ECOLOGICAL FACTORS AFFECTING THE ESTABLISHMENT OF THE BIOLOGICAL CONTROL AGENT *Gargaphia decoris* DRAKE (HEMIPTERA: TINGIDAE)

A thesis submitted in partial fulfilment of the requirements
for the degree of

Doctor of Philosophy
in
Plant Science

at Massey University, Manawatu, New Zealand

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2017

ABSTRACT

The Brazilian lace bug (*Gargaphia decoris* Drake (Hemiptera:Tingidae)) was released in New Zealand in 2010 for the biological control of the invasive weed woolly nightshade (*Solanum mauritianum* Scopoli (Solanaceae)). Currently there is scarce information about the potential effect of ecological factors on the establishment of this biological control agent. This study investigated: 1) the effect of maternal care and aggregation on nymphal survival and development; 2) the effect of temperature, photoperiod and humidity on *G. decoris* performance; and 3) the effect of light intensity on *S. mauritianum* and *G. decoris* performance.

Maternal care and aggregation are characteristic behaviours of *G. decoris*. These behaviours have an adaptive significance for the offspring and are key determinants for the survival of the species under natural conditions. Maternal care is reported to increase the survival and development of offspring under field conditions, and higher aggregations to increase the survival of the offspring. However, in this study, maternal care negatively affected the survival and development of the offspring, and higher aggregations had no significant impact on offspring survival. The availability of host plants under laboratory conditions may have influenced the expression of these behaviours.

Climate is a factor that constrains insect development and therefore establishment. In this study, temperature affected the survival, nymphal development, life cycle, adult longevity, female reproductive success (i.e. total number of eggs, number of eggs laid per female, number of egg batches, number of eggs per batch, pre-oviposition period, percent females that oviposited successfully, number of eggs in the first batch and percentage of eggs that hatched from the first batch) and population growth parameters (i.e. life table). Temperatures between 20 – 25 °C were the optimal temperatures for *G. decoris* establishment. Photoperiod affected the mean percentage of egg hatch (i.e. emergence of nymphs in egg batch collected from colony) and total nymphal survival (i.e. egg to adult emergence), adult longevity and population growth parameters. The photoperiod 16L:8D was the optimal photoperiod for insect establishment. Humidity affected the mean percentage of egg hatch, adult longevity and population growth parameters. *G. decoris*

population growth was highest at 70 ± 10% RH but the population growth was faster at 50 ± 10%.

The CLIMEX model predicted that *G. decoris* could occupy broader regions not only on its native range (i.e. Brazil and Argentina) but also other regions where *S. mauritianum* is considered invasive (i.e. New Zealand and South Africa). *G. decoris* is predicted to be able to establish optimally in most of New Zealand North Island, except in regions with altitudes higher than 1300 meters above sea level. Most of the South Island is considered unsuitable for *G. decoris* establishment, except parts of the West Coast, Nelson and the Tasman region, which are predicted to be moderately to marginally suitable.

Light intensity and plant age (i.e. day of harvest) affected host plant quality and had an indirect impact on insect establishment. Light intensity and plant age affected key physiological, morphological and defensive traits of *S. mauritianum.* Three compounds appeared to be involved, and were positively identified as glycoalkaloids: α-solamargine/β-solamarine, solauricine/solasonine, and unknown-954. The reproductive performance of *G. decoris* was affected because females avoided ovipositing on unshaded plants. The presence of trichomes and an increase in concentration of glycoalkaloids in the second harvest affected the nymphal performance and was reflected in adults, which had smaller bodies and wings.

The results of my study have implications for using the Brazilian lace bug *G. decoris* in biological control programmes. The ecological factors included in this study work synergistically rather than independently and are important to consider when deciding the best locations in which the insect could be liberated.

ACKNOWLEDGEMENTS

First of all I would like to thank the New Zealand Ministry of Foreign Affairs and Trade for granting me a New Zealand Development Scholarship (NZAID) which allowed me to complete my PhD studies. I would like to acknowledge the support provided by NZAID coordinators at Massey University.

I express my sincere appreciation to my supervisors Masha Minor, Kerry Harrington, Adriana Najar-Rodriguez and Quentin Paynter for their patience, their professional guidance and emotional support during my research. Special thanks to Masha and Kerry for accepting being my supervisors under special circumstances and ensuring the successful completion of my PhD studies. I would like to mention Gonzalo Avila (Plant and Food Research) for teaching me how to use the software CLIMEX and reviewing my research and Sarah Cordiner (Plant and Food Research) for teaching me how to process and prepare my samples for a HPLC-MS analysis and for all the time spent answering all my questions and reviewing my thesis. Also, thank you to Qiao Wang and Xiong He for their earlier contribution to my thesis.

To my partner Sam McColl who played an important role in the completion of my studies. His positiveness, emotional support, and professional guidance helped me surpass any obstacle during my PhD studies. Thank you for giving me technical assistance in GIS. To all my friends that made my PhD very enjoyable and particularly my friends and colleagues Diwas Khatri, Kambiz Esfandi and Jana Müller for their advice and technical support. To my family, specially my parents for always supporting my dreams, believing in me and their unconditional love. I would like to thank several people that assisted me during my research and provided technical, administrative support or provided information for my research. For that I would like to mention Steven Ray, Lindsay Silva, Lesley Taylor and Georgina Hamilton, Chris Rawlingson, Denise Stewart, Cory Matthew, Chris Winks, Matthew Savoian, Andrew Blayney, Terry Olckers, Daleen Strydom and Debbie Muir.

Thanks to Landcare Research for sponsoring the C/N analysis as well as Plant and Food Research for collaborating in the HPLC-MS analysis, opening the Chemistry Lab and providing all the materials and equipment for me to be able to process my samples.

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CHAPTER 1: INTRODUCTION

1.1. Background

Weeds are plant species which grow where they are not wanted (WSSA, 2016). The weeds that have been introduced as a result of human activity into environments where they are not native, and are able to establish, persist and spread outside their native range are catalogued as invasive weeds (Richardson et al., 2000; WSSA, 2016). Invasive weeds can directly or indirectly cause problems to agriculture (Muniappan et al., 2009), natural resources, wildlife, public health or environment (Macdonald, 1983; Samways *et al.*, 1996; WSSA, 2016; Muniappan *et al.*, 2009; Mack *et al.*, 2000), and many of them are also classified as noxious weeds (WSSA, 2016).

In New Zealand, the total expenditure by the central government and private sector to protect the country agriculture and forestry sector against noxious weeds, is about NZ$578 million per year (Williams and Timmins, 2011). In the case of public conservation land, the Department of Conservation (DOC) increased its total expenditure on weed control from NZ$1.76 million in 1994-1995 to NZ$20.27 million in 2008-2009 (Williams and Timmins, 2011). However, while these control costs are not insubstantial, they are small compared to the potential economic cost that invasive weeds pose to native biodiversity. Of the 8,593,371 ha of public conservation land over 1,476, 788 ha (17%) is formally reported under weed threat. Reports by Reid (1998) listed 61 (55%) of the 125 indigenous species under strong threat by weeds therefore requiring ongoing weed control to protect biodiversity values (Williams and Timmins, 2011). In New Zealand, the total (direct, indirect, and passive) value of land-based biodiversity was estimated to be NZ$43 billion in 1994 (not adjusted for inflation). Considering that 17% of the land managed by DOC is under weed threat, it was conservatively estimated that weeds cause a loss of native biodiversity of around NZ$ 4.42 billion (as at 1994, not adjusted for inflation) (Patterson and Cole, 1999; Williams and Timmins, 2011).

Solanum mauritianum Scopoli (Solanaceae) commonly known as woolly nightshade, constitutes a major invasive weed in native forest margins, scrub and shrubland areas and pasture land (Stanley, 2003). Conventional weed control techniques, such as

mechanical and chemical controls, are effective for controlling this weed. However, because they are expensive, labour intensive, and require repeated application, they are impractical for managing widespread plant invasions especially in ecologically-fragile conservation areas (Culliney, 2005). Because of drawbacks associated with conventional weed control methods, classical biological control was considered as an alternative to address the control of *S. mauritianum* (Olckers, 2000; Culliney, 2005).

The first country to start a biological control programme against *S. mauritianum* was South Africa (Olckers, 2009). Those agents that reduce fruiting and vegetative growth were prioritized (Olckers, 2009). As a result, the insect selected for further consideration as a biological control agent against this weed was the lace bug *Gargaphia decoris* (Hemiptera: Tingidae). The attributes that make this insect ideal as a biocontrol agent include its high fecundity and longevity, good adult dispersal abilities, overlapping generations and high feeding rates, all of which could potentially result in high levels of damage to the leaves of the plant, and therefore to its vegetative growth (Olckers, 2009).

The lace bug was introduced in South Africa following a single collection made near Iguazu Falls, Misiones, Argentina in 1995 (Olckers *et al.*, 2002). Later on, in 2002, fresh specimens of *G. decoris*, comprising a much broader genetic base, were introduced to South Africa from Paraná in southern Brazil (Pedrosa-Macedo *et al.*, 2003). After its successful establishment in South Africa, the lace bug was considered for importation to New Zealand (Withers *et al.*, 2002; Borea, 2006). However, additional experiments had to be completed to show that this insect does not colonize non-target *Solanum* species (Olckers, 2000; Olckers and Lotter, 2004; Olckers and Borea, 2009; Hope and Olckers, 2011) in order to be approved for importation.

In 2009, the New Zealand Environmental Risk Management Authority (ERMA) granted permission to release *G. decoris*. The first shipment, of a Brazilian provenance, was imported in 2010 from South Africa where the lace bug has been used as a biocontrol agent for the past decade. The first releases began in 2010 in the Bay of Plenty (Plant Research, 2014).

The lace bug was introduced to New Zealand despite little success in South Africa in controlling *S. mauritianum.* Early reports from releases of *G. decoris* in South Africa showed that the biocontrol agent had become established and was dispersing, but the

damage to *S. mauritianum* populations was negligible and was not making a significant contribution to the management of the weed (Zimmermann *et al.*, 2004). Damage to weed populations in South Africa has mostly been moderate because lace bug populations have remained at low densities, not reaching the outbreak levels that are needed to inflict severe damage (Olckers, 2009). Recent findings show that shading has an effect on *G. decoris* dispersal in the field, with the insect having difficulties establishing in open, sunny areas, which might explain the restricted establishment in South Africa (Patrick and Olckers, 2014). Similar shading effects have been recently observed by the author in some of the release sites in New Zealand. Patrick and Olckers (2014) have suggested that this phenomenon could be due to temperature related effects, plant quality or greater abundance of generalist predators in unshaded localities, but these potential drivers are yet to be explored.

In addition to the shading effects, there are a number of other factors that may influence the establishment of *G. decoris* that have not yet been explored along with basic information useful for monitoring establishment. For instance, there is limited information on the biology and behaviour of *G. decoris*. The first attempts to describe the life cycle and behaviour of *G. decoris* were made by Olckers (2000) and Guidoti *et al.* (2015) respectively. The study performed by Olckers (2000) was useful; however, the range of temperatures under which the experiments were conducted was limited. The maternal care study performed by Guidoti *et al* (2015) is the first study that addresses maternal care in *G. decoris,* but little is known about the implications of this behaviour for insect survival, and therefore its establishment.

To date there is insufficient information on the thermal requirements of *G. decoris* to construct meaningful life tables as those described by Carey (1993), which can be used for predicting population dynamics. Further, little is known about the potential distribution of the insect in the field based on ecoclimatic factors, namely temperature and precipitation, in New Zealand (or elsewhere). This information could be used to identify the locations that would be suitable for insect establishment.

In summary, there is a large array of poorly quantified factors that could affect the success of *G. decoris* as a control agent in New Zealand. Addressing the influence of these factors on the performance and fitness of this insect providing better models of

establishment, will help to optimise insect releases in the field, i.e. where, when, and how often releases should be made, and where alternative methods are required.

With this context in mind, **the aim of the present study** is to investigate some potential ecological factors affecting the successful establishment of *G. decoris* populations in New Zealand. To achieve this, the following objectives have been identified:

1.2. Objectives

i) To study the effect of temperature, photoperiod and humidity on insect performance. Insect performance is a concept that is used to measure the fitness of offspring throughout their life cycle and lifespan (Fellowes *et al.*, 2005; Panizzi and Parra, 2012). In this study, the survival and developmental time of all juvenile stages, adult longevity and female reproductive success (e.g. mean total number of eggs, number of eggs laid per female and number of egg clutches) was recorded and life tables constructed to explore the population dynamics of the lace bug.

ii) Determine the current global distribution of *S. mauritianum* and to match it with the developmental biology of *G. decoris* to develop a model of the potential global distribution of *G. decoris*.

iii) Investigate the effect of maternal care on nymphal development and survival and its effect on adult morphology.

iv) Investigate the effect of aggregation behaviour on nymphal development and survival and its effect on adult morphology.

v) Explore the effect of light intensity on (a) plant growth and defence (i.e. physical structures and chemical components including primary and secondary metabolites) parameters, and (b) to explore the relationship of these parameters with *G. decoris* performance (survival) and adult fitness (number of egg batches, number of eggs, biomass and body measurements).

CHAPTER 2: LITERATURE REVIEW

2.1. Overview

This chapter starts with a general description of the host plant *Solanum mauritianum* Scopoli (woolly nightshade) and its ecological importance followed by a general description of the biology of the biological control agent *Gargaphia decoris* Drake. Special emphasis has been given to maternal care and aggregation behaviour, both behaviours displayed by *G. decoris* and considered important for insect establishment.

The effect of environmental stressors (such as temperature, humidity and photoperiod) on insect performance has been reviewed and several useful management tools have been described to predict insect establishment. In addition, a section is dedicated to the effect of another environmental stressor (light intensity) on plant-insect interactions. This section starts with general information about plant-insect interactions, followed by a review emphasising the influence of light intensity on plant growth (e.g. leaf area, leaf thickness, chlorophyll content) and plant defence (i.e. trichomes and secondary metabolites). There is also a general description of secondary metabolites present in plants and their importance, especially in *Solanum* species and woolly nightshade. The chapter ends with a general review of the trade-off between growth and defence experienced by the plant and how this is related to the carbon-nitrogen hypothesis.

2.2. *Solanum mauritianum*: an invasive weed

2.2.1. Taxonomical classification of *Solanum mauritianum*.

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Subfamily: Solanoideae

Tribe: Solanae

Subgenus: Brevantherum

Genus: *Solanum*

Species: *Solanum mauritianum* Scopoli

Solanum mauritianum was first described by Scopoli in 1788. Since then, it has been described under the names *S. erianthum* D.Don, *S. auriculatum* Aiton 1789, *S. tabaccifolium* Vell. 1829, *S. verbascifolium* Jacq., and *S. carterianum* Rock, 1913. Currently all these descriptions are considered as synonyms (Ruschel and Nodari, 2011). *S. mauritianum* is commonly known as woolly nightshade in New Zealand but goes by other common names in other parts of the world (Table 2.1).

Table 2.1. Common names of *S. mauritianum.*

Common name	Countries
Bugweed, wild-tobacco, flannel, kerosene plant, tobacco weed	Australia and South Africa
Woolly nightshade	New Zealand, Australia, South Africa
Groot-bitterappel, luisboom, igayintombi, umbanga-banga, isigwayana	Kwazulu-Natal, South Africa
Fumo-bravo	Brazil
Pua-nãnã-honua	Hawaii
Pula	Tonga
Rau 'ava' ava	Cook Islands-Polynesia

2.2.2. Botanical description

Woolly nightshade is a fast-growing shrub to small tree that can reach heights of up to 12 m, a trunk diameter of up to 30 cm, and lives for up to 30 years (Roe, 1972; Stanley, 2003). The leaves and stems of the plant are densely-covered with overlapping branched and stellate hairs (Roe, 1972; Healy, 1974; BoPRC, 2005) (Fig. 2.1a). The leaves, which have an ovate to elliptic shape, can range from 10-40 cm long by 3.5 to 10 cm wide (Healy, 1974). The leaves are grey-green or dark-green on the upper surface and white to yellowish-green on the underside. They have a very strong smell, especially when rubbed or crushed (Stanley, 2003). On the base of each stem, the leaves commonly have two small stipulate-like rounded leaves (Healy, 1974) (Fig. 2.1b).

Woolly nightshade is an autogamous plant that produces clusters of light purple inflorescences (Rambuda and Johnson, 2004). The flowers, which are about 1-2 cm in diameter, each have five lobes with a yellow centre, and appear in clusters at the end of the branches (Stanley, 2003) (Fig. 2.1c). The plant blooms throughout the year, although most fruiting occurs in late spring-summer (Roy *et al.*, 1998). Each inflorescence can produce between 20-80 green berries that mature into yellow, globose, pubescent berries of about 1.5 cm diameter (Healy, 1974; BoPRC, 2005; Ruschel and Nodari, 2011). Each berry

contains approximately 150 to 250 seeds that are each 1-2 mm long with 98% viability (Webb *et al.*, 1988; van Den Bosch *et al.*, 2004) (Fig. 2.1d).

Figure 2.1. Woolly nightshade tree (a), stipulate leaves at the base of stems (b), inflorescence (c), and fruits in clusters (d).

2.2.3. Geographic distribution

S. mauritianum is indigenous to northeastern Argentina, Uruguay, Paraguay and southern Brazil (Olckers and Zimmermann, 1991; Ruschel and Nodari, 2011). It is thought to have migrated via Portuguese sea-trade routes in the early sixteenth century (van Den Bosch *et al.*, 2004), either accidentally by human colonists or through deliberate importations for ornamental purposes. Currently the plant is considered as an invasive or an introduced species in the American continent (México, USA), Fiji, Polynesian Islands, Hawaii, New Caledonia, Solomon Islands, Tonga, Mauritius, Madagascar, Australia, New Zealand, and several African countries, including South Africa (Roe, 1972; Ruschel and Nodari, 2011).

In New Zealand, *S. mauritianum* was first recorded as early as 1883, growing wild around Auckland (BoPRC, 2005) and is now considered invasive in Northland, Auckland, Waikato, Bay of Plenty, Hawkes Bay, Manawatu-Wanganui, Wellington, Nelson district, Tasman and Marlborough regions (ERMA, 2009). It thrives in a range of habitats, including plantations and forest margins, riparian strips, pastures on poorer country, and is causing increasing concern in some districts with its invasion of hill country grassland and formation of woody communities (Healy, 1974).

2.2.4. Ecological and economic importance

Woolly nightshade was the first *Solanum* in New Zealand to have status as a noxious weed under the Noxious Weeds Act 1950 (Healy, 1974), and currently is listed on the National Pest Plant Accord, meaning that it cannot be sold, propagated or distributed in New Zealand (ERMA, 2009).

It is an aggressive weed that has the ability to grow in dense stands and, as a consequence, suppress other plants (Webb *et al.*, 1988). A preliminary laboratory study showed that the leaves contain water soluble phyto-inhibitors that cause allelopathic effects (i.e. suppresses growth of surrounding plants) (Florentine and Westbrooke, 2003). This finding is consistent with the field observation that very few seedlings of other species occur underneath mature plants (Florentine *et al.*, 2003). van Den Bosch *et al.* (2004) suggested that leaf drop from mature *S. mauritianum* plants may be the mechanism preventing the establishment of other plants. When woolly nightshade prevents the establishment of other plants, it has the capacity to influence the diversity of invertebrates. Samways *et al.* (1996) found that the dense thickets produced by woolly nightshade prevented the flight of insects and as a result he found no presence of wasps (Pompilidae) where these might otherwise be expected. Macdonald (1983) found that woolly nightshade suppressed an indigenous plant favoured by bees.

In Brazil, bees are attracted to woolly nightshade but this is considered problematic for beekeeping because it provides honey with a spicy aroma not desirable by consumers (Ruschel and Nodari, 2011).

The foliage is toxic upon ingestion by humans, is unpalatable to wild and domestic animals and is suspected of poisoning stock in New Zealand (Connor, 1977; Macdonald,

1983). This toxicity is thought to be related to the presence of alkaloids in the leaves and fruits. However, steroidal alkaloids within the leaves and green berries have been seen as a potential resource of pharmaceutical interest (Vieira, 1999).

In addition to being an aggressive and toxic plant, woolly nightshade also has a high seed production (Webb *et al.*, 1988). Florentine *et al.* (2003) found that each mature plant can produce on average 40,000 seeds per year. Most seeds fall close to the parent plant but some are dispersed by birds and bats over relatively long distances (Symon, 1979; Olckers, 1999; Stanley, 2003; Witkowski and Garner, 2008). Some birds even prefer the fruit of woolly nightshade over those of indigenous plants (Macdonald, 1983; Olckers, 1999). For example, in South Africa, the Rameron fruit pigeon (*Columba arquatrix* Temminck) no longer disperses seeds of the native forest species because it prefers the abundant introduced woolly nightshade (Macdonald, 1983). In New Zealand, the seeds are thought to be dispersed by bird species such as silvereyes (*Zosterops lateralis* Latham) and kereru (*Hemiphaga novaeseelandiae* Gmelin), as well as possums (*Trichosurus vulpecula* Kerr) (Stanley, 2003).

The fruit provides alternative hosts for several species of fruit flies (Tephritidae) that are pests of cultivated fruit in Africa, and serve as reservoirs for these pests throughout the year (Olckers and Zimmermann, 1991; Copeland and Wharton, 2006).

Woolly nightshade is a serious environmental weed not only in New Zealand but in other parts of the world (McGregor, 1999; Olckers, 2009). The easily dispersed seeds and high germination rate makes mechanical and chemical control unsustainable because both methods do not prevent re-infestation nor invasion of new areas (McGregor, 1999; Stanley, 2003). Biological control was considered the best option for the long-term solution of the problem weed (McGregor, 1999; ERMA, 2009). The well advanced South African programme to control woolly nightshade via the control agent, the lace bug, *Gargaphia decoris*, gave to New Zealand an opportunity to evaluate the possibility of success to control woolly nightshade with the same biological control agent (McGregor, 1999).

2.3. Taxonomical classification of *Gargaphia decoris*

Order: Hemiptera

Suborder: Heteroptera

Infraorder: Cimicomorpha

Family: Tingidae

Subfamily: Tinginae

Genus: *Gargaphia*

Species: *Gargaphia decoris* Drake

The family Tingidae, known as lace bugs because of the delicate lace-like feature of their forewings (Drake and Ruhoff, 1965), has around 2500 species of true bugs (Heteroptera) (Guilbert *et al.*, 2014). The genus *Gargaphia* comprises around 85 species distributed around the world (Drake and Ruhoff, 1965).

2.3.1. Geographic distribution

G. decoris is known to be naturally distributed through parts of Argentina and Brazil (Drake and Ruhoff, 1965), where its preferred host is woolly nightshade. The insect has been introduced to South Africa and New Zealand as part of a pest management program to control woolly nightshade (ERMA, 2009).

The insects introduced to New Zealand came from stock in South Africa that had been collected from Curitiba in Paraná state, Southwestern Brazil (25°22'S 49°16'W). The Brazilian stocks were selected over Argentinian specimens for use in South Africa because of their greater tolerance to cooler conditions (Hope and Olckers, 2011). The climate of the natural habitat of *G. decoris* is classified according to the Köppen classification as subtropical highland climate (Cfb) (Alvarez *et al.*, 2013). The region of Curitiba, Brazil, where the New Zealand stock originates from, has a mean annual maximum temperature of 21.8°C and a mean annual minimum temperature of 14.3°C. The highest temperatures (22-24°C) are usually between the months of October and February. The maximum number of

days when temperatures range between 25-30°C is 13.3 days. The annual rainfall is about 1314 mm, with September and February the months with the highest average precipitation (100-152 mm/month) (Meteoblue, n.d.).

2.3.2. *Gargaphia decoris* biology

The life cycle of *G. decoris*, from egg to adult emergence, takes about 30 days. The eggs hatch after 9-14 days, then the immature stages take about 17-19 days before moulting into adults (Olckers, 2000) (Fig. 2.2 a,b). The eggs are deposited in irregular batches, usually on the underside of the leaf and very rarely on the upper side (Olckers, 2000). The eggs are small (0.5 mm in length) and their dark colour contrasts with the light-green colour of the underside of the leaves. The eggs are firmly fixed in the leaf epidermis and they rely on the moisture of the leaves to survive; the eggs deposited on the leaf will not hatch if the leaf desiccates or abscises (Olckers, 2000).

Figure 2.2. *G. decoris* adults, ventral side of female (left) and male (right). Scale bar represents 50 µm.

During the immature development stages, *G. decoris* undergoes five nymphal instars (Olckers, 2000) (Fig. 2.3). The nymphs in the first instar are transparent without any visible separation between the thorax and abdomen. The thorax and abdomen are defined in the second instar with broader and more visible spines (Olckers, 2000). By the third instar, the insects develop darker spines on the head and thorax with the presence of a black semi-circular patch on the thorax (Olckers, 2000). The wings start to develop in the fourth instar and the semi-circular patch develops into two block-shaped markings (Olckers, 2000). In the fifth instar, the wings are three-quarters of the length of the abdomen and the thorax has several black markings (Olckers, 2000). The characteristic lace-like appearance of the adults appears after the final moult (Olckers, 2000).

The adults start mating when 11 days old (i.e. 11 days after the final moult) and eggs are laid when females are about 24 days old (i.e. 24 days after final moult) (Olckers, 2000). *G. decoris* adults are long-lived, reaching up to 145 days old when reared on *Solanum mauritianum*. As a result of their longevity, there is an overlap of generations because the female can lay consecutive egg batches during her lifetime (Olckers, 2000). Both adults and nymphs feed on the upper and lower leaf surfaces, however most of the feeding is on the underside of the leaves (Olckers, 2000).

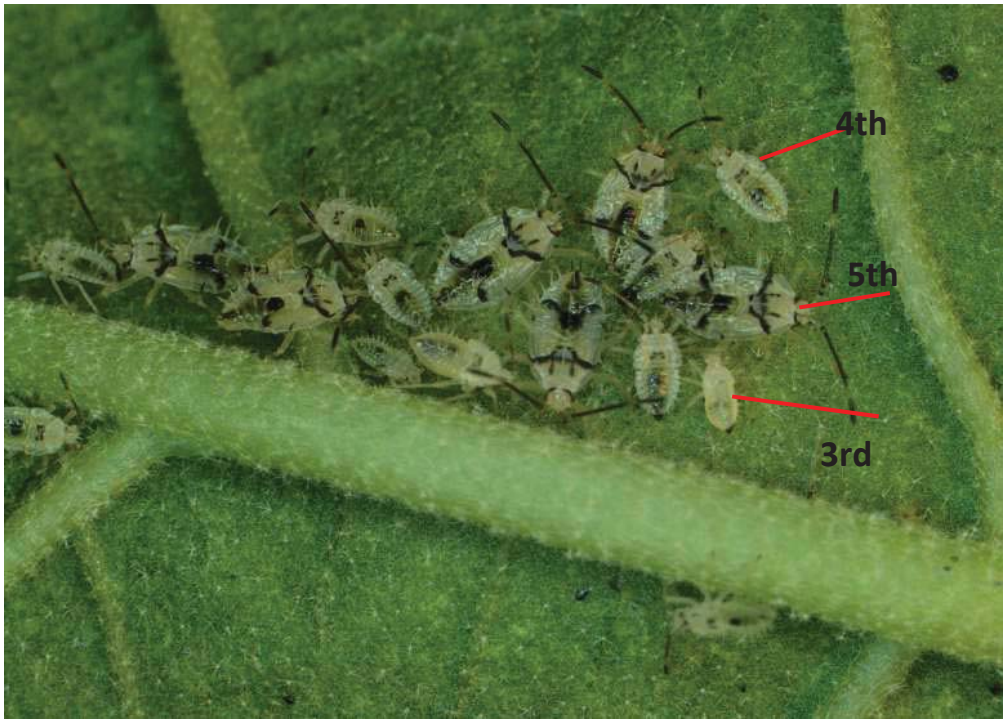

Figure 2.3. *G. decoris* (3rd, 4th, and 5th) nymphal instars.

Lace bugs of the genus *Gargaphia* feed directly on the chloroplasts of leaf mesophyll cells (Tallamy and Denno, 1981a). In leaves of many plants (especially dicotyledons), the chloroplast-bearing mesophyll cells include the palisade layer cells and spongy mesophyll (Fahn, 1990) (Fig. 2.4).

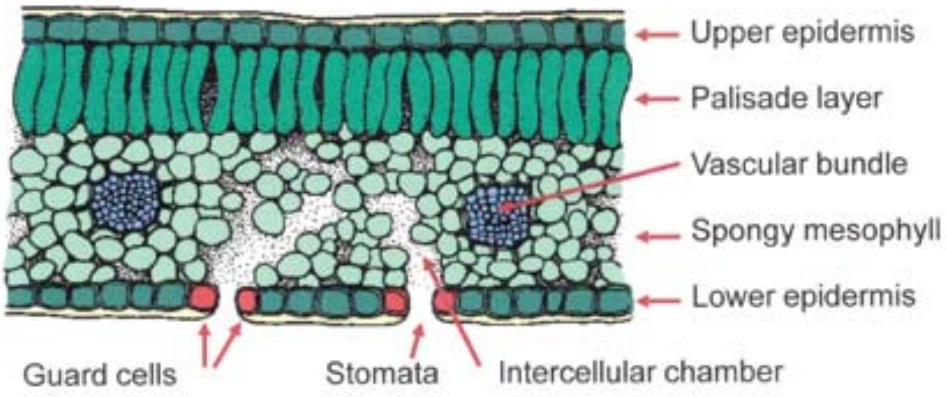

Figure 2.4. Leaf structure displaying the palisade and spongy mesophylls, both of which contain chloroplasts that lace bugs feed on (reproduced from Johnson and Whiting, 2015).

Adults and nymphs are thought to access the cellular tissues (chloroplasts) by piercing the epidermis of the leaf blades; although some tingid species, for example the azalea lace bug *Stephanitis pyrioides* Scott, are known to access the cellular tissues through the stomata (Ishihara and Kawai, 1981; Buntin *et al.*, 1996) on the underside of the leaf. The chloroplasts are extracted using a feeding method called lacerate-and-flush, which is common to many tingids. The lacerate-and-flush method consists of repeated insertion and withdrawal of the stylet (mouth piece) into the plant (Sharma *et al.*, 2014). The chlorophyll cells from the palisade layer are pierced by the stylets, they are liquefied, and saliva is then used to flush the ruptured cell contents into the sucking mouthpiece for ingestion (Sharma *et al.*, 2014). The feeding marks of lace bugs are seen as white chlorotic spots on the upper side of the leaves. If the plant is subjected to extensive feeding, the leaves tend to discolour, desiccate and abscise prematurely (Olckers, 2000). In addition to the feeding injury inflicted on the plant, lace bugs also excrete dark coloured faeces that often coat the lower surface of infested leaves. It has been suggested that the excretion could impair the gas exchange of the host plant by clogging the stomata (Putshkov, 1966; as cited in Wheeler, 2001). Similar brown or black excrement is deposited near the edges of the upperside of the leaf by *G. decoris* (Olckers, 2000) (Fig. 2.5).

Figure 2.5. Chlorotic leaf after extensive feeding by *G. decoris*. Black excrement (red arrow) is deposited on the upperside of *Solanum mauritianum* leaves.

A preliminary laboratory study by Olckers (2009) revealed that high levels of feeding damage from the lace bug *G. decoris* affected the growth of *S. mauritianum* plants. Those plants that were damaged contained 33% less biomass than the undamaged controls.

2.4. Parental care in insects

Parental care occurs in a wide variety of insects (Zeh and Smith, 1985; Zink, 2002) (Fig. 2.6), including lace bugs (Kearns and Yamamoto, 1981; Tallamy, 1982; Tallamy and Iglay, 2004; Faeth, 1989; Loeb and Bell, 2006; Guidoti *et al.*, 2015).

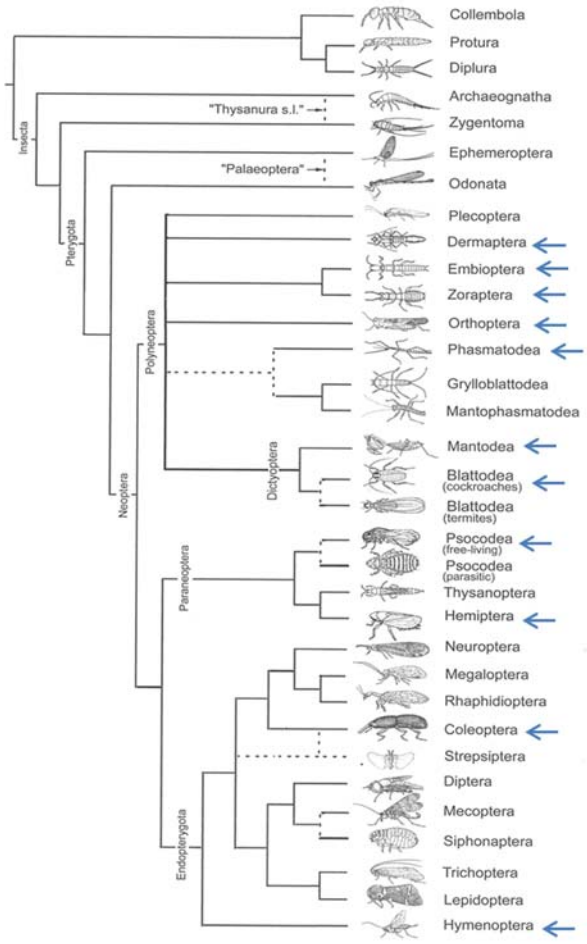

Figure 2.6. Insect orders (Gullan and Cranston, 2010) showing, with the blue arrows, those that have been previously identified to have at least some species that exhibit parental care (Zeh and Smith, 1985).

Parental care is a behaviour characteristic of subsocial and eusocial insects, however the way in which insects take care of their offspring varies (Wilson, 2000). In eusocial insects sterile or less fecund individuals of the same species cooperate for the caring of the young of few reproductive individuals. In subsocial insects parental care involves the physical protection of eggs ("egg guarding"), which can be done by either parent, and further maternal care after hatching (Tallamy, 1984; Queller, 1994). The way parental care is displayed can differ, starting from passive egg guarding to complex grooming, feeding, protective, and nesting behaviours (Tallamy and Wood, 1986; Ang *et al.*, 2008; Hilker and

Blum, 2008). This behaviour promotes the survival, growth and development of offspring (Tallamy and Wood, 1986).

Parental care behaviour is more likely to evolve when parents are in close proximity to their offspring (Lion and van Baalen, 2007). Hence, it has been suggested that parental care will be more prevalent in populations that have low dispersal (Hamilton, 1964). In addition, in order for parental care to be beneficial for the population, it requires that the parents live long enough to benefit one or more batches of offspring (Tallamy and Wood, 1986).

Egg guarding by both parents occurs at the oviposition site after egg laying (Smiseth *et al.*, 2012). After egg hatching, the extended care of the offspring is done mainly the female, who either stays in a fixed position with her offspring, and/or actively defends them against predators (Wood, 1975; Smiseth *et al.*, 2012), or escorts them as they move around (Smiseth *et al.*, 2012).

A number of studies have explored the costs and benefits of parental care (e.g. (Tallamy and Horton, 1990; Zink, 2002; Gilbert *et al.*, 2010). There is little doubt that the subsocial interactions between parents and offspring reduce reproductive losses by predation (Melber and Schmidt, 1975; Tallamy and Denno, 1981a). However, in some cases, parental care benefits are only evident when there is a plausible threat. For instance, in the experimental removal of the lace bug *Gargaphia solani* Heidemann, there was no difference in the offspring survival regardless of whether the female parent was removed or not when predators were not present. However, in presence of predators, maternal care increased offspring survival (Tallamy and Denno, 1981a).

The increased survival and growth of offspring via parental care can entail a cost to the parent's own survival, mating and reproductive success (Tallamy and Denno, 1982; Zink, 2003; Stiver and Alonzo, 2009; Gilbert *et al.*, 2010; Gilbert and Manica, 2010; Smiseth *et al.*, 2012; Klug *et al.*, 2012). For example, in the assassin bug *Rhinocoris carmelita* Stäl, females lost significant weight while guarding eggs proving that maternal care is costly and will have future impacts on their fecundity (Gilbert and Manica, 2010). Because of the reproductive costs experienced by the parents, Trivers (1972) considered parental care as an investment. Trivers (1972) describes the term *parental investment* as "any investment by the parent in an individual offspring that increases the offspring's

chance of survival (and hence reproductive success) at the cost to the parent's ability to invest in other offspring". The way parents protect their offspring will be influenced by (a) the environment, (b) parent fitness and (c) offspring fitness.

The local environment that a female inhabits can imprint on the phenotype, and therefore fitness, of her future offspring (Bernardo, 1996; Mousseau and Fox, 1998). For instance, when mothers are subjected to harsh environmental conditions, their future offspring tend to be better suited to those same harsh local environmental conditions (Wilson, 2000; Clutton-Brock, 1991). Hence, this is a way of protecting offspring from environmental stressors (Mousseau and Dingle, 1991; Agrawal *et al.*, 1999). However, female parents can also act in a 'selfish' way when faced with harsh environmental conditions, by improving their own survival, at the expense of a reduced quantity or quality of offspring (Marshall and Keough, 2004).

Milinski (1980) and Emlen (1970) predicted that in many organisms the parent's fitness has an influence on the time invested in offspring care. Young parents have higher reproductive rates and therefore invest less in offspring care but maximise their own survival (Tallamy, 1982; Tallamy, 1984). The older parents, whose reproductive rate is lower and whose potential contribution to future generations is reduced, while less fit than younger parents, tend to allocate their residual energy into caring for current offspring, rather than investing in producing new offspring (Clutton-Brock, 1991; Klug and Bonsall, 2010). Therefore, the fitness of a female does not necessarily reflect on an offspring's fitness (Marshall and Uller, 2007; Creighton et al., 2009).

The fitness of an offspring influences the female parental choice to care for those offspring. For instance, the female parent is better-off more energetically defending those offspring that are nearer reproductive maturity, compared to eggs, because of the higher probability of returns on the parental investment (Tallamy, 1982). However, in the case that egg mortality is high in the absence of care, egg guarding may be the preferred parental investment (Webb *et al.*, 2002; Klug and Bonsall, 2010), especially when the duration of the egg stage is relatively long (Klug and Bonsall, 2010). This scenario is consistent with the Dale *et al.* (1996) 'harm to offspring' hypothesis which suggests that parents should provide more care to poor quality and/or younger offspring because they are the ones that will benefit the most from care. Ultimately, the investment in care will only be profitable if

the cared-for offspring survive in greater numbers and/or reach maturity quicker than if they were not cared for (Tallamy and Denno, 1981b).

2.4.1. Maternal care in lace bugs

The description of maternal care and studies of this behaviour in lace bug species have been very scarce, despite the number of species identified in this family (Tingidae) (Drake and Ruhoff, 1965). Maternal care has been observed to be displayed in some lace bugs: *Gargaphia solani* (Fink, 1915; Tallamy, 1982); *G. tiliae* (Weiss, 1919); *G. irridiscens* (De La Torre-Bueno, 1942); *Corythucha bulbosa* (Sheeley and Yonke, 1977); *Leptobyrsa decora* (Melksham, 1984); *C. hewitti* (Faeth, 1989); *Compseuta picta* (Tallamy and Iglay, 2004); *G. decoris* (Guidoti et al., 2015) but only in *G. solani* are there extensive studies about the implications of this behaviour. There are some species that do not appear to display maternal care (Tallamy and Denno, 1981b) but this might be more related to the absence of predation pressure, rather than to the inability for this behaviour to be expressed (Faeth, 1989).

Asocial lace bugs (that do not display parental care) have same behaviours as those used by subsocial lace bugs in their parental care activities (such as flapping wings). However, in asocial lace bugs these behaviours are used for different purposes, such as deterring predators. Tallamy and Wood (1986) suggest that maternal care in subsocial lace bugs could be an extension of asocial behaviour in all lace bugs. Recently, Guilbert *et al.* (2014) included 46 species of Tingidae in a combination of molecular phylogenetic analysis and morphological parameters. The results show that three lace bugs species (*G. solani*, *G. arizonica* and *G. tiliae*) are closely related, supporting the monophyly of the genus (Guilbert *et al.*, 2014). Two of aforementioned lace bug species exhibit maternal care, but not *G. arizonica*, nevertheless they have similar morphology and behaviour. Guidoti *et al.* (2015) suggested that maternal care behaviour could be a defining trait in a subsection of *Gargaphia* and that it could be included as a characteristic for further phylogenetic analysis.

All lace bugs that display maternal care will guard their egg batches and care for newly emerged nymphs throughout their development (Tallamy and Wood, 1986) (Fig.2.7). However, other approaches to protecting their offspring differ between species.

For instance, the African lace bug *Compseuta picta* and *G. solani* aggressively push away intruders from the eggs or raise their wings towards the intruder (Fink, 1915; Tallamy and Iglay, 2004). Alternatively, the lace bug *Corythucha bulbosa* flaps its wings and is thought to use a chemical repellent to deter predators (e.g. jumping spiders) (Shelley and Yonke, 1977).

Figure 2.7. *G. decoris* mother guarding an egg batch.

How maternal defensive behaviour is triggered is unknown for most of the subsocial lace bugs but in the case of *Gargaphia* species, maternal behaviour is triggered not only by visual cues, but also by alarm pheromones released by the disturbed or injured nymphs (Kearns and Yamamoto, 1981).

Maternal care in lace bugs is costly in terms of total clutch production and individual survival (Loeb and Bell, 2006) because of time investment (Tallamy and Denno, 1982). According to observational studies performed in *G. solani*, it takes about 39% of the adult average life span (19 days) to guard an egg batch and provide care throughout the five nymphal stages (Tallamy and Denno, 1982). During this time, females interrupt their reproductive activity. This shift in fecundity causes less production of egg batches at a later period in the adult life (Tallamy and Denno, 1982). In view of the cost experienced by the

guarding female, there is pressure to develop communal associations in which the costs can be shared (Tallamy, 2005).

2.4.2. "Egg dumping" behaviour in lace bugs and its ecological importance

Egg dumping is an example of a communal association that enables some cost sharing during parental care. It is a mechanism in which ovipositing females abandon their brood to the care of a female that already initiated an egg mass (Tallamy and Horton, 1990). This provides an opportunity for younger females to increase their fecundity, especially at a younger age (Tallamy and Horton, 1990). Therefore, in these communal associations there are caring females that have the advantage to live longer but invest more in offspring survival, and there are females that allocate their energy to reproduction and die at a younger age (Tallamy and Denno, 1982).

Egg dumping is a facultative behaviour that is only expressed when the dumping female has an immediate egg batch where she can place her eggs. Otherwise, the gravid female will be forced to create her own egg batch and care for it, or face losses by predators (Tallamy, 1985). Usually those females that initiate their own masses remain to protect their offspring (Tallamy, 1985). These females are frequently approached by egg dumpers, which they either accept passively or very rarely reject aggressively (Tallamy, 1985). A guarding female produces biochemical compounds which are spread over her eggs. These compounds act as an attractive cue for egg dumpers, to help guide a potential egg dumper to a specific egg batch (Tallamy and Denno, 1981a; Loeb and Bell, 2006). The compounds also help the guarding female to distinguish her own eggs from those that have been dumped (Monaco *et al.*, 1998). This could be how the lace bug *G. solani*, is able to selectively provide more attention to its own eggs and nymphs while also guarding dumped eggs (Tallamy, 1985).

The egg dumpers lay their eggs around the periphery of the target egg mass or close by (Monaco *et al.*, 1998). There is a critical size of egg mass that is advantageous for the guarding female, and egg dumpers will avoid those egg masses that are too large because there is a risk that the guarding female may abandon the egg mass if it becomes too large (Tallamy, 1985; Tallamy and Horton, 1990).

Egg dumping is beneficial not only for the female egg dumper but also for the guarding female. The eggs on the periphery of an egg mass are more susceptible to predation, and therefore the dumping of eggs around the periphery of the egg mass helps protect the guarding female's eggs in the centre of the egg batch. This means that accepting dumped eggs provides a buffer against predators and dilutes the losses from predation (Monaco *et al.*, 1998; Balshine, 2012).

With egg dumping being a facultative behaviour, lace bugs are able to change their strategy according to the ecological pressure to which they are subjected. When there are enough quality resources, the lace bug population is stable, and there are no seasonal constraints, the egg dumping strategy is favoured, whereas in the opposite situation, when there are not enough resources and there are seasonal constraints, own egg guarding may be favoured (Tallamy and Denno, 1982; Tallamy and Horton, 1990).

2.4.3. Aggregation behaviour in insects

Aggregation is the tendency of an insect to position itself with others in a way that they are in contact with each other or nearly so, making their distribution in the environment patchy (Vulinec, 1990). Aggregating insects can be found in many of the major orders (Table 2.2). The degree of aggregation has been shown to influence nymphal survival and rate of development (Tsubaki, 1981; Breden and Wade, 1987; Matsumoto, 1989; Lawrence, 1990). An increase in nymphal survival and decrease in developmental rate as aggregation increases has been shown to be as a result of: (1) feeding facilitation (Ghent, 1960; Tallamy and Denno, 1981b; Denno and Benrey, 1997); (2) overcoming physical plant defences (Rathcke and Poole, 1974); (3) thermoregulation (Seymour, 1974); (4) predation minimization (Tostowaryk, 1972; Chew and Robbins, 1984; Damman, 1987); and (5) synchronized development (Long, 1953).

For example, the first instar larvae of the moth *Pryeria sinica* Moore suffered higher mortality when reared in isolation or small groups due to unsuccessful feeding. This resulted in a prolonged larval period and difficulties for establishment (Ghent, 1960; Tsubaki, 1981). On the other hand, the larvae of the butterfly *Mechanitis polymnia isthmia* Bates aggregates in order to avoid trichomes by feeding in groups and forming together a web over the trichomes to facilitate the movement of the larvae (Rathcke and Poole, 1974).

Table 2.2. List of orders where aggregating insects can be found (excerpt from Vulinec, 1990)

Order	Life stage	Example
Collembola	Adults and juveniles	*Hypogastura viatica*
Thysanoptera	Adults and juveniles	*Batnalliella yuccae*
Orthoptera	Adults and juveniles	*Ceuthopilus secretus*
Homoptera	Adults and juveniles	*Schizolachnus pineti*
Hemiptera	Overwintering adults	*Blissus leucopterus*
	Nymphs	*Nezara viridula*
	Adults and juveniles	*Velia caprai*
	Adults	*Roscius illustris*
Neuroptera	Larvae	*Ascaloptynx furciger*
Diptera	Larvae	*Tipula simplex*
Coleoptera	Adults	*Epilachna varivestis*
	Larvae	*Plagiodera versicolora*
	Adults and larvae	*Odontotaenius disjunctus*
	Overwintering adults	*Coleomegilla maculate*
Trichoptera	Overwintering larvae and pupae	*Dicosmoecus atripes*
	Larvae	*Nymphalis antipa*
Lepidoptera		
	Adults at night	*Heliconius spp.*
	Adults at daytime	*Smyrna karwinski*
Hymenoptera	Larvae	*Perga sp.*
	Adults	*Melissodes obluqua*

In spite of the advantages of aggregation behaviour, there are also some disadvantages. Large aggregations may be detrimental when resources are scarce due to intense feeding competition pressure (Ito *et al.*, 1982; Weis *et al.*, 1983). This is accentuated when there is an optimal population density that yields the optimal growth in accordance with the resources available (Beck, 1971; Fitzgerald, 1976; Beckage and Ridifford, 1978; Colbo and Porter, 1979). For example, a study with the larvae of the beetle *Trogoderma glabrum*, showed that both container size (rearing area) and population density have an effect on the pupation rate (Beck, 1971). In addition, aggregation of large numbers of insects can be a more powerful attractant for predators and parasitoids (Tostowaryk, 1972; Subinprasert and Svensson, 1988). For example, the pentatomid *Podisus modestus* Dallas tends to be more attracted to aggregated larvae of the sawflies *Neodiprion swainei* Middleton and *N. banksianae* Roh. When the larva is wandering by itself on a branch, the pentatomid has to use a combination of techniques to be able to encounter the larvae whereas the searching time is reduced when the larvae are aggregated (Tostowaryk, 1972).

