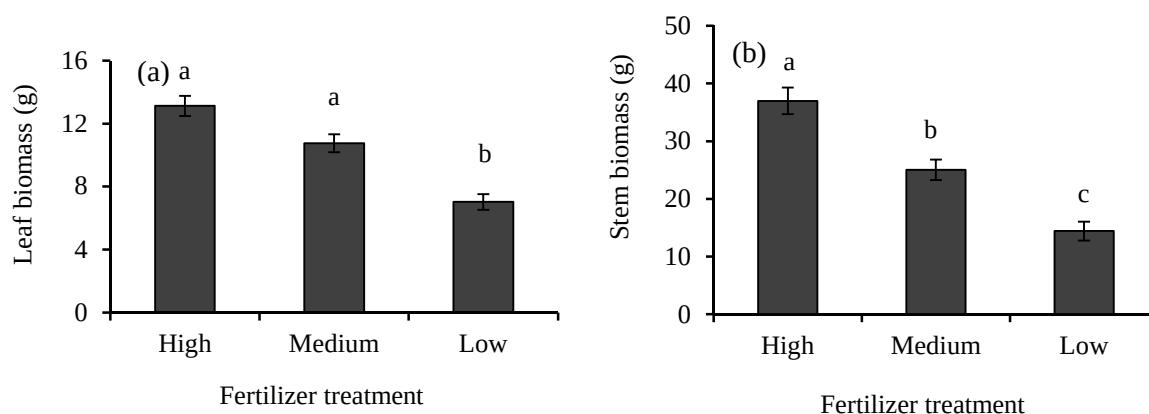


Figure 7.2 Effects of fertilization on leaf (a) and stem (b) biomass of *Chromolaena odorata*. F-ratios of one-way ANOVA with fertilization treatments as factors are given in Table 1. Means ($\pm$ SE) with different letters above bars are significantly different ($P < 0.05$) after Tukey's Honestly Significant Difference (HSD) test.

7.3.2 Effect of fertilization on the performance of *Pareuchaetes insulata*

Total immature survival (first instar to adult eclosion) was high (between 90 and 97%) and was not significantly influenced by plant fertilization (Pearson $\chi^2 = 3.96$, d.f. = 2, $P = 0.138$) (Figure 7.3). While adult longevity did not significantly differ (GLM ANOVA: $F_{2,279} = 1.77$, $P > 0.173$) according to fertilizer treatments, development duration (GLM ANOVA: $F_{2,279} = 67.70$, $P < 0.001$), pupal mass (GLM ANOVA: $F_{2,279} = 4.58$, $P = 0.011$) and the fecundity of the adult progeny (GLM ANOVA: $F_{2,48} = 4.70$, $P = 0.014$) were significantly influenced by fertilizer treatments (Figure 7.4 a-d). Individuals that were fed on leaves from high- or medium-fertilizer treatments developed faster and had greater pupal mass and improved fecundity compared with those that fed on leaves from the low-fertilizer treatment.

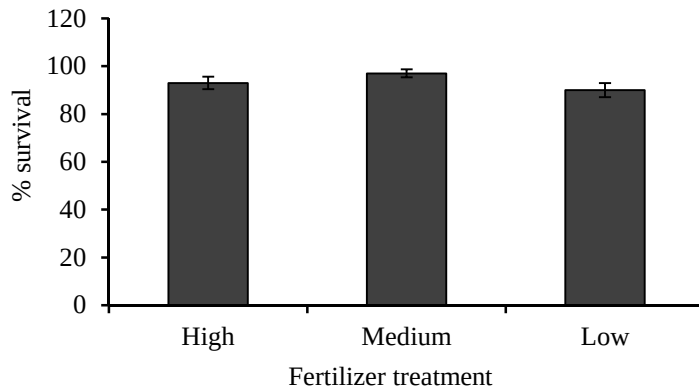


Figure 7.3 Effect of fertilization of *Chromolaena odorata* plants on the percentage survival (mean $\pm$ SE) of combined immature stages of *Pareuchaetes insulata*.

