INVESTIGATIONS INTO THE INCIDENCE AND ECOLOGY OF *BILOBATA SUBSECIVELLA* (ZELLER) (LEPIDOPTERA: GELECHIIDAE), A NEW PEST OF GROUNDNUT IN SOUTH AFRICA

By

N.M. Buthelezi

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the Discipline of Entomology, School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, Republic of South Africa

2015

Thesis abstract

The leaf-mining moth, *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae), thought to be an invasion from Indo-Asia (where it is known as *Aproaerema modicella* (Deventer); but hereafter referred to as *B. subsecivella*) has become a major pest of groundnut (*Arachis hypogaea* L.) and soya bean (*Glycine maxi* (L.) Merr.) in South Africa and Africa as a whole. Following the sudden outbreaks of *B. subsecivella* as a new pest of groundnut in a number of African countries, the continent has been confronted with the problem of having no information on the biology and ecology of the pest that can be used for its management/control. In this context, the main aim of the research for this thesis was to study the biology and ecology of *B. subsecivella* in South Africa with the main objective of obtaining information that will assist in its management as a novel pest of groundnut. To achieve this objective, several studies were carried out.

First, a detection survey of *B. subsecivella* infestation was conducted on groundnut, soya bean and lucerne (*Medicago sativa* L.), the common host crops for *B. subsecivella* in India, at six widely separated sites in South Africa during the 2009/2010 growing season. The sites included the Agricultural Research Council research stations at Potchefstroom and Brits as well as the farms surrounding the Brits research farm in the North West province, Vaalharts Research Station in the Northern Cape province, the Department of Agriculture Lowveld Agricultural Research Station near Nelspruit in Mpumalanga province, and Bhekabantu and Manguzi in the northern part of the KwaZulu-Natal province. The study had three objectives. The first was to build a complete host crop/plant list and record damage symptoms caused by *B. subsecivella* in South Africa. The second was to identify the pest to species level. The third was to determine its inter- and intra-population genetic diversity by analysing in, both cases, the mitochondrial DNA (mtDNA) COI gene of specimens collected from these sites. Sixty specimens comprising 24 larvae, 24 pupae and 12 moths were collected from the six survey sites, and their mtDNA COI were sequenced and compared with those from the Barcode of Life Data System (BOLD) gene bank. Infestation by *B. subsecivella* was observed on groundnut and soya bean, but not on lucerne. The mtDNA COI from all specimens of the pest, irrespective of whether they were from groundnut or soya bean, matched 100% with the sequences in BOLD belonging to a *B. subsecivella* population occurring in Australia (referred to as *Aproaerema simplexella* (Walker)) and known as the soya bean moth in that country).

There was very little genetic diversity between and within the populations from the six sites, which suggested that the populations were maternally of the same origin.

Further molecular and phylogenetic studies were also completed to determine the evolutionary relationships between *B. subsecivella* populations collected from Australia, Africa and India. These studies involved sequencing and analysing five gene regions of mitochondrial and nuclear DNA, including COI, cytochrome oxidase II (COII), cytochrome *b* (cytb), 28 ribosomal DNA (28S rDNA), and intergenic spacer elongation factor-1 alpha (EF-1 ALPHA). The mtDNA COI analysis also included *B. subsecivella* (but called *A. simplexella*) sequences downloaded from the National Center for Biotechnology Information (NCBI) GeneBank collected from different areas in Australia. In four phylogenetic trees (COI, COII, cytb and EF-1 ALPHA), sequences of *B. subsecivella* personally sampled from Australia were grouped separately from the others, whereas sequences of *B. subsecivella* from South Africa, India and Mozambique were clustered in one group in most cases. Furthermore, in the mtDNA COI phylogenetic tree, one Australian sequence of *B. subsecivella* that was downloaded from the NCBI GeneBank was grouped with other sequences from South Africa, India and Mozambique. Moreover, one sequence of *B. subsecivella* personally sampled from Australia was grouped with the other two sequences of *B. subsecivella* from Australia that were downloaded from the NCBI GeneBank. Based on these results, it could be hypothesized that there is genetic diversity within *B. subsecivella* populations in Australia. The mtDNA COI gene analysis in the current study revealed that there are *B. subsecivella* populations in Australia that are similar to the *B. subsecivella* populations in South Africa, Mozambique and India. Phylogenetic analysis of the 28S gene region revealed a lack of genetic diversity between sequences of *B. subsecivella* from India, South Africa, Mozambique and Australia. Genetic pairwise distances between the experimental sequences ranged from 0.97 to 3.60% (COI), 0.19% to 2.32% (COII), 0.25 to 9.77% (cytb) and 0.48 to 6.99% (EF-1 ALPHA).

Field experiments were then conducted at Vaalharts, Brits, Nelspruit, Manguzi and Bhekabantu during the 2010/2011 and 2011/2012 growing seasons. These experiments pursued three objectives. The first one was to determine *B. subsecivella* infestation levels on groundnut, soya bean, lucerne, pigeon pea (*Cajanus cajan* L.) and lablab bean (*Lablab purpureus* L.) under field conditions. The second was to develop a host plant list for *B. subsecivella* and the third was to determine the effect of cypermethrin application on damage

by *B. subsecivella* to groundnut and soya bean plants. In the 2010/2011 season, larval infestation was monitored on groundnut crops planted in November 2010 and January 2011. In the 2011/2012 season, larval infestation was monitored on groundnut, soya bean, lucerne, pigeon pea and lablab bean planted in November 2011 and January 2012. Wild host plants were inspected for damage symptoms and the presence of larvae. An experiment which examined the effect of cypermethrin application on *B. subsecivella* damage to groundnut and soya bean plants was completed in the 2011/2012 season at Vaalharts and Nelspruit. A survey for wild plant hosts of *B. subsecivella* was conducted in the proximity of the field experiments during the 2011/2012 growing season, as well as in winter. Amongst the host crops tested, soya bean was highly infested by *B. subsecivella* followed by groundnut, at all sites. The pest was also observed on pigeon pea at all sites, but the infestation was very low, while lucerne had very low larval infestation. No infestation was observed on lablab bean across these sites. Groundnut and soya bean crops planted in January were severely infested by *B. subsecivella*, compared to the crops planted in November; however, *B. subsecivella* infestation on crops was observed 5-6 weeks after crop emergence. Sprays of cypermethrin on groundnut and soya bean reduced larval infestation in both crops to very low levels. Wild plant hosts identified were from five families which included three species in the Leguminosae, two species in the Convolvulaceae, two species in the Malvaceae and one species each in the Lamiaceae and Asteraceae.

Seasonal monitoring of the flight activity of *B. subsecivella* moths was completed at Manguzi, Bhekabantu, Nelspruit, Brits and Vaalharts over a two-year period (from November 2010 to December 2012). The objective of this study was to monitor the flight activity of *B. subsecivella* in order to understand its dispersal and off-season survival tactics and to predict its initial occurrence. Pheromone traps were used to monitor the moths' flight activity. Information collected included climatic data (rainfall, temperature and humidity) that were obtained from ARC weather stations placed at four planting sites. Pearson's test for correlation was performed to assess the relationship between *B. subsecivella* moth catches and environmental factors (rainfall, temperature and humidity). Results from this study showed variation in *B. subsecivella* populations throughout the monitoring period. The highest peak in *B. subsecivella* catches was between January and April/May for both seasons. Though low in numbers, *B. subsecivella* moths were caught in winter at Manguzi, Nelspruit, Vaalharts and Bhekabantu. No *B. subsecivella* moths were trapped during the winter months at Brits. Pearson's test for correlation indicated that there was a significant negative

association between temperature and *B. subsecivella* catches in pheromone traps at Nelspruit, whereas at Vaalharts there was a significant positive association between humidity and *B. subsecivella* catches. There was no correlation between environmental factors and *B. subsecivella* catches at Manguzi and Brits. Furthermore, it was found that *B. subsecivella* in Australia (moths collected for DNA analysis in the current study) responded to the species-specific lure that was developed from the sex pheromone of *B. subsecivella*, referred to as *A. modicella* in India.

Overall, the study revealed important ecological and genetic information on *B. subsecivella* populations occurring in southern Africa. More importantly, this study established the genetic connection between *B. subsecivella* populations from Australia, India and Africa. Hence, the species conforming to these populations were tentatively synonymized as *B. subsecivella* in this thesis.

Declaration

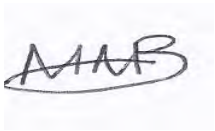
I, **Nokubekezela Makhosi Buthelezi**, declare that:

i. The research reported in this thesis is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other persons' data, writing, pictures, graphs or information, unless acknowledged as being sourced from other persons.

iv. This thesis does not contain text, graphics or tables copied and pasted from the internet, except where specifically acknowledged, and the source being detailed in the thesis and in the References section.



N.M. Buthelezi (Candidate)

Dr. D.E. Conlong (Supervisor)

Dr. T. Olckers (Co-supervisor)

Prof. G.E. Zharare (Co-supervisor)

Acknowledgements

I wish to express my earnest gratitude to the following people/organisations:

To my supervisors, Prof. Desmond Conlong, Dr. Terence Olckers (both from the University of KwaZulu-Natal) and Prof. Godfrey Zharare (from the University of Zululand) for their guidance, encouragement, commitment and support through the whole journey; they have been an inspiration and pillar of my strength.

Researchers and field assistants of the Agricultural Research stations at Brits and Vaalharts, Department of Agriculture Lowveld Agricultural Research Unit at Nelspruit and farmers at Manguzi and Bhekabantu for assisting with maintaining research experiments and allowing us to use their fields for this study.

Dr. Domingos Cugala (Eduardo Mondlane University, Mozambique) for assisting with the collection of *B. subsecivella* specimens in Mozambique.

Dr. G.V. Ranga Rao (ICRISAT-Hyderabad) for assisting with the collection of *B. subsecivella* specimens in India.

Mr. Roy Cockin (Brisbane, Australia) for assisting with the collection of *B. subsecivella* specimens in Australia.

Mr. Dudley Farman (Natural Resources Institute, University of Greenwich, United Kingdom) for supplying pheromone lures for *B. subsecivella*.

Prof. Jennifer Lamb (University of KwaZulu-Natal) for assisting with the analysis of the phylogenetics data.

Dr. Rosemary Ntuli (University of Zululand) for assisting with the identification of *B. subsecivella* host plants.

Dr. Devan Nadasan (Mangosuthu University of Technology) for assisting with the statistical analyses of the data for *B. subsecivella* seasonal monitoring field experiments.

Dr. Klaus Sattler (Natural History Museum, London, UK) for providing information on species names by which the GLM is known.

Ms Lauren Martin (South African Sugarcane Research Institute (SASRI)) for assisting with the analyses of the mtDNA COI sequences.

Mr. Mike Way (SASRI) for taking pictures of the *B. subsecivella* moth.

Stellenbosch University – Central Analytical Facilities, DNA Sequencing Facility and Inqaba Biotech Industries (South Africa) for DNA sequencing.

National Research Foundation (NRF), University of Zululand and Mangosuthu University of Technology for financial support.

Agricultural Research Council for providing climate data.

All my friends for their support, encouragement and for being there whenever I needed them.

My family for their love, encouragement, continued support and for always believing in me. I would not have reached this far without them, and for that I will always be thankful.

Above all, I thank God for everything through it all.

List of papers resulting from this thesis that have been published in or accepted by peer reviewed journals

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2012.The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soybean moth (*Aproaerema simplexella* PS 1) (Walker) (Lepidoptera: Gelechiidae). *African Journal of Agricultural Research* **7**(38): 5285-5292. (Chapter 2)

BUTHELEZI, N.M, CONLONG, D.E. & ZHARARE, G.E. 2013. A comparison of the infestation of *Aproaerema simplexella* on groundnut and other known hosts for *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *African Entomology* **21**(2): 183-195. (Chapter 5)

BUTHELEZI, N.M., ZHARARE, G.E. & CONLONG, D.E. In press. Behavioural and molecular evidence suggesting the re-examination of the taxonomy of *Aproaerema simplexella* Walker, *Aproaerema modicella* Deventer and *Stomopteryx subsecivella* Zeller. (Accepted for publication by *African Entomology* and will appear in Vol. 24(1), which is scheduled to be published in March 2016). (Chapter 3)

Table of contents

Chapter Three

Molecular and behavioural evidence suggest a re-examination of the taxonomy of *Aproaerema simplexella* (Walker) and *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae)

Chapter Four

Phylogenetic relationships of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) populations collected from India, Australia and Africa based on mitochondrial and nuclear DNA gene sequences

Chapter Five

A comparison of the infestation of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) on groundnut and other known hosts, and the impact of insecticide applications on its populations in groundnut and soya bean

Chapter Six

Seasonal monitoring of the incidence and flight activity of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) at five sites in South Africa

Thesis introduction

Groundnut (*Arachis hypogaea* L.) is an important annual, self- pollinated legume crop that is grown worldwide on some 24 million hectares (ha) for protein and the extraction of its edible oil (Janila *et al.* 2013). Insect pests represent a major yield constraint in groundnut production, either as a result of direct damage or as vectors of viral diseases (Ghewande & Nandagopal 1997). Currently, the production of groundnut in Africa is threatened by the groundnut leaf miner (GLM) which has generally been referred to as *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae), a major pest of groundnut and soya bean (*Glycine max* (L.) Merr.) in Indo-Asia (Shanower *et al.* 1993). The groundnut leaf miner is a small moth whose larvae create mines in between the upper and lower epidermis of the green leaf, thereby reducing the photosynthetically active leaf area. This adversely affects the growth and yield of the crop. A single larva destroys from 34.8 to 179.3 cm^{-2} of leaf area in its lifetime (Islam *et al.* 1983; Shanower 1989). The damaged leaves eventually become brownish, rolled and desiccated, which results in early defoliation (Kenis & Cugala 2006), and this further negatively impacts on the growth and yield of the groundnut plants. Groundnut leaf miner can cause up to a 90% loss in total yield of groundnut (Reddy *et al.* 1978; Sumithramma 1998), and where there are no natural enemies, an epidemic can result in total crop loss (Wightman & Ranga Rao 1993).

Previously confined to the Indo-Asian continent, the groundnut leaf miner pest problem was first noticed on the African continent in Uganda in 1998 (Epieru 2004). The problem has since raised considerable alarm and concern in the groundnut production industries of Malawi (Subrahmanyam *et al.* 2000), Uganda (Page *et al.* 2000; Epieru 2004), Mozambique (Kenis & Cugala 2006), Democratic Republic of the Congo (Munyuli *et al.* 2003) and South Africa (Du Plessis 2002). In South Africa, GLM was first noticed on groundnut in 2000 within the Vaalharts irrigation scheme in the Northern Cape Province (Du Plessis 2002). Since then, it has spread over the entire groundnut production areas of the country, including the Free State, Northern Cape, North West and Mpumalanga provinces (Du Plessis 2003), and has become a major pest that is threatening the viability of groundnut production in the country. In KwaZulu-Natal, the pest was identified at Manguzi in the northern part of the province during the 2008/2009 season where it caused total crop losses in late plantings (Zharare, pers.

comm.).[1] The severity of the pest's occurrence, however, appears to differ from location to location and from year to year. Generally, the occurrence of GLM is highly sporadic (Kennis & Cugala 2006), and this might pose difficulties in predicting GLM incidence.

In the rural areas of South Africa, as in other rural African areas, groundnut is a basic staple crop of small-holder farmers and is grown both for subsistence and as a cash crop (Janila *et al.* 2013). Therefore, GLM poses a serious threat to the food security of these areas. There are some insecticides that are used to provide control of GLM (Kenis & Cugala 2006), but these are largely unaffordable for small-holder farmers. There is thus a necessity to find cheaper alternative methods for managing GLM for small-holder farmers. As a relatively new pest in South Africa, there is not much information on the ecology and ecophysiology of the pest that might help to predict its incidence and potential for outbreaks, and to facilitate control measures.

Integrated Pest Management (IPM) involves employing different management approaches against pests (e.g. cultural control, biological control, chemical control), which either reduce the incidence or delay the build-up of the insect pest complex (Nandagopal & Ghewande 2004). Furthermore, they play a vital role in maintaining pest populations at levels below those causing economic injury (Kogan 1998). In order to develop an effective IPM program, it is crucial to have ecological information about pests and their crop environments beforehand (Kogan 1998). Kenis & Cugala (2006) suggested various integrated approaches which can be employed in controlling GLM, such as intercropping, manipulation of planting dates, utilization of less suitable crop genotypes, trap crops, botanical pesticides and the bacterial biopesticide *Bacillus thuringiensis* (Berliner). One of the examples of an integrated approach that has proven to be efficient in controlling GLM in India is described by Nandagopal & Ghewande (2004). This approach involves trap crops (soya bean), pheromone traps, specific planting patterns (plant groundnut with a suitable variety of soya bean), base spray schedules (spraying at 30-35, 45-50, and 60-65 days after planting) and botanical insecticide treatments (2% crude neem oil).

Since the identification of the GLM problem in South Africa in 2000, there have been several collaborative studies conducted on the pest by the Agricultural Research Council – Summer Grain Crops Institute and the North West University, both at Potchefstroom in the North

[1]GE Zharare, University of Zululand, Private Bag x 1001, KwaDlangezwa 3886, Empangeni, Tel: +2735 9026072, Email: ZharareG@unizulu.ac.za

West Province. These studies included: a) the development of a control strategy for GLM on groundnut; b) distinguishing male from female larvae; c) surveys on GLM infestation levels in groundnut and the rates of parasitism of the pest in South Africa and; d) monitoring GLM flight activity at the borders of groundnut fields using pheromone traps. However, there still remain several important ecological questions with respect to the control of the pest in South Africa, which include:

(i) Where did the GLM in South Africa originate from?
(ii) How does the pest move from one area to another? Has it become naturalized in and adapted to different climatic environments?
(iii) How does the pest survive from one season to another?
(iv) Is the GLM in South Africa the same species as the one in India?

The groundnut leaf miner in Africa was thought to be a recent invasion of *A. modicella* from the Asian continent (Du Plessis 2002; Kenis & Cugala 2006). However, there are reports dating back to the 1950s of a moth similar to *A. modicella*, but referred to as *Stomopteryx subsecivella* (Zeller) [= *Gelechia* (*Brachmia*) *subsecivella* (Zeller)], being recorded but being considered of non-economic importance in Africa (Janse 1954; Mohammad 1981). Also, in Australia there is a congeneric soya bean pest (*Aproaerema simplexella* (Walker) [= *Gelechia simplexella* (Walker)] which is morphologically similar to *A. modicella* (Bailey 2007). The use of different names for GLM worldwide is also reflected in the work of Shanower *et al*. (1993), who described the GLM in India as *Anacampsis nerteria* (Meyrick), the one in Africa as *S. subsecivella* (Zeller) and another in India-Indonesia as *A. modicella* (Van Deventer). Further literature searches revealed several synonyms that have been applied to this insect. These include *Aproaerema nerteria* (Meyrick) (Fletcher 1914, 1917, 1920), *Stomopteryx nerteria* (Meyrick) (Anon 1941; Cherian & Basheer 1942), *Stomopteryx subsecivella* (Zeller) (Abdul Kareem *et al.* 1972-73) and *Biloba subsecivella* (Zeller) (Anon 1977; Dean 1978).

The current pool of knowledge cannot answer all of the above questions. Therefore, the current study was conducted to provide further information on the ecology of the pest in South Africa. It was also envisaged that molecular studies (DNA analysis) might provide answers about the origin of GLM in South Africa, as they offer more precise options for species identification (Scheffer 2000), and thus provide a lead in determining the best integrated management plan for the control of this pest in South Africa. The overall aim of this study was to obtain information on the ecology and the genetics of GLM in South Africa

with a view towards finding ways to deal with it as a pest of groundnut. The objectives embedded within this overall aim were:

(i) To investigate the incidence, population dynamics and behaviour of GLM in relation to geographic area, season and climatic conditions.
(ii) To determine the genetic diversity of GLM from the different agro-ecological regions of South Africa through DNA analysis.
(iii) To determine the relatedness between GLM in Africa and Indo-Asia and the soya bean moth in Australia.

The work on GLM which is reported in this thesis has indicated that *A. modicella*, *A. simplexella* and GLM in Africa are very closely related to each other; consequently, these 'species' have been tentatively synonymized as *Bilobata subsecivella* (Zeller) based on the analyses of mitochondrial and nuclear DNA. However, further studies including both molecular and morphological analyses of the genitalia of the specimens will be conducted to reinforce the synonymization status of the species. The literature on the *B. subsecivella* population in India (previously called *A. modicella*) has been reviewed for the purpose of this thesis, as it is the *B. subsecivella* population that has been extensively studied.

The thesis is represented in the form of separate chapters using a setup of complete distinct papers. Consequently, there might be duplication of some information between chapters, especially in the introduction and reference sections. Some of these papers have already been published in peer reviewed journals. For consistency of referencing, the style of the journal *African Entomology* has been used throughout the thesis. The layout of the thesis is as follows:

a) Thesis introduction.
b) Literature review (Chapter 1).
c) The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soya bean moth (*Aproaerema simplexella* (Walker)) (Lepidoptera: Gelechiidae) (Chapter 2).
d) Molecular and behavioural evidence suggesting a re-examination of the taxonomy of *Aproaerema simplexella* (Walker) and *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) (Chapter 3).

e) Phylogenetic relationships of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) based on mitochondrial and nuclear DNA gene sequences (Chapter 4).

f) A comparison of the infestation of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) on groundnut and other known hosts and the impact of insecticide applications on its populations in groundnut and soya bean (Chapter 5).

g) Seasonal monitoring of the incidence and flight activity of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) at five sites in South Africa (Chapter 6).

h) Thesis overview.

References

ABDUL KAREEM, A., NACHIAPPAN, R.W., SADAKTHULLA, S. & SUBRAMANIAM. T.R. 1972-73. An account of the recent insecticidal control of surulpoochi, *Stomopteryx subsecivella* Zeller, on groundnut. Annamalai University Agricultural Research Annual **4&5**: 150-153.

ANONYMOUS. 1941. Agriculture and Animal Husbandry in India. 1938-39. Delhi, 422 pages.

ANONYMOUS. 1977. Outbreak of Pest and Diseases. Quarterly Newsletter F.A.O. Plant Protection Committee for South East Asia and Pacific Region **20** (4): 3-5.

BAILEY, P.T. 2007. Pests of field crops and pastures: Identification and control. CSIRO Publishing, Australia. 221-222.

CHERIAN, M.C. & BASHEER, M. 1942. Studies on *Stomopteryx nerteria* Meyr.- A pest of groundnut in the Madras Presidency. *Madras Agricultural Journal* **30**: 379-381.

DEAN, G.J. 1978. Insects found on economic plants other than rice in Laos. *Pest Articles & News Summaries* **24**: 129-142.

DU PLESSIS, H. 2002. Groundnut leaf miner *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48 - 49.

DU PLESSIS, H. 2003. First report of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut, soya bean and lucern in South Africa. *South African Journal of Plant & Soil* **20**: 48.

EPIERU, G. 2004. Participatory evaluation of distribution, status and management of the groundnut leaf miner in the Teso and Lango farming systems. Final Technical Report, Serere Agricultural and Animal Production Research Unit (SAARI), Soroti, Uganda. 30.

FLETCHER, T.B. 1914. Some South Indian Insects and Other Animals of Importance-considered especially from an economic point of view. Government Press, Madras, 565 pages.

FLETCHER, T.B. 1917. Groundnut Pests. *Proceedings of the Second Entomological Meeting.* Pusa. 43.

FLETCHER, T.B. 1920. Life histories of Indian Insects: Microlepidoptera. Memoirs, Department of Agriculture, India, Pusa. *Entomology Series* **6**: 77-79.

GHEWANDE, M.P. & NANDAGOPAL, V. 1997. Integrated pest management in groundnut (*Arachis hypogaea* L.) in India. *Integrated Pest Management Reviews* **2**:1-15.

ISLAM, W., AHMED, K.N., NARGIS, A. & ISLAM, U. 1983. Occurrence, abundance and extent of damage caused by insect pests of groundnuts (*Arachis hypogaea). Malaysian Agricultural Journal* **54**: 18-24.

JANILA, P., NIGAM, S.N., PANDEY, M.K., NAGESH, P. & VARSHNEY, R.K. 2013. Groundnut improvement: use of genetic and genomic tools. *Frontiers in Plant Science* **4**: 1-16.

JANSE, A.J.T. 1954. The Moths of South Africa. V. Gelechiidae. Pretoria, Transvaal Museum, South African Museum **5**(4): 301-464, pls. 137–202.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural* Resources **1**: 1-9.

KOGAN, M. 1998. Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology* **43**: 243-270.

MOHAMMAD, A. 1981. The groundnut leaf miner, *Aproaerema modicella* (Deventer) (= *Stomopteryx subsecivella* (Zeller) (Lepidoptera: Gelechiidae). A review of world literature. Occasional paper 3, Groundnut Improvement Program, ICRISAT.

MUNYULI, T.M.B., LUTHER, G.C., KYAMANYWA, S. & HAMMOND, R. 2003. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda and Democratic Republic of Congo. *African Crop Science Conference Proceedings* **6**: 231-237.

NANDAGOPAL, V. & GHEWANDE, M.P. 2004. Use of Neem products in groundnut pest management in Uganda. *Natural Product Radiance* **3**(3): 150-155.

PAGE, W.W., EPIERU, G., KIMMINS, F.M., BUSOLO-BULAFU, C. & NALYONGO, P.W. 2000. Groundnut leaf miner *Aproaerema modicella*: a new pest in eastern districts of Uganda. *International Arachis Newsletter* **20**: 64-6.

REDDY, N.M., HAVANAGI, G.V. & HEGDE, B.R. 1978. Effect of soil moisture level and geometry of planting on the yield and water use of groundnut. *Mysore Journal of Agricultural Sciences* **12**: 50-55.

SCHEFFER S.J. 2000. Molecular evidence of cryptic species within *Lyriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* **93**: 1146–1151.

SHANOWER, T.G. 1989. The Biology, Population Dynamics, Natural enemies, and Impact of the Groundnut Leafminer (*Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut in India. PhD dissertation, University of California, Berkeley, USA.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993. Biology and control of the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**: 3-10.

SUBRAHMANYAM, P., CHIYEMBEKEZA, A.J. & RAO, G.V.R. 2000. Occurrence of groundnut leaf miner in northern Malawi. *International Arachis Newsletter* **20**: 66-67.

SUMITHRAMMA, N. 1998. Bio-ecology of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) and its natural enemy complex in Southern Karnataka. Ph.D. Thesis, University of Agricultural Sciences, GKVK, Bangalore.

VAN DEVENTER, W. 1904. Microlepidoptera van Java. *Tijdschrift voor Entomologie* **47**:1-42, pls. 1-2.

WIGHTMAN, J.A. & RANGA RAO, G.V. 1993. Groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). In: A Groundnut Insect Identification Handbook for India. *ICRISAT Information Bulletin* **39**: 18-21.

Chapter One

LITERATURE REVIEW

1.1 Groundnut

1.1.1 Origin of groundnut

The natural existence of the genus *Arachis* is believed to be restricted to Argentina, Bolivia, Brazil, Paraguay, and Uruguay, with the headwaters of the Paraguay River in the region of Mato Grosso (Brazil) considered to be the center of origin of the genus (Rao 1987). The cultivated groundnut (peanut), *Arachis hypogaea* L. is believed to have arisen in an area of southern Bolivia and northwestern Argentina, on the eastern slopes of the Andes (Krapovickas 1969). The species is comprised of several subspecies and botanical varieties that have a specific geographic distribution in South America (Rao 1987). The crop was introduced to Asia, Europe, several Pacific Islands and Africa during the discovery voyages of the Portuguese, Spanish, British and Dutch during the 16th and 17th Century (Cumo 2013).