In subsocial insects such as lace bugs, aggregation facilitates effective care for the family unit by the parents. When maternal care is extended beyond the egg stage, the mother adopts behaviours that maintain aggregation of the offspring (Wood, 1975; Kearns and Yamamoto, 1981; Balshine, 2012). The female can efficiently guide the aggregated nymphs to more suitable parts of the host plant (Kearns and Yamamoto, 1981) (Fig. 2.8), resulting in an increase in nymphal survival and developmental rate. In the lace bug *G. solani*, aggregation behaviour was first described by Fink (1915). He observed that, while the nymphs migrated from one leaf to another, the guarding mother moved back and forth from one end of the leaf to the other in an attempt to keep the nymphs together while also urging them in the right direction. Similar behaviour was also described by Faeth (1989) for the lace bug *Corythucha hewitti*, with the female guiding nymphs to fresh food, but additionally, the female was observed to reunite nymphs after they become separated. If the mother was not present, the nymphs in the brood would disappear and wander to other leaves, which is likely to increase the mortality of the nymphs.

Figure 2.8. *G. decoris* mother near aggregated nymphs.

In *G. solani,* as long as the nymphs remain aggregated throughout their development, they have no problems moving from one leaf to another without the guidance of the mother (Tallamy and Denno, 1981a). However, in the absence of the guarding mother, when the nymphs are faced with a choice of paths, the nymphal aggregation may divide. When the mother is present, she blocks one route and forces the nymphs to stay together. By maintaining cohesion, it eases the female guarding effort (Kearns and Yamamoto, 1981; Tallamy and Denno, 1981a).

Feeding facilitation improves when lace bug nymphs are aggregated (Tallamy and Denno, 1981b; Denno and Benrey, 1997). For example, the nymphs of the lace bug *G. decoris* tend to act synchronously during feeding (i.e. act of consuming the food) and foraging (i.e. behaviours involved in obtaining food). When one individual in the aggregation withdraws its stylets, others in the aggregation follow. When part of the aggregation starts to move, the nymphs will bump to signal each other until the whole aggregation is activated (Kearns and Yamamoto, 1981). Signalling between aggregating nymphs has been observed not only for feeding facilitation but also as a warning for predator threat. In the presence of a predator, aggregated nymphs can minimize predation by producing a synchronous vibration that informs the caring mother of the threat (Ramaswamy and Cocroft, 2009).

The lace bug *G. decoris* has been observed to display maternal care (Guidoti *et al.*, 2015) and aggregation behaviour (Olckers, 2000), similar to other lace bugs (e.g. *Leptobyrsa decora* and *G. solani*) (Melksham, 1984; Kearns and Yamamoto, 1981). However, nothing is known about the implications that maternal care and aggregation behaviour have on nymphal survival and developmental time. The implications of maternal care have been thoroughly studied in the lace bug *G. solani* (Kearns and Yamamoto, 1981; Tallamy and Denno, 1981a; Tallamy and Horton, 1990; Monaco *et al.*, 1998; Tallamy, 1985; Tallamy and Denno, 1982). Implications of aggregation behaviour on nymphal survival and developmental time have been documented in the lace bugs *Corythucha cydoniae* Fitch (Braman and Pendley, 1993), *C. ciliata* (Wei *et al.*, 2013) and *Tingis cardui* L. (Eguagie, 1974).

Maternal care and aggregation are heritable behavioural traits that affect the demography of insect populations by contributing to population outbreaks (Wallner, 1987). These traits can determine the future of the offspring (hence the population) by enhancing their survival and fitness (Kearns and Yamamoto, 1981; Braman and Pendley, 1993; Hunter, 2002). The lace bug *G. decoris* expresses both traits. Thus, knowing the implications of these traits on *G. decoris* populations will help with understanding the key determinants that influence distribution in a small-case scenario (Hui *et al.*, 2010). The distribution in a small-case scenario will help to predict the distribution in a large-scale scenario (metapopulations) (Hortal *et al.*, 2010).

2.5. The effect of environment on insect life history: temperature, humidity, photoperiod

The environmental conditions to which an insect population is subjected have an impact on performance and fitness, both of which strongly influence the establishment of the population (Aysal and Kivan, 2008; Dhileepan *et al.*, 2010; Saavedra *et al.*, 2015). There are several approaches that have been developed to predict how different environmental conditions will affect population growth. Some of these approaches are discussed here.

2.5.1. Temperature effects on insect development

Temperature is one of the most important environmental factors influencing the life of insects (Omkar and Pervez, 2004). It influences developmental rate (Briere *et al.*, 1999) and can limit biological activity above or below critical thresholds (Huffaker, 1990). The temperature threshold for development and survival varies among insect species (Omkar and Pervez, 2004; Karlsson and Wiklund, 2004; Morimoto *et al.*, 2007), populations of the same species (Hope and Olckers, 2011), and even different developmental stages within a population (Neal and Douglass, 1988).

The relationship between developmental rate (1/development time) and temperature (at different constant temperatures set during laboratory experiments) tends to be linear within more optimal range of temperatures, and becomes non-linear at the extreme ends (Briere *et al.*, 1999) (Fig. 2.9). Development does not occur at temperatures that fall below a low-temperature threshold. Rises in temperature above this threshold lead to increasing rates of development, but only until a critical point (the optimum temperature) is reached. When the temperature continues to rise above this optimum temperature, the development rate decreases rapidly to zero (Briere *et al.*, 1999) (Fig. 2.9).

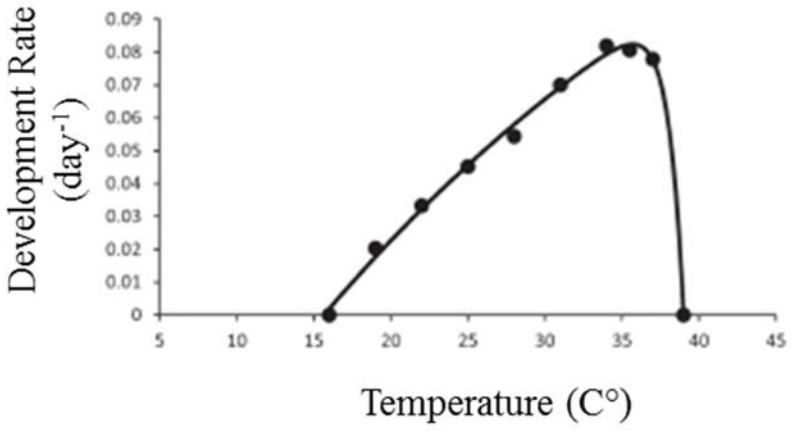

Figure 2.9. Development rate of immature stages at ten constant temperatures for *Monoscoteira unicostata* (Hemiptera: Tingidae) (Sánchez-Ramos et al., 2015).

The increase in development rate with increasing temperatures (below the optimum temperature threshold) is influenced by enzyme-dependent reactions that positively affect physiological processes with increasing temperatures. Beyond the optimum temperature, proteins and membranes start to disintegrate, resulting in a rapid reduction in insect performance (Harrison *et al.*, 2012).

Several approaches have been used to model developmental rates under different temperature conditions. The earliest approach (and still extensively used) is based on the degree-day model (Bonnet, 1779; Stratchey, 1878; Murray, 2008; Gómez *et al.*, 2009). The degree-day model is applied to poikilothermic organisms (i.e. those organisms, including insects, whose development is dependent on the ambient temperatures), and assumes that the relationship between the organism's development and the temperature where it is found is linear over a range of temperatures (Speight *et al.*, 1999; Gómez *et al.*, 2009).

A degree day is a measurement of heat units over time (Murray, 2008) calculated from daily maximum and minimum temperatures. Degree days are usually calculated for a 24-hour period time but of most importance is the cumulative heat experienced by the insect starting from a given point (a biological event or a calendar date) (Jones and Brunner, 1993; Murray, 2008). The degree day value for a 24-hour period is added to the prior days' values and this is done consecutively through the insect's development (Murray, 2008). The information gathered from degree-day models helps to predict development rate and time certain life stages of insects (i.e. an approximation of a future event), thus aiding in planning activities such as scouting (when to place traps or sample populations), and timing pesticide application (Murray, 2008) (Fig.2.10).

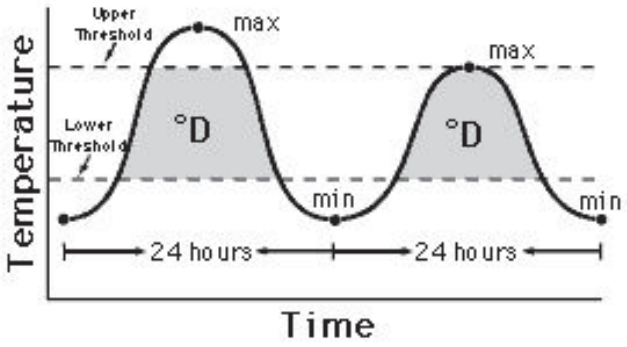

Figure 2.10. Visual representation of degree-days using the sine wave calculation. This method includes the daily minimum, maximum, and baseline temperatures (lower threshold and upper threshold for insect development). The sections in grey color under the curve represent the number of degree days that fall between the upper and lower threshold for a 24-hour day (Murray, 2008).

However, because the relationship between development and temperature is non-linear above or below critical thresholds, several non-linear models (e.g. those listed in Table 2.3) have been proposed to describe developmental rate response curves for scenarios in which the temperature range can exceed these thresholds (Briere *et al.*, 1999; Damos and Savopoulou-Soultani, 2012).

A complementary approach to modelling insect development at different temperatures is through construction of life table parameters (Pearl and Parker, 1921; Carey, 1993). A life table is a record made of population fitness characteristics, taken at either a single point in time (static) or for the life cycle of a group (cohort) within the population. *Static* life tables are a record of the number of living individuals of each age in a population and their reproductive output at a given time. One of the drawbacks of the static life table is that it assumes that age-specific survival and fertility rates remain constant from when the oldest members of the population were born (Donovan and Welden, 2002). *Cohort* life tables remove this assumption by following the survival and reproduction of all members of a group from birth to death. In order to build this type of table, it is necessary to record the survival in each stage of the life cycle and the fecundity (number of offspring born to females of each age class) (Donovan and Welden, 2002).

Table 2.3. Common non-linear models used to describe insect development rate over a range of temperatures (modified from Damos and Savopoulou-Soultani, 2012).

Non-linear model	Description/Reference
$1/D = c/(1 + e^{(a+b*T)})$, if $T \leq T_{opt}$	"Stinner" (Stinner *et al.*, 1974)
$1/D = c/(1 + e^{[(a+b*(2*T_{opt}-T)]})$, if $T > T_{opt}$	
$1/D = \Psi * [1/(1 + k * e^{-((Tmax-T)/\Delta)}]$	"Logan 10" (Logan *et al.*, 1976)
$1/D = a* T^3 + b * T^2 + c * T + d$	"3rd-order polynomial" (Harcourt and Yee, 1982)
$1/D = e^{\rho*T} - e^{\rho*T\max -(T\max -T/\Delta))} + \lambda$	"Lactin" (Lactin *et al.*, 1995)
$1/D = a * T * (T- T_{min}) * (\sqrt{T_{max}-T})^{(1/m)}$	"Briere 2" (Briere *et al.*, 1999)
$1/D = a * T * (T - T_{min}) * (\sqrt{T_{max} - T})$	"Briere 1" (Briere *et al.*, 1999)
$1/D = \rho * (a - T/10) * (T/10)^{\beta}$	"Simplified beta type" (Damos and Savopoulou-Soultani, 2008).
$1/D = a/(1 + bT + cT^2)$	"Inverse second-order polynomial 1" (Damos and Savopoulou-Soultani, 2012)

Life tables were first used as a tool for human demographic studies and used extensively to ascertain the probability of death (Harcourt, 1969). Later on, they were used for insect populations such as the fruit fly *Drosophila melanogaster* (Pearl and Parker, 1921) and have become one of the most useful tools for the study of insect population dynamics, especially for understanding the causes of mortality and predicting future growth or decline of populations (Donovan and Welden, 2002). The growth and decline of a population is tightly dependent on the reproductive rate (clutch size), age (when females begin to have their young) and lifespan (for how long the females produce young), which are the characteristics that life tables capture (Harcourt, 1969; Huffaker, 1990; Donovan and Welden, 2002; Omkar and Pervez, 2004; Kakde *et al.*, 2014).

2.5.2. Effect of temperature on insect performance

High temperatures may interfere indirectly and directly with certain reproductive processes (Eguagie, 1972; Dominguez da Silva, 2004; Aysal and Kivan, 2008). Fecundity (defined as the biological capacity of male and females to reproduce) (Awmack and Leather, 2002)) and fertility (defined as the number of viable offspring produced (Awmack and Leather, 2002)) are some of the reproductive processes that are affected by temperature. For example, in the moth *Panolis flammea* Denis and Schiffermüller, females mate less frequently at high temperatures than at the optimal one and, as a result, they lay fewer eggs (Zwölfer, 1931 as cited in Engelmann, 1970) with the possibility that a greater proportion of them may turn out to be infertile (Menusan, 1935; Hinton, 1981). In males, higher temperatures can impede the transfer of sperm (Katsuki and Miyatake, 2009) or even induce sterility (McMullen, 1967).

Higher temperatures can also increase fecundity by affecting oviposition rates. Higher temperatures generally cause higher oviposition rates (Legaspi and Legaspi, 2005; Aysal and Kivan, 2008). Therefore, insects can produce more generations per season and have higher population growth rates (Frazier *et al.*, 2006). The specific response in oviposition patterns, however, differs among even closely related species (Subba Rao and Gopinath, 1961; Engelmann, 1970). While increased oviposition rate brings increased fecundity, this can be offset by the reduction in fertility associated with higher temperatures (Menusan, 1935).

Temperature can influence how long an insect lives, with lifespan usually negatively correlated with temperature (Speight *et al.*, 1999; Matadha *et al.*, 2004; Gould *et al.*, 2005; Legaspi and Legaspi, 2005; Gómez *et al.*, 2009). Insects can survive in cool environments for longer periods, even when food is in short supply, because their metabolic energy demands are low (Speight *et al.*, 1999). For example, the female sycamore lace bug *Corythucha ciliata* Say displayed a significantly higher lifespan at lower temperatures (16 °C, 58.9 days) compared to higher temperatures (33 °C, 17. 3 days) (Ju *et al.*, 2011).

The lifespan of males and females does not always change equally when subjected to higher temperatures. In a study by Dominguez da Silva (2004), the males of the lace bug *Gargaphia torresi* Lima lived longer than females when subjected to higher temperatures.

It was suggested that the variations in lifespan between sexes could be attributed to different ways of allocating energy (such as oviposition in females).

Currently the information regarding the life history of *G. decoris* is limited. Olckers (2000) recorded the duration and lifespan of the insect cohort at a given temperature (22 ± 3 °C). However, the Olckers (2000) study did not include records of survival from each life stage of the insect, the proportion of females produced, fecundity of the females during their life duration and lifespan over a range of temperatures. Temperature related studies will help us build the life table of *G. decoris* and provide useful information for enhancing the mass rearing (Zhang *et al.*, 2011), as well as providing a quantitative basis for predicting development and activity of *G. decoris* in pest management programmes (Matadha *et al.*, 2004). In addition, the optimal temperature for *G. decoris* performance can be applied to changing scenarios (i.e. climate change) to predict how likely the establishment may be in a particular region (Saavedra *et al.*, 2015).

2.5.3. Effect of humidity on insect performance

Relative humidity (RH) is the ratio of actual water vapour present in the air to that necessary for saturation of the air, at a particular temperature. Relative humidity can affect insect development and reproductive success (Holdaway, 1932; Ahmad, 1936; Gullan and Cranston, 2010) by affecting growth rate (Fraenkel and Blewett, 1944), activity (Bursell, 1956), mating, fecundity and fertility (Holdaway, 1932). The effects of humidity on insect development are less evident than the effects of most other environmental parameters, and as such have received less attention (Holdaway, 1932). Nonetheless, humidity plays an important role (Engelmann, 1970). The loss of water is a serious hazard for insects which can affect their physiology and, consequently, affect development, longevity and oviposition of many insects (Gullan and Cranston, 2010).

There is an optimum humidity for insect development and this optimum humidity differs between species (Bell, 1975; Hagstrum and Milliken, 1988). Usually the optimal relative humidity at which egg maturation and egg laying are observed is related to the species' natural environment (Engelmann, 1970). However, there are cases in which insects have been observed to be relatively unaffected by differences in humidity. For example, the groundnut bruchid (Coleoptera: Bruchidae) *Caryedon gonagra* Fabricius laid

the highest numbers of eggs between the 27-39 °C range and this numbers appeared to be independent of RH in the 50-90% range (Cancela da Fonseca, 1964). It has been observed that insects subjected to higher humidity become more aroused and this increases their sexual activity leading to more fertile eggs (Holdaway, 1932). In other cases, higher humidity has led to insects and their eggs drowning or being infected more easily by pathogens (Gullan and Cranston, 2010).

The biology of *G. decoris* has been studied under a RH of 70-80% (Olckers, 2000) but nothing is known about the effects of this particular humidity or a lower humidity (50-60%) on the performance of the insect. Currently the insect is confirmed to have established in the northern part of the North Island in New Zealand. One region where the insect has successfully established, the Bay of Plenty, displays an average annual RH of 78.8% (NIWA, 2016). However, the host plant, woolly nightshade, is present not only in New Zealand but in other parts of the world where the mean annual RH can reach as low as 59.8% (e.g. South Africa) (Olckers and Zimmermann, 1991). Knowing the effect of different humidity on *G. decoris* performance will allow us to select optimal conditions for its establishment and therefore aid in control of woolly nightshade.

2.5.4. Effect of photoperiod on insect performance

Photoperiod is the length of the daily light phase, also known as day length (Gullan and Cranston, 2010). It is the most reliable long-term cue to future conditions (Tauber *et al.*, 1986) and as a result, insects have evolved the ability to take advantage of the photoperiod progression (Tauber *et al.*, 1986) by synchronizing their life cycles according to the daily photoperiod (Beck, 1980).

The importance of photoperiod on insect behaviour varies with latitude (i.e. the degree of seasonality). For equatorial and tropical locations that do not experience seasonal differences in day length, temperature and moisture have more influence than photoperiod (Tauber *et al.*, 1986). Photoperiod is much more important in temperate locations, where there are strong seasonal changes in day length; however, temperature and other environmental factors can modify the effects of photoperiod (Prakash, 2008).

Larval and/or nymphal growth rates tend to be primarily determined by temperature and food quality and availability (Leimar, 1996), but photoperiod plays a role in the insect's

ability to time its development according to favourable conditions (Leimar, 1996). Photoperiod can serve as a cue for an insect to enter diapause (i.e. a quiescence period), which can help the insect to survive hostile conditions, for example, summer heat, drought, or winter cold (Nealis *et al.*, 1996; Speight *et al.*, 1999).

Photoperiod, in combination with temperature, humidity and light intensity, also influences developmental times (Beck, 1980; Tauber *et al.*, 1986), along with some other periodic activities that include locomotion, feeding, adult emergence, mating and oviposition (Beck, 1980; Taylor *et al.*, 1995; Neal *et al.*, 1992).

Currently, there are no records that *G. decoris* is affected by differences in photoperiod. The effect of photoperiod has been studied in the lace bug *Corythucha cydoniae* (Braman and Pendley, 1993). Results from their study show that the proportion of females ovipositing within 30 days reached its lowest point at a 13 hours daylength under constant temperature. In a separate study, Neal et al. (1992) reported that *C. cydoniae* reared in the lab at a daylength of 14 hours did not diapause but when reared in a glasshouse under natural declining light, adults diapaused resulting in a delayed preoviposition period.

The developmental and reproductive biology of the lace bug *Gargaphia decoris* in relation to photoperiod have not been studied in a set of different photoperiods. This information will be important for the development and implementation of information-based pest management strategies in the field.

2.6. Plant-insect interactions

In natural ecosystems, plants and insects continuously interact in a complex way (Mello and Silva-Filho, 2002). This plant-insect interaction is a dynamic system, subjected to continuous variations (Mello and Silva-Filho, 2002). Insect attack has selected for plants that possess a variety of defensive traits including chemical (Baldwin, 2001) and physical barriers (Fordyce and Agrawal, 2001). This has resulted in a co-evolutionary 'arms race' whereby insect herbivores that are capable of overcoming plant defences are more likely to pass on their genes (Zangerl, 1990; Scott and Wen, 2001; Nishida, 2002).

Host plant quality is a term used to describe the different components of plants (e.g. levels of nitrogen, carbon, trace elements, and defensive compounds) and their suitability

for optimal performance of herbivorous insects (Awmack and Leather, 2002). The host plant quality can be affected by environmental factors which can play a crucial role in morphogenesis and resource allocation in plants (Begna *et al.*, 2002). Changes in resource allocation can alter growth, leaf chemistry, leaf defence (Bryant *et al.*, 1983; Schaffer and Mason, 1990; Moran and Showler, 2005; Ingersoll *et al.*, 2010) and herbivore activity (Aide and Zimmerman, 1990; Muth *et al.*, 2008; Diaz *et al.*, 2011).

One of the environmental factors that influences host plant quality is light intensity (Holt, 1995). Light intensity (or irradiance) is the rate at which light energy reaches a unit of surface (Forbes and Watson, 1992). Variations in light intensity can be of a predictable nature like those that accompany the time of the day, season and latitude (Roberts and Paul, 2006) or unpredictable like those resulting from the shade of plant canopies (McDonald *et al.*, 1999).

Light intensity has been suggested to affect the establishment of the lace bug *Gargaphia decoris*. In a study performed by Patrick and Olckers (2014) in South Africa, they observed that there is an optimal shade condition for the persistence of adults and nymphs of *G. decoris*. They attributed the higher persistence of *G. decoris* in shaded conditions to a lower presence of predators. However, they do recognize that differences in adult persistence may also have been influenced by the varying plant quality caused by the shade treatments.

2.6.1. Effect of light intensity on plant growth and implications for herbivory

Low light (i.e. shaded) conditions tend to limit plant biomass, with a reduction in the biomass of leaves, stems, and fruits (Diaz *et al.*, 2011). This has been documented for different functional groups of plants (Wilkens *et al.*, 1996b; Crone and Jones, 1999; Schooler, 2008; Moran and Showler, 2005). The reduction in biomass occurs because shaded plants generally cannot acquire sufficient resources to support rapid growth (Bryant *et al.*, 1983; Rajcan *et al.*, 2002), and tend to modify their height, weight, number of leaves, leaf morphology, and shoot-to-root ratio (Clough *et al.*, 1979; Begna *et al.*, 2002; Rajcan *et al.*, 2002; Bentz, 2003; Curt *et al.*, 2005; Diaz *et al.*, 2011). The key driver behind all of these changes is to increase the photosynthetic efficiency (Fahn, 1990).

Light energy absorbed by a plant can be used to drive photosynthesis (photochemistry), be released as heat, or be re-emitted as light by means of chlorophyll fluorescence (Maxwell and Johnson, 2000). Plants exposed to different light intensities express differences in their photosynthetic activity (Sarijeva *et al.*, 2007). These differences in photosynthetic activity have been attributed to differences in pigment ratios (e.g. chlorophyll a to chlorophyll b (Chl a/b)) (Lichtenthaler *et al.*, 1981), light harvesting Chl a/b-proteins (i.e. LHCII) (Sarijeva *et al.*, 2007) and chloroplast ultrastructure (i.e., grana stacks inside thylakoids) (Boardman, 1977; Lichtenthaler *et al.*, 1981).

Leaves grown in sunlight have a higher photosynthetic capacity per leaf area and total chlorophyll basis; they exhibit higher levels of chlorophylls (Chl) and carotenoids, and higher values for the ratio Chl a/b, despite having a much lower level of light harvesting Chl a/b proteins (LHCII), lower values for the ratio Chl/carotenoids and a lower stacking degree of thylakoids than shade leaves (Sarijeva *et al.*, 2007). The differences in pigment ratios between sun and shade plants reveal that sun plants have evolved an adaptive response to irradiance (Sarijeva *et al.*, 2007). The higher photosynthetic capacity in sun leaves despite having lower LHCII is counterbalanced by a greater amount of reactions centers (i.e. multisubunit membrane protein complexes) to total chlorophyll content basis (Lichtenthaler *et al.*, 1982).

Shade leaves display higher fluorescence intensity than sun leaves when dark-adapted leaves are artificially illuminated with high levels of light (Lichtenthaler *et al.*, 1986). One of the reasons for this is that shade leaves invest more in light-harvesting pigments that can allow them to utilize the limited amount of sunlight received (Salisbury and Ross, 1992). This explains the higher levels of light harvesting Chl a/b binding proteins (LHCII) in shade leaves which has been associated with higher and broader grana thylakoid stacks within chloroplasts and a higher proportion of thylakoid membranes (Boardman, 1977; Lichtenthaler *et al.*, 1981).

Many plant species adjust the anatomy and morphology of their leaves to optimize the amount of radiation received by the sun, thus increasing their photosynthetic efficiency (Kubínová, 1999; Stanton, 2010). The reported differences in the anatomy and morphology of sun and shade leaves are not always consistent (Friend and Pomeroy, 1970; Dale and Felippe, 1972; Lichtenthaler, 1985); thus, they are thought to be species-specific

(Kubínová, 1999). For instance, species that have evolved to become shade-tolerant may have developed different physiological and morphological adaptations than shade-intolerant species (Boardman, 1977); these adaptations allow species to respond in a different manner to changes in light environment. Regardless, typical responses to changes in light environment exhibited by a wide variety of plant species include changes in the leaf area and leaf thickness (Regnier *et al.*, 1988; Sims and Pearcy, 1992).

Specific leaf area (SLA) (leaf area per unit leaf mass) has been reported to be higher at low-light levels (Friend and Pomeroy, 1970; Longstreth *et al.*, 1985; James and Bell, 2000; Stanton, 2010). The increase in SLA is considered an acclimatory response to maximise the amount of chlorophyll exposed to the light available (Regnier *et al.*, 1988; Sims and Pearcy, 1992). Often associated with higher SLA is a reduction in the thickness of the leaf, which also helps to concentrate the chlorophyll towards the top surface of the leaf (Sims and Pearcy, 1992; Regnier *et al.*, 1988). These morphological changes allow greater interception of light per unit of leaf tissue (Regnier and Harrison, 1993), and higher potential photosynthetic leaf area relative to leaf biomass (James and Bell, 2000). This resource optimization strategy (Vile *et al.*, 2005; Clough *et al.*, 1979) has been utilised as an indicator of the productivity of a species and/or cultivar (White and Montes-R, 2005) or ecological performance (Diaz *et al.*, 2004).

In addition to high SLA and reduced thickness, another adaptation of shaded leaves to maximise photosynthesis potential is to change the distribution of chloroplasts within the palisade cells (Sousa-Paiva *et al.*, 2003). The chloroplasts conglomerate together, creating large gaps between each conglomeration. The gaps between the chloroplast conglomerations permit light transmission into the spongy mesophyll. The spongy mesophyll itself is more dispersed, with large air spaces between the cells (Fig. 2.11). These air spaces in turn cause more of the transmitted light to reflect and refract back towards the conglomerated chlorophyll in the palisade cells.

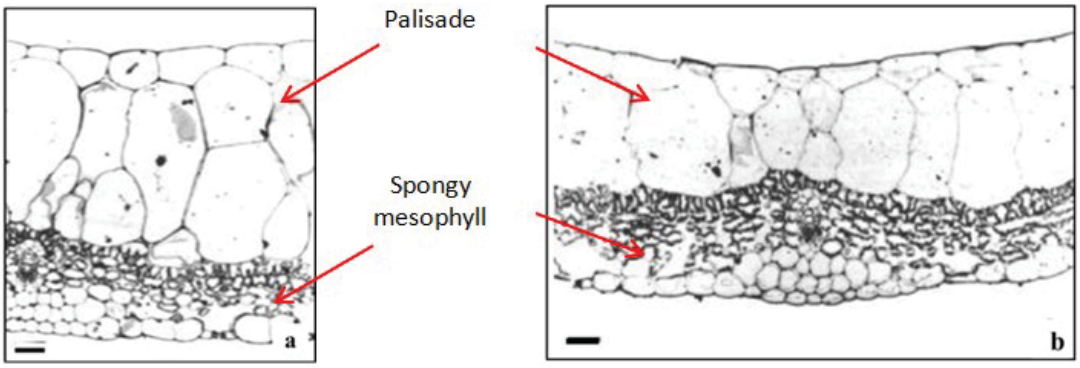

Figure 2.11. *Tradescantia pallida* (Rose) Hunt cv. *purpurea* Boom (Commelinaceae) leaves subjected to different light intensities, (a) 800 µmol m^{-2} s^{-1} (b) 80 µmol m^{-2} s^{-1}. Figures modified from Sousa-Paiva et al (2003).

As well as optimizing the amount of sunlight received per unit leaf area, the rearrangement of leaf cells also helps to control water evaporation in sun-grown leaves by enhancing epidermal resistance of water to vapour (Xu *et al.*, 2009). Leaves exposed to high light conditions appear to have lower water content than shaded leaves (Louda and Rodman, 1996; Henriksson *et al.*, 2003; Moran and Showler, 2005), regardless of the abundance of water at the roots (Marsh, 1941). The decrease in water content results in an increase in dry weight per unit area (Bentz, 2003). This increase in dry weight is an adaptation of the plant to prevent water vapour loss through the cuticles under high light intensities and water stress. Therefore, creating an epidermal resistance enables the plant to increase its leaf lifespan under adverse conditions (Xu *et al.*, 2009).

The morphological and structural changes which occur in the leaves of plants subjected to different light environments have both direct and indirect effects on herbivorous insects. Changes in foraging behaviour and oviposition have been implicated as potential causes for the difference in plant damage or insect abundance observed in sun versus shade environments (Collinge and Louda, 1988; Moore *et al.*, 1988; Potter, 1992; Trumbule and Denno, 1995; Rossi and Stiling, 1998; Sipura and Tahvanainem, 2000; Bentz, 2003; Connor, 2006). Factors associated with shading, such as temperature, irradiation, and humidity, play a crucial role in host plant selection, consumption, development and fecundity (Tingle and Copland, 1988; Moore *et al.*, 1988; Sipura and

Tahvanainem, 2000; Niesenbaum and Kluger, 2006), and the distribution and abundance (Capuccino and Root, 1992) of herbivorous insects.

Herbivorous insects have different responses to changes in light conditions. Some species perform better (i.e., show higher feeding rate, reproductive rate, growth rate, survival and longer lifespan) on shade-grown plants (Trumbule and Denno, 1995; Trumbule *et al.*, 1995; Folgarait *et al.*, 1996; Jansen and Stamp, 1997; Rossi and Stiling, 1998; Braman *et al.*, 2000; Sipura and Tahvanainem, 2000; Henriksson *et al.*, 2003; Connor, 2006; Roberts and Paul, 2006; Muth *et al.*, 2008; Diaz *et al.*, 2011), while others perform better in partial shade (Patrick and Olckers, 2014). Yet others perform best on plants in full sun conditions (Lincoln and Mooney, 1984; Raupp, 1984; MacGarvin *et al.*, 1986; Capuccino and Root, 1992; Levesque *et al.*, 2002; Bentz, 2003; Kay *et al.*, 2007; Barber, 2010). Other insects perform equally well on shade and sun-grown plants (Rowe and Potter, 2000; Potter, 1992; Rowe and Potter, 1996; Connor, 2006; Diaz *et al.*, 2011). Because of the different ways insect herbivores respond to variations in light conditions, it has been suggested that these responses are species-specific rather than habitat related (Louda *et al.*, 1987; Bultman and Faeth, 1988; Connor, 2006).

Besides differences in responses to light environment among insect species, variations in response can be expressed within the same species of herbivorous insects. Different stages of an insect's life history might have different preferences for light conditions (Diaz *et al.*, 2011). For example, the beet armyworm *Spodoptera exigua* Hübner larvae preferred to feed on plants in full sunlight, but adult females preferred shade conditions for feeding and oviposition (Moran and Showler, 2005). The optimal light conditions for larval feeding, larval performance, and adult oviposition may differ (Berdegué *et al.*, 1998).

Light intensity can influence the behaviour of insects indirectly by increasing warming (Alonso, 1997) and by affecting chemical traits of host plants. A good example of warming effect on insect behaviour is found in the western tent caterpillar *Malacosoma californicum pluviale* Dyar, which prefers to oviposit and build tents on south-facing branches rather than on shaded-north facing branches of host trees (Connor, 2006).

Plant chemical traits are affected by light intensity and can influence herbivory by changing the total nitrogen concentration, carbon/nitrogen ratio, or protein or amino acid

concentrations (Aide and Zimmerman, 1990; Roberts and Paul, 2006; Muth *et al.*, 2008). For example, the spicebush *Lindera benzoin*, had greater percent nitrogen (N) and soluble protein in the shade compared to greater percent carbon (C) in the sun (Muth *et al.*, 2008) The higher percent of nitrogen found in *L. benzoin* shade plants increased herbivory activity of the larva of the moth *Epimecis hortaria* Fabricius (Muth *et al.*, 2008).

The effect of light intensity on host plant suitability has been addressed as part of a management tool to control lace bug *Stephanitis pyrioides* populations in ornamental plants (i.e. chrysanthemum) (Bentz, 2003). The results showed that lace bugs appear to inflict more damage as shade progresses; shade also increased the levels of chlorophyll, mean leaf area, and leaf moisture; in contrast the biomass and leaf thickness decreased (Bentz, 2003). The resource allocation within the plant due to the effect of light intensity can have an effect on the insect feeding (Buntin *et al.*, 1996) and oviposition selection (Neal and Bentz, 1997). The effects of insect feeding and oviposition selection can be related to the rearrangement of chloroplasts within the cells, and higher leaf moisture, which could increase the leaf acceptability (Bentz, 2003).

Studies explaining the light-induced changes in the invasive weed *Solanum mauritianum* and related changes in its suitability as a host to the lace bug *Gargaphia decoris* are non-existent. Exploring the changes in resource (C and N) allocation in *S. mauritianum* due to changes in light intensity will help further understanding of the effects of these plant metabolic changes on *G. decoris* performance and, therefore, help to explain the failure of this insect to impact on plants growing in full sun.

2.6.2. Effect of light intensity on plant defences

Biotic stressors, such as changes in light intensity, play an important role in plant performance. Plants use several mechanisms to deal with biotic stressors in order to balance resource allocation. Plants must grow fast enough to avoid being outcompeted by other plants and at the same time, they need to defend their tissues from herbivorous insects (Ballaré, 2014). Some of the mechanisms used by plants to defend against herbivorous insects are mechanical and structural barriers (Levin, 1973; Coley, 1983; Coley, 1987; Lucas *et al.*, 2000), and secondary metabolites (defensive compounds) (Rosenthal and Janzen, 1979; Mithen, 1992).

2.6.2.1. Mechanical and structural barriers

Some examples of mechanical and structural barriers include leaf thickness, toughness and hardness, possession of thorns, spines or hairs, and surface waxes, all of which can affect plant-herbivore interactions (Roberts and Paul, 2006). Trichomes (i.e. hair-like structures) are a characteristic feature of *S. mauritianum* which are likely to be a significant barrier to herbivory, as has been documented in other *Solanum* plants (Nihoul, 1993; Wilkens *et al.*, 1996a).

A trichome is a unicellular or multicellular hair-like appendage that originates from epidermal cells only and extends outwards from the surface of various plant organs (Levin, 1973; Werker, 2000). Trichomes can be found on the vegetative and reproductive parts of the plant (Werker, 2000). They are classified in two major groups: (1) glandular, and (2) non-glandular. Glandular trichomes contain glands from which they produce, store and secrete secondary metabolites (Glas *et al.*, 2012). Each type of glandular trichome is differentiated by the type of secretory materials, as well as the trichome morphology (e.g. stellate, T-shaped, rounded or spiral; (Payne, 1978)). Non-glandular trichomes are differentiated solely by their morphology (Werker, 2000).

The distribution of trichomes is variable; they can be spread evenly on the surface, or be more localized (Werker, 2000). In some plants, leaf glandular trichomes are concentrated in the intervein areas, while the non-glandular type is abundant on the veins and leaf margins (Ascensão *et al.*, 1995). In other plants, non-glandular trichomes are concentrated around stomata. These trichomes play a role in protecting the endodermal cells which prevent apoplastic water leakage from stomata (Fahn, 1986; Fahn, 1990).

Trichome development commences at an early stage of leaf development (Werker, 2000). They can be fixed in density during the life span of a leaf (Ascensão and Pais, 1987) or vary with leaf age (Unzelman and Healey, 1974; Turner *et al.*, 1980; Croteau *et al.*, 1981), environmental conditions (Bentz, 2003) and the presence of herbivores (Traw and Dawson, 2002).

Light intensity affects the density and size of trichomes that develop on a leaf (Bentz, 2003; Diaz *et al.*, 2011), with trichome density and size generally decreasing with shading (França and Tingey, 1994; Bentz, 2003; McGuire and Agrawal, 2005). It is thought that the total number of trichomes per leaf is constant, so a more expansive leaf

area in shaded leaves results in lower density of trichomes (Unzelman and Healey, 1974; Bentz, 2003). Alternatively, or in addition, lower density and size of trichomes in shade could result from the leaf investing more energy into producing chlorophyll pigments to maximize light absorption (Salisbury and Ross, 1992) at the expense of the energy required by the epidermal cells to produce new trichomes (Unzelman and Healey, 1974).

Plants that are exposed to higher sunlight suffer more water and irradiance stress than shaded plants (Liakoura *et al.*, 1997). These plants are stimulated to produce more trichomes to protect themselves from excessive water loss, sometimes by creating a dense mat over the leaf (Werker, 2000) or by concentrating around the stomata (Fahn, 1990).

Trichomes have been shown to affect insect herbivore survival, feeding, oviposition, locomotion, growth and fecundity, and influence their interactions with natural enemies (Butler *et al.*, 1991; Goertzen and Small, 1993; Nihoul, 1993; Haddad and Hicks, 2000; Andres and Connor, 2003). Trichomes appear to be particularly effective in lowering insect survival in the initial stages of an insect's life cycle (Haddad and Hicks, 2000); also, by covering the leaves or other plant structures, the trichomes prevent adult insects from laying eggs (Norris and Kogan, 1980). The production of trichomes on the new growth of host plants can be induced by previous feeding by herbivores on older leaves, with adverse consequences not only for the inducer, but also for the community of other herbivores feeding on the plant (Traw and Dawson, 2002).

However, not all herbivores are negatively impacted by trichomes (Norris and Kogan, 1980). Some herbivores prefer plants with dense trichomes for oviposition, and can perform better on such plants (Price *et al.*, 2011).

2.6.2.2. Chemical defence compounds (secondary metabolites)

Secondary metabolites are derived from metabolites that participate in primary physiological processes (i.e. primary metabolites) (Berenbaum, 1995). Thus, they share common precursors and intermediates with primary metabolites (Lindsey and Yeoman, 1983; Berenbaum, 1995). Because they share common pathways, concentrations of one compound may increase at the expense of the other (Chew and Rodman, 1979; Baldwin *et al.*, 1987; Berenbaum and Zangerl, 1988). For example, if a plant is directing its resources primarily to growth (using primary metabolites, such as nitrogen and carbon, for this

process), less of these resources will be used towards defensive compounds (i.e. secondary metabolites). Although plant secondary compounds can be difficult to classify, they are often divided into two main groups: (1) qualitative defensive compounds; and (2) quantitative defensive compounds.

Qualitative defensive compounds are small molecules present in low concentrations in plant tissues (< 2% dry weight): these include alkaloids, pyrethrins, glucosinolates, cardenolides, cyanogenic compounds and non-protein amino acids. They are dynamic compounds which means that they possess properties that make them easy to synthesize, store, transport or re-synthesize into other compounds (Rhoades, 1983). This dynamism and low concentration allow these molecules to be produced at low cost to the plant and without a huge demand on the total secondary metabolism (Herms and Mattson, 1992). However, while being easy to produce, and effective against many polyphagous herbivores, these compounds are thought to provide limited protection against co-evolved specialist herbivores that have developed detoxification or tolerance mechanisms (Price *et al.*, 2011).

Quantitative defensive compounds are large molecules that occur in relatively large concentrations (5-20% dry weight), and include tannins, resins, latexes, fibres, lignin, oils, and waxes. They are thought to be more costly to produce than the qualitative defensive compounds and are more stable end products (Herms and Mattson, 1992). Even though these compounds are costly to produce, they are thought to provide plants better protection against both specialized and polyphagous herbivores (Price *et al.*, 2011).

Plant secondary metabolites are a diverse group of chemicals that show both spatial and temporal variation. The spatial variation can be within individual species, between plants in a population and between plant populations (Hunter *et al.*, 1996; Hartman, 2007) Temporal variation can be between seasons (Forkner *et al.*, 2004) and between various stages of ontogeny (e.g. between seedling and adult tree, or at different stages of leaf development) (Langenheim *et al.*, 1981; Schoonhoven *et al.*, 2005; Price *et al.*, 2011).

The types of defence chemicals can be different between plant parts and within plant parts (e.g. leaf parts) (Schoonhoven *et al.*, 2005). For example, the fruits of wild parsnip *Pastinaca sativa* L. contain higher concentrations of furanocoumarin (i.e. phenolic) than leaves and roots (Schoonhoven *et al.*, 2005), and in tobacco *Nicotiana tabacum* L.

leaves nicotine concentration increases from the basal to the apical portion (Schoonhoven *et al.*, 2005).

Quantitative defences generally increase in concentration with leaf age (McKey, 1979; Rhoades, 1979; Swain, 1979; Norris and Kogan, 1980) and qualitative defences decline with leaf age (Smeathers *et al.*, 1973; Lawton, 1976; Woodhead and Bernays, 1978). The compounds that are present in newly developing tissues, reproductive organs such as seeds, and in succulent herbaceous plants are the most toxic (Mattson, 1980). On the other hand, the compounds that are associated with nitrogen-deficient plant tissues (such as mature leaves, stems, bark, and slow-growing woody perennials) are known to be of reduced digestibility (Mattson, 1980).

Because secondary metabolites can experience turnover, changes in the concentrations of individual secondary metabolites are not always correlated with overall changes in total secondary metabolism (Gilmore, 1977; Berenbaum *et al.*, 1986; Hegerhorst *et al.*, 1988). For example, individual compounds varied in their changes during the growing season of rubber rabbitbrush *Ericameria nauseousus* (Pall.) Britton. Limonene was negatively correlated with rubber production whereas β-cubebene was positively correlated (Hegerhorst *et al.*, 1988).

In newly developing tissue, secondary metabolite production can be constrained by the lack of enzymatic machinery necessary for their synthesis and by the lack of fully developed structures required for secondary metabolite compartmentation (such as cell walls, vacuoles, idioblasts, resin ducts, laticifers, and other specialized structures) (Collin, 1987; Aerts *et al.*, 1991; Cotton *et al.*, 1991). Despite the lack of fully developed compartmentation systems, concentrations of some secondary metabolites (particularly low-molecular-weight phenolics, terpenes and alkaloids) are highest during early stages of seedling growth and leaf expansion, and/or they are synthesized in young tissue only (Fujimori *et al.*, 1991; Porter *et al.*, 1991; Singh *et al.*, 1991).

Many secondary metabolites are highly multifunctional, with broad defensive and ecological roles as well as roles in an array of primary plant functions (de Vos *et al.*, 2008; Bednarek and Osbourn, 2009; Gould *et al.*, 2010; Møller, 2010). Secondary metabolite multifunctionality may explain why some studies have reported a positive correlation between secondary metabolite concentration and plant growth (King *et al.*, 2006). For

example, the secondary metabolite canavanine (non-protein amino acid) is an effective defensive compound against predation, disease and even competition with other plants. Despite this diversion of the compound to defence purposes, canavanine does not pose a significant burden to the plant normal processes because the catabolism of canavanine generates essential primary metabolites necessary for plant growth (Rosenthal, 1990).

A given chemical may serve as a toxin or nutrient, depending on many factors including its environment, concentration, and the physiological state of the receiver (van Emden, 1978; Reese, 1979). For example, canavanine is lethal to armyworm *Prodernia eridania* Cramer, but is a source of nitrogen for bruchid beetles (Rehr *et al.*, 1973; Rosenthal *et al.*, 1978).

It is known that secondary metabolites can be affected by environmental factors (e.g. drought, temperature, humidity, light intensity, minerals and CO_2) (Ramakrishna and Ravishankar, 2011; Neilson *et al.*, 2013; Ninemets, 2016). As a result, plant productivity and resistance are affected with further effects on herbivore performance and population dynamics (Price, 1991; Braman *et al.*, 2000; Whittaker, 2001; Awmack and Leather, 2002; Throop and Lerdau, 2004). One of the environmental factors that affect plant secondary metabolites is light intensity (Price *et al.*, 2011). Light intensity can induce an increase of some secondary metabolites in some plants (e.g. tannins in acacia trees and ferns; resins in legumes; phenolics in willow leaves) (Cooper-Driver *et al.*, 1977; Langenheim *et al.*, 1981; Larsson *et al.*, 1986; Mole *et al.*, 1988). In other plants, light intensity may cause a reduction in secondary metabolites, e.g. cyanogenic glycoside in ferns, monoterpenoids in mint (Cooper-Driver *et al.*, 1977; Lincoln and Langenheim, 1978), and there are some plants in which secondary metabolites are unaffected by changes in light (Lincoln and Langenheim, 1978; Lincoln and Mooney, 1984; Potter, 1992; Muth *et al.*, 2008). These contrasting responses indicate that plants differ physiologically, chemically and morphologically when exposed to different light intensities (Bultman and Faeth, 1988).

When plants are exposed to high light intensities, there is an over-flow production of carbohydrates (Herms and Mattson, 1992; Rowe and Potter, 2000). The excess of carbohydrates is expected to increase carbon-based metabolites (Ingersoll *et al.*, 2010) and decrease nitrogen-based metabolites in herbaceous plants as well as woody species (Jansen and Stamp, 1997; Crone and Jones, 1999; Briskin and Gawienowski, 2001; Henriksson *et*

al., 2003; Muth *et al.*, 2008). However, there are many observations that are inconsistent with this prediction (Bryant *et al.*, 1987; Lincoln *et al.*, 1993; Gershenzon, 1994; Ohnmeiss and Baldwin, 1994).

Light intensity affects secondary metabolites independently of other environmental factors, but it has been documented that when combined with temperature, light intensity can influence the type of metabolite the plant will produce (Lincoln and Langenheim, 1978). In addition, there is evidence that concentration of some secondary metabolites is under genetic control (Fajer *et al.*, 1992; Orians *et al.*, 2003). This means that the degree of induction or suppression of certain types of metabolite could vary among genotypes (Hamilton *et al.*, 2001). However, even though the patterns of variation of secondary metabolites is a result of a tight genetic control, environmental factors such as light intensity and temperature superimpose to create variation upon the type of metabolite (Lincoln and Langenheim, 1978).

For example, low day temperatures stimulated the formation of pulegone in young leaves and isomenthone in mature leaves of *Clinopodium douglassi* (Benth) Kuntze. In contrast, greater amounts of piperitenone were found in younger leaves and piperitone in older leaves when the plant was exposed to high temperatures (Lincoln and Langenheim, 1978). In addition, regardless of the day temperature, low light regimes increased the amounts of monoterpenoids per leaf weight compared to high light regimes (Lincoln and Langenheim, 1978).

Changes in plant host secondary compounds induced by environmental stressors can affect host-plant preference, and growth and abundance of herbivores (Louda and Rodman, 1996; Jansen and Stamp, 1997; Schoonhoven *et al.*, 2005). However, the effect on insect herbivores is not always predictable (Lindroth *et al.*, 1993; Kinney *et al.*, 1997; Hemming and Lindroth, 1999; Roslin and Salminen, 2008). For example, in a study performed by Blau *et al* (1978), the cabbage white butterfly *Pieris rapae* L. and the black swallowtail *Papilio polyxenes* Fabricius reacted differently to allyl glucosinolates concentrations. *P. rapae* performance was unaffected by allyl glucosinolate concentrations that were higher than normal, while *P. polyxenes* died when exposed to lower than normal concentrations of the same metabolite.