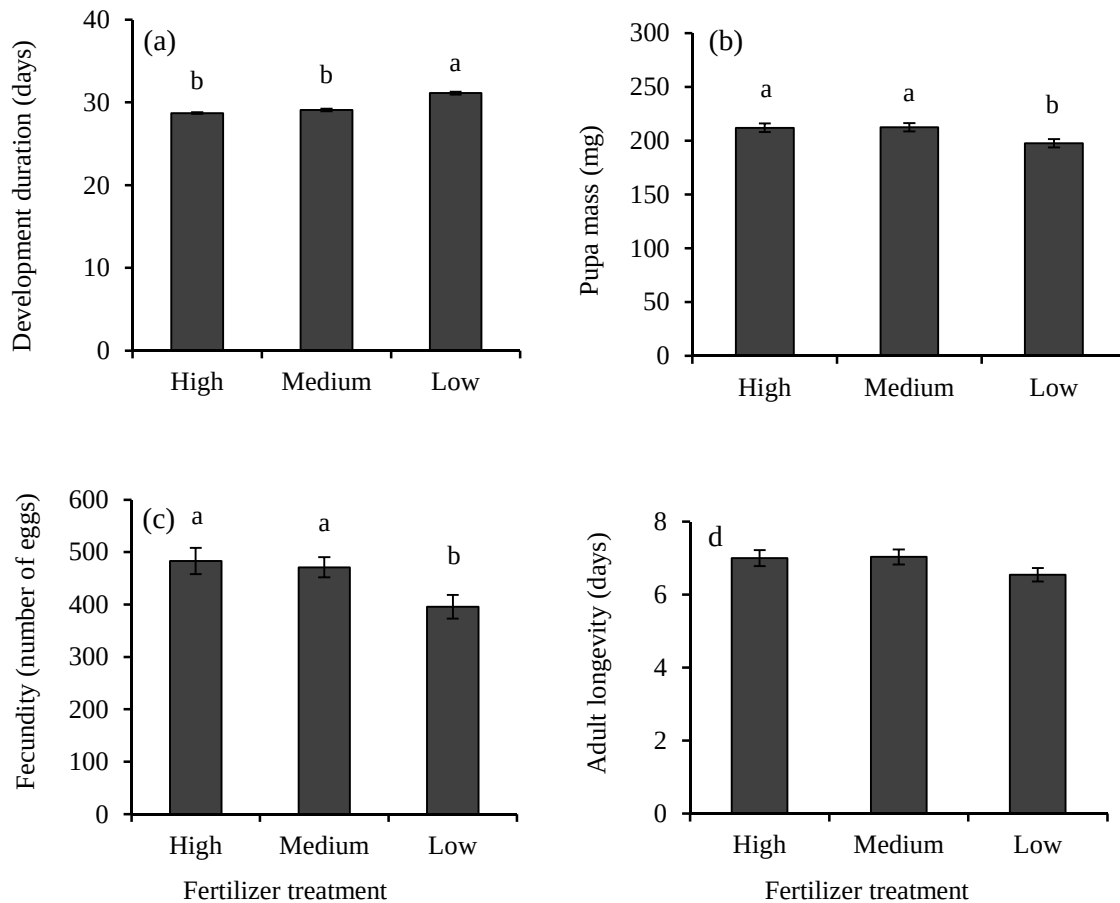


Figure 7.4 Effects of fertilization of *Chromolaena odorata* plants on development duration (a), pupal mass (b), female fecundity (c) and adult longevity (d) of *Pareuchaetes insulata*. Means ($\pm$ SE) with different letters above bars are significantly different ($P < 0.05$) after Tukey-Kramer test.

7.4 DISCUSSION

This study showed that fertilization significantly influences characteristics of *C. odorata*. For example, plants that received high- or medium-fertilizer treatments had higher foliar nitrogen concentrations, greater basal stem diameter, longer shoots, longer leaves and increased leaf- and stem biomass compared with low-fertilizer treatment. Although no previous published studies exist on the response of *C. odorata* to varying nitrogen levels, it is widely known that plants respond positively to fertilizer addition. Experimentally, nitrogen addition has been linked with improved plant traits or leaf characteristics such as longer shoots and leaves, increased number of shoots and leaves, high nitrogen concentration, and increased flowering in a number of plants including Scentless Chamomile, *Tripleurospermum perforatum* (Mérat) Lainz (Asteraceae) (Hinz and Müller-Schärer, 2000). The improved performance of *C. odorata* in the high- or medium-fertilization treatments in this study is analogous to the performance of *C. odorata* under shaded field conditions documented in Chapter 5. For example, the foliar nitrogen concentrations of 4.18% recorded for the high-fertilizer treatment is similar to the 4.03% recorded on plants growing under shaded conditions in autumn.

While there were no significant differences in immature survival and adult longevity of *P. insulata*, development time was faster, pupal mass was heavier and improved egg production was evident in individuals that fed on leaves from high-fertilized plants. The similarity in total survival of individuals that fed on plants from the three fertilizer treatments suggests that nitrogen levels were still too high in the low fertilizer treatment (3.1%) to negatively affect survival. The equally high survival (84-98%) recorded in individuals that fed on leaves (obtained fresh from sunny habitat field sites) of low nitrogen concentration (2.8%) in an earlier study (Uyi *et al.*, submitted, Chapter 5) support this conjecture. Although increased foliar nitrogen concentrations have been reported to improve insect survival (including

biological control agents) (e.g. Wheeler, 2003; Huberty and Denno, 2006), studies have also demonstrated that increased nitrogen can have a neutral or negative effect on insect survival and population levels (Chapter 5).

Medium or high fertilization reduced the total development duration by 2 to 2.5 days and increased pupal mass by 8% compared with low fertilization. It is assumed that a faster development time is advantageous for offspring. Possible advantages could be that a fast development time on nitrogen-rich foliage might promote fitness in growing a population with overlapping generations. Another advantage of fast development might be a higher chance of survival as predicted by the “slow-growth-high-mortality hypothesis” (Williams, 1999). This hypothesis suggests that slow-growing larvae suffer greater mortality from natural enemies, unfavourable environmental conditions and other mortality factors (Benrey and Denno, 1997; Williams, 1999; Fordyce and Shapiro, 2003). Faster development duration in insects in response to increased fertilization or increased foliar nitrogen has been previously documented (Heisswolf *et al.*, 2005; Huberty and Denno, 2006; Chapter 5).

Most importantly, the 8% increase in pupal mass in individuals that fed on leaves from high- or medium-fertilization treatment led to improved fecundity of the resultant adult females. Increased pupal mass and female fecundity has been previously linked with increased foliar nitrogen or high fertilization of host plants (Hinz and Muller-Schärer, 2000; Wheeler, 2003; van Hezewijk *et al.*, 2008; also see Chapter 5).