1.1.2 Groundnut production and its importance

Groundnut is grown in areas between 40 degrees South and 40 degrees North of the equator, where the average rainfall is 500 to 1200 mm with mean daily temperatures higher than 20˚C (Krapovickas 1973; Hammons & Branch 1982; Isleib *et al.* 1994). At present, India, China, Nigeria, Senegal, Sudan, Burma and the United States of America are the major groundnut producing countries in the world, accounting for about 69% of the area under cultivation and 70% of the total production (Madhusudhana 2013). Worldwide, some 18.9 million hectares (ha) are under groundnut cultivation with around 17.8 million tons produced annually (Madhusudhana 2013). In Africa, groundnut is mostly grown by small-holder farmers under rain-fed conditions with low inputs. It thus serves as a cash crop, providing income and livelihoods to the farmers (Janila *et al.* 2013). In South Africa, groundnut is mainly produced in the north-western regions of the country, namely the western and north-western Free State (40%), Northern Cape (31%) and the North West Province (25%) (Department of Agriculture, Forestry and Fisheries 2011-2012). Groundnut is also produced in Limpopo, KwaZulu-Natal and Mpumalanga provinces; however, production in these provinces is considerably lower (Department of Agriculture, Forestry and Fisheries 2011-2012).

Groundnut is valued as a rich source of energy as it provides 564 kcal of energy from 100 g of kernels, which contain oil and protein (48–50% and 25–28% of the kernels, respectively) (Jambunathan 1991). Groundnut kernels can be consumed in an unprocessed state, but more commonly they provide raw materials for the manufacturing of various products such as peanuts, peanut butter, sweets and cooking oil (Department of Agriculture, Forestry and Fisheries 2011-2012). In addition to contributing to human nutrition through the consumption of energy- and protein-rich groundnut kernels, groundnut also provides nutritious fodder (haulms) for livestock (Janila *et al.* 2013). Therefore, groundnut cultivation contributes to the sustainability of mixed crop-livestock production systems, the most predominant agricultural system of the semi-arid areas of the world (Janila *et al.* 2013).

1.1.3 Pests of groundnut

Insect pests which are known to attack groundnut worldwide include: lepidopteran defoliators such as groundnut leaf miner (GLM) *Aproaerema modicella* (Deventer)[2] (Lepidoptera: Gelechiidae), red hairy caterpillar *Amsacta albistriga* (Walker) (Lepidoptera: Arctiidae), tobacco bud worm *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), gram pod borer *Helicoverpa armigera* (Hübner) (Hübner) (Lepidoptera: Noctuidae) and bihar hairy caterpillar *Spilosoma obliqua* (Walker) (Lepidoptera: Arctiidae); sap-sucking insects such as thrips (Insecta: Thysanoptera) and aphids such as *Aphis craccivora* (Koch) (Hemiptera: Aphididae); soil insects such as white grubs *Apogonia rauca* (Fabricius) (Coleoptera: Melolonthidae) and termites (Isoptera); and mite pests such as two spotted white spider mite *Tetranychus urticae* (Koch) (Arachnida: Tetranychidae) (Ghewande & Nandagopal 1997). Among these, GLM is regarded as one of the most important pests of groundnut in India (Kapadia *et al.* 1982; Ghewande & Nandagopal 1997). Recently, GLM has become a major pest of groundnut in African countries including Uganda, Malawi, Democratic Republic of the Congo, Mozambique and South Africa (Page *et al.* 2000; Subrahmanyam *et al.* 2000; Du Plessis 2002; Munyuli *et al.* 2003; Epieru 2004; Kenis & Cugala 2006).

1.2 Classification of GLM

The groundnut leaf miner belongs to the lepidopteran family Gelechiidae and the subfamily Anacampsinae. There are some twelve species in the genus *Aproaerema*. They include *A.*

[2] South African GLM, *A. modicella* and *A. simplexella* have been tentatively synonymised as *Bilobata subsecivella* (Zeller) in this thesis.

alfalfella (Amsel), *A. anthyllidella* (Hübner), *A. aureliana* (Capuse), *A. brundini* (Benander), *A. crotalariella* (Busck), *A. lerauti* (Vives), *A. mercedella* (Walsingham), *A. modicella* (Deventer), *A. nerteria* (Meyrick), *A. nigritella* (Stainton), *A. simplexella* (Walker) and *A. sparsiciliella* (Barrett) (Hall *et al.* 1993; Jyothi *et al.* 2008). Of these, *A. modicella* has been the most studied, because of its pest status on groundnut.

1.3 The biology of GLM

The adult GLM is a grey mottled moth, with a full wing span of up to 18 mm. The eggs are small (<1.0 mm) shiny white and oval shaped (Shanower *et al.* 1993a). The larvae are grey-green with a shiny black head (Shanower *et al.* 1993a). Different numbers of larval instars have been reported in the literature, ranging from three (Kapadia *et al.* 1982) to four (Gujrati *et al.* 1973), five (Amin 1987; Shanower 1989) and six (Islam *et al.* 1983). The first of the GLM's five larval instars has an average length of 0.56 mm, while the final instar is approximately 6.0 mm long and very active (Shanower *et al.,* 1993a; Subrahmanyam *et al.,* 2000). Shanower *et al.* (1993a) reported that the first instar larvae feed within the epidermis, reaching the leaf mesophyll and creating winding mines between the upper and lower epidermis. The mines extend outwards from an initial serpentine shape to become blotch like and they enlarge as the larvae grow (Chanthy *et al.* 2010). Later, when the larvae become too large to occupy the mines, they emerge onto the leaf surface and either fold over a single leaf and hold it down with silk, or web together two or more leaflets, and thereafter live and feed in the shelter they have constructed until they pupate (Shanower *et al.* 1993a; Kenis & Cugala 2006). The pupae rarely exceed 8 mm in length (Shanower *et al.* 1993a). Shanower *et al.* (1993a) reported that the presence of pink coloured gonads in the region of the sixth and seventh abdominal segments is a distinguishing characteristic of male larvae.

The life cycle begins when the adult females lay eggs directly on the undersides of groundnut leaflets, stems and petioles (Shanower *et al.* 1993a; Kenis & Cugala 2006). The number of eggs laid by each female ranges from about 87 to 473 (Cherian & Basheer 1942; Gujrati *et al.* 1973). The duration of development from egg to adult is dependent on environmental conditions, particularly temperature. The entire life cycle generally takes 15 to 28 days in the warmer conditions of southern India (Cherian & Basheer 1942), compared to 37 to 45 days in northern India where temperatures are cooler (Sandhu 1978). Shanower *et al.* (1993b) reported that fewer eggs were produced at 15 ^{o}C than at 30 °C and at low temperatures, GLM

may take as long as 80 days to complete its life cycle, compared to only 23 days at high temperatures.

Under field conditions, eggs generally hatch in 3-4 days, but at lower temperatures may require 6-8 days (Kapadia *et al.* 1982; Shanower *et al.* 1993a). Larval development requires 9 to 28 days under field conditions and ambient temperatures (Cherian & Basheer 1942; Sandhu 1978; Kapadia *et al.* 1982). Shanower *et al.* (1989) reported that larval development to the adult stage requires approximately 325 degree-days above a threshold temperature of 11.3 °C. Pupation occurs in the webbed leaflets (Kenis & Cugala 2006), requires 72 degree-days (Shanower 1989) and can be completed in 3 to 10 days at ambient temperatures (Cherian & Basheer 1942; Sandhu 1978). Adults eventually emerge from the pupa and the cycle repeats.

1.4 Host crops for GLM

Shanower *et al.* (1993a) stated that GLM is polyphagous and has been reported to feed on a variety of host plants, mostly leguminous crops. However, *Borreria hispida* (L.) K. Schum (= *Spermacoca hispida* L.; http://wfo.kew.org) (Rubiaceae) is a notable exception. GLM host plants in the Fabaceae that are listed by Shanower *et al.* (1993a) include *Arachis hypogaea* L. (groundnut), *Glycine max* (L.) Merr. (soya bean), *Vigna radiata* (L.) Willzeek (= *Phaseolus aureus*) (mung bean), *Cajanus cajan* (L.) Millsp. (pigeon pea), *Medicago sativa* L. (lucerne), *Psolarea corylifolia* L. (babchi), *Indigofera hirsuta* L. (hairy indigo), *Vigna umbellata* (Thunb) Ohwi and Ohashi (= *Phaseolus calcaratus*) (rice bean), *Glycine soja* Sieb. & Zucc. (wild soya bean), *Trifolium alexandeium* L. (berseem clover), *Teramnus labiolis* (L.) Spreng (blue wiss), *Lablab purpureus* L. (lablab bean), *Rhynchosia minima* DC. (jumby bean) and *B. hispida* (shaggy button weed).

1.5 Economic importance of GLM and yield loss

Groundnut leaf miner is a major pest of groundnut and soya bean in the semi-arid tropics (Wightman *et al.* 1990). Amin (1983) referred to GLM as the most important groundnut pest in India, while Wightman *et al.* (1990) considered it to be the most serious pest of groundnut and soya bean in South and South-East Asia. More recently, Kenis & Cugala (2006)

described GLM as the most important groundnut pest to have recently invaded Africa (Uganda, Malawi, DRC, and Mozambique).

A single larva, in its lifetime, destroys from 34.8 to 179.3 cm^2 of leaf area (Islam *et al.* 1983; Shanower 1989). Additionally, the mined leaves become distorted within a few days. Three or four mines per groundnut leaflet can cause so much distortion that an infested leaf exposes as little as 30% of its potential photosynthetic area to the sun, which further affects the growth and yield of the crop (Kenis & Cugala 2006). The damaged leaves eventually become brownish, rolled and desiccated, resulting in early defoliation that aggravates yield losses (Kenis & Cugala 2006). Infestations are usually detected by the presence of small brown blotches on (or in) the leaves (Wightman & Ranga Rao 1993) and the webbing of leaflets (Kenis & Cugala 2006).

1.6 Effects of climatic factors on GLM infestation

The level of infestation is largely dependent on environmental conditions. Rainfall, humidity and temperature are the most important climatic factors that affect GLM populations. Amin (1987) suggested that heavy rainfall reduces GLM populations. However, Wheatley *et al.* (1989) found that water from an overhead irrigation system did not lower GLM densities. In southern India, GLM infestations are intense during drought periods, especially when no rain is recorded for 21 days or more (Gadgil *et al.* 1999; Narahari Rao *et al.* 2000). Ranga Rao *et al.* (1997) also observed GLM infestations to be severe when the groundnut crop suffers from moisture stress. It is generally accepted that the conditions most favorable for the growth of the GLM are long dry spells in association with high temperature and low humidity (Amin & Reddy 1983; Ranga Rao *et al.* 1997; Gadgil *et al.* 1999; Narahari Rao *et al.* 2000; AICRPAM 2001). Shanower *et al.* (1989) reported that GLM egg production is lower at 15 and 35°C than at 30°C. In addition to affecting egg production and duration of development, temperature also influences the survival of GLM in its immature stages, especially the larval stage. For example, hatching is slower at 15°C than at higher temperatures and larval mortality approaches 100% at 15°C (Shanower *et al.* 1993b).

Because of modulation by climatic conditions, the number of annual generations per crop is highly variable and has been reported to range from two to seven (Yang & Liu 1966; Campbell 1983; Logiswaran & Mohanasundaram 1986; Wheatley *et al.* 1989; Shanower *et al.* 1993a; Kenis & Cugala 2006). In the absence of natural mortality factors, GLM

numbers can increase by a factor of up to 20 per generation so that by the crop's pod-filling stage, they are present in high numbers (Wheatley *et al.* 1989; Shanower *et al.* 1993a), resulting in severe leaf defoliation and reduction of the leaf surface area exposed to the sun for photosynthesis (Kenis & Cugala 2006). Board *et al.* (2010) found that leaf defoliation during the pod-filling period on soya bean reduced the assimilate supply of nutrients and consequently seed size was reduced, which drastically reduced yields (Shew *et al*. 1995). Shanower *et al.* (1993a) reported that population densities of more than 320 larvae per plant may occur in some seasons. The impact of GLM on the growth and yield of groundnut is, however, determined by the time of infestation in relation to the growth stages of the crop as well as by the presence of natural enemies. In groundnut, the GLM can cause up to a 90% loss in total yield (Reddy *et al.* 1978; Sumithramma 1998), and where there are no natural enemies, an epidemic can result in total crop loss (Wightman & Ranga Rao 1993; Lavanya 2009). In most groundnut growing areas of India, because of the presence of natural enemies (Kenis & Cugala 2006), groundnut pod yield loss ranges between 30% and 60% (Shanower *et al*. 1993a; Muthiah & Kareem 2000). In South Africa, crop loss assessments have not yet been conducted but Zharare (pers. comm.) noted total crop losses in the Manguzi area of KwaZulu-Natal.

1.7 Economic threshold levels for GLM

The economic threshold levels for GLM differ between regions and with the growth/development stage of the crop (Shanower *et al.* 1993a). Those reported in the literature range from two larvae per plant (Ghewande & Nandagopal 1997) to 38 larvae per plant (Muthiah & Kareem 2000; Epieru 2004; Kenis & Cugala 2006; Van der Walt *et al*. 2009). In Uganda, control action against the pest is initiated when the GLM infestation levels reach 5 and 10 larvae per plant at 30 and 50 days, respectively, after crop emergence (Epieru 2004). In southern Mozambique, Kenis & Cugala (2006) reported that the infestation levels that cause economic damage range from 29 to 38 larvae per plant, whereas in South Africa, the threshold has been set at between 2 and 10 larvae per plant (Van der Walt *et al.* 2009). However, the critical plant growth stages at which these infestation levels reach the economic threshold levels have not been specified for Mozambique or South Africa.

1.8 Control measures for GLM

There are various methods available for the control of GLM. These include cultural, biological as well as chemical control.

1.8.1 Cultural control

Cultural control involves crop husbandry activities that modify the relationships between a pest population and its natural environment. Thus, cultural control methods are also known as ecological control methods (Zethner 1995; Abate & Ampofo 1996). Cultural methods used to control GLM include crop rotation, intercropping, timing of planting dates, resistant varieties, and irrigation.

a) Crop rotation

Crop rotation is the practice of growing a series of different types of crops in the same area in sequential seasons, in order to avoid the build-up of pathogens and pests, improve soil health, and avoid pesticide resistance issues that often occur when one species is continuously cropped (Lozano & Belloti 1980). Crop rotation is one of the oldest and most effective cultural control methods for both insect pests and diseases (Paine & Harrison 1993). Growing a single groundnut crop year after year in the same field (i.e. monocultures) gives GLM pest populations sufficient time to become established and build up to damaging levels (Ghewande & Nandagopal 1997). Therefore, it is recommended that crop rotation with non-leguminous crops should be considered, as the GLM utilizes mostly legume crops (Shanower *et al.* 1993a).

b) Irrigation

Evidence from the literature suggests that drought-stressed groundnut plants are much more suitable to GLM attack than irrigated plants, because the growth of the GLM is favoured by dry conditions/drought (Amin & Reddy 1983; Ranga Rao *et al.* 1997; AICRPAM 2001). Therefore, GLM incidence can also be reduced by irrigating the groundnut crop, so as to avoid periods of water stress (Ghewande & Nandagopal 1997).

c) Intercropping

Intercropping, also known as mixed cropping, is the agricultural practice of cultivating two or more crops in the same space at the same time (Andrews & Kassam 1976). In some cases,

intercropping lowers the overall attractiveness of the environment to a pest, as occurs when host and non-host plants are mixed together in a single planting (Meyer 2009*)*. Intercropping therefore offers another way to reduce GLM pest populations by increasing biological diversity. Intercrops such as pearl millet and sorghum have been used to suppress GLM populations, as these plants act as traps or barriers, thus reducing GLM pest incidence on groundnut (Logiswaran & Mohanasundaram 1986; Ghewande & Nandagopal 1997). Muthiah (2000) also reported that intercropping of groundnut with black gram, pigeon pea, green gram and pearl millet reduced GLM infestation levels in Tindivanam, India.

d) Planting dates

One of the ways of managing certain pests is to adjust crop planting dates to take advantage of the growth stages of the crop and pest life cycles (Pilcher & Rice 2001). Rusch *et al.* (2010) stated that the timing of planting dates affects the level of damage resulting from insect pest attacks and the ability of the plants to compensate for this damage. For example, Bajwa & Kogan (2004) demonstrated that early-sown corn (maize) is less suitable to the stem borer, *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae). This lower susceptibility results from the tendency of *D. grandiosella* to lay fewer eggs on more mature plants, which have already passed their critical growth stage before most of the larvae begin to feed (Bajwa & Kogan 2004).

Results from the survey which was undertaken at Tshiombo Irrigation Scheme in the Limpopo Province of South Africa during the 2006/2007 season, indicated that farmers who planted in the months of July and August experienced lower GLM infestation levels than those who planted in the months of September to October (ARC 2007). This was also confirmed by Zharare (pers. comm.) at Manguzi in northern KwaZulu-Natal, where GLM was particularly active on crops planted after December, with devastating effects. It is currently not known why early planted groundnut crops are able to suffer lower GLM damage. It could be hypothesized that pest pressure during the growing season varies according to the environmental conditions of the area. Environmental variables such as temperature affect developmental times in the life cycle of the pest, which in turn affect the progression of infestation during the growing season (Shanower *et al.*1993b).

f) Integrated Pest Management (IPM) methods

Examples of integrated approaches that have proven to be efficient in reducing GLM infestation in India include the use of trap crops (soya bean), pheromone traps, planting patterns (sow groundnut with a suitable variety of soya bean), timed insecticide applications (spraying at 30-35, 45-50, 60-65 days after sowing) and the use of botanical insecticide mixtures (2% crude neem oil) (Nandagopal & Ghewande 2004).

1.8.2 Biological control

Biological control of pests (natural control) in agriculture relies fully on predation, parasitism, or other natural mechanisms (Shanower *et al.* 1993a), and therefore can be an important component of IPM programs. A number of parasitoids, predators, pathogens and nematodes have been recorded as natural enemies of the GLM in Asia, where the pest is presumed to be indigenous (Shanower *et al.* 1993a).

a) Predators

Several invertebrate taxa that prey on GLM have been reported in India. These include larvae of the ground beetle *Chlaenius* sp. (Bonelli) (Coleoptera: Carabidae) (Shanower & Ranga Rao 1990), various robber flies (Diptera: Asilidae) (Srinivasan & Siva Rao 1986), the predatory wasp *Odynerus punctum* (Fabricius) (Hymenoptera: Eumenidae), the ladybirds *Cheilomenes sexmaculata* (Fabricius) and *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae) and the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Crop Pest Compendium 2005). Curently, there is little information on the rates of predation and the impact of predators on GLM populations in Africa (Kenis & Cugala 2006). Furthermore, all predators feeding on GLM in Asia have been reported to be polyphagous (Kenis & Cugala 2006), and thus would not be suitable for use as classical biocontrol agents outside Asia (e.g. in Africa).

b) Parasitoids

Several parasitoids of GLM have been recorded in Asia (Shanower *et al.* 1993a). Of these, hymenopteran parasitoids are most effective on GLM larvae, parasitizing more than 90% of the available hosts (Khan & Raodeo 1978; Shanower *et al.* 1992). In Mozambique, several parasitoid species from the families Braconidae, Ichneumonidae, Chalcididae, Eulophidae and Bethylidae were observed, with parasitism rates varying between 0 and 23.2%, although the parasitoids were not identified further (Kenis & Cugala 2006). Van der Walt *et al.* (2009) confirmed that parasitic Hymenoptera, primarily attacking the larval stages of GLM, formed

an important part of its natural enemy complex in South Africa. Parasitoids in the families Eulophidae (*Diglyphus* sp. (Walker) and *Asecodes* sp. (Förster)) and Pteromalidae (*Pteromalus* sp. (Swederus)), with parasitism rates that varied from 1.4 to 4.5%, were reported from Uganda and the Democratic Republic of the Congo (Munyuli *et al.* 2003; Kenis & Cugala 2006). Concluding remarks by Kenis & Cugala (2006) in their review of the prospects for biological control of GLM emphasized that the use of parasitoids for the control of GLM in Africa appears to have potential. However, the biology of the parasitoid complex attacking GLM is not well known. Therefore, there is a need to explore the biology of these parasitoids before they are introduced in a biological control program.

c) Pathogens and nematodes

In India, several diseases and nematodes that attack GLM have been recorded. Rajagopal *et al.* (1988) reported infections of the bacterium *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) and the fungus *Beauveria bassiana* Bals. Criv. (Hypocreales: Cordycipitaceae). Shanower *et al.* (1992) observed that viral and fungal pathogens killed up to 30% of the larvae; however, there was no identification of the species involved and it was not mentioned which larval instars were infected, nor where they were found. Rao & Reddy (1997) isolated the fungus *Metarhizium anisopliae* (Metschn.) from dead larvae and tested it successfully in the laboratory. They suggested that it could be used as a biological control agent for GLM.

1.8.3 Chemical control

In India, several insecticides have been screened for use against GLM, most of which are applied to the foliage either as liquid sprays or as dust (Praveen 2010). Systemic insecticides have been tested both as seed dressings and as granules that are incorporated into the soil during planting (Shanower *et al.* 1993a). The pesticide DDT was the first systemic insecticide to be recommended for GLM control (Ramakrishna Ayyay 1940). For India, the following chemicals and rates of application have more recently been recommended for the control of GLM: dimethoate 30 EC 0.03%; monocrotophos 36 EC 0.5% (both applied at 500-700l $ha^{-1)}$; carbaryl 50 WP 0.1% and 0.2%; and endosulfan 35 EC 0.05% (Rajput *et al.* 1984; Ghewande *et al.* 1987; Ghule *et al.* 1987; Shrivastava *et al.* 1988). The threshold populations recommended for application of these chemicals are when five or more active larvae per plant are found up to 30 days after seedling emergence (DAE), 10 larvae per plant at 50 DAE, or 15 larvae per plant at 75 DAE or later (Shanower *et al.* 1993a).

Kenis & Cugala (2006) stated that the use of insecticides such as cypermethrin or dimethoate was the only available control method for GLM in Africa at that time. In Mozambique, Cugala *et al.* (2010) reported that spraying cypermethrin on groundnut reduced the population densities of GLM and increased groundnut grain yields. In Uganda, the effectiveness of cypermethrin in controlling GLM was confirmed by Epieru *et al.* (2004). Though several insecticides are recommended for the control of GLM in India and Africa, they are not a sustainable option for Africa as they are unaffordable for small-holder farmers (Kenis & Cugala 2006). Therefore, there is a need to explore biological control methods.

The observations of varying GLM populations between years had been reported in India and Africa (Shanower *et al.* 1993a; Van der Walt 2007). Kenis & Cugala (2006) suggested that the frequent decreases of GLM populations were due to natural enemies controlling the pest. Furthermore, Kenis & Cugala (2006) stated that even though there are several GLM parasitoids listed in India, their identity, biology and ecology is not well understood. Therefore, a biological control programme against GLM in Africa should begin with studies involving a proper identification of GLM parasitoids; this could be achieved by employing methods such as molecular techniques which offer complementary, faster and more precise options for species identification (Scheffer 2000). In addition, molecular techniques can provide answers on the identity of GLM as Shanower *et al.* (1993a) reported that there is a degree of uncertainty as to the correct classification of GLM in India, Indonesia and Africa.

1.9 DNA analysis as a research tool in entomology

DNA fingerprinting is a molecular research tool which assists in the identification of the unique DNA pattern of an organism by the genetic polymorphism in its DNA, which constitutes the genetic material (Crawford *et al.* 1993). The individual specific DNA patterns render DNA fingerprinting possible, and the technology is being widely used for the identification of biological entities (Crawford *et al.* 1993; Jayarao & Oliver 1994; Peng *et al.* 2003; Saez *et al.* 2004). Techniques for DNA fingerprinting include either the analysis of nuclear DNA (nDNA) or mitochondrial DNA (mtDNA), depending on the purpose of the analysis. Those based on nDNA, such as restriction fragment length polymorphism analysis and short tandem repeats analysis (Butler 2001), are most suitable for discrimination between individuals, which allows the identification of individuals, and hence are suitable for within-population genetic diversity (Crawford *et al.* 1993; Peng *et al.* 2003). Mitochondrial DNA is

maternally inherited. Consequently, its analysis can provide insights into population genetic structure, gene flow and between-population, biogeographic and intraspecific relationships (Moritz *et al.* 1987; Danforth *et al.* 1998; Sperling *et al.* 1999; Simmons & Scheffer 2004). Techniques involved in mtDNA analysis are therefore most commonly used to determine genetic relationships between populations (Sperling *et al.* 1999; Scheffer 2000; Scheffer & Lewis 2001; Segraves & Pellmyr 2001; King *et al.* 2002; Simmons & Scheffer 2004). These techniques are also able to reveal cryptic lineages that represent distinct species within geographically widespread and apparently morphologically homogeneous organisms (Scheffer 2000).

Generally, DNA techniques are based on a procedure known as the polymerase chain reaction (PCR) (Saiki *et al.* 1988). This procedure allows creations of millions of precise DNA replications from a single sample of DNA; enough to allow genetic variation to be analysed in a number of ways (Saiki *et al.* 1988). Furthermore, PCR analysis has the advantage of analyzing very small sample sizes, even if they are degraded; although, they must not be contaminated with DNA from other sources during the collection, storage and transport of the sample (Jayaro & Oliver 1994; Saez *et al.* 2004).

For the study reported in this thesis, mtDNA analysis using the Cytochrome oxidase I (COI) gene was used in the identification of GLM occurring in South Africa. This method was selected on the basis that it is useful in identifying species which have similar morphological characteristics (Scheffer 2000); as is the case with the GLM entities that have been described as *A. modicella* and *A. simplexella*. In addition to mtDNA analysis, nDNA analysis was used in a phylogenetic relationships study of GLM in Africa and material from India and Australia that have been described as *A. modicella* and *A. simplexella*, respectively.

1.10 References

ABATE, T. & AMPOFO, J.K.O. 1996. Insect pests of common beans in Africa. *Annual Review of Entomology* **41**: 45–73.

AICRPAM. 2001. Annual Report, All India Coordinated Research Project on Agrometeorology, Hyderabad, India.

AMIN, P.W. 1983. Major field insect pests of groundnut in India and associated crop losses. *Indian Journal of Entomology* **2**: 337-344 (special issue).

AMIN, P.W. 1987. Insect pests of groundnut in India and their management. In: Veerabhadra Rao, M. & Sithanantham, S. (Eds.) *Plant Protection in Field Crops*. 219-333. Plant Protection Association of India, Hyderabad.

AMIN, P.W. & REDDY, D.V.R. 1983. Annual progress reports of All India Coordinated Research Project on Oil Seeds. Directorate of Oil Seeds Research, Hyderabad.

ANDREWS, D.J. & KASSAM, A.H. 1976.The importance of multiple cropping in increasing world food supplies. In: Papendick, R.I., Sanchez, A. & Triplett, G.B. (Eds.) Multiple Cropping.1-10. ASA Special Publication 27. American Society of Agronomy, Madison, WI.

ARC-GRAIN CROPS INSTITUTE. 2007. Development of a control strategy for groundnut leaf miner on groundnut. Online at: http://www.arc.agric.za (Accessed on 15 March 2014).

BAJWA, W. I. & M. KOGAN. 2004. Cultural practices: Springboard to IPM. In: Koul, O., Dhaliwal, G.S. & Cuperus, G.W. (Eds.) *Integrated pest management: Potential, constraints and challenges*. 21–38. Wallingford, UK: CABI.

BOARD, J.E., KUMUDINI, S., OMIELAN, J. PRIOR, E. & KAHLON, C.S. 2010. Yield response of soybean to partial and total defoliation during the seed filling period. *Crop Science* **50**:703-712.