Secondary metabolites that deter colonization and feeding are primary reasons why plants escape the vast majority of herbivores occurring in their environments (Dethier, 1980; Ikonen *et al.*, 2002). The metabolites influence the plant's nitrogen availability by affecting the herbivore's feeding behaviour and its life span (Mattson, 1980; Bernays, 1981). The herbivore might be killed or incapacitated quickly after initial feeding, or it might be affected further in non-feeding stages (e.g. pupae) (Reese and Beck, 1976). For example, phenolics are known to limit herbivore consumption by lowering palatability and causing gut lesions (Bernays, 1981) and interfering with digestive enzymes in the herbivore gut (Larsson *et al.*, 1986; Waterman and Mole, 1994).

Determining the contribution of individual secondary compounds to insect performance can be difficult, because sometimes they co-vary with nutritional effects in such a way that nutritional differences may have more impact on insect's performance when it is simultaneously challenged with secondary compounds (McDonald *et al.*, 1999). There are some herbivores that have evolved mechanisms to counteract the effects of secondary metabolites in their host plant (Whittaker and Feeny, 1971). For example, some caterpillars contain microsomal oxidases (enzymes) in their midgut that help them metabolize certain types of toxins (Krieger *et al.*, 1971).

The production and concentrations of some secondary metabolites, such as alkaloids, glucosinolates and furanocoumarins, can be induced by herbivory (Karban and Baldwin, 1997). For example, it was shown that an increase in the abundance of insect herbivores on *Datura stramonium* (Solanaceae) induced the increase in concentration of two tropane alkaloids (Shonle and Bergelson, 2000). Many kinds of herbivores can affect plant chemistry both directly and indirectly (Mattson, 1980). Some species of sucking insects secrete salivary juices directly into plant tissues, modifying plant metabolism. For example, some aphids can cause attacked leaves to become darker green and to develop higher nitrogen content than the rest of the leaf tissue (Osborne, 1973; Mattson, 1980). In addition, when herbivores remove or damage tissue, they may stimulate plant growth and nitrogen yields (Jameson, 1963; Dyer and Bokhari, 1976; Mattson, 1980).

The most investigated secondary metabolite in *Solanum* species is the glycoalkaloid solasodine owing to the potential use as a precursor for the synthesis of medicinal steroids (Lancaster and Mann, 1975; Drewes, 1993; Jayakumar and Murugan, 2016) (Fig. 2.12).

Most glycoalkaloids in the Solanaceae species are classified as either the solanidane or spirosolane type (Fig. 2.13). Solasodine is classified as a spirosolane type.

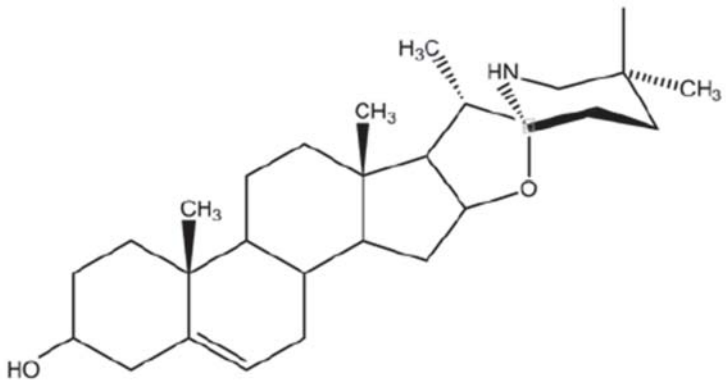

Figure 2.12. Chemical structure of solasodine (retrieved from Al-Sinani and Eltayeb, 2013).

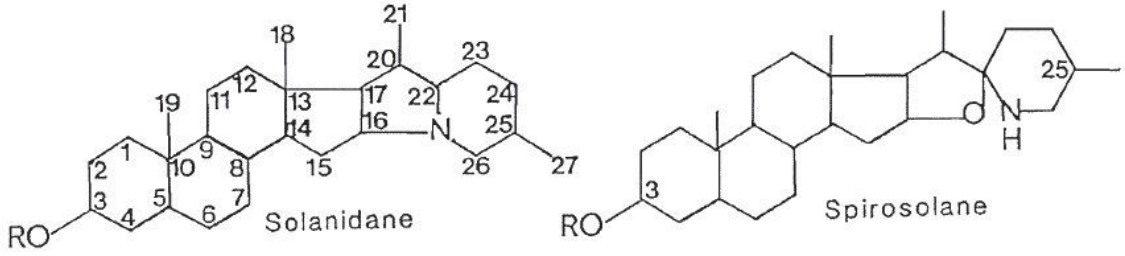

Figure 2.13. Class of glycoalkaloids most commonly found in Solanaceae (retrieved from Drewes, 1993).

Glycoalkaloids are affected by unfavourable climatic conditions such as extreme temperatures (Papathanasiou *et al.*, 1999), or by exposure to artificial or natural light (Percival, 1996; Percival, 1999). Other triggers of glycoalkaloid production include insect damage, and the creation of hybrid species (i.e. grafting) (van Gelder and Scheffer, 1991; Hlywka, 1994).

Most of the glycoalkaloids that have been identified to date have come from various potato species (Milner *et al.*, 2011). The two glycoalkaloids most frequently found in Solanaceous species are solasonine and solamargine (Osman, 1980). Other glycoalkaloids are notable for their presence in commercial crops, such as α-solanine and α-chaconine in potato, and tomatine in tomato (Milner *et al.*, 2011).

The ratios of these compounds can vary between tissues, cultivars and species. For example, the toxicity of glycoalkaloids in potato is much higher than in other *Solanum* species (e.g. tomato and eggplant) (Milner *et al.*, 2011). In tomato, glycoalkaloids are found in all parts of the plant but in eggplant they are concentrated in the fruit skin (Milner *et al.*, 2011).

Solanum mauritianum has been reported to contain steroidal glycoalkaloids (Gresshof (1890) as reported in Bell *et al.*, 1942). Gresshof (1890) reported that the green berries of the plant were rich in solanine (Bell *et al.*, 1942). Bell and Briggs (1942) identified compounds that agree with that of solanine – s (solanine) and its corresponding free base, solanidine – s (solasodine), and suggested that the same base could be united to different sugars, giving rise to different glycosidic alkaloids. This could be an explanation why Anderson and Briggs (1937) obtained aglycone with the same colour reactions as solasodine. They concluded that the aglycone was a dimorphic form of solasodine, but later research showed that the aglycone isolated from woolly nightshade and solasodine are neither identical nor dimorphic forms, but are isomeric and extremely closely related (Bell et al., 1942) and can be found in the fruits of woolly nightshade. Bell et al (1942) suggested the names solauricine and solauricidine for the glucoside and aglycone, respectively.

In more recent analysis, woolly nightshade has been shown to contain 0.5% solasodine in the leaves and 2.0 to 3.5% (dry weight) in the green fruits (Schreiber, 1968; Vieira, 1989). The concentration of solasodine in woolly nightshade is higher than average compared to other *Solanum* species (Drewes, 1993; Jayakumar and Murugan, 2016).

2.6.3. Trade-offs between growth and defence

A number of physiological trade-offs between growth rate, structural defences and secondary metabolism have been documented in both wild and crop plant species

(Hrutfiord and Gara, 1989; Lightfoot and Whitford, 1989; Margna *et al.*, 1989; Björkman and Anderson, 1990).

When plants are subjected to abiotic stress, there is a drop in plant growth, and as a result carbohydrates accumulate in excess of growth requirements (McDonald *et al.*, 1986; Wardlaw, 1990). This occurs because net photosynthesis, being not as sensitive to resource limitation (Daie and Patrick, 1988), allows carbohydrates to continue being produced as normal, but nutrient or water deficiency prevents the plant from being able to consume the carbohydrate for growth (Daie and Patrick, 1988; Körner, 1991). The carbon-nutrient balance (CNB) hypothesis suggests that the excess carbohydrates are then allocated to the production of secondary metabolites with little or no significant trade-off with growth (Charles *et al.*, 1990; Horner, 1990; Lerdau and Coley, 2002). Consequently, because of the production of secondary metabolites, these plants can be more resistant to herbivores than plants experiencing no environmental limitations upon growth (Herms and Mattson, 1992). When environmental conditions are favourable, vegetative growth generally receives resource priority over secondary metabolism and storage (Bazzaz *et al.*, 1987), thus, herbivore resistance declines (Bryant *et al.*, 1983; Waring and Pitman, 1985).

It has been suggested that there is a fraction of secondary metabolism that is genetically determined and occurs independent of resource availability (McDonald *et al.*, 1999). Thus, when conditions allow for carbohydrate surpluses, secondary metabolites increase above the minimum levels (McDonald *et al.*, 1999). The CNB hypothesis has been criticised because it fails to adequately predict defence differences between species and genotypes (Lerdau and Coley, 2002). However, limitations aside, the hypothesis provides a useful first step for explaining the plastic responses of a particular genotype to variation in resource supply (Lerdau and Coley, 2002).

2.7. Summary

The lace bug *Gargaphia decoris* was released in New Zealand as a biocontrol agent for the invasive weed woolly nightshade. The lace bugs released at Ngapeke, Bay of Plenty, displayed a differential pattern of distribution. Lace bugs seemed to establish better on woolly nightshade plants grown in the shade compared to the plants in full sunlight. Lace bugs are gregarious insects and some species display maternal care. Both behaviours are

key determinants for the successful establishment of the insect. *G. decoris* display both of these behaviours, increasing its suitability as a biocontrol agent.

However, factors other than intrinsic insect behaviour can influence the establishment of the insect. Abiotic stressors to which the host plant and the insect are subjected will have an impact on the insect performance and fitness, and therefore affect its establishment. Temperature, humidity, photoperiod and light intensity are some of the abiotic stressors that affect the establishment of herbivorous insects. The amount of light received by a plant can affect the insect directly or indirectly. Plants have multiple mechanisms to cope with abiotic stressors and resource allocation. Some of these mechanisms are the production of physical, mechanical or chemical barriers.

The presence of chemical barriers also needs considering since it is known that woolly nightshade contains the glycoalkaloid solasodine. Solasodine has been thoroughly studied not only in woolly nightshade but in other *Solanum* plants because of the presence of steroidal glycoalkaloids, relevant to the pharmaceutical industry. Addressing all these factors will contribute to a better understanding of the plant-insect relationships between a biocontrol agent and its preferred host, and provide useful information for pest management programs not only in New Zealand but in other parts of the world where woolly nightshade is classified as invasive.

CHAPTER 3: EFFECTS OF MATERNAL CARE AND AGGREGATION ON GROWTH AND SURVIVAL OF *GARGAPHIA DECORIS* (HEMIPTERA: TINGIDAE)

ABSTRACT. The lacebug *Gargaphia decoris* Drake has been reported to express maternal care and aggregation behaviours (Olckers, 2000; Guidoti *et al.*, 2015). To explore the effect of maternal care on life cycle duration and nymphal survival, an experiment was set up with two treatments: 1) nymphs with their mother and 2) nymphs without their mother. Subsequently another experiment was set up with four treatments, with ten nymphs and ten replicates, to test the effect of maternal care and handling with a fine brush on life cycle duration, nymphal survival and adult morphology.

To explore the effect of aggregation densities on each of the nymphal development stages, total nymphal development, nymphal survival and adult morphology, an experiment was set up with five treatments which consisted of different aggregation densities: 1, 5, 10, 20 and 30 nymphs which were transferred to a 0.75 L potted *S. mauritianum* plant with two mature leaves and placed on the underside of the biggest leaf.

Results show that there was no significant effect of the mother being present on the life cycle duration and nymphal survival when all of the nymphs were touched. When the effects of maternal care and of handling the newly emerged nymphs with a fine brush were tested separately, results showed higher nymphal survival when the mother was not present (87.74 ± 3.82%) than when the mother was present (57.5 ± 5.57%). Life cycle duration was shorter with the presence of the mother (23.22 ± 0.65 days) than without the mother (25.42 ± 0.85 days). Touching the nymphs significantly affected nymphal survival – touched nymphs had a lower survival (65.74 ± 5.57%) that those that were not touched (78.5 ± 5.04%). Nymphs that were touched had shorter life cycle duration (21.85 ± 0.42 days) than those that were not touched (26.79 ± 0.66 days). There was a significant overall effect of maternal care and handling the nymphs on the morphology of *G. decoris* adults (pseudo-$F_{3,36}$ = 1.98, $p < 0.05$). Significant differences in morphology were observed between the treatment "nymphs without mother, not touched" and the rest of the treatments (pseudo-$F_{1,36}$ = 2.81, $p < 0.05$). No significant differences were observed amongst the other three treatments. Adult pronotum width and antennae II length contributed most to the

observed separation between treatments. There was a significant positive relationship between antennae II length and the % of eggs that hatched and no relationship was found between pronotum width and adult weight, number of eggs laid and fertility. There was no effect of aggregation on survival and adult morphology of *G. decoris,* but significant effects were found in the development duration of the nymphal instars. Nymphs developed faster when they were single or in groups of 5 in most of the instars (except second instar). These results have increased our understanding of the maternal and aggregation behaviour of *G. decoris*.

3.1. Introduction

Maternal care is a behaviour that occurs post-fertilization and is directed toward offspring (Tallamy, 1986). It has been reported to increase offspring's survival, growth and lifetime reproductive success in insects (Klug *et al.*, 2012; Smiseth *et al.*, 2012). The most basic form of maternal care is limited to the physical protection of eggs from predators and parasites (Tallamy, 1984; Tallamy and Wood, 1986), but although rare, it can be extended after egg hatching by either staying with the newly hatched progeny in a fixed position or escorting them as they move around (Tallamy, 1984; Smiseth *et al.*, 2012).

Maternal care behaviour has been reported in lace bugs (Tingidae): *Leptobyrsa decora* Drake (Melksham, 1984; Loeb and Bell, 2006), *Compseuta picta* Schouteden (Tallamy and Iglay, 2004), *Corythucha hewitti* Drake (Faeth, 1989), and *Corythucha bulbosa* Osborn and Drake (Sheeley and Yonke, 1977). Within the genera *Gargaphia*, maternal care was reported in *G. tiliae* Walsh (Weiss, 1919; Tallamy, 1982; Tallamy and Horton, 1990; Monaco *et al.*, 1998), *G. iridescens* Champion (Tallamy, 1985) and *G. solani* Heidemann (Fink, 1915; Kearns and Yamamoto, 1981; Tallamy and Denno, 1981a; Tallamy, 1982; Tallamy and Horton, 1990; Monaco *et al.*, 1998). Recently, Guidoti *et al.* (2015) described for the first time maternal care behaviour in *G. decoris*.

G. decoris appears to have maternal care similar to what has been described in other *Gargaphia* species; when the egg batch was threatened by performing movements towards the egg batch using a needle, the female moved aggressively towards the needle and then fanned her wings (Guidoti *et al.*, 2015). Despite Guidoti *et al.* (2015) having described

maternal care, more experiments are needed to fully characterize the behaviour and its effects on *G. decoris*.

Maternal care behaviour in *G. decoris* is facilitated by the gregarious behaviour that adults and nymphs express (Olckers, 2000; Guidoti *et al.*, 2015). Gregarious behaviour is known to facilitate maternal care in other lace bugs as well (Kearns and Yamamoto, 1981; Faeth, 1989; Loeb and Bell, 2006). For example, in *G. solani* it was observed that the female went ahead of the moving nymphal aggregation to guide them to a new leaf and had several behavioural interactions that allow aggregated nymphs to understand her message (i.e. pointing the tip of her abdomen and fanning her wings) (Kearns and Yamamoto, 1981).

Besides facilitating maternal care, aggregation behaviour is known to increase development rate and survival in insects (Ghent, 1960; Breden and Wade, 1987; Matsumoto, 1989; Denno and Benrey, 1997). Among *Gargaphia* species, the effects of aggregation on survival have been reported in *G. solani* (Tallamy and Denno, 1981a), where solitary nymphs, despite reaching maturity, suffered significantly lower survivorship rates than nymphs in groups of 5 or 30 individuals. It was suggested that groups of nymphs are able to feed on a wider range of leaf types (i.e. young or old leaves) than solitary nymphs.

Currently, the effect of maternal care and nymphal aggregation on the development rate, survival and reproductive fitness of *G. decoris* is unknown. Therefore, the objectives of this study were to determine the effect of maternal care and aggregation on nymphal development rate, survival and reproductive performance of subsequent adults. I hypothesized that: (1) there would be an increase in nymphal development rate and survival with the presence of the mother; (2) there would be differences in the morphological parameters between the newly emerged adults developed in the presence of the mother and in the absence of the mother; (3) there would be an increase in developmental rate and survival with higher nymphal densities; (4) there would be differences in the morphological parameters of adults that developed alone and in nymphal aggregations of higher densities.

The results of this study will provide a better understanding of the maternal-offspring interactions and the benefits of maternal care and nymphal aggregation on *G. decoris* performance.

3.2. Materials and methods

3.2.1. General methodology

Prior to the beginning of the experiment, woolly nightshade *S. mauritianum* seeds were sown in a 60-cell growing tray (45 ml plug size) with Daltons Premium seed mix. When seedlings developed a strong root system and cotyledons reached a length of approximately 3 cm (10 days after germination), they were transplanted to 0.75 L pots using a soil mixture which included Woodace ® longterm fertilizer (18% nitrogen, 2.2 % phosphate, 8.3 % soluble potash and micronutrients). These potted woolly nightshade plants were kept in a glasshouse until two fully mature leaves had developed.

The insect colony was reared on woolly nightshade 5 L potted plants inside a controlled environment room at 20 ºC and 16L:8D (light: dark) hours. Fifth instar nymphs were then collected from the insect colony and placed on separate 5 L potted woolly nightshade plant under the same conditions as the colony. The newly emerged adults (1 day old) were sexed and males were placed on a separate potted plant from the females. Then, the pots were covered with white tulle fabric (40 openings/cm^2) to avoid mating prior the beginning of the experiment. Later, 15-day-old adults were collected, coupled and each couple was placed on 0.75 L potted woolly nightshade plants with two fully matured leaves. Each potted plant was covered with modified plastic containers using a fine metal mesh to allow ventilation. Three days later, daily observations were started to check for deposited eggs and to record the oviposition date.

3.2.2. Maternal care

To explore the effect of maternal care on life cycle duration (i.e. the number of days that it takes for the nymphs to develop from egg to adults) and total nymphal survival (i.e. the percentage of first instar nymphs that reach adulthood) an experiment was set up with two treatments: 1) nymphs with their mother and 2) nymphs without their mother. To set up this experiment, one female and a male were placed together on a 0.75 L potted woolly nightshade plant and left to mate. As soon as an egg batch was observed, males were removed and the eggs deposited by each *G. decoris* female were observed daily for egg hatch. After hatching, 10 newly emerged nymphs from each egg batch were collected with the aid of a soft fine brush and each group of 10 nymphs was placed on a fresh 0.75 L potted woolly nightshade plant either with the mother or without the mother. This procedure was repeated until 15 replicates per treatment have been completed. Subsequently, to determine the effect of handling the newly emerged nymphs with a fine brush on life cycle duration (i.e. days that it takes the nymphs to develop from egg to adult), nymphal instar survival (i.e. percentage of nymphs that survive within each development stage) and total nymphal survival (i.e. percentage of first instar nymphs that reach adulthood), another experiment was set up to determine both the effect of maternal care and handling the nymphs. For this, four treatments were set up: a) "nymphs with mother, touched" – 10 nymphs and their mother were transferred to a new 0.75 L potted *S. mauritianum* plant using a fine brush; b) "nymphs without mother, touched" – only 10 nymphs were transferred to a new plant using a fine brush; c) "nymphs with mother, not touched" – newly emerged 10 nymphs were kept with their mother and they were not transferred to a new plant; d) "nymphs without mother, not touched" – newly emerged 10 nymphs were not kept with their mother and they were not transferred to a new plant. For "not touched" treatments, the soft brush was only used to remove those nymphs, from each of the egg batches, that were not included in the experiment. Extra care was taken to avoid the bristles of the brush touching the experimental nymphs. Sometimes, the mother was on top of the newly emerged nymphs, thus when the treatment required the mother to be present, it was necessary to move the mother to the side to be able to remove the extra nymphs that were not included in the experiment. However, females were not moved far to avoid making it difficult for them to find their way back to their egg batch. The data on life

cycle duration, nymphal instar survival and total nymphal survival of *G. decoris* were obtained by recording the oviposition date and hatching date of the first 10 nymphs that emerged from each of the egg batches. After the nymphs were transferred to a *S. mauritianum* pot, daily observations were made to record nymphal survival every time nymphs moulted to a new instar; this was repeated until nymphs emerged to adults, when total nymphal survival and life cycle duration were recorded. Ten replicates per treatment were obtained.

The newly emerged adults were sexed and one male and one female from each replicate in each treatment were collected for morphological measurements. The morphometric measurements used in this experiment are shown in Fig. 3.1.

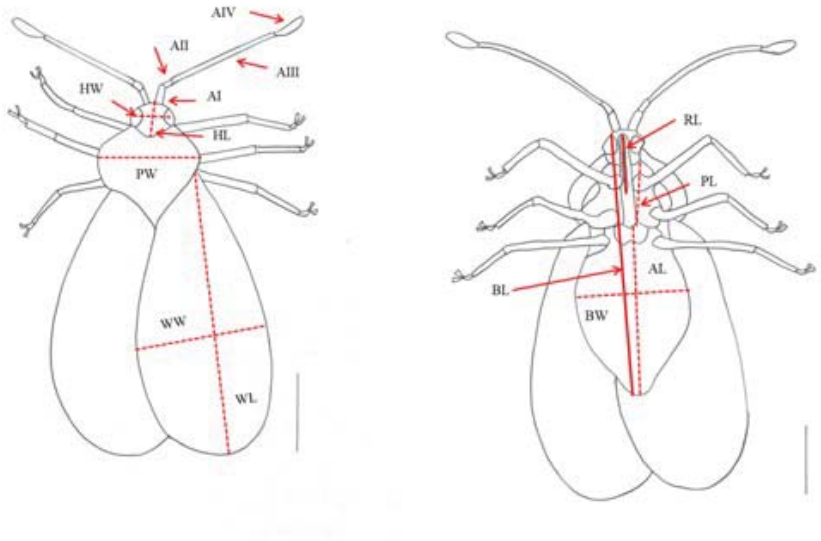

Figure 3.1. Dorsal view (left) and ventral view (right) of the female lace bug *Gargaphia decoris* adult. Lines represent morphometric measurements performed on the insect: AI – Antennae I, AII – Antennae II, AIII – Antennae III, AIV – Antennae IV, HW – Head Width, HL – Head Length, PW – Pronotum Width, PL – Pronotum Length, WL – Wing Length, WW – Wing Width, RL – Rostrum Length, BL – Body Length, AL – Abdomen Length, BW – Body Width. Scale bar represents 1 mm.

In the experiment described above, morphological measurements were performed on *G. decoris* adults, but no information about female weight and reproductive parameters (i.e. number of eggs oviposited and fertility) was collected. Therefore, to determine if morphometric measurements were correlated with the reproductive performance of the lace bug adult females, an additional experiment was done. A set of 20 females aged 15-days-old were weighed before placing each of them with a 15-day-old male to mate on a 0.75 L potted woolly nightshade plant. After the females oviposited, the eggs of the first egg batch were counted and later on, when the eggs hatched, the hatching day and the number of newly emerged nymphs was recorded. After nymphs emerged to adults, the female adults were collected to perform morphological measurements as described above.

3.2.3. Aggregation effects

The experiment was set up to determine the effect of aggregation densities on nymphal instar development duration (i.e. the number of days that each nymphal development stage takes), total nymphal development duration (i.e. days that it takes for the first instar nymphs to reach adulthood) and total nymphal survival (i.e. percentage of first instar nymphs that reach adulthood). The hatching date was recorded, and the newly emerged nymphs were allocated to the following aggregation treatments: 1, 5, 10, 20 and 30 nymphs. Mother and nymphs were transferred using a fine brush to a new 0.75 L potted woolly nightshade plants, ensuring that both mother and nymphs were close together when placed underneath the leaf. This procedure was repeated until ten replicates per treatment were obtained. The nymphs were observed daily to record nymphal development. When the nymphs moulted to adults, the total nymphal development duration and total nymphal survival were recorded. Newly emerged adults were sexed and their morphological measurements were performed as described in section 3.2.2.

3.2.4. Statistical analyses

To determine the effect of maternal care and handling of newly emerged nymphs with a fine brush on the life cycle duration, nymphal instar survival and total nymphal survival of *G. decoris* nymphs, data were analysed using one and two-way Analyses of Variance (ANOVA) on untransformed or transformed data (square root and log transformations) All data were tested for normality using a qqplot and Shapiro-Wilk's test. This was followed by post-hoc Tukey's Honest Significant Difference (HSD) test to locate pairwise differences ($\alpha = 0.05$) when overall ANOVA tests were significant. If the assumptions of normality were violated, data was analysed using Kruskall-Wallis test with a chi-square approximation to determine the p-values and significance level. To determine the effect of maternal care and handling on morphological parameters of *G. decoris* adults, data was analysed using Permutational Multivariate Analysis of Variance (PERMANOVA). A Linear Discriminant Analysis (LDA) was performed on the morphological measurements using the software R packages MASS (Venables and Ripley, 2002) and ggplot2 (Wickham, 2009) to detect which morphological parameters contribute most to the separation across treatments. Linear regression analyses were performed to explore relationships between morphological measurements, weight, and reproductive parameters of lace bug adult females.

The effect of aggregation densities on nymphal instar development duration, total nymphal development duration, and total nymphal survival was analysed using one-way ANOVA. This was followed by the post-hoc Tukey's Honest Significant Difference (HSD) test ($\alpha = 0.05$) when overall ANOVA tests were significant. To explore the effect of aggregation densities on morphological parameters measured on *G. decoris* adults, data were analysed using PERMANOVA. A Linear Discriminant Analysis was not performed on the morphological measurements, since PERMANOVA showed no significant treatment effect.

All data were tested for normality using a qqplot and Shapiro-Wilk's test. If the assumptions were violated, data were analysed using a Kruskall-Wallis test followed by the post-hoc Dunn's test for means comparison. All statistical analyses were performed using the software R (R Core Team, 2002) and Primer 7 for PERMANOVA (Clarke and Gorley, 2015).

3.3. Results

3.3.1. Maternal care

The results indicate that there was no significant effect of maternal care on life cycle duration ($F_{1,27} = 2.56$, $p = 0.121$) and total survival ($H = 1.85$, $p = 0.174$) when all nymphs were touched. When the effects of maternal care and of handling the newly emerged nymphs were explored separately in a new experiment, results indicated a small but significant increase in the duration of the life cycle when nymphs developed without the mother (25.42 ± 0.85 days) compared to when they developed with the mother (23.22 ± 0.65 days) ($F_{1,36} = 10.29$, $p < 0.01$) (Fig. 3.2). In addition, there was a significant decrease in the life cycle duration when the nymphs were "touched" (21.85 ± 0.42 days) compared to when the nymphs were "not touched" (26.79 ± 0.66 days) ($F_{1,36} = 51.71$, $p < 0.01$) (Fig. 3.2). There was no significant interaction effect between maternal care and "touching" the nymphs on life cycle duration ($F_{1,36} = 3.02$, $p = 0.091$).

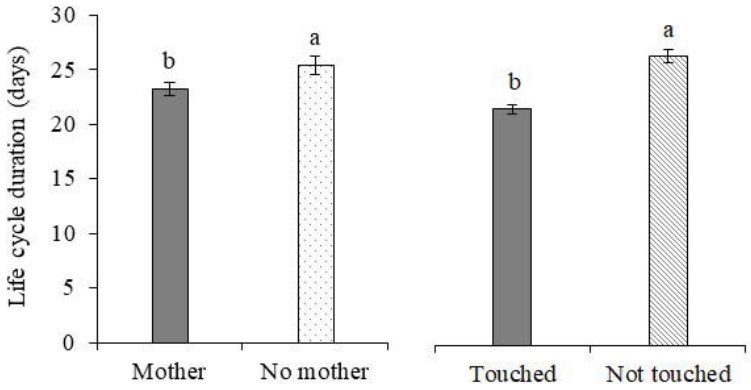

Figure 3.2. Effect of maternal care (left) and "touching" the nymphs with a fine brush (right) on *Gargaphia decoris* life cycle duration (days). Error bars represent standard errors. Means with the same letter indicate no significant difference between treatments (two-way ANOVA, $\alpha = 0.05$).

Total nymphal survival (i.e. percentage of the first instar nymphs that reach adulthood) was significantly higher in nymphs that developed without the mother (86.7 ± 3.8%) compared with the nymphs that developed in the presence of the mother (57.5 ± 5.6%) ($F_{1,36}$ = 21.04, p < 0.01) (Fig. 3.3). Nymphs that were not touched had higher survival (78.5 ± 5.0%) than nymphs that were touched (65.7 ± 5.6%) (Fig. 3.3); the effect of touching on total survival approached statistical significance ($F_{1,36}$ = 4.01, p = 0.053). There was no significant interaction between maternal care and "touching" the nymphs on nymphal survival ($F_{1,36}$ = 2.58, p = 0.117).

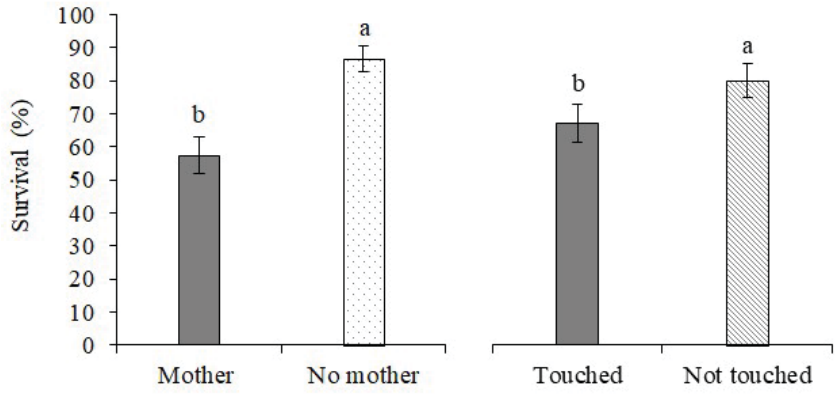

Figure 3.3. Effect of maternal care (left) and "touching" the nymphs with a fine brush (right) on *Gargaphia decoris* nymphal survival (%). Error bars represent standard errors. Mean with the same letter indicate no significant difference between treatments (two-way ANOVA, α = 0.05).

Maternal care did not significantly affect the survival of the first (H = 0.79, p = 0.373), second (H = 0.58, p = 0.445), third (H = 0.11, p = 0.742), fourth (H = 1.74, p = 0.188) and fifth instars (H = 2.35, p = 0.125). Touching the nymphs significantly decreased the survival of the first instar (H = 14.06, p < 0.01), but the survival of the second (H = 0.05, p = 0.820), third (H= 0.11, p = 0.742), fourth (H = 1.74, p = 0.188) and fifth instars (H = 2.35, p = 0.125) was unaffected (Fig. 3.4).

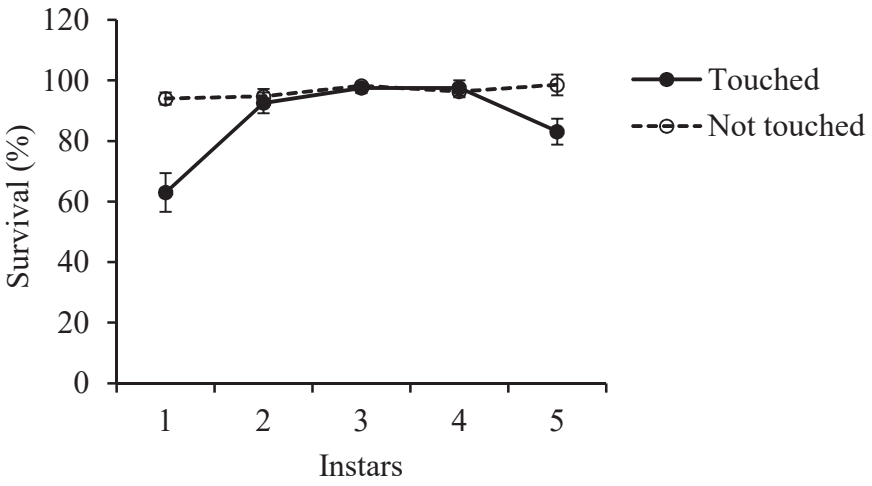

Figure 3.4. Mean survival (%) of *Gargaphia decoris* nymphs across the nymphal instars when they were "touched" or "not touched" with a fine brush. Error bars represent standard errors.

There was a significant overall effect of maternal care and handling on the morphology of *G. decoris* adults (PERMANOVA pseudo-$F_{3,36}$ = 1.98, $p < 0.05$). Significant differences were observed between the treatment "nymphs without mother, not touched" and the rest of the treatments (pseudo-$F_{1,36}$ = 2.81, $p < 0.05$), while no significant differences were observed among other three treatments. Sex of the adult bug had a significant effect on body measurements, as expected (pseudo-$F_{1,36}$ = 2.95, $p < 0.05$). There was no significant interaction between sex and the treatments. In a linear discriminant analysis (LDA) (Fig. 3.5), most of the variation between treatments was due to the pronotum width and antennae II followed by the pronotum length. The antennae II were larger and pronotum had less width and more length in adults that were "not touched" as nymphs. "Touched" nymphs as adults had wider and shorter pronotum and shorter antennae II.

After knowing that antennae II and pronotum width contributed the most to the variation between treatments in the maternal care and handling experiment, these morphological parameters were then selected from the set of morphological parameters measured on 20 females to explore the relationship between morphological parameters and female weight, number of eggs and fertility, in order to relate the changes in morphology

with the reproductive performance of *G. decoris*. The results showed no significant relationship between antennae II vs. weight (R^2 = -0.04, p = 0.567) and number of eggs (R^2 = -0.05, p = 0.776), but showed a significant relationship with % eggs that hatched (R^2 = 0.355, $p < 0.01$). There was no significant relationship between pronotum length vs. weight (R^2 = -0.003, p = 0.347), number of eggs (R^2 = -0.05, p = 0.832) and % eggs that hatched (R^2 = 0.13, p = 0.07).

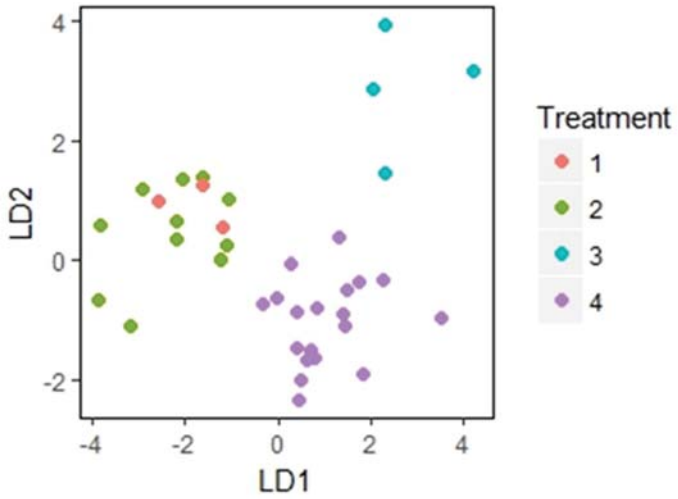

Figure 3.5. Linear discriminant analysis (LDA) (LD1-59.67%, LD2-22.60%) displaying the grouping of morphological parameters measured on *Gargaphia decoris* females in maternal care experiment. Treatments: 1 – nymphs with mother, touched, 2 – nymphs without mother, touched, 3 – nymphs with mother, not touched, 4 – nymphs without mother, not touched. The variables that contributed most toward the separation of treatments along LD1 were Pronotum Width (negative) and Antennae II Length (positive).

3.3.2. Aggregation effects

Aggregation did not significantly affect nymphal survival (H = 4.48, p = 0.345) but it did affect the development duration of the nymphal instars. The first instar nymphs had the shortest development duration when they were aggregated in groups of five; the development of third, fourth and fifth instars took longer when nymphs were aggregated in groups ≥ 10 (Table 3.1). The total developmental duration was significantly different

amongst treatments, being significantly longer when nymphs were in groups of 30 compared with 1, 5, 10, and 20 nymphs (Table 3.1).

The effect of aggregation densities on morphological measurements of *G. decoris* adults was tested and no significant effect was found (PERMANOVA pseudo-$F_{4,69} = 1.47$, $p = 0.187$). The treatment "1 nymph" was compared to the other aggregation densities and no significant differences were found (pseudo-$F_{4,69} = 0.44$, $p = 0.660$). Sex had a significant effect on the morphometric measurements of adults, as expected (pseudo-$F_{1,69} = 33.55$, $p < 0.01$).

3.4. Discussion

3.4.1. Maternal care

Maternal care occurs when the mother increases the survival and growth of her offspring (Smiseth *et al.*, 2012) and has been considered one of the most important influences on offspring phenotype (Marshall and Uller, 2007). In this study, the effect of maternal care on *Gargaphia decoris* nymphal survival, development and adult phenotype has been explored. Results showed that maternal care significantly decreased the total nymphal survival (i.e. percentage of nymphs that reached adulthood) and life cycle duration (i.e. the number of days it took the nymphs to develop from egg to adult). This result was unexpected, because it was expected that maternal care would either increase or not have a significant effect on nymphal survival (Tallamy and Denno, 1981a). The lower total survival observed when the mother was present could be a result of the mother trying to search for better food quality. Lace bugs feed on the chloroplasts, located inside the parenchyma cells of leaves. Under a constrained amount of leaves on which the female and nymphs can feed, is possible that chloroplasts were exhausted during the month that it typically takes the nymphs to reach adulthood (Olckers, 2000). The nymphal stage is where most of the feeding takes place, and where more damage is inflicted to the plant (Olckers, 2000).

Table 3.1. Average nymphal instar development duration (days) and total nymphal development duration (days) of *Gargaphia decoris* nymphs at different aggregation densities. Pairwise differences were obtained using the post-hoc Dunn's test with an alpha level of 0.05. Significant differences between aggregations densities (within each column) are represented by different letters. Data were analyzed with one-way ANOVA or Kruskall-Wallis test. Data analyzed with Kruskall-Wallis test are identified with an asterisk (*).

Aggregation densities	Instars					
	First	Second	Third	Fourth	Fifth	Total all nymphal stages
1	4.67 ± 0.25 a	3.54 ± 0.24 a	3.17 ± 0.11 c	4.18 ± 0.12 b	7.18 ± 0.12 b	21.56 ± 0.26 b
5	3.00 ± 0.00 b	4.00 ± 0.29 a	3.56 ± 0.37 bc	4.00 ± 0.29 b	7.00 ± 0.29 c	22.71 ± 0.42 b
10	5.00 ± 0.21 a	3.80 ± 0.36 a	4.05 ± 0.35 abc	5.15 ± 0.37 a	8.50 ± 0.37 a	21.5 ± 0.64 b
20	4.78 ± 0.15 a	3.38 ± 0.32 a	4.94 ± 0.48 a	5.06 ± 0.33 a	9.05 ± 0.36 a	22.05 ± 0.78 b
30	4.80 ± 0.20 a	3.30 ± 0.30 a	4.70 ± 0.32 ab	5.27 ± 0.38 a	9.64 ± 0.29 a	27.71 ± 0.58 a
$F_{4,46}/H_4^2$	25.33 *	0.94	5.45	14.61 *	16.59	21.85
P	< 0.01	0.447	< 0.01	< 0.01	< 0.01	< 0.01

It has been reported that *G. solani* nymphs feeding in groups move to new feeding sites after approximately an hour, when 75% of the leaf has been exhausted (Kearns and Yamamoto, 1981). These feeding movements guided by the mother have been reported not only in *G. solani* but in *Corythucha hewitti,* where the female seemed to initiate the movements of nymphs, but mainly during early instars (Kearns and Yamamoto, 1981; Faeth, 1989). However, these observations were made on lace bug aggregations with mixed nymphal stages and higher number of nymphs aggregated as opposed to my study, where aggregations were maintained with ≤ 10 nymphs of the same instar. Thus, in my study the 75% of the leaf would be exhausted after a longer period than that reported by Kearns and Yamamoto (1981). Nevertheless, in my study, after most of the chloroplasts of the leaves of *S. mauritianum* were exhausted, it could be expected that the mother would attempt to guide her progeny to a new feeding site. This nymphal movement would have been unsuccessful because of the inability of the female and nymphs to move to another plant when the quality of the current feeding site deteriorates. During these constant unsuccessful movements in search for a new feeding site, it is possible that some nymphs failed to keep up with the aggregation. This could have negative repercussions for the well-being of the nymphs because it has been observed that the nymphs that were left behind don't settle down until they find the aggregation (Kearns and Yamamoto, 1981). Therefore, during these feeding movements some *G. decoris* nymphs could have wandered around the plant searching for sufficient physical contact with other nymphs and not feeding appropriately or acquiring enough water and nutrients for their development. As a result, these nymphs die of desiccation or starvation. This could help to explain the negative effect of the mother on nymphal survival and development in my experimental setup.

There are many reports of maternal care in lace bugs, and the way it is expressed varies across species (Sheeley and Yonke, 1977; Tallamy and Denno, 1981a; Faeth, 1989). Maternal care behaviour has been extensively studied in the eggplant lace bug *Gargaphia solani* Heidemann (Kearns and Yamamoto, 1981; Tallamy and Denno, 1981a; Tallamy and Denno, 1981b; Tallamy, 1982; Tallamy, 1985; Monaco *et al.*, 1998). The *G. solani* mother interacts with her progeny (nymphs) by directing them to new leaves and uses its long antennae to keep the nymphs together (Tallamy and Denno, 1981a). Fink (1915) and

Kearns and Yamamoto (1981) have reported that when a potential predator (i.e. ladybirds, anthocorids and ants) came close to an aggregation of feeding nymphs, the *G. solani* female, with slightly raised wings, chased away the intruder. A study performed by (Tallamy and Denno, 1981a), showed that in the absence of predators, nymphal survival was not significantly affected when nymphs where raised without their mother. But when nymphs were left alone under normal field conditions, only 3% survived to adulthood. In my study, the effect of predators on maternal care and its effects on nymphal survival were not tested but it is possible that under natural conditions, with no constraints in food resources (i.e. plants), maternal care would show benefits to nymphal survival, due to the mother providing protection from predators. In a study performed by Hardin and Tallamy (1992), maternal care effects were compared between the lace bugs *G. solani* and *G. tiliae.* Results showed that *G. solani* displayed a more aggressive defensive behaviour than *G. tiliae* and this was attributed to the higher amount of predators found in horsenettle habitats (where *G. solani* feeds) compared to basswood habitats (where *G. tiliae* feeds). These results suggest that maternal defensive behaviour, being an ecologically costly behaviour, will be developed proportionally to the intensity of predation (Hardin and Tallamy, 1992).

The handling of *G. decoris* nymphs using a fine brush significantly decreased the percentage of nymphs that reached adulthood, as well as reducing development duration. This has not been reported in other studies that involved handling lace bug nymphs with fine brushes (Rogers, 1977; Tallamy and Denno, 1981a). First instar nymphs could have been more susceptible to handling, possibly because at this stage *G. decoris* has not developed a strong integument and spines compared to later nymphal stages (Olckers, 2000), which could protect them from the bristles of the brush. However, handling immature stages with a fine brush is a common practice and this method has been reported not only for lace bugs (Rogers, 1977; Tallamy and Denno, 1981a) but for other insect species (Morales-Ramos *et al.*, 2014).

Even if the newly emerged nymphs were injured by the brush bristles, the negative impact could have been outweighed by the positive effects of transferring the newly emerged nymphs to a fresh plant, where there were more chloroplasts available. In my experiment, those nymphs which were “not touched” were kept on the same plant on which their mother oviposited, throughout their life cycle. Even though these nymphs were not

exposed to handling with a brush and their aggregative formation was not disrupted, the plant quality could have been inferior compared to the new plants on which the "touched" nymphs were transferred. This could explain why nymphs that were "touched" developed faster than those that were "not touched.

Experimental treatment of nymphs affected *G. decoris* morphological parameters. Changes in the morphology of the insect can have an impact on adult fitness – for example, in a study performed by Mappes and Kaitala (1994) with the parent bug *Elasmusha grisea* L. (Acanthasomatidae), results showed that the body size and shape are key determinants of maternal success. Mothers adjust their egg batch sizes according to the surface area a female can cover with its body, and therefore defend. Small females that were experimentally provided with an enlarged egg batch lost all the additional eggs. There was an evidence that *E. grisea* lays the largest eggs in the central, safest part of the egg mass. However, Mappes and Kaitala (1994) suggest that other ecological factors should be examined in detail to determine their effect on the evolution of the female body size. In my study, pronotum width and antennae II were the morphological parameters that showed the most variation between treatments. These changes in morphology can have implications in the expression of maternal care in lace bugs. For example, the lace bug *G. solani* mother relies on the size of her body and wings to increase its offensive behaviour against potential predators (Tallamy and Denno, 1981a).

In the work described in this chapter, "touching" the nymphs affected the morphological parameters of *G. decoris*. Nymphs that were not touched resulted in adults with longer but less wide pronotum and longer antennae II. The antennae in lace bugs may be important for guarding behaviour. In a study performed by Parr et al. (2002) in *G. solani*, removing the antennae of adults disrupted the communication with nymphs, terminating maternal care and inducing a new cycle of egg production. However, this disruption was only observed when all antennal segments were removed. Adults with few segments were able to guard their young normally. Therefore, there is no evidence that "touched" female *G. decoris* nymphs emerging as adults with smaller antennae will be unable to perform their maternal care duties.

In some insects, body size and weight are good indicators of potential fecundity, survival (e.g. the stinkbug *Podisus rostralis* Stäl (Zanuncio *et al.*, 2002), the speckled wood

butterfly *Pararge aegeria* L. (Berger *et al.*, 2008) and the diamondback moth *Plutella xylostella* L. (Nethononda *et al.*, 2015)) and mating success (Krishna and Hegde, 2003).

In my study, a positive significant relationship was found only between antennae II length and % eggs that hatched. Due to the time constraints of a PhD, it was not possible to test whether changes in adult morphology due to nymphs being "touched" could have long-term effects on the effectiveness of females in expressing maternal care, i.e. during the second generation, or on their mating success and the fitness of their offspring; this could be worth exploring in the future.

As mentioned earlier, the nymphs that were touched were feeding on fresh plant material and developed faster compared to those nymphs that were not touched. There are studies that show that an increased investment in larval development could alter resource allocation (Nijhout and Emlen, 1998; Stevens *et al.*, 1999). For example, in caddis flies *Odontocerum albicorne* Scopoli (Trichoptera: Odontoceridae) the increased resources allocated for protection during immature stages resulted in a reduction in the size of the thorax and wings in adults (Stevens *et al.*, 1999). In my study, it is possible that the presence of fresh resources served as a cue to the nymphs to allocate more energy to develop faster to take advantage of the fresh resources while they are available. For example, in the oak leaf roller moth *Tortrix viridiana* L. (Lepidoptera; Tortricidae) developmental synchrony to host plant is important for the successful development of the larvae (Ivaslov *et al.*, 2002). Similarly, in the lace bug *G. tiliae*, leaf age and timing of adult eclosion determines the reproductive future of the females. If the lace bugs reach sexual maturity prior to leaves losing their nutritional quality, they will be able to enter a reproduction phase immediately, unlike those females that mature after the foliage has begun to deteriorate, which delays reproduction (Hardin and Tallamy, 1992). These trade-offs are also observed in lace bug reproduction (e.g. *G. solani*): when there are scarce resources, female allocate their energy to guarding their existing eggs, but when there are enough resources, there are more females that "dump" their eggs so they can spend their energy to mate and produce more eggs (Hardin and Tallamy, 1992).