Although this study demonstrated that *P. insulata* responded positively (in terms of reduction in development time and increased fecundity) to increased nitrogen or fertilization, other studies have reported negative effects of high nitrogen concentrations on insect performance

metrics (e.g. development time, pupal mass) (e.g. Hartley and Lawson, 1992; Birch *et al.*, 1992; Clissold *et al.*, 2006). Boesma and Elser (2006) postulated the concept of high plant-nutrient concentration being energetically costly to herbivores. In situations where nutrient concentrations (e.g C:N) in the host plant (due to nitrogen addition) exceed herbivore threshold elemental ratios (TER) (i.e. if host plant elemental content is higher than the level that satisfies herbivore requirement), the excess elemental composition would have to be excreted (Boesma and Elser, 2006). If excretion is energetically costly, then herbivores that consume foliage which exceeds TER will exhibit slower growth, reduced reproduction and reduced population growth rates. Results from several studies have supported this. For example, Lee *et al.* (2002) showed a decrease in pupal mass of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) on a diet with high protein to carbohydrate ratio, while Clissold *et al.* (2006) showed that excess nitrogen relative to carbohydrate led to elongation in development time in the nymphs of Australian plague locust, *Chortoicetes terminifera* Walker (Orthoptera: Acrididae). The lack of significant differences in insect performance metrics between high- and medium-fertilizer treatments in the current study suggests that a foliar nitrogen content of leaves of 3.84% in the medium-fertilized plants satisfied the nitrogen requirement of *P. insulata* and that foliar nitrogen content in both high and medium treatments did not exceed herbivore TER.

7.4.1 Implications for biological control

Although the biological control programme against *C. odorata* in South Africa is no longer mass-rearing and releasing *P. insulata*, other countries in Asia or Africa with *C. odorata* infestations may benefit from the findings of this research. This study showed that the use of 9.6 g (for medium-fertilizer treatment) not only increased foliar nitrogen content in *C. odorata*, it also led to a reduced development time and resulted in improved pupal mass and

number of eggs laid in *P. insulata*, thus suggesting that the mass-rearing of the moth would benefit from increased foliar nitrogen content up to a certain level. These results also suggest that when larvae are reared on leaves with more than a 3.84% nitrogen content, there might be no additional increase in benefits and that foliar nitrogen content above 4.1% in *C. odorata* might be associated with a fitness cost (e.g. Lee *et al.*, 2002; Clissold *et al.*, 2006) – presumably because of elevated metabolic costs related to catabolising protein (for use as an energy source) and excreting excess nitrogen (Schroeder, 1986; Boersma and Elser, 2006; Clissold *et al.*, 2006). The findings of Chapter 5 support this because individuals of *P. insulata* that fed on leaves with nitrogen concentration of 4.8% experienced prolonged development and reduced pupal mass as well as reduced fecundity compared to their counterparts that fed on leaves with 4.0% nitrogen.

The positive response of *C. odorata* to fertilization and the corresponding improved insect performance on high- and medium-fertilized plants have several important management implications. First, the mass production of *P. insulata* at high- and medium-fertilizer levels will reduce development time and increase female fecundity. Second, when considering factors for the selection of agent release sites, nascent populations of this agent will establish and build up populations more rapidly at sites where the foliar nitrogen content of host plants is high (or in high-fertilization sites) because of a reduction in development time and improved fecundity. Finally, if *P. insulata* is to be reared in a small open field for mass production and redistribution, fertilization of *C. odorata* will result in rapid increase in population growth rates and fitness of the colony.

This study showed that *C. odorata* positively responded to fertilization and that the specialist herbivore performed better on high or medium fertilized plants compared to low fertilizer

treatment. The inability to demonstrate significant differences in survival of *P. insulata* among all three fertilizer treatments and the lack of differences in insect performance between high- and medium-fertilization treatments reiterate the fact that relationships between nitrogen levels in host plants and phytophagous insect performance are not simple. This study is the first report of the effect of fertilization on *C. odorata* and on the performance of *P. insulata*, thus contributing to our understanding of the nutritional ecology of erebid moths in the subfamily Arctiinae.

CHAPTER 8

8.1 GENERAL DISCUSSION

The increasing attempts to limit the spread and/or reduce the negative impacts of invasive alien plant species in their adventive ranges occasionally result in the introduction of natural enemies, especially insects, as biological control agents, from the native range of the plant by biological control practitioners. The success of biological control agents in their introduced range is sometimes constrained by a multiplicity of biotic (e.g. predators, parasitism, plant genetic variation) and abiotic (e.g. climate, habitat variation, soil nutrients) factors which may limit their efficacy and even result in the failure of some agents to establish. Weed biological control has been relatively successful, but there is room for improvement in certain areas. These include (i) early-programme work such as genetic/biotype matching of the origin of the weed (e.g. Goolsby *et al.*, 2006; Paterson and Zachariades, 2013), and climatic similarity between the native and introduced ranges of an agent (Robertson *et al.*, 2008), both of which can have a knock-on effect on agent performance (e.g. Dray *et al.*, 2004) and consequently on the success of the biological control programme; (ii) laboratory-based assessment of the biological attributes of the species in question which may make it a promising or poor biological control agent, including measures of its fecundity, mating behaviour, development time and survival, thermal physiology, and relative performance on different genotypes of the target weed; and (iii) post-release evaluation of an established agent, conducted in-field and at several levels or stages: the eventual distribution and population levels of the agent, which could be influenced by climatic factors or mortality due to predation and parasitism; the impact it has on the target weed at individual and population levels; and the agent's indirect positive impact on resources that had been negatively impacted by the weed, and had motivated the weed's control in the first place.

Such ‘non-core’ studies are often not undertaken, primarily due to a lack of resources (Syrett *et al.*, 2000, Thomas and Reid 2007, Morin *et al.*, 2009), and because in the case of post-release evaluations they are long-term and difficult. There is also often pressure on biological control practitioners to move on to other agents and weeds. In particular, biological control practitioners do not often adequately assess agents that have established but have seemingly performed inadequately. It is important to assess such agents for various reasons, such as to allow improved agent selection in future; to better quantify the impact of a biocontrol project on the target weed and thereby decide whether further agents are needed; and to assess the cost-effectiveness of weed biological control compared to other control methods.