BUTLER, J.M. 2001. *Forensic DNA Typing*. Elsevier. ISBN 978-0-12-147951-0. OCLC 45406517 223032110 45406517. 14 –15.

CAMPBELL, W.V. 1983. Management of arthropods on peanut in Southern Asia. *Annual Report Peanut Collaboration Research Support Program (CRSP),* University of Georgia, Georgia Experiment Station, USA.

CHANTHY, P., BELFIELD, S. & MARTIN, R. 2010. Insects of upland crops in Cambodia. A field guide to identifying insect pests and beneficial insects and spiders in the upland

cropping systems of Cambodia. Australian Centre for International Agricultural Research (ACIAR) Monograph 143. ISBN 9781921615887.

CHERIAN, M.C. & BASHEER, M. 1942. Studies on *Stomopteryx nerteria* Meyr.- A pest of groundnut in the Madras Presidency. *Madras Agriculture Journal* **30**: 379-381.

CRAWFORD, D.J., COSNER, B.S. & STUESSEY, T.F 1993. Use of RAPD markers to document the origin of intergeneric hybrid *Margyracaena skottsbergii* (Rosaceae) on the Juan Fernandez Islands. *American Journal of Botany* **80**: 89-92.

CROP PEST COMPENDIUM (Monograph on CD-ROM). 2005. Wallingford, UK: CAB International.

CUGALA, D., SANTOS, L., BOTAO, M., SOLOMONE, A. & SIDUMO, A. 2010. Assessment of groundnut yield loss due to the groundnut leaf miner, *Aproaerema modicella,* infestation in Mozambique. Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda.

CUMO, C. 2013. Encyclopaedia of Cultivated Plants: From Acacia to Zinnia. ABC-CLIO, ISBN13: 9781306088008.

DANFORTH, B.N., MITCHELL, P. & PARKER, L. 1998. Mitochondrial DNA differentiation between two cryptic *Halictus* species. *Annals of the Entomological Society of America* **91**: 387-391.

DEPARTMENT OF AGRICULTURE, FORESTRY & FISHERIES.2011-2012. Groundnut Market Value Chain Profile. Pretoria, South Africa.

DU PLESSIS, H. 2002. Groundnut leaf miner *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48–49.

EPIERU, G. 2004. Participatory evaluation of distribution, status and management of the groundnut leaf miner in the Teso and Lango farming systems. Final Technical Report, Serere Agricultural and Animal Production Research Unit (SAARI), Soroti, Uganda. 30.

GADGIL, S., RAO, P.R.S. & SRIDHAR, S. 1999. Modelling impact of climate variability on rain-fed groundnut. *Current Science* **76**: 557-569.

GHEWANDE, M.P, NANDAGOPAL, V. & REDDY, P.S. 1987. Plant Protection in Groundnut. *International Committee for Animal Recording Technical Bulletin*. Indian Council of Agricultural Research, Junagadh, Gujarat.

GHEWANDE, M.P. & NANDAGOPAL, V. 1997. Integrated pest management in groundnut (*Arachis hypogea* L.) in India. *Integrated Pest Management Reviews* **2**: 1–15.

GHULE, B.D., JAGTAP, A.B., DHUMAL, V.S. & DEOKAR, A.B. 1987. Determination of critical time for protection against leaf miner, *Stomopteryx subsecivella* (Lepidoptera: Gelechiidae). *Journal of Maharashtra Agricultural University* **12**: 292-293.

GUJRATI, J.P., KAPOOR, K.N. & GANGRADE, G.A. 1973. Biology of soybean leaf miner, *Stomopteryx subsecivella* (Lepidoptera: Gelechiidae). *Entomologist* **106**: 187-191.

HALL, D.R., BEEVOR, P.S., CAMPON, D.G., CHAMBERLAIN, D.J., CORK, A., WHITE, R., ALMESTRE, A., HENNEBERRY, T.J., NANDAGOPAL, V., WIGHTMAN, J.A. & RAO, G.V.R. 1993. Identification and synthesis of new pheromones. *Bulletin OILB SROP* **16**:1-9.

HAMMONS, R.O. & BRANCH, W. D. 1982. Pedigreed natural crossing to identify peanut testa genotypes. *Peanut Science* **9**(2): 90-93.

ISLAM, W., AHMED, K.N., NARGIS, A. & ISLAM, U. 1983. Occurrence, abundance and extent of damage caused by insect pests of groundnuts (*Arachis hypogaea). Malaysian Agriculture Journal* **54**: 18-24.

ISLEIB, T.G., WYNNE, J.C. & NIGAM, S.N. 1994. Groundnut breeding. In: Smartt, J. (Ed.) The groundnut crop: a scientific basis for improvement. 552– 623. Chapman and Hall, London.

JAMBUNATHAN, R. 1991. Groundnut quality characteristics, in uses of tropical grain legumes. *Proceedings of a Consultants Meeting, March 27–30* (Patancheru: ICRISAT) 267–275.

JANILA, P., NIGAM, S.N., PANDEY, M.K., NAGESH, P. & VARSHNEY, R.K. 2013. Groundnut improvement: use of genetic and genomic tools. *Frontiers in Plant Science* **4**: 1-16.

JAYARAO, B.M. & OLIVER, S.P. 1994. Polymerase chain reaction-based DNA fingerprinting for identification of *Streptococcus* and *Enterococcus* species isolated from bovine milk. *Journal of Food Protection* **57**(3): 240-245.

JYOTHI, K.N., PRASUNA, A.L., PRASAD, A.R. & YADAV, J.S. 2008. Electrophysiological responses of both sexes of groundnut leaf miner, *Aproaerema modicella* (Lepidoptera: Gelechiidae) to synthetic female sex pheromone blend. *Current Science* **94**: 629-633.

KAPADIA, M.N., BHARODIA, R.K. & VORA, V.J. 1982. Biology and estimation of incidence of groundnut leaf miner, *Stomopteryx subsecivella* Zell. (Gelechiidae: Lepidoptera). *Gujarat Agricultural University Research Journal* **8**: 37-39.

KRAPOVICKAS, A. 1969. The origin, variability and spread of the groundnut (*Arachis hypogaea*). In: Ucko, R.J. & Dimbledy W.C. (Eds.) The domestication and exploitation of plant and animals. 427-441. Greald Duckworth Co. Ltd, London.

KRAPOVICKAS, A. 1973. Evolution of the genus *Arachis.* In: Moav, R. (Ed.) Agricultural Genetics-Selected Topics. 135-151. John Wiley & Sons, New York, USA.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural* Resources **1**: 1–9.

KHAN, M.I. & RAODEO, A.K. 1978. Importance of larval parasites in the control of *Stomopteryx subsecivella* Zeller. *Journal of Maharashtra Agricultural University* **3**: 261–263.

KING, H., CONLONG, D.E. & MITCHELL, A. 2002. Genetic differentiation in *Eldana saccharina* (Lepidoptera: Pyralidae): evidence from the mitochondrial cytochrome oxidase I and II genes. *Proceedings of the South African Sugar Technologists' Association* **76**: 321-328.

LAVANYA, 2009. Groundnut Leaf miner E:\GROUNDNUT LEAFMINER\Groundnut Leaf miner. Online at: http://agropedia.iitk.ac.in/content/groundnut-leaf-miner (Accessed on 16 May 2014).

LOGISWARAN, G. & MOHANASUNDARAM, M. 1986. Influence of weather factors on the catches of moths of groundnut leaf miner *Aproaerema modicella* (Lepidoptera: Gelechiidae) in the light trap. *Entomon* **12**: 147-150.

LOZANO, J.C. & BELLOTI, A.C. 1980. Control integrado de enfermedades y pestes de lay yucca (*Manihot esculenta* Crantz). *Fitopatologia Colombiana* **2**: 97-104.

MADHUSUDHANA, B. 2013. A survey on area, production and productivity of groundnut crops in India. *IOSR Journal of Economics and Finance* **1**(3) (Sep. – Oct. 2013): 01-07.

MEYER, J.R. 2009. Cultural control. 8 April 2009. *General Entomology.* Online at: http://www.cals.ncsu.edu/course/ent425/library/tutorials/applied_entomology/cultural_control.html - (Accessed on17April 2014).

MORITZ, C., DOWLING, T.E. & BROWN, W.M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics* **18**: 269-292.

MUNYULI, T.M.B., LUTHER, G.C., KYAMANYWA, S. & HAMMOND, R. 2003. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda

and Democratic Republic of Congo. *African Crop Science Conference Proceedings* **6**: 231-237.

MUTHIAH, C. 2000. Effect of intercropping on the incidence of leaf miner (*Aproaerema modicella*) in groundnut (*Arachis hypogaea*). *Indian Journal of Agricultural Sciences* **70**: 559-561.

MUTHIAH, C. & KAREEM, A.A. 2000. Survey of groundnut leaf miner and its natural enemies in Tamil Nadu, India. *International Arachis Newsletter* **20**: 62-63.

NANDAGOPAL, V. & GHEWANDE, M.P. 2004. Use of Neem products in groundnut pest management in Uganda. *Natural Product Radiance* **3**(3): 150-155.

NARAHARI RAO, K., GADGIL, S., RAO, P.R.S. & SAVITHRI, K. 2000. Tailoring strategies to rainfall variability – The choice of sowing window. *Current Science* **78** (10): 1216-1230.

PAGE, W.W., EPIERU, G., KIMMINS, F.M., BUSOLO-BULAFU, C. & NALYONGO, P.W. 2000. Groundnut leaf miner *Aproaerema modicella*: a new pest in eastern districts of Uganda. *International Arachis Newsletter* **20**: 64-6.

PAINE, L.K. & HARRISON, H. 1993.The historical roots of living mulch and related practices. *Horticultural Technology Journal* **3**:137-143.

PENG, W.K., LIN, H.C., CHEN, C.N. & WANG, C.H. 2003. DNA identification of two laboratory colonies of the weevils, *Sitophilus oryzae* (L.) and *S. zeamais* Motschulsky (Coleoptera: Curculionidae) in Taiwan. *Journal of Stored Products Research* **39** (2): 225-235.

PILCHER, C.D. & RICE, M.E. 2001. Effect of planting dates and *Bacillus thuringiensis* corn on the population dynamics of European corn borer (Lepidoptera: Crambidae). *Journal of Economic Entomology* **94:** 730-742.

PRAVEEN, Y.V. 2010. Studies on groundnut leaf miner *Aproaerema modicella* Deventer in Northern dry zone of Karnataka. M.Sc. Thesis, University of Agricultural Sciences, Dharwad, India.

RAJAGOPAL, D., MALLIKARJUNAPPA, S. & GOWDA, J. 1988. Occurrence of natural enemies of the groundnut leaf miner (*Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae)). *Journal of Biological Control* **2**: 129-130.

RAJPUT, S.G., DALAYA, V.P. & AWATE, B.G. 1984. Efficacy of different insecticides for the control of groundnut leaf miner. *Journal of Maharashtra Agricultural University* **9**: 336-337.

RAMAKRISHNA AYYAR, T.V. 1940. A Handbook of Economic Entomology for South India. Macmillan (India), New Delhi.

RANGA RAO, G.V., REDDY, D. & SHANOWER, T.1997. Status of groundnut leaf miner in Peninsular India: management options. Meeting Abstracts of Pest Management Research Unit, United States Department of Agriculture (USDA).

RAO, V.R. 1987. Origin, Distribution, and Taxonomy of *Arachis* and Sources of Resistance to Groundnut Rust (*Puccinia arachidis Speg.*). *In: Groundnut Disease Proceedings of a Discussion Group Meeting, 24 - 28 September 1984*, ICRISAT Patanceru, India.

RAO, G.V.R. & REDDY, P.M. 1997. *Metarhizium anisopliae* (Metschn.): a potential biocontrol agent for groundnut leaf miner. *International Arachis Newsletter* **17**: 48-49.

REDDY, N.M., HAVANAGI, G.V. & HEGDE, B.R. 1978. Effect of soil moisture level and geometry of planting on the yield and water use of groundnut. *Mysore Journal of Agricultural Sciences* **12**:50-55.

RUSCH, A., VALANTIN-MORISON, M., SARTHOU, J. & ROGER-ESTRADE, J. 2010. Biological control of insect pests in agroecosystems: effects of crop management, farming systems, and seminatural habitats at the landscape scale: A review. *Advances in Agronomy* **109**: 219-259.

SAEZ, R., SANZ, Y. & TOLDRÁ, F. 2004. PCR-based fingerprinting techniques for rapid detection of animal species in meat products. *Meat Science* **66** (3): 659-665.

SAIKI, R.K., GELFAND, D.H., STOFFEL, S., SCHARF, S.J., HIGUCHI, R., HORN, G.T. MULLIS, K.B. & ERLICH, H.A. 1988. Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science* **239**: 487-491.

SANDHU, G.S. 1978. Note on the incidence of *Stomopteryx subsecivella* (Zell.) (Lepidoptera: Gelechiidae) on lucerne at Ludhiana. *Indian Journal of Agricultural Sciences* **48**: 53-54.

SCHEFFER, S.J. 2000. Molecular evidence of cryptic species within *Lyriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* **93**: 1146–1151.

SCHEFFER, S.J. & LEWIS, M.L. 2001.Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America* **94**: 648–653.

SEGRAVES, K.A. & PELLMYR, O. 2001. Phylogeography of the yucca moth *Tegeticula maculata*: the role of historical biogeography in reconciling high genetic structure with limited speciation. *Molecular Ecology* **10**: 1247–1253.

SHANOWER, T.G. 1989. The Biology, Population Dynamics, Natural enemies, and Impact of the Groundnut Leafminer *(Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut in India. PhD dissertation, University of California, Berkeley, USA.

SHANOWER, T.G. & RANGA RAO, G.V. 1990. *Chlaenius* sp. (Coleoptera: Carabidae): a new predator of groundnut leaf miner larvae. *International Arachis Newsletter* **8**: 19-20.

SHANOWER, T.G., WIGHTMAN, J.A., GUTIERREZ, A.P., & RANGA RAO, G.V. 1992. Larval parasitoids and pathogens of the groundnut leaf miner, *Aproaerema modicella* (Lepidoptera: Gelechiidae) in India. *Entomophaga* **37**: 419–427.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993a. Biology and control of the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**:3–10.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993b. Effect of temperature on development rates, fecundity and longevity of the groundnut leaf miner, *Aproaerema modicella* (Lepidoptera: Gelechiidae), in India. *Bulletin of Entomological Research* **83**: 413–419.

SHEW, B.B., BEUTE, M.K. & STALKER, H.T. 1995. Towards sustainable peanut production: Progression in breeding for resistance to foliar and soilborne pathogens of peanut. *Plant Diseases* **79**: 1259-1261.

SHRIVASTAVA, K.K., SRIVASTAVA, B.K. & DEOLE, J.Y. 1988. Studies on chemical control and varietal resistance of soybean against leaf miner, *Stomopteryx subsecivella* Zeller. *Indian Journal of Plant Protection* **176**: 147-151.

SIMMONS, R.B. & SCHEFFER, S.J. 2004. Evidence of cryptic species within the pest *Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae). *Annals ofthe Entomological Society of America* **97**: 675-680.

SPERLING, F.A.H., RASKE, A.G. & OTVOS, I.S. 1999. Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera: Geometridae). *Insect Molecular Biology* **8**: 97-106.

SRINIVASAN, S. & SIVA RAO, D.V. 1986. New reports of parasites of groundnut leaf webber, *Aproaerema modicella* (Deventer) (=*Stomopteryx subsecivella* Zell.) (Lepidoptera: Gelechiidae) in Andhra Pradesh. *Entomologist* **12**: 117-119.

SUBRAHMANYAM, P., CHIYEMBEKEZA, A.J. & RAO, G.V.R. 2000. Occurrence of groundnut leaf miner in northern Malawi. *International Arachis Newsletter* **20**: 66-67.

SUMITHRAMMA, N. 1998. Bio-ecology of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) and its natural enemy complex in Southern Karnataka. Ph.D. Thesis, University of Agricultural Sciences, GKVK, Bangalore.

VAN DER WALT, A. 2007. Small holder farmers' perceptions, host plant suitability and natural enemies of the groundnut leafminer, *Aproaerema modicella* (Lepidoptera: Gelechiidae) in South Africa. M.Env. Sci. dissertation, North-West University, Potchefstroom, South Africa.

VAN DER WALT, A., DU PLESSIS, H. & VAN DEN BERG, J. 2009. Infestation of groundnut by the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) and rates of parasitization of this pest in South Africa. *Crop Protection* **28**(1):53-56.

WHEATLEY, J.A. WHEATLEY, A.R.D, WIGHTMAN, J.A., WILLIAMS, J.H. & WHEATLEY, S.J. 1989.The influence of drought stress on the distribution of insects on four groundnut genotypes grown near Hyderabad, India. *Bulletin of Entomological Research* **79**: 567–577.

WIGHTMAN, J.A., DICK, K.M., RANGA RAO, G.V., SHANOWER, T.G. & GOLD, C.S. 1990. Pests of groundnut in the semi-arid tropics. In: Singh, S.R. (Ed.) Insect Pests of Food Legumes. 243-322. John Wiley & Sons, New York.

WIGHTMAN, J.A. & RANGA RAO, G.V. 1993. Groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). In: A Groundnut Insect Identification Handbook for India. *ICRISAT Information Bulletin* **39**: 18-21.

YANG, C. & LIU, H. 1966. Biological observations on *Stomopteryx subsecivella* Zell. in Den Bei District, Kwantug (in Chinese). *Acta Entomologica Sinica* **15**:39-46 (English summary in *Review of Applied Entomology Series* **54**: 483).

ZETHNER, O. 1995. Practice of integrated pest management in tropical and sub-tropical Africa: an overview of two decades (1970–1990). In: Mengech, A.N., Saxena, K.N. & Gopalan, H.N.B. (Eds.) Integrated Pest Management in the Tropics.1–68.Wiley, Chichester, UK.

Chapter Two

The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soya bean moth (*Aproaerema simplexella* (Walker) (Lepidoptera: Gelechiidae)

Abstract

Although the leaf miner attacking groundnut in Africa has been widely reported as *Aproaerema modicella* (Deventer)[3], a common groundnut (*Arachis hypogaea* L.) and soya bean (*Glycine max* (L.) Merr.) pest in Indo-Asian countries, a proper taxonomic identification of the pest has not been completed. A detection survey for the pest was conducted on groundnut, soya bean and lucerne (*Medicago sativa* L.), the common host crops for *A. modicella*, at six widely separated sites in South Africa during the 2009-2010 growing season. Sixty specimens comprising 24 larvae, 24 pupae and 12 moths of what was thought to be *A. modicella* (54 from groundnut; six from soya bean) were collected from the six survey sites, and their mitochondrial DNA (mtDNA) COI were sequenced and compared with those from the BOLD gene bank. Infestation by GLM was observed on groundnut and soya bean, but not on lucerne. The mtDNA COI from all specimens of the pest, irrespective of whether they were from groundnut or soya bean, matched 100% with the sequences in BOLD belonging to *Aproaerema simplexella* PS1, a species occurring in Australia, and known as the soya bean moth in that country. There was very little genetic diversity between and within the populations from the six sites, which suggested that the populations were maternally of the same origin.

***Key words*:** *Arachis hypogaea*, *Aproaerema modicella*, *Glycine max*, lucerne, mitochondrial DNA.

2.1 Introduction

The identity of the groundnut leaf miner (GLM) in Africa, including South Africa, has generally been assumed to be *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae)

[3] GLM occurring in South Africa, *A. modicella* and *A. simplexella* has tentatively been synonymised as *Bilobata subsecivella* (Zeller).

(Page *et al.* 2000; Subrahmanyam *et al.* 2000; Du Plessis 2002; Munyuli *et al.* 2003; Epieru 2004; Kenis & Cugala 2006), although Shanower *et al.* (1993) hinted that it might be a different species. Since no proper taxonomic identification has been done on this new pest in southern Africa, the adoption of the name *A. modicella* was probably based on morphological characteristics of the larvae and adults, crop damage symptoms similar to those of *A. modicella* and the strong prevalence of the pest on groundnut (Du Plessis 2002, 2003; Kenis & Cugala 2006). Van der Walt *et al.* (2008) examined the gonads of the female and male larvae of GLM specimens collected in South Africa, and concluded that they were similar to those reported for *A. modicella* in Asia by Shanower *et al.* (1993), which reinforced the assumption that the pest was *A. modicella*. Because of its sudden appearance in Africa, GLM was thought to be a recent invasion from the Indo-Asian continent (Kenis & Cugala 2006) where *A.modicella* is considered to be native and infests groundnut and soya bean (Shanower *et al.* 1993). Although this is possible, an alternative hypothesis is that the pest may have evolved and spread within Africa.

Morphological studies have been the keystone of insect pest identification in the past, and continue to be in the present, although modern molecular techniques offer complementary, faster and more precise options for species identification (Scheffer 2000). These are especially useful in differentiating between related species that share similar morphological characteristics. In addition, molecular techniques (e.g. DNA finger printing), especially those involving mitochondrial DNA (mtDNA), are reliable in pinpointing or tracing the geographical origin/links of pests and their paths of spread (Scheffer 2000; Simmons & Scheffer 2004).

The study reported in this chapter had three objectives. The first was to compile a complete host crop/plant list and record damage symptoms on these caused by GLM occurring in South Africa. The second was to identify the pest to species level and the third was to determine its inter- and intra-population genetic diversity by analysing in, both cases, the mtDNA COI gene of specimens collected from widely separated sites.

2.2 Materials and methods

2.2.1 Detection survey and specimen collection sites

A detection survey involving three visits to each site was undertaken to determine the presence of GLM on groundnut and alternative host crops/plants at six locations (Table 2.1) in the North West, Northern Cape, Mpumalanga and KwaZulu-Natal provinces of South Africa. The first visit was done between 11 and 16 January 2010, the second between 22 and 27 March 2010 and the last between 24 and 29 October 2010. In the North West province, the sites included the Agricultural Research Council research stations at Potchefstroom and Brits as well as farms surrounding the Brits research farm. In the Northern Cape, the inspection site was the Vaalharts Research Station. In Mpumalanga, the inspection site was the Department of Agriculture Lowveld Agricultural Research Station near Nelspruit. In KwaZulu-Natal, the inspections were done at Bhekabantu and Manguzi in the northern part of the province. The latter site is unique from the other sites in that it is warm throughout the year and groundnut can thus be planted continuously. Being coastal, Manguzi is also expected to have higher humidity than the other sites.

Table 2.1. Survey sites where groundnut leaf miner inspections and sample collections were conducted.

Province and Inspection site	**Climatic description**			**Crops inspected**
	Mean annual rainfall (mm)	Summer temperatures (^{o}C)	Winter temperatures (^{o}C)	
Northern Cape Province				
Vaalharts (27º95’761’’S ; 24º83’991’’E)	300- 450	16 - 32	1 - 18	groundnut, lucerne
North West Province				
Brits (25º59’135’’S ; 27º76’875’’E)	300-700	22 - 34	15 - 20	groundnut, soya bean, lucerne
Potchefstroom (26 º73’607’’S ; 27º 07’553’’E)	360- 507	18 - 34	2 - 18	groundnut, soya bean
KwaZulu-Natal Province				
Manguzi (26º 95’532’’S ; 32º82’356’’E)	600-700	23 - 35	17 - 27	groundnut
*Bhekabantu (27°01’12.38” S; 32°19’18.29” E)	-	-	-	groundnut
Mpumalanga Province				
Nelspruit (25º45’452’’S; 30º97’154’’E)	500- 780	24 - 30	17 - 24	groundnut, lucerne

*****Climatic data were not available because there is no weather station near the site.

2.2.2 Infestation recognition and specimen collection

The survey included visual inspection of old and young leaves of groundnut, soya bean, lucerne (*Medicago sativa* L.) and any other known hosts of *A. modicella* for infestation by the pest, and the collection of GLM larvae and pupae for DNA analyses. During the survey, the presence of the pest and the damage symptoms on the crop were searched for. In the first survey visit, in addition to visual inspection of the groundnut plants and other host plants at each site, five specimens each of larvae and pupae were removed from infested groundnut (all survey sites) or soya bean (Potchefstroom) plants and immediately placed in 10-ml polycarbonate vials containing absolute ethanol and closed with press-on plastic lids. The vials were taken to a laboratory and stored at -80°C in a cryogenic freezer until DNA sequencing. In addition, about 20 pupae per site were placed in a clear 250-ml plastic bottle that was perforated by a dissecting needle in many places to allow free air movement into and out of the bottle. The holes were small (≤ 2 mm in diameter) and not large enough to allow the GLM moths out of the bottles, which were closed with screw-on polycarbonate lids. The bottles containing the pupae were stored in a laboratory at room temperature at the University of Zululand until moths emerged. After visual inspection of the emerged moths, five of the moths from each site were placed in 10-ml polycarbonate vials containing absolute ethanol and the vials were closed with press-on lids. As with vials containing larval and pupal specimens, the vials containing moths were stored at -80°C until DNA sequencing.

2.2.3 DNA analyses

2.2.3.1 DNA extraction

From the specimens collected at the six survey sites, a total of 60 specimens (9 to 12 specimens per site) comprising pupae, larvae and adults (Table 2.2) were used for DNA analyses. All specimens processed for DNA analyses were from groundnut, except for three larvae and three pupae that were collected from soya bean at Potchefstroom. The specimens were identified in relation to the area from which they were collected, as shown in Table 2.2. The DNA was extracted from the specimens following the method of McPherson *et al.* (1991). The specimens were added individually to 500 µl Buffer PL1 of the NucleoSpin PlantII kit (Macherey-Nagel) and 2 µl of 10 mg/ml proteinase K (Sigma-Aldrich), homogenised using the TissueLyser (Qiagen) and incubated overnight at 60°C. The samples were then centrifuged at 6.0 relative centrifugal force for 20 min. The rest of the protocol was performed on a robotic platform Genesis RMP200 (Tecan). A total of 400 µl supernatant was

mixed with 450 µl binding buffer PC and transferred to a silica membrane plate. The mixture was pulled through the membrane by a vacuum system. The bound DNA was washed to remove proteins and salts with 400 µl buffer PW1 and twice with 700 µl buffer PW2. The bound DNA was eluted twice with 100 µl volumes of elution buffer preheated to 70°C.

Table 2.2. Labelling system used in the assigning of specimen identity.

Area	Specimen identity	Specimen description
Manguzi	Man 1	A-Adult
Bhekabantu	Man 2	L- Larva
Vaalharts	Vaal, VD	P- Pupa
Potchefstroom	Pot	
Brits	Brits	
Nelspruit	Nel	

2.2.3.2 DNA amplification and sequencing

DNA amplification by PCR was performed with the primers Ron and Nancy. The PCR conditions were as follows: 1x KAPA Robust Ready Mix (KAPA Biotech), 1x Enhancer A, 0.4 µM of each primer and 20ng DNA. The PCR was performed in a verity PCR-cycler (Applied Biosystems) with the following conditions: 95°C for 5 min followed by 40 cycles of 95°C at 30s, 55°C at 60s and 72°C for 90s and a final extension of 72°C for 10 min. Post-PCR purification was done using the NucleoFast Purification System (Separations). Sequencing was performed with each primer and BigDye Terminator V1.3 (Applied Biosystems) followed by electrophoresis on the 3730xl DNA Analyser (Applied Biosystems). Sequences were analysed using the Sequencing Analysis Version 5.3.1 software (Applied Biosystems).

2.2.3.3 Editing of DNA sequences

DNA sequences were manually edited (for base calling errors), pruned and aligned by ClustalW using the BioEdit Sequence Alignment Editor (Hall 1999) to create consensus sequences which were saved in the fasta format in MEGA5 (Hall 1999).