3.4.2. Aggregation effects

Aggregation has been reported to increase the survival and growth of certain insects, including lace bugs (Ghent, 1960; Kearns and Yamamoto, 1981; Breden and Wade, 1987; Faeth, 1989; Matsumoto, 1989; Denno and Benrey, 1997; Olckers, 2000; Loeb and Bell, 2006). In my study, the survival of *G. decoris* was not statistically different between aggregation densities, but the mean nymphal survival was lower when the nymph was solitary (35.0 ± 10.9%) vs. at high aggregation densities i.e. 30 nymphs (38.8 ± 6.4%). Higher survival at higher aggregation densities has been reported in the lace bug *G. solani* (Tallamy and Denno, 1981a). Aggregation behaviour has evolved in insects to sidestep physical and chemical plant defences (Rathcke and Poole, 1974; Fitzgerald, 1976; Tallamy and Denno, 1981a). For example, in the aphid *Aphis fabae* Scopoli and in the milkweed bug *Oncopeltus fasciatus* Dallas, communal feeding is advantageous because there is an increase in ingestion rates through a more economical use of their saliva (Dixon and Wratten, 1971; Bongers and Eggerman, 1971). These insects inject saliva into the plants they feed upon (Forrest and Noordink, 1971), and the saliva is known to contain many substances which affect the metabolism of plants (Schäller, 1968), improving the quality of the food available. In other insect species, gregariousness is an adaptation to overcome plant trichomes (Rathcke and Poole, 1974).

In the work described in this chapter, it was unexpected that solitary nymphs and nymphs in higher density aggregations (30 nymphs) had similar survival rates. However, it is possible that nymphs aggregated in groups of ≥ 10 could have experienced a shortage of plant resources. *G. decoris* nymphs that were solitary were feeding on the same amount of plant resources as nymphs that were aggregated in higher numbers. Nonetheless, under natural conditions, when there is no competition amongst individuals due to a higher amount of plant resources, aggregation could have positive effects through feeding facilitation when nymphs are in larger groups, rather than solitary (Tallamy and Denno, 1981a). Also, when exposed to predators, the chances of survival could be higher when larger numbers of nymphs are aggregated by reducing the individual probability of predation amongst the group members (Vulinec, 1990).

I observed that third, fourth and fifth instar nymphs had a longer development duration when they were aggregated in numbers >10. It is possible that by the time nymphs

reached these instars, leaves of the food plant had lost most of their nutritional quality and nymphs had to feed for longer periods of time to acquire the nutrients necessary for them to be able to moult to the next developmental stage. In another insect species, Eastern forktail *Ischunura verticalis* Say (Odonata: Coenagrionidae) the larvae exposed to temporary food shortage suffered from a reduced growth rate compared to those that were fed without limitations (Dmitriew and Rowe, 2005). In my study, nymphs that were isolated or in groups of 5, had proportionally more resources available and were able to reach maturity without facing a loss in plant quality. Longer developmental duration could be beneficial or detrimental for the insect. In the field, it is possible that spending more time in each developmental stage can expose the nymphs to predators for a longer time, thus affecting their survival (Price *et al.*, 1980; Benrey and Denno, 1997).

The results presented in this chapter are a step forward toward understanding maternal care and aggregation behaviour in the biological control agent *G. decoris,* and I speculate that these behaviours might not have been fully expressed under constrained plant resources and controlled conditions (no predation pressure). These behaviours are inherent in these insects and the expression and benefits of these behaviours might be fully displayed when females and nymphs are exposed to natural conditions.

3.5. Concluding remarks

Maternal care had a negative effect on the development and survival of nymphs and morphology of *G. decoris* adults. I speculate that the mothers' guiding the nymphs in search of fresh resources under a constrained amount of food indirectly affected the nymphal development and survival. In natural conditions, where lace bugs have more plant resources, and are exposed to predators, it is possible that maternal care will increase nymphal survival, as reported in another study with lace bugs (Tallamy and Denno, 1981a). Handling newly emerged nymphs with a soft brush positively affected nymphal development and survival, and affected adult morphology. I suggest that this reflects not the direct effect of handling, but the effect of having been transferred to a new feeding plant, while the un-handled nymphs were not transferred. However, I recommend that nymphal handling should be made after the first instar, when nymphs have developed stronger integument and spines have grown bigger. The pronotum width and antennae II

were the morphological parameters that most contributed to the differences among treatments in maternal care experiment. A positive significant relationship was found only between antennae II and % of eggs that hatched. However, due to time constraints, it was not possible to know if the morphological changes observed in first generation females (F_1) will have an effect on maternal care behaviour, mating success or fertility of second generation females (F_2).

Higher aggregation densities did not increase *G. decoris* survival and had no effect on the morphology of the adult insects, but it did affect developmental duration. The third, fourth and fifth nymphal instars took longer to develop when nymphs were aggregated in numbers > 10. This increase in developmental duration could be a result of spending more time eating to compensate for the loss of nutritional quality of the plant and to be able to molt to the next developmental stage (Dmitriew and Rowe, 2005). Solitary nymphs had similar survival to aggregations of 30 nymphs. Similar survival between solitary and groups of 30 nymphs could be a result of a higher feeding rate in higher aggregations, as opposed to lower amounts of plant resources (i.e. leaves) on which these nymphs could feed compared to solitary nymphs. Results of this study are a step further toward understanding maternal care and aggregation behaviour in *G. decoris* and opens new questions to further explore and help characterize these behaviours.

CHAPTER 4: EFFECT OF ENVIRONMENT ON LIFE HISTORY AND POTENTIAL DISTRIBUTION OF *Gargaphia decoris*

ABSTRACT. The lace bug *Gargaphia decoris* Drake is a biological control agent released in New Zealand to control the invasive weed *Solanum mauritianum*, commonly known as woolly nightshade. The aim of this study was to determine the effects of temperature, photoperiod and humidity on nymphal development, survival, longevity, fecundity and population growth of *G. decoris* reared on potted woolly nightshade plants. Insects were grown under four different temperatures (15, 20, 25 and 27.5 ºC), three constant photoperiods (8L:16D, 14L:10D and 16L:8D) and two relative humidity (RH) levels (50 and 70 ± 10%). The climate modelling software CLIMEX (Hearne Scientific Software Pty Ltd, Australia) was used to predict the potential distribution of *G. decoris* both in New Zealand and worldwide.

There was a significant effect of temperature on *G. decoris* reproductive parameters with the mean total number of eggs laid per female and egg clutches significantly higher at 25 °C than at 15 ºC and 27.5 °C. The mean number of females that oviposited and the number of eggs in the first clutch were significantly higher at temperatures above 15 °C. No significant differences were found in the mean number of egg clutches and percentage of egg hatch between the temperatures tested. These changes in fecundity were reflected in life table parameters. Life cycle duration was longest at 15 ºC (71.73 days) and shortest at 27.5 ºC (24.29 days). The fifth instar was the longest nymphal stage at all temperatures, with development time ranging from 5.40 days (27.5 ºC) to 15.39 days (15 ºC). The eggs and fifth instar nymphs were found to have the higher thermal requirements (119DD and 93.46DD, respectively). Both females and males survived longer at lower temperatures (68.95 and 41.94 days, respectively) compared to higher temperatures (44.4 and 30.62 days, respectively). Females survived longer than males at all temperatures except at 20 ºC. Photoperiod affected the percentage of egg hatch and total survival. The mean percentage of egg hatch was significantly higher at 8L:16D and 16L:8D, and the total nymphal survival was significantly higher at 16L:8D. Female and males survived the longest at 16L:8D (75.79 and 76.26 days, respectively) and died sooner at 14L:10D photoperiod than

at any other photoperiod tested. Life table parameters were affected by photoperiod were R_o, r_m and λ, were higher at 16L:8D, and T and DT which were both lower at 16L:8D photoperiod. The effect of RH was different according to the parameter being measured. Thus, whereas mean percent of egg hatch was significantly higher at 50 ± 10% RH than at 70 ± 10% RH, adult survival was significantly longer at 70 ± 10%. The Ro, T, DT were significantly higher at 70 ± 10% RH, whereas the r_m and λ were significantly higher at 50 ± 10% RH.

Predictions from the CLIMEX model coincided with the known distribution of *G. decoris* and woolly nightshade in Brazil, Argentina, South Africa and New Zealand. The model also predicted a possible expansion of *G. decoris* to tropical and Mediterranean regions. In New Zealand, the model predicted that the lace bug will be able to populate most of the North Island and the districts of Malborough, Buller, Grey, Hurunui, Waimakariri and north of Westland in the South Island, provided *S. mauritianum* is present. The results from this study will contribute to a better understanding of the climatic requirements and susceptibility of *G. decoris* to environmental conditions, and the likelihood of dispersal of the lace bug into new areas where the host *Solanum mauritianum* is present.

4.1. INTRODUCTION

Biological control (biocontrol) of weeds is a method which aims to reduce and control invasive species using host-specific natural enemies (Culliney, 2005). The classical biological control programs frequently involve the importation and release of species to emulate the ecological interactions found between the natural enemies and their hosts in their native environments (McFadyen, 1998; Bellows, 2001; Hope and Olckers, 2011). Although biocontrol is one of the most cost-effective practices for the management of invasive species when it is successful (Culliney, 2005), it has some constraints. One of these is the environment into which the biological control agent is to be released. The environment (e.g. temperature, photopheriod and humidity) influences the insect life history (development, fecundity and longevity), hence affecting its establishment and limiting its potential distribution (Kriticos *et al.*, 2012). The Brazilian lace bug *Gargaphia decoris* is a biological agent that has been released in New Zealand to control the invasive

weed woolly nightshade. Despite the importance of environmental parameters to insect survival and establishment, studies of the effect of the environment on *G. decoris* life history traits are scarce. Olckers (2000) tested the performance of this insect under a limited range of temperatures (22 ± 3 °C), photoperiods (14L:10D) and humidity (70-80% RH. Although Olckers (2000) information is useful, is not sufficient to perform life history and population dynamic studies that would allow us to determine temperature thresholds and to predict population outbreaks over the seasons.

The native range of *G. decoris* is the southern states of Brazil (Hope and Olckers, 2011; Pedrosa-Macedo, personal communication, 2017) and Misiones Province of Argentina, the regions classified according to the Köppen-Geiger climate classification as subtropical highland and humid subtropical climates, respectively (Alvarez *et al.*, 2013). However, *G. decoris* is now established in the regions of Eastern Cape, Kwazulu-Natal, Mpumalanga, Pretoria and Limpopo of South Africa and Swaziland (Henderson, 2001 cited in Cowie, 2016; T. Olckers, D. Strydom and D. Muir, personal communication, 2017). The reported establishment of *G. decoris* in South Africa and Swaziland indicates that the lace bug has the potential to establish itself in other areas (i.e. semiarid regions) that differ from their native range. In New Zealand, the lace bug has been released and is known to be established in the northern regions of the North Island (i.e. Bay of Plenty, Auckland and Bay of Islands) which have climates similar to the native range of *G. decoris* (pers. communication, Paynter, 2017)

CLIMEX (Hearne Scientific Software Pty Ltd, Australia) has been used to undertake risk assessments for arthropod pests, weeds, and diseases (Kriticos *et al.*, 2003; Yonow *et al.*, 2004; Saavedra *et al.*, 2015). Using climate as a factor that limits the potential geographical distribution of a species, CLIMEX estimates the level of similarity between the native ranges or known established areas of a given species (i.e. a pest or a biocontrol agent) and predicts the likelihood of establishment of this species into new areas by mapping its potential global distribution (Sutherst *et al.*, 2007; Kriticos *et al.*, 2015). To date, no model of potential global distribution of *G. decoris* has been conducted.

The main objectives of this chapter are: (1) to investigate the effects of temperature, photoperiod and humidity on *G. decoris* development duration, survival, fecundity and longevity; (2) develop a CLIMEX model that predicts its potential geographic distribution

and likelihood of establishment into new areas, not only in New Zealand but also on a worldwide scale.

Environment-dependent development studies like the one presented here are important to understand the dynamics of insects' populations and establishment patterns. Such studies help to enhance the efficiency of insect mass rearing in the case of beneficial insects such as biocontrol agents, and provide a quantitative basis for predicting insect development and activity in the case of damaging insects, both of which will be useful in the development of effective pest management programs.

4.2. MATERIALS AND METHODS

4.2.1. Colony initiation and maintenance

The *G. decoris* colony used in this study was derived in 2013 from a glasshouse-colony based at Landcare Research, Auckland, NZ. The colony was maintained in a bioassay room at Massey University, Palmerston North, NZ. Additional *G. decoris* adults were collected from Ngakepe (Bay of Plenty district, NZ) and added to the colony to introduce fresh genetic stock. Insects were maintained on potted *Solanum mauritianum* plants (in 0.75L pots) at 25 °C with a 14:10 (L:D) hours and 50 ± 10% RH. Plants were watered every other day and replaced once a month or sooner if considerable feeding damage was observed on the plants.

4.2.2. Plant material

S. mauritianum seedlings growing in the wild were collected from Atawhai Rd, Palmerston North, NZ and reared in a glasshouse (Massey University, Plant Growth Unit (PGU), Palmerston North, NZ). Fruits were collected and seeds were removed, washed, dried and sown in Daltons^{TM} Premium seed mix in a seed tray (60 cells x 45 ml). When seedlings developed a strong rooting system they were transplanted to 0.75L pots and after a week they were thinned to allow only one plant per pot. Plants were maintained under daily average minimum and maximum temperatures of 17.1 ºC and 24.05 °C, respectively, and were watered twice a day during summer and once a day in winter. Plants that reached a height of approximately 15 cm from the top of the pot were used for the experimental work.

4.2.3. Environmental parameters: temperature, photoperiod and humidity

4.2.3.1. Temperature

G. decoris development, from egg to adult, was monitored at constant temperatures of 10, 15, 20, 25, 27.5 and 30 ± 1 ºC, with a photoperiod of 14L:10D (light:dark) hours and 50 ± 10% relative humidity (%RH) in environmental chambers (Sanyo MLR-350T; Japan) and bioassay rooms. Eggs from a different set of parents were collected from the colony by cutting a portion of the leaf where the egg batch was located. The leaf pieces were then placed in plastic Petri dishes (8.5 cm diameter) lined with moist paper towel and placed for incubation inside controlled environment rooms at their respective treatment temperatures. Each egg batch was considered the sampling unit with ten pseudo-replications.

The eggs were checked daily and the number of nymphs emerging each day was recorded until no nymphs emerged for more than two consecutive days. Eggs kept at 10 °C failed to hatch, and nymphs that emerged at 30 °C, died after one day, therefore, these two temperatures were excluded from further analyses. Based on these results, 30 ºC was considered the upper temperature threshold for eggs and nymphs. To obtain the lower temperature threshold for nymphs, non-replicated observational trials were performed with 10 newly emerged nymphs of each of the five instars. A temperature threshold of 7 ºC was chosen because nymphs failed to develop and/or survive within three consecutive days of observations.

To evaluate nymphal development, ten newly emerged nymphs from each egg batch (i.e. first day of emergence) were collected using a fine brush and placed individually on separate potted *S. mauritianum* plants (0.75 L), which had at least two fully matured leaves. Plastic lids (11 cm diameter) were modified by cutting a hole in the centre of approximately 1 cm dia., and cutting through to the edge to allow the plastic lid to surround the stem. The hole in the centre of the plastic lid was bigger than the diameter of the plant stem. Therefore, transparent tape was used to cover the space between the stem and the plastic lid. By doing this, the upper section of the plant (i.e. leaves and stem) was separated from the soil. This modification restrained the lace bugs from wandering into the soil. Each potted plant was covered with an inverted modified plastic container, which had a fine metal mesh placed in the bottom to allow ventilation. Nymphs were observed daily and the

duration and survival of each nymphal stage (instar) was recorded. Observations continued until the last surviving nymph emerged as an adult.

The proportion of adult females (i.e. the number of females, identified as F_1, that emerged from each egg batch), the life cycle duration (i.e. number of days it takes to develop from egg to adult), and nymphal instar survival (i.e. percentage of surviving nymphs at each nymphal stage) were recorded at each temperature tested. The incubation of egg batches and the recording of nymphal development data was replicated three times (three replicates of the experiment in time).

To compare adult reproduction and lifespan across temperatures, three lace bug couples (i.e. F_1 parents) of the same age, from a different set of parents, where collected among the adults resulting from the previous nymphal development experiment. Each couple was placed on separate potted *S. mauritianum* plants kept under the same environmental conditions as those used to evaluate nymphal development. This procedure was replicated three times. Plants were checked daily for the presence of egg batches. Once an egg batch was observed, it was collected and placed in a plastic Petri dish for incubation under similar environmental conditions as the parents. When nymphs emerged, they were counted, placed on a new potted *S. mauritianum* plant and followed until they reached adulthood. The F_2 adults were sexed and survival (i.e. percentage of nymphs that moulted to adults) was recorded. Lace bug parents were monitored daily to record lifespan. For F_1 females, several reproductive parameters were recorded: a) the number of females that oviposited; b) the length of pre-oviposition period (i.e. number of days counted from the day the female was placed with the adult male until the presence of an egg batch); c) number of eggs laid per female; d) number of egg batches oviposited per female; e) number of eggs per batch; f) number of eggs in the first batch; and g) number of eggs that hatched from the first batch. During the experiment, plants were renewed when at least 75% of the leaves were damaged.

4.2.3.2. Photoperiod and humidity

To evaluate the effect of photoperiod on *G. decoris* life history, the development from egg to adult was monitored at three different photoperiods 8L:16D, 14L:10D and 16L:8D, at a constant temperature of 20 ºC and 50 ± 10% RH. In a separate experiment, the effect of humidity was evaluated at 50 ± 10% and 70 ± 10% RH in environmental chambers (Sanyo MLR-350T, Japan) and bioassay rooms under a temperature of 20 °C and a photoperiod of 16L:8D. The life cycle duration (i.e. days that it took to develop from egg to adult) and survival (i.e. percentage of nymphs that moulted to adults) was recorded. The procedure and recorded variables (total number of eggs, number of eggs laid per female, number of egg batches, number of egg per batch, pre-oviposition period (days), number of females that oviposited successfully (i.e. produced egg batches that hatched), number of eggs in the first batch, percentage of egg hatch (first batch) were as described for the temperature experiment. Three replicates were performed for the photoperiod and humidity experiment.

4.2.4. Life table calculations

To examine the effect of temperature, photoperiod and humidity on *G. decoris* population growth, fertility parameters were calculated for each female that laid eggs: (Table 4.1).

Table 4.1. Description and formulae of life table reproductive parameters (Carey, 1993; Begon et al., 1996).

Parameters	Description	Formula
x	Age (day when each F_1 female oviposited)	
lm	Proportion of females that oviposited at each age times the number of female offspring per female that oviposited.	
Net reproductive rate (R_o)	Total number of female offspring produced by a female at each age.	$\sum_{x=\alpha}^{\beta} l_x m_x$
Intrinsic rate of natural increase (r_m)	The change in population size in a non-limiting environment per individual per unit of time (days).	$\sum_{x=\alpha}^{\beta} e^{-rx} l_x m_x$
Mean generation time (T)	Period elapsing between the birth of the female parent and the birth of her offspring.	$(log_e R_o)/r$
Doubling time (DT)	Time required for the population to increase twofold	$(log_e 2)/r$
Finite rate of increase (λ)	Ratio of population size at each time step (age)	e^r

4.2.5. Statistical analysis

The *G. decoris* development time, nymphal survival, fecundity, fertility and life table parameters were compared across the different temperature, photoperiod and humidity levels tested using general linear models (GLM) and Analysis of Variance (ANOVA) followed by Fishers Least Significant Difference (LSD) for comparison of means at $p <$

0.05 when the overall ANOVA test were significant. All data were tested for normality and homogeneity of variances using a qqplot and Shapiro-Wilks test. A linear regression using developmental rate ($1/d$) with temperature as a predictor variable (15 ºC – 27.5 ºC) was also conducted. Following the assumption that development rate is proportional to temperature, the degree days (DD) required for development were calculated as the reciprocal of the slope (1/slope) of a regression line fitted to the developmental data (Capinera, 2008).

The estimation of the distribution of survival times was obtained by calculating the Survival Distribution Function (SDF). The SDF evaluated at t is the probability that an experimental unit from the population will have a lifetime exceeding t,

$$S(t) = \Pr(T > t)$$

The LIFETEST PROCEDURE (SAS 9.3, USA) was used to compare survival curves using the non-parametric Wilcoxon test. The test compared k-sample tests based on the weighed sum of observed minus expected numbers of failure under the null hypothesis of identical survival curves to estimate the hazard rate of the individual population. The hazard function, denoted $h(t)$, is defined as $f(t)/S(t)$, where $f(t)$ is the derivative of $F(t)$ and $F(t)$ is the cumulative distribution function (CDF) (SAS Institute Inc., 2009).

4.2.6. CLIMEX modelling

The CLIMEX model, first described by Sutherst and Maywald (1985), is based on the assumption that climate determines the distribution of species, and therefore by knowing the actual distribution of a species it is possible to predict the potential geographical distribution. The model uses several indices to estimate potential population growth (GI_A); these indices are either growth-related or stress-related (see Appendix, Table S1 for explanation of growth and stress-related indices). All indices are calculated on a weekly basis or combined into an annual value of the index (Sutherst *et al.*, 2007). The annual growth index (GI_A) describes the potential for population growth as a function of soil moisture and temperature during favourable conditions and of up to eight stress indices (cold, wet, hot, dry, cold-wet, cold-dry, hot-wet and hot-dry) to simulate the ability of a

population to survive under unfavourable conditions (Sutherst *et al.*, 2007). The GI_A is calculated as:

$$GI_A = \frac{100}{52} \sum_{w=1}^{52} \mathrm{TI}_w \text{ x } \mathrm{MI}_w$$

where *w* is the week of the year, TI_w is the temperature index for week *w* and MI_w is the moisture index for week *w*. The temperature index (TI) describes the response of the species to the daily temperature cycle; the moisture index (MI) is based on the assumption that microclimatic conditions are a result of the vegetation moisture that itself is influenced by the soil moisture (Kriticos et al., 2015) (see Appendix , Table S1 for further description).

CLIMEX combines the growth and stress indices into an overall annual index of climatic suitability, the Ecoclimatic Index (EI). This index provides an overall measure for the potential of a given location to support a permanent population of the species (Sutherst *et al.*, 2007). The EI is calculated as:

$$\mathrm{EI} = \mathrm{GI_A} \text{ x } \mathrm{SI} \text{ x } \mathrm{SX}$$

where GI_A is the annual growth index, SI is the total stress and SX is the interaction between stresses (see Appendix , Table S2 for further description).

The EI is scaled between 0 to 100, with EI close to zero indicating that the location is not favourable for the long-term survival of the population and values close to 100 are ideal conditions, mostly met under controlled conditions (i.e. incubators) (Sutherst *et al.*, 2007). It has been found that EI values ≥ 20 indicate that the location is able to support significant population densities (Sutherst, 2003; Sutherst and Maywald, 2005).

4.2.7. CLIMEX model parameters and meteorological data

The CLIMEX model parameters values were determined using data obtained from the experiments on the effects of temperature on nymphal development, survival, longevity, fecundity and population growth of *G. decoris*. The information collected from this

experiment was used to determine the upper and lower developmental threshold as well as the optimal temperatures for population growth. The data collected from the laboratory combined with the known observations in its native range and locations in other parts of the world where this insect is known to be established was used to fit the projected distribution of this species in New Zealand and other parts of the world where woolly nightshade is a problem. To fit the projected distribution to the known distribution of *G. decoris*, stress parameters were adjusted manually until the best fit was obtained. The criterion to determine best fit was that the native range and those locations were *G. decoris* has been reported as established should have EI > 25, otherwise, adjustments had to be made to one or multiple stress parameters. The CliMond global gridded climate dataset with a 10 min spatial resolution was used for modelling (Kriticos *et al.*, 2012) (Table 4.2). The model grid output of ecoclimatic indices (EI, see above) were exported to ArcMap 10.4 (ESRI®, 2017) to construct the final maps. An EI > 25 was considered as optimal suitability, 6-25 moderate suitability, 1-5 marginal suitability and 0 as unsuitable.

Temperature index: Temperature parameters were estimated from the bioassays performed on adults, nymphs and eggs under constant controlled temperatures. The lower temperature threshold (DV0) for eggs was found to be at 10 ºC because all eggs failed to hatch at this temperature. However, the DV0 was set to 7 °C because at this temperature nymphal death took place before any growth could be observed. The lower and upper optimal temperatures for development (DV1 and DV2) were set to 20 °C and 27.5 °C, because at these temperatures growth was observed, but below and above these temperatures insect development was negatively affected (i.e. no development observed). The upper temperature threshold (DV3) for growth, at which newly emerged nymphs survived less than a day, was set to 30 °C.

Moisture index: The soil moisture is a dimensionless index which is based on the assumption that soil moisture determines the moisture availability to plants and will influence the microclimatic conditions (i.e. relative humidity) (Sutherst *et al.*, 2007; Kriticos *et al.*, 2015). The lower soil moisture threshold, SM1 was set as 0.1 to represent a value close to 0. The value 0 is catalogued as no soil moisture content according to

Sutherst et al (2007) and is when plants reach permanent wilting point and will die. Values higher than 1 indicates water content greater than the soil holding capacity, represented by wet tropical and subtropical climates. The upper soil moisture threshold was set as 2 to represent the subtropical climate found in the lace bug native range.

Cold stress: The cold stress threshold (TTCS) was adjusted taking into account the thermal requirements of *G. decoris*. Its accumulation rate (THHS) was iteratively adjusted to fit the insect's establishment records in the Bay of Plenty, New Zealand and Limpopo, South Africa, which are the coldest regions where *G. decoris* has been reported to be established.

Heat stress: The heat stress threshold (TTHS) was adjusted taking into account the thermal requirements of *G. decoris.* The accumulation rate (THHS) was iteratively adjusted to fit the hottest locations known to be suitable for *G. decoris* within the semiarid and subtropical distribution in its native or established range in the southern states of Brazil (Paraná, Santa Catarina and Rio Grande do Sul), Misiones province in Argentina, and Kwazulu-Natal and Gariepdam in South Africa.

Dry stress: The limiting dry stress parameter (SMDS) was set to 0.1 to match the permanent wilting point and the lower soil moisture threshold (SM0).

Wet stress: The native distribution of *G. decoris* is known to be in subtropical climates of Brazil and Argentina, therefore the wet stress threshold (SMWS) and accumulation rate (HWS) were adjusted iteratively to fit the non-native distribution of *G. decoris* in semiarid regions of South Africa.

Table 4.2. CLIMEX parameter values used for modelling the predicted distribution of *Gargaphia decoris*, as derived from laboratory data (constant temperature rearing) and from known distributions localities in Brazil, Argentina, South Africa and New Zealand.

Index	Parameter	Values	Units*
Temperature	DV0 = lower threshold	7	°C
	DV1 = lower optimum temperature	20	°C
	DV2 = upper optimum temperature	27.5	°C
	DV3 = upper threshold	30	°C
Moisture	SM0 = lower soil moisture threshold	0.1	
	SM1 = lower optimum soil moisture	0.7	
	SM2 = upper optimum soil moisture	1.3	
	SM3 = upper soil moisture threshold	2	
Cold stress	TTCS = temperature threshold	7	°C
	THCS = stress accumulation rate	-0.001	$week^{-1}$
Heat stress	TTHS = temperature threshold	30	°C
	THHS = stress accumulation rate	0.0025	$week^{-1}$
Dry stress	SMDS = soil moisture dry stress threshold	0.1	°C
	HDS = stress accumulation rate	-0.005	$week^{-1}$
Wet stress	SMWS = soil moisture wet stress threshold	2	°C
	HWS = stress accumulation rate	0.0015	$week^{-1}$
PDD	Number of degree-days above DV0 necessary to complete one generation	384	ºC days

* Values without units are dimensionless indices. See Appendix , Table S1 for further explanation of parameters.

4.3. Results

4.3.1. Effect of temperature on *G. decoris* development and population growth

Temperature significantly affected the mean (± SE) developmental duration of eggs ($F_{3,8}$ = 180, $p < 0.01$) and the life cycle duration ($F_{3,8}$ = 240.2, $p < 0.01$) (15-27.5 ºC) (Table 4.3). Nymphal development did not occur at 10 or 30 ºC, therefore, only those temperatures where nymphs developed to adults were included for further analysis. Developmental duration decreased with increasing temperatures. Thus, *G. decoris* had the shortest life cycle at 27.5 ºC (24.3 ± 0.4 days) compared with 15 ºC (71.7 ± 1 days). A similar trend was observed for the duration of the egg stage. No significant differences were observed for both the egg stage duration and life cycle duration at 25 ºC and 27.5 ºC. The egg stage took approximately 27-29% of the life cycle duration and required 119 DD. The egg stage and life cycle were approximately three times longer at 15 ºC than at 25 ºC or 27.5 ºC (Table 4.3).

Nymphal development was significantly affected by temperature ($F_{3,8}$ = 6.58, $p <$ 0.01 for first instar; $F_{3,8}$ = 19.64, $p < 0.01$ for the second instar; $F_{3,8}$ = 45.2, $p < 0.01$ for the third instar; $F_{3,8}$ = 50.15, $p < 0.01$ for the fourth instar; $F_{3,8}$ = 19.67, $p < 0.01$ for the fifth instar), however significant pair-wise differences varied between nymphal stages (Table 4.3).

To complete the life cycle (egg to adult), *G. decoris* required 384 DD (Table 4.3). The thermal requirement for the nymphal period was 312.5 DD, with the second instar being the fastest to develop requiring 48.31DD (20-27.5 °C). The fifth instar took the longest to develop across all temperatures and required 93.43DD.

Higher temperatures resulted in higher development rates, which was adequately described by the linear regression $Y = a + bx$. The adjusted coefficients of determination R_a^2 ranged from 0.78 to 0.95 for the nymphal stages and the life cycle (Fig. 4.1). The data could have been fitted to many different equations, however, a simple linear regression was chosen because the higher temperature range selected in this study did not result in a decline in development rate. Therefore, it was not necessary to use non-linear models such as Brière or Logan models (Logan *et al*., 1976; Briere *et al.*,1999).

Table 4.3. Mean development time in days (± SE) of *Gargaphia decoris* at four constant temperatures: 15, 20, 25 and 27.5 °C, with a constant photoperiod of 14L:10D and relative humidity of 50 ± 10%. Means followed by the same letter within a column are not significantly different (Tukey's HSD test, $\alpha = 0.05$). The K-factor, represents the degree days calculated as 1/slope of a regression line fitted to the development data.

Temp. (°C)	Eggs	Instar					Nymphal period	Life cycle
		1º	2º	3º	4º	5º		
15	20.56 ± 0.39 a	7.58 ± 0.54 a	8.50 ± 0.54 a	9.21 ± 0.34 a	10.39 ± 0.29 a	15.39 ± 0.33 a	51.77 ± 0.82 a	71.73 ± 1 a
20	11.32 ± 0.79 b	4.84 ± 0.21 ab	4.32 ± 0.20 b	4.75 ± 0.21 b	6.12 ± 0.20 b	9.28 ± 0.32 a	29.76 ± 0.54 b	41.32 ± 0.61 b
25	7.7 ± 0.25 c	3.78 ± 0.20 b	2.78 ± 0.20 b	3.5 ± 0.20 b	3.4 ± 0.20 c	5.78 ± 0.15 b	18.51 ± 0.53 c	29.97 ± 0.56 c
27.5	6.47 ± 0.27 c	3.10 ± 0.14 b	2.89 ± 0.10 b	3.27 ± 0.15 b	3.42 ± 0.12 c	5.40 ± 0.14 b	20.24 ± 0.79 c	24.29 ± 0.39 c
K (DD)	119	69.93	48.31	62.11	58.82	93.46	312.5	384

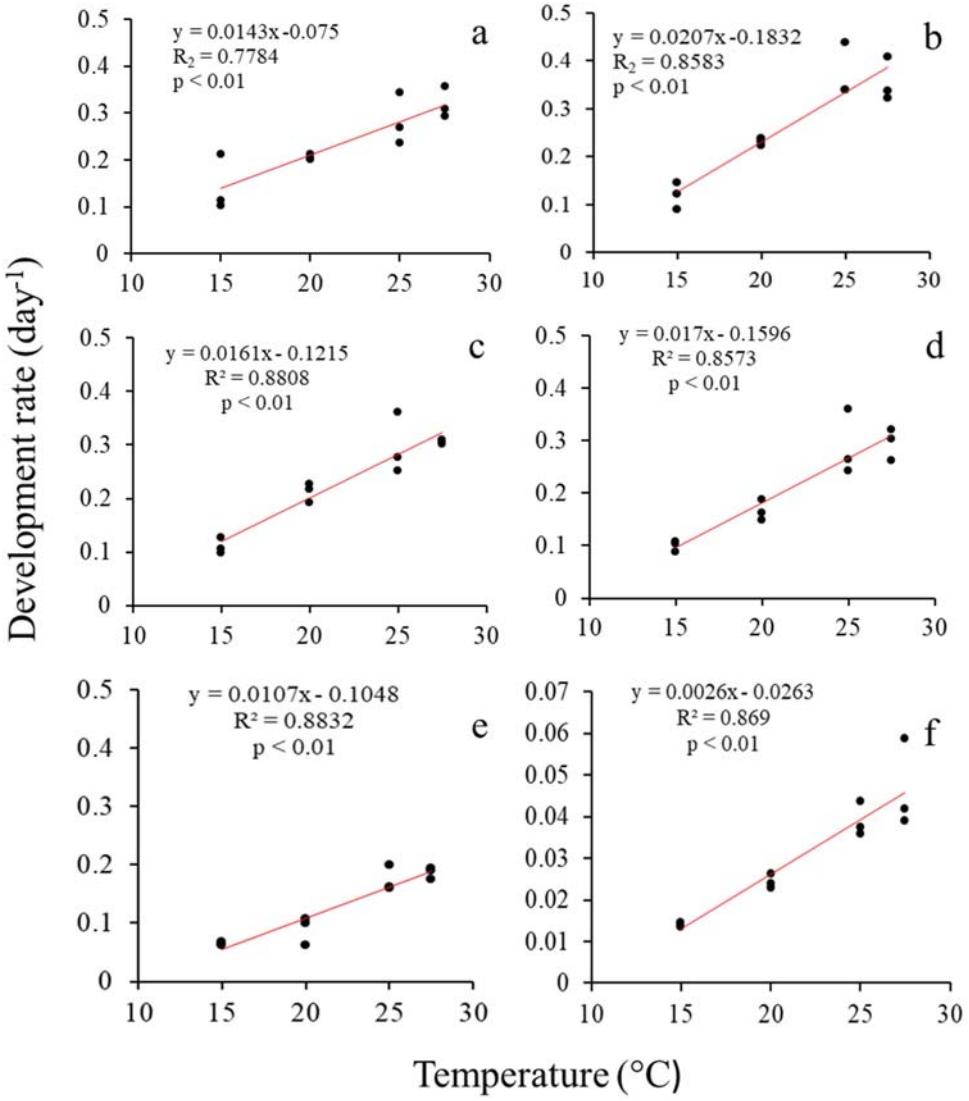

Figure 4.1. Development rates (day^{-1}) for *Gargaphia decoris* nymphal stages at four different constant temperatures: 15 °C; 20 °C; 25 °C; and 27.5 °C, as predicted by a linear regression model; a) first instar; b) second instar; c) third instar; d) fourth instar; e) fifth instar; f) the whole life cycle. Closed circles represent the mean development rates recorded in experiments, and the solid red lines represent the fitted linear regression model.

4.3.2. Effect of temperature on *G. decoris* survival

No significant differences were observed in the survival of nymphal instars and total survival across temperatures ($F_{3,8}$ = 1.75, p = 0.239 for first instar; $F_{3,8}$ = 0.918, p = 0.475 for second instar; $F_{3,8}$ = 1.351, p = 0.325 for third instar; $F_{3,8}$ = 3.632, p = 0.06 for fourth instar, $F_{3,8}$ = 1.943, p = 0.201 for fifth instar; $F_{3,8}$ = 1.39, p = 0.314 for total survival). The lowest and highest total survival (from egg to adult) were observed at 15 and 25 °C (27.6 ± 4.1% and 39.8 ± 4.3%, respectively).

There was a significant effect of gender on *G. decoris* longevity across different temperatures tested. Females lived significantly longer than males across all temperatures tested except at 20 °C, where no significant difference was observed between males and females (Wilcoxon test: χ^2 = 9.48, p < 0.01 for 15 °C; χ^2 = 2.08, p = 0.149 for 20 °C; χ^2= 17.83, p < 0.01 for 25 °C; χ^2 = 7.51, p < 0.01 for 27.5 °C) (Fig. 4.2). Regardless of gender, survival was higher at lower temperatures than at higher temperatures (Wilcoxon test: χ^2 = 18.5, p < 0.01 for females; χ^2 = 13.7, p < 0.01 for males) (Fig. 4.3).

4.3.3. Effect of temperature on *G. decoris* fecundity and life table parameters

Temperature significantly affected some of the reproductive parameters of *G. decoris*. The total number of eggs was significantly higher at 25 °C (703.33 ± 283.18) compared to 15 °C (37 ± 25.11) (Table 4.4). The number of eggs per female, per batch and the percentage of females that oviposited was higher at 25 °C. The pre-oviposition period increased as the temperature decreased ($F_{3,8}$ = 5.16, p < 0.05) and the percent of eggs that hatched in the first batch was higher at 20 °C. However, no significant differences were found across the other temperatures tested (15-27.5 °C). Temperature did not affect significantly the number of egg batches laid per female ($F_{3,8}$ = 2.07, p = 0.18) (Table 4.4).

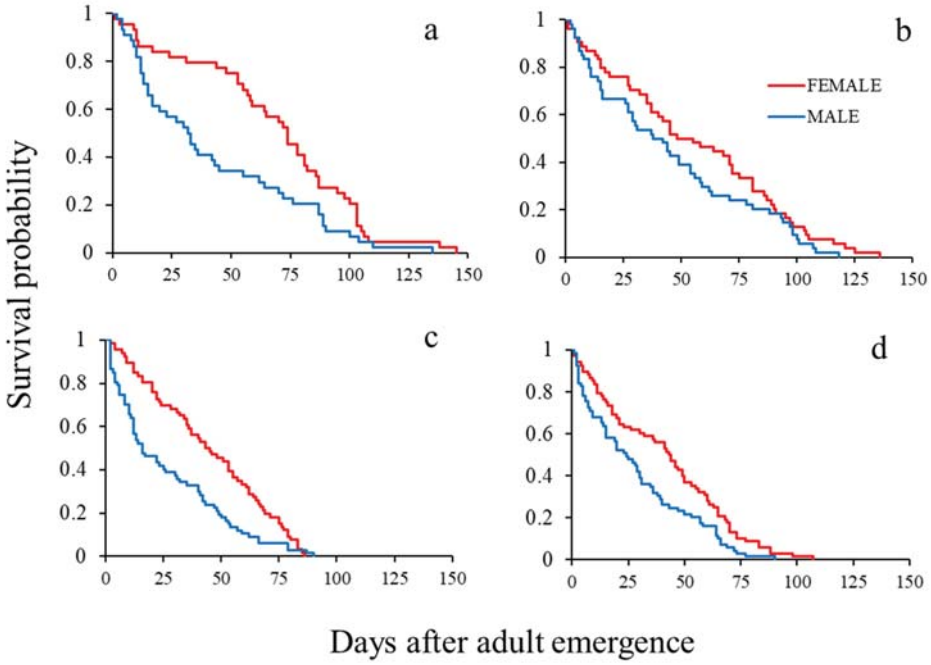

Figure 4.2. Survival probability of *Gargaphia decoris* females and males across four constant temperatures: a) 15 °C; b) 20 °C; c) 25 °C; and d) 27.5 °C. Survival probability is the probability that an experimental unit from the population will have a lifetime exceeding *t*, described by the survival distribution function (SDF) denoted as S(*t*) = Pr (T > *t*).

Most of the life table parameters measured for *G. decoris* were affected by temperature (Table 4.5). Net reproductive rate (R_0) was highest at 25 °C (54.30 ± 0.3) and lowest at 15 °C (3.5 ± 0.5). All temperatures displayed a positive net reproductive rate denoting an increasing population. The intrinsic rate of natural increase (r_m) and finite rate of increase (λ) significantly decreased at 15 °C and the values were highest at 20-25 °C. Doubling time (DT) and mean generation time (T) were lowest at 25 °C and highest at 15 °C (Table 4.5).

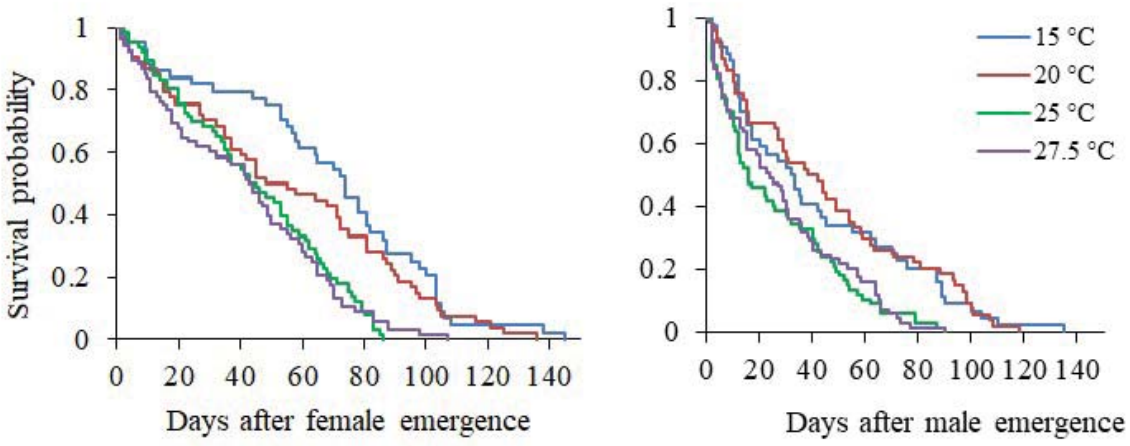

Figure 4.3. Survival probability of *Gargaphia decoris* females (left) and males (right) across four constant temperatures: 15 °C; 20 °C; 25 °C; and 27.5 °C. Survival probability is the probability that an experimental unit from the population will have a lifetime exceeding *t*, described by the survival distribution function (SDF) denoted as S(*t*) = Pr (T > *t*).

4.3.4. Effect of photoperiod on *G. decoris* life history traits

Photoperiod significantly affected the mean (±SE) percentage of egg hatch (i.e. emergence of nymphs in egg batches collected from the colony) ($F_{2,6}$ = 8.43, $p < 0.05$) and total nymphal survival (i.e. nymph to adult emergence) ($F_{2,6}$ = 5.47, $p < 0.05$). The highest percentage of egg hatch and total survival (88.15% and 69.44%, respectively) were observed at 16L:8D photoperiod and the lowest at 14L:10D (61.74% and 41.19%, respectively) (Fig. 4.4). However, photoperiod did not significantly affect life cycle duration ($F_{2,6}$ = 1.62, p = 0.273), the percentage of females that emerged ($F_{2,6}$ = 0.91, p = 0.451), the percentage of females that oviposited successfully ($F_{2,6}$ = 3.78, p = 0.087), the total number of egg laid by females ($F_{2,6}$ = 4.21, p = 0.072), the number of eggs laid per female ($F_{2,6}$ = 0.97, p = 0.431), the number of egg batches ($F_{2,6}$ = 2.61, p = 0.153), the number of eggs per batch ($F_{2,6}$ = 0.76, p = 0.508), the number of eggs in the first batch ($F_{2,6}$ = 2.32, p = 0.180), or the percentage of eggs that hatched (first oviposition) ($F_{2,6}$ = 2.46, p = 0.166).

Table 4.4. Effect of four constant temperatures: 15, 20, 25 and 27.5 °C on female fecundity parameters of *Gargaphia decoris* (± SE), at a constant photoperiod of 14L:10D and relative humidity of 50 ± 10%. Means followed by the same letter within a row are not significantly different (Fisher's LSD test, $\alpha = 0.05$).

Factor	Temperature (°C)				Statistics
	15	20	25	27.5	
Total No. eggs	37 ± 25.11	560 ± 239.40	703.33 ± 283.18	249.67 ± 101.83	$F_{3,8} = 2.44$, $p = 0.140$
No. eggs laid per female	15.25 ± 3.99 c	60.16 ± 11.11 ab	62.09 ± 13.05 a	29.72 ± 8.28 bc	$F_{3,8} = 5.64$ $p < 0.05$
No. egg batches	2 ± 1	13.67 ± 5.04	16 ± 6.08	9 ± 3.21	$F_{3,8} = 2.07$, $p = 0.183$
No. eggs per batch	15.25 ± 3.99 c	37.88 ± 4.27 ab	40 ± 2.55 a	25.2 ± 5.74 bc	$F_{3,8} = 7.29$, $p < 0.05$
Pre-oviposition period (days)	55 ± 3.61 a	40.63 ± 12.30 ab	24.81 ± 1.86 b	23.97 ± 0.88 b	$F_{3,8} = 5.16$, $p < 0.05$
Females that oviposited successfully*(%)	15.06 ± 5.16	47.55 ± 16.19	41.54 ± 14.84	27.52 ± 1.81	$F_{3,8} = 1.66$, $p = 0.252$
No. eggs in first batch	16 ± 4.62 b	43.05 ± 5.64 a	39.23 ± 6.27 ab	26.43 ± 5.69 ab	$F_{3,8} = 4.90$, $p < 0.05$
% egg hatched (first batch)	36.69 ± 5.49	60.76 ± 15.66	55.97 ± 10.27	63.91 ± 6.32	$F_{3,8} = 0.80$, $p = 0.529$

* "Oviposited successfully" refers to a female producing an egg batch that hatched.

Table 4.5. Life table parameters (mean ± SE) of *Gargaphia decoris* at four constant temperatures: 15, 20, 25 and 27.5 °C, at a constant photoperiod of 14L:10D and a relative humidity of 50 ± 10%. Means with the same letter within a row are not significantly different (Dunn´s test, $\alpha = 0.05$). The letter n represents the number of females used to calculate each of the parameters.

Parameters	Temperatures (°C)				Statistics
	15 (n = 2)	20 (n = 24)	25 (n = 33)	27.5 (n = 19)	
Net reproductive rate (R_0)	3.5 ± 0.5 c	48.50 ± 1.567 b	54.30 ± 0.281 a	24.91 ± 1.104 bc	$x_3^2 = 58.31$, $p < 0.01$
Intrinsic rate of natural increase (r_m)	0.037 ± 0.005 c	0.154 ± 0.001b	0.198 ± 0.001 a	0.140 ± 0.002 c	$x_3^2 = 64.51$, $p < 0.01$
Finite rate of increase (λ)	1.04 ± 0.005 c	1.17 ± 0.001 b	1.22 ± 0.001 a	1.15 ± 0.002 c	$x_3^2 = 64.51$, $p < 0.01$
Mean Generation time (T)	34.07 ± 0.82 a	25.20 ± 0.07 a	20.26 ± 0.069 b	22.90 ± 0.174 c	$x_3^2 = 67.74$, $p < 0.01$
Doubling time (DT)	19.32 ± 2.693 a	4.52 ± 0.035 b	3.51 ± 0.016 c	4.97 ± 0.074 a	$x_3^2 = 64.51$, $p < 0.01$

Adult longevity was significantly affected by photoperiod. Females (χ^2_2 = 12.05, p < 0.01) and males (χ^2_2 = 7.66, p < 0.01) lived longer at 16L:8D photoperiod (75.79 ± 0.54 and 76.26 ± 3.82 days, respectively) and both died earlier at 14L:10D photoperiod (67.33 ± 17.31 and 50.83 ± 13.89, respectively). No significant differences were observed in the longevity of females between 8L:16D and 16L:8D photoperiods (p = 0.43) or in the longevity of males between 8L:16D and 14L:10D photoperiods (p = 0.19) (Fig. 4.6).

Photoperiod significantly affected the population growth parameters (Table 4.6). The net reproductive rate (R_o) was positive in all photoperiods tested indicating population growth. Population growth increases at a faster rate during the 16L:8D photoperiod. The net reproductive rate (R_o), intrinsic rate of natural increase (r_m) and finite rate of increase (λ) were higher at 16L:8D photoperiod and lower at 14L:10D photoperiod. The time to produce one generation (T) and to double it (DT) were lower at 16L:8D photoperiod and higher at 14L:10D photoperiod (Table 4.5).