The studies in this thesis were conducted in order to better understand the apparently poor performance of the specialist herbivore *P. insulata* on *C. odorata* in north-eastern South Africa, following the establishment of the biological control agent there in 2004. Of the thirty sites where large numbers (about 1.9 million individuals) of the insect were released, it is thought to have established at only one site, from where it has since spread to other areas (although recent outbreaks of the insect in northern KZN (Strathie and Zachariades, 2014) have cast some doubt on this). Apart from occasional outbreaks of *P. insulata* (Zachariades *et al.*, 2011a; Strathie and Zachariades, 2014; pers. obs.), its population has generally remained very low and consequently its impact has been variable and generally thought to be low. Its performance was thought to be poorer than that of its congener *P. pseudoinsulata* in many of the areas where the latter has established.

Although questions of the origin of the biotype of *C. odorata* invading southern Africa, and the most climatically similar areas of the Americas from which to collect candidate biological control agents have been addressed (Robertson *et al.*, 2008, Paterson and Zachariades 2013),

the response of individual agents to imperfect genetic or climatic matches can best be assessed through controlled laboratory trials. The series of studies comprising this thesis has done this for *P. insulata*, addressing specifically (i) the relative performance of the insect on *C. odorata* from Florida, USA, from where it was collected, and the SA biotype of the weed, on which it established, and which is genetically identical only to plants from Jamaica and Cuba; (ii) various aspects of the thermal physiology of the insect, from which predictions of its performance in the climatic conditions across South Africa were made; and (iii) its responses to variation in plant condition, which was achieved through manipulation of fertilization regime, and sampling across season and habitat. The biology of the insect was also studied in greater detail than previously, and possible implications of this for its success as a biological control agent assessed. Time and logistical constraints precluded conducting life-table studies in the field to assess mortality rates and causes.

This concluding chapter discusses some of the contributing factors (biotic and abiotic) suspected to be responsible for the apparent poor performance of *P. insulata* as a biological control agent in South Africa. As it was not possible to address all possible reasons for poor performance of *P. insulata* during the course of these studies, future useful areas of research on *P. insulata* are discussed. Further, the implications of this research for the biological control programme against *C. odorata* and the direction of future research for the control of *C. odorata* are discussed, as is the contribution of these studies to the discipline of weed biological control in general. Some of the studies within this thesis were not of great relevance to biocontrol, but rather to the ecology of plant-herbivore interactions; these results are discussed in that context.

8.1.1 The biology of *Pareuchaetes insulata*

In the laboratory, *P. insulata* displayed good biological attributes such as high female mating success, fecundity, egg hatchability and survival of immature life stages (Chapter 2). It thus appears unlikely that the basic biological attributes of *P. insulata* prevent population increases in the field. In contrast, Boughton and Pemberton (2012) showed that aspects of its life history traits (e.g. fecundity) may explain the establishment failure of the biological control agent, *Austromusotima camptozonale* (Hampson) (Lepidoptera: Crambidae) released against the old world climbing fern, *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae) in Florida, USA. The only biological trait of *P. insulata* which may negatively impact field populations is the absence of protandry and the possible presence of protogyny, because adult females emerge before males. The absence of protandry in small populations or in insects with a short-lived adult stage (e.g. *P. insulata*) can lead to matelessness and can even result in local extinction (Calabrese and Fegan, 2004; Fauvergue, 2013). However, further speculation on the consequences of the absence of protandry on the population dynamics of this moth should be deferred until field studies on adult emergence phenology and mating behaviour of this insect are conducted. Also, data concerning mate finding and the relationship between male moth density and female mating success will be needed to elucidate further, how protandry and protogyny influence female mating success or failure.

8.1.2 The influence of climatic factors on the performance of *Pareuchaetes insulata*

The effects of low temperatures on the life history traits of classical weed biological control agents have explained the failure of some biological control programmes (McClay and Hughes, 1995; Byrne *et al.*, 2002; Coetzee *et al.*, 2007) partly because temperature is a critical factor affecting the performance and behaviour of insects (Bale *et al.*, 2002; Chown and Terblanche, 2007; Mazzi and Dorn, 2012). As ectothermic organisms, the physiological

processes of insects are highly sensitive to temperature. Development rates, longevity, fecundity and locomotion abilities are strongly affected by environmental temperature (Bale *et al.*, 2002; Ferrer *et al.*, 2014). Consequently, life-cycle events and population decline or profusion in time and space are modified by exposure to extreme low or high temperatures (Ward and Masters, 2007; Robinet and Roques, 2010).

Exposures to low temperatures at short or long timescales have both direct and indirect effects on *P. insulata* (Chapter 4). At constant temperatures below 25 °C, survival declined while development time was prolonged. Direct mortality and the indirect effects of low temperatures such as increased mortality resulting from slow development and increased exposure time to natural enemies may prove to be catastrophic to the population levels of this insect in the field during winter in the distribution range of *C. odorata* in KZN province. Furthermore, slow development of the *P. insulata* over winter would result in a lower rate of population increase and this may consequently reduce the impact of the moth on the weed population.

The results obtained from iButtons placed 60 cm above ground level in Sappi Cannonbrae plantation, Umkomaas, suggest that absolute minimum temperature can be as low as 4.6, 6.6 and 5.1 °C in the winter months of June, July and August respectively compared with the moth's developmental threshold of 11.3 °C. Temperatures close to or below the developmental threshold often retard development and in many cases increase mortality (Savopoulou-Soultani *et al.*, 2012). The mean daily temperature (suspended iButtons) at the Sappi Cannonbrae plantation, Umkomaas, was 15.1 and 15.9 °C in June and July respectively. Given that only 10.0% of the moths survived to adult emergence in the laboratory when reared at a constant temperature of 15 °C and that development was almost

four times longer compared with those reared at a constant temperature of 25 °C, this suggests that low winter temperature could be a key factor responsible for low populations of *P. insulata* in South Africa. Several other studies have shown that the potential distribution of biological control agents is associated with their ability to tolerate cold temperatures, which is essential for permanent population establishment (Byrne *et al.*, 2003; Coetzee *et al.*, 2007; Lapointe *et al.*, 2007; May and Coetzee, 2013).