2.2.3.4 Determining evolutionary relationships

The evolutionary history was inferred using the Neighbour-joining method (Saitou & Nei 1987) with bootstrap analysis based on 1000 replicates (Felsenstein 1985). A phylogenetic tree was constructed based on the Neighbour-joining method. The evolutionary distances were computed using the Kimura 2- parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The rate variation among sites was modelled with a

gamma distribution (shape parameter =1). The analysis involved 60 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 363 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.* 2011). Additionally, all consensus sequences were entered in the Barcode of Life Data System (BOLD) to positively identify the species. All specimens were identified as from the same species, except one sample, which was identified as a different species, and was therefore used as an out group in the analysis. Additionally, the sequences were also exposed to Multiple Sequence Alignment by ClustalW (http://www.genome.jp/tools/clustalw/) to verify the level of similarity between samples.

2.3 Results

2.3.1 Host plants

All of the groundnut crops inspected at the six survey sites were infested by GLM, and so were the soya bean crops inspected at Vaalharts, Potchefstroom and Brits. In contrast, GLM infestation was absent from all lucerne crops inspected at Nelspruit, Brits and Vaalharts. At Vaalharts, this was despite volunteer lucerne plants growing on the edges of groundnut fields that were infested by GLM. At Bhekabantu, only two GLM larvae were observed on an *Indigofera* L. species, even though there were a number of these plants within 5m of a groundnut crop that was heavily infested with GLM.

2.3.2 Crop damage symptoms

The symptoms of damage found on the groundnut leaves mirrored those described for GLM in Mozambique and elsewhere (Kenis & Cugala 2006; Lavanya 2009). The symptoms varied with season and growth stage of the crop (Figure 2.1). Early in the growth season, the mines are relatively small and the larvae produce small necrotic areas, mostly in the middle of the leaflets (Figure 2.1A and B), or a slight folding at the end of a leaflet. Leaf folding and webbing (Figure 2.1B) may be less visible compared to the mid and late season symptoms. In late growth stages of the groundnut crop, the affected leaves are severely necrotic and distorted (Figure 2.1C). In severely affected plants, almost all leaflets are affected/infested (Figure 2.1D) or there is complete defoliation (Figure 2.1E).

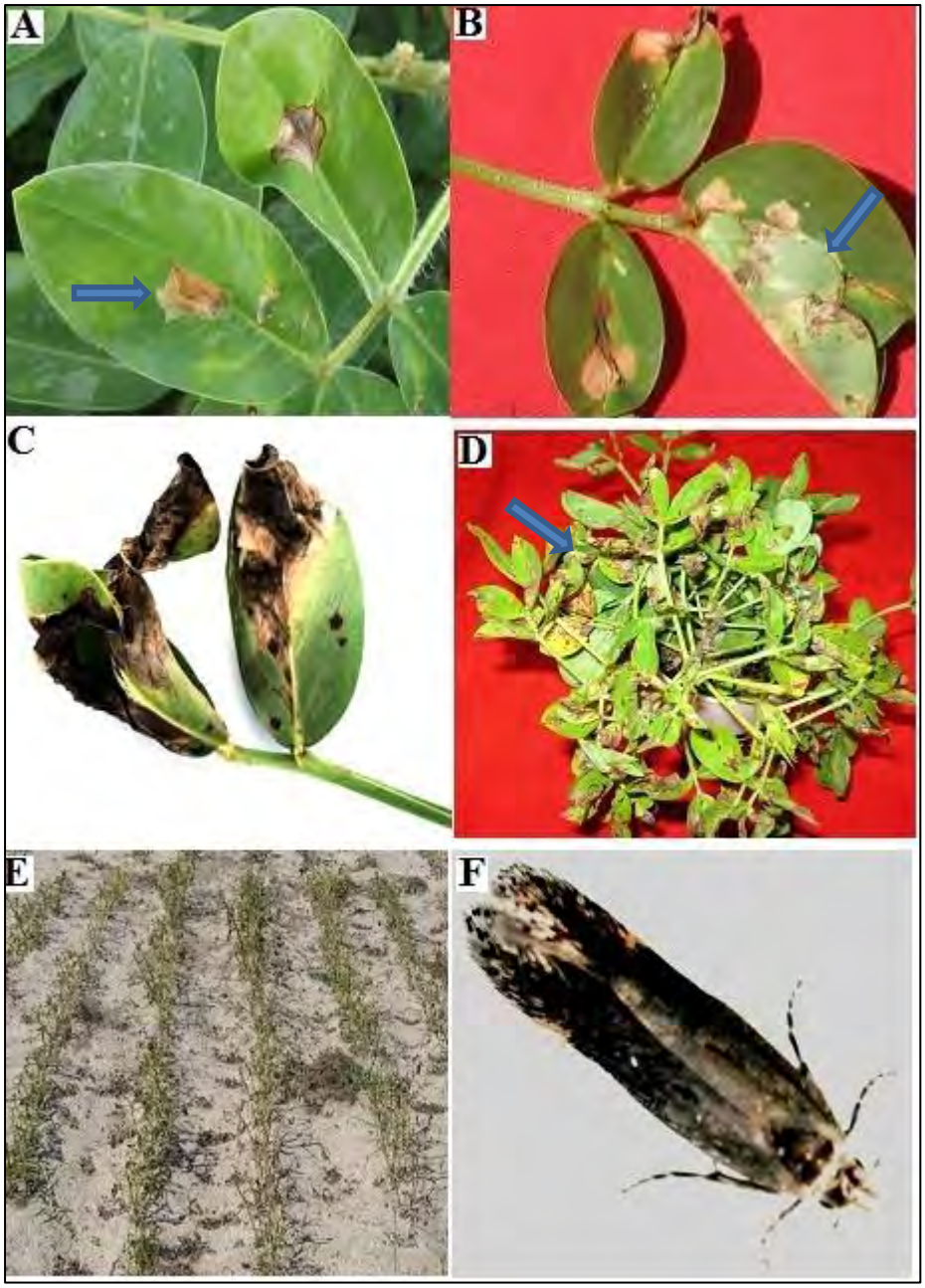


Figure 2.1. Pictogram showing (i) symptoms of groundnut leaf miner infestation in groundnut; early season leaf symptoms (A, B), late season symptoms (C), whole plant symptoms (D), crop defoliation (E), and (ii) the adult groundnut leaf miner moth (F). Note the necrotic bubble/blotches in the middle of leaflets in (A), the folding and webbing of leaflets in (B) and the extensive necrosis of leaflets in C.

2.3.3 Pest description and morphology

The moths, when newly emerged from the pupae, have light-grey coloured wings. As they age, they turn dark grey or brownish and mottled; with dark brown forewings and pale brown hind wings covered with scales and whitish towards the lower part (Figure 2.1F). The moth is about 4 to 5 mm long. Eggs are oval in shape, small shiny and white. Larvae are pale green when small and became dark-green when larger in size, with a shiny black head capsule. Larvae became cream coloured towards pupation. The pupa is enclosed in a thin silken cocoon inside the folded leaflets. Pupae were light brown when they were newly emerged, but later became dark brown. The moths lived for about 6 to 9 days inside the perforated plastic bottles with screw-on lids at room temperature.

2.3.4 Species identification by mtDNA (COI)

Based on comparisons with published sequences from the BOLD gene bank, one sample was identified as possibly *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (99.3% match), but the remaining samples (59) were identified as *Aproaerema simplexella* PS1 (Walker) (100% match). In addition, the topmost 15 matches after *A. simplexella* PS1 among the sequences available in the BOLD gene bank (Table 2.3) included 11 *A. simplexella* (93.53 to 98.08% match), one *Aproaerema lerauti* (Vives) (Lepidoptera: Gelechiidae) (93.53% match), two *Aproaerema isoscelixantha* (Lower) (Lepidoptera: Gelechiidae) (92.81 to 93.05% match) and one *Aproaerema captivella* (Herrich-Schäffer) (Lepidoptera: Gelechiidae) (92.33% match). There was very little genetic diversity within and between the specimens from the six surveyed sites (Figure 2.2).

Table 2.3. The 16 topmost matches of mtDNA of groundnut leaf miner specimens with sequences from the BOLD GeneBank.

Phylum	Class	Order	Family	Genus	Species	Specimen similarity (%)
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella* PS1	100
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	98.08
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.84
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.84
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.84
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.84
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.84
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.79
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.73
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	97.36
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*simplexella*	93.53
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*lerauti*	93.53
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*isoscelixantha*	93.05
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*isoscelixantha*	92.81
Arthropoda	Insecta	Lepidoptera	Gelechiidae	*Aproaerema*	*captivella*	92.33

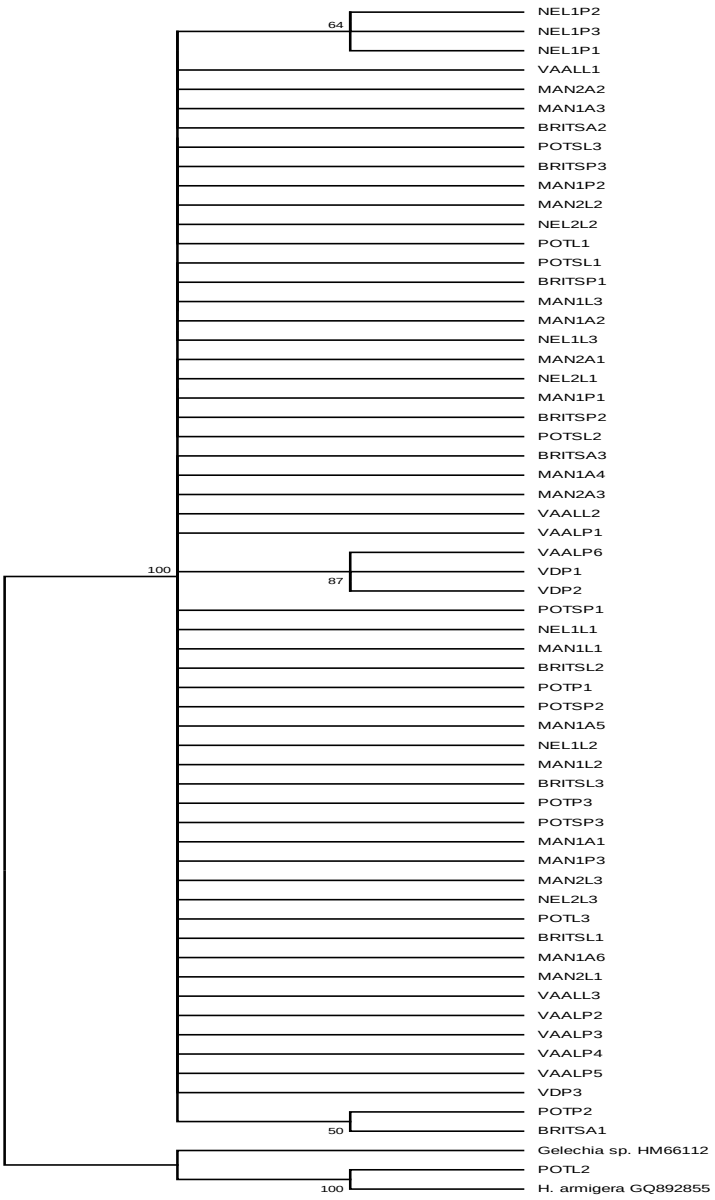


Figure 2.2.The phylogenetic relationships based on mtDNA COI regions of groundnut leaf miner identified as *Aproaerema simplexella* PSI from specimens collected from six survey sites in South Africa. The names on taxa positions reflect the sampling areas (Vaal and VD denote Vaaharts; Brits, Pot, Nel, Man 1 and Man 2 denote Brits, Potchefstroom, Nelspruit, Manguzi and Bhekabantu respectively) and whether the specimen was a larva (L), pupa (P) or adult (A). Numbers at the nodes represent bootstrap proportions (50% or more; 1000 replicates). Numbers after species names preceded with HM or GQ indicate GeneBank accession numbers.

2.4 Discussion

It has generally been assumed that GLM occurring on groundnut in Africa had its origins in Asia, with all reports from the African continent assuming the name *A.modicella* (Deventer) for the pest (Kenis & Cugala 2006; Du Plessis 2002, 2003). Contrary to this assumption,

irrespective of the place or crop (groundnut or soya bean) from which the specimens in this study were taken, the mtDNA COI sequences of the GLM specimens examined matched 100% with those of *A. simplexella* PS1 (previously *Stomopteryx subsecivella* (Ziller)) (Bailey 2007). This particular species, *A. simplexella*, is native to Australia where it is reported to be a pest of soya bean (Common 1990; Bailey 2007). The evidence obtained from the mtDNA COI analysis in the present study suggests that the GLM in South Africa is this Australian species. This is supported by the finding that all GLM specimens taken from the six widely separated sites in South Africa were identified as *A. simplexella* PS1, with *A. modicella* not listed in the most closely related species (Table 2.3). This infers that all infestations of GLM in Africa may be caused by the former, and not the latter species. Based on morphological characteristics, Shanower *et al.* (1993) suggested that the species found in Africa may be different from that found in India or Indonesia, describing the GLM in India as *Anacampsis nerteria* (Meyr.) (Meyrick 1906), the one in Africa as *Stomopteryx subsecivella* and another in India-Indonesia as *A. modicella* (Deventer). It is thus clear that a large degree of uncertainty has always existed as to the correct classification of GLM in Africa. No attempt has, however, been made to discriminate between the species genetically.

Previous to the present DNA analysis, *A. simplexella* PS1 was known to be present only in Australia (Common 1990; Bailey 2007). Now, given the presence of *A. simplexella* in Africa, it is at present difficult to conclude which of Africa and Australia is the native continent of the pest. However, the sudden visibility of the pest in Africa points to the possibility that it is a recent invasion to Africa, and this appears to be confirmed by the lack of intra- and inter-population diversity in the mtDNA COI gene amongst the specimens collected in the present study (Figure 2.2). The distribution range of *A. simplexella* PS1 in Australia covers almost all of the country (Common 1990; Bailey 2007). However, even though groundnut is a major crop in Australia, *A. simplexella* PS1 has not been reported to attack this crop in that country. In Australia, *A. simplexella* PS1 is generally regarded as a minor pest of soya bean, and is commonly known there as the soya bean moth (Common 1990; Bailey 2007). This suggests that the pest has a stronger preference for soya bean than for groundnut in Australia. In contrast, although it has been noted to infest soya bean (in this study), the pest has so far not been reported to be problematic on this crop in South Africa, or elsewhere on the African continent. In South Africa, this is despite the fact that soya bean production (515,000 ha) far exceeds that of groundnut (47,000 ha) (The Crop Site 2013). It is therefore surprising that, unlike in Australia, the pest has caused severe problems with groundnut rather than soya bean

in South Africa and in the rest of Africa. Also, whilst lucerne was expected to be one of the moth's alternative hosts (Du Plessis 2003), the present study suggests that it may not be a preferred host as it was not recorded on that crop at Vaalharts, Brits and Nelspruit, despite its presence on groundnut crops nearby. Nonetheless, lucerne and other host plants may play an important role in maintaining small moth populations, between seasons when the groundnut crop is not present.

2.5 Conclusion

Mitochondrial DNA COI analysis identified GLM in South Africa as *A. simplexella* PS1 (100% match on the BOLD system), native to Australia, which suggested that Australia may be the origin of the pest. It is most likely that GLM being reported on groundnut in other parts of Africa is also *A. simplexella* PS1. The phylogenetic tree based on specimens of *A.simplexella* PS1 obtained from the six widely separated sites in South Africa indicated that there was very little genetic diversity between and within the populations, suggesting that the pest might be from the same origin and could be a recent introduction to South Africa. Given that the sequences of GLM in South Africa matched those of *A. simplexella* PS1 and that the damage symptoms of the pest on groundnut are similar to those of *A. modicella* found in Asia, there is a need to determine if the two species are indeed genetically different. This has a bearing on the development and use of groundnut lines that are resistant to GLM, in countries where it is a problem. For the purpose of formulating strategies for managing the pest, there is also a need to determine its correct identity, its host range as well as its in-between season survival tactics in Africa.

2.6 References

BAILEY, P.T. 2007. Pests of field crops and pastures: Identification and control. CSIRO Publishing, Australia. 221-222.

COMMON, I.F.B. 1990. Moths of Australia, Melbourne University Press. 259.

DU PLESSIS, H. 2002. Groundnut leaf miner *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48-49.

DU PLESSIS, H. 2003. First report of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut, soya bean and lucerne in South Africa. *South African Journal of Plant & Soil* **20**: 48.

EPIERU, G. 2004. Participatory evaluation of the distribution, status and management of the groundnut leaf miner in the Teso and Lango farming systems. Final Technical Report of a project supported by NARO/DFID, Saari Kampala, Uganda.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783-791.

HALL, T.A. 1999. BioEdit: A user friendly biological sequence alignment editor and analysis program for window 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**: 1-9.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111-120.

LAVANYA, 2009. Groundnut Leaf miner.E:\GROUNDNUT LEAFMINER\Groundnut Leaf miner. Online at: http://agropedia.iitk.ac.in/content/groundnut-leaf-miner (Accessed on 16 May 2014).

MCPHERSON, M.J., QUIRKE, P. & TAYLOR, G.R. 1991. PCR: A practical approach. Oxford: IRL Press. 253.

MEYRICK, E. 1906. Description of Indian Microlepidoptera. *Journal of Bombay Natural History Society* **17**:139-140.

MUNYULI, T.M.B., LUTHER, G.C., KYAMANYWA, S. & HAMMOND, R. 2003. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda and Democratic Republic of Congo. *African Crop Science Conference Proceedings* **6**: 231-237.

PAGE, W.W., EPIERU, G., KIMMINS, F.M., BUSOLO-BULAFU, C. & NALYONGO, P.W. 2000. Groundnut leaf miner *Aproaerema modicella*: a new pest in eastern districts of Uganda. *International Arachis Newsletter* **20**: 64-66.

SAITOU, N. & NEI, M. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

SCHEFFER, S.J. 2000. Molecular evidence of cryptic species within *Lyriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* **93**: 1146-1151.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993. Biology and control of the groundnut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**: 3-10.

SIMMONS, R.B. & SCHEFFER, S.J. 2004. Evidence of cryptic species within the pest *Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **97**: 675-680.

SUBRAHMANYA, M.P., CHIYEMBEKEZA, A.J. & RAO, G.V.R. 2000. Occurrence of groundnut leaf miner in northern Malawi. *International Arachis Newsletter* **20**: 66-67.

TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M. & KUMAR, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* (http://www.genome.jp/tools/clustalw/).

THE CROP SITE. 2013. USDA GAIN: South Africa Oilseeds and Products Annual 2013 (http://www.thecropsite.com/reports/?id=1794#sthash.YLBWzqaH.dpuf (Accessed on 29 October 2014).

VAN DER WALT, A., DU PLESSIS, H. & VAN DEN BERG, J. 2008. Using morphological characteristics to distinguish between male and female larvae and pupae of the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *South African Journal of Plant & Soil* **25**(3): 182-184.

Chapter Three

Molecular and behavioural evidence suggest a re-examination of the taxonomy of *Aproaerema simplexella* (Walker) and *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae)

Abstract

Since 2000, the groundnut leaf miner has increasingly become a pest of groundnut and soya bean on the African continent. The origin of the pest in Africa is uncertain. Early reports in South Africa assumed it to be an invasion of *A. modicella* from the Asian continent, but subsequent mitochondrial DNA COI gene (mtDNA COI) fingerprinting matched it to *Aproaerema simplexella* (Walker) from Australia. Prior to this, reports in the 1950s recorded the pest in Africa under the name *Stomopteryx subsecivella* (Zeller 1852). Furthermore, it was found that *A. simplexella* responded to the species specific lure developed from the sex pheromone of *A. modicella*. As a result of these apparent anomalies, we examined the genetic relatedness of the above species from Africa, India and Australia. Mitochondrial DNA COI analyses were performed on 44 specimens collected from South Africa, four from Mozambique, and three each from single locations in India and Australia. In the BOLD gene bank, 70% of the specimens analyzed matched the *A. simplexella* sequences from Australia (99%-100%), including all three specimens from both India and Australia, and two from Mozambique. The match for the remaining specimens was 98-99%. Two specimens, later linked with parasitoid sequences, did not match with any of the sequences in the BOLD gene bank. In the NCBI gene bank, 81% of the sequences matched 99-100%, and a further 15% matched 92-98% with *A. simplexella* sequences. Based on these mtDNA COI analyses, and the similarities of the behavioural responses originally noted between the species, I believe that I am dealing with a single species and suggest tentative synonymisation of the names of the three taxa from the three continents, under the name of *Bilobata subsecivella* (Zeller).

Key words: Africa, Australia, India, mitochondrial DNA, pheromone response.

3.1 Introduction

Groundnut (*Arachis hypogaea* L.) and soya bean (*Glycine max* (L.) Merr.) production on the African continent is threatened by what is commonly known as the groundnut leaf miner (GLM) a name originally associated with *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae), occurring on the Indo-Asian continent as a pest of groundnut and soya bean. In Africa, the GLM became a major pest of both crops around 2000 (Du Plessis 2002; Kenis & Cugala 2006). The pest is a small moth whose larva mine between the upper and lower epidermis of the leaf, thereby reducing the photosynthetically active leaf area, which adversely affects the growth and yield of the crop. A single larva destroys from 34.8 to 179.3 cm-2 of leaf area (Islam *et al.* 1983; Shanower 1989). The damaged leaves eventually become brownish, rolled and desiccated, which results in early defoliation (Kenis & Cugala 2006), and this further negatively impacts on the growth and yield of the groundnut plants. In groundnut, GLM can cause up to a 90% loss in total yield (Reddy *et al.*1978; Sumithramma 1998), and where there are no natural enemies, an epidemic can result in total crop loss (Wightman & Ranga Rao 1993).

The GLM in Africa is thought to be a recent invasion of *A. modicella* from the Asian continent (Du Plessis 2002; Kenis & Cugala 2006). However, there are reports dating back to the 1950s that describe a moth similar to *A. modicella*, which was referred to as *Stomopteryx subsecivella* (Zeller) [= *Gelechia (Brachmia) subsecivella* Zeller 1852] and was recorded as being of non-economic importance in Africa (Janse 1954; Mohammad 1981). In Australia, there is a soya bean pest (*Aproaerema simplexella* (Walker) [= *Gelechia simplexella* Walker 1864]), which is morphologically similar to *A. modicella* in Asia (Bailey 2007) and the GLM found in Africa (Buthelezi *et al.* 2012). The complexity of names, and thus apparently confused taxonomy, for GLM worldwide is reflected by Shanower *et al.* (1993), who described the GLM in India as *Anacampsis nerteria* (Meyrick 1906), the one in Africa as *Stomopteryx subsecivella* (Zeller 1852) and another in India-Indonesia as *Aproaerema modicella* (Van Deventer 1904). Further literature searches revealed several synonyms applied to the GLM. These include *Aproaerema nerteria* (Meyrick) (Fletcher 1914; 1917; 1920), *Stomopteryx nerteria* (Meyrick) (Anon 1941; Cherian & Basheer 1942), *Stomopteryx subsecivella* (Zeller) (Abdul Kareem *et al.* 1972-73; Litsinger *et al.* 1978) and *Biloba subsecivella* (Zeller) (Anon 1977; Dean 1978).

To add to this complexity, Buthelezi *et al.* (2012), using mtDNA COI analysis on GLM specimens collected from six widely separated sites in South Africa, showed that all of the specimens matched 100% with *A. simplexella* (the Australian species) on the Barcode of Life Data System (BOLD). This caused more confusion, as there is no record of *A. simplexella* having been previously recorded from Africa, and it is not known to be a groundnut pest anywhere in the world. In an attempt to resolve this taxonomic complexity, specimens of GLM were hand collected from groundnut crops in India and Mozambique, and through pheromone traps in Australia. These were added to those collected from South Africa by Buthelezi *et al.* (2012). The mitochondrial DNA COI (mtDNA COI) gene regions of these specimens were sequenced and analysed to determine their genetic relatedness to each other, and to the named specimens in the BOLD and NCBI gene banks. This chapter presents these results, and relates them to the previous published literature on the cosmopolitan species making up the complex known as the GLM, and proposes that this taxonomic group should be revisited and revised.

3.1.1 History of the species previously described under different names on three continents.

3.1.1.1 The Australian connection

Gelechia simplexella (Walker 1864) (Lepidoptera: Gelechiidae) = *Aproaerema simplexella* (Walker 1864)

Aproaerema simplexella was described in Australia in 1864 (Walker 1864) and is thought to be native to that country (Bailey 2007). In 1904, Meyrick made an unjustified emendation of *G. simplexella* Walker to *Anacampsis simplicella* (Lepidoptera: Gelechiidae) (Meyrick 1906). The distribution range of *A. simplexella* in Australia covers almost all of the country (Common 1990; Bailey 2007), where it is generally regarded as a minor pest of soya bean, and is commonly known as the soya bean moth (Common 1990; Bailey 2007). Even though groundnut is a major crop in Australia, *A. simplexella* has not been reported from it, suggesting that *A. simplexella* has a stronger preference for soya bean than for groundnut in Australia. However, during recent pheromone trapping in a garden in the Brisbane area of Australia, using lures baited with *A. modicella* sex pheromones, adults of *A. simplexella* were caught for the subsequent mtDNA analyses.

3.1.1.2 The Indian connection

Anacampsis nerteria Meyrick 1906 (Lepidoptera: Gelechiidae)

Meyrick (1906) first described the GLM in India as *Anacampsis nerteria*. This name was also used by Maxwell-Lefroy and Howlett (1909) and by Maxwell-Lefroy (1923). *Anacampsis nerteria* was subsequently synonymized with *Gelechia (Brachmia) subsecivella* (Meyrick 1925). The moth is a serious pest of groundnut in the Indian States of Andhra Pradesh (Channabasavanna 1957; Krishnamurthy Rao *et al.* 1962), Karnataka (Channabasavanna 1951; 1954; 1957; Krishnamurthi & Appanna 1951; Usman & Puttarudraiah 1955), Maharashtra and Tamil Nadu (Anon 1941; 1950; 1963; Cherian & Basheer 1942; Fletcher 1914; 1917) and Gujarat (Mohammad 1981). It is also a major pest of soya bean in Madhya Pradesh and Karnataka (Rai *et al.* 1973; Kapoor *et al.* 1975; Rawat & Singh 1979) and a pest of lucerne in Punjab (Sandhu 1978).

Aproaerema modicella Deventer 1904 (Lepidoptera: Gelechiidae)

Aproaerema modicella was originally described as *Xystophora modicella* in 1904 by Van Deventer from Java (Indonesia) (Van Deventer 1904). In 1980, *A. modicella* (Deventer) was proposed as the scientific name for the Indian-Indonesian groundnut leaf miner, with the synonyms *Xystophora modicella*, *Anacampsis nerteria* and *Stomopteryx subsecivella* (Mohammad 1981). As *A. nerteria* is now synonymised with *A. modicella* (Mohammed 1981), it was thought that the two species of Gelechiidae attacking groundnut and soya bean in India, may comprise a single species.

3.1.1.3 The link with African species

Gelechia (Brachmia) subsecivella Zeller 1852 (Lepidoptera: Gelechiidae)

= *Stomopteryx subsecivella* Zeller 1852 = *Bilobata subsecivella* (Zeller 1852)

Stomopteryx subsecivella (Zeller) was originally described by Zeller in 1852 (Zeller 1852). Meyrick and Fletcher (1932) ascribed the Indian *Xystophora modicella* (Deventer) and *Stomopteryx nerteria* (Meyrick) as synonyms of the South African *S. subsecivella* (Mohammad 1981). Janse (1954) was the first to revise *S. subsecivella* and his conception

was that it was very different and could be congeneric with *A. modicella.* He also proposed a new genus, *Biloba* Janse, for *S. subsecivella*. However, the name *Biloba* was unavailable as it was preoccupied (Mohammad 1981). In 1986, *Bilobata* (Vári) (Lepidoptera: Gelechiidae) was established as an objective replacement name for *Biloba*, a junior homonym (Vári 1986).