4.3.5. Effect of relative humidity on *G. decoris* life history traits

Relative humidity significantly affected the mean (±SE) percentage of egg hatch (i.e. emergence of nymphs from each egg batch collected from the colony) ($F_{2,6}$ = 8.21, p < 0.05). The percentage of egg hatch was higher at 50 ± 10% RH (88.10 ± 0.34) than at 70 ± 10% RH (75.03 ± 4.57). In contrast, no significant effect of humidity was found in life cycle duration ($F_{1,4}$ = 0.11, p = 0.759), total survival (nymphs to adult) ($F_{1,4}$ = 4.66, p = 0.100), pre-oviposition period ($F_{1,4}$ = 0.40, p = 0.562), percentage of females that oviposited ($F_{1,4}$ = 0.01, p = 0.919), total number of eggs ($F_{1,4}$ = 0.18, p = 0.692), number of eggs per female ($F_{1,4}$ = 1.28, p = 0.322), number of egg batches ($F_{1,4}$ = 0.97, p = 0.379), number of eggs per batch ($F_{1,4}$ = 1.78, p = 0.253), number of eggs in the first batch ($F_{1,4}$ = 0.01, p = 0.925), and the percentage of eggs that hatched (first batch) ($F_{1,4}$ = 2.55, p = 0.186). Adult longevity was significantly affected by humidity. Adults lived longer at 70% RH (χ^2 = 10.74, p < 0.01), but females lived longer than males at both RH (χ^2 = 3.96, p < 0.05) (Figs. 4.6, 4.7).

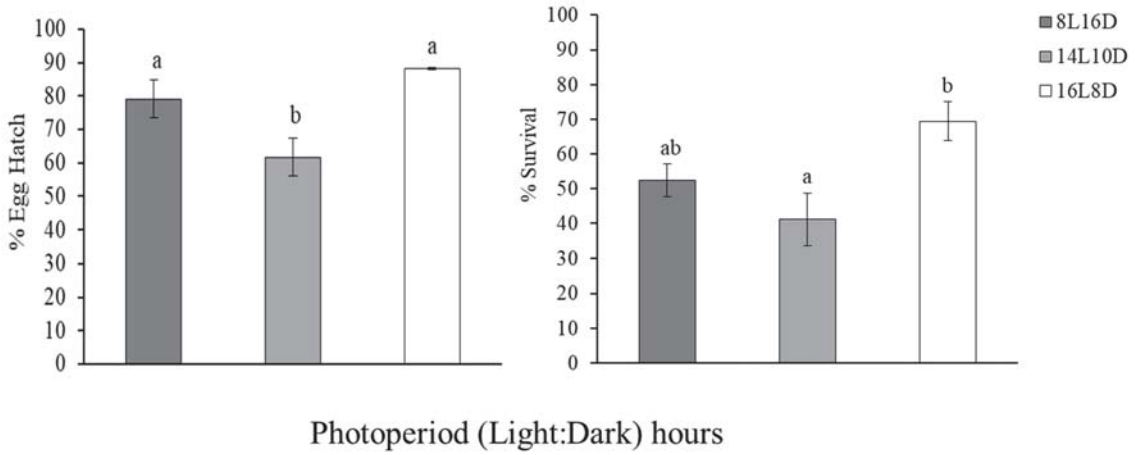

Figure 4.4. Mean (± SE) percentage of egg hatch (i.e. emergence of nymphs from each egg batch collected from the colony) (left) and total survival (nymph to adult) (%) (right) of *Gargaphia decoris* individuals grown at three different photoperiods and at a constant temperature of 20 °C and relative humidity of 50 ± 10%. Error bars represent standard errors. Means with the same letter are not significantly different (Fisher's LSD test, α = 0.05).

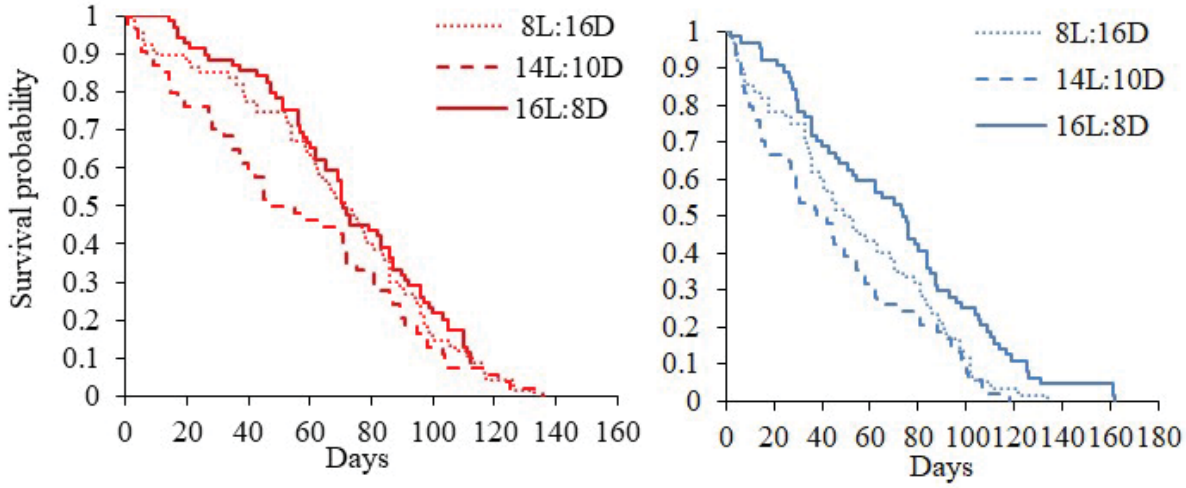

Figure 4.5. Survival probability of *Gargaphia decoris* females (left) and males (right) when grown under three different photoperiods: 8L:16D, 14L:10D, 16L:8D. Survival probability is the probability that an experimental unit from the population will have a lifetime exceeding *t*, described by the survival distribution function (SDF) denoted as S(*t*) = Pr (T > *t*).

Table 4.6. Life table parameters (mean ± SE) of *Gargaphia decoris* at three constant photoperiods: 16L:8D, 14L:10D and 8L:16D at a constant temperature of 20 °C, and a relative humidity of 50 ± 10%. Means with the same letter within a row are not significantly different (Dunn's test, α = 0.05). The letter n represents the number of females used to calculate the parameters.

Parameters	Photoperiod			Statistics
	16L:8D (n = 98)	14L:10D (n = 24)	8L:16D (n = 40)	
Net reproductive rate (R_o)	146.78 ± 0.71 a	48.50 ± 1.57 b	64.16 ± 0.88 c	$x_2^2 = 120.29$, $p < 0.01$
Intrinsic rate of natural increase (r_m)	0.52 ± 0.001 a	0.15 ± 0.001 b	0.18 ± 0.0006 c	$x_2^2 = 1.32$, $p < 0.01$
Finite rate of increase (λ)	1.68 ± 0.002 a	1.17 ± 0.0015 b	1.20 ± 0.0007 c	$x_2^2 = 1.32$, $p < 0.01$
Mean generation time (T)	9.60 ± 0.02 c	25.20 ± 0.07 a	23.05 ± 0.05 b	$x_2^2 = 122.46$, $p < 0.01$
Doubling time (DT)	1.33 ± 0.003 c	4.52 ± 0.03 a	3.84 ± 0.01 b	$x_2^2 = 132.18$, $p < 0.01$

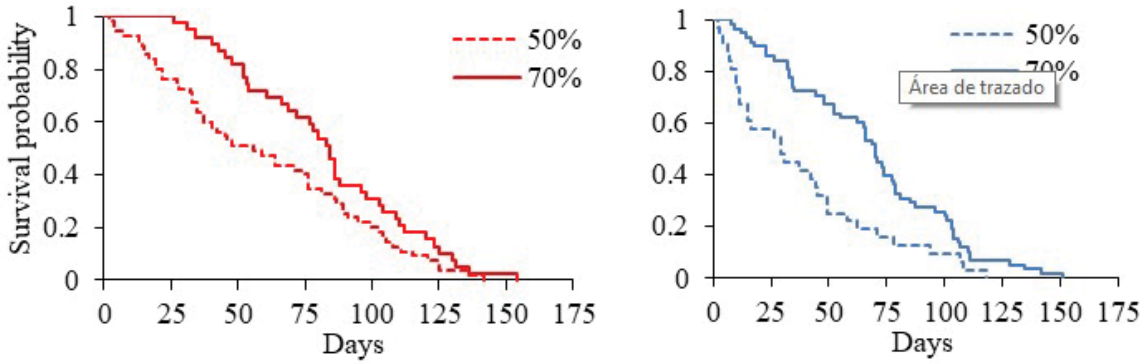

Figure 4.6. Survival probability of *Gargaphia decoris* females (left) and males (right) among the relative humidity tested: 50 ± 10% and 70 ± 10%. Survival probability is the probability that an experimental unit from the population will have a lifetime exceeding t, described by the survival distribution function (SDF) denoted as $S(t) = Pr(T > t)$.

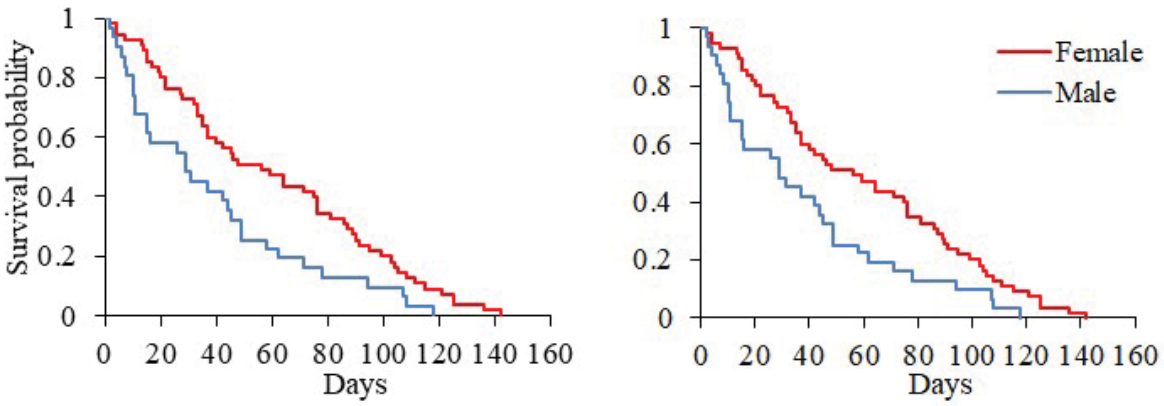

Figure 4.7. Survival probability of *Gargaphia decoris* adults at 50 ± 10% (left) and 70 ± 10% relative humidity (right). Survival probability is the probability that an experimental unit from the population will have a lifetime exceeding t, described by the survival distribution function (SDF) denoted as $S(t) = Pr(T > t)$.

Relative humidity affected *G. decoris* population growth parameters (Table 4.7). The positive net reproductive rate (R_0) indicates that population growth took place at both 50 and 70% relative humidity. The population growth was higher at 70% RH, but *G. decoris* population increased at a higher rate (r_m) at 50% compared to 70% RH. Therefore, the mean generation time (T) and doubling time (DT) were both shorter at 50% than at 70% RH (Table 4.7).

Table 4.7. Life table parameters (mean ± SE) of *Gargaphia decoris* at 50 ± 10% or 70 ± 10% relative humidity at a constant temperature of 20 °C and 16L:8D photoperiod. Means followed by the same letter within a row are not significantly different (Kruskall-Wallis test, α = 0.05). The letter n represents the number of females used to calculate the parameters.

Parameters	*Humidity (%)*		*Statistics*
	50 (n = 24)	70 (n =75)	
Net reproductive rate (Ro)	146.78 ± 0.71b	157.57 ± 0.12 a	x^2= *110.99, p < 0.01*
Intrinsic rate of natural increase (rm)	0.52 ± 0.001 a	0.21 ± 0.004 b	x^2= *153.73, p < 0.01*
Finite rate of increase (λ)	1.68 ± 0.002 a	1.24 ± 3.93e-05 b	x^2 = 153.73 , p < 0.01
Mean generation time (T)	9.60 ± 0.02 b	23.89 ± 0.004 a	x^2 = 144.02 , p < 0.01
Doubling time (DT)	1.33 ± 0.003 b	3.27 ± 0.0005 a	x^2 = 153.73 , p < 0.01

4.3.6. CLIMEX model fit and projections

The CLIMEX model predicted the eco-climatic suitability for *G. decoris* populations for those regions with a Köppen-Geiger climate classification of tropical rainforest climate, tropical monsoon climate, tropical savannah climate, hot-summer Mediterranean climate, warm-summer Mediterranean climate, humid subtropical climate, subtropical highland climate and oceanic climate. The projected global potential distribution of *G. decoris* using the parameter values from Table 4.2, is shown in a worldwide map (Fig. 4.8) and in detailed maps of New Zealand (Fig 4.9), Americas (4.10) and Africa (Fig. 4.11).

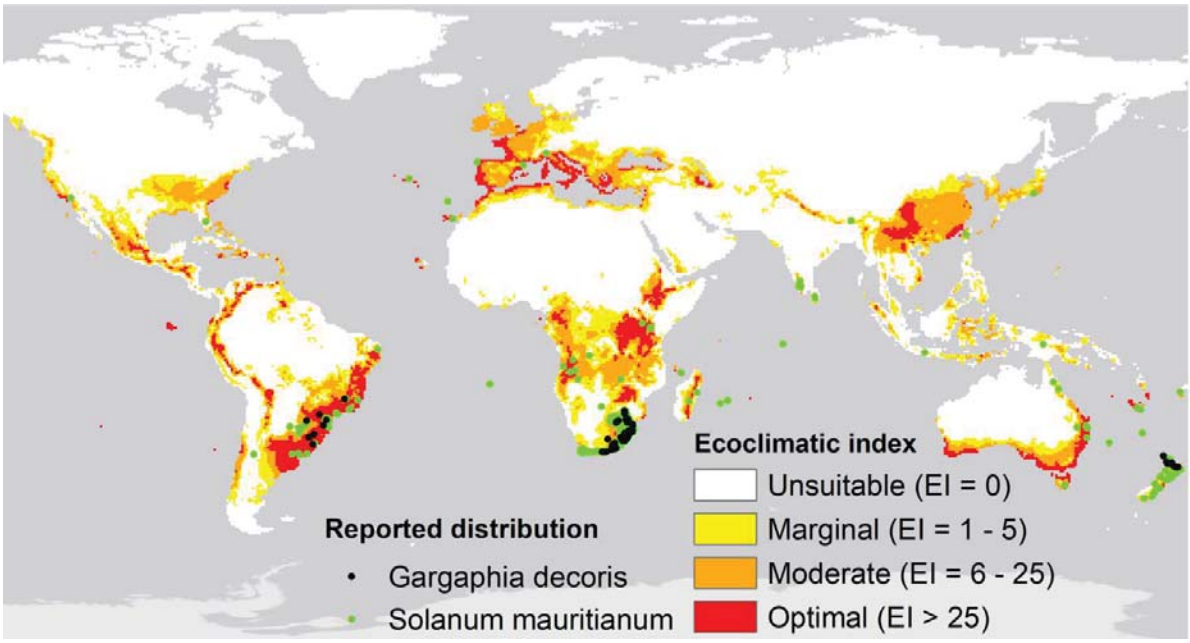

Figure 4.8. Global climatic suitability for *Gargaphia decoris* as modelled using CLIMEX. The map includes the predicted and the known distribution of the biological control agent, *G. decoris*, and the known distribution of the host, *Solanum mauritianum*. The known distribution of *S. mauritianum* and *G. decoris* is represented by light green and black dots, respectively.

4.3.7. Model validation and predictions

The CLIMEX model was validated by comparing climatic suitability with the actual distribution of *G. decoris*. The projected distribution fitted the native and non-native occurrences where the insect has established. All non-native occurrences in New Zealand and South Africa were projected to be climatically suitable for the development of *G. decoris*.

New Zealand

In New Zealand, the potential distribution of *G. decoris* covers most of the North Island and the northern regions of the South Island (Fig. 4.9). The North Island was projected to be optimally suitable for *G. decoris* establishment. The areas that are projected to have optimal suitability are the regions of Northland, Auckland, North of Waikato, including Coromandel and Hamilton, Bay of Plenty, including Tauranga, Whakatane, coasts of

Opotiki; Gisborne, including Waihau Bay, Hicks Bay and Tokomaru Bay; Hawkes Bay (specifically Napier), Taranaki, including New Plymouth, Stratford, South Taranaki, South Manawatu-Whanganui, and Wellington, including Kapiti coast and Lower Hutt. The areas that are deemed not suitable are located at higher altitudes in Hawkes Bay, Gisborne and Waikato, and Central Manawatu-Whanganui: Huiarau range, Kaweka range, Ruahine range and Mt. Ruapehu (approx. above 1300 m a.s.l.). In the South Island the areas that were predicted to have moderate to marginal suitability were the coast of the districts of Malborough, Tasman, Buller, Grey, Hurunui, Waimakariri and North of Westland. The rest of the districts in the South Island: Central and South Canterbury, South and Central Westland, Otago and Southland appear to be unsuitable for *G. decoris* populations, but not so for woolly nightshade establishment, as the weed has been reported to be present in these areas (Fig. 4.9).

South America

The predicted distribution model of *G. decoris* in its native range in Brazil and Argentina (Fig. 4.10) is consistent with the known native geographical range of this insect. The CLIMEX model predicted the presence of *G. decoris* in regions in the Southern states of Brazil: Rio Grande do Sul, Santa Catarina and Paraná and Misiones, Argentina, with subtropical and oceanic climate, where this insect has been reported (Hope and Olckers, 2011; Pedrosa-Macedo, personal communication, 2017). In addition, CLIMEX predicted that this species could optimally survive in tropical and temperate climates in the Southeastern and Eastern states of São Paulo, Rio de Janeiro, Espirito Santo, Minas Gerais, coasts of Bahía, Paraíba and Pernambuco and in the state of Goiás, the capital Brasilia. In Argentina, the model predicted that the insect could also survive optimally in the provenance of Buenos Aires, Entre Rios and Corrientes. The known distribution of the host plant *Solanum mauritianum* also converges with the known and predicted climatic suitability of its biological control agent *G. decoris.*

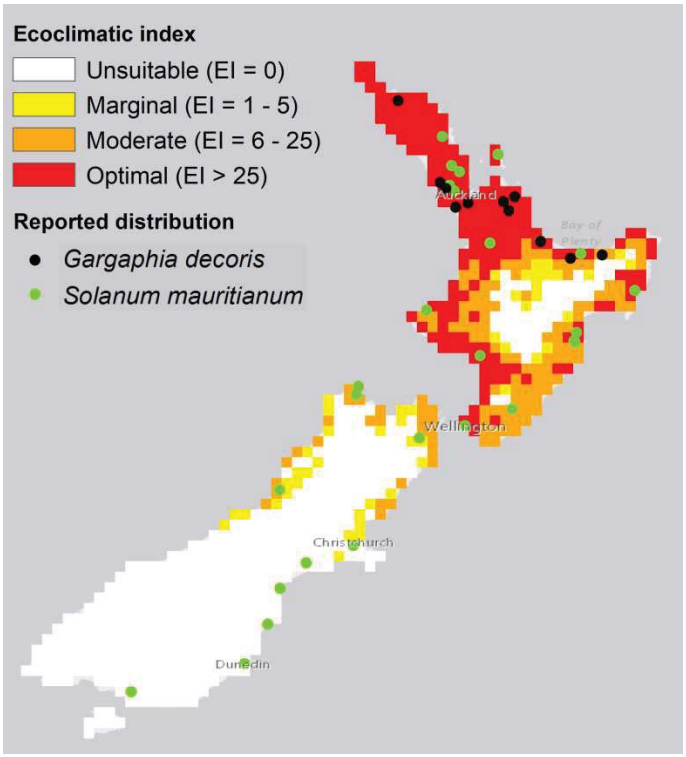

Figure 4.9. Modelled habitat suitability for the lace bug *Gargaphia decoris* in New Zealand as predicted using CLIMEX. Also showing the known distribution of *G. decoris* (black dots) and of the host plant *Solanum mauritianum* (green dots).

Besides the potential distribution in its native range (Brazil and Argentina), other regions in the South American continent are predicted to be optimally- to moderately-suitable for *G. decoris* establishment including: Uruguay, East of Paraguay (provinces of Canindeyú, Parts of San Pedro, Caaguazú, Alto Paraná, Itapúa, Guaira and Caazapá); National Parks Isiboro Secure, Carrasco and Amboró in Bolivia's subtropical highlands, Peru, Ecuador and Colombia's central tropical highlands, and Venezuela, specifically the National Park Canaima and Parina Tapirapecó in the Guayara region, and parts of Los Andes and Lara-Falcon region (Fig. 4.10).

Africa

The CLIMEX model projection for South Africa (Fig. 4.11) also fits all the known establishment records of *G. decoris* in South Africa. The optimal suitability was predicted in the regions of Limpopo, Pretoria, Pietermaritzburg (humid subtropical climate),

Mpumalanga and parts of the Eastern Cape (oceanic climate), Johannesburg (subtropical highland climate) and Kwazulu-Natal (humid subtropical climate without dry season).

The known report of *G. decoris* in Gariepdam was predicted to be marginally suitable (semi-arid climate). Besides the aforementioned regions in South Africa, a number of regions with tropical, temperate and Mediterranean climates were predicted to be optimally suitable in other parts of Africa including: Zambia, Equatorial Guinea, Gabon, Northeast Ethiopia, Uganda, Tanzania, parts of Congo, Rwanda, Burundi, Kenya, specifically locations close to Nairobi, East Madagascar, and coasts of Morocco, Algeria and Tunisia (Fig. 4.11).

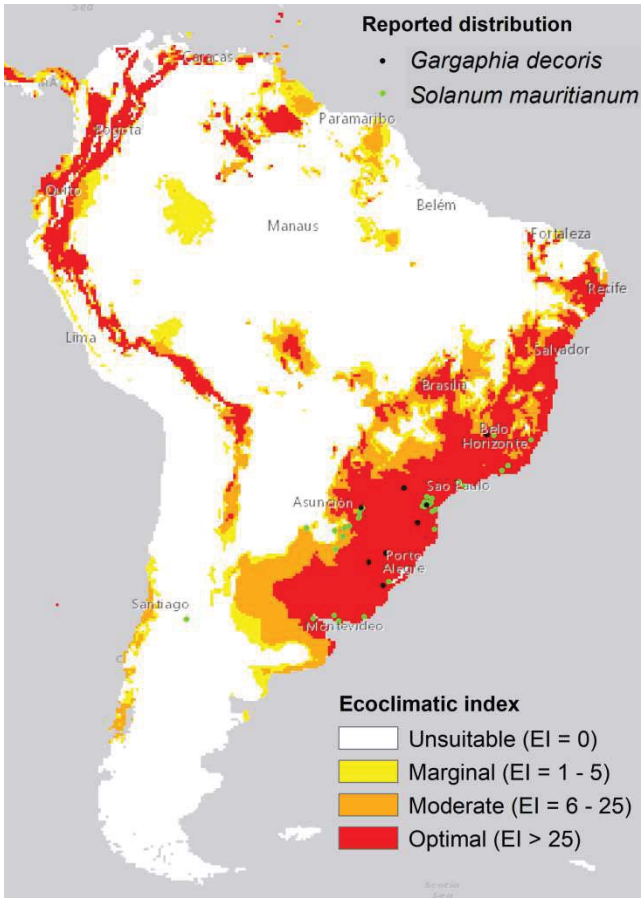

Figure 4.10. Modelled potential distribution of the lace bug *Gargaphia decoris* in South America as predicted using CLIMEX. Also showing the known distribution of *G. decoris* (black dots) and of the host plant *Solanum mauritianum* (green dots).

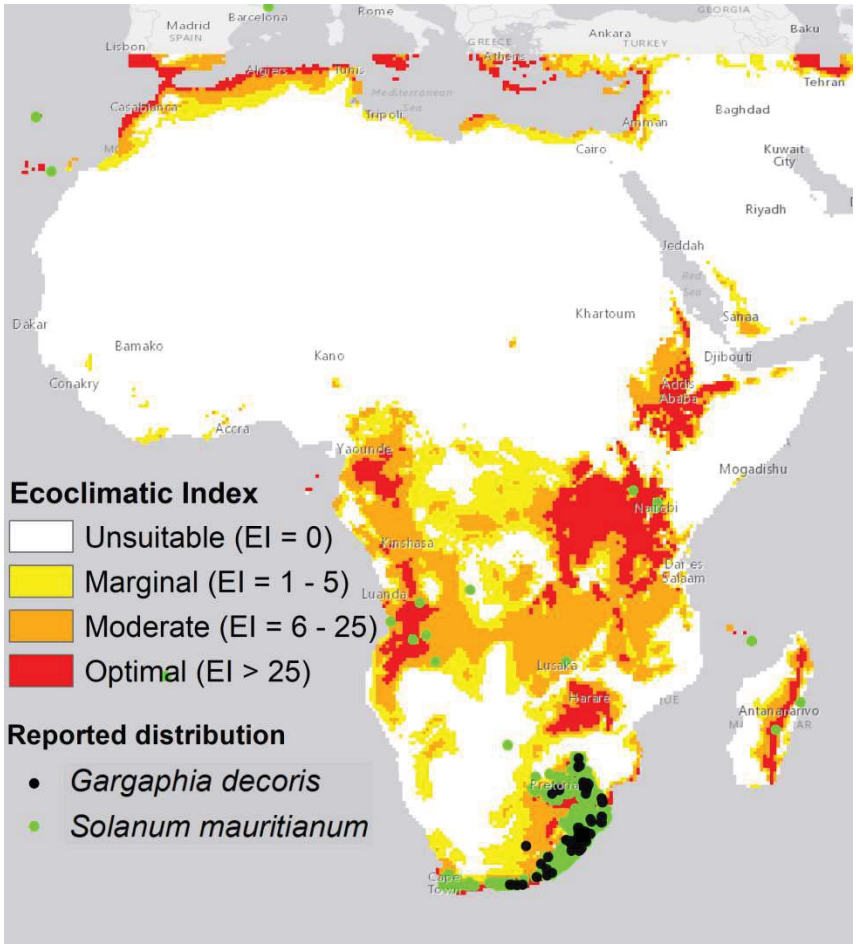

Figure 4.11. Modelled potential distribution of the lace bug *Gargaphia decoris* in the African continent as predicted using CLIMEX. Also showing the known distribution of *G. decoris* (black dots) and of the host plant *Solanum mauritianum* (green dots).

4.4. Discussion

4.4.1. Effect of temperature on *G. decoris* development

The effect of temperature on juvenile development has been studied in several tingid species and results have shown that some lace bug species have a broader range of temperatures at which they can develop, compared to the more restrictive range of others. Studies performed in other lace bugs show that they are able to develop at higher temperatures (30 – 39 °C) (*Tingis ampliata* Herrich-Shäffer, Eguagie, 1972; *Corythucha morrilli* Osborn and Drake, Rogers, 1977; Stone and Watterson, 1985; *Corythucha*

cydoniae Fitch, Neal and Douglass, 1990; Braman and Pendley, 1993; *Stephanitis pyrioides* Scott, Braman *et al.*, 1992; *Leptopharsa heveae* Drake and Poor, Cividanes *et al.*, 2004; *Gargaphia torresi* Lima, Dominguez da Silva, 2004; *Carvalhotingis visenda* Drake and Hambleton, Dhileepan *et al.*, 2010; *Corythucha ciliata* Say, Ju et al., 2011; *Leptodictya plana* Heidemann, Carr and Braman, 2012; *Monosteira unicostata* Mulsant and Rey, Sánchez-Ramos *et al.*, 2015). In my study, it was observed that *Gargaphia decoris* is susceptible to temperatures ≥ 30 °C. Under laboratory conditions, eggs hatched but first instar nymph survived for less than a day under such conditions, thus no development was observed. Despite the susceptibility to higher temperatures, *G. decoris* is able to develop in colder temperatures compared to other lace bug species, in which eggs did not hatch or nymphs emerged but died shortly when exposed to temperatures < 20 °C (*Corythucha cydoniae*, Stone and Watterson, 1985; Braman and Pendley, 1993; *Gargaphia torresi*, Cividanes *et al.*, 2004; *Corythucha ciliata*, Ju *et al.*, 2011; *Leptodictya plana*, Carr and Braman, 2012; *Monosteira unicostata*, Sánchez-Ramos *et al.*, 2015).

As predicted, temperature affected *G. decoris* development. The juvenile stages were prolonged at colder temperatures (71.73 days at 15 °C vs. 24.29 days at 27.5 °C). At all temperatures, the fifth instar required the longest time, and the second instar required the least, similar to reports in other lace bugs (*Stephanitis takeyai*, Tsukada, 1994; *Carvalhotingis visenda*, Dominguez da Silva, 2004; *Leptodictya plana*, Carr and Braman, 2012; *Tingis americana*, Moreira et al., 2013). In addition, the cumulative thermal requirement the thermal constant of the fifth instar was the highest of the juvenile stages (93.46 DD) and the second instar - the lowest (48.31 DD). The longer development time of fifth instar nymphs, together with their higher thermal requirements could be the result of higher energy consumption and longer time needed to cope with the physiological changes necessary for the transformation from nymph to adult stage.

The lower threshold temperature for *G. decoris* egg development was set to 10 °C. At 10 °C, eggs failed to hatch after one month of observations. The lower egg development threshold observed in *G. decoris* was only comparable with the lace bug *Tingis ampliata* which had a threshold of about 9 °C (Eguagie, 1972). In other lace bugs, egg development stops at 16 °C (Ju et al., 2011; Sánchez-Ramos et al., 2015).

The cumulative thermal requirement needed for the complete development of *G. decoris* (384 DD) was within the thermal constant range (318.2 – 398.4 DD) reported in other lace bug species: *Leptopharsa heveae* (370.4 DD) (Cividanes *et al.*, 2004), *Corythucha ciliata* (370.57 DD) (Ju *et al.*, 2011), *Corythucha cydoniae* (318.2 DD) (Braman *et al.*, 1992), *Stephanitis pyrioides* (398.4 DD) (Braman *et al.*, 1992) but was higher than that reported in the lace bug *Gargaphia torresi* (209.4-220.3 DD) (Dominguez da Silva, 2004).

The highest total development survival (first instar to adult) observed in *G. decoris* was at 25 °C (39.77%) and the lowest was at 15 °C (27.59%). The survival observed at 25 °C was higher than that observed in the lace bug *Corythucha cydoniae* (30.9%) (Braman *et al.*, 1992), but lower than the survival observed in the lace bug *Leptopharsa heveae* (62.5%) (Cividanes *et al.*, 2004). The low survival observed in *G. decoris* in my experiment could be attributed to the reduced amount of plant resources available for the insect to acquire the necessary nutrients before the foliage began to deteriorate. In my experiment, plants were renewed when leaves had at least 75% damage, but it is possible that plant renewal should have been more frequent, even if visually the plant had less than 75% damage. However, the disadvantage of changing plants too often is the increase in handling, which could have increased nymphal mortality either by damaging the nymphs or dropping the nymphs while transferring them to a new *Solanum mauritianum* plant.

G. decoris females outlived males at each temperature, with the highest mean lifespan of sexes 68.95 and 41.94 days, respectively. These results are opposite from the lifespan trends observed in the lace bugs *S. pyrioides, C. cydoniae, C. ciliata and G. torresi*, in which males lived longer than females at all temperatures (Braman *et al.*, 1992; Braman and Pendley, 1993; Cividanes *et al.*, 2004; Dominguez da Silva, 2004; Ju *et al.*, 2011). However, a slightly higher mean female lifespan was observed in the lace bug *Leptoypha hospita* (60.1 days for males and 79.9 days for females) when tested at 24-26 °C (Zhang *et al.*, 2011). The higher lifespan observed in female *G. decoris* could be due to the energy that males have to expend for courting and copulating. For example, copulation reduced male but not female lifespan in the fly *Saltella sphondylli* (Diptera: Sepsidae) (Martin and Hosken, 2004). It was observed that in *G. decoris* males are the ones that approach *G. decoris* females to copulate (C. Falla, own data). The courtship starts by a

male fluffing its wings in front of the female, moving towards the female at a quick pace until both male and female are front to front. The male exerts some frontal pressure that lightly lifts the female body. Afterwards the male directs his abdomen in a 45-90 degrees angle to be able to copulate. This courtship often lasts more than 15 minutes without the male being successful when the female was reluctant to mate, and the male can perform this courtship rituals several times even when the female is reluctant (C. Falla, own data). Therefore, these interactions are costly for the male and could negatively affect its lifespan.

4.4.2. Effect of temperature on *G. decoris* fecundity

Unlike *L. heveae*, and *Carvalhotingis visenda*, *G. decoris* fecundity was affected by the temperature (Cividanes *et al.*, 2004; Dhileepan *et al.*, 2010). Fecundity was highest at 25 °C and lowest at 15 °C. The number of laid eggs per female ranged from 15.25 - 62.09 and was lower than that reported for *Tingis americana* (310) (Moreira *et al.*, 2013), *L. hospita* (240) (Zhang *et al.*, 2011), *Oncochila simplex* (175) (Pecora *et al.*, 1992), *C. morrilli* (97-330) (Stone and Watterson, 1985) and *C. ciliata* (87.7 – 286.8) (Ju *et al.*, 2011). The highest number of eggs laid per female observed in my study (62.09) is similar to the lowest number (61) observed by Dominguez da Silva (2004) in his study with *G. torresi.*

Under field conditions, it is expected that *G. decoris* would have a higher fecundity. Under experimental conditions, females were paired and allowed to copulate and lay eggs during their lifetime. However, when males died during the experiment, they were not replaced and females remained unpaired until they died. It is possible that in field conditions, females will have a higher number of eggs laid because they will be exposed to an unlimited supply of males that come from overlapping generations. The population growth parameters reported in this study show that there is an increase in population growth in all temperatures tested. The net reproductive rate (R_0) was higher and females reproduced at a higher rate at 25 °C, followed by 20 °C, and lowest at 15 °C. Lower temperatures (15 °C) increased the doubling time and mean generation time of *G. decoris* population. Similar reports were observed in the pear lace bug *Stephanitis pyri*, when population growth parameters were tested under 20-32 °C (Aysal and Kivan, 2008).

According to the results in my study, *G. decoris* had the highest survival and fecundity at 25 °C, however, 20 °C was used as the constant temperature to test differences in development and fecundity under different photoperiods and relative humidity.

4.4.3. Effect of photoperiod and humidity on *G. decoris* development and fecundity

Besides temperature, photoperiod and humidity play an important role in the development, survival and reproduction of insects (Tauber *et al.*, 1986). Results from my study show significantly higher percentage of egg hatch, total survival and total number of laid eggs under longer photoperiods. However, other variables measured were not influenced by the photoperiods tested. *G. decoris* appears to behave as a "long-day" insect, a term that was given to those insects that develop without interruption under the influence of 24-hour photoperiods containing long photophase (>15 hours) or short photophase (< 9 hours) (Beck, 1962). Despite the ability to develop continuously across 24-hour photoperiod, there are critical photoperiods that induce a short diapause in some "long-day" insects. For example, when European corn borer *Ostrinia nubilalis* (Hübner) was subjected to long and short photoperiods it was expected that short photoperiods would induce diapause effects. However, percent mortality was higher and similar in both long and short photoperiods and photophase between 9-15 hours increased *O. nubilalis* mortality (Beck, 1962). The higher survival of *G. decoris* adults under long and short photoperiods could be related to species-specific particular time for locomotion, feeding, mating and oviposition (Beck, 1980). The population growth parameters show that *G. decoris* is able to sustain a positive population growth under all photoperiods tested, but there is higher population growth at a faster rate at 16L:8D photoperiod. Under the photoperiods tested there was no evidence of reproductive diapause as observed in other hemipterans (Wheeler, 2001; Mourao and Panizzi, 2002; Hou *et al.*, 2016). However, it is possible that changes in temperature and humidity modify the effects of photoperiod, particularly in temperate species like *G. decoris* (Tauber *et al.*, 1986) and that reproductive diapause (i.e. females delaying oviposition) might be induced at temperatures lower than 15ºC.

Humidity can affect insect fecundity and fertility (Leather *et al.*, 1995) and in many insects, increasing humidity increases the oviposition and egg hatch (Eguagie, 1972;

Leather and Awmack, 1998). Humidity was not a factor that affected the reproductive parameters of *G. decoris*, however, higher humidity (70 ± 10%) decreased the percentage of egg hatch. The high mortality observed in eggs had been attributed to an increase in susceptibility to fungal pathogens (Leather and Awmack, 1998) and this is in accordance to observations in my experiment. Many of the egg clutches incubated under high relative humidity were covered with a white fungus that prevented eggs from hatching and those nymphs that emerged got entangled between the hyphae (fungal branching filaments) and died. Thus, the higher mortality during egg stage under high relative humidity could explain the higher egg hatch observed in lower humidity (50 ± 10%).

G. decoris adults survived longer under higher humidity; this in accordance with studies performed in three dominant pest species of the alfalfa plant bug *Adelphocoris* spp. (Pan *et al.*, 2014). The population growth parameters were affected by humidity. Despite having a higher net reproductive rate (R_0) under high humidity, the rate of increase was higher at low humidity, thus, the doubling time (DT) and mean generation (T) time were shorter under low humidity. The higher rate of increase (r_m) at low humidity could be because low humidity conditions being better for feeding, as has been observed in a study with spider mites (Boudreaux, 1958). Spider mites need water-loss evaporation from their cuticle to efficiently utilize the ingested liquid cell contents. Low relative humidity could favour *G. decoris* by allowing the ingestion of liquid cell contents with a much higher concentration of nutrients and a much more rapid utilization. This rapid utilization of food could stimulate production of eggs at a much higher rate than happens under moist conditions (Boudreaux, 1958).

4.4.4. CLIMEX modelling

The introduction of selective exotic natural enemies as a part of classical biological control method has been implemented as an alternative option to conventional weed control methods (Culliney, 2005). The classical biological control of weeds involves the importation, colonization and establishment of exotic natural enemies (McFadyen, 1998). This method relies on an inoculative strategy, where natural enemies are liberated over a period of time to ensure their establishment (Culliney, 2005). Climate (i.e. temperature and humidity) is a determinant factor for the establishment of species (Byrne et al., 2002) and in

order to ensure the successful establishment of biological control agents, software packages (e.g. CLIMEX, NAPPFAST) are used to incorporate knowledge of an organism's response to environmental variables, particularly climate data, retrieved from direct observations (Ireland et al., 2013). These models use the information on the species survival and development as a function of climatic factors, in order to estimate the potential of the species to establish in sites where suitable conditions are available.

In 2009, Environment Bay of Plenty, applied to the Environmental Risk Management Authority for a permit to import and release the lacebug *G. decoris* as a biological control agent in New Zealand for the weed woolly nightshade *Solanum mauritianum* (ERMA, 2009). The petition was approved and since then, multiple liberations have been completed in the North Island using the climatic region of its reported native range (i.e. southeastern states of Brazil and Misiones, Argentina (Hope and Olckers, 2011)) as a guideline.

The CLIMEX model developed in this study suggests that *G. decoris* could occupy broader regions than what is currently reported. However, this increase in distribution will depend on the availability of the host plant woolly nightshade. It is also likely that even if the climate is appropriate for *G. decoris* development, the population could be limited by the presence of predators (invertebrates and vertebrates), pathogens and other phytophagous invertebrates that compete for the same resource (Pedrosa-Macedo *et al.*, 2003).

Regarding the global predicted distribution of *G. decoris*, the model was well validated. Experimental data for temperature were used to construct the model, and from the native range I established the moisture index. I used distribution data from South America and my own data to predict the distribution of *G. decoris* in South Africa and New Zealand. Predicted distribution well matched the known distribution records of *G. decoris* in its native range and in those countries where the lace bug was introduced and has been established as a biological control agent, i.e. South Africa and New Zealand.

The Brazilian stock of *G. decoris* was selected because of its cold tolerance being able to match New Zealand and South Africa field conditions (Hope and Olckers, 2011). Despite its cold tolerance, the CLIMEX model predicted that the lace bug will fail to establish optimally in the New Zealand South Island, due to the lower temperatures during

summer in these regions (i.e. Invercargill (8.8-9.8 °C), Milford Sound (8.2 – 9 °C), and lowest temperatures recorded of -6.1 °C; Macara, 2013). The temperatures in the South Island are below the lower temperature threshold for *G. decoris* development obtained in this study, thus it is expected that eggs and nymphs will not be able to develop under these climatic conditions.

Currently, the potential for *G. decoris* establishment is promising in New Zealand with recent reports of establishment in Northland (Paynter, Q., pers. communication). It is unknown how the establishment of this insect may be affected in the future due to climate change. In my study predictions of establishment due to climate change were not investigated, but this could be worth exploring in the future, because regions that are deemed unsuitable now could become suitable if the global temperature rise. Alternatively, establishment can be contracted or limited due to changes in climatic conditions (Sutherst et al., 2007).

The CLIMEX model uses climate-related features and meteorological data but does not incorporate other factors, for example the presence of predators, host presence and possible plant-insect interactions. It has been suggested that predators and host plant quality could be the factors that affect *G. decoris* establishment (Patrick and Olckers, 2014). These variables could be significant factors influencing survival, dispersal and establishment of the species and if incorporated, can affect the CLIMEX model predictions (Verkerk, 2004). Despite these limitations, the CLIMEX model in my study has delivered important information about the potential distribution of *G. decoris*. In addition, for management purposes, it is practical to liberate the biological control agent in those areas that are not only climatically suitable but also with a strong presence of the weed.

4.5. Concluding remarks

Temperature, photoperiod and humidity play an important role in *G. decoris* development, nymphal survival, longevity, fecundity and population growth parameters. Compared to other lace bugs that have been studied so far, *G. decoris* seems to be able to withstand cooler temperatures, but is not able to survive warmer temperatures (> 30 ºC). *G. decoris* has a preference for long days (16L:8D hours) followed by short days (8L:16D hours), suggesting that this insect is a "long-day" insect and the insect life cycle benefits from a

combination of long and short periods of light. Humidity was not a factor that affected *G. decoris* reproductive parameters; however, it affected the percentage of egg hatch, longevity and population growth parameters. It seems that at higher relative humidity the eggs are more prone to being affected by fungus than at lower relative humidity, but adults survive longer at higher humidity. Temperature development data was used to create a model using CLIMEX (Hearne Scientific Software Pty Ltd, Australia). The model predictions fits the current records of *G. decoris* in its native range in Brazil and Argentina, and the reported adventive records in South Africa and New Zealand, where it has been released as a biocontrol agent against woolly nightshade. The model's predicted distribution of *G. decoris* also agrees well with the reported distribution of woolly nightshade worldwide, suggesting that *G. decoris* has strong potential as a biocontrol agent for this weed. Globally, the model predicted that *G. decoris* could potentially establish in tropical and Mediterranean climates in addition to temperate climates. The results reported in this chapter may prove useful for the identification of areas suitable for the release of *G. decoris,* and will help to understand the thermal requirements and susceptibility of this lace bug to environmental conditions.

CHAPTER 5: EFFECT OF LIGHT INTENSITY ON THE PERFORMANCE OF WOOLLY NIGHTSHADE (SOLANACEAE) AND ITS BIOLOGICAL CONTROL AGENT *Gargaphia decoris* (HEMIPTERA: TINGIDAE)

ABSTRACT. The lace bug *Gargaphia decoris* Drake has been released in New Zealand for the biological control of woolly nightshade *Solanum mauritianum* Scopoli. At one of the release sites it was observed that *G. decoris* more commonly infests woolly nightshade growing in shaded woody locations, compared to woolly nightshade plants growing in open, sunny fields. To explore the mechanisms for this preferential selection, the objectives of this study were to determine the effect of light intensity on the performance of woolly nightshade and *G. decoris* under glasshouse conditions. One hundred ten-day old plants were placed under shaded and unshaded treatments (Day 1). Sub-samples of plants were harvested and physical and chemical plant traits were measured at Day 30 (first harvest) before plants were infested with *G. decoris.* Half of the remaining plants were infested with lace bugs after the first harvest (Day 30). Physical and chemical plant traits were measured again on Day 48 and Day 90 of the experiment (2^{nd} and 3^{rd} harvest, respectively). Insect performance indicators were measured on Day 40, Day 65, and Day 80 after infestation and compared between the shaded and unshaded treatments.

Results show that new leaves of older plants under unshaded conditions were smaller, thicker, had longer trichomes, lower water content and lower specific leaf area compared to shaded leaves. Shaded plants were taller, with higher shoot-to-root ratios, and new leaves of older plants had higher photosynthetic efficiency compared to unshaded plants. At the second harvest, leaves of unshaded plants had higher C:N ratio and total glycoalkaloid concentration. At the second harvest *G. decoris* showed no discrimination between shade and unshaded leaves for ovipositing or feeding. However at the third harvest, when a lower C:N ratio and total glycoalkaloid concentration was observed in leaves from both shaded and unshaded plants, first generation *G. decoris* females failed to oviposit on unshaded plants and displayed smaller bodies compared to the females that developed feeding on shaded plants.

It is concluded that light intensity did affect woolly nightshade and *G. decoris* performance, but it remains unclear which is the key factor that explains the differential performance of *G. decoris* under contrasting light conditions.

5.1. Introduction

Host selection by a herbivorous insect is based not only on physical and chemical traits of the host plant itself but also on the environment in which the plant grows (Awmack and Leather, 2002; Barber, 2010). Light intensity is an abiotic factor that is known to affect quality traits of the plant host and performance of the insect herbivore (Potter, 1992; Trumbule and Denno, 1995; Connor, 2006; Barber, 2010; Diaz *et al.*, 2011). High light intensity has been reported to limit the establishment of a biological control agent (the lace bug *Gargaphia decoris* Drake) introduced to South Africa to control the weed woolly nightshade (*Solanum mauritianum* Scopoli) (Patrick and Olckers, 2014). The lace bug has also been released in New Zealand to control woolly nightshade, and it was observed at one of the lace bug release sites that the insects have preferentially established on woolly nightshade plants growing in shade (C. Falla, own data). It has been suggested that high light intensity may reduce the host quality of the woolly nightshade plant through the production of physical and chemical defences (Mattson, 1980; Coley, 1987; Herms and Mattson, 1992). To explore whether physical and chemical defences can explain the preferential selection of woolly nightshade in shaded conditions, the objectives of this study were to determine the effect of light intensity on the performance of woolly nightshade and *G. decoris* under glasshouse conditions.

Based on the observations in the field, the following research questions were proposed: (1) To what extent are key physical and chemical characteristics of woolly nightshade affected when plants are grown under contrasting light conditions (i.e. sun vs. shade)? (2) Do light-mediated changes in plant quality affect the performance of *G. decoris*? (3) Does the presence of *G. decoris* affect key physical and chemical characteristics of woolly nightshade?

I hypothesized that: (1) woolly nightshade plants will perform better in full sunlight; (2) light intensity will affect plant resource allocation (carbon and nitrogen), influencing the production of physical (e.g. trichomes) and/or chemical (e.g. glycoalkaloids) defences; (3)

total concentrations of glycoalkaloids will be higher in woolly nightshade plants grown in full sunlight compared to shade-grown plants; (4) *G. decoris* survival, fecundity and fertility will be higher on shade-grown woolly nightshade; (5) the presence of *G. decoris* will affect resource allocation in woolly nightshade, and as a result affect the production of physical and chemical defences.

The results from this study will help to understand how plant-insect interactions are influenced by light intensity, and in the case of *G. decoris*, help to determine if light intensity plays a role in insect establishment. This information can be useful for decision making in biological control programmes that target woolly nightshade through optimization of insect releases in the field.