Reductions in development time and body size at higher temperatures are not uncommon in ectotherms. This plastic response to variation in temperature is referred to as the temperature-size rule (TSR) and holds true for more than 80% of all the ectothermic species (Atkinson, 1994; 1995; Kingsolver and Huey, 2008). Such reaction norms also apply to several Lepidoptera species (Davidowitz *et al.*, 2004; Fischer and Karl, 2010). While the current study (Chapter 4) did not explicitly test the TSR, it demonstrated support for this rule, as development time was prolonged at low temperature. Although pupal mass was not measured, reduced egg production was evident in female adults that were exposed to low temperatures. Because the females used for this study were reared at a constant temperature of 25 °C, it is inappropriate to relate the low egg production to the TSR. The low number of eggs produced at extreme low and high temperatures might be due to physiological damage suffered by the insect. This result suggests that female adults of *P. insulata* incur fitness costs at low temperature in terms of prolonged development times and reduced lifetime egg production.

Models that use both laboratory developmental data and long-term climate records can be useful for predicting the geographic distribution of, and favourable areas for, permanent establishment of biological control agents (May and Coetzee, 2013). In this study the number

of generations per year for *P. insulata* varied from 1 to 8.9 generations across the four provinces in South Africa where *C. odorata* is present. Clearly, in areas where less than three generation per year is possible, permanent establishment might be precluded. The model predicted a range of 3.1 - 7.5 generations per year for *P. insulata* throughout KZN province, which indicates the existence of favourable conditions (especially along the coastal belt) for establishment of this agent. The predicted number of generations in all areas in KZN where the moth is currently present ranged between 6.4 and 7.5 generations. This suggests that the moth may readily establish at locations in Limpopo and Mpumalanga provinces with more than five generations per year. As a separate issue to the numbers of generations of the moth per year, establishment may be precluded in areas with a pronounced temperate climate because of low temperatures.

In addition to the direct mortality (discussed earlier), thermal stress can also cause neuronal damage (Chown and Terblanche, 2007). Although this study showed that direct mortality due to low temperature for a short term exposure (0.5 to 4 hours) might be trivial, it is evident that low temperatures (6 and 11 °C) negatively affected locomotion performance of larvae, suggesting that the insect would be unable to feed or move during winter when temperatures falls below 6 °C . This might have a significant impact on the population levels of the insect because increased exposure to low temperatures may lead to starvation and inability to escape from natural enemies. While the above hypothesis can be validated by the low temperatures recorded at the suspended microsite (at Sappi Cannonbrae plantation, Umkomaas), further studies on the effects of temperature on the flight performance (e.g. Ferrer *et al.*, 2014) and on mating behaviour of *P. insulata* are desirable.

The findings of this study partially support the beneficial acclimation hypothesis (BAH), as there was a clear beneficial effect of acclimation to low temperature (when exposed to low temperatures, locomotion performance was much improved in cold acclimated individuals). Only few studies using lepidopterans have tested the BAH (Sibly *et al.*, 1997; Woods and Harrison, 2001; Chidawanyika and Terblanche, 2011; Ferrer *et al.*, 2014). With the exception of Ferrer *et al.* (2014) who studied the effect of temperature experienced during development (thermal acclimation) on flight performance of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), other workers investigated the effect of acclimation in terms of plasticity in thermal limits through the evaluation of survival. The current study investigated the effect of acclimation on locomotion performance in *P. insulata* larvae. The result emphasizes that locomotion abilities of the larvae of the moth are affected by the climatic conditions of individual development, and hence may differ according to seasonal fluctuation. This result suggests that biological control agents scheduled for release in winter or late autumn should be mass reared at low temperatures to enhance or optimise the performance of such agents in the field. The result also implies that the harmful effects of low temperatures in winter may not be as great as expected, because larvae become gradually acclimatized to low temperatures.

Low winter temperature may also have indirect negative effects (through variation in host plant quality, expressed as increased leaf nitrogen in winter) on the performance of *P. insulata* as has been suggested in Chapter 5, because there might be a fitness cost associated with excess foliar nitrogen (e.g. Boersma and Elser, 2006; Clissold *et al.*, 2006).

Other climatic factors such as relative humidity and rainfall can also directly or indirectly influence the distribution and establishment of biological control agents and other insect

species (Dempster, 1971; Byrne *et al.*, 2002). For example, the effect of desiccation as a result of low humidity and rainfall has been shown to cause egg failure and rapid death of first instar larvae of *P. pseudoinsulata* in Trinidad (Cruttwell, 1972). The contribution of these factors to the variable performance of *P. insulata* needs to be explored.

Zachariades *et al.* (2013) documented the presence of the AWAB *C. odorata* in a number of eastern and southern African countries such as Kenya, Tanzania and possibly Malawi and Mozambique. These areas are more tropical, sometimes with higher rainfall and a less-pronounced winter compared to South Africa. *Pareuchaetes pseudoinsulata* is from a tropical origin (Trinidad) whereas *P. insulata* and *P. aurata aurata* were collected in the more subtropical areas of Florida/Jamaica/Cuba and northwest Argentina respectively, and would therefore be expected to maybe perform better in South Africa. Tropical climates in the aforementioned eastern and southern African countries may be more suitable for *Pareuchaetes* species, as has been reported in West Africa and other tropical regions (see Zachariades *et al.*, 2009) where *P. pseudoinsulata* has established. With the current spread of *P. insulata* in South Africa (Zachariades *et al.*, 2011; Strathie and Zachariades, 2014), it is not impossible that this species may eventually spread into one of the aforementioned countries. If *P. insulata* eventually spread into these countries, the climate may well support its performance and consequently help to reduce the populations of the AWAB *C. odorata* present there.

