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

Cecilia María Falla

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.
# TABLE OF CONTENTS

**LIST OF FIGURES** ........................................................................................................................................... i

**LIST OF TABLES** ............................................................................................................................................. xi

**CHAPTER 1: INTRODUCTION** .......................................................................................................................... 1

1.1. Background .................................................................................................................................................. 1

1.2. Objectives .................................................................................................................................................... 4

**CHAPTER 2: LITERATURE REVIEW** ................................................................................................................. 5

2.1. Overview .................................................................................................................................................... 5

2.2. *Solanum mauritianum*: an invasive weed ................................................................................................. 6

2.2.1. Taxonomical classification of *Solanum mauritianum* ........................................................................... 6

2.2.1. Botanical description ............................................................................................................................ 7

2.2.2. Geographic distribution ......................................................................................................................... 8

2.2.3. Ecological and economic importance .................................................................................................... 9

2.3. Taxonomical classification of *Gargaphia decoris* ..................................................................................... 11

2.3.1. Geographic distribution ....................................................................................................................... 11

2.3.2. *Gargaphia decoris* biology .................................................................................................................. 12

2.4. Parental care in insects ............................................................................................................................... 16

2.4.1. Maternal care in lace bugs .................................................................................................................... 20

2.4.2. “Egg dumping” behaviour and its ecological importance ..................................................................... 22

2.4.3. Aggregation behaviour in insects .......................................................................................................... 23

2.5. The effect of environment on insect life history: temperature, humidity, photoperiod ............................. 26

2.5.1. Temperature effects on development and predicting the establishment of the lace bug *Gargaphia decoris* .......................................................................................................................... 28

2.5.2. Effect of temperature on insect performance ......................................................................................... 32

2.5.3. Effect of humidity on insect performance ............................................................................................. 33

2.5.4. Effect of photoperiod on insect performance ......................................................................................... 34

2.6. Plant-insect interactions ............................................................................................................................. 35

2.6.1. Effect of light intensity on plant growth and implications to herbivory ................................................. 36

2.6.2. Effect of light intensity on plant defences .............................................................................................. 41
5.2.8. Lace bug performance ................................................................. 131
5.2.9. Statistical analysis ........................................................................ 132
5.3. Results ........................................................................................... 133
  5.3.1. Environmental variables ............................................................. 133
  5.3.2. Plant performance ................................................................. 135
  5.3.3. Plant physical defences ............................................................ 142
  5.3.4. Plant chemical defences ............................................................ 152
  5.3.5. Lace bug performance ............................................................. 159
5.4. Discussion ...................................................................................... 161
  5.4.1. Plant performance and leaf adaptations to light intensity .......... 161
  5.4.2. Plant performance: physical defenses ...................................... 164
  5.4.3. Effect of light intensity on woolly nightshade chemical defences ............................................................ 165
  5.4.4. Gargaphia decoris performance .............................................. 166
  5.5. Concluding remarks ........................................................................ 168

CHAPTER 6: GENERAL DISCUSSION ......................................................... 169
CONCLUSIONS AND RECOMMENDATIONS ........................................ 175
APPENDIX .......................................................................................... 179
BIBLIOGRAPHY ......................................................................................... 181
LIST OF FIGURES

Figure 2.1. Woolly nightshade tree (a), stipulate leaves at the base of stems (b), inflorescence (c), and fruits in clusters (d). .............................................................................................................. 8

Figure 2.2. *G. decoris* adults, underside of female (left) and male (right). Scale bar represents 50 μm................................................................................................................................. 12

Figure 2.3. *G. decoris* (3rd, 4th, and 5th) nymphal instars.................................................................................. 14

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). .............................................................................................................................................. 14

Figure 2.5. Chlorotic leaf after extensive feeding by *G. decoris*. Black excrement (red arrow) is deposited on the upperside of *Solanum mauritianum* leaves. ................................. 16

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). .............................................................................................................................................. 17

Figure 2.7. *G. decoris* mother guarding an egg batch. .......................................................................................... 21

Figure 2.8. *G. decoris* mother near aggregated nymphs.................................................................................... 26

Figure 2.9. Development rate of immature stages at five constant temperatures for *Monoscoteira unicostata* (Hemiptera: Tingidae) (Sánchez-Ramos et al., 2015). .......... 28

Figure 2.10. Visual representation of degree-days. The sections in black under the curve represent the number of degree days that fall between the upper and lower threshold for a 24-hour period (Murray, 2008). .............................................................................................................................................. 30
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). ................................................................. 39

Figure 2.12. Chemical structure of solasodine (retrieved from Al-Sinani and Eltayeb, 2013). .................................................................................................................................... 49