5.2. Materials and Methods

5.2.1. Plant material

Woolly nightshade seeds were sown at the beginning of September 2015 in a 60-cell growing tray (45 ml plug size) with Daltons Premium seed mix. When seedlings developed a strong root system, and cotyledons reached a length of approximately 3 cm (10-day old after germination), they were transplanted to 5 L pots one day before the experiment started (October 2nd) using a soil mixture which included Woodace® Longterm fertilizer (18% nitrogen, 2.2% phosphate, 8.3% soluble potash and micronutrients). All pots had four holes of the same size underneath to standardize the amount of water they absorbed and retained. The pots were positioned on tables covered with capillary matting (Lysdrain Plus; Cosio Industries Ltd) in a glasshouse located in the Plant Growth Unit of Massey University, Palmerston North, New Zealand. A drip irrigation system provided water underneath the pots. The drip irrigation system was programmed to deliver water at a flow rate of 8 L/hour for two minute durations at 08:00, 11:00, 14:00, and 17:00 hours daily. Seedlings that were allocated for insect breeding were transplanted to 0.75 L pots with the same soil mixture used for the experiment and placed in a neighbouring glasshouse where irrigation was performed manually twice a day (at 08:00 and 16:00 hours) daily.

5.2.2. Experimental setup in the glasshouse

A 10 x 10 Latin square design was used to arrange the positioning of experimental treatments in the glasshouse (Fig. 5.1). The two blocking factors were shade treatments: "Unshaded" (< 10% light reduction, see 5.2.3) and "Shade" (70-80% light reduction) placed in rows next to each other and the subjects were 100 pots with woolly nightshade plants, arranged in columns of ten. Each shade treatment was replicated five times, making fifty plants allocated per shade treatment.

Subsamples of plants were randomly selected to be harvested for plant growth measurements (section 5.2.5) and/or chemical analysis (section 5.2.7) (Fig. 5.1). Three harvests were undertaken. Twenty plants were destructively sampled during the first harvest on 2 November 2015 (day 30 after the shade treatments were allocated). Out of 80 plants remaining after the first harvest, half (40 plants) were randomly infested with lace bugs, providing a total of twenty infested plants in each of the second and third harvests, and twenty infested plants per shade treatment. Forty plants (ten from each treatment) were randomly allocated to the second harvest on 30 November 2015 (Day 48). The remaining forty plants were harvested on 11 January 2016 (Day 90).

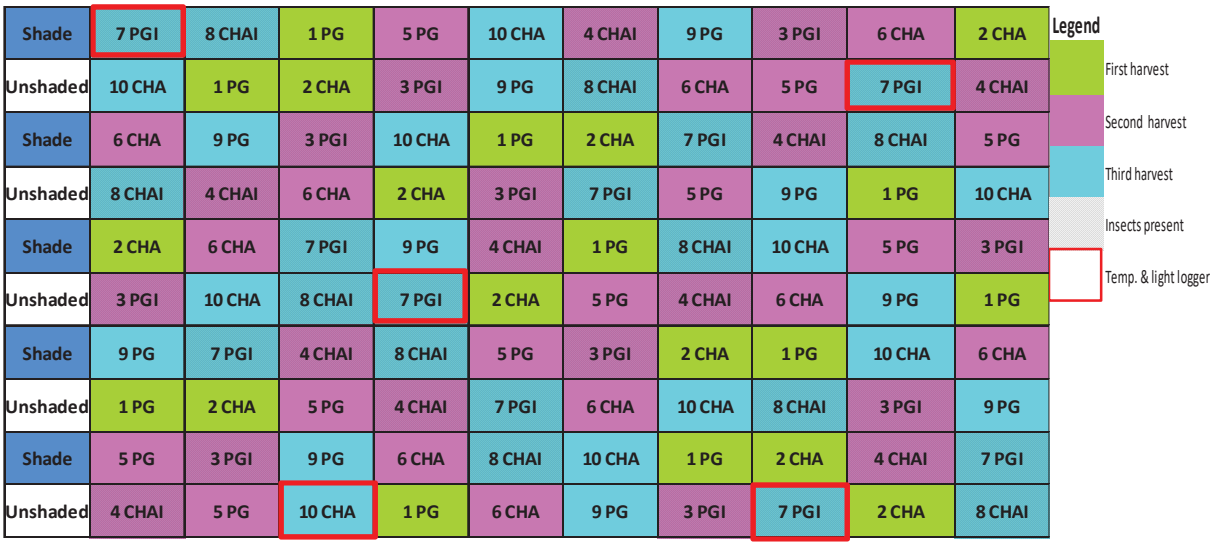

Figure 5.1. A 10 x 10 Latin square experimental design showing the shade treatments as rows and the woolly nightshade plants as subjects in columns. The shade treatments (first column) are "shade" (blue), representing 70-80% shade, and "unshaded" (white) representing < 10% shade. Plants randomly allocated for plant growth measurements and chemical analyses are represented with the letters PG and CHA (no-insect) and PGI and CHAI (insects present), respectively. The location of temperature and light intensity loggers is represented by red boxes.

5.2.3. Shade treatments

To guide the appropriate level of shade applied in the experiment, natural light conditions were measured at the first release site of *G. decoris* in New Zealand (released in 2010), where preferential establishment had been observed. The release site is located near Ngapeke Road, Bay of Plenty (E1883871 N5818164; NZTM2000). Within the release site, two areas were identified according to their light condition and abundance of *G. decoris*. In the first area, woolly nightshade was growing under pine trees, where *G. decoris* was abundant and in the second area, it was growing in the open, under full sunlight and *G. decoris* was largely absent. These areas were denominated as "shaded" and "unshaded" conditions for future reference. Light intensity, measured as photosynthetically active radiation (PAR; 400-700 nm), was recorded at shaded and unshaded conditions at the release site in 2015 using a portable quantum sensor (LI-250; LICOR®, Lincoln, Nebraska,

USA) (Fig. 5.2). At both shaded and full sun locations, light intensity was measured at five point locations adjacent to the top fully expanded mature woolly nightshade leaves. The mean light intensities observed were 59.05 ± 27.25 µmol m^{-2} s^{-1} for shade and 1602.93 ± 88.5 µmol m^{-2} s^{-1} for full sun conditions, indicating that shade conditions received about 3% sunlight compared with full sun conditions.

Figure 5.2. Measuring photosynthetically active radiation (PAR) among woolly nightshade plants growing in unshaded (left) and shaded conditions (right).

The PAR measurements obtained in the field were used as a guide to determine the shade treatments to be used for the glasshouse experiment. The glasshouse light measurements performed in a single clear day every hour starting from 09:00 to 15:00 (835.30 ± 76.70 µmol m^{-2} s^{-1} without cover) represented 52% less light than was recorded in the field for full sun conditions. However, this value represents full sunlight condition for the purpose of this experiment. The "full sunlight" (no-covering) light treatment could not be used in the experiment because some plants were going to be infested with lace bugs, which needed to be contained. The shading provided by tulle netting and two shade cloths, with stated levels of shading of < 10%, 50-60%, and 70-80%, respectively, was tested over a period of one day (Fig. 5.3). The tulle netting cut out more than its stated nominal level of shading (< 10%), cutting out approximately 63.44% of the light (average light intensity under tulle 529.95 ± 62.45 µmol m^{-2} s^{-1}, Fig. 5.3). The tulle cloth was, therefore, considered to represent moderate intensity light, rather than full sun conditions,

but for convenience is referred to as the "unshaded" treatment in the remainder of this chapter.

The 50-60% and 70-80% shade cloths permitted average light intensities of 255.62 ± 67.62 µmol m^{-2} s^{-1} and 142.29 ± 40.80 µmol m^{-2} s^{-1} respectively, which represented 30.6% and 17.03% with respect to the full sunlight (no-covering) in the glasshouse. The 70-80% shade cloth was selected for "shade" treatment because it more closely approached the lower level of shade found in the field conditions.

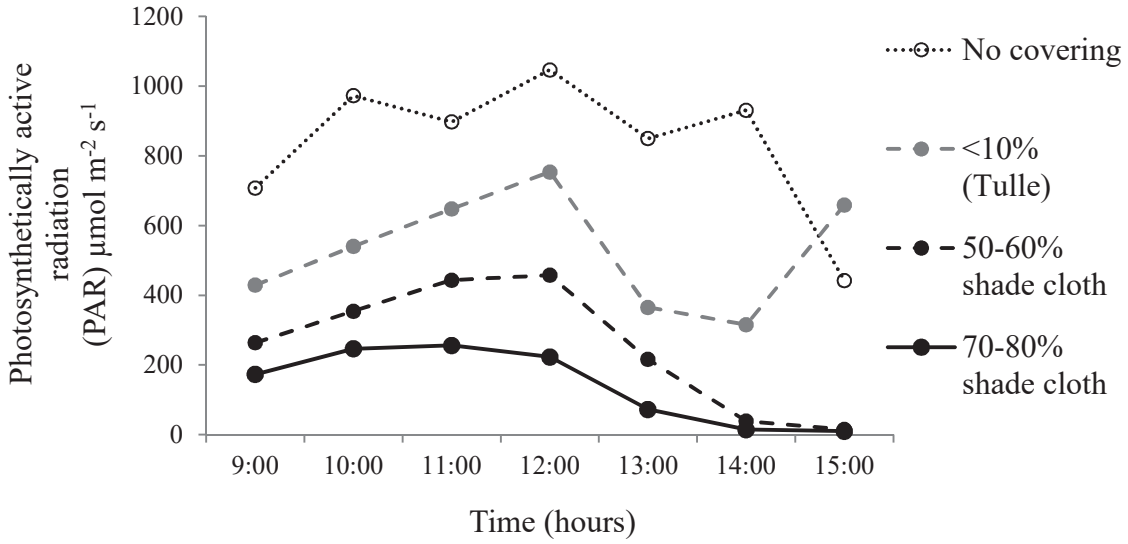

Figure 5.3. Photosynthetically active radiation (PAR) (400-700 nm) under different shade conditions, assessed in the glasshouse during a single day between the hours of 09:00-15:00.

Cages for plants (60.5 cm high x 20.6 cm long x 20.6 cm wide) were made using stainless steel rods and timber. The cages allocated for "unshaded" treatment were covered with a tulle fabric; the "shaded" treatment cages were covered with 70-80% shade cloth. The cages were placed over 10-day old woolly nightshade plants on benches inside the glasshouse (Fig. 5.4).

Figure 5.4. Experimental set-up inside the glasshouse. The green cages and the white cages represent the 70-80% and < 10% shade treatment, respectively.

Temperature and humidity in the glasshouse were recorded using Tinytag Plus 2 data loggers (TGP-4500; Gemini Data Loggers, Chichester, UK) and light intensity was recorded using Hobo® 8k pendant (Onset Computer Corporation, Bourne, MA, USA). Both types of loggers were programmed to record every 15 minutes. The light intensity loggers were placed inside randomly selected cages at close proximity to the top leaves of the plant, while trying to avoid the shade produced by the closest leaf. The temperature loggers were positioned in the same cages to avoid direct sunlight. The position of the loggers with respect to the experimental design is shown in Figure 5.1. In addition to the temperature loggers that were placed inside the cages, a temperature logger (HortPlus™) was installed inside the glasshouse (attached to the ceiling) and was programmed to record temperature every 10 minutes.

5.2.4. Plant measurements

For each randomly selected plant, at each harvest, the following parameters were measured (see details below): a) plant height; b) shoot-to-root ratio; c) moisture content; d) specific leaf area; e) chlorophyll content; h) leaf thickness; i) trichome density; and j) trichome length. Measurements of net photosynthesis were only performed in the first harvest and chlorophyll fluorescence measurement only in the third harvest.

The height of each plant was assessed with a measuring tape before positioning the cages on Day 1 of the experiment and on the day of each harvest (Day 30, 48 and 90). The height was measured from the soil surface to the top of the newest shoot. Roots were washed in a dark plastic container, using a hand held shower hose. Particular care was taken to extract as many roots as possible from the plastic container. However, really fine roots were excluded because as reported by Berhongaray *et al.* (2013), the recovered fine roots after one hour picking only represent 2% of the total root biomass. Washed roots were placed in paper bags and oven-dried in a Watvic convection oven (Watson Victor Ltd; Wellington, NZ) for 72 hours. The shoot-to-root ratio (S:R) was calculated as *S:R = dry weight of the shoot/dry weight of the roots.*

Water content (WC) was calculated using the fresh and dry biomass of all leaves, petioles, stems, flowers and fruits of the plant. After each harvest, each plant was separated into leaves, stems, petioles, flowers, and fruits, which were then placed in sealed plastic bags, separate for each plant. The fresh material was then weighed using precision scales (Sartorius Universal, Göttingen, Germany; ± 0.01 g). The samples were then removed from plastic bags, placed in individual paper bags, and oven dried (Watson Victor Ltd; Wellington, NZ) at 60 °C for 72 hours then weighed again. The moisture content was calculated as: *MC = ((Initial fresh weight – Oven-dry weight)/ Initial fresh weight)*100.*

To determine the specific leaf area (the ratio of leaf area to dry mass), the area of the third and fourth mature leaves from the top of each plant was measured with a leaf area meter (LI-3100; LICOR® Biosciences, Lincoln, NE, USA) in a laboratory with a 0.1 mm^2 resolution (Fig. 5.5). These leaves were then dried and weighed. The specific leaf area was calculated as: *Specific leaf area (SLA) = one-sided area of a fresh leaf/dry-oven weight* (Ackerly *et al.*, 2002).

Figure 5.5. Leaf area meter used to measure woolly nightshade leaf area.

The chlorophyll content was determined using a chlorophyll meter (CCM-200 plus; Opti-Sciences, Inc., Hudson, NH, USA). The chlorophyll meter uses the transmittance of light to estimate the chlorophyll content (*a* and *b*) in leaf tissue and for this, the absorbance of two wavelengths is used. One wavelength falls within the chlorophyll absorbance (653 nm) and the other wavelength (931 nm) is used to compensate for mechanical differences (e.g. leaf thickness). The device measures the transmittance of both wavelengths and calculates a chlorophyll content index (CCI) that is proportional to the amount of chlorophyll in the sample (Opti-Sciences, Inc. 2005). Chlorophyll content measurements for each plant were performed in four different locations on the third and fourth intact mature leaves of the plant, avoiding the midrib and major veins (Fig. 5.6.).

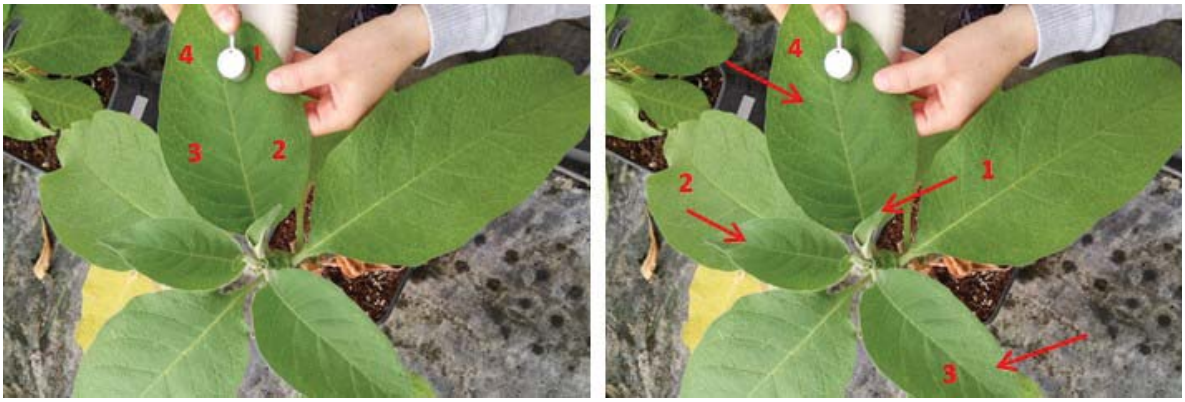

Figure 5.6. Leaf chlorophyll measurements performed on leaves of woolly nightshade. LEFT: numbers (1-4) represent points on the leaf where measurements were taken. RIGHT: arrows and numbers represent the leaf age (1 –the youngest leaf); 3rd and 4th leaves were selected to perform measurements.

The net CO_2 exchange rate (net photosynthesis, P_n) was measured on the day of first harvest prior to collecting leaves on the third and fourth mature leaves of the plant using a portable photosynthesis system (CIRAS-2; PP System; MA, USA). Measurements were performed under a PAR of 1000 µmol m^{-2} s^{-1} and a CO_2 concentration of 420 ppm.

Chlorophyll fluorescence was measured on the day of the third harvest prior to collecting leaves on every plant allocated to plant growth measurements using a field fluorescence monitoring system (FMS 2; Hansatech Instruments Ltd, Norfolk, England). The field fluorescence system is a pulse-modulated instrument, designed to measure photosynthesis under ambient light conditions. The third and fourth mature leaves of every plant were dark-adapted using leaf clips for 30 minutes prior to measurements. The measurements were initiated by switching on the measuring light giving a measure of the fluorescence origin (F_o) (minimal level of fluorescence). Afterwards, a saturating flash of light allowed the measurement of the maximum level of fluorescence (F_m) in the dark-adapted state. The difference between F_0 and F_m fluorescence relates to the maximum capacity for photochemical energy quenching of the leaf (F_v). The maximum quantum efficiency of the photosystem II (PSII) was obtained by $F_v/F_{m..}$ Following these measurements, actinic light was applied with saturating flashes using a halogen saturation lamp. From each of these flashes the maximum fluorescence in the light (F'_m) was measured. The steady state before the flash (F_t) and the state after the removal of the flash

(F'_o) were used to calculate the efficiency of the photosystem II: $\phi_{PSII} = (F'_m - F_t)/ F'_m$ (Maxwell and Johnson, 2000; Baker and Oxborough, 2004).

5.2.5. Physical defences

Leaf thickness was measured using a digimatic thickness gauge (Absolute; Mitutoyo, UK). The measurements were performed in four different locations on the third and fourth mature leaf of each plant, avoiding the midrib and major veins.

In addition, leaf thickness measurements were performed using fluorescence microscopy. Thin cuts (< 1 mm) were made on the fourth mature leaf with a single edge blade under a microscope, avoiding major veins. The plant tissue was then placed in a concave microscope slide with a MgCl 0.2N buffer and covered with a microscope cover slip. Fluorescence microscopy was performed using a Leica TCS SP5 scanning confocal system attached to a Leica DM6000B upright microscope. All imaging was performed with a 10x (NA 0.40) lens. To visualise the chloroplasts, chlorophyll A and B were concurrently excited using 405 nm and 458 nm light and the fluorescence detection window was set for 620-750 nm. Leaf thickness was measured on the images using the image processing software ImageJ (Schneider *et al.*, 2012) (Fig. 5.7).

The density of trichomes on the third and fourth mature leaves of each plant was measured. Leaf images were taken with an Olympus SC100 camera attached to an Olympus SZX7 stereomicroscope, in four different sample leaf locations (avoiding major veins) on the lower and upper surface of each leaf. The trichome density was estimated by counting the number of stellate trichomes (i.e. trichomes with a central stalk and several radiating spikes) in each of four 4 mm^2 sample areas (Fig.5.8) using the cell counter ImageJ plugin (https://imagej.nih.gov/ij/plugins/cell-counter.html). An average trichome density was calculated from all four sample areas, for the upper and lower side of each leaf.

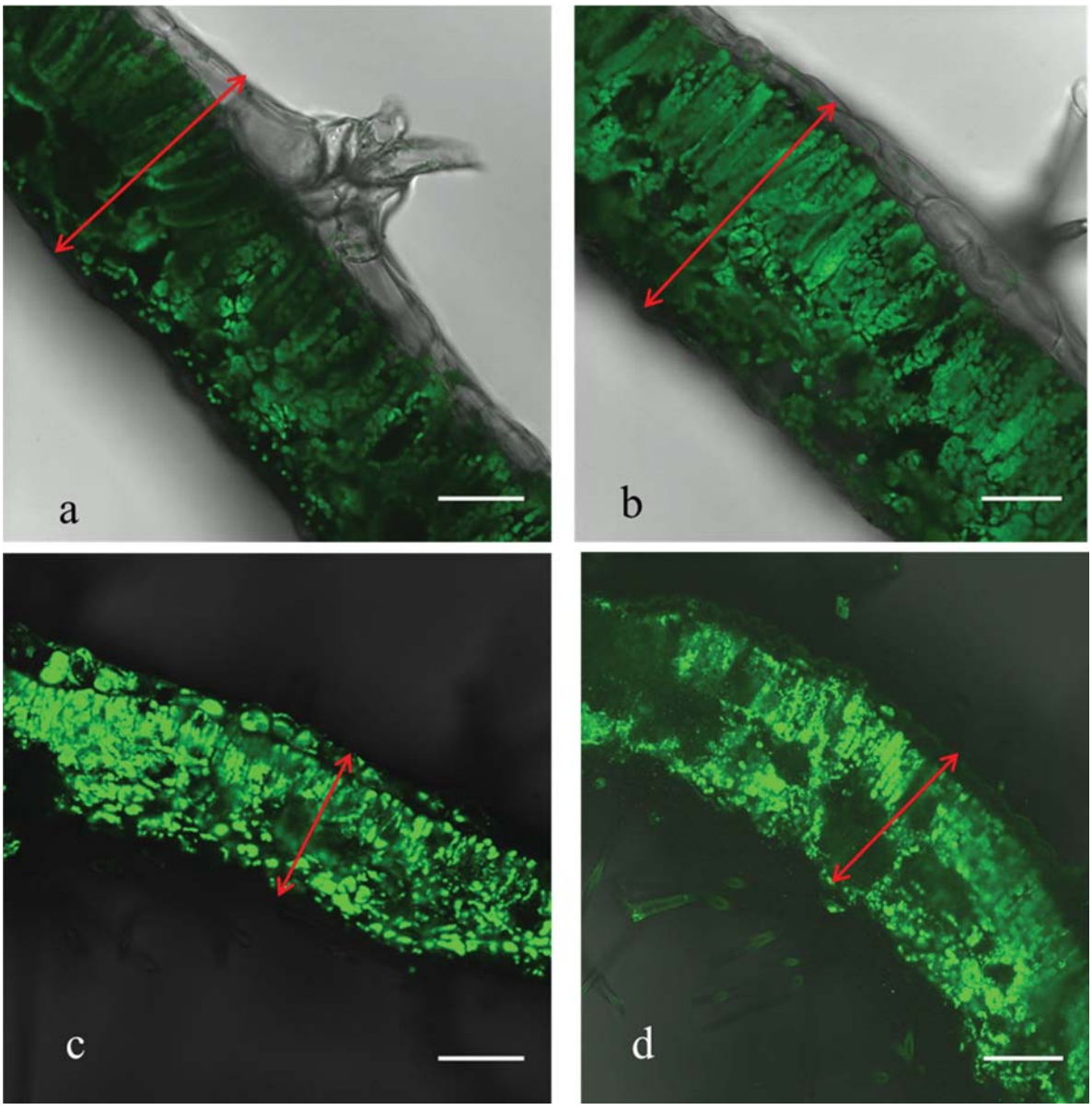

Figure 5.7. Cross-section of woolly nightshade leaves seen under confocal microscope images using a 10x (NA 0.40) lens. Differences in leaf thickness can be observed between shaded (a) and unshaded (b) leaves from the first harvest (plants 30-day old), and shaded (c) and unshaded (d) leaves from the third harvest (plants 90-day old). Double arrows (red) show the points from where leaf thickness measurements were taken. Scale bar represents 60 µm.

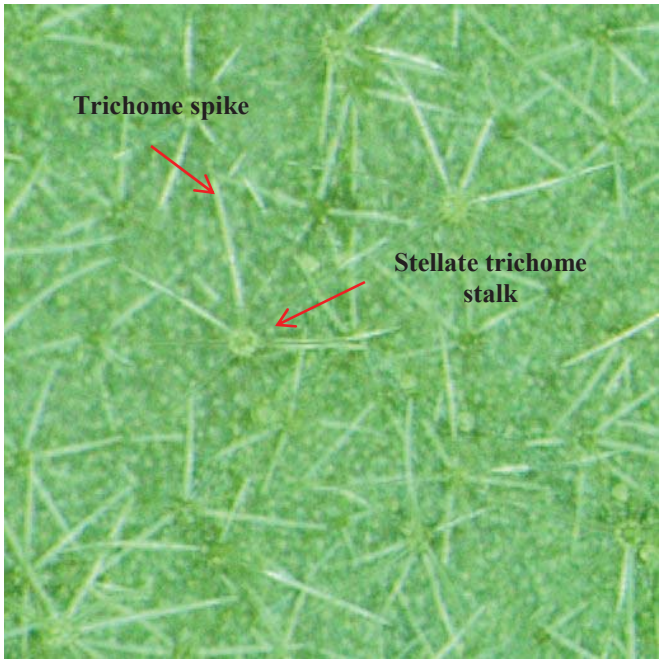

Figure 5.8. Stellate trichomes on the underside of a woolly nightshade leaf observed in a cropped 4 mm^2 sample area. Each stellate trichome consists of a central stalk with several radiating spikes.

Trichome stalk density was measured on leaves collected from all three harvests. However, only data from the first and second harvests were used in further statistical analysis, due to the difficulty in reliably counting trichomes amongst the very dense trichome mats observed on leaves from the third harvest. The average trichome spike length was estimated by averaging the lengths measured for the three longest visible spikes found in every 4 mm^2 sample area on the leaf.

5.2.6. Chemical defences

For each randomly selected plant, at each harvest, the following chemical analyses were performed: a) C/N ratio, and b) glycoalkaloid analysis.

The third mature leaf of each plant was oven-dried for 72 hours at 60 °C (Watson Victor Ltd; Wellington, New Zealand). The dried samples were pulverized for 30 seconds using a Laboratory Mixer mill (MM200, Retsch®; Haan, Germany). The C/N analysis was outsourced to Landcare Research Ltd. (Palmerston North, New Zealand). The dried samples were re-heated at 80 °C to avoid any re-absorbed moisture prior to the analysis.

The analysis was performed using a Leco TruMac Analyzer. The methodology was performed as described in Landcare Research plant testing guidelines (Landcare Research, 2018).

Glycoalkaloid analyses were performed on the fourth mature leaves from woolly nightshade plants from the three harvests. Leaves were cut, snap-frozen and loaded in a freeze drier (Cuddon FD18; Blenheim, NZ) at approximately -14 °C with a condenser set up to -30 °C. Once the vacuum was sufficient (approximately 1 millibar), the temperature was adjusted to 20 °C to speed up the sublimation process. After 72 hours of freeze-drying, the samples were kept at -80 °C until chemical analyses were performed.

Samples were ground for 60 seconds using a laboratory mixer mill. Then, 100 mg of powder/sample was weighed into a 10 ml plastic test tube to which 5 ml MeOH/MQ (80/20) was then added. All samples were vortexed and mixed on a rotary shaker for 60 minutes. Afterwards, samples were incubated at 4 °C overnight. Samples were centrifuged at 3000 rpm for 10 minutes and stored at -20 °C. One hundred µL aliquots were taken from each of the 50 centrifuged samples and then combined to make a composite sample. Twenty-fold dilutions of samples were made in HPLC (High Performance Liquid Chromotography) tubes by adding 50 µL of sample to 950 µL MeOH/MQ (80/20).

Liquid chromatography (LC) was performed using a HPLC instrument (Dionex Ultimate 3000 system; SRD-3400 degasser, HPG-34000RS pump module, WPS-3000TRS autosampler, TCC-3000RS column oven; Thermo Scientific™) coupled with Electro-Spray Ionization Mass Spectrometer (ESI-MS) (micrOTOF-QII™, Bruker Daltonics). Samples were analyzed using a Hypersil GOLD™ (Thermo Scientific™, Waltham MA, USA) column with 200 mm length, 2.1 mm internal diameter and 1.9 µm particle size, maintained at 40 °C. Glycoalkaloids were separated using solvents A and B which correspond to 0.2% formic acid and 100% acetonitrile, respectively, with a flow rate of 400 µL/min and the solvent gradient outlined in Table 5.1.

Table 5.1. Solvent programme and separation time (minutes) of glycoalkaloids.

Time (min)	Percentage of B (Acetonitrile)
0 – 0.5	10% B hold
0.5 - 7	10 – 30% B
7 - 10	30 – 35% B
10 -12	35 -75% B
12 - 19	75 – 100% B
19 - 26	100%B

*After 26 min the glycoalkaloid separation gradient returned to the starting conditions over 0.5 min and maintained that condition for 1.5 min giving a total run time for each analysis of 28 min.

Mass spectrometry (MS) was performed in positive-ion mode, using the following conditions: electrospray source endplate offset voltage = -500V; capillary voltage = -4500V; nebulizer = 21.8 psi; dry gas flow = 6.0 L/min; dry gas temperature = 225 °C. The resulting glycoalkaloid concentrations were represented as α-solanine equivalents due to the lack of appropriate standards to compare against.

5.2.7. Insect material

Prior to the experiment, *G. decoris* nymphs (5^{th} instar) were sourced from a colony maintained under controlled conditions (20 ± 0.5 °C; 14 h light: 10 h dark) in a bioassay room at Massey University. Then, the nymphs were transferred to a glasshouse located in the Plant Growth Unit, and were reared on woolly nightshade potted plants (5 L) under the same environmental conditions as the plant material prepared for this experiment. After nymphs emerged to adults, male and female lace bugs were placed separately on potted woolly nightshade plants (to avoid mating prior the experiment) and each plant was covered with a cage with stainless steel and timber framing with tulle fabric (40 openings/cm^2). Olckers (2000) reported that adult lace bugs start mating when they are approximately 11 days old. Thus, insects were kept separated for 17 days to make sure sexual maturity in both male and female was reached. After that, the insects were used for the experiment.

5.2.8. Lace bug performance

Five male and five female adult lace bugs were placed on each 'infested' treatment plant using a fine brush one day after the first harvest; these adults will be referred to as $F_{o.}$ The F_o adults were left to mate and oviposit. Two days before the second harvest (Day 38-39), the total number of eggs laid by each F_o female, the number of eggs per egg batch and the total eggs/batch/F_o female were recorded in all infested plants allocated to the second and third harvest. Then, one egg batch per plant was selected and marked with a black dot nearby (to be easily identified and to be able to follow until emergence) in those plants allocated to the third harvest. The remaining egg clutches and the F_o adults were discarded (Day 38-39) to avoid F_o adults overlap with the future adults that would emerge from the selected egg batch. At this point, no nymphs had emerged from any egg batch.

When nymphs emerged from the selected egg batches, they were allowed to complete their development into adulthood. When nymphs reached adulthood (F_1 adults), adult survival and sex ratio were recorded on Day 65. F_1 adults were left to mate and oviposit and on Day 80 and Day 87, the number of eggs per batch and number of egg batches was recorded. To count the number of eggs per batch, digital images were taken using a Canon 700D SLR camera**.** Digital images were processed in ImageJ using the plugin cell counter to count the eggs on the leaves. The F_1 adults of both sexes from each shaded and unshaded treatment were collected on Day 87 (before the third harvest) to explore the possibility that host plant quality, affected by light intensity, could affect the morphology of the insect. For this, the following morphological parameters were measured: 1) wing length; 2) wing width; 3) antennae length; 4) body length; 5) abdomen length; 6) abdomen width; 7) pronotum length; 8) pronotum width; 9) head length; 10) head width; and 11) rostrum length (for description of the morphological parameters see Chapter 3, section 3.2.2). Differences in the anatomical parameters between shade and unshaded conditions were tested using a MANOVA analysis. However, only the morphological parameters that had a correlation coefficient higher than 0.60 were included in this analysis.

5.2.9. Statistical analysis

To determine the effect of time (i.e. harvest), shade and insect presence on plant and insect performance and plant chemical composition (C/N and glycoalkaloids), data was analysed using three-way, two-way, and one-way analysis of variance (ANOVA) tests on untransformed or transformed data (square root and log transformations). This was followed by the post-hoc Tukey's Honest Significant Difference (HSD) test, to locate pairwise differences ($p \leq 0.5$ level) when overall ANOVA tests were significant.

All data were tested for normality using a qqplot and Shapiro–Wilk´s test. If the assumptions of normality were violated, data was analysed using a Kruskall-Wallis test followed by the post-hoc Dunn's test. Correlation analysis was used to test the relationship between some physical and chemical plant traits. To compare the differences in plant performance and plant chemical composition between Leaf 3 and Leaf 4 of each plant, data were analysed using a t-test for independent samples. To compare the effects of time and shade on insect performance, the fecundity of the F_o females was compared with the fecundity of the first generation females (F_1), using a paired t-test analysis. Results from the insect morphological measurements were analysed using a multivariate analysis of variance (MANOVA). Principal component analysis (PCA) was performed using ggfortify and ggplot packages, to visualize insect morphological measurements (Horikoshi and Tang, 2016; Wickham, 2009). To carry out PCA, a variance-covariance matrix was used without rotation. All statistical analyses were conducted using the software R (R Core Team, 2016).

5.3. Results

5.3.1. Environmental variables

Temperature and humidity was monitored throughout the experiment and the results show no significant difference in temperatures recorded inside shaded and unshaded framed cages (Kruskal-Wallis test, $H = 1.32$, $p = 0.858$). Similarly, no significant differences in humidity were recorded between treatments ($H = 5.99$, $p = 0.199$). Temperatures higher than 25°C were observed between the 10:00 and 16:00 hrs and during that same period of time, relative humidity (RH) lower than 60% was observed (Fig 5.9, 5.10). The data logger close to the ceiling of the glasshouse recorded a maximum average temperature of 35 ºC and a minimum average temperature of 15 ºC during January 2016, which corresponds with the third harvest. (Fig. 5.11). Light intensity was significantly different between shade and unshaded framed cages ($H = 39.95$, $p < 0.01$) with unshaded cages with an average photosynthetically active radiation (PAR) of 429.84 ± 24.74 µmol m^{-2} s^{-1} and shaded ones with 108.2 ± 7.13 µmol m^{-2} s^{-1} (Fig 5.12).

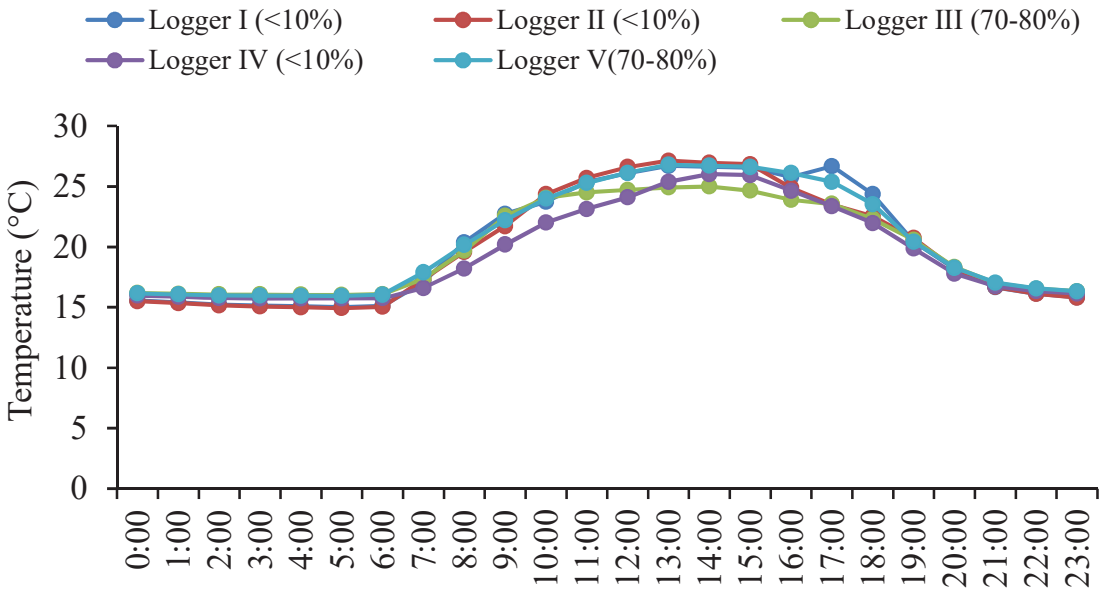

Figure 5.9. Average hourly temperature (°C) recorded from loggers placed inside shaded (70-80%) and unshaded (< 10%) framed cages, during the entire duration of the trial (October 2015-January 2016).

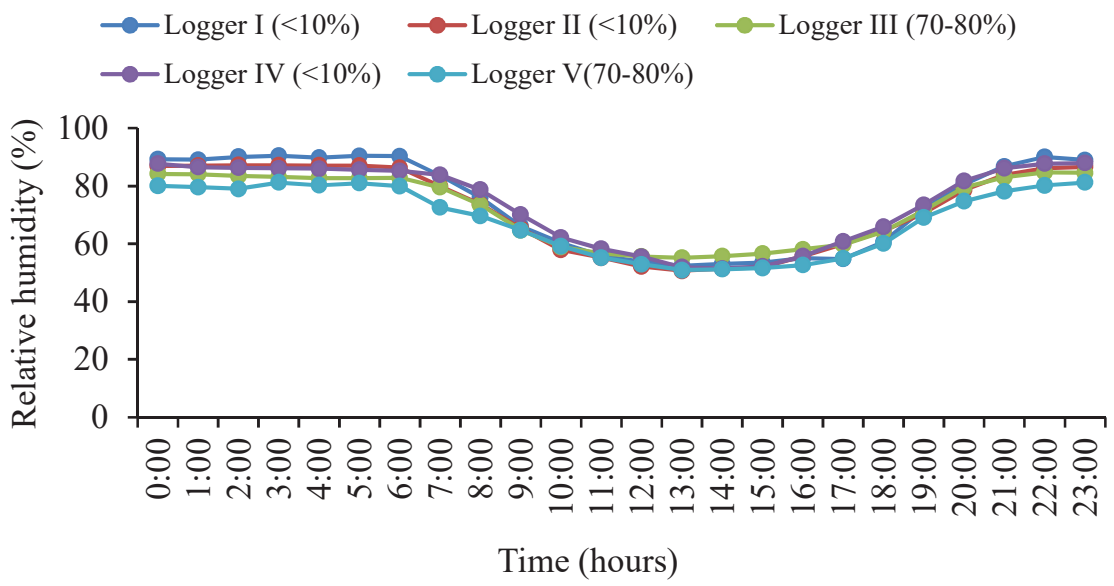

Figure 5.10. Average hourly relative humidity (%) recorded from loggers placed inside shaded (70-80%) and unshaded (< 10%) framed cages, during the entire duration of the experiment (October 2015-January 2016).

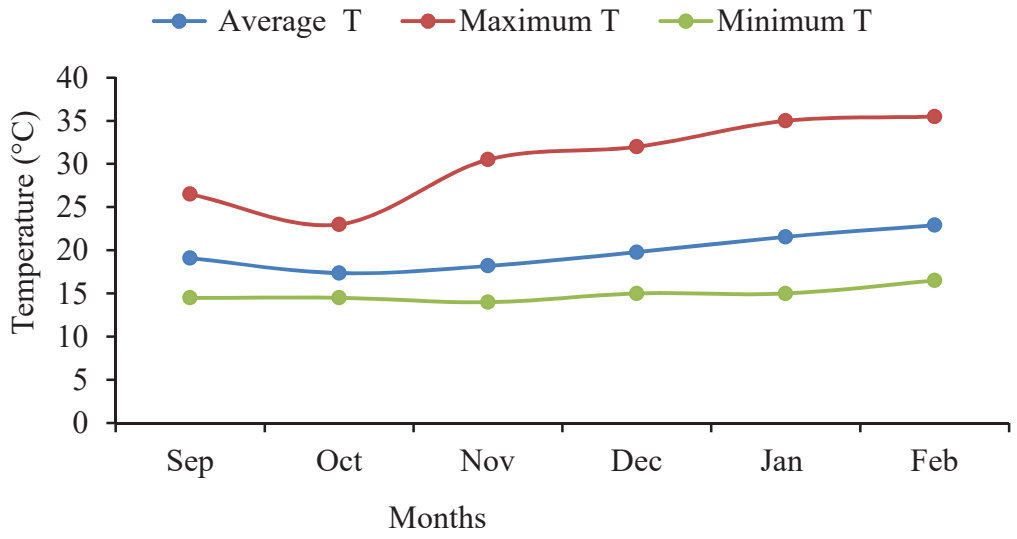

Figure 5.11. Average total, maximum and minimum monthly temperature (°C) from the months September 2015-February 2016, recorded from a temperature logger inside the glasshouse. Seedlings emerged inside the glasshouse on mid-September 2015, shade treatments began on October 2nd 2015, and the final harvest on January 11th 2016.

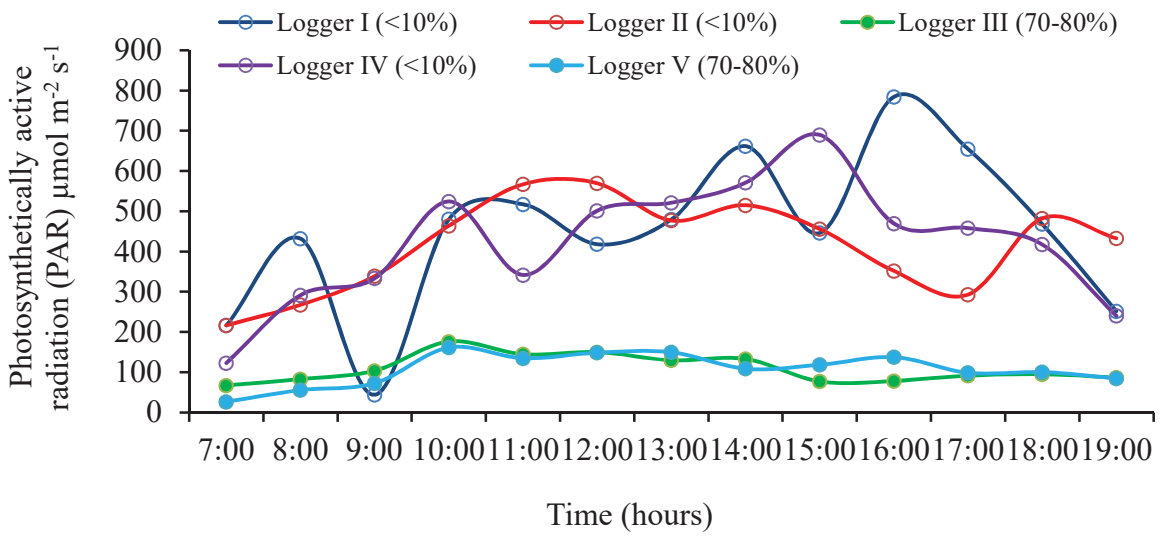

Figure 5.12. Average hourly photosynthetically active radiation (PAR) µmol m^{-2} s^{-1} recorded from loggers placed inside shaded and unshaded framed cages during the months of November 2015 to January 2016.

5.3.2. Plant performance

Growing woolly nightshade plants under shaded vs unshaded conditions did not significantly affect plant height at the first harvest (one-way ANOVA, $F_{1,8} = 1.01$, $p = 0.344$) but did so at the second ($F_{1,18} = 11.44$, $p < 0.01$) and third harvests ($F_{1,18} = 40.77$, $p < 0.01$) with shade plants from the third harvest 20.6% taller than unshaded plants ($F_{1, 44} = 40.12$, $p < 0.01$) (Fig. 5.13). Plant height was significantly different between harvests ($F_{2, 47} = 222.9$, $p < 0.01$) and was not affected by the presence of lace bugs ($F_{1,36} = 2.638$, $p = 0.113$).

Shoot-to-root ratio (S:R) was significantly higher in woolly nightshade plants under shade conditions ($F_{2,44} = 0.965$, $p < 0.05$) (Fig. 5.14). However no significant differences were observed between harvests ($F_{2,44} = 0.965$, $p = 0.389$) and no significant effect was observed due to the presence of lace bugs ($F_{1,32} = 0.696$, $p = 0.410$). The increased shoot-to-root ratios observed in woolly nightshade plants growing in the shade were largely influenced by the lower dry matter content of roots ($\bar{x}$ ± se: 5.64 ± 0.87 g) compared to the roots of unshaded plants ($\bar{x}$ ± se 14.79 ± 1.71 g).

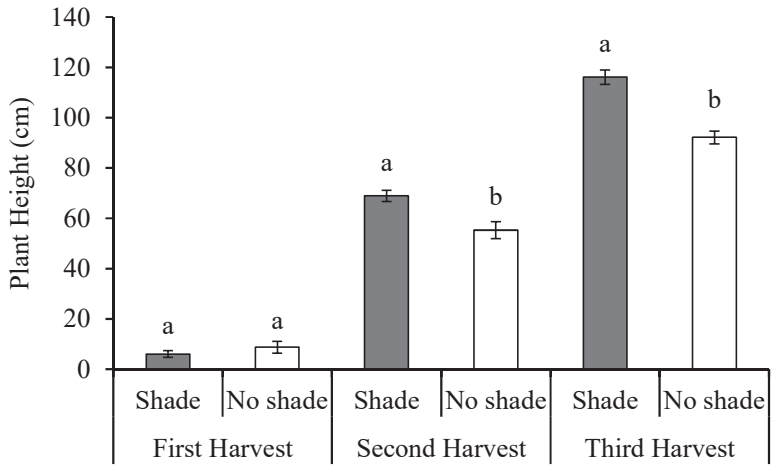

Figure 5.13. Mean height (cm) of shaded and unshaded woolly nightshade plants at the first (plants 40-days old), second (plants 58-days old) and third (plants 100-days old) harvests. Shade conditions began when plants were 10-days old. Error bars represent standard errors. Mean with the same letter indicate no significant difference between shaded and unshaded treatments (one-way ANOVA, $\alpha = 0.05$). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

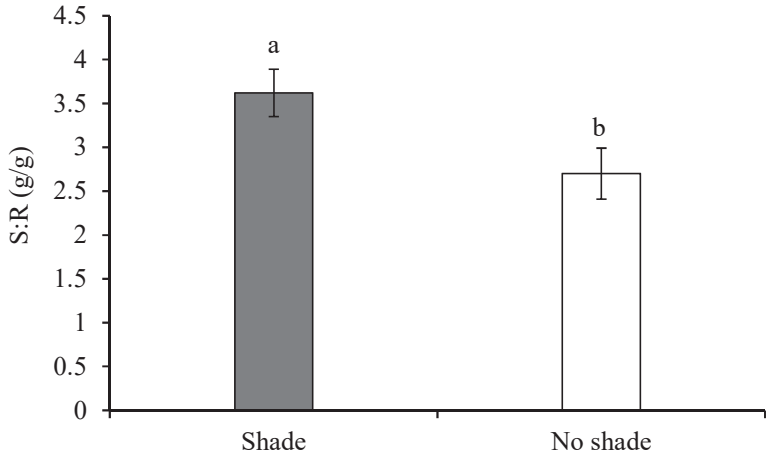

Figure 5.14. Mean shoot-to-root ratio (S:R) of shaded and unshaded woolly nightshade plants. Error bars represent standard error. Means with the same letter indicate no significant difference (two-way ANOVA, $\alpha = 0.05$).

Plants from the first harvest allocated most of their resources for the production of leaves but this changed as the plants got older. By the third harvest, fewer resources were directed to the leaves and more to stems in both shade and unshaded plants, and also unshaded plants began to produce flowers and fruits, unlike shade plants (Table 5.2).

Woolly nightshade plants (i.e. leaves, petioles, stems, flowers and fruits) subjected to shaded conditions had significantly higher water content than unshaded plants (H = 18.97, $p < 0.01$) (Fig. 5.15). Irrespective of shade condition, there was a decrease in the moisture content as the plant aged beyond the first harvest (H = 20.25, $p < 0.01$). No significant effect in water content was observed due to the presence of lace bugs (H= 0.14, $p = 0.706$).

Table 5.2. Aboveground plant dry matter (mean ± SE) from shaded and unshaded woolly nightshade plants from the first (plants 40-days old), second (plants 58-days old), and third harvest (plants 100-days old).