8.1.3 The influence of host-plant factors on the performance of *Pareuchaetes insulata*

A great deal of literature has accumulated and continues to be generated which links the quality of food to the performance of insect herbivores (e.g. Preszler and Price, 1988; Price, 2003; van Hezewijk *et al.*, 2008). The suitability of the plant as a food source for a

monophagous or oligophagous insect is influenced by both chemical factors in the form of both nutrients and deleterious chemicals; and physical attributes such as the presence of trichomes and the physical toughness of tissues. In turn these are influenced by a variety of factors such as the genetic make-up of the plant, soil nutrient levels and other soil properties, light levels and weather or climatic conditions (see references in Chapters 3, 5, 6 and 7).

Ecologists have long debated the relative importance of bottom-up (i.e. plant quality) and top-down (predation and parasitism) effects on the population dynamics of phytophagous insects (Dempster, 1971; Isaacson, 1973; Briese, 1986; Preszler and Price, 1988; Price, 1990; Price *et al.*, 1990; Cornell and Hawkins, 1995; Price, 2003). Over the past decades, the debate has moved away from arguments about which factors, top-down or bottom-up processes have the biggest influence on insect herbivore toward an increasing recognition that both processes can influence insect population dynamics across a number of taxa (e.g. Cornell and Hawkins, 1995; Hunter *et al.*, 1997; Hunter, 2001). In particular, bottom-up factors such as increased foliar nitrogen can result in dramatic improvement of herbivore performance (Room and Thomas, 1985; Heard and Winterton, 2000; van Hezewijk *et al.*, 2008; Moran and Goolsby, 2014), but very high levels are known to be detrimental (Hartley and Lawton, 1992; Clissold *et al.*, 2006; Lee *et al.*, 2006; Zehnder and Hunter, 2009). Therefore, Chapters 3, 5, 6 and 7 investigated several bottom-up effects in the bitrophic interactions between *C. odorata* and *P. insulata*.

Genetic or morphological variations in host plants have been shown to affect the performance of certain biological control agents (e.g. Dray *et al.*, 2004; Wheeler, 2006). Although *C. odorata* from Florida is morphologically and genetically distinct to that from South Africa, *P. insulata* performed equally well on both plants in the laboratory, even if there was the

suspicion of larval preference for its original native plant from Florida (Chapter 3). Similarly, Paterson *et al.* (2012) found no effect of genetic variation in *Pereskia aculeata* Miller (Cactaceae) on the fitness of the monophagous biological control agent, *Phenrica guérini* Bechyné (Coleoptera: Chrysomelidae). Variable tolerance by insect species of genetically-based variations in their host plant species can be attributed to variability between insect species in their degree of host-specificity (biological control agents only vary between stenophagy and oligophagy). Both *P. insulata* and *P. pseudoinsulata* appear to have slightly oligophagous host ranges; in addition, Dube *et al.* (2014) demonstrated that *P. insulata* in Jamaica, Cuba and Florida are genetically similar, which implies that their performance on plants from these three regions may be similar.

However, the equal performance of *P. insulata* from Florida on SAB and Floridian *C. odorata* plants is not a criterion to argue that determining the ancestral origin of invasive alien weed populations should not be prioritized when collecting biological control agents in the native range. Because the *C. odorata* biotype invasive in South Africa is genetically similar to the Jamaican and Cuban *C. odorata* (Paterson and Zachariades, 2013), pre-release studies should be conducted on potential agents collected elsewhere in the Americas (i.e. other than Cuba or Jamaica) to compare insect preference for, and performance on, the genotype of *C. odorata* from which it was collected and the SAB genotype.

Studies on seasonal changes, changes mediated by sunlight, changes mediated through fertilizer and variability depending on feeding history showed that *P. insulata*'s development time, survival and fecundity (in some cases) was influenced by plant quality (Chapters 5, 6 and 7). Generally, shaded habitat provided a more nutritionally balanced diet. The better performance of individuals that fed on shaded leaves in autumn compared to those that fed on

the same leaves in winter is probably due to the elevated nitrogen levels (and to an extent the high magnesium content) of shaded leaves in winter, because excess foliar nitrogen is known to negatively impact herbivore performance (see Lee *et al.*, 2002; Clissold *et al.*, 2006; Zehnder and Hunter, 2009). The findings of Chapter 5 suggest that low winter temperatures can cause a nutritional imbalance in *C. odorata* leaves growing in shaded habitats and that individuals feeding on such foliage would experience a prolonged development time and reduced fecundity. This would reinforce the negative effects of low winter temperature on the performance and population dynamics of this insect. Previous studies have also documented that variations in abiotic conditions can have effects on the abundance and performance of herbivores both directly (e.g. Shreeve, 1986) and indirectly through host plant effects (e.g. Crone and Jones, 1999). In a natural environment, seasonal changes and variation in microhabitats can influence the leaf characteristics (concentrations of primary and secondary metabolites, physical defence structures etc.) and this can affect the performance of the associated herbivores (Scriber and Slansky, 1981; Mattson and Scriber, 1987).

Behaviourally, two possible scenarios may explain the superior larval and adult performance on shaded foliage compared to full sun foliage; (i) adult females might have evolved a preference to lay eggs on shaded foliage because of the benefits their offspring would receive from such foliage or (ii) larvae may migrate from full sun to shaded habitat either to escape adverse conditions or possible predation. The first scenario suggests that increased nutrients (i.e. nitrogen) in a shaded environment relative to full sun might have resulted in the evolution of an ovipositional preference – larval performance link (Jaenike, 1978; Price, 1991) for *P. insulata*. This concept is often referred to as the preference – performance hypothesis (Jaenike, 1978; Price, 1991) and it states that females should oviposit on individuals of plant species that enhances the performance of their offspring. The fact that

larvae and adults of this moth are nocturnal suggests that the direct impact of light might be inconsequential (because the proximal light environment encountered by females or larvae may not differ at night, irrespective of whether the plant is growing in the shade or sun) when selecting feeding or oviposition sites; rather, the light-mediated changes in leaf attributes may drive their choices.