Figure 2.13. Class of glycoalkaloids most commonly found in Solanaceae (retrieved from Drewes, 1993). ...................................................................................................................... 49

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 ....................................................... 58

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, α = 0.05). .............................................................................................................. 61

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). .............................................................................................................. 62

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. .................................................................................................................................... 63

Figure 3.5. Linear discriminant analysis (LDA) biplot (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
between treatments along LDA were Pronotum Width (negative) and Antennae II length (positive). ................................................................. 64

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; and 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. ................................................................. 90

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 \( S(t) = \Pr (T > t) \). ............. 92

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 \( S(t) = \Pr (T > t) \). ............. 93

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, \( \alpha = 0.05 \)). ................................................................................................................................................................. 97

Figure 4.5. Survival probability of *Gargaphia decoris* females (left) and males (right) when grown at 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 \( S(t) = \Pr (T > t) \). ................................................................................................................................................................. 97

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 \( S(t) = \Pr (T > t) \). ............. 98
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 $S(t) = Pr(T > t)$. 99

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. 101

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). 103

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). 104

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). 105

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. 119

Figure 5.2. Measuring photosynthetically active radiation (PAR) among woolly nightshade plants growing in unshaded (left) and shaded conditions (right). 120
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.

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.

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

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.

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 mm² sample area. Each stellate trichome consists of a central stalk with several radiating spikes.

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 2\textsuperscript{nd} 2015, and the final harvest on January 11\textsuperscript{th} 2016. 

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

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, $\alpha = 0.05$). 

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. 

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, $\alpha = 0.05$). ......................................................... 140

Figure 5.19. Net photosynthesis ($P_n$) (CO$_2$ $\mu$mol m$^{-2}$ s$^{-1}$) 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, $\alpha = 0.05$). ............................................................................................ 141

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................................................................. 141

Figure 5.21. 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. ....................................................................... 143

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, $\alpha = 0.05$). The letters a and b only apply within a harvest. ............................................................................................................................................ 143

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, $\alpha = 0.05$). ......................................................................................................................... 144

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). ......................................................................................................................... 145

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). ......................................................................................................................... 146

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, α = 0.05). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest ........................................................................................................ 147

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, α = 0.05) ........................................................................................................ 147

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 ........................................................................................................ 148

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, α = 0.05). ........................................................................................................ 149

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) ........................................................................................................ 149

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, α = 0.05). The graph represents 3 one-way ANOVAs; letters a and b only apply within a harvest.......................................................................................... 150

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 ...................................................... 152

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 x10⁵. The peaks surrounded by a dotted box represent: a – α-solamargine or β-solamarine; b – solauricine or solasonine; and c – Unknown 954. .............................................................. 153

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 10⁵ ................................………………………………………………………………………………………… 154

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). .................................................................................................................... 155

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
Figure 5.37. PCA biplot 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%).

Figure 5.38. Differences in the mean number of eggs/batch/female in the lace bug *Gargaphia decoris* between initial (F₀) and first generation (F₁) females that fed on shaded woolly nightshade plants. F₀ females lived on second harvest plants (plants 58-day old) and F₁ on third harvest plants (plants 100-days old).

Figure 5.39. PCA of body measurements performed on lace bugs *Gargaphia decoris* (F₁ 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%).
LIST OF TABLES

Table 2.1. Common names of *S. mauritianum* ................................................................. 7

Table 2.2. List of orders where aggregating insects can be found (excerpt from Vulinec, 1990) ..................................................................................................................................... 24

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)................. 31

Table 3.1. Average nymphal instar development duration (days) and total nymphal development duration (days) of *Gargaphia decoris* nymphs at different aggregation densities. Significant differences between aggregations densities (within each column) are represented by different letters. Chi-square values ($\chi^2$) are represented with an asterisk (*); one-way ANOVA or Kruskal-Wallis test, $\alpha = 0.05$. ........................................................................ 66

Table 4.1. Description and formulae of life table reproductive parameters (Carey, 1993; Begon et al., 1996) .................................................................................................................. 82

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. 87

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 row are not significantly different (Fisher’s HSD test, $\alpha = 0.05$). ................................................................................ 89

Table 4.4. Effect of four constant temperatures: 15, 20, 25 and 27.5 °C on 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, $\alpha = 0.05$). ................................................................................ 94

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. ........................................................................................................... 95
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. .................................................................98

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.................................................................100

Table 5.1. Solvent programme and separation time (minutes) of glycoalkaloids. .......... 130

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). ............................................................................................. 137

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 (ULET), 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. ....................................... 151

Table 5.4. Concentration of glycoalkaloid compounds (μg/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, α = 0.05. .................................................... 156

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. .................................................................160