		Aboveground plant dry matter (%)			
Harvest	Shade	Leaves	Stem	Petioles	Flowers/fruits
First	Yes	81.85 ± 1.4	14.05 ± 1.23	4.1 ± 0.31	-
	No	81.86 ± 1.63	15.02 ± 1.43	3.42 ± 0.31	-
Second	Yes	51.53 ± 0.68	43.68 ± 0.65	4.79 ± 0.19	-
	No	57.99 ± 2.17	38.64 ± 2.2	3.37 ± 0.22	-
Third	Yes	42.92 ± 5.58	60.31 ± 0.65	2.81 ± 0.5	-
	No	35.94 ± 1.3	59.79 ± 1.3	1.44 ± 0.08	2.82 ± 0.53

Leaf 4 was significantly larger than Leaf 3 (U = -4.87, $p < 0.01$) in all harvests. No significant differences were found in leaf area between shade and unshaded treatments in Leaf 3 ($F_{1,46}$ = 2.84, p = 0.099) and Leaf 4 ($F_{1,46}$ = 2.83, p = 0.100). However, significant differences between harvests were found in both leaves ($F_{2,46}$ = 21.10, $p < 0.01$ (Leaf 3); $F_{2,46}$ = 6.95, $p < 0.01$ (Leaf 4)), where Leaf 3 and Leaf 4 got smaller as the plant got older (Fig. 5.16).

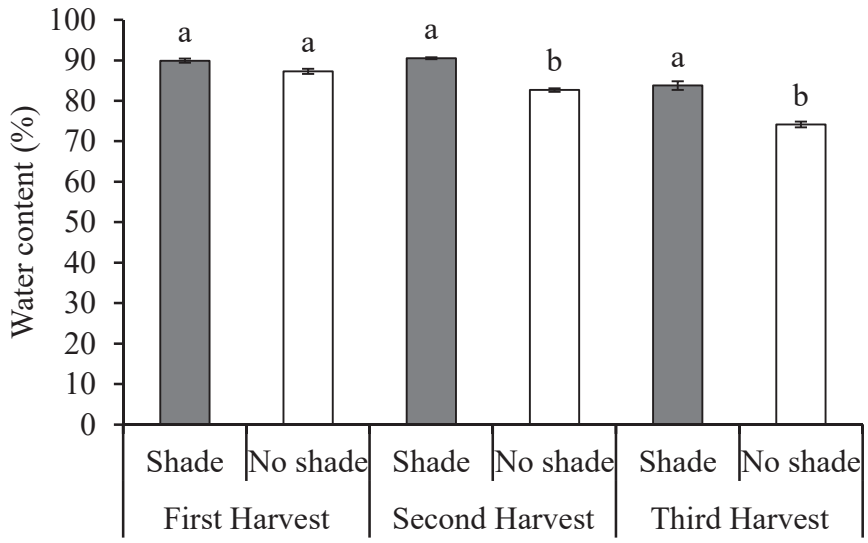

Figure 5.15. Water content (%) of shaded and unshaded woolly nightshade plants from the first (plants 40-days old), second (plants 58-day old), and third harvest (plants 100-days old). Error bars represent standard errors. Means with the same letter indicate no significant difference between shaded and unshaded treatments (one-way ANOVA, $\alpha = 0.05$). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

Specific leaf area (SLA; fresh leaf area/dry mass) was significantly lower for unshaded compared to shaded (H= 47.53, $p < 0.01$) leaves, and it also became lower as the plants got older (H = 36.68, $p < 0.01$) (Fig. 5.17). This means that unshaded leaves had lower leaf area for light capture per unit of biomass compared to shaded leaves. Regardless of shade conditions and harvest, woolly nightshade plants had larger specific leaf areas when insects were not present ($F_{1,72}$ = 11.54, $p < 0.01$) (Fig. 5.18). When SLA measurements were performed, insects had been feeding on these plants for 15 days (second harvest) and 60 days (third harvest). SLA between Leaf 3 and Leaf 4 was not significantly different (U = 1407, p = 0.281).

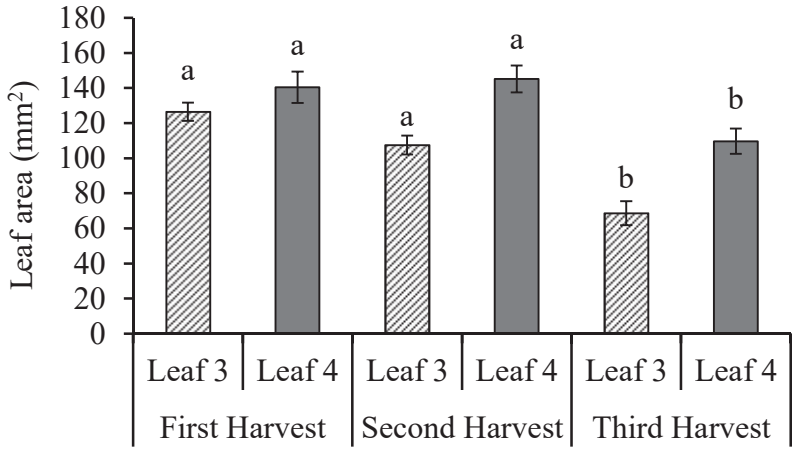

Figure 5.16. Leaf area (mm^2) of Leaf 3 and Leaf 4 from woolly nightshade plants of first (plants 40-day old), second (plants 58-day old), and third (plants 100-days old) harvest. Error bars represent standard error. Means with the same letter indicate no significant difference in leaf area of Leaf 3 and Leaf 4 between harvests (one-way ANOVA, $\alpha = 0.05$). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

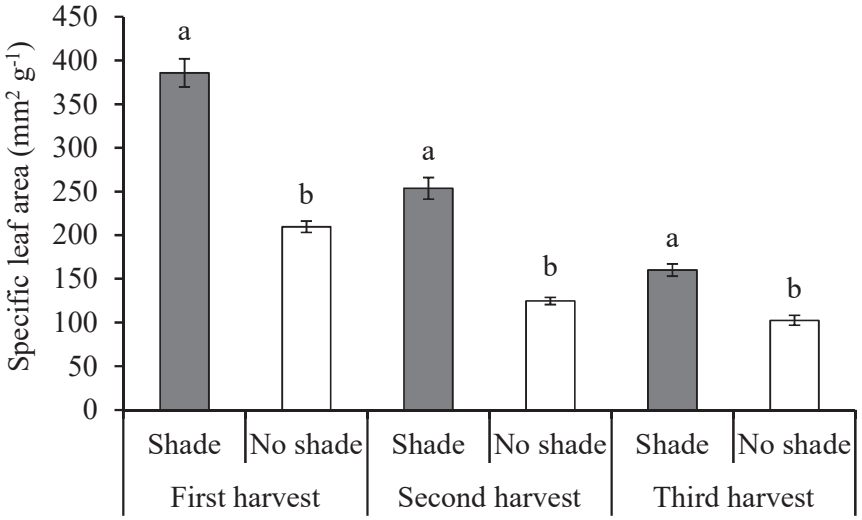

Figure 5.17. Specific leaf area ($mm^2\ g^{-1}$) of shaded and unshaded woolly nightshade leaves from the first (plants 40-days old), second (plants 58-days old), and third harvest (plants 100-days old). Error bars represent standard errors. Means with the same letter indicate no significant difference in specific leaf area between shaded and unshaded treatments within each harvest (one-way ANOVA, $\alpha = 0.05$). The graph represents 3-one-way ANOVAs; letters a and b only apply within a harvest.

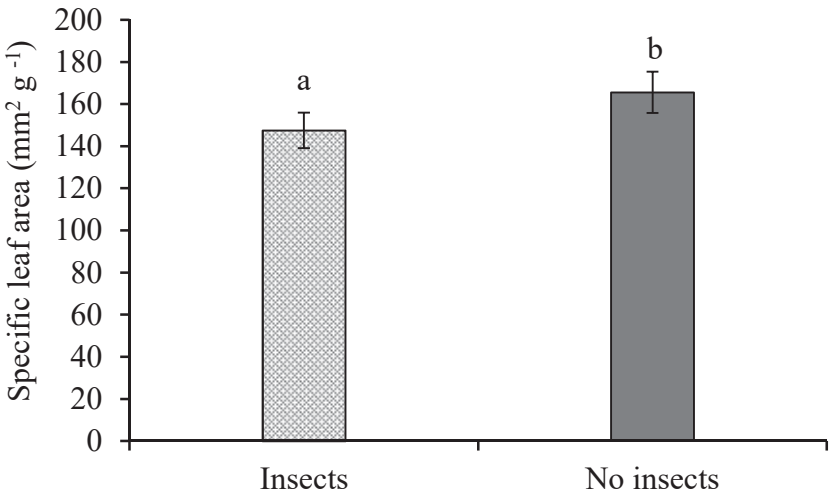

Figure 5.18. Mean specific leaf area (mm^2 g^{-1}) between woolly nightshade leaves with and without insects. Error bars represent standard errors. Means with the same letter indicate no significant difference (one-way ANOVA, $\alpha = 0.05$).

Net photosynthesis (P_n) from first harvest plants was significantly higher in unshaded leaves (Kruskal-Wallis test, $H= 4.49$, $p < 0.05$) (Fig. 5.19). This difference in net photosynthesis between shaded versus unshaded leaves can be attributed to differences in leaf chlorophyll content. Leaf chlorophyll content index (CCI) was significantly higher in unshaded leaves ($F_{1,18} = 18.75$, $p < 0.01$) at the first harvest. When plants reached the second harvest, no significant difference in the CCI between shaded and unshaded leaves was observed ($F_{1,36} = 2.64$, $p = 0.113$). However, a significant decrease in the CCI of unshaded leaves was observed as the plants became older (at third harvest) ($F_{1,36} = 55.10$, $p < 0.01$) (Fig. 5.20). The CCI of woolly nightshade leaves was not significantly affected by the presence of lace bugs ($F_{1,72} = 0.85$, $p = 0.360$).

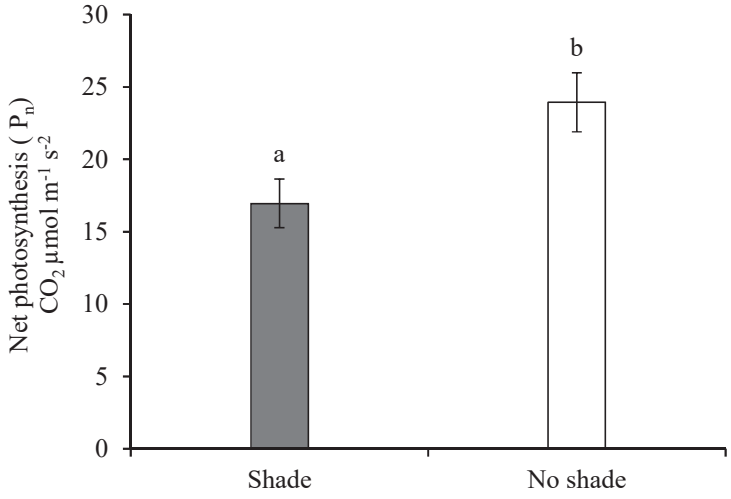

Figure 5.19. Net photosynthesis (P_n) (CO_2 µmol m^{-1} s^{-2}) in shaded and unshaded woolly nightshade leaves from the first harvest (plants 40-days old). Error bars represent standard errors. Means with the same letters indicate no significant difference between treatments (one-way ANOVA, $\alpha = 0.05$).

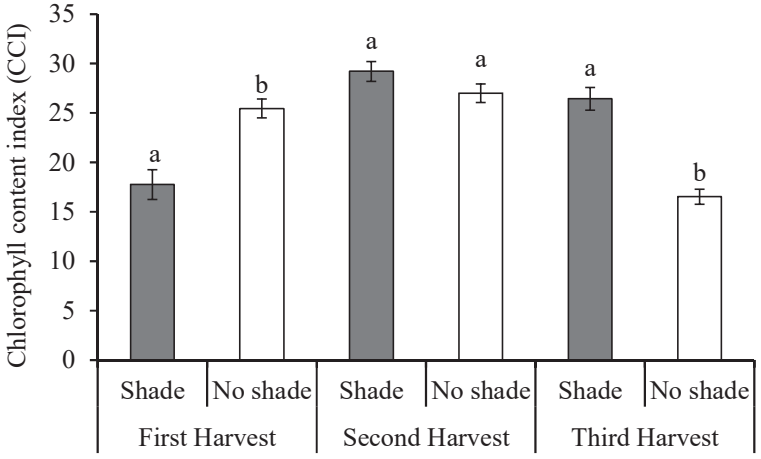

Figure 5.20. Chlorophyll content index (CCI) of shaded and unshaded woolly nightshade leaves from the first (plants 40-days old), second (plants 58-days old) and third harvest (plants 100-days old). Error bars represent standard errors. Means with the same letter indicate no significant difference between shaded and unshaded treatments (one-way ANOVA, $\alpha = 0.05$). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

The Chl fluorescence ratio (F_v/F_m), or maximum quantum efficiency exhibited significantly higher values in woolly nightshade shade leaves compared to unshaded leaves (shaded: $\bar{x}$ ± se = 0.87 ± 0.01, unshaded: $\bar{x}$ ± se = 0.81 ± 0.01) ($F_{1,16}$ = 14.38, p < 0.01). The maximal fluorescence from dark adapted leaves, F_m was significantly higher in shade leaves ($F_{1,16}$ = 19.55, p < 0.01). The variable fluorescence from dark adapted leaves, F_v, contributed to the differences observed between shade and unshaded leaves in the maximum quantum efficiency of photosystem II (PSII) (Fig. 5.21).

The maximal fluorescence from light adapted leaves (F_m') was significantly higher in shade leaves compared to unshaded leaves ($F_{1,16}$ = 20.13, p < 0.01). The observed differences in the maximal fluorescence (F_v') led to the significant differences obtained in the quantum efficiency of photosystem II photochemistry ϕPSII ($F_{1,16}$ = 13.82, p < 0.01), where shade leaves had slightly higher values than unshaded leaves (shade: $\bar{x}$ ± se = 0.84 ± 0.003, no-shade: $\bar{x}$ ± se = 0.81 ± 0.01) (Fig. 5.21).

The Chl fluorescence ratio and the maximum quantum efficiency were not significantly affected by the presence of lace bugs (F_v/F_m: $F_{1,16}$ = 1.84, p = 0.194; øPSII: $F_{1,16}$ = 3.21, p = 0.09).

5.3.3. Plant physical defences

Leaf thickness measured using confocal microscope images was significantly different between harvests ($F_{2,46}$ = 37.51, p < 0.01) and between light conditions (H = 12.816, p < 0.01). The difference between shaded and unshaded conditions was not observed at the first harvest ($F_{1,7}$ = 0.001, p = 0.982). Woolly nightshade leaves from the third harvest grown under unshaded conditions were 23.08% thicker than the leaves grown in shaded conditions. Leaves of shaded and unshaded plants (both Leaf 3 and Leaf 4) became thinner overall as the plants got older (first vs. third harvest): with a 42.2% and 29.73% thickness reduction in shaded and unshaded leaves, respectively, from first to third harvest (Fig. 5.7 and 5.22). Leaf thickness was not affected by the presence of lace bugs ($F_{1,37}$ = 0.77, p = 0.385).

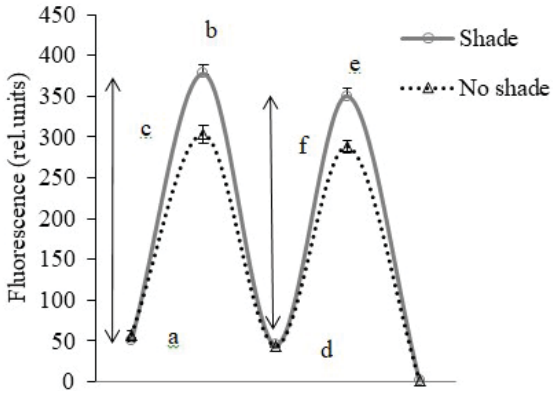

Figure 5.21. Average fluorescence trace of shade and unshaded woolly nightshade leaves (a) F_0 – minimum level of fluorescence from dark-adapted leaf; (b) F_m – maximal level of fluorescence from dark-adapted leaf; (c) F_v – variable fluorescence from dark-adapted leaf; (d) F_0' – minimal fluorescence from light-adapted leaf; (e) F_m' – maximal fluorescence from light-adapted leaf; and (f) F_v' – variable fluorescence from light-adapted leaves (Baker, 2008). Error bars represent standard errors.

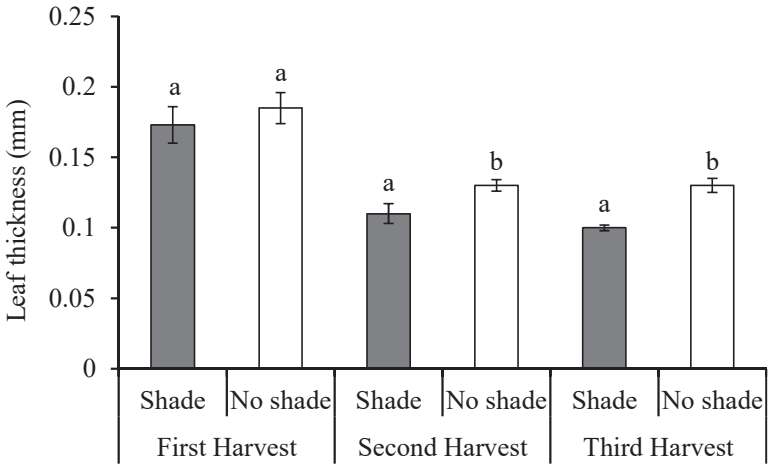

Figure 5.22. Leaf thickness (mm) of shaded and unshaded woolly nightshade leaves at the first (plants 40-days old), second (plants 58-days old), and third harvest (plants 100-days old) measured using confocal microscope images. Error bars represent standard errors. Means with the same letter indicate no significant difference between shaded and unshaded treatments (Kruskall-Wallis test, $\alpha = 0.05$). Letters a and b only apply within a harvest.

Leaf thickness measurements performed using confocal imagery showed no significant correlation with leaf thickness measurements performed with a digital gauge (r_{47} = -0.20, p = 0.162) (Fig 5.23). The differences between two leaf thickness measuring methods were not related to differences between measuring points on the leaves; the location on the leaf was not a significant source of variation when measuring with a digital gauge, as no significant differences were found between measuring points (χ^2_3 = 3.66, p = 0.301).

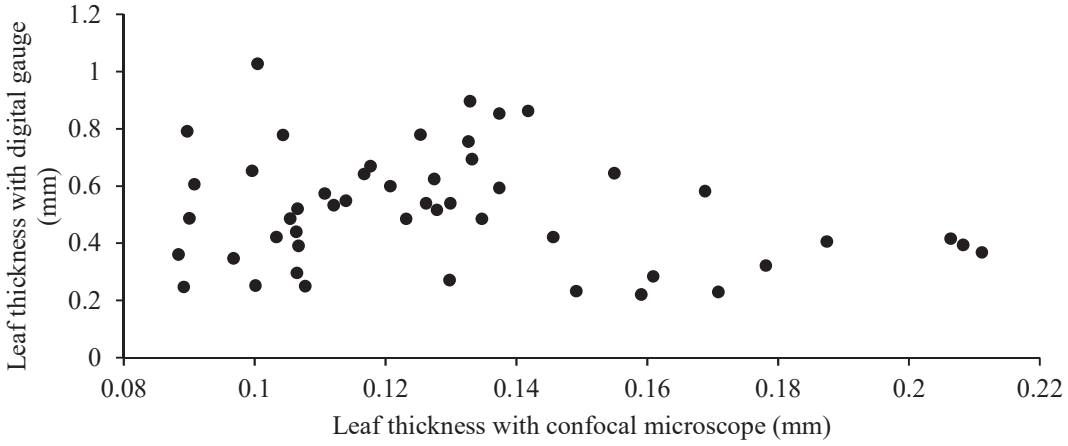

Figure 5.23. No relationship (r_{47} = -0.20, p = 0.162) between leaf thicknesses measured with a digital gauge versus measured using a confocal microscope imagery (Pearson's correlation, α = 0.05).

I hypothesize that the observed differences between the two-leaf thickness measuring methods (confocal vs. gauge) were largely due to the trichomes affecting the measurement of the leaf thickness when using a digital gauge. The digital gauge measures the total thickness of a leaf inclusive of its trichomes (total leaf thickness = thickness of leaf excluding trichomes + trichome thickness), and the relationship between trichome density and leaf thickness (digital gauge) shows a positive correlation (r_{47} = 0.68, p < 0.01) (Fig 5.24).

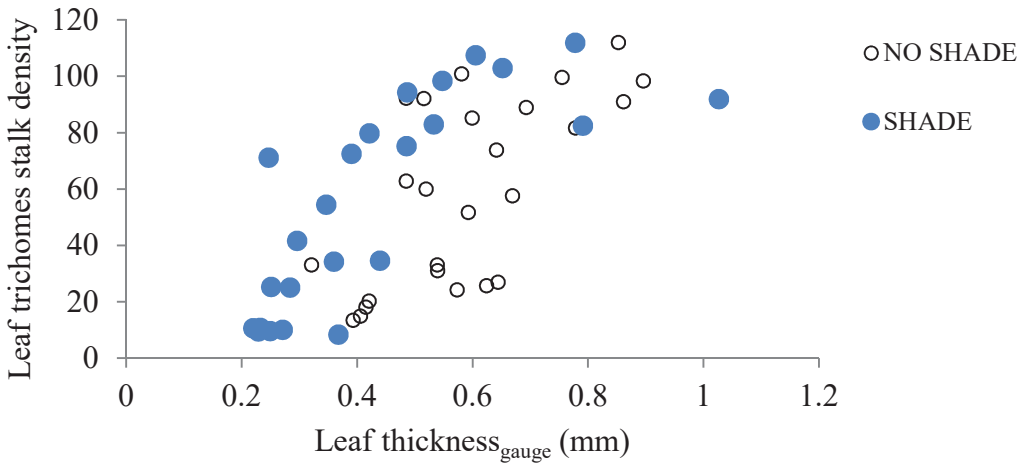

Figure 5.24. Relationship (r_{47} = 0.68, p < 0.01) between leaf trichome stalk density/4 mm^2 and leaf thickness measured with a digital gauge (mm). These data are from Leaf 4 of woolly nightshade plants, and measurements were separated according to shade treatment (Pearson's correlation, α = 0.05).

Specific leaf area (SLA) together with the ratio of leaf dry mass to saturated fresh mass (LDMC) have been reported as predictors of leaf thickness (Wilson *et al.*, 1999; Vile *et al.*, 2005). The expression: $(SLA \times LDMC)^{-1}$ has been used as an estimate of leaf thickness of laminar leaves, where LDMC is calculated as [*1- leaf water content*] (Vile *et al.*, 2005). In woolly nightshade in this experiment, the SLA and leaf thickness measured with the gauge were negatively correlated (r_{47} = -0.74, $p < 0.01$), and no correlation was found between SLA and leaf thickness measured using confocal microscope images (r_{47} = 0.24, $p = 0.10$). The lack of relationship between SLA and leaf thickness measured with confocal microscope could be because SLA includes the dry matter of the internal structure of the leaf (e.g. parenchyma cells) as well as the external structure (e.g. trichomes). When leaf thickness was normalized for trichomes, a positive relationship was found between SLA and measured leaf density (r_{47} = 0.70, $p < 0.01$) (Fig. 5.25).

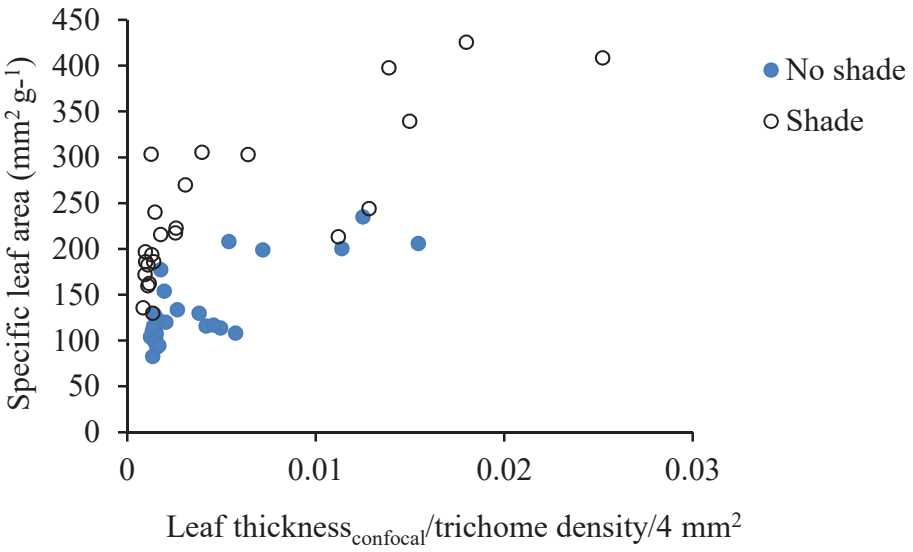

Figure 5.25. Relationship (r_{47} = 0.70, p < 0.01) between leaf thickness measured via confocal microscope images (normalized for trichome density) and specific leaf area (Pearson's correlation, α = 0.05).

Leaf thickness measured with a digital gauge showed significant differences between harvests ($F_{2,96}$ = 66.86, p < 0.01). There was a 52.46% and 45.59% increase in leaf thickness in shaded and unshaded leaves, respectively, from first harvest to third harvest plants, contrasting with a significant decrease in leaf thickness measured through confocal images. This is also probably due to the layer of trichomes getting thicker as the plants got older. There was a significant difference in leaf thickness between shaded and unshaded leaves ($F_{1,96}$ = 52.82, p < 0.01), where unshaded leaves were 10.29% thicker than shaded leaves (Fig 5.26). In addition, leaf thickness appeared to be affected by the presence of lace bugs when measured with a digital gauge ($F_{1,72}$ = 4.74, p < 0.05) (Fig. 5.27).

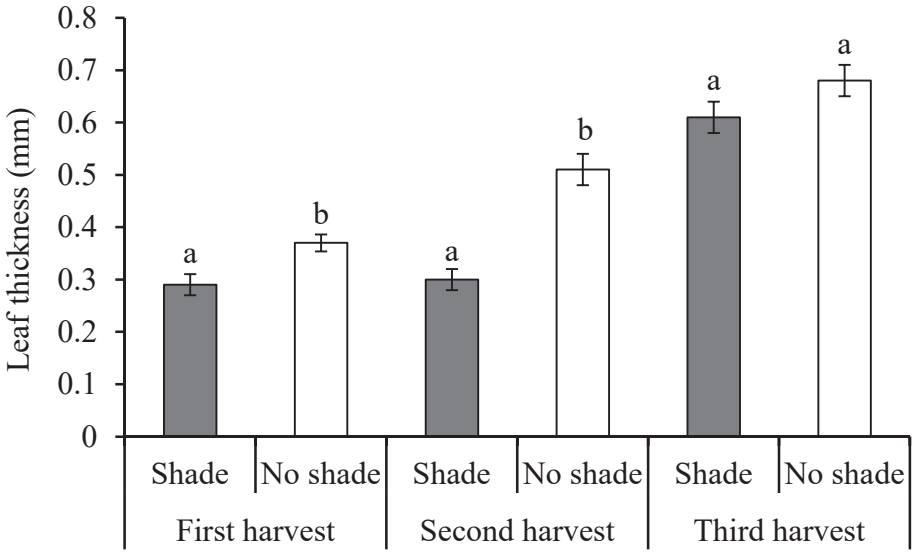

Figure 5.26. Leaf thickness (mm, measured with a digital gauge) of shaded and unshaded woolly nightshade leaves, at the first (plants 40-day old), second (plants 58-days old), and third harvest (plants 100-days old). Error bars represent standard errors. Means with the same letter indicate no significant difference between shaded and unshaded treatments (one-way ANOVA, $\alpha = 0.05$). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

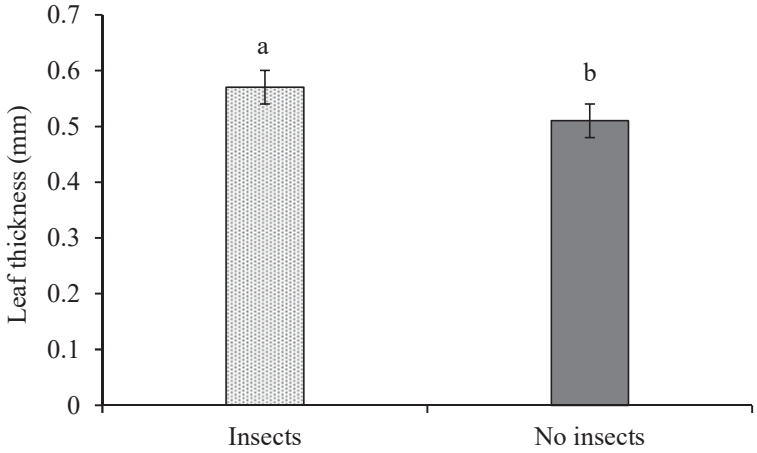

Figure 5.27. Mean leaf thickness (mm, measured with a digital gauge) of leaves with and without insects. Error bars represent standard errors. Mean with the same letter indicate no significant difference (two-way ANOVA, $\alpha = 0.05$).

At first harvest, unshaded leaves had significantly higher trichome stalk density than shade leaves ($F_{1,18}$ = 6.00, p < 0.05) (Fig. 5.28). However, no significant difference in trichome density between light intensities was observed in second harvest leaves (H = 1.35, p = 0.245) (Fig. 5.28). There was a significant increase in trichome stalk density between first and second harvest (H = 26.30, p < 0.01) (Fig. 5.29). First harvest leaves displayed small trichomes with short hairs on the underside of the leaf and dark green circled spots without any apparent trichome growth on the upper side of the leaves. By the third harvest, trichomes increased in size on upper leaf surfaces, developing from the dark green spots observed in the first and second harvest leaves (Fig. 5.30).

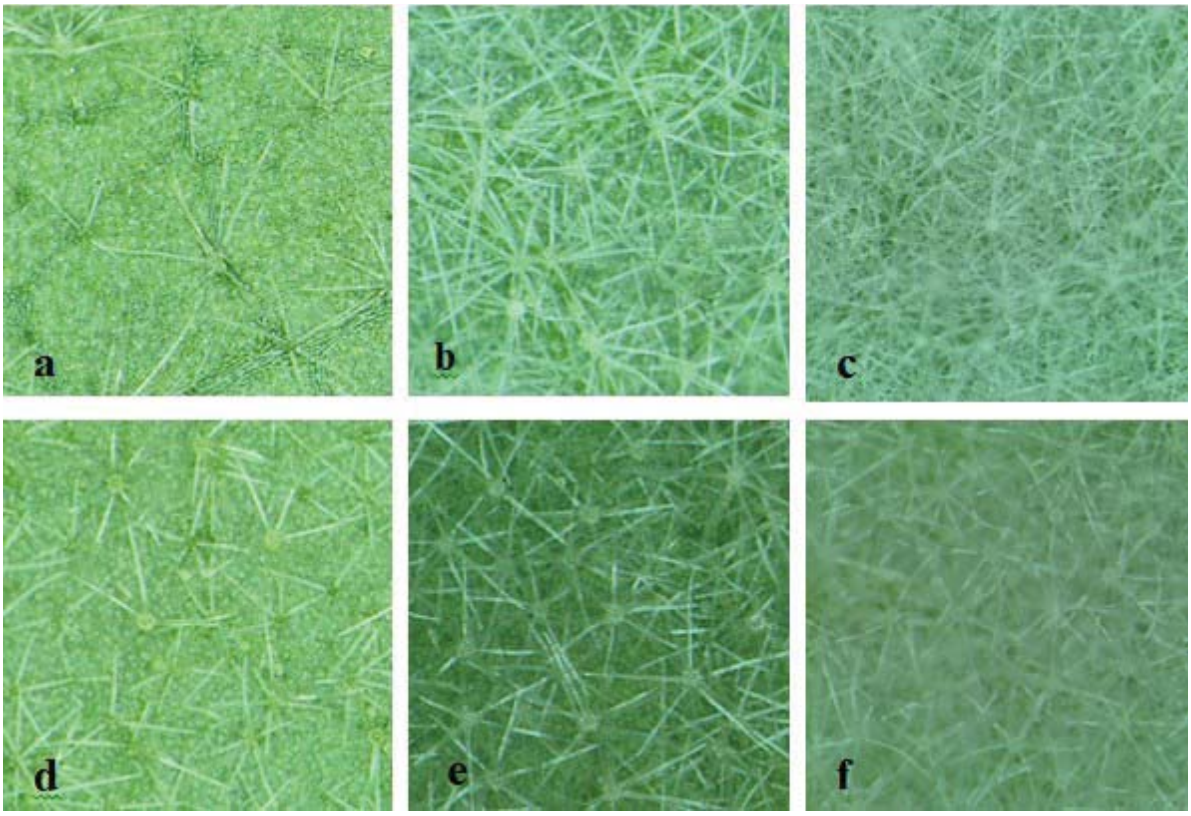

Figure 5.28. Visual comparison of trichome density on the underside of woolly nightshade leaves. Upper row: shade leaves at (a) first (plants 40-days old), (b) second (plants 58-day old), and (c) third harvest (plants 100-days old). Lower row: unshaded leaves from (d) first, (e) second, (f) third harvest.

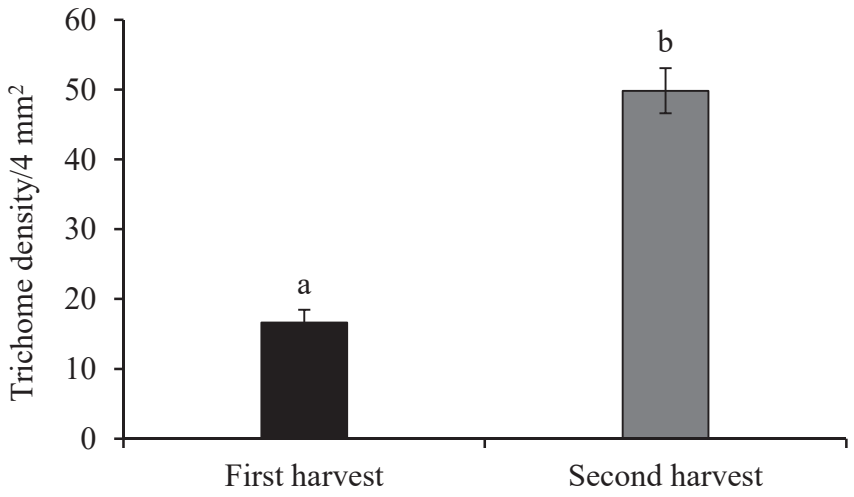

Figure 5.29. Mean trichome density (stalks/4 mm^2) measured on the underside of woolly nightshade leaves collected on the first (plants 40-days old) and second harvest (plants 58-days old). Means represent the trichome density of both Leaf 3 and Leaf 4 for each harvest. Error bars represent standard errors. Means with the same letter are not significantly different (one-way ANOVA, $\alpha = 0.05$).

Figure 5.30. Leaf trichomes on the upper side of woolly nightshade shade leaves at (a) first (plants 40-day old), (b) second (plants 58-days old) and (c) third harvest (plants 100-days old)

Visually, there was a much greater density of trichomes by the third harvest, but no statistical analysis was performed to quantify this difference, because of difficulties in reliably counting the trichomes on third harvest leaves.

The average trichome spike length was significantly different between harvests ($F_{2,96} = 21.99$, $p < 0.01$). Significant differences were not observed between the first and the second harvest. There was a significant difference between shaded and unshaded conditions ($F_{1,96} = 60.00$, $p < 0.01$), where trichome spikes were longer in unshaded leaves than in shaded leaves of second and third harvest plants (Fig. 5.31). Lace bugs had no significant effect on the trichome spike length ($F_{1,78} = 2.19$, $p = 0.143$).

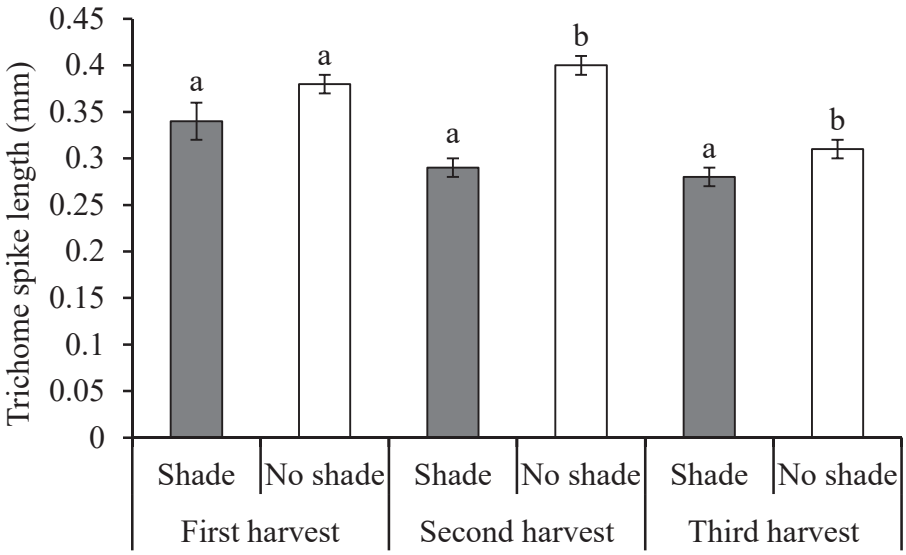

Figure 5.31. Mean trichome spike length in shaded and unshaded woolly nightshade leaves (Leaf 3 and Leaf 4 combined) at the first (plants 40-days old), second (plants 58-days old) and third harvest (plants 100-days old). Error bars represent standard errors. Means with the same letter indicate no significant difference between shaded and unshaded conditions (one-way ANOVA, $\alpha = 0.05$). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

Woolly nightshade leaves showed significant differences in their anatomy between shaded and unshaded conditions and between harvests, which could represent leaf adaptations to changes in the environment (i.e. light intensity). Unshaded leaves (Leaf 4)

from first ($F_{1,6}$ = 11.77, p < 0.05) and third harvest ($F_{1,14}$ = 11.02, p < 0.01) had thicker lower epidermis layers than shade leaves. In addition, unshaded leaves from the third harvest had thicker palisade layer ($F_{1,16}$ = 41.48, p < 0.01) than shade leaves (Table 5.3). Significant differences between leaf anatomical measurements were evident between first and third harvest. Irrespectively of light condition, leaves from the first harvest had a thicker palisade layer ($F_{1,24}$ = 74.2, p < 0.01), wider palisade cells ($F_{1,23}$ = 105.7, p < 0.01), and thicker upper ($F_{1,24}$ = 24.26, p < 0.01) and lower epidermis layers ($F_{1,22}$ = 16.02, p < 0.01) than leaves from the third harvest (Table 5.3).

Table 5.3. Average anatomical measurements (μ) performed on cross-sections of unshaded and shade woolly nightshade leaves from the first (plants 40-days old) and third harvest (plants 100-days old). Anatomical features: palisade layer thickness (PLT), palisade cell width (PCW), upper epidermal layer thickness (UELT), lower epidermal layer thickness (LELT). Significant differences between shaded and unshaded condition within each harvest are represented by different letters (a,b); significant differences between harvests are represented with an asterisk (*); one-way ANOVA, α = 0.05.

Treatments	*PLT**	*PCW**	*UELT**	*LELT**
First Harvest				
Shade	75.75 ± 9.94 a	23.19 ± 2.72 a	20.92 ± 1.82 a	10.78 ± 0.9 a
No shade	90.42 ± 4.07 a	16.02 ± 0.42 a	21.39 ± 1.04 a	14.78 ± 0.74 b
Third Harvest				
Shade	35.32 ± 0.86 a	7.9 ± 0.43 a	15.02 ± 1.04 a	7.9 ± 0.43 a
No shade	48.58 ± 1.78 b	8.32 ± 0.4 a	15.86 ± 0.81 a	8.32 ± 0.4 b

5.3.4. Plant chemical defences

There was a significant difference in the Carbon:Nitrogen ratio between harvests (H = 224.24, $p < 0.01$) and between shaded and unshaded leaves across all harvests ($F_{1,44}$ = 224.24, $p < 0.01$) ($F_{1,44}$ = 224.24, $p < 0.01$). Differences in the C:N ratio were even more evident at the second harvest, when unshaded leaves had much higher C:N ratios than shaded leaves ($\bar{x}$ ± se: Shade = 6.64 ± 0.13; Unshaded = 14.36 ± 0.62; $F_{1,18}$ = 149.4, $p <$ 0.01) (Fig. 5.32). Presence of lace bugs did not significantly affected the C:N ratio (H = 0.32, p = 0.570).

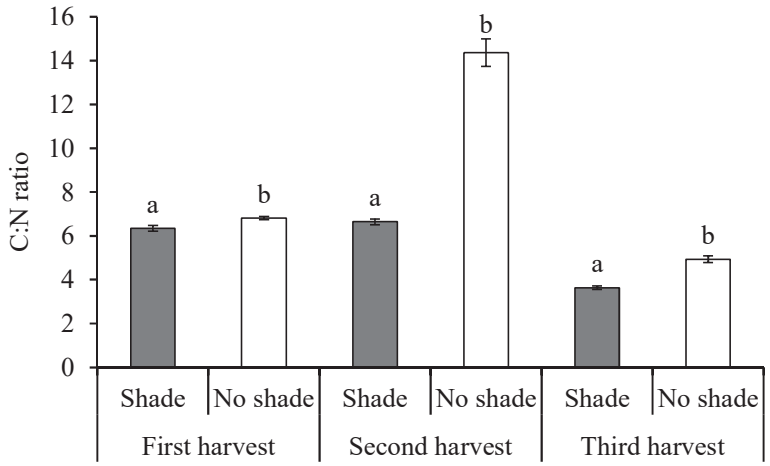

Figure 5.32. Mean C:N ratio of shaded and unshaded woolly nightshade leaves (Leaf 3) for the first (plants 40-days old), second (plants 58-days old) and third harvest (plants 100-days old). Error bars represent standard errors. Means with the same letter indicate no significant difference within harvest (one-way ANOVA, α = 0.05). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) analysis of glycoalkaloid extracts from woolly nightshade leaf samples (see section 5.2.6) showed multiple chromatographic peaks, with three peaks having fragmentation patterns consistent with glycoalkaloids, and elution time similar to the standards α-solanine, α-chaconine, and α-tomatine (Fig. 5.33). Fragment ions (MS fragmentation) of each of the glycoalkaloid chromatographic peaks of woolly nightshade leaf samples were compared with other known glycoalkaloids fragment ions to find a possible match. The fragment ions of two chromatographic peaks matched other known glycoalkaloids (Fig. 5.34). The first peak (A) is consistent with either α-solamargine or β-solamarine and the second peak (B) is consistent with either solauricine or solasonine (Claeys *et al.*, 1996; Bianco *et al.*, 2002; Distl and Wink, 2009; Altesor *et al.*, 2014; El-Hawary *et al.*, 2016; Jared *et al.*, 2016; Lelario *et al.*, 2016). No identification was possible for the third peak; therefore, it was named "Unknown 954" (Fig. 5.34).

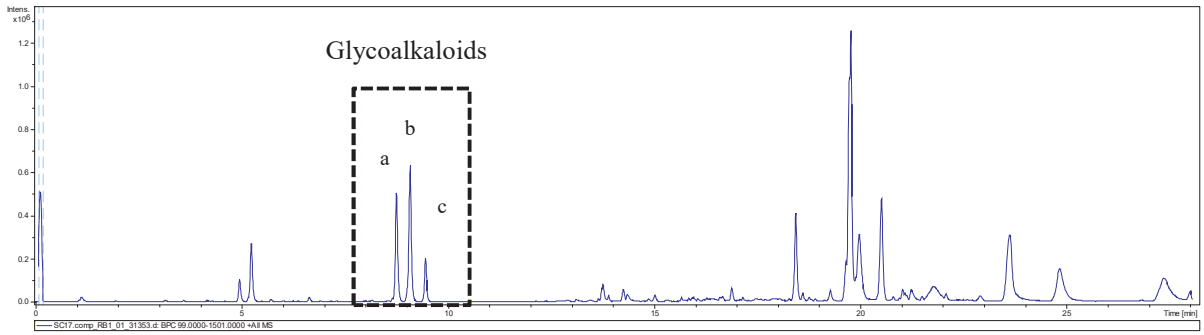

Figure 5.33. Base peak chromatogram (column Thermo Hypersil GOLD 2.1x200 mm, 1.9 µm) displaying the peaks of the main glycoalkaloids found in woolly nightshade leaf extracts. The x-axis shows retention time and the y-axis shows the intensity $x10^5$. The peaks surrounded by a dotted box represent: a – α-solamargine or β-solamarine; b – solauricine or solasonine; and c – Unknown 954.

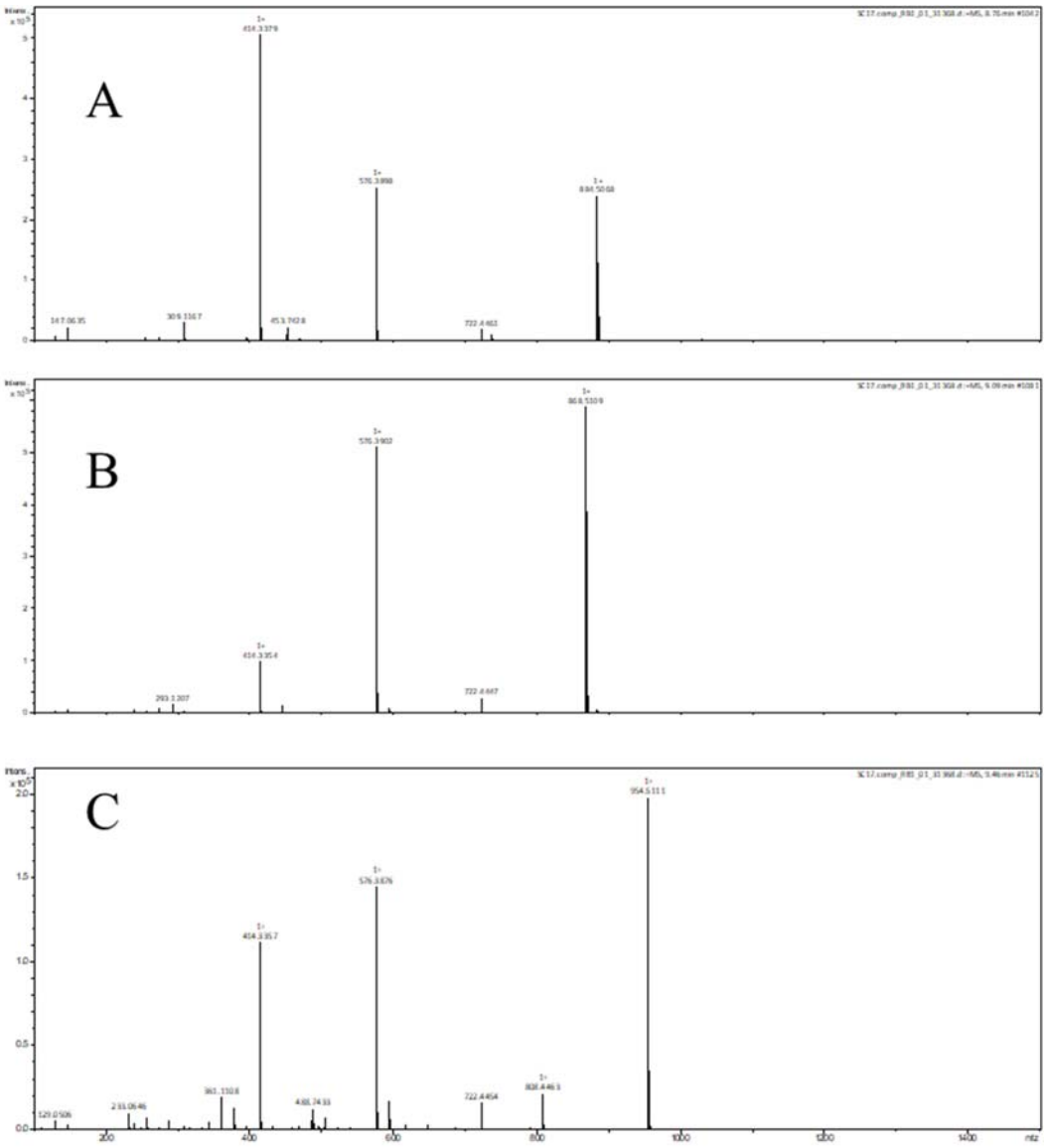

Figure 5.34. Mass spectra of compounds positively identified as glycoalkaloids in woolly nightshade leaves from the first (plants 40-days old), second (plants 58-days old) and third harvest (plants 100-days old) under shaded and unshaded conditions and with the presence or absence of insects. The composite sample included a mixture of the 50 samples (each sample represented one leaf), was subjected to mass spectrometry and the retention time of each of the positively identified glycoalkaloids was recorded: A – α-solamargine or β – solamarine, (m/z 884.506), B – solauricine or solasonine (m/z 868.5109), C – Unknown 954 (954.5111). The x- axis represents the retention time (m/z) and the y-axis represents the intensity x 10^5.