Although larvae and adults of *Pareuchaetes* species are thought to prefer high-quality host plants (with high water and high nitrogen contents) or plants growing in a moist climate and semi-shaded habitat in the field (pers. obs.), field studies are required to determine if there is microhabitat preference in the field. Such studies would also assist to disentangle the mechanism underlying the habitat preference of *P. insulata*.

The outbreaks of the insect in moist microhabitats within dry, hotter areas in recent months in KZN province (Strathie and Zachariades, 2014) suggests that the insect survives and performs better in such microhabitats and that the moth is able to identify or choose host plants of high food quality because the leaves of such host plants remain green all year and may contain increased leaf water and nitrogen contents. Increased water and nitrogen can lead to a reduction in development time, increased survival and improved fecundity in insects (e.g. van Hezewijk *et al.*, 2008; Münzbergová and Skuhrovec, 2013; Uyi *et al.*, submitted). The high temperatures in the current outbreak sites in northern KZN (Strathie and Zachariades 2014) might have also improved the fitness of the moth – as high (non-lethal) temperatures help to accelerate the development of immature stages. It is not impossible that the combined effect of high temperatures coupled with improved food availability and quality may have resulted in the outbreaks recorded in 2014.

In outbreak situations, it is plausible that insect-induced defence exists in the bi trophic relationship between *C. odorata* and *Pareuchaetes* species as has been previously documented for *P. pseudoinsulata* feeding on *C. odorata* on Guam (Marutani and Muniappan, 1991). These authors found that feeding of larvae caused the leaves of *C. odorata* plants to turn yellow with higher concentrations of nitrate nitrogen (compared with green leaves). Further, they found that *P. pseudoinsulata* preferred to feed on, and performed better on green leaves, and when exclusively fed on yellow leaves, they exhibited prolonged development and high mortality. Although this thesis did not address the importance of insect-induced defence in *C. odorata*, it is not impossible that insect-induced defence in this plant plays a major role in the population dynamics of *P. insulata* in South Africa, following outbreaks.

The performance of *P. insulata* offspring whose parents fed on foliage different from that which is available for offspring consumption was investigated in pure- and cross-feeding experiments (Chapter 6). The results suggests that a ‘negative switch in diet’ (i.e. when progeny from parents reared on shaded leaves are fed on full sun leaves) resulted in 40% direct mortality, prolonged development time and reduced fecundity. The poor performance of offspring on the negative switched diet points to full sun leaves as a poor quality food source for *P. insulata*. The findings of this chapter suggest that the clearing of vegetation that exposes *C. odorata* to direct sunlight could have a catastrophic effect on the population dynamics of the moth due to a sudden change in diet quality. Schoonhoven and Meerman (1978) suggested that metabolic costs may be associated with a change in diet from suitable quality to a nutritionally unbalanced diet.

Although the metabolic costs associated with the negative switch in diet by *P. insulata* need further investigation, the results of this chapter highlight the importance of dialogue among biological control practitioners, weed ecologists and weed controllers prior to any clearing (mechanical or chemical) operations. Such discussions may not only help to prevent the destruction of the most suitable habitats for biological control agents, but may also help to prevent the local extinction of the agents. Following the release of agents, practitioners should monitor their establishment and spread and study their nutritional ecology over a spatio-temporal spectrum so as to be knowledgeable about the habitat preference and the behaviour of the released agents. One major finding of Chapter 6 is that differences in mortality and other performance indices of *P. insulata* between parents and progeny were not apparent until cross-feeding experiments were performed. This further buttresses the earlier call for studies on the nutritional ecology of insects used as weed biological control candidates.

The findings of Chapter 7 suggest that performance was positively correlated with increased foliar nitrogen up to a point above which herbivore performance did not improve but rather remained unchanged (e.g. van der Meijden *et al.*, 1984). Increased nitrogen will not only improve aspects of the moth's fitness and performance, but also the insect's impact on the target weed - as the populations of biological control agents are known to increase with increasing foliar nitrogen (Room and Thomas, 1985; Heard and Winterton, 2000; Hinz and Müller-Schärer, 2000; van Hezewijk *et al.*, 2008).

Most insects used in biological control programmes are "latent species" (Price, 2000) which usually remain at stable population densities and do not have the potential to erupt and cause significant damage to host plant populations. An eruptive species has two phases, one of low

density and low damage and one of high density, that impacts substantially on host plant populations (Price *et al.*, 1990). Price (2000; 2003) discusses how phytophagous insects with typically latent population dynamics can become eruptive in response to vigorous plants of high quality. Therefore it is expected that if a plant such as *C. odorata* is growing under conditions of high nitrogen availability (e.g. shaded habitats), their biological control agents (e.g. *P. insulata*) may have the potential to have eruptive population dynamics if they respond positively to vigorous plants of high quality. *Pareuchaetes insulata* showed a positive response to *C. odorata* plants with medium and high nitrogen content in terms of their development time, pupal mass and fecundity, therefore *C. odorata* plants growing in shaded habitat may create ideal conditions for this species to become eruptive and reach high population densities. The usefulness of nitrogen in mass-rearing of latent insects and weed biological control agents (Wheeler, 2001; Wheeler, 2003), improving the population establishment of biological control agents (Heard and Winterton, 2000; Hinz and Müller-Schärer, 2000; van Hezewijk *et al.*, 2008) and in initiating insect outbreaks in weed biological control (Room and Thomas, 1985; Price, 2000) has been documented. Although a few population outbreaks of *P. insulata* have been reported in South Africa, the reasons for these remain speculative, but it is not impossible that the outbreaks are related to increases in foliar nitrogen concentrations and increased water levels of the host plant. Although increased foliar nitrogen concentrations have been reported to improve the performance of biological control agents, studies have also demonstrated that increased nitrogen can have a neutral or negative effect on insect performance and population levels (see Chapter 5; Hartley and Lawson, 1992; Birch *et al.*, 1992; Clissold *et al.*, 2006; Zehnder and Hunter, 2009). The demonstration that the performance of a specialist herbivore can be explained by light mediated changes, seasonal changes, changes mediated through fertilizer and variability depending on feeding history in host plant, is not only of ecological and evolutionary