However, it seems that the species name for GLM in South Africa and Mozambique was accepted as *A. modicella* (Du Plessis 2002; Kenis & Cugala 2006), until the recent study of Buthelezi *et al.* (2012) which on the basis of mtDNA analyses, found that all the analysed South African specimens aligned 100% with the Australian *A. simplexella*. This has caused confusion as to where the GLM occurring in South Africa originated, and whether it was named correctly in the first place.

The information gathered from the literature divulges that even though GLM is known by different names on three continents, there are some anomalies with these species. For example, *A. simplexella* in Australia is known as the soya bean moth (Bailey 2007) whereas in South Africa it has been regarded as a pest of groundnut and soya bean, with soya bean being more infested than groundnut (Buthelezi *et al.* 2013). In Asia, *A. modicella* is regarded as the most important pest of groundnut and soya bean (Shanower *et al.* 1993).To add to this confusion, in a recent study, mtDNA COI analysis of the GLM collected from South Africa matched it with *A. simplexella* sampled from Australia (Buthelezi *et al.* 2012). Molecular techniques such as DNA fingerprinting offer complementary, faster and more precise options for species identification (Scheffer 2000), and are especially useful in discriminating between related species that share morphologically similar features (Scheffer 2000).

3.1.2 DNA fingerprinting as a research tool in entomology

Mitochondrial DNA analysis is the most commonly used technique for determining genetic relationships amongst animal and plant populations (King *et al.* 2002; Simmons & Scheffer 2004). It has also proven capable of highlighting cryptic lineages representing distinct species within geographically widespread and apparently morphologically homogeneous organisms (Scheffer 2000). In the current study, this technique was used to examine the genetic relatedness of GLM species in Africa, India and Australia, in an attempt to resolve the complex taxonomic status of the GLM grouping.

3.2 Material and methods

3.2.1 Specimen collection

Specimens were collected from four widely separated sites in South Africa namely; Brits (25°59'1.35" S 27°76'8.75" E), Nelspruit (25°45'4.52" S 30°97'1.54" E), Manguzi (26°95'5.32" S 32°82'3.56" E) and Vaalharts (27°95'7.61" S 24°83'9.91" E); two sites in Mozambique; namely Pambara EP1 (21°94'33" S 35°10'06" E) and Pambara Produtor (21°99'84" S 35°15'13" E); and one site each in India, Hyderabad- ICRISAT (17°21'57" N 78°28'33" E) and Australia, Brisbane (27°32'08" S 152°51'35" E). In South Africa, the specimens comprised larvae that were collected from groundnut, soya bean, pigeon pea (*Cajanus cajan* L.) and lucerne (*Medicago sativa* L.). In India and Mozambique, the specimens comprised larvae that were collected from groundnut. Specimens from Australia comprised moths which were captured using pheromone traps installed (see installation procedures in Chapter 6, Section 6.2.4) in natural vegetation containing wild soya bean. All specimens were placed in 10-ml polycarbonate vials containing absolute ethanol and closed with press-on plastic lids. The vials were taken to a laboratory at the University of Zululand, Empangeni, KwaZulu-Natal and stored at minus 80°C in a cryogenic freezer until DNA sequencing commenced.

3.2.2 DNA extraction and polymerase chain reaction amplification

The DNA sequencing was performed using the mtDNA COI gene of 44 specimens from South Africa, four from Mozambique, and three each from Australia and India. The DNA was extracted from the specimens using the Tissue mini Prep kit from Zymo Research following the manufacturer's protocol. Polymerase Chain Reaction (PCR) was conducted with Lara C1 Seq primers in DreamTaq, 25 µl reactions with 10pmol primer and around 30 ng of gDNA. The cycling protocols used were as follows: 95 ºC for 5min, 95 ºC for 30sec, 50 ºC for 30sec, 45 cycles, 72 ºC for 1min, 72 ºC for 10min, 4 ºC hold. Successful amplicons were then purified using ExoSap following the manufacturer's protocol. The purified templates were sequenced using the ABI Big Dye kit V3.1. Sequenced products were cleaned with the Zymo sequencing clean-up kit before injection into ABI 3500 Xl genetic analysers with a 50 cm array and POP7.

3.2.3 Editing of DNA sequences and species identification by mtDNA COI in BOLD and NCBI gene banks

DNA sequences were manually edited (for base calling errors), pruned and aligned by ClustalW using the BioEdit Sequence Alignment Editor (Hall 1999) to create consensus sequences which were saved in the fasta format in MEGA5 (Hall 1999). All consensus sequences were entered into the BOLD and NCBI gene banks to identify positively the species that comprised the various specimens.

3.3 Results

3.3.1 Species identification by mtDNA COI in BOLD and NCBI gene banks

In the BOLD system, the majority (70%) of the specimens analyzed matched 99-100% with the *A. simplexella* sequences from Australia. These included all three specimens from both India and Australia and two from Mozambique. A further 26% of the specimens analyzed matched 98-99% with the *A. simplexella* sequences in the BOLD gene bank. However, in two specimens from South Africa (4%), the sequences did not match with any sequences on the BOLD gene bank (Table 3.1). In the NCBI gene bank, 81% of the specimens analyzed matched 99-100% with the *A. simplexella* sequences and 15% matched 92-98% with these sequences. The two specimens (4%) which did not match with any sequences from the BOLD gene bank, displayed a distant but inaccurate match (87%) with *Euplectrus* sp. (Hymenoptera: Eulophidae) (Table 3.1) on the NCBI gene bank, presumably as a result of parasitism of the larval specimens.

Table 3.1. Percentage match of the mitochondrial DNA COI sequences from specimens of groundnut leaf miner collected during the present study from South Africa, Mozambique, India and *A. simplexella* from Australia, with sequences of *A. simplexella* already published in the BOLD and NCBI gene banks.

		BOLD gene bank			NCBI gene bank		
Location	**Number of specimens analysed**	**99.1-100% match with *A. simplexella***	**98-99% match with *A. simplexella***	**No match**	**99-100% match with *A. simplexella***	**92-98% match with *A. simplexella***	**87% match with *Euplectrus* sp.**
South Africa	44	30	12	2	12	30	2
India	3	3				3	
Australia	3	3				3	
Mozambique	4	2	2		1	3	
Percebrage of matched specimens		70%	26%	4%	15%	81%	4%

3.3.2 Aligned nucleotide sequences for mtDNA COI

Nucleotide sequences for mtDNA COI, which were aligned by ClustalW using the BioEdit Sequence Alignment Editor (Hall 1999), are presented in Table 3.2. For the specimens collected from South Africa, India and Australia, a 100% match with *A. simplexella* was obtained from the BOLD gene bank; while for the Mozambique specimens, a 100% match with *A. simplexella* was obtained from the NCBI gene bank. In addition, one aligned nucleotide sequence of *A. simplexella* from the BOLD and one from the NCBI gene banks are included in Table 3.2.

Table 3.2. Selected mtDNA COI sequences of GLM collected from South Africa, Mozambique and India and mtDNA COI sequences of *A. simplexella* collected from Australia that matched 100% with those of *A. simplexella* sequences from Australia in the BOLD gene bank (South Africa, India and Australia) or in the NCBI gene bank (Mozambique and South Africa).

Country	**DNA Sequences**
South Africa	CATTCCCCCGTATAAATAATATAAGATTTTGACTTTTACCTCCATCTTTAACCTTACTAATTTC AAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTGTACCCCCCACTATCATCTA ATATTGCCCATGGAGGAAGTTCAGTAGATTTAGCTATTTTTTCATTACATTTAGCAGGTATTTC TTCAATTCTTGGAGCAATTAATTTTATTACTACTATTATCAATATGCGAATTAATGGTATAATA TTTGATCAAATACCTTTATTTGTATGAGCTGTAGGAATTACAGCTTTATTATTATTATTATCAT TACCTGTATTAGCAGGAGCTATTACAATATTATTAACAGATCGAAACCTTAATACATCATTTT TTGACCC
India	TCCGTGGGCCGAMTAGCATTCCCCCGWATAAATAATATAAGATTTTGACTTTTACCTCCATCT TTAACCTTACTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTGTA CCCCCCACTATCATCTAATATTGCCCATGGAGGAAGTTCAGTARATTTAGCTATTTTTTCATTA CATTTAGCAGGTATTTCTTCAATTCTKGGAGCAATTAATTTTATTACTACTATTATCAATATGC GAATTAAKGGTATAATATTTGATCAAATACCTTTATTTGTATGAGCTGTAGGAATTACAGYTT

	TATTATTATTATTATCATTACCTGTATTAGCAGGAGCTATTACAATATTATTAACAGATCGAA ACCTTAATACATCATTTTTTGACCA
Australia	ACCASCCTGAMAGCATTCCCCCGTATAAATAATATAAGATTTTGACTTTTACCTCCATCTTTA ACCTTATTAATTTCAAGAAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTGTACCC CCCACTATCATCTAATATTGCCCATGGAGGAAGTTCAGTARATTTAGCTATTTTTTCATTACAT TTAGCAGGTATTTCTTCAATTCTTGGAGCAATTAATTTTATTACTACTATTATCAATATACRAA TTAAKGGTATAATATTTGATCAAATACYTTTWTTTGTATGAGCTGTAGGAATTACAGCTTTAT TATTATTATTATCATTGCCCGTATTAGCTGGAGCTATCACAATATTACTAACAGATCGAAACC TTAATACATCWTTTTTTGACC
Mozambique	CCCSGGTAATAAATAATATAAGATTTTGACTTTTACCTCCATCTTTAACCTTACTAATTTCAAG AAGAATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTGTACCCCCCACTATCATCTAATA TTGCCCATGGAGGAAGTTCAGTAGATTTAGCTATTTTTTCATTACATTTAGCAGGTATTTCTTC AATTCTTGGAGCAATTAATTTTATTACTACTATTATCAATATGCGAATTAATGGTATAATATTT GATCAAATACCTTTATTTGTATGAGCTGTAGGAATTACAGCTTTATTATTATTATTATCATTAC CTGTATTAGCAGGAGCTATTACAATATTATTAACAGATCGAAACCTTAATACATCATTTTTTG ACCC
A. simplexella sequences from BOLD gene bank	AACATTATATTTTATTTTTGGTATTTGAGCAGGTATAGTGGGAACATCATTAAGTTTACTAATT CGAGCTGAATT AGGAAATCCGGGTCAATTAATTGGAGATGACCAAATTTATAATACTATTGTAACCGCTCATG CTTTTATTATAAT TTTTTTTATAGTAATGCCAATTATAATTGGAGGATTTGGTAATTGATTAGTACCATTAATATTA GGAGCCCCTGA TATAGCATTTCCTCGAATAAATAACATAAGATTTTGACTTTTACCTCCATCTTTAACCTTATTA ATTTCAAGAA
A. simplexella sequences from NCBI gene bank	AACATTATATTTTATTTTTGGTATTTGAGCAGGAATAGTAGGAACATCTCTTAGTTTATTAATT CGAGCAGAATTAGGAA ATCCAGGACAATTAATTGGAGACGATCAAATTTATAATACTATTGTTACAGCTCATGCCTTCA TTATAATTTTTTTTATA GTAATGCCAATTATAATTGGGGGATTTGGTAATTGATTAGTGCCTTTAATACTAGGAGCCCCC GATATAGCATTCCCCCG TATAAATAATATAAGATTTTGACTTTTACCTCCATCTTTAACCTTACTAATTTCAAGAAGAATT GTAGAAAATGGAGCAG GAACTGGATGAACAGTGTACCCCCCACTATCATCTAATATTGCCCATGGAGGAAGTTCAGTA GATTTAGCTATTTTTTCA TTACATTTAGCAGGTATTTCTTCAATTCTTGGAGCAATTAATTTTATTACTACTATTATCAATA TGCGAATTAATGGTAT AATATTTGATCAAATACCTTTATTTGTATGAGCTGTAGGAATTACAGCTTTATTATTATTATTA TCATTACCTGTATTAG CAGGAGCTATTACAATATTATTAACAGATCGAAACCTTAATACATCATTTTTTGACCCAGCTG GAGGAGGTGACCCAATTTTATACCAACATTTATTC

3.4 Discussion

A literature review completed for the present study revealed the current taxonomic conundrum for GLM. The correct scientific name of GLM was first questioned by Mohammad (1981) and consultations were made with the Commonwealth Institute of Entomology and the British Museum (Natural History), London (UK) on the issue. From the discussions, it was highlighted that uncertainties on the correct scientific name of GLM emanated from the fact that there were two separate species known as GLM. Unfortunately these species were not mentioned by Mohammad (1981). During these discussions, a

consultation was made with Dr. Klaus Sattler, a British Museum (Natural History) specialist of Lepidoptera. A resolution was reached between them that the synonyms of the GLM *A. modicella* (Deventer) are *X. modicella* (Van Deventer 1904), *A. nerteria* (Meyrick 1906), and *S. subsecivella* (Zeller 1852) (Mohammad 1981). Janse (1954) revised *S. subsecivella* and considered it to be a species that was different from *A. modicella*, but likely to be congeneric with *A. modicella*. Janse's argument was based on the fact that the specimen from Barberton in South Africa (which he studied) and the specimen originally described from Rondebosch in South Africa, and previously identified by Meyrick, were not white at the end of the second joint of the palpi, as described for *A. modicella* (Janse 1954). However, Janse (1954) also mentioned that there was only one specimen each in the collections of the Transvaal Museum (Pretoria) and South African Museum (Cape Town) which he studied, and that the specimen was inconspicuous in general appearance; therefore, a mistake could easily have been made when describing the specimen. Bailey (2007), furthermore, reported that there was a species similar to *A. simplexella* occurring in Asia. However, it was never concluded whether they were the same species. The mtDNA COI analyses completed for the present study revealed that all specimens analysed, irrespective of their geographic origin or host plants are almost identical to each other (52 specimens out of 54 matched between 98.41% and 100 % and between 92-100% with *A. simplexella* in the BOLD and in the NCBI gene banks respectively). The very close relatedness of these specimens in terms of mtDNA COI are thus evident, indicating that even though the species are from very distinct geographic areas, they are (those matching 99-100% with *A. simplexella*) either the same species, or very closely related (98-99% match). This relatedness is further indicated by the similar behaviours of the populations from the different geographic regions.

In a seasonal monitoring study of the GLM in South Africa (see Chapter 6), pheromone trapping of the pest was successfully carried out using traps baited with polyethylene vials containing the female sex pheromone blend of *A. modicella* [(Z)-7,9-decadienyl acetate, (E)-7-decenyl acetate and (Z)-7-decenyl acetate in the ratio10:2:1.4] as described by Hall *et al.* (1994) and supplied by the Natural Resources Institute (NRI) of the University of Greenwich, United Kingdom. In India, the same sex pheromone blend was used by Das (1999) to monitor the seasonal activity of *A. modicella* in groundnut fields in the west Nimer Valley. Also, the same blend was used to trap *A. simplexella* adult specimens in Australia, (i.e those used for the mtDNA COI analyses in this study). In all cases, the GLM species in the different continents (Africa – *A. modicella*/*A. simplexella*, India – *A. modicella* and Australia – *A.*

simplexella) responded positively to the same lures. Pheromone lures are species specific (Megido *et al.* 2013) so it is unlikely that two different species would have been trapped using the same *A. modicella* pheromone blend supplied by the NRI. These observations thus support the motivation supplied by the mtDNA study for a re-investigation into the uncertain taxonomic status of the GLM complex.

3.5 Conclusion

Mitochondrial DNA COI results presented in this study have suggested that *A. simplexella* found in Australia and *A. modicella* and *A. modicella/A. simplexella* found in India and Africa, respectively, are the same species. Further evidence for this conclusion was provided by these 'species' responding positively to the *A. modicella* pheromone blend that is used in commercially available lures, which are generally species specific. We thus tentatively synonymize these 'species' based on the results of the mtDNA COI gene analyses. However, further studies including both molecular and morphological analysis of the genitalia of the different 'species' will be conducted to reinforce this proposed synonymy. The synonymization should be under the Genus *Bilobata* (Gelechiidae: Lepidoptera) and the species name should be formalized as *subsecivella* as it is the original name which was first described by Zeller in 1852. This will reduce the current taxonomic confusion related to GLM, and possibly allow the identification of the area of origin of the species. More importantly, it will allow more effective control measures to be developed for this pest, as correct identification of a pest species is the foundation on which good integrated pest management techniques are built. If the three taxa from Africa, India and Australia should indeed be referable to a single species, the resulting classification should be as follows:

Bilobata Vári, 1986

Biloba Janse, 1954, nom. praeocc.

Bilobata subsecivella (Zeller, 1852)

Gelechia (*Brachmia*) *subsecivella* Zeller, 1852

Gelechia simplexella Walker, 1864, **syn. nov.**

Xystophora modicella Deventer, 1904, **syn. rev.** (Synonymized with *G.* (*B.*) *subsecivella* by Meyrick, 1925: 111, but subsequently recalled from synonymy).

Anacampsis simplicella Meyrick, 1904 (An unjustified emendation of *G. simplexella* Walker).

Anacampsis nerteria Meyrick, 1906 (Synonymized with *G*. (*B*.) *subsecivella* by Meyrick, 1925: 111).

3.6 References

ABDUL KAREEM, A., NACHIAPPAN, R.W., SADAKTHULLA, S. & SUBRAMANIAM. T.R. 1972-73. An account of the recent insecticidal control of surulpoochi, *Stomopteryx subsecivella* Zeller, on groundnut. Annamalai University Agricultural Research Annual **4&5**: 150-153.

ANONYMOUS. 1941. Agriculture and Animal Husbandry in India. 1938-39. Delhi, 422 pages.

ANONYMOUS. 1950. Pests and Diseases of Groundnut in Madras Province. Indian Farming 11-71.

ANONYMOUS. 1963. Groundnut Leafminer. *Plant Protection Bulletin* (pest and disease number) 11-121.

ANONYMOUS. 1977. Outbreak of Pest and Diseases. Quarterly Newsletter F.A.O. Plant Protection Committee for South East Asia and Pacific Region **20**(4): 3-5

BAILEY, P.T. 2007. Pests of Field Crops and Pastures: Identification and Control. CSIRO Publishing, Collingwood, Victoria 3066, Australia.

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2012. The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soya bean moth (*Aproaerema simplexella*) (Walker) (Lepidoptera: Gelechiidae). *African Journal of Agricultural Research* **7**(38): 5285–5292.

BUTHELEZI, N.M, CONLONG, D.E. & ZHARARE, G.E. 2013. A comparison of the infestation of *Aproaerema simplexella* on groundnut and other known hosts for *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *African Entomology* **21**(2): 183-195.

CHANNABASAVANNA, G.P. 1951. "The surulpuchi" (leafminer) of groundnut. *Mysore Agricultural Journal* **26**: 67-68.

CHANNABASAVANNA, G.P. 1954. Insect pests of some cash crops – Nature of damage and control. Mysore Agricultural College Year Book. 89.

CHANNABASAVANNA, G.P. 1957. The groundnut leaf miner and its control. Mysore Agricultural College Year Book (1956-1957). 123-124.

CHERIAN, M.C. & BASHEER, M. 1942. Studies on *Stomopteryx nerteria* Meyr. – A pest of groundnut in the Madras Presidency. *Madras Agricultural Journal* **30**: 379-381.

COMMON, I.F.B. 1990. Moths of Australia. Melbourne: Melbourne Univ. Press. 217-266.

DAS, S.B. 1999. Seasonal activity of groundnut leafminer, *Aproaerema modicella* in west Nimer Valley, Madhya Pradesh. *Insect Environment* **5**: 69.

DEAN, G.J. 1978. Insects found on economic plants other than rice in Laos. *Pest Articles & News Summaries* **24**: 129-142.

DU PLESSIS, H. 2002. Groundnut leaf miner *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48-49.

FLETCHER, T.B. 1914. Some South Indian Insects and Other Animals of Importance – considered especially from an economic point of view. Government Press, Madras, 565 pages.

FLETCHER, T.B. 1917. Groundnut Pests. *Proceedings of the Second Entomological Meeting*, Pusa, 43.

FLETCHER, T.B. 1920. Life histories of Indian Insects: Microlepidoptera. *Memoirs, Department of Agriculture, India, Pusa, Entomology Series* **6**: 77-79.

HALL, T.A. 1999. BioEdit: A user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symposium Series* **41**: 95-98.

HALL, D.R., BEEVOR, D.G., CAMPION, D.J., CHAMBERLAIN, D.J., CORK, A., WHITE, R., ALMESTRE, A., HENNEBERRY, T.J., NANDAGOPAL, V., WIGHTMAN, J.A. & RANGA RAO, G.V. 1994. Identification and synthesis of new pheromones. p.1- 9. In: McVeigh, L.J., Hall, D.R. & Beevor, P.S. (Eds.) *Proceedings of OILB meeting, NRI, Chathman Maritime, Kent, UK*.

ISLAM, W., AHMED, K.N., NARGIS, A. & ISLAM, U. 1983. Occurrence, abundance and extent of damage caused by insect pests of groundnuts (*Arachis hypogaea*). *Malaysian Agricultural Journal* **54**: 18-24.

JANSE, A.J.T. 1954. The Moths of South Africa. V. Gelechiidae. Pretoria, Transvaal Museum **5**(4): 301–464, pls. 137–202.

KAPOOR, K.N., GUJRATI, J.P. & GANGRADE, GA. 1975. Chemical control of soya bean leaf miner, *Stomopteryx subsecivella* Zeller. (Lepidoptera: Gelechiidae). *Indian Journal of Entomology* **37**(3): 286-291.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa. CAB Reviews: *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**:19.

KING, H., CONLONG, D.E. & MITCHELL, A. 2002. Genetic differentiation in *Eldana saccharina* (Lepidoptera: Pyralidae): evidence from the mitochondrial cytochrome

oxidase I and II genes. *Proceedings of the South African Sugar Technologists' Association* **76**: 321-328.

KRISHNAMURTHI, B. & APPANNA, M. 1951. Occurrence, distribution and control of major insect pests of some important crops in Mysore. *Mysore Agricultural Journal* **27**: 1-23.

KRISHNAMURTHY RAO, S., RANGACHARULU, P. & YESUDA, T. 1962. Aerial spraying against surulpoochi (*Stomopteryx nerteria*) on summer irrigated groundnut. *The Andhra Agricultural Journal* **9**: 202-206.

LITSINGER, J.A, QUIRINO, C.B., LUMANAN, M.D. & BANDONG, J.P. 1978. The grain legume pest complex of rice-based cropping systems at three locations in the Philippines. In: Singh, S.R., Van Emden, H.F. & Ajibola Taylor, T. (Eds.) Pests of Grain Legumes: Ecology and Control. 309-320. London, UK: Academic Press.

MAXWELL-LEFROY, H. & HOWLETT, F.M. 1909. Indian Insect Life: A Manual of the Insects of the Plain (Tropical India).Today and Tomorrow's Publishers, New Delhi (reprinted 1971).

MAXWELL-LEFROY, H. 1923. *Anacampsis nerteria.* Manual of Entomology – Longmans, Green & Co. New York.

MEGIDO, R.C., HAUBRUGE, E. & VERHEGGEN, F.J. 2013. Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A review, *Base* [En ligne], numéro 3, **17** (2013), 475-482 URL : http://popups.ulg.ac.be/1780-4507/index.php?id=10229.

MEYRICK, E. 1906. Description of Indian Microlepidoptera. *Journal of Bombay Natural History Society* **17**:139-140.

MEYRICK, E. 1925. Lepidoptera Heterocera. Fam. Gelechiidae. *Genera Insectorum* **184:** 1-290, pls 15.

MOHAMMAD, A. 1981. The groundnut leaf miner, *Aproaerema modicella* Deventer (= *Stomopteryx subsecivella* Zeller) (Lepidoptera: Gelechiidae*).* A review of world literature. Occasional paper 3, Groundnut Improvement Program, ICRISAT.

RAI, P.S., REDDY, K.V.S. & GOVINDAN, R. 1973. A list of insect pests of soya bean in Karnataka State.*Current Research* **2**(11): 97-98.

RAWAT, R.R. & SINGH, O.P. 1979. Final report on "Studies on the chemical control of major insect pests of soya bean, groundnut and rice at Jabalpur, Madhya Pradesh". Period rabi 1977-78 and kharif 1978-79, Dept. of Entomology- Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur- (MP) India.

REDDY, N.M., HAVANAGI, G.V. & HEGDE, B.R. 1978. Effect of soil moisture level and geometry of planting on the yield and water use of groundnut. *Mysore Journal of Agricultural Sciences* **12**: 50-55.

SANDHU, G.S. 1978. Note on the incidence of *Stomopteryx subsecivella* (Zell.) (Lepidoptera: Gelechiidae) on lucerne at Ludhiana. *Indian Journal of Agricultural Sciences* **48**: 53-54.

SCHEFFER, S.J. 2000. Molecular evidence of cryptic species within *Lyriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* **93**: 1146-1151.

SHANOWER, T.G. 1989. The Biology, Population Dynamics, Natural enemies, and Impact of the Groundnut Leafminer (*Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut in India. PhD dissertation, University of California, Berkeley, USA.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993. Biology and control of the groundnut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**: 3-10.

SIMMONS, R.B. & SCHEFFER, S.J. 2004. Evidence of cryptic species within the pest *Copitarsia decolora* (Guene´e) (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **97**: 675-680.

SUMITHRAMMA, N. 1998. Bio-ecology of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) and its natural enemy complex in Southern Karnataka. Ph.D. Thesis, University of Agricultural Sciences, GKVK, Bangalore.

USMAN, S. & PUTTARUDRAIAH, M. 1955. A list of insects of Mysore including mites. Department of Agriculture Mysore State – *Entomology Series Bulletin* **16**:194 Pages.

VAN DEVENTER, W. 1904. Microlepidoptera van Java. - *Tijdschrift voor Entomologie* **47**: 1–42, pls. 1–2.

VÁRI, L. 1986. Introduction. In: Vári, L. & Kroon, D. (1986). *Southern African Lepidoptera – a series of cross-referenced indices.* The Lepidopterists' Society of Southern Africa & The Transvaal Museum, Pretoria. pp. vii-x.

WALKER, F. 1864. List of the specimens of lepidopterous insects in the collection of the British Museum **30**: 837–1096.

WIGHTMAN, J.A. & RANGA RAO, G.V. 1993. Groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). In: A Groundnut Insect Identification Handbook for India. *ICRISAT Information Bulletin* **39**: 18-21.

ZELLER, P.C. 1852. Lepidoptera Microptera, quae J.A. Wahlberg in Caffrorum Terra Collegit.1–120.

Chapter Four

Phylogenetic relationships of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) populations collected from India, Australia and Africa based on mitochondrial and nuclear DNA gene sequences

Abstract

In recent molecular work, the South African groundnut leaf miner (GLM) was found to be very closely related to both the Australian soya bean moth *Aproaerema simplexella* (Walker) and the Indian GLM *A. modicella*; consequently, these 'species' were tentatively synonymized as *Bilobata subsecivella* (Zeller). However, there are differences in the plant species utilized in the different countries. Therefore, I investigated the evolutionary relationships of specimens collected from these three continents by comparing sequences of five different gene regions of mitochondrial and nuclear DNA (COI, COII, cytb, 28S and EF-1 ALPHA). Sequenced samples included 44 collected from four sites in South Africa, four from Mozambique and three each from India and Australia. Evolutionary history was assessed using Maximum Parsimony (MP) and Neighbour Joining (NJ) analyses. In the phylogenetic tree for region 28S, all sequences, irrespective of the country from where they were sampled, gathered and formed one group. In the phylogenetic trees for regions COI, COII, cytb and EF-1 ALPHA, a similar pattern was observed in the way that the sequences assembled into different groups; i.e. some sequences of *B. subsecivella* from Australia (i.e. *A. simplexella*) were grouped separately from the others, but some Australian sequences grouped with those of the *B. subsecivella* from South Africa, India and Mozambique. Genetic pairwise distances between the experimental sequences ranged from 0.97 to 3.60% (COI), 0.19% to 2.32% (COII), 0.25 to 9.77% (cytb) and 0.48 to 6.99% (EF-1 ALPHA). Phylogenetic analysis results of the current study indicate that *B. subsecivella* populations in Africa, India and Australia are genetically related and presumably constitute a single species, with the Australian population showing the greatest genetic diversity.

***Key words**:* groundnut leaf miner, mitochondrial DNA, nuclear DNA, soya bean moth

4.1 Introduction

The lepidopteran family Gelechiidae comprises *ca.* 4700 described species in about 500 genera (see Karsholt *et al.* 2013). The family is distributed worldwide and is amongst the most diverse lepidopteran taxa in many regions and habitats (Karsholt *et al.* 2013). Gelechiidae are small to medium-sized, often grey or brown moths whose larvae exhibit a wide range of feeding tactics which include leaf mining, gall induction and stem boring (Karsholt *et al.* 2013). Some Gelechiidae are species of agricultural importance. In Chapter 3, African groundnut leaf miner (GLM), Australian *Aproaerema simplexella* (Walker) and Indian *A. modicella* (Deventer) were tentatively synonymized as *Bilobata subsecivella* (Zeller) based on the analysis of the mtDNA COI gene. However, *B. subsecivella* populations in Africa and India attack groundnut and soya bean whereas the *B. subsecivella* population in Australia attacks soya bean. The existence of these different host utilization patterns has raised some uncertainty regarding the identity and the origin of *B. subsecivella* in Africa (see Chapter 3). Hence, there is a need for further DNA analyses of these 'species'.

Previously, insect identification was based only on morphological and taxonomic studies (Mandal *et al.* 2014). Subsequently, difficulties in morphological identification led to the use of molecular techniques in the identification and characterization of different taxa (Mandal *et al.* 2014). According to Scheffer (2000), modern molecular techniques offer complementary, faster and more precise options for species identification. Phylogenetics (the study of evolutionary relationships) can be useful in describing the relationships between insect species (Miller *et al.* 2003; Johnson *et al.* 2009; Utsugi *et al.* 2011). Phylogenetic analyses can apply both molecular and morphological data in order to classify organisms. Molecular methods involve studies of gene sequences, on the basis that similarities between genomes of organisms will help to develop an understanding of the taxonomic relationships amongst the species. In contrast, morphological methods use the phenotype of organisms as the basis of phylogeny. However, these two methods are related since the genome strongly contributes to the phenotype of the organism (Ptaszyńska *et al.* 2011).

According to Avise (2004), mitochondrial DNA (mtDNA) has been one of the most widely used molecular markers for phylogenetic studies in animals. Mitochondrial DNA has many advantages in phylogenetic studies since it involves strict maternal transmission (San *et al.* 2006). Nuclear DNA (nDNA) has a slow rate of evolution; consequently, its use in phylogenetics has often been restricted to intraspecific studies (Buburuzan *et al.* 2007).

Mitochondrial DNA cytochrome oxidase 1 (COI) was found to be the best molecular marker for evolutionary studies (Mandal *et al.* 2014). Cases where mtDNA and nDNA have proven to be efficient in phylogenetic studies in insects were reviewed by Mandal *et al.* (2014). Mitochondrial ND5 and mtDNA CO1 gene regions were successfully used in phylogenetic studies of flies (Diptera) (Mandal *et al.* 2014). Mitochondrial cytochrome *b* (cytb) was used to determine the molecular phylogeny of crickets (Orthoptera: Gryllidae) (Gray *et al.* 2006). Cruickshank (2002) reported that the nuclear ribosomal genes 18S and 28S are an equally powerful tool for phylogenetic analyses at the deepest levels within the Acari. The Intergenic spacer elongation factor-1 alpha (EF-1 alpha) has also been used widely in arthropods; e.g. mites (Acari) (Cruickshank 2002).

The study reported in this chapter was conducted to determine the evolutionary relationships between *B. subsecivella* populations from Africa, India and Australia by comparing the sequences of five different gene regions of their mitochondrial and nuclear DNA (COI, COII, cytb, 28S and EF-1 ALPHA).

4.2 Material and methods

4.2.1 Collection of GLM specimens

Specimen collections and procedures for DNA analyses (DNA extraction, amplification, sequencing and editing of DNA sequences) are as described in Chapter 3. Description of locations and sample identity are presented in Table 4.1.

Table 4.1. Description of locations, sample identities and host plants of *B. subsecivella* populations collected for the study and accession numbers of existing NCBI sequences.

Country	Location	Coordinates	Sample identity	Host plant	NCBI accession number
South Africa	Vaalharts	27°95'7.61" S 24°83'9.91" E	VAL PP A	Pigeon pea	N/A
			VAL PP B	Pigeon pea	N/A
			VAL PP C	Pigeon pea	N/A
			VAL PP D	Pigeon pea	N/A
			VAL S A	Soya bean	N/A
			VAL S B	Soya bean	N/A
			VAL S C	Soya bean	N/A
			VAL S D	Soya bean	N/A
			VAL G A	Groundnut	N/A
			VAL G B	Groundnut	N/A

			VAL G C	Groundnut	N/A
			VAL G D	Groundnut	N/A
	Nelspruit	25°45’4.52” S 30°97’1.54” E	NEL PP A	Pigeon pea	N/A
			NEL PP B	Pigeon pea	N/A
			NEL PP C	Pigeon pea	N/A
			NEL PP D	Pigeon pea	N/A
			NEL S A	Soya bean	N/A
			NEL S B	Soya bean	N/A
			NEL S C	Soya bean	N/A
			NEL S D	Soya bean	N/A
			NEL L A	Lucerne	N/A
			NEL L B	Lucerne	N/A
			NEL L C	Lucerne	N/A
			NEL L D	Lucerne	N/A
			NEL G A	Groundnut	N/A
			NEL G B	Groundnut	N/A
			NEL G C	Groundnut	N/A
			NEL G D	Groundnut	N/A
	Brits	25°59’1.35” S 27°76’8.75” E	BRIT PP A	Pigeon pea	N/A
			BRIT PP B	Pigeon pea	N/A
			BRIT PP C	Pigeon pea	N/A
			BRIT PP D	Pigeon pea	N/A
			BRIT S A	Soya bean	N/A
			BRIT S B	Soya bean	N/A
			BRIT S C	Soya bean	N/A
			BRIT S D	Soya bean	N/A
			BRIT L A	Lucerne	N/A
			BRIT L B	Lucerne	N/A
			BRIT L C	Lucerne	N/A
			BRIT L D	Lucerne	N/A
			BRIT G A	Groundnut	N/A
			BRIT G B	Groundnut	N/A
			BRIT G C	Groundnut	N/A
			BRIT G D	Groundnut	N/A
	Manguzi	26°95’5.32” S 32°82’3.56” E	MAN G A	Groundnut	N/A
			MAN G B	Groundnut	N/A
			MAN G C	Groundnut	N/A
			MAN G D	Groundnut	N/A
			MAN PP A	Pigeon pea	N/A
			MAN PP B	Pigeon pea	N/A
			MAN PP C	Pigeon pea	N/A
			MAN PP D	Pigeon pea	N/A
			MAN S A	Soya bean	N/A
			MAN S B	Soya bean	N/A
			MAN S C	Soya bean	N/A
			MAN S D	Soya bean	N/A
India	Hyderabad-ICRISAT	17°21’57” N 78°28’33” E	SAT 1	Groundnut	N/A
			SAT 2	Groundnut	N/A
			SAT 3	Groundnut	N/A
Australia	Brisbane	27°32’08” S 152°51’35” E	GLM 1	Wild soya bean	N/A
			GLM 2	Wild soya bean	N/A
			GLM 3	Wild soya bean	N/A
Mozambique	Pambara EP1	21°94’33” S 35°10’06” E	MOZA A	Groundnut	N/A
			MOZA B	Groundnut	N/A

	Pambara Produtor	21°99'84" S 35°15'13" E	MOZA C	Groundnut	N/A
			MOZA D	Groundnut	N/A
Australia	Queensland	18° 21' 0" S 138° 1' 48" E	*A. simplexella*	?	KF394619
Australia	Queensland	35° 14' 24" S 149° 13' 47" E	*A. simplexella*	?	KF390882
Australia	New South Wales	21° 2' 13" S 149° 9' 28" E	*A. simplexella*	?	KF388723
Australia	Queensland	35° 27' 0" S 149° 33' 36" E	*A. simplexella*	?	KF389952
Australia	New South Wales	26° 32' 23" S 151° 50' 20" E	*A. simplexella*	?	KF391769
Australia	Queensland	26° 32' 23" S 151° 50' 20" E	*A. isoscelixantha*	?	KF388320
Australia	Queensland	?	*A. isoscelixantha*	?	KF392065
Finland	?	41° 31' 47" N 70° 39' 16" W	*A. anthyllidella*	?	JX984182
USA	Massachusetts	55° 27' 54" N 127° 48' 21" W	Gelechiidae	?	HQ964474
Canada	?	35° 14' 24" S 149° 13' 47" E	*Anacampsis innocuella*	?	HM866839
Australia	New South Wales	?	*Ardozyga acroleuca*	?	JN270831
?	?	?	*Xylophanes porcus*	?	AN749417
Tajikistan	?	?	*Hyles hippophaes*	?	FN386566
China	?	?	*Clossiana gong*	?	JQ924425
China	Xiaolongmen, Beijing	?	*Parnassius bremeri*	?	HM243588
China	?	?	*Trichoplusia ni*	?	EU771090
USA	?		*Hemileucua* sp. L6	?	AF423922

N/A- not applicable
?- information not available

4.2.2 Primers used to sequence DNA regions of *B. subsecivella* specimens in the current study

Primers used to sequence all gene regions (COI, COII, cytb, 28S and EF-1 ALPHA) were designed by Inqaba Biotech Industries, South Africa and are presented in Table 4.2. The sequencing process included both forward and reverse primer sequences for all gene regions.

Table 4.2. A list of primers used to sequence five DNA gene regions (COI, COII, cytb, 28S and EF-1 ALPHA) of *B. subsecivella* populations collected from South Africa, Mozambique, India and Australia.

Region	Gene location		Primer Sequence
Mitochondrial	COI	Forward	GRTCHCCWCCTCCTCYHGGRTC
		Reverse	GATTTTGATCAGGWATAC
Mitochondrial	COII	Forward	GGCTACTTGATCAAATCTTA
		Reverse	CCGGGTTAGCATCAACTTTT
Mitochondrial	Cytb	Forward	GCGTCTACCTACACATTGG
		Reverse	CGAGCTCCGATTCATGTTA
Nuclear	28S	Forward	ATCGCTACGGTCCTCCA
		Reverse	GCATGTGTGCGAGTCATT
Nuclear	EF-1 ALPHA	Forward	ACGAGACGACGATGAAGAAGGA
		Reverse	AACGTGTCTGCAACTGAGC

4.2.3 Sequence analyses and phylogenetic reconstruction

Phylogenetic analyses were conducted using the Maximum Parsimony (MP) and Neighbour Joining (NJ) methods. All of the datasets of the five DNA gene regions that were sequenced were also analysed using the Basic Local Alignment Search Tool (BLAST) to find the closest matches for inclusion in the phylogenetic trees as out groups and for purposes of information. However, phylogenetic trees constructed for all gene regions did not include all sequences of the specimens analysed because some of the sequences were bad (not usable) and some were missing. Maximum parsimony is not based on a model of evolution. JModeltest was used to find the correct nucleotide substitution model to use in neighbour joining analyses (a phenetic method) (Posada & Crandall 1998). The models used, and the parameters of the datasets are presented in Table 4.3. Genetic pairwise distances were calculated using PAUP* 4.0b10 (Swofford 2003).

4.2.1.1 Maximum Parsimony analyses

Maximum Parsimony analyses of the total data matrix (i.e. data from all five genes) were conducted using PAUP* 4.0b10 (Swofford 2003). Starting trees were obtained via stepwise addition. The addition sequence was random, with 10 replicates and one tree held at each step during the stepwise addition. The tree-bisection-reconnection (TBR) branch-swapping

algorithm was used. Nodal support was estimated by bootstrap re-sampling analysis (1000 iterations).

4.2.1.2 Neighbour Joining analyses

Neighbour Joining analyses were carried out in PAUP* 4.0b10 (Swofford 2003). Nucleotide substitution models were determined using JModeltest 3.7 (Posada & Crandall 1998) (Table 4.3). Nodal support was estimated using bootstrap re-sampling analysis (1000 iterations).

Table 4.3. A summary of nucleotide substitution models used in neighbour joining analyses of the five DNA gene regions (COI, COII, cytb, 28S and EF-1 ALPHA) sequenced from *B. subsecivella* populations collected from South Africa, Mozambique, India and Australia.

DNA region	Model	Alignment length	Parsimony informative characters
28S	GTR+I*	180	5
COI	GTR+I	308	44
COII	HKY+I**	431	30
cytb	GTR+I	399	52
EF-1 ALPHA	HKY+I	209	13

*GTR+1- General Time Reversible substitution model

**HKY+1- Hasegawa, Kishino and Yano substitution model

4.3 Results

4.3.1 Mitochondrial DNA COI gene

4.3.1.1 Phylogenetic tree

The phylogenetic tree (Figure 4.1) is based on 308 nucleotides of the mtDNA COI gene and eight sequences of *Aproaerema* species (*A. simplexella, A. isoscelixantha* (Lower) and *A. anthyllidella* (Hübner)), one species of Gelechiidae and two out-groups (*Anacampsis innocuella* (Zeller) (Lepidoptera: Gelechiidae) and *Ardozyga acroleuca* (Meyrick) (Lepidoptera: Gelechiidae)) downloaded from the National Center for Biotechnology Information (NCBI) gene bank. The phylogenetic tree shows an arrangement of sequences into different groups. Sequences of *B. subsecivella* samples from South Africa (41), all three from India (SAT 1, SAT 2 and SAT 3), two from Mozambique (MOZA G C and MOZA G B) and one from Australia (KF394619) (obtained from NCBI gene bank) are grouped

together. This is the largest group, comprising 75% of the total sequences with 84-96% bootstrap support. One *B. subsecivella* sequence from Vaalharts (VAL PP B) and one from Mozambique (MOZA G D) are grouped together separately. It should be noted that one sequence belonging to *B. subsecivella* sampled personally from Australia (GLM 1) and two additional Australian *B. subsecivella* sequences (KF388723 and KF389952), downloaded from NCBI gene bank, grouped together and separately from the other Australian *B. subsecivella* sequences. Furthermore, the two other sequences of *B. subsecivella* collected personally from Australia (GLM 2 and GLM 3) are grouped together, separate from the former group. Two additional Australian *B. subsecivella* sequences (KF388320 and KF 392065) downloaded from NCBI formed a third group, separately from all the other sequences.

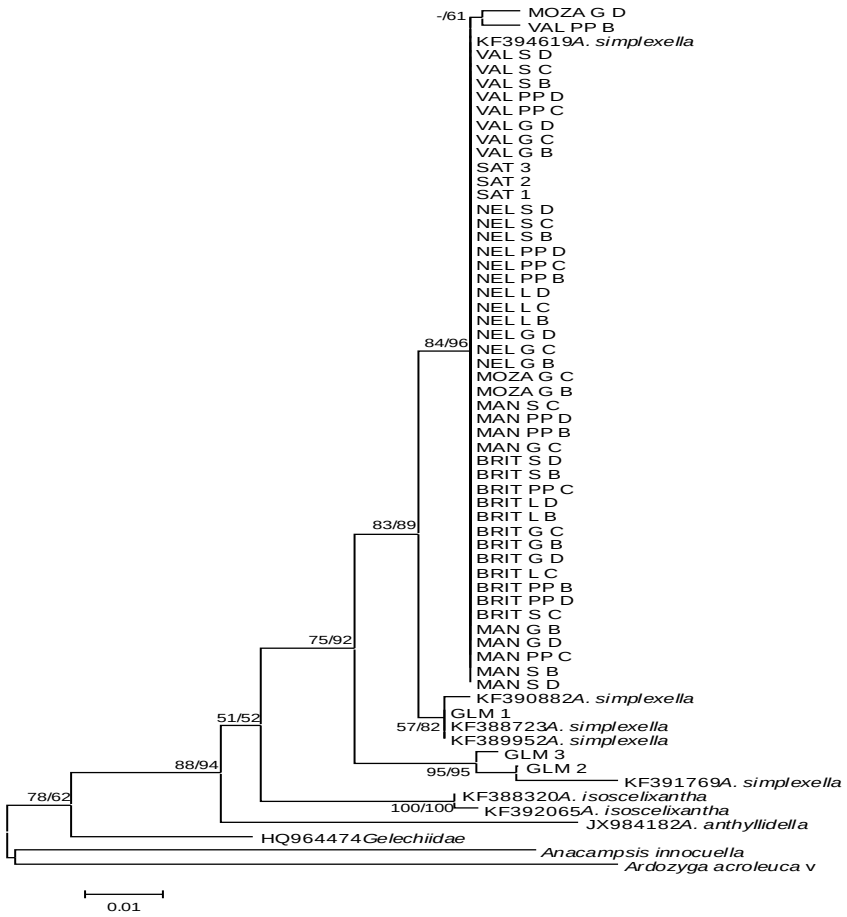


Figure 4.1. A phylogenetic tree showing the relationship between the *B. subsecivella* populations from Africa, India, Australia and NCBI sequences based on the mtDNA COI gene. Geographic origins and descriptions of the samples are presented in Table 4.1. The tree is based on congruent neighbour joining and maximum parsimony analyses; node support is indicated as [nj bootstrap %/ mp bootstrap %]. The names on taxon positions reflect the sampling areas, host plants as well as replications (Vaal, Brits, Nel, Man, Moza, SAT,

GLM; denote Vaalharts, Brits, Nelspruit, Manguzi, Mozambique, India and Australia, respectively) and whether the South African specimens were collected from groundnut (G), soya bean (S), lucerne (L) or pigeon pea (PP). For the South African and Mozambican specimens, A, B, C, D indicates replications whereas for India and Australia, replications are indicated by 1, 2 and 3. The tree also included eight sequences of other *Aproaerema* species (*A. simplexella, A. isoscelixantha* and *A. anthyllidella*), one other Gelechiidae and two out-groups (*Anacampsis innocuella* and *Ardozyga acroleuca*) downloaded from the NCBI gene bank. The numbers next to the species name indicate the gene bank accession number. *A. simplexella* refers to the *B. subsecivella* population from Australia.

4.3.1.2 Genetic pairwise distances

The different groups in the COI genetic pairwise distance analysis correspond with the different groups on the COI phylogenetic tree (Figure 4.1). Group one in Table 6.4 consists of all sequences from the samples collected in South Africa, India and Mozambique. The sequences in group one comprise the main clade in the COI phylogenetic tree (Figure 4.1). Genetic pairwise distances between the experimental sequences ranged from 0.97 to 3.60%. Experimental sequence GLM 1 is identical to two sequences of *B. subsecivella* from Australia, KF388723 and KF389952, and is not separated from them at all (Table 4.4).

Table 4.4. Genetic pairwise distances (%) in the mtDNA COI gene within *B. subsecivella* populations collected from South Africa, Mozambique, India and Australia (1-4) and NCBI samples (5-10). *A. simplexella* refers to the *B. subsecivella* population from Australia.

		1	2	3	4	5	6	7	8	9
1	Group 1									
2	GLM1	1.00								
3	GLM2	3.60	3.25							
4	GLM3	3.27	3.57	0.97						
5	KF388723 *A. simplexella*	1.00	0.00	3.25	3.57					
6	KF394619 *A. simplexella*	0.03	0.97	3.57	3.25	0.97				
7	KF389952 *A. simplexella*	1.00	0.00	3.25	3.57	0.00	0.97			
8	KF390882 *A. simplexella*	1.33	0.32	3.57	3.90	0.32	1.30	0.32		
9	KF391769 *A. simplexella*	4.90	4.55	1.30	1.95	4.55	4.87	4.55	4.87	
10	KF388320 *A. simplexella*	5.22	4.22	6.17	5.84	4.22	5.19	4.22	4.55	7.14

4.3.2 Mitochondrial COII gene

4.3.2.1 Phylogenetic tree

The phylogenetic tree (Figure 4.2) is based on 431 nucleotides of the mtDNA COII gene from specimens of *B. subsecivella* collected for the study, and two out-groups (*Xylophanes porcus* (Hübner) (Lepidoptera: Sphingidae) and *Hyles hippophaes* (Esper) (Lepidoptera: Sphingidae)) downloaded from the NCBI gene bank. No other *B. subsecivella* sequences were downloaded from NCBI for this analysis. The phylogenetic tree displays five groups. The first group includes six sequences from South Africa while the second group includes another three different sequences from South Africa. The first and the second groups have weak bootstrap support of only 60-65%. The third group includes a further 43 sequences from South Africa, two from Mozambique (MOZA C and MOZA D) and India (SAT 2 and SAT 3) and comprises the largest group consisting of 78% of the total sequences with strong bootstrap support of 93-94%. The fourth group has one sequence from India (SAT 1) and the fifth group has one sequence from Australia (GLM 1).

4.3.2.2 Genetic pairwise distances

The different groups in the COII genetic pairwise distance analysis (Table 4.5) correspond with the different groups on the COII phylogenetic tree (Figure 4.2). Group one consists of six sequences from South Africa, group two consists of another three different sequences from South Africa, group three consists of the remaining 43 sequences from South Africa with two each from India (SAT 2 and SAT 3) and Mozambique (MOZA C and MOZA D), group four consists of one sequence from India (SAT 1) and group five consists of one sequence from Australia (GLM 1). Genetic distances between the groups ranged from 0.19% to 2.32% (Table 4.5).

Table 4.5. Genetic pairwise distances (%) in the mtDNA COII gene within *B. subsecivella* populations collected from South Africa, Mozambique, India and Australia.

	1	2	3	4
1 Group 1				
2 Group 2	0.27			
3 Group 3	0.19	0.46		
4 Group 4	0.66	0.93	0.47	
5 Group 5	2.05	2.32	1.86	2.32

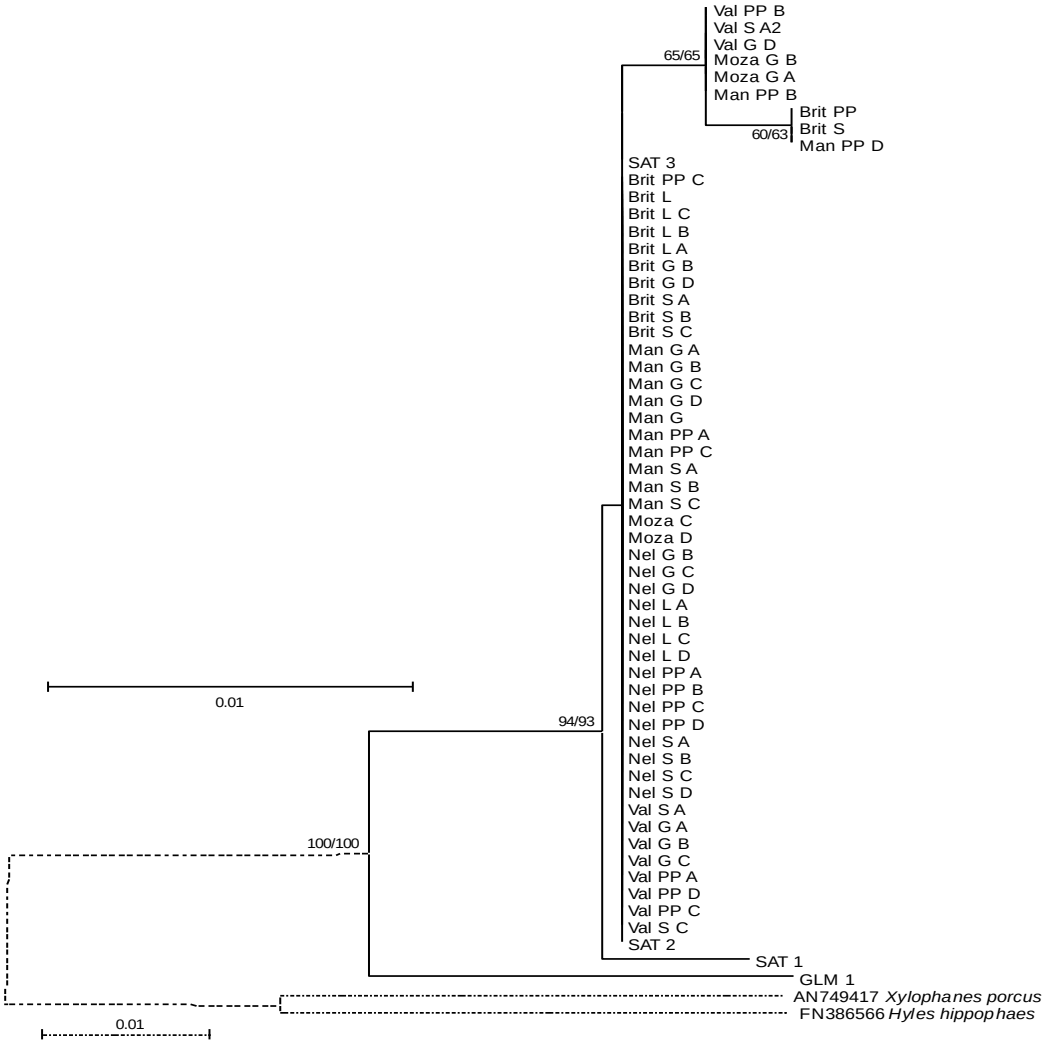


Figure 4.2. A phylogenetic tree showing the relationship between mtDNA COII gene sequences of *B. subsecivella* populations from Africa, India and Australia. Geographic origins and descriptions of the samples are presented in Table 4.1. The tree is based on congruent neighbour joining and maximum parsimony analyses; node support is indicated as [nj bootstrap %/ mp bootstrap %]. The names on taxon positions reflect the sampling areas, host plants as well as replications; full descriptions are shown in Table 4.1 (Vaal, Brit, Nel, Man, Moza, SAT, GLM; denote Vaalharts, Brits, Nelspruit, Manguzi, Mozambique, India and Australia, respectively) and whether the South African specimens were collected from groundnut (G), soya bean (S), lucerne (L) or pigeon pea (PP)). For the South African and Mozambican specimens, A, B, C, D indicate replications whereas for India and Australia, replications are indicated by 1, 2 and 3.The tree also included two out-groups (*Xylophanes porcus* and *Hyles hippophaes*) downloaded from the NCBI gene bank and the numbers next to the species name indicate the gene bank accession number.

4.3.3 Mitochondrial cytb gene

4.3.3.1 Phylogenetic tree

The phylogenetic tree (Figure 4.3) is based on 399 nucleotides of the mtDNA cytb gene and two out-groups (*Clossiana gong* (Oberthür) (Lepidoptera: Nymphalidae) and *Parnassius bremeri* (Latreille) (Lepidoptera: Papilionidae) downloaded from the NCBI gene bank. The phylogenetic tree shows three groups. The first group includes 31 sequences from South Africa, all three sequences from India (SAT 1, SAT 2 and SAT 3) and two from Mozambique (MOZA G B and MOZA G C). This is the largest group comprising 87% of the total sequences with very strong bootstrap support of 99-100%. The second group includes one specimen's sequence from Australia (GLM 1). The third group includes the sequences of the two remaining specimens collected from Australia (GLM 2 and GLM 3).

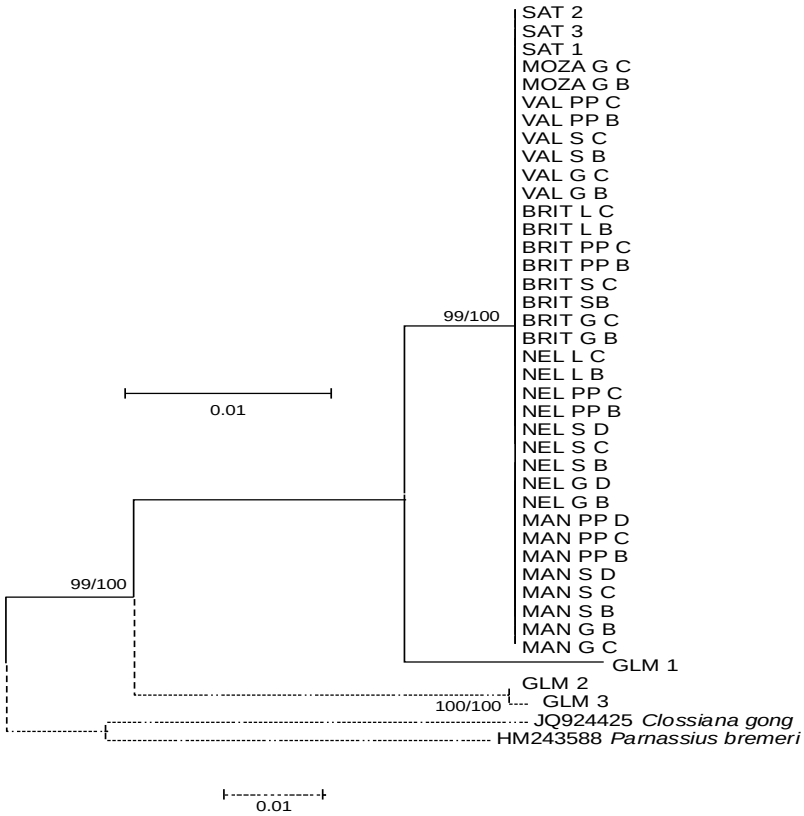


Figure 4.3. A phylogenetic tree showing the relationship between *B. subsecivella* populations from Africa, India and Australia based on the mtDNA cytb gene sequences. Geographic origins and descriptions of the samples are presented in Table 4.1. The tree is based on congruent neighbour joining and maximum parsimony analyses; node support is indicated as [nj bootstrap %/ mp bootstrap %]. The names on taxon positions reflect the sampling areas, host plants as well as replications; full descriptions are shown in Table 4.1 (Vaal, Brit, Nel, Man, Moza, SAT, GLM; denote Vaalharts, Brits, Nelspruit, Manguzi, Mozambique, India and Australia, respectively) and whether the South African specimens were collected from groundnut (G), soya bean (S),

lucerne (L) or pigeon pea (PP). For the South African and Mozambican specimens, A, B, C, D indicate replications, whereas for India and Australia, replications are indicated by 1, 2 and 3. The tree also included two out-groups (*Clossiana gong* and *Parnassius bremeri*) downloaded from the NCBI gene bank and the numbers next to the species name indicate the gene bank accession number.

4.3.3.2 Genetic pairwise distances

The different groups in the cytb genetic pairwise distance analysis (Table 4.6) correspond with the different groups on the cytb phylogenetic tree (Figure 4.3). Group one consists of all sequences from South Africa, India and Mozambique. Group two to four consists of one *B. subsecivella* sequence from Australia in each group. Genetic distances between the groups ranged from 0.25% to 9.77% (Table 4.6).

Table 4.6. Genetic pairwise distances (%) in the mtDNA cytb gene within *B. subsecivella* populations collected from South Africa, Mozambique, India and Australia.

		1	2	3
1	Group 1			
2	GLM1	1.50		
3	GLM2	8.77	9.52	
4	GLM3	9.02	9.77	0.25

4.3.4 Nuclear ribosomal 28S gene

4.3.4.1 Phylogenetic tree

The phylogenetic tree (Figure 4.4) is based on 180 nucleotides of the 28S rDNA gene and two out-groups (*Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and *Hemileucua* sp. L6 (Walker) (Lepidoptera: Saturniidae) downloaded from the NCBI gene bank. The phylogenetic tree shows that all sequences of the samples from South Africa (41) and three each from Australia (GLM 1, GLM 2 and GLM 3), India (SAT 1, SAT 2 and SAT 3) and Mozambique (MOZA G B, MOZA B C and MOZA G D) assembled together to form one large group with very strong bootstrap support of 100%. This result also shows that there was no genetic diversity between *B. subsecivella* populations from South Africa, Australia, India and Mozambique, based on the 28S rDNA gene region.

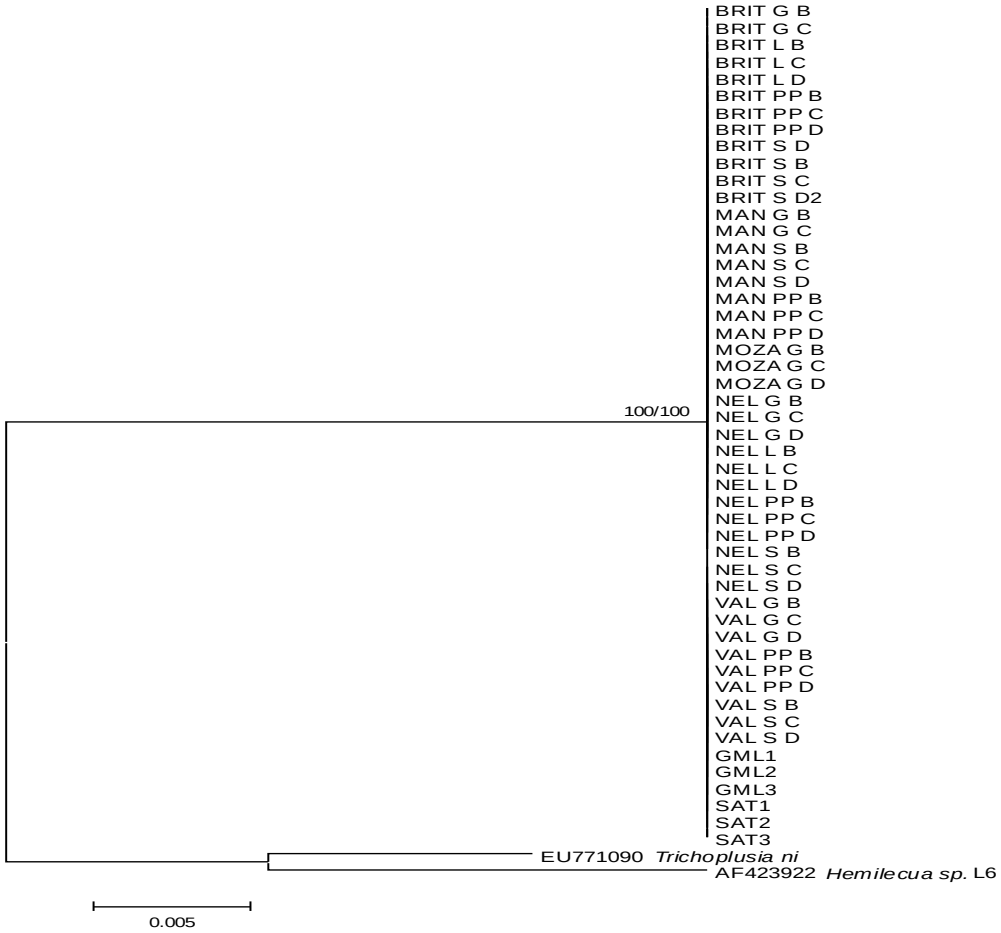


Figure 4.4. A phylogenetic tree showing the relationship between *B. subsecivella* populations from Africa, India and Australia and NCBI sequences based on the rDNA 28S gene. Geographic origins and descriptions of the samples are presented in Table 4.1. The tree is based on congruent neighbour joining and maximum parsimony analyses; node support is indicated as [nj bootstrap %/ mp bootstrap %]. The names on taxon positions reflect the sampling areas, host plants as well as replications; full descriptions are shown in Table 6.1 (Vaal, Brit, Nel, Man, Moza, SAT, GLM; denote Vaalharts, Brits, Nelspruit, Manguzi, Mozambique, India and Australia, respectively) and whether the South African specimens were collected from groundnut (G), soya bean (S), lucerne (L) or pigeon pea (PP)). For the South African and Mozambican specimens, A, B, C, D indicate replications whereas for India and Australia, replications are indicated by 1, 2 and 3. The tree also included two out-groups (*Trichoplusia ni* and *Hemilecua* sp. L6) downloaded from the NCBI gene bank and the numbers next to the species name indicate the gene bank accession number.

4.3.4.2 Genetic pairwise distances

Genetic pairwise distances for 28S rDNA were not calculated since the phylogenetic tree constructed for this DNA gene region did not display distinct groups of the sequences of *B. subsecivella* populations. The tree comprises only one group comprising all sequences of the specimens of *B. subsecivella* collected from South Africa, Mozambique, India and Australia.

4.3.5 Intergenic spacer elongation factor-1 alpha (EF-1 ALPHA) gene

4.3.5.1 Phylogenetic tree

The phylogenetic tree is based on 209 nucleotides of the EF-1 ALPHA region (Figure 4.5). The search for out-groups from the gene bank yielded no matches, so it was not possible to include out-groups in the tree. The phylogenetic tree shows three groups. The first group includes 37 sequences from South Africa, all three sequences from India (SAT 1, SAT 2 and SAT 3) and three from Mozambique (MOZA G B, MOZA G C and MOZA G D). This is the largest group comprising 93% of the total sequences with 63-64% bootstrap support. The second group includes one sequence from Australia (GLM 1), while the third group includes the remaining two sequences from Australia (GLM 2 and GLM 3).

4.3.5.2 Genetic pairwise distances

The different groups in the EF-1 ALPHA genetic pairwise distance analysis correspond with the different groups on the EF-1 ALPHA phylogenetic tree (Figure 4.5). Group one consists of all sequences from South Africa, India and Mozambique. Group two to four consists of one *B. subsecivella* sequence from Australia in each group. Genetic distances between the groups ranged from 0.00% to 6.99% (Table 4.7).

Table 4.7. Genetic pairwise distances (%) in the EF-1 ALPHA gene within *B. subsecivella* populations collected from South Africa, Mozambique, India and Australia.

		1	2	3
1	Group 1			
2	GLM1	0.48		
3	GLM2	6.99	6.45	
4	GLM3	6.99	6.45	0.00

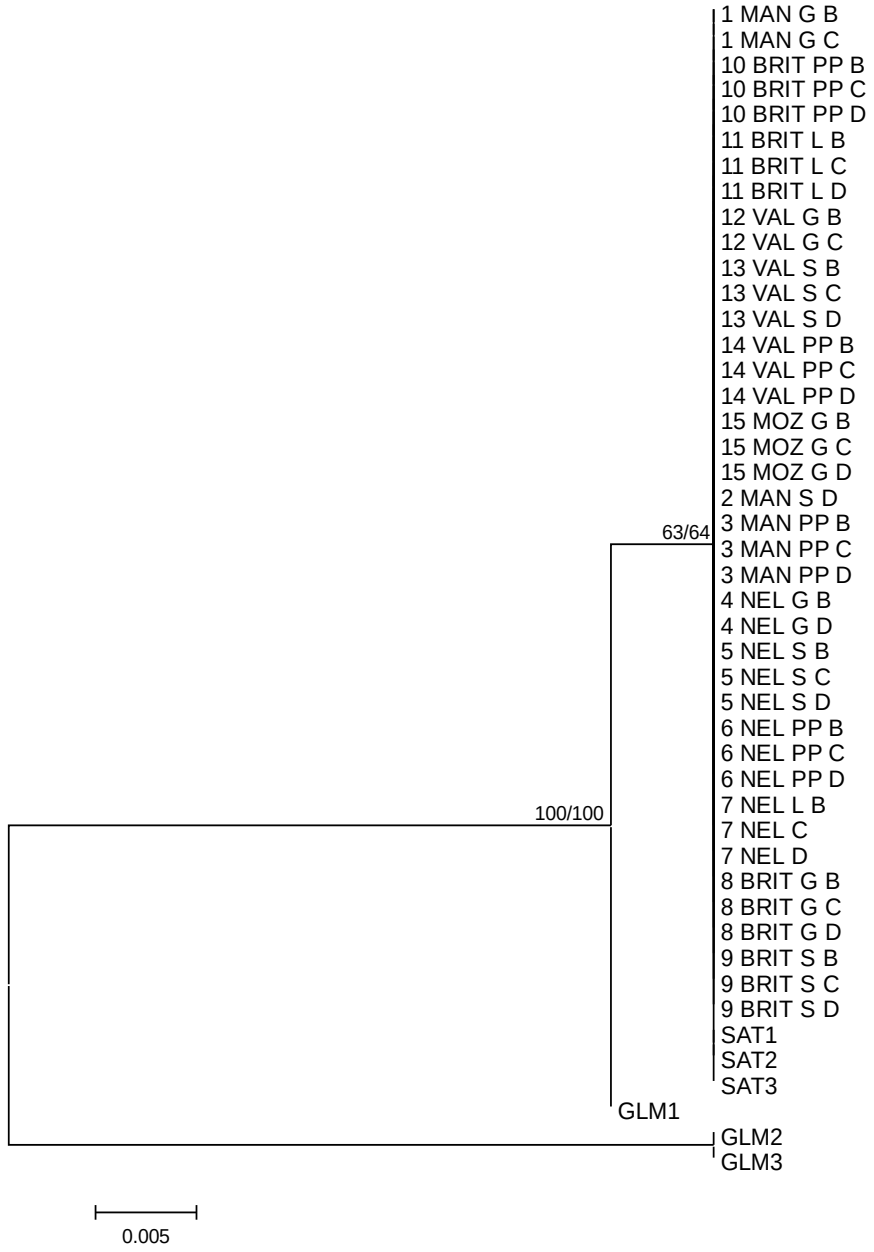


Figure 4.5. A phylogenetic tree showing the relationship between *B. subsecivella* populations from Africa, India, Australia and NCBI sequences based on the nDNA EF-1 ALPHA gene. Geographic origins and descriptions of the samples are presented in Table 4.1. The tree is based on congruent neighbour joining and maximum parsimony analyses; node support is indicated as [nj bootstrap %/ mp bootstrap %]. The names on taxon positions reflect the sampling areas, host plants as well as replications; full descriptions are shown in Table 4.1 (Vaal, Brit, Nel, Man, Moza, SAT, GLM; denote Vaalharts, Brits, Nelspruit, Manguzi, Mozambique, India and Australia, respectively) and whether the South African specimens were collected from groundnut (G), soya bean (S), lucerne (L) or pigeon pea (PP)). For the specimens from South Africa and Mozambique, A, B, C, D indicate replications whereas for India and Australia, replications are indicated by 1, 2 and 3. Numbers in front of the names of specimens are laboratory identification codes.

4.4 Discussion

The presence of three *B. subsecivella* populations feeding on different hosts in Asia (i.e. *A. modicella*), Australia (i.e. *A. simplexella*) and Africa (Bailey 2007; Buthelezi *et al.* 2012, 2013) has raised uncertainty regarding the identity and the origin of *B. subsecivella* in Africa. There are also more recent findings, linking *B. subsecivella* populations in Australia, Africa and India (Buthelezi *et al.* 2012, 2013). Firstly, adults of *B. subsecivella* in Australia and Africa responded to the species specific sex pheromone lure developed for the *B. subsecivella* population in India (see Chapter 3). Secondly, *B. subsecivella* populations in Africa, India and Australia share some plant hosts (Buthelezi *et al.* 2013). Thirdly, *B. subsecivella* symptoms of damage found on the groundnut leaves in South Africa mirrored those of the *B. subsecivella* described on groundnut in Mozambique and India as well as those of *B. subsecivella* described on soya bean in Australia (Kenis & Cugala 2006; Bailey 2007; Buthelezi *et al.* 2012). Finally, mtDNA COI gene analysis carried out on the specimens of *B. subsecivella* collected from South Africa, India, Mozambique and Australia gave similar percentage matches which varied between 98-100% and 92-100% with the Australian *B. subsecivella* sequences in the BOLD and NCBI gene banks, respectively (see Chapter 3). In this context, we examined the genetic and evolutionary relationships of *B. subsecivella* populations in Australia, India and Africa by sequencing five gene regions of nuclear and mitochondrial DNA (COI, COII, cytb, 28S and EF I). It was anticipated that this study will provide more evidence to support the tentative synonymization of these three populations from three continents which was proposed in Chapter 3. DNA analysis methods, especially those including mtDNA, are believed to be capable of identifying species which have similar characteristics (Scheffer 2000). Furthermore, literature searches revealed that molecular analyses have been successfully used in identifying Gelechiidae species. For example, Adamski *et al.* (2014) used morphological together with mtDNA COI gene analyses to identify three new species of leaf-mining Gelechiidae: *Xenolechia ceanothiae* (Priest), *Gnorimoschema shepherdiae* (Priest), and *Scrobipalpula manierreorum* (Priest). Karsholt *et al.* (2013) used DNA sequence data which included mtDNA COI and nDNA EF-1 ALPHA genes to re-examine the higher level phylogeny and evolutionary affinities of the Gelechiidae.

In all phylogenetic trees constructed from five DNA gene regions analysed in the current study, sequences of the specimens of *B. subsecivella* from Australia, South Africa,

Mozambique and India displayed different grouping patterns which indicate genetic variation amongst these populations. Genetic variation in insect species from different geographic areas has been reported in the literature and is believed to be associated with specialization on different host plant species (Mitter *et al.* 1988; Farrell 1998) and isolation due to geographic barriers (Arctander *et al.* 1999; Alpers *et al.* 2004). However, the findings from the current study indicated the absence of any host-plant associated genetic relationship between *B. subsecivella* populations sampled from groundnut, soya bean, lucerne and pigeon pea in four locations in South Africa, as there were no groupings of sequences according to host plants recorded across all the gene regions analysed. However, populations could be separated on an isolation basis, as they were linked to different geographic regions. This finding is similar to that of Assefa (2006) on *Eldana saccharina* Walker (Lepidoptera: Pyralidae). According to Assefa (2006), *E. saccharina* populations attacking different hosts within geographically similar areas lacked genetic differentiation, but those from different geographic areas were genetically different.

In the phylogenetic tree constructed from the 28S gene region (Figure 4.4), all sequences of *B. subsecivella* from all four countries (South Africa, Mozambique, India and Australia) assembled together to form one group. This result suggests a lack of genetic diversity between *B. subsecivella* populations from Australia, Africa and India based on this rDNA gene region. The nuclear ribosomal gene 28S is recognised as a powerful tool for phylogenetic analyses at the deepest levels within the Acari (Cruickshank 2002). However, phylogenetic trees constructed from COII, cytb and EF-1 ALPHA displayed different grouping patterns among the sequences of *B. subsecivella* populations collected from different countries (Figures 4.2, 4.3 and 4.5). In the phylogenetic trees for these three DNA gene regions, sequences of *B. subsecivella* populations sampled from South Africa, Mozambique and India assembled together while the sequences of *B. subsecivella* personally sampled from Australia were not assembled in the groups which included sequences of the *B. subsecivella* populations from the other three countries. These observations indicate that *B. subsecivella* populations from South Africa, Mozambique and India are from the same origin.

The phylogenetic tree constructed from the COI gene, which included *B. subsecivella* sequences from Australia downloaded from the NCBI gene bank, showed different grouping patterns between the sequences of *B. subsecivella* from Australia and Africa compared to the other four DNA gene regions (Figure 4.1). In this gene region, one Australian sequence of *B. subsecivella* downloaded from the NCBI gene bank (KF394619) was grouped with other

sequences from South Africa, India and Mozambique. This result shows that there are *B. subsecivella* populations in Australia that are similar to the *B. subsecivella* populations in southern Africa and India. Moreover, two sequences of *B. subsecivella* (GLM 1 and GLM 2) personally sampled from Australia were grouped with other *B. subsecivella* sequences from Australia that were downloaded from the NCBI gene bank (Figure 4.1). Genetic pairwise distances showed that the sequence of *B. subsecivella* (GLM 2) personally sampled from Australia was identical to *B. subsecivella* sequences KF389952 and KF388723 (downloaded from the NCBI gene bank) and is not separated from them at all, as the genetic pairwise distances between these sequences is 0.00% (Table 4.4). Based on these results, it is clear that there is considerable genetic diversity within the *B. subsecivella* populations in Australia, and that some of these Australian populations are very similar genetically to the Indian and African *B. subsecivella* populations. This is further supported by the findings in Chapter 3 which showed that the similarity percentage matches between *B. subsecivella* specimens (collected from South Africa, India, Mozambique and Australia) and *B. subsecivella* sequences in the BOLD and NCBI gene banks matched closely (between 98-100% and 92-100%, respectively).

Origin of the South African GLM

In all five DNA gene regions analysed, sequences of the *B. subsecivella* samples from South Africa, Mozambique and India grouped together and showed a lack of genetic diversity between them, indicating that they are from the same origin. However, in some gene regions (COI and 28S), some sequences of *B. subsecivella* from Australia indicated that they were very closely related to the other *B. subsecivella* populations from Africa and India. It is therefore difficult at present to determine the origin of the South African *B. subsecivella* population, i.e. from India or Australia. Furthermore, the Indian and Australian specimens that were sequenced in the current study were very few (only three from each country) and were only collected from a single site in each country. However, the mtDNA COI analysis, which included sequences of *B. subsecivella* downloaded from the NCBI gene bank, indicated genetic diversity within *B. subsecivella* populations in Australia which included other populations matching closely with the South African and Indian populations. Therefore, at present, it could be suggested that Australia is the origin of the *B. subsecivella* that invaded Africa and not India.

4.5 Conclusion

Populations of *B. subsecivella* in Australia, Africa and India are genetically very closely related, indicating that they constitute a single species, possibly originating in Australia. Based on these findings, further molecular and morphological analyses are proposed, which include collecting and sequencing more samples from key geographic areas, notably in Australia and India, to confirm these hypotheses.

4.7 References

ADAMSKI, D., LANDRY, J. F., NAZARI, V. & PRIEST, R. J. 2014.Three new species of leaf-mining Gelechiidae (Lepidoptera) from Canada and northeastern United States. *Journal of the Lepidopterists' Society* **68**: 101-123.

ALPERS, D.L., VAN VUUREN, B.J., ARCTANDER, P. & ROBINSON, T.J. 2004. Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. *Molecular Ecology* **13**: 1771-1784.

ARCTANDER, P., JOHANSEN, C. & COUTELLEC-VRETO, M. 1999. Phylogeography of three closely related African bovids (tribe Alcelaphini). *Molecular Biology and Evolution* **16**:1724-1739.

ASSEFA, Y. 2006. Sugarcane stemborers in Ethiopia: Ecology and phylogeography. PhD dissertation.University of KwaZulu-Natal, Pietermaritzburg, South Africa.

AVISE, J.C. 2004. Molecular markers, natural history, and evolution. 2nd edition. Sinauer, Sunderland, USA.

BAILEY, P.T. 2007. Pests of Field Crops and Pastures: Identification and Control. CSIRO Publishing, Collingwood, Victoria 3066, Australia.

BUBURUZAN, T., MAY, G., MELIA, T., MODEKER, J. & WETTERWALD, M. 2007. Integration of broadcast technologies with heterogeneous networks – an IEEE 802. 21 centric approach. In: *Consumer Electronics,* ICCE 2007.

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2012.The groundnut leaf miner collected from South Africa is identified by mtDNA CO1 gene analysis as the Australian soybean moth (*Aproaerema simplixella* PS 1) (Walker) (Lepidoptera: Gelechiidae). *African Journalof Agricultural Research* **7**(38): 5285–5292.

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2013. A comparison of the infestation of *Aproaerema simplexella* on groundnut and other known hosts for *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *African Entomology* **21**(2): 183-195.

CRUICKSHANK, R. H.2002. Molecular markers for the phylogenetics of mites and ticks. *Systematic & Applied Acarology* **7**: 3-14.

DAS, S.B. 1999. Seasonal activity of groundnut leafminer, *Aproaerema modicella* in west Nimer Valley, Madhya Pradesh. *Insect Environment* **5**: 69.

FARRELL, B.D. 1998. "Inordinate fondness" explained: why are there so many beetles? *Science* **281**: 555-559.

GRAY, D.A., BARNFIELD, P., SEIFRIED, M. & RICHARDS, M.H. 2006. Molecular divergence between *Gryllus rubens* and *Gryllus texensis*, sister species of field crickets (Orthoptera: Gryllidae). *Canadian Entomologist* **138**: 305-313.

JOHNSON, G.D., PAXTON, J.R., SUTTON, T.T., SATOH, T. P., SADO, T. & NISHIDA, M. 2009. Deep-sea mystery solved: astonishing larval transformations and extreme sexual dimorphism unite three fish families. *Biology Letters* **5**: 235-239.

KARSHOLT, O., MUTANEN, M., LEE, S. & KAILA, L. 2013. A molecular analysis of the Gelechiidae (Lepidoptera: Gelechioidea) with an interpretative grouping of its taxa. *Systematic Entomology* **38**: 334-348.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa. CAB Reviews: *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**:19.

MANDAL, D.S., CHHAKCHHUAK, L., GURUSUBRAMANIAN, G. & KUMAR, N.S. 2014. Mitochondrial markers for identification and phylogenetic studies in insects – A Review. *DNA Barcodes* **2**: 1–9.

MILLER, K.B., ALARIE, Y., WOLFE, G.W. & WHITING, M. F. 2003. Association of insect life stages using DNA sequences: the larvae of *Philodytes umbrinus* (Motschulsky) (Coleoptera: Dytiscidae). *Systematic Entomology* **30**: 499-509.

MITTER, C., FERRELL, B., WIEGMANN, B. 1988.The phylogenetic study of adaptive zones: Has phytophagy promoted diversification? *American Naturalist* **132**(1): 107-128.

PTASZYŃSKA, A.A., ŁĘTOWSKI, J., GNAT, S. & MAŁEK, W. 2011. Application of COI sequences in studies of phylogenetic relationships among 40 Apionidae species. *Journal of Insect Science* **12**: 16.

POSADA, D. & CRANDALL, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

SAN, M.D., GOWER, D.G., ZARDOYA, R. & WILKINSON, M. 2006. A hot spot of gene order rearrangement by tandem duplication and random loss in the vertebrate mitochondrial genome. *Molecular Biology and Evolution* **23**: 227-234.

SCHEFFER S.J. 2000. Molecular evidence of cryptic species within *Lyriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology* **93**: 1146–1151.

SWOFFORD, D.L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).Version 4. Sinauer Associates, Sunderland, Massachusetts.

UTSUGI, J.I.N.B.O., TOSHIHIDE, K.A.T.O. & MOTOM, I.T.O. 2011. Current progress in DNA barcoding and future implications for entomology. *Entomological Science* **14**: 107-124.

Chapter Five

A comparison of the infestation of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) on groundnut and other known hosts, and the impact of insecticide applications on its populations in groundnut and soya bean

Abstract

The GLM occurring in South Africa has recently been shown by mitochondrial and nuclear DNA analysis to be the same species as the Australian *A. simplexella* and Indian *A. modicella* and the three texta were tentatively synonymized as *B. subsecivella*. Two experiments were conducted during the 2011–2012 growing season at five sites in South Africa. The first experiment examined *B. subsecivella* infestation levels between groundnut, soya bean, lucerne, pigeon pea and lablab bean (common known host crops for *B. subsecivella* in India). The second experiment examined the effect of cypermethrin application on damage by *B. subsecivella* to groundnut and soya bean plants at two sites. Wild host plants were inspected for damage symptoms and the presence of larvae. Amongst the host crops tested, soya bean was highly infested by *B. subsecivella* followed by groundnut, at all sites. The pest was also observed on pigeon pea at all sites, but the infestation was very low. Lucerne had very low larval infestation by *B. subsecivella*. No infestation was observed on lablab bean at any of the sites. Sprays of cypermethrin on groundnut and soya bean reduced infestation in both crops to very low levels. In the unsprayed plots, the high infestation levels significantly reduced crop yields.

Key words: alternative hosts, *B. subsecivella,* cypermethrin, scouting

5.1 Introduction

Although groundnut (*Arachis hypogaea* L.) has been grown on the African continent for decades, the crop has been free from leaf miner pests until recently when a number of major groundnut-producing countries on the continent started to report severe incidences of a groundnut leaf miner (GLM) (Page *et al.* 2000; Subrahmanyam *et al.* 2000; Du Plessis 2002; Munyuli *et al.* 2003; Epieru 2004; Kenis & Cugala 2006). The new pest is a small moth

whose larva mines in between the lower and upper epidermal layers of the green leaf. Initial reports on the incidence of the pest in Africa assumed its identity as *Aproaerema modicella* (Deventer) (Page *et al.* 2000; Subrahmanyam *et al.* 2000; Du Plessis 2002; Munyuli *et al.* 2003; Epieru 2004; Kenis & Cugala 2006), which had presumably invaded Africa from Indo-Asian countries (Du Plessis 2002; Kenis & Cugala 2006) where *A. modicella*, also a small moth, is a serious pest of groundnut and soya bean (*Glycine max* (L.) Merr.) (Shanower *et al.* 1993). However, recent mitochondrial DNA (COI gene) analyses (see Chapter 2 and 3) have determined that the GLM occurring in Africa is the same species as the GLM in Indo-Asia (*A. modicella*) and the soya bean moth *A. simplexella* (Walker) native to Australia (Common 1990; Bailey 2007), and the three 'species' were synonymized under the name *Bilobata subsecivella* (Zeller) (see Chapter 3).

The widespread prevalence of *B. subsecivella* in many of the African countries where groundnut is grown suggests that it has successfully established itself on the continent in areas that differ widely in climate. For the purpose of formulating strategies for managing the pest, there is a need to determine how the pest has spread so widely and successfully in the different agro-ecological regions of the African continent. Generally, the geographical spread of a new insect pest and its survival is dependent on various factors such as permissive climatic conditions and the availability of the appropriate plant hosts (Sujay *et al.* 2010). Overall, climatic conditions set the boundary of habitat range (Panizzi & Niva 1994). However, successful establishment of a new species involves complex interactions with the new environment (Kolar & Lodge 2001), some of which involve the adaptation of the new species to prevailing abiotic conditions (Holt *et al.* 2005; Travis *et al*. 2005; Gomulkiewicz *et al.* 2010), which may demand certain survival tactics at certain times of the year, *e.g.* hibernation or diapause through winter (Panizzi & Niva 1994). Amongst the biotic factors, the pest's host plant range and that of its natural enemies are extremely important in determining the persistence and success of a pest in a given locality (Panizzi 1997). In this respect, knowledge of natural enemies and host range, including wild and crop plants, is important in managing insect pests (Panizzi 1997). In relation to host range, the presence of alternative wild plant hosts enables the pest to survive during periods when the suitable crop is not available (Panizzi 1997), whilst rotations of suitable crops on the same piece of land facilitates a build-up of the pest (Ghewande & Nandagopal 1997). In some cases, wild plant hosts are indispensable for the completion of a pest's life cycle. In northern Paraná, Brazil, nymphs of *Nezara viridula* L. (Hemiptera: Pentatomidae) feed and may complete their life

cycle on several wild hosts such as wild soya bean (*Glycine wightii* L. Merr.) and hairy indigo (*Indigofera hirsuta* L.). Nymphs will feed on these hosts throughout the year, even during the less favourable "winter" season (June –August), and late instars may be found on wild hosts (Panizzi 1997). Other than identifying the host plants that support the successful establishment of the new insect pest, it is also essential to monitor the pests on these hosts to determine their impact on the build-up of pest populations (Jones 1979; Panizzi 1992).

Currently, the host range of the *B. subsecivella* population occurring in Africa is not well established, but some reports on this pest in Africa have assumed the same host range as that of the *B. subsecivella* population occurring in India (Du Plessis 2003). For example, lucerne (*Medicago sativa* L.), a well-known host for *B. subsecivella* in India, is strongly suspected to be a refuge host in the winter months when groundnut is not growing (Du Plessis 2003). However, during a detection survey of *B. subsecivella* in South Africa that included the inspection of lucerne in winter and summer months, Buthelezi *et al.* (2012) failed to find the moth on lucerne in an area that is generally characterized by heavy infestation of the pest on groundnut crops in summer.

The study reported here pursued three objectives. The first one was to examine *B. subsecivella* infestation levels between groundnut, soya bean, lucerne, pigeon pea (*Cajanus cajan* L.) and lablab bean (*Lablab purpureus* L.), the common known host crops for *B. subsecivella* in India (Shanower *et al.* 1993). The second was to augment the host plant list for *B. subsecivella* in South Africa and the third was to determine the efficacy of cypermethrin in controlling the pest on soya bean and groundnut crops.

5.2 Materials and methods

The study was conducted in South Africa and involved (a) an experiment (with two planting dates) that examined *B. subsecivella* infestation levels on host plants at five sites, (b) a survey of wild plant hosts for *B. subsecivella*, (c) an experiment which examined the efficacy of cypermethrin for the control of the pest on soya bean and groundnut and, (d) monitoring of *B. subsecivella* infestation on groundnut at Manguzi, where farmers generally plant groundnut from the end of June to August.

5.2.1 Experiment 1: Infestation levels of *B. subsecivella* on different crops under field conditions in South Africa

Experiment 1 examined the infestation levels of *B. subsecivella* in South Africa on soya bean, groundnut, lablab, pigeon pea and lucerne, five of the known crop hosts for *B. subsecivella* in India, at five widely separated sites. The test sites included the Agricultural Research Council research station at Brits in the North West Province, the Agricultural Research Council research station at Vaalharts Irrigation Scheme in the Northern Cape Province, the Department of Agriculture Lowveld Agricultural Research Unit near Nelspruit in Mpumalanga Province and two sites (Manguzi and Bhekabantu) in KwaZulu-Natal in the northeast coastal part of the province (See climatic characteristics and geographic locations of the sites in Chapter 2, Table 2.1). The sites were chosen for their wide variation in climatic conditions, which varied from a warm coastal subtropical climate at Manguzi to one characterized by hot summers and cold winters at Brits (Buthelezi *et al.* 2012). At Vaalharts, there were two planting dates of the five test crops during the 2011–2012 growing season. The first planting (early season planting) was done in mid-November 2011 and the second planting (late season planting) was done during the second week of January 2012. At Nelspruit, Brits, Manguzi and Bhekabantu there was only one planting, which was done between mid-November 2011 and mid-January 2012 (Table 5.1). The five test crops were planted in a Randomized Complete Block Design (RCBD) with three replicates. Each crop plot had four rows that were 4 m long with an inter-row spacing of 45 cm. The intra-row spacing was 15–20 cm and the planting depth was 5 cm with exception of lucerne, which was dribbled in 2 cm deep furrows.

Table 5.1. Sites, planting dates and scouting dates for Experiment 1.

Site	GPS reading	Planting dates	Scouting dates
Vaalharts	27º 95' 761" S; 24º 83' 991" E	17 November 2011 13 January 2012	26 January 2012, 05 March 2012, 02 April 2012, 02 May 2012
Nelspruit	25º 45' 452" S; 30º 97' 154" E	29 November 2012	27 January 2012, 07 March 2012, 04 April 2012
Manguzi	26º 95' 532" S; 32º 82' 356'"E	19 January 2012	24 February 2012, 05 April 2012, 26 April 2012

Bhekabantu	27 º01'12.38" S; 32º19'18.29"E	19 January 2012	09 February 2012, 23 February 2012, 05 April 2012, 27 April 2012
Brits	25º 59' 135" S; 27º 76' 875" E	28 November 2011	25 January 2012, 06 March 2012, 03 April 2012

5.2.1.1 Land preparation

The experimental land was mouldboard ploughed and then disked to facilitate a fine seedbed. At planting, hand hoes were used to level the soil after the experimental area was marked. Basic fertilizer 2:3:2 (202 g per plot) was applied by broadcasting to the whole plot and turning in by hoes at planting. Soya bean seeds were inoculated with Soygro™ inoculant and planted immediately after inoculation. The groundnut was top-dressed with gypsum (405 g per plot) applied by dusting to plants immediately after irrigation or rainfall at first flower. At Nelspruit and Brits, sprinkler irrigation was used to provide supplementary irrigation, whilst at Vaalharts, flood irrigation was used. At Bhekabantu and Manguzi, the test crops were rain-fed. At all sites, weeding was done manually using hand hoes.

5.2.1.2 Field scouting for B. subsecivella infestation

The level of infestation on the crops was assessed through field scouting. The frequency and dates of scouting varied among the sites (Table 5.1). Scouting for *B. subsecivella* infestation was done five times at Vaalharts, which had two plantings (early and late season plantings) of the test host plants. At the rest of the sites, which had only one planting date, scouting was done three or four times. Roughly, the first scouting took place about two weeks after planting and thereafter was approximately a month apart. In the first scouting, the infestation was very low; hence 30 plants per plot were chosen at random and inspected for infestation by *B. subsecivella*, and the number of infested plants and infested leaves per plant were recorded. In subsequent scouting, 10 plants per plot were chosen at random and inspected for infestation by *B. subsecivella*. For each of the 10 plants selected per plot, the number of infested leaflets and the total number of leaflets were counted, and the proportion of infested leaflets were expressed as a percentage of the total number of leaflets. In pigeon pea, which had rare infestations of *B. subsecivella*, the infested leaflets (about one to three leaflets per plant on one to six plants per plot) were removed at each scouting to enable proper tracking of new infestations. The monitoring of *B. subsecivella* infestation on this perennial plant as well as on lucerne, another perennial plant, was continued until mid-December 2012 after the

dry groundnut and soya bean crops had been removed in May 2012. This was done to determine if the perennial pigeon pea and lucerne served as carryover hosts of *B. subsecivella* during May to September when groundnut and soya bean plantings were absent. In addition to monitoring *B. subsecivella* infestation on the experimental crop at Manguzi, where farmers traditionally plant their groundnut from the end of June to mid-August, infestation of the farmers' groundnut crop was monitored to determine the start of infestation. Infestation levels are, however, not discussed in this Chapter.

5.2.2 Experiment 2: Effect of cypermethrin application on damage by *B. subsecivella* to groundnut and soya bean plants

5.2.2.1 Treatments and crop management

Groundnut and soya bean were planted at Vaalharts and Nelspruit on 13 and 25 January 2012, respectively, in two blocks; each block having three plots of each of the two crops. The crops were planted with an inter-row spacing of 45 cm in four rows per plot that were 4 m long. The intra-row spacings were 15 cm and 20 cm, respectively, for soya bean and groundnut. The planting depth was 5 cm for both crops. Plants in one of the two blocks were sprayed three times with 20 % E.C. cypermethrin at a concentration of 2 ml/l of water, using a knapsack sprayer (20 litres). Chemical applications were conducted four weeks apart. The management of the crops and the scouting for *B. subsecivella* were carried out as described for Experiment 1. The first scouting was conducted on 05 March at Vaalharts and 07 March at Nelspruit. The second scouting was conducted on 02 April at Vaalharts and 03 April at Nelspruit. At both sites, the crops received supplementary irrigation which was provided as flood irrigation at Vaalharts and as overhead irrigation at Nelspruit.

5.2.2.2 Groundnut and soya bean harvesting and processing

At maturity, five plants of soya bean or groundnut per plot were randomly selected and pulled from the soil by hand. The pods were separated from the plant and the number of pods per plant counted. The pods were air-dried and weighed until constant weight was achieved. The total number of pods per plant was counted, then the number of single seeded and double seeded pods were also counted. The pods were shelled, the number of seeds per plant was determined, as was the seed mass per plant. The percentage of ovarian cavities that were occupied by seeds in each pod class was calculated by dividing the total number of cavities by the total number of seeds.

5.2.3 Survey for wild host plants

A survey for wild hosts of *B. subsecivella* was conducted in the proximity of Experiments 1 and 2 during the growing season, as well as in winter. Plants in the proximity of the experiments were inspected for larvae, pupae and the leaf mines characteristic of *B. subsecivella* (see Chapter 2). Plants were declared primary hosts for *B. subsecivella* if actively feeding larvae were present on the plants, in addition to the presence of pupae and signs of feeding by the larvae. They were declared secondary hosts if only pupae were found, with no sign of feeding by the larvae. The plants with larvae or pupae were pressed and submitted for identification by a Botanical Systematist at the University of Zululand, KwaDlangezwa, Empangeni, South Africa.

5.3 Data analysis

Yield data collected from experiment 2 were subjected to one-way Analysis of Variance using the Genstat 12ed software. Means were separated using LSD post-hoc tests, when the *F*-values were significant at $P < 0.05$.

5.4 Results

5.4.1 Experiment 1: Infestation levels by *B. subsecivella* on different crops under field conditions

Vaalharts

At Vaalharts, *B. subsecivella* infestation was observed on groundnut, soya bean and pigeon pea, but not on lucerne and lablab (Table 5.2). On pigeon pea, the infestation occurred only in the late-planted crop (January 2012 planting), whereas it was present on groundnut and soya bean in both the early (November 2011 planting) and late crops. In the early-planted crop, the proportions of infested groundnut (16.7 %) and soya bean (6.7%) plants was low in the first scouting done in January 2012 (60 days after planting) but increased substantially to 73% and 100% in groundnut and soya bean, respectively, in April 2012 (83 days after planting) (Table 5.2). Although the proportion of infested groundnut plants was markedly higher than that of soya bean in scouting during January 2012 and March 2012, all soya bean plants sampled in April 2012 towards the end of the season were infested, compared with 73% infested groundnut plants. In contrast to the early-planted crops, all plants sampled in the late-planted crops at all three scouting times were infested by *B. subsecivella*. However, soya bean had a

consistently higher proportion of damaged leaves per plant than observed for groundnut, as was the case in the second and third scouting of the early-planted crops (Table 5.2). Infestation on pigeon pea was only observed towards the end of the growing season in April 2012 in the late-planted crop. Moreover, the infestation on pigeon pea was very low (observed only on two pigeon pea plants compared with 100% of plants for soya bean and groundnut) (Table 5.2). Furthermore, there was no new *B. subsecivella* infestation on pigeon pea from May to mid-December 2012.

Table 5.2. Infestation of *B. subsecivella* on groundnut, soya bean, pigeon pea, lucerne and lablab at Vaalharts.

Planting date	Scouting date	Crop	% of sampled plants that were infested	Average number of infested leaflets per plant	% of leaflets that were infested
17 November 2011	26 January	Groundnut	16.7	2.2	-
		Soya bean	6.7	1.5	-
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	05 March	Groundnut	86.7	3.53	1.42
		Soya bean	66.7	3.33	6.83
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	02 April	Groundnut	93.33	5.92	8
		Soya bean	80	7.56	8.93
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
13 January 2012	05 March	Groundnut	100	13.86	10.9
		Soya bean	100	5.26	24.0
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	02 April	Groundnut	100	33.66	15.43
		Soya bean	100	23.8	84.06
		Pigeon pea	(2)*	1	-
		Lucerne	0	0	0
		Lablab	0	0	0
	02 May	Groundnut	100	-	100
		Soya bean	100	-	100
		Pigeon pea†	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0

- Not recorded

* Actual number of pigeon plants (2) that were infested in the entire experimental crop.

†No new infestation by *B. subsecivella* occurred on pigeon pea from May 2012 until the termination of infestation monitoring in mid-December 2012.

Nelspruit

As observed at Vaalharts, *B. subsecivella* infested groundnut, soya bean and pigeon pea, but not lucerne and lablab, at Nelspruit. In the two scouting assessments that were undertaken at this site, soya bean had a higher infestation compared with groundnut in terms of both the proportion of plants and leaves per plant that were infested (Table 5.3). In the entire period in which *B. subsecivella* was observed on pigeon pea (January-December 2012), infestation on this perennial plant only occurred in March and April 2012, and the infestation was extremely low compared to that observed for soya bean and groundnut.

Table 5.3. Infestation of *B. subsecivella* on groundnut, soya bean, pigeon pea, lucerne and lablab at Nelspruit.

Planting date	Scouting date	Crop	% of sampled plants that were infested	Average number of infested leaflets per plant	% of leaflets that were infested
29 November 2011	27 January	Groundnut	3.3	1	-
		Soya bean	10	1.25	-
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	07 March	Groundnut	73.3	3.13	0.96
		Soya bean	93.3	5.63	24.6
		Pigeon pea	40	1.25	-
		Lucerne	0	0	0
		Lablab	0	0	0
	04 April	Groundnut	100	-	100
		Soya bean	100	-	100
		Pigeon pea*	73.3	2.8	-
		Lucerne	0	0	0
		Lablab	0	0	0

- Not recorded

*No new infestation occurred on pigeon pea after April, 2012.

Brits

At Brits, *B. subsecivella* infestation was observed on lucerne in addition to groundnut, soya bean and pigeon pea (Table 5.4). *Bilobata subsecivella* was present on groundnut and soya bean at all scouting occasions, but the infestation on lucerne was observed only during March 2012 and that on pigeon pea was observed only in March and April 2012 (Table 5.4). The infestation of *B. subsecivella* on lucerne and pigeon pea was very low (Table 5.4). In pigeon pea, the infestation occurred on one to two leaflets per plant on an average of two plants per plot. In lucerne, it was observed on only three plants of the entire crop. The *B. subsecivella* infestation on groundnut and soya bean increased from January to April and all plants that were inspected in April were infested (Table 5.4). Soya bean had a consistently higher

infestation than groundnut throughout the monitoring period in terms of both the proportion of plants and leaves that were infested (Table 5.4).

Table 5.4. Infestation of *B. subsecivella* on groundnut, soya bean, pigeon pea, lucerne and lablab at Brits.

Planting date	Scouting date	Crop	% of sampled plants that were infested	Average number of infested leaflets per plant	% of infested leaflets per plant
28 November 2011	25 January 2012	Groundnut	10	1.5	-
		Soya bean	14.4	1.2	-
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	06 March 2012	Groundnut	93.3	6.25	2.31
		Soya bean	100	11	19.53
		Pigeon pea	(1)*	1	-
		Lucerne	(3)**	1	-
		Lablab	0	0	0
	03 April 2012	Groundnut	100	100	-
		Soya bean	100	100	-
		Pigeon pea**†	2.2	1.33	-
		Lucerne†	0	0	0
		Lablab	0	0	0

- Not recorded.
*Actual number of pigeon pea plants (1) infested in the entire experimental crop.
** Actual number of lucerne plants (3) infested in the entire experimental crop.
† Groundnut and soya bean had defoliated after reaching maturity, and so no data on leaf damage are available.
†† No new infestations occurred on pigeon pea after April.

Manguzi

At Manguzi, *B. subsecivella* infestation was observed on groundnut, soya bean and pigeon pea in all of the scoutings undertaken. The infestation of *B. subsecivella* on pigeon pea was observed in February and April 2012 (Table 5.5), and no further infestation was observed on pigeon pea after April 2012. On both scouting dates (February and April), infestation of pigeon pea on the inspected plants was lower compared to that on groundnut and soya bean (Table 5.5). In the first scouting done in February, groundnut had slightly higher *B. subsecivella* infestation in terms of the proportion of plants infested amongst the inspected plants compared to soya bean, but soya bean had a higher proportion of infested leaflets. However, during the second sampling all inspected plants from both crops were infested (Table 5.5). Both lucerne and lablab remained free of *B. subsecivella* for the duration of the experiment.

Table 5.5. Infestation of *B. subsecivella* on groundnut, soya bean, pigeon pea, lucerne and lablab at Manguzi.

Planting date	Scouting date	Crop	% of sampled plants that were infested	Average number of infested leaflets per plant	% of infested leaflets per plant
19 January 2012	24 February	Groundnut	100	4.46	9.03
		Soya bean	96.7	7.42	47.5
		Pigeon pea	20	1	-
		Lucerne	0	0	0
		Lablab	0	0	0
	05 April 2012	Groundnut	100	-	100
		Soya bean	100	-	100
		Pigeon pea	86	3.2	-
		Lucerne	0	0	0
		Lablab	0	0	0
	26 April 2012		**Comments**		
		Groundnut	All plants were dead and blackened due to severe attack by *B. subsecivella*. The dead blackened leaves contained pupae or empty shells of pupae.		
		Soya bean	All leaves had been defoliated due to severe attack by *B. subsecivella*.		
		Pigeon pea	No new infestation was recorded until the termination of monitoring in mid-December 2012.		
		Lucerne	Plants remained free of *B. subsecivella* until monitoring was terminated in mid-December 2012.		
		Lablab	Plants remained free of *B. subsecivella* until monitoring was terminated in mid-December 2012.		

- Not recorded.

Bhekabantu

At the initial scouting undertaken in January 2012 at Bhekabantu, *B. subsecivella* infestation was observed only on groundnut and soya bean, with low infestations (Table 5.6). During the second scouting, *B. subsecivella* infestation on the inspected plants increased drastically, with higher infestation on groundnut compared to soya bean (Table 5.6). By the end of April 2012, all plants inspected for both crops had all leaflets infested by *B. subsecivella*. *Bilobata subsecivella* was also observed on pigeon pea later in April. However, the infestation was very low compared to groundnut and soya bean (Table 5.6). No new infestation was recorded on pigeon pea from May until the termination of monitoring in mid-December 2012. There was no infestation recorded in lucerne and lablab for the duration of the experiment. The farmers' groundnut crops planted from the end of June to August 2011 remained free of the pest until mid-December 2011, when infestations started to appear on the crop.

Table 5.6. Infestation of *B. subsecivella* on groundnut, soya bean, pigeon pea, lucerne and lablab at Bhekabantu.

Planting date	Scouting date	Crop	% of sampled plants that were infested	Average number of infested leaflets per plant	% of leaflets that were infested
January 2012	09 February	Groundnut	6.7	1	-
		Soya bean	11.7	1.5	-
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	23 February	Groundnut	96.7	3.96	5.9
		Soya bean	73.3	3.16	9.5
		Pigeon pea	0	0	0
		Lucerne	0	0	0
		Lablab	0	0	0
	05 April	Groundnut	100	-	100
		Soya bean	100	-	100
		Pigeon pea	20	2	-
		Lucerne	0	0	0
		Lablab	0	0	0
	26 April 2012		**Comments**		
		Groundnut	All plants and leaflets were infested, and the mines either carried a pupae or shells of pupae from which adults had emerged.		
		Soya bean	All leaves had been defoliated due to severe attack by *B. subsecivella*.		
		Pigeon pea	No new infestation was recorded until the termination of monitoring in mid-December 2012.		
		Lucerne	Plants remained free of *B. subsecivella* until monitoring was terminated in mid-December 2012.		
		Lablab	Plants remained free of *B. subsecivella* until monitoring was terminated in mid-December 2012.		

- Not recorded.

5.4.2 Experiment 2: Effect of cypermethrin application on damage by *B. subsecivella* to groundnut and soya bean plants

5.4.2.1 Effect of cypermethrin on plant infestation by B. subsecivella

At Nelspruit and Vaalharts, both groundnut and soya bean were infested by *B. subsecivella*, irrespective of whether they were sprayed with cypermethrin or not (Table 5.7). Generally, the infestation was higher in soya bean than it was in groundnut (Table 5.7). At Nelspruit, spraying the crops with cypermethrin markedly reduced the percentage of infested plants and leaflets per plant compared with the unsprayed plots, but more so in the groundnut crop (Table 5.7). Compared with unsprayed plots, the reductions in *B. subsecivella* infestation on

groundnut at Nelspruit were 14.9- and 7.5-fold in contrast to 1.8- and 1.3-fold for soya bean in the first and second scouting, respectively. Nonetheless, although the proportion of infested soya bean plants was considerably higher in plots sprayed with cypermethrin, the proportion of infested leaflets per plant was very low (Table 5.7), down to 4% and 6% at the first and second scouting, respectively; this compared with 40% and 100% of infested plants in the first and second scouting, respectively, in the unsprayed plots. At Vaalharts, spraying with cypermethrin had little effect in reducing the proportion of plants that were infested by *B. subsecivella*, but markedly reduced the proportion of infested leaflets per plant in those plants that were infested.

Table 5.7. Effect of cypermethrin application on *B. subsecivella* infestation in groundnut and soya bean at Nelspruit and Vaalharts.

Planting date	Scouting date	Crop	% of plants infested		% of leaflets damaged in infested plants	
			Unsprayed	Sprayed	Unsprayed	Sprayed
			Nelspruit			
25 January 2012	07 March 2012	Groundnut	100	20	7.8	0.2
		Soya bean	100	80	40.0	4.0
	04 April 2012	Groundnut	100	20	13.7	1
		Soya bean	100	80	100	6
			Vaalharts			
13 January 2012	05 March 2012	Groundnut	100	100	13.6	6.8
		Soya bean	100	100	55.9	19.2
	02 April 2012	Groundnut	100	93.3	16.6	3.9
		Soya bean	100	100	89.5	20.5

5.4.2.2 Effect of cypermethrin on soya bean yield at Vaalharts and Nelspruit

In the plots in which the infestation of soya bean by *B. subsecivella* was not controlled, the pest had a deleterious effect on the yield performance of the crop. Damage to plants by *B. subsecivella* did not increase further after an application of cypermethrin. At both Nelspruit and Vaalharts**,** spraying the soya bean crop with cypermethrin significantly improved the mean numbers of pods and seeds per plant as well as the mean pod mass and seed mass per plant (Table 5.8). Furthermore, the proportion of rotten seed per plant was reduced from 9.7% in unsprayed plots to 2.0% in sprayed plots at Vaalharts and from 19.3% in unsprayed plots to 0.2% in sprayed plots at Nelspruit (Table 5.8).

Table 5.8. Effects of cypermethrin application on soya bean yield performance at Nelspruit and Vaalharts.

Yield parameter	Unsprayed	Sprayed	Mean	$LSD_{0.05}$
		Nelspruit		
Pod number per plant	6.60	9.77	8.19	1.866
Number of seeds per plant	14.4	22.7	18.5	4.68
Percentage of rotten seeds per plant	19.3	0.2	9.7	5.76
Pod weight per plant (g)	3.99	6.02	5.01	1.368
Seed weight per plant (g)	2.79	4.34	3.56	0.924
		Vaalharts		
Pod number per plant	8.15	11.16	9.65	2.295
Number of seeds per plant	15.6	23.8	19.7	5.23
Percentage of rotten seeds per plant	9.7	2.0	5.9	4.32
Pod weight per plant (g)	4.47	7.24	5.85	1.380
Seed weight per plant (g)	2.97	4.99	3.98	0.922

5.4.2.3 Effect of cypermethrin on yield performance of groundnut at Vaalharts

At Vaalharts there were marked differences in yield performance between plants that were sprayed with cypermethrin and those that were not sprayed (Table 5.9). The percentage of ovarian cavities that successfully produced seed and the mean numbers of pods, seeds and kernels per plant as well as the mean pod weight and kernel weight per plant were all significantly lower in the unsprayed than in the sprayed