The total concentration of measured glycoalkaloids per 100 gr of dried woolly nightshade leaves was 37.39% higher in unshaded leaves compared to shaded leaves ($F_{1,44}$ = 17.04, $p < 0.01$). However, this significant difference in glycoalkaloid concentration was only detected at the second harvest ($F_{1,18}$ = 27.63, $p < 0.01$). There was a significant decrease in the total glycoalkaloid concentration of new leaves (Leaf 4) between harvests, regardless of whether the leaves were grown in shade or no-shade conditions ($F_{2,44}$ = 95.07, $p < 0.01$) (Fig. 5.35). The presence of lace bugs did not affect the total concentration of glycoalkaloids ($F_{1,38}$ = 0.95, p = 0.336).

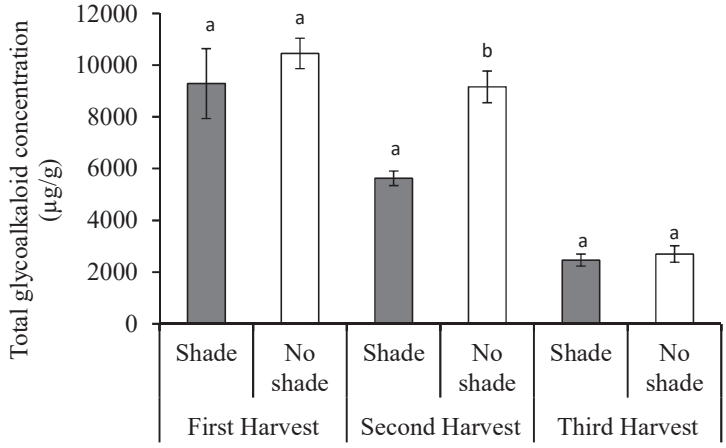

Figure 5.35. Mean total glycoalkaloid concentration (µg/g as α-solasonine equivalents) from shaded and unshaded woolly nightshade leaves from the first (plants 40-days old), second (plants 58-days old) and third harvest (plants 100-days old). Error bars represent standard errors. Mean with the same letter indicate no significant difference within harvest (one-way ANOVA, α = 0.05). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.

The concentrations of the three glycoalkaloids present in woolly nightshade leaves varied in a way similar to each other. Regardless of harvest time, significant differences in all three glycoalkaloid compounds between shaded and unshaded leaves were observed (Table 5.4). Significant differences in the glycoalkaloid concentration between shaded and unshaded leaves were evident at the second harvest (Fig. 5.36a-c). Regardless of light intensity, the concentration of each glycoalkaloid decreased as the plants got older (Figs. 5.36a-c). The possibility was explored that total glycoalkaloid concentration is influenced by the leaf area, but no significant relationship was observed between these two variables ($r_{48} = -0.07$, $p = 0.62$).

The increase of C:N ratio has been shown to be positively correlated with the increase in secondary metabolites when these are carbon-based (Herms and Mattson, 1992). Despite the fact that the positively identified glycoalkaloids are all nitrogen-containing compounds (Milner *et al.*, 2011), the proportion of nitrogen compounds (14.0067 g/mol) is small compared to the total molecular weight of the carbon molecules (540.4815 g/mol). This explains why the C:N ratio was strongly positively correlated with the total glycoalkaloid concentration ($r_{18} = 0.84$, $p < 0.01$).

Table 5.4. Concentration of glycoalkaloid compounds (mg/g) in woolly nightshade leaves of second harvest plants (plants 58-days old) grown in shaded and unshaded conditions. The differences in concentration of each glycoalkaloid between shaded and unshaded conditions was tested in a one-way ANOVA, $\alpha = 0.05$.

Glycoalkaloid	Shade	No-shade	$F_{1,18}$	P
α-solamargine/β-solamarine	3.48 ± 0.12	4.49 ± 0.17	28.07	< 0.01
solauricine/solasonine	1.39 ± 0.12	2.22 ± 0.21	11.82	< 0.01
Unknown 954	0.76 ± 0.08	2.35 ± 0.28	30.29	< 0.01

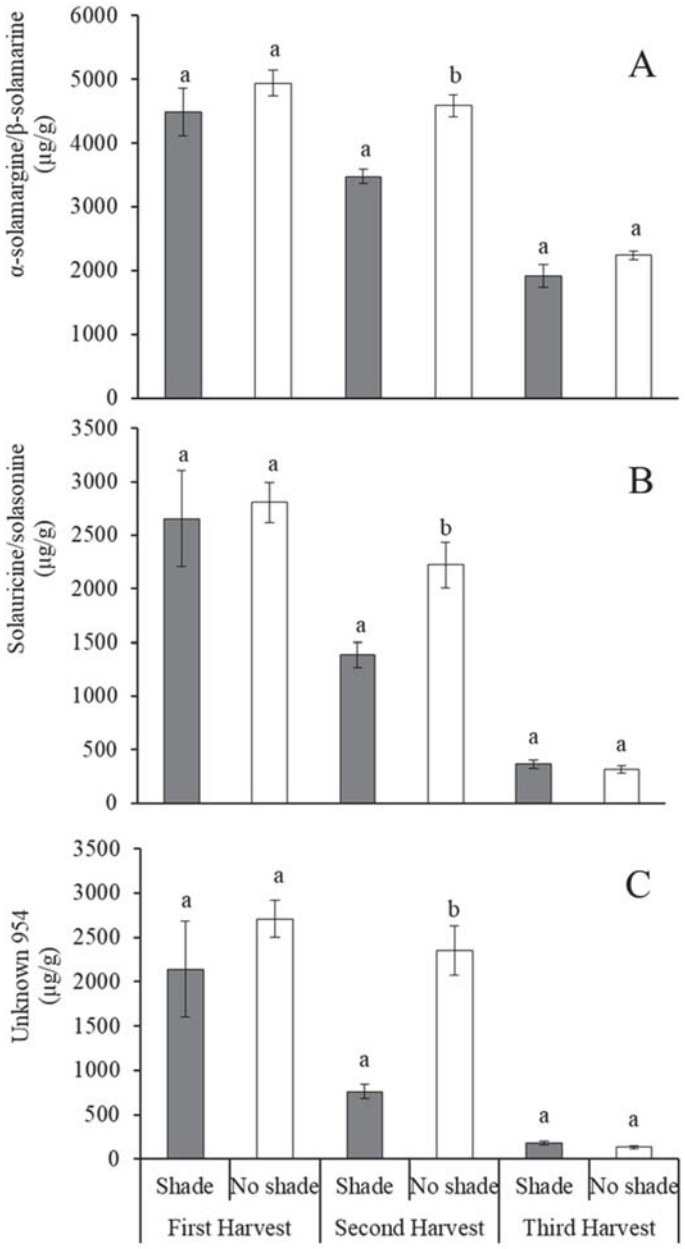

Figure 5.36. Concentration (µg/g) of glycoalkaloids in woolly nightshade leaves grown in shaded and unshaded conditions until first (plants 40-days old), second (plants 58 –days old) and third harvest (plants 100-days old): A – α-solamargine/β-solamarine, B – solauricine/solasonine, and C – Unknown 954. Error bars represent standard errors. Means with the same letter indicate no significant difference within harvest (one-way ANOVA, α = 0.05). The graph represents 3 one-way ANOVAs; the letters a and b only apply within a harvest.

The presence of lace bugs significantly affected the concentration of α-solamargine/β-solamarine ($F_{1,42}$ = 7.09, p < 0.05), but did not affect the concentration of solauricine/solanine ($F_{1,42}$ = 3.97, p = 0.053) and of Unknown 954 ($F_{1,42}$ = 1.94, p = 0.053). Even though the presence of lace bugs significantly affected one of the glycoalkaloids (α-solamargine or β-solamarine), in the principal component analysis most of the variation was observed between harvests (PC1, 95.34%) followed by the light conditions (PC2, 3.43%) (Fig. 5.37). Only during the second harvest (green color in Fig. 5.37) was it possible to observe a separation in glycoalkaloids between shaded and unshaded conditions.

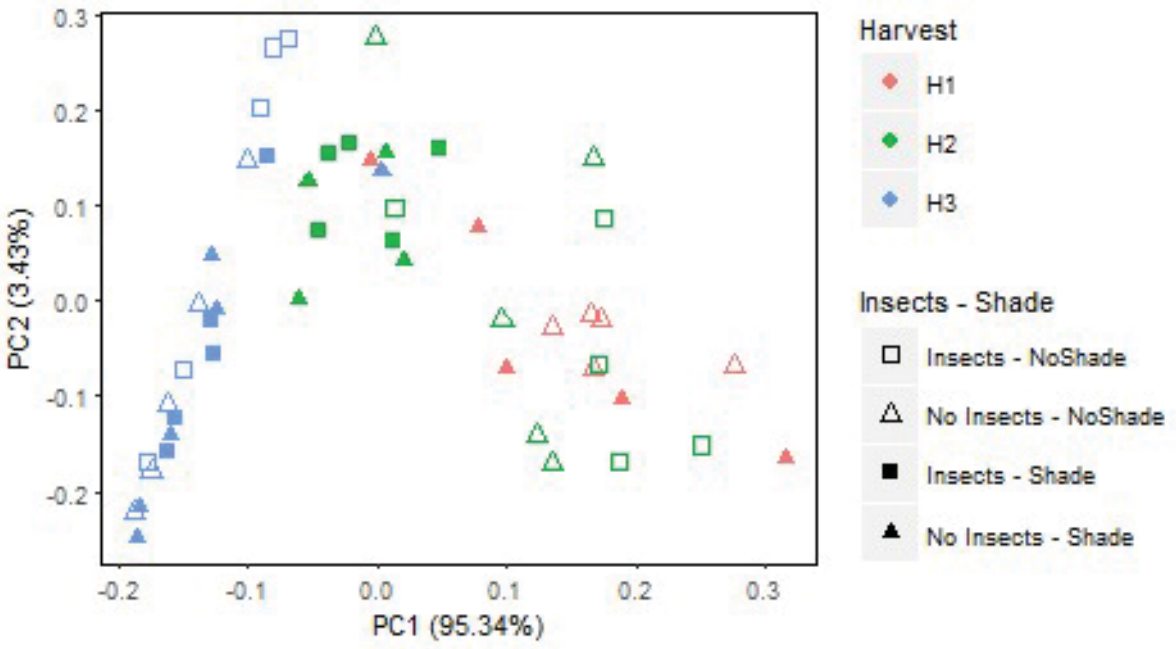

Figure 5.37. PCA of the total glycoalkaloid concentration in woolly nightshade leaves from three different harvests, subjected to shade and unshaded conditions, with lace bugs or without lace bugs. The factors that contributed most toward separation along each of the PC axes were the age of plant (harvest) (PC1, 95.34%) and light conditions (PC2, 3.43%).

5.3.5. Lace bug performance

F_0 females feeding on either shaded or unshaded plants produced similar numbers of egg batches (Kruskal-Wallis test, $H = 0.03$, $p = 0.857$), mean number of eggs per batch ($F_{1,37} = 1.85$, $p = 0.182$) and mean number of eggs per female ($H = 2.89$, $p = 0.090$) for F_o females at the second harvest. At the third harvest, there were no significant differences in the survival of F_1 adults of both sexes ($H = 0.70$, $p = 0.403$) and in the percentage of surviving F_1 females ($F_{1,17} = 0.12$, $p = 0.737$) between shaded and unshaded leaves. However, F_1 females had laid eggs only under shade conditions ($p < 0.01$, Fisher's exact test). Among females feeding on shaded leaves, there was a significant decrease in the number of eggs per female from F_o females (second harvest) to F_1 females (third harvest) ($t_7 = 3.06$, $p < 0.05$) (Fig. 5.38).

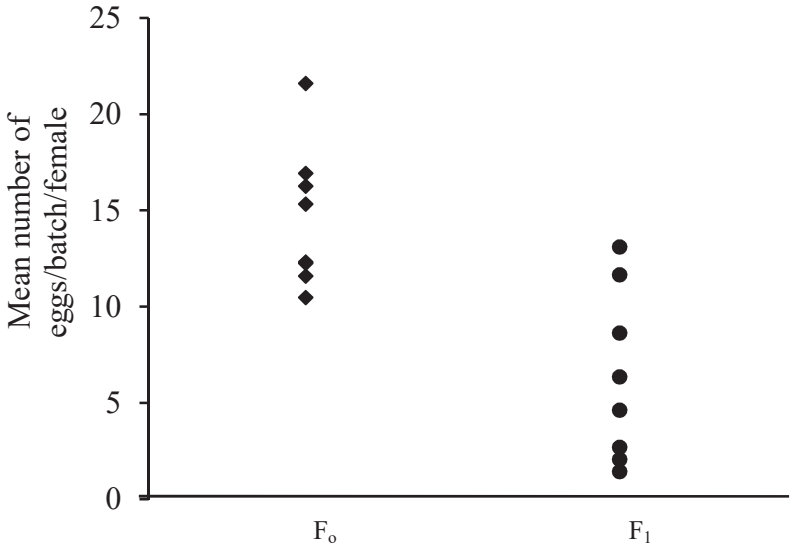

Figure 5.38. Differences in the mean number of eggs/batch/female in the lace bug *Gargaphia decoris* between initial (F_0) and first generation (F_1) females that fed on shaded woolly nightshade plants. F_0 females lived on second harvest plants (plants 58-day old) and F_1 on third harvest plants (plants 100-days old).

There was a significant difference in the overall morphology between F_1 female and male lace bugs that fed on plants subjected to shade conditions vs. those fed on unshaded plants (Table 5.5). In the principal component analysis (PCA) on morphological data most of the variation between PC1 and PC2 was due to the body length parameter – the lace bug F_1 females that developed and fed on shade plants had longer bodies than lace bugs that fed on unshaded plants (Fig. 5.40).

Table 5.5. Morphological measurements (mm) (mean ± se) of *Gargaphia decoris* first generation males and females fed on woolly nightshade leaves grown in either shaded or unshaded conditions.

Parameter (all in mm)	Shade	No-shade	F / H	p
Body length	3.73 ± 0.05	3.41 ± 0.05	23.94	< 0.01
Pronotum length	1.03 ± 0.04	0.97 ± 0.04	1.4173	0.24
Head length	0.50 ± 0.01	0.48 ± 0.01	1.26	0.26
Antennae I	0.34 ± 0.01	0.31 ± 0.01	2.15	0.15
Antennae II	0.14 ± 0.01	0.13 ± 0.004	1.95	0.17
Antennae IV*	0.59 ± 0.03	0.58 ± 0.02	0.79*	0.37*
Rostrum length	0.96 ± 0.06	1 ± 0.05	0.34	0.56
MANOVA for all parameters	$F_{1,30}$ = 3.976, Pillai´s Trace = 0.4883, p = < 0.01			

Values with an asterisk (*) were analyzed using the Kruskall-Wallis test with a chi-square approximation.

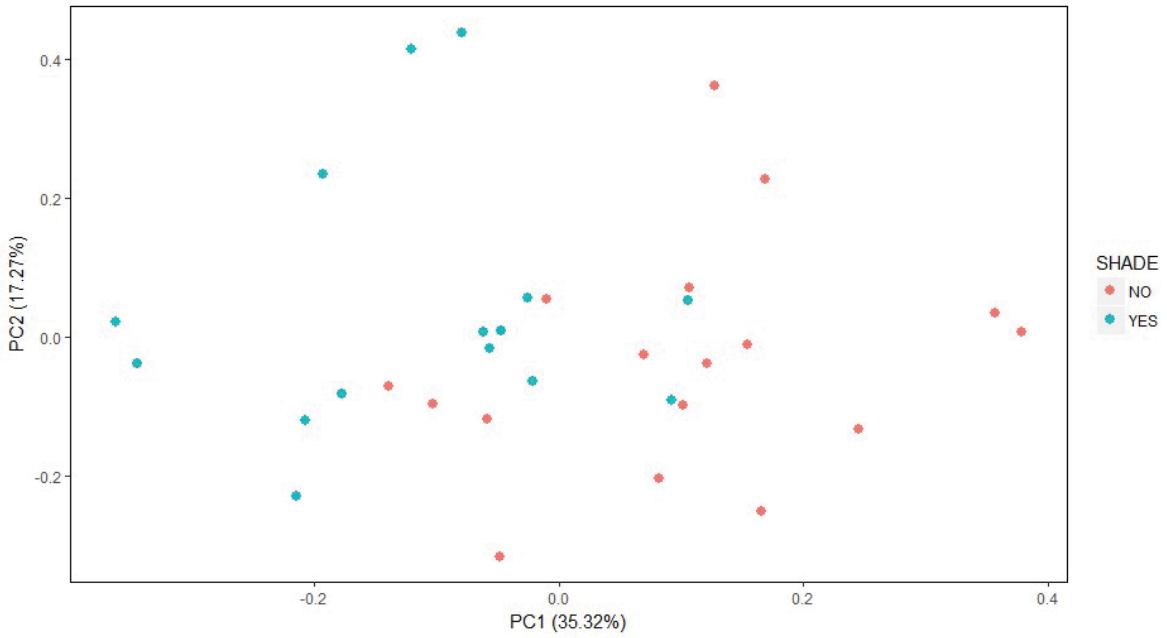

Figure 5.39. PCA of body measurements performed on lace bugs *Gargaphia decoris* (F_1 male and females) grown on shaded and unshaded woolly nightshade plants of first (plants 40-day old), second (plants 58-days old) and third (plants 100-days old). The variables that contributed most toward separation along each of the PC axes is the body length (PC1, 35.32%) and pronotum length (PC2, 17.27%).

5.4. Discussion

5.4.1. Plant performance and leaf adaptations to light intensity

Woolly nightshade plants differed in key physiological and morphological characteristics when subjected to different light conditions. Plants subjected to shade conditions were taller, displayed higher shoot-to-root ratio, thinner leaves, higher water content and larger specific leaf area than unshaded plants. Similar responses to shade conditions have been observed in other plant species (Bultman and Faeth, 1988, Aquifoliaceae; Collinge and Louda, 1988, Brassicaceae; Regnier and Harrison, 1993, Solanaceae; França and Tingey, 1994, Asteraceae; James and Bell, 2000, Solanaceae; Bentz, 2003, Myrtaceae; Crotser *et al.*, 2003, Solanaceae; Curt *et al.*, 2005, Fagaceae); these responses have been considered as some of the major adaptations of plants to low light conditions (Corré, 1983).

The response of plants to low light conditions (i.e. shade) has been explained previously by the "functional equilibrium" of biomass allocation. This concept predicts that plants will increase their growth by allocating more biomass to vegetative tissues and organs where it will enhance the uptake of resources that are more limited (Poorter *et al.*, 2012). In other words, plants change their biomass allocation in response to shade to compensate and maximize the capture of one of the major growth limiting resources, light (Lambers *et al.*, 2008).

Specific leaf area (ratio of leaf area to leaf dry mass; SLA), has previously been used to deduce the leaf thickness of a plant, and it has been demonstrated that SLA and leaf thickness are negatively correlated (Niinemets, 1999). Similar results have been observed in this study, where SLA of woolly nightshade was negatively correlated with leaf thickness when measured with a digital gauge.

The reduction in thickness of shaded leaves has been documented in other plant species (Regnier *et al.*, 1988; Buisson and Lee, 1993; Pereira *et al.*, 2009). However, thickness differences between shaded and unshaded plants in my experiment were not observed in first harvest leaves, and thus it is possible that the adaptation of the plant to the light conditions took longer than the time (28 days) allowed between start of the shading treatment and the first harvest.

Leaf thickness of new leaves (Leaf 3 and Leaf 4) of woolly nightshade plants subjected for a longer time to shade conditions, displayed contrasting results when measured by either confocal microscope images or digital gauge (with no relationship found between the measuring methods). Leaf 3 and Leaf 4 measured via confocal microscope images appeared to be thinner as the plants got older and they appeared to be thicker when measured with a digital gauge. The increase in leaf thickness when measuring with a digital gauge was attributed to the presence of trichomes on woolly nightshade leaves (see section 5.4.2 below).

The increased leaf thickness in sun-exposed leaves has been related to changes in the internal structure of the leaf (Shields, 1950; Friend and Pomeroy, 1970; Kubínová, 1999; Crotser *et al.*, 2003; Sousa-Paiva *et al.*, 2003; Pereira *et al.*, 2009) and these changes, triggered by light exposure, are a result of adaptations of the leaf due to changes in leaf size and water deficit (higher evaporation) (Xu *et al.*, 2009). The thicker palisade mesophyll

layer observed in new unshaded leaves (Leaf 4) of woolly nightshade plants at the third harvest (plants 100 days old) could be attributed to changes in leaf size but not to water deficit. First harvest leaves were larger than third harvest leaves, and this could have affected the arrangement of cells within the leaf. Sun-exposed leaves display elongated palisade cells, which facilitate the equal distribution of light to chloroplasts by allowing light to penetrate deeper into the thicker leaves (Osborne and Raven, 1986; Vogelman and Martin, 1993; Sousa-Paiva *et al.*, 2003). There is no evidence in my study that water deficit contributed to the increase in mesophyll palisade layer. Plants in unshaded conditions had a lower water content than shaded plants, but observed water content levels were still higher than the range that is considered typical for woody trees or shrubs (60-70%) (Goss, 2013).

Besides the elongation of palisade layers, the movement of chloroplasts and the production of light harvesting pigments is an adaptation that enables the efficient use of light either in shaded or in unshaded leaves (Salisbury and Ross, 1992; Taiz and Zeiger, 1998). Unshaded woolly nightshade leaves displayed a higher chlorophyll content index (CCI) than shade leaves in the first harvest, but when the plants reached the third harvest, shade leaves had higher CCI than unshaded leaves. Increased chlorophyll content in response to reduced light has been associated with shade-adapted species (Regnier *et al.*, 1988). Shade-adapted species display higher photosynthetic rates at low light intensities (Regnier *et al.*, 1988). For example, studies on the Eastern black nightshade *Solanum ptychanthum* Dunal indicated that this weed is more photosynthetically efficient when grown under reduced light conditions and that this greater efficiency resulted in greater net photosynthesis (Crotser *et al.*, 2003). Results from my study show a higher net photosynthesis in unshaded leaves from first harvest plants. However, net photosynthesis measurements were not performed in third harvest plants, thus, it is unknown if shade leaves will exceed the values from unshaded leaves.

Despite not having information about net photosynthesis in the third harvest plants, it was possible to perform chlorophyll *a* fluorescence measurements. Photosynthetic function (usually measured by CO_2 assimilation rates) can also be judged by the chlorophyll induction kinetics of dark-adapted leaves (Lichtenthaler *et al.*, 1986; Maxwell and Johnson, 2000; Sarijeva *et al.*, 2007). One of the most commonly used Chl

fluorescence ratios is F_v/F_m, which indicates the maximum quantum efficiency of the photosystem II (Sarijeva *et al.*, 2007) and provides information about the underlying processes which have altered efficiency (Maxwell and Johnson, 2000).

For most plant species, F_v/F_m is a sensitive indicator of plant photosynthetic performance, and Fv/Fm values result in higher light utilization efficiency and stronger ability of plants to adapt to low-light conditions (Fu *et al.*, 2012). Under normal physiological conditions, F_v/F_m for most plant species is between 0.80-0.83 (Björkman and Demmig, 1987; Johnson *et al.*, 1993; Maxwell and Johnson, 2000; Fu *et al.*, 2012). When the F_v/F_m value is below this range, the plant is considered to be exposed to environmental stress (Maxwell and Johnson, 2000). Shaded woolly nightshade leaves had higher F_v/F_m ratios than unshaded leaves, however, values for unshaded leaves were within the range of normal physiological conditions.

5.4.2. Plant performance: physical defences

Leaf trichome stalk density was significantly affected by the light environment; however this was only evident for first harvest plants (40 day old), where there was higher trichome density under unshaded conditions; in second harvest the difference was not significant, and in third harvest the trichome density was too dense to quantify in both shaded and unshaded treatments. Kennedy *et al.* (1981) reported that in wild tomato *Lycopersicum hirsutum* Dunal (Solanaceae) significantly higher trichome density under high light condition was observed in older plants.

Light intensity also affected the trichome spike length of woolly nightshade leaves. Leaves grown under shade conditions had shorter spikes than unshaded leaves. This increase in trichome spike length contributed to creating a thick hair mat on the surface of the leaves. The production of this hair mat is a mechanism used by plants to reduce water evaporation from the cuticle of leaves, to protect leaf tissues by reducing the solar ultraviolet-B radiation that penetrates inside the leaf, and to act as a deterrent for herbivorous insects (Haddad and Hicks, 2000; Werker, 2000).

The trichome stalk density observed in leaves from the first and second harvests showed a positive relationship with leaf thickness measured with a digital gauge; this is

probably because digital gauge measures the total leaf thickness inclusive of trichomes. Differentiation processes that result in structural reinforcement (e.g. trichome production) could have influenced the resource allocation to the internal structure of the leaf (Herms and Mattson, 1992) and therefore, showing a decrease in leaf thickness via confocal microscope images while there is an increase in trichome stalk density.

The increase in trichomes, whether stimulated by light or not, has conferred resistance to pests in other plant species. For example in tomato *Solanum lycopersicum*, more mites were found trapped in the trichomes of unshaded leaves (Nihoul, 1993) and in the hawthorn lace bug *Corythucha cydoniae* (Fitch), trichomes reduced oviposition and nymphal survival (Schultz and Coffelt, 1987). In my study, nymphal survival and adult fecundity of the lace bug *G. decoris* were unaffected by the increase of trichome production in second harvest and third harvest plants. Even though females were reluctant to oviposit on unshaded third harvest plants, their counterparts on shaded plants exhibited no problem ovipositing on leaves that appeared to be similar to leaves of third harvest plants, although the exact trichome stalk density at third harvest leaves could not be quantified. However, it could be that it was not trichome stalk density but rather the increase in trichome spike length that affected the oviposition selection of *G. decoris.*

5.4.3. Effect of light intensity on woolly nightshade chemical defences

Light intensity, besides influencing the adaptive physiological and morphological traits of plants, has been recognized as one of the ecological factors that affect plant defences (Roberts and Paul, 2006). It does so by stimulating the plant to produce mechanical defences (e.g. trichomes), as well as causing a variation in chemical traits within the plant (i.e. primary and secondary metabolites).

Woolly nightshade plants had higher C:N ratio when growing in unshaded conditions. This result agrees with other studies, which demonstrated a similar effect (Trumbule and Denno, 1995; Shrewsbury and Raupp, 2000; Diaz *et al.*, 2011). The C:N ratio has been used as an indication of how well the plant is defended (Hoffland *et al.*, 1999; Sipura and Tahvanainem, 2000; Lerdau and Coley, 2002; Bentz, 2003; Muth *et al.*, 2008). The resource allocation to defence has been explained by the carbon nutrient

balance (CNB) hypothesis (Bryant *et al.*, 1983). Higher availability of soluble sugars (carbohydrates) allows the plant to direct some of the excess sugars to the production of carbon-based secondary metabolites (Herms and Mattson, 1992; Bryant *et al.*, 1983; Larsson *et al.*, 1986; Price *et al.*, 2011). The results from my study are inconsistent with the CNB hypothesis: the nitrogen-containing metabolites (glycoalkaloids) responded to C:N, following the pattern of carbon-based secondary compounds. These results are similar to those of Hoffland *et al.* (1999) and Royer *et al.* (2013) in their studies with tomato. These results are unexpected because with a high C:N ratio, the production of nitrogen-containing molecules should be restricted (Bryant *et al.*, 1983). However, it is important to note that in all positively identified glycoalkaloids in my study (α-solamargine/β-solamarine, solauricine/solasonine), 63% of the total molecular weight corresponds to carbon, compared to 1.58% of nitrogen. Thus, the higher content of carbon explains the increase in the plant C:N ratio. The increase in both C:N ratio and glycoalkaloids could reflect sharing of common metabolic pathways, processes that have been observed in potato steroidal glycoalkaloids (Ginzberg *et al.*, 2012).

5.4.4. *Gargaphia decoris* performance

Light intensity affected the host quality and as a result affected *G. decoris* performance. Lace bug females oviposited on unshaded second harvest plants and nymphs developed successfully up to adults. This means that even though glycoalkaloid content was higher in these plants, females and nymphs were still able to feed and develop. However, it is possible that glycoalkaloids could have affected performance of the resulting adults. Even though unshaded plants were not exposed to full sunlight due to glasshouse conditions and shade covers, lace bugs from third harvest plants behaved in similar way as in the field: females were reluctant to oviposit in unshaded conditions. Moreover, adults that were collected from unshaded third harvest plants were smaller than their counterparts from shaded plants. This reduction in adult size could be the result of them developing on younger plants that had higher glycoalkaloid content, as higher glycoalkaloid content was observed in leaves of unshaded second harvest plants (50 days old).

The glycoalkaloids present in Solanaceae are known to have a detrimental effect on insect physiology (Chowánsky *et al.*, 2016). There is evidence that glycoalkaloids present

in some Solanaceae species affect enzyme activity in insects (Buyukguzel *et al.*, 2013; Adamski *et al.*, 2014) and one of the most important insecticidal effects is the decrease in reproduction (Chowánsky *et al.*, 2016). For example, the beetle *Leptinotarsa undecimlineata* Stål reabsorbed smaller and immature oocytes (immature egg cell) as a response to inadequate diet conditions when feeding on Solanaceae glycoalkaloids (Lopez-Carretero *et al.*, 2005). Therefore, wooly nightshade glycoalkaloids could have affected the capability of *G. decoris* to absorb nutrients necessary for development, thus preventing them from growing bigger and simultaneously affecting their fecundity.

The glycoalkaloid concentration was highest in first harvest plants compared to second and third harvest plants, independently of light condition. It is unknown if *G. decoris* performance was affected by these glycoalkaloid concentrations, because plants were infested after the acclimation period of the plants to the light treatments (30 days after the experiment started).

In my study, the focus was on the glycoalkaloid defence mechanisms, which are known to be present in other *Solanum* species (Chowánsky *et al.*, 2016), but it is possible that compounds other than glycoalkaloids, such as soluble carbohydrates in plants (represented by a high C:N ratio) could have affected *G. decoris* performance more than glycoalkaloids. For example, carbon-based compounds are known to affect insect fecundity in the cotton bollworm and in migratory grasshoppers (Wu and Li, 1992; Joern and Behmer, 1998; Awmack and Leather, 2002). Higher soluble carbohydrates could have negatively affected the fecundity of *G. decoris* and, as a result, first generation females (F_1) were unable to oviposit on unshaded third harvest plants.

Another possibility is that water deficit could have deterred *G. decoris* from ovipositing on unshaded leaves. There is some evidence that mesophyll-feeding lace bugs and leafhoppers avoid feeding and perform poorly on water-stressed plants (Connor, 1988; Hoffman and Hogg, 1991; Trumbule and Denno, 1995). In my study, unshaded leaves of woolly nightshade plants had lower water content than shaded leaves. The decrease in water content has been attributed to an increase in light intensity and consequently higher temperatures (Forbes and Watson, 1992). However, there was no significant difference in ambient temperature between shaded and unshaded plants in my study. In addition, lower water content observed in unshaded leaves from third harvest plants was within a range that

is considered normal for woody shrubs (Goss, 2013). There is no evidence that unshaded woolly nightshade was under water deficit, and that this caused *G. decoris* to avoid ovipositing on unshaded plants. However, it could be that *G. decoris* has a particularly high sensitivity to water content, choosing plants for oviposition that have water content above 80%

5.5. Concluding remarks

Results obtained in this experimental study coincide with results of field observations. Despite light intensities inside the glasshouse and in the field being not similar, there was an effect of light vs. shade on physical, morphological and chemical characteristics of woolly nightshade. Plants growing in the shade were taller, had higher shoot-to root ratio, thinner leaves, and larger specific leaf area. High C:N ratio and glycoalkaloid content in unshaded leaves was mostly evident at the second harvest. Trichome spikes were longer in unshaded leaves (observed after the first harvest). The morphology and reproductive performance of *G. decoris* were affected by light conditions. First generation females were smaller on unshaded plants, and avoided ovipositing on third harvest unshaded plants.

It is known that glycoalkaloids present in Solanaceae act as bioinsecticides and one of their most important insecticidal effects are malfunctions and malformations of the reproductive system (Lopez-Carretero *et al.*, 2005). The reluctance of *G. decoris* to oviposit in unshaded leaves of third harvest plants could be because feeding on leaves with high glycoalkaloids content during their nymphal stages affected the maturity of their reproductive system, which could be investigated in the future. Alternatively, it is possible that higher soluble carbohydrates (indicated by high C:N ratio) could have negatively affected *G. decoris* fecundity.

CHAPTER 6. GENERAL DISCUSSION

The objectives of this study have been to observe the ecological factors that influence the development of an insect, *G. decoris*, and in doing so learn more about the factors that are likely to influence establishment of introduced or invasive insect populations. The findings of this study suggest that behaviour (i.e. maternal care and aggregation), climate (i.e. temperature, photoperiod and humidity), and host plant quality (i.e. plant performance and defence) are all factors that will likely affect the establishment of *G. decoris*. It is also demonstrated that these factors are intertwined and need to be considered as a whole to assess or predict the successful establishment of *G. decoris* as a biological control agent in New Zealand and elsewhere.

In New Zealand, woolly nightshade lacks specialist natural enemies (Winks et al., 2001) but in its native range in South America (e.g., Argentina and Paraguay) eight insect species were found feeding on *S. mauritianum*, including the lace bug *Gargaphia decoris* (Olckers et al., 2002). Specialist herbivores, such as *G. decoris*, have a tight relationship with their host plant and have evolved adaptations to cope with the abiotic and biotic environment where the plant thrives. Some of the adaptations to biotic environment are maternal care and aggregation behaviours.

In this study, it was found that maternal care negatively affected nymphal survival and higher nymphal aggregations did not result in an increase in nymphal survival. These results are counter-intuitive and surprising since previous reports in other *Gargaphia* species (i.e. *G. solani*), have shown that there was an increase in nymphal survival when the mother was present (under field conditions) and the chances of survival were higher when the nymphs were aggregated in higher numbers (Tallamy and Denno, 1981a). It is thought that the results observed in this study can be explained by a restricted plant resource available to the insects interferring with the normal movement and feeding behaviour of mother and nymphs when the plant becomes exhausted. These results could be a representation of what could happen under field conditions when the mother and nymphs face restricted plant resources. Nevertheless, it is possible that the negative effects of maternal care due to competition for restricted resources would be offset by the benefits of maternal care against predators (Tallamy and Denno, 1981a).

Higher aggregative formations have been reported to increase nymphal survival of lace bugs under field conditions (Tallamy and Denno, 1981a). The results of this study show that higher aggregations affected nymphal survival and that isolated nymphs have similar chances to survive than groups of thirty nymphs. It is thought that the availability of the host plant in the experimental setup influenced the results of this study. Under field conditions, limited host availability could impair the benefits of aggregative formation by increasing competition between mother and offspring, thus diminishing survival. However, if there is sufficient plants available, higher aggregation has been proved to be necessary for the survival of lace bugs nymphs by facilitating maternal care, feeding facilitation and increasing the chances of survival when facing predators (Ghent, 1960; Denno and Benrey, 1997, Ramaswany and Cocroft, 2001). Due to the aggregative formation of nymphs and maternal care behaviour, there is a possibility that not only host plant availability (i.e. amount of plants in the field) but as well as the disposition of the plants in the field affects the expression of this behaviours. Experimental observations in this study led to the hypothesis that at those release sites in which woolly nightshade plants are interconnected (i.e. leaves of one bush touching the leaves of another bush), or close together, are more likely to support *G. decoris* than in sites where the weed only grows in patches (McClay and Balcinus, 2005). This could be worth exploring in the future.

In addition to the availability of the host plant, the success of a biological control agents is known to be partly determined by climatic factors such as temperature, photoperiod and humidity, which are critical for their establishment and effectiveness (Speight et al., 1999; Byrne et al., 2004 as cited in Dhileepan et al., 2010) and are deemed important to consider when importing a biological control agent (Sun et al., 2017). Often the effects of these environmental parameters are augmented when they interact with each other (Corkum and Hanes, 1992; Kreppel et al., 2016). In this study, the trials performed in the laboratory indicated that the range of temperatures (20-27.5 ºC) and the photoperiod (16L:8D) are most optimal for *G. decoris* establishment in laboratory conditions. At all temperatures and photoperiods tested there was no evidence of reproductive diapause in *G. decoris* females but rather development and population increase slowed down. At the lower temperature threshold (7 ºC- for nymphs and 10 ºC- eggs) nymphs were not able to survive after three days exposed to constant low temperature and eggs did not hatch. At the

upper temperature threshold (30 ºC- nymphs and eggs), emerging nymphs die after hours of being subjected to higher temperatures and eggs failed to hatch due to dessication. These results suggest that *G. decoris* is likely to establish in the North Island of New Zealand if there are sufficient plants available.

Humidity levels were shown to influence the percentage of eggs hatched. The 70 – 80% RH caused an increase in the mortality of eggs due to pathogens compared to the 50-60% RH. This suggests that in field conditions with high humidity levels, egg survival may be limited; for example in Northland where the relative humidity reaches 77-89% during summer months,this could increase pathogen activity. However, in field conditions, a higher natural air circulation (i.e. windy conditions, compared to enclosed environmental chambers) might help to reduce prolonged conditions of high humidity and pathogen development. Nonetheless, despite the influence on egg hatching, the relative humidity tested in this experiment had no significant impact on the development and reproduction of *G. decoris*.

Based on the experimental data collected in this study and the climate conditions of New Zealand the distribution of *G. decoris* modelled by CLIMEX suggests that establishment in New Zealand should occur successfully in most of the North Island, but shows limited potential to establish in the South Island where conditions are cooler. In the areas of New Zealand where there is predicted to be limited or no establishment of the insect, wooly nightshade is found only sporadically rather than prolifically (WCRC, 2016), and in these places is considered only a moderately serious weed (DOC, 2001). This lends some support to the modelling as G. *decoris* tends to establish in similar climatic conditions as woolly nightshade. However, woolly nightshade has a slightly broader range of temperatures in which it can establish compared to *G. decoris*. Therefore in areas of woolly nightshade infestation that are modelled to be unsuitable for *G. decoris* establishment, the control of the weed would need to rely entirely on other control measures.

Host plant quality is the third factor shown to influence establishment. As mentioned earlier, specialist insects who have a tight bond with their host, are a preferred choice for biological control agents of undesirable pests (Greathead, 1995). However, due

to this host specificity, any ecological process that cause physiological changes to the host can have an undesirable effect on the biological control agent (Brodeaur, 2012).

In this study, the environment (i.e. light intensity) was shown to affect the physiology and morphology of the host plant *S. mauritianum*. The plant displayed adaptations to maintain its photosynthetic efficiency and prevent photoinhibition when subjected to higher light intensity (Powles, 1984). These changes in the physiology of the plant altered the C/N balance within the plant and stimulated an increase in the physical and chemical defences (i.e. trichomes and glycoalkaloids). Glycoalkaloids are known to act as bioinsecticides for several *Solanum* insect pests (e.g. potato aphid *Macrosiphum euphorbiae* Thom. and tobacco hornworm *Manduca sexta* L.) (Chowánski et al., 2016). The lack of oviposition observed in *G. decoris* adults when exposed to higher light intensity has been attributed to a combination of an increase in trichome defence (War et al., 2012) and the increase in glycoalkaloid concentration, which could have affected the physiology of nymphs that fed on these plants. The females that were observed in this study to develop on unshaded plants as immatures were smaller that those that developed on shaded plants, and it is possible that their reproductive capacity could have been affected by glycoalkaloids, either through malformation in their reproductive apparatus (Friedman, 2006), or through a delay in sexual maturity (Weissenberg et al., 1998). While it is possible that other reasons such as predation may also explain this observation, further study could be undertaken to investigate the role of reproductive effects. The finding could explain the failure of *G. decoris* to establish in unshaded plants in the release site in Tauranga and this could suggest that the control of woolly nightshade in open sunny sites would need to rely entirely on other control methods.

High concentrations of glycoalkaloids in the weed plant is not a desirable trait, as it could reduce the effectiveness of a biological control agent (Brodeaur, 2012). The glycoalkaloids detected in *S. mauritianum* are those commonly found in other *Solanum* species (i.e. solamargine, solasonine), which have toxic effects on insects and animals (War et al., 2012). It is possible that *G. decoris* has evolved adaptations to thrive in *S. mauritianum* even with the presence of glycoalkaloids. Some specialist insects have evolved highly specialized enzymes that are able to metabolize specific substrates (Lampert, 2012). However, other specialist insects are negatively affected by the defensive

compounds present in their host plant. Metabolizing large amounts of consumed compounds is energetically expensive no matter the enzyme system specificity (Lampert, 2012).

Due to the specificity of *G. decoris* to *S. mauritianum*, changes in the plant physiology and availability of the plant as a result of changes in the environmental conditions, can have considerable impact on the behaviour and therefore establishment of the insect. The results of this study goes a step further to understand how behaviour, environment and plant interact and the significance for insect establishment. This will aid in decision making in the current biological control program of *S. mauritianum* in New Zealand and worldwide and for other biological control agents that are being considered for importation.

CONCLUSIONS AND RECOMMENDATIONS

Maternal care and aggregation effects:

1. Maternal care negatively affected the total nymphal survival (i.e. percentage of nymphs that reached adulthood) and life cycle duration (i.e. number of days it took the nymphs to develop from egg to adult). However, the restricted amount of host plant and the absence of predators might explain this results; maternal care might prove beneficial under field conditions.

2. Handling *G. decoris* nymphs using a fine brush negatively affected the percentage of nymphs that reached adulthood, but at the same time reduced development duration. The nymphs that were not handled resulted in adults with longer antennae II and narrower longer pronotum than those nymphs that were handled. The trade-offs between reducing development duration and reducing morphological parameters could be a strategy of *G. decoris* to be able to take advantage of fresh resources when they are available. It is recommended to avoid if possible handling first instar nymphs with a fine brush because they are susceptible to damage due to their soft body tissues.

3. Aggregation did not increase the survival and morphology of *G. decoris* but affected nymphal development duration. The lower survival observed in higher aggregation could be a result of reduced host plant availability.

Effect of temperature, photoperiod and humidity on G. decoris performance and potential distribution

1. The optimal temperature and photoperiod for *G. decoris* development, survival, reproduction and population growth was within the range of 20-25 ºC and a photoperiod of 16L:8D. The lower developmental threshold for nymphs was 7 ºC and for eggs 10 ºC, and the upper development threshold for nymphs and eggs was 30 ºC.

2. There is no evidence of reproductive diapause in *G. decoris* at the temperatures and photoperiods tested, rather development and reproduction slowed down. Given the experimental results obtained in this experiment, the CLIMEX model predicted that *G. decoris* is likely to establish in most of the North Island, except in the mountain ranges (e.g. Kaweka and Ruahine Ranges). Most of the South Island was considered unsuitable for *G. decoris* establishment, except parts of the West Coast, Nelson and the Tasman region which are predicted to be moderately to marginally suitable.

3. *G. decoris* population growth was higher at 70 ± 10% RH, despite higher mortality of eggs due to presence of pathogens due to the lack of aeration in enclosed environmental chambers.

4. The CLIMEX model predicted that *G. decoris* could occupy broader regions not only in its native range but in other regions where *Solanum mauritianum* is considered invasive (i.e. New Zealand and South Africa). The predicted establishment of *G. decoris* coincides with the reported distribution of the weed worldwide. However, the weed can withstand to survive in lower temperatures compared to *G. decoris*. Therefore in the South Island were woolly nightshade is present, other control measures should be considered. In the future is recommended to include a climate change scenario in the CLIMEX model. Areas that now are deemed suitable might be unsuitable or viceversa after a period of ten years.

Effect of light intensity on the performance of woolly nightshade and its biological control agent G. decoris

1. Light intensity and plant age (i.e. day of harvest) affected key physiological and morphological aspects of *S. mauritianum.* Plants subjected to shade conditions (70-80% shade) were taller, displayed higher shoot-to-root ratio, thinner leaves, higher water content, larger specific leaf area and higher photosynthetic efficiency than unshaded plants (< 10% shade).

2. Light intensity and plant age affected physical (i.e. trichomes) and chemical defences (i.e. glycoalkaloids) of *S. mauritianum*. Trichome spike density and trichome hair length increased as the plants got older combined with an increase in trichome hair length when plants were exposed to the sun.

3. Three compounds were positively identified as glycoalkaloids: α-solarmagine/β-solamarine, solauricine/solasonine, and unknown-954. The glycoalkaloid highest peak concentration coincided with an increase in C/N ratio during the second harvest.

3. *G. decoris* avoided ovipositing on third harvest unshaded *S. mauritianum* plants. The results indicate that the combination of trichome density and the bioinsecticide effect of the glycoalkaloids in *S. mauritianum* during the nymphal stages (on second harvest plants) affected *G. decoris* adult performance. This might explain observations that *G. decoris* outbreaks in New Zealand are currently confined to shaded plants. However, more studies need to be performed to confirm these results.

APPENDIX

Table S1. CLIMEX indices used to estimate the potential growth and survival of a population at a given location. These indices are grouped into growth-related indices and stress-related indices. Indices are defined as reported by Kriticos et al. (2015).

Growth-related indices	Description
Temperature Index (TI)	Response of the species to the daily temperature cycle. It varies between 0 and 1. Population growth is maximized when TI = 1, and is zero when TI = 0. Four parameters define the range of suitability for temperature, which are used to calculate TI: DV0 = *lower temperature threshold*, DV1 = *lower optimum temperature*, DV2 = *upper optimum temperature*, DV3 = *upper temperature threshold.*
Moisture Index (MI)	Is calculated with a hydrological model from the stored soil moisture from the previous week and the current week's rainfall and evapotranspiration (moisture loss from plants and soil surface). SM = 0 indicates no soil moisture; SM = 0.5 indicates soil moisture content is 50% of capacity; SM = 1 indicates that the soil moisture content is 100% of capacity; SM > 1 indicates a water content greater than the soil holding capacity. The four parameters used in calculating soil moisture to determine population growth include: SM0 = *lower soil moisture threshold*, SM1 = *lower optimal soil moisture*, SM2 = *upper optimal soil moisture*, SM3 = *upper soil moisture threshold.*
Stress-related indices	**Description**
Cold Stress (CS)	Occurs when a threshold number of degree days above the developmental temperature

	threshold (DVCS) are not reached. The cold stress temperature threshold (TTCS) represents the average weekly minimum temperature (°C) below which cold stress accumulates. Cold stress accumulates at a given rate (THCS) when the average weekly minimum temperature (T_{min}) drops below a given threshold value (TTCS).
Heat stress (HS)	Relates to species that fail to survive when exposed to excessively high temperatures. The threshold temperature model for heat stress has two parameters: TTHS is the threshold temperature (°C) above which heat stress accumulates, and THHS is the rate at which stress accumulates.
Dry stress (DS)	Dry stress accumulates when the soil moisture level falls below the Dry Stress Threshold (SMDS). The difference between SMDS and the soil moisture level (SM) is multiplied by the Dry Stress Rate (HDS) to give the resultant dry stress for the week.
Wet stress (WS)	Wet stress accumulates if the soil moisture level exceeds the Wet Stress Threshold (SMWS). The difference between SMWS and the soil moisture level (SM) is multiplied by the Wet Stress Rate (HWS) to give the resultant wet stress.

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