relevance in the context of insect plant interaction in shaded versus full sun habitats, it also helps in further integrating ecological theories into the applied field of weed biological control.

8.2 Biological control of *Chromolaena odorata*

Retrospective analyses of biological control programmes, such as those presented in this thesis (Chapters 2, 3, 4, 5, 6 and 7), are valuable ways to examine factors (intrinsic and extrinsic) which may have affected the success or failure of biological control agents post-release. Such factors may have been neglected because practitioners do not often prioritize pre-release studies on the nutritional ecology and thermal physiology of insects used as biological control agents. A detailed analysis of these factors could result in improved agent selection in future because results are likely to be applicable to other biological control programmes.

The variable performance of *P. insulata* in South Africa necessitated the introduction and release of other biological control agents such as *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae), *L. aemulus* and *D. odorata*. While *C. eupatorivora* readily established in South Africa, its impact on *C. odorata* remains negligible (Nzama *et al.*, 2014). Further, the establishment of *L. aemulus* in southern KZN remains problematic, possibly because aspects of its biology, physiology or ecology preclude the establishment of the insect. Therefore detailed investigations on several aspects of the life history traits of the released biological control agents are warranted (Chapter 2) so as to determine whether any of these aspects are responsible for the variable performance (e.g. non-establishment). Also, detailed life history of the agents currently undergoing host-range testing in quarantine (e.g. *Polymorphomyia*

basilica Snow (Diptera: Tephritidae)) should be investigated because a proper understanding of the biology and ecology of such agents might help in predicting establishment.

Although *P. insulata* performed equally well on Floridian and SAB *C. odorata* (Chapter 3), future surveys for potential biological control agents should be conducted in Jamaica and Cuba because plants in these countries are genetically more similar to the SAB plants (Paterson and Zachariades, 2013). Biological control agents may also be collected from regions in the Americas with a climate similar to that in South Africa. The results of this study (Chapter 4) suggest that low winter temperatures might have negatively impacted the survival, development time, fecundity, locomotion abilities and on the population dynamics of *P. insulata* in the field. Hence, more cold-tolerant biological control agents may establish and perform better in South Africa because of the seasonally cold winter period. Although thermal physiology studies conducted post-release can help in explaining the poor performance or establishment failure of biological control agents (e.g. Chapter 4; Byrne, 2002), such studies conducted prior to release are important tools in biological control programmes (e.g. May and Coetzee, 2013), particularly those in resource-limited countries, to prevent wasting efforts in getting an agent established. Therefore studies on the thermal physiology of released biological control agents (e.g. *C. eupatorivora*, *L. aemulus* or *D. odorata*) and agents undergoing host-range tests in quarantine (e.g. *P. basilica*) are warranted because results from such studies may help to improve biological control programmes.

The role of bottom-up factors such as resource limitation in the population dynamics of insects (including biological control agents) is only beginning to be appreciated (e.g. Chapters 5, 6 and 7; Prezsler and Price, 1988; Price, 2003). While it is important to identify microhabitats that can support optimal performance of biological control agents prior to

release and post-release, practitioners often do little to understand the nutritional and behavioural ecology of insects used as weed biological control agents. Price (2000) discusses how biological control of weeds can be improved by manipulating the nutrient levels in host plants so that phytophagous insects with typically latent population dynamics can become eruptive in response to vigorous plant of high quality. The influence of sunlight and season on the leaf characteristics of *C. odorata* and the corresponding effect of these factors on the performance of *P. insulata* suggest that bottom-up effects can significantly affect the population dynamics of this insect, as has been shown by Prezsler and Price (1988) and van Hezewijk *et al.* (2008). Globally, weed biological control practitioners should conduct studies on the nutritional ecology and behaviour of established agents (Chapters 5, 6 and 7) and agents in quarantine. This might help to explain the differential or variable performance of biological control agents.

8.3 CONCLUSION

Studies such as this thesis are valuable for the purpose of determining factors that might be responsible for the poor establishment and performance of biological control agents. However, conducting such studies at the pre-release stage would also be very useful because it might inform practitioners on how well biological control agents would perform after their eventual release. From this study, it is demonstrably evident that the poor performance of *P. insulata* on *C. odorata* in South Africa is caused by multiple factors such as low temperatures as well as spatio-temporal variations in the characteristics of *C. odorata* leaves. Further, the study also showed that the performance of *P. insulata* is not affected by plant biotype (variation in host plant), although the preference (and mate finding and selection in the field) may be affected by plant biotype. Top-down factors such as predation, parasitism or disease are other possibilities regarding the poor performance of *P. insulata* in South Africa.

Although the PAs sequestered by *Pareuchaetes* species may act as defences against predators and parasitoids as has been reported for other erebid moths (e.g. Eisner *et al.*, 2000; Bezzerides *et al.*, 2004), the role of top-down factors (e.g. predation and parasitism) on the different life stages of this insect across a spatio-temporal spectrum should be the focus of future research. This study shows the complexity of understanding the causes of low populations and apparent low impact of biological control agents, and herbivorous insects generally, in the field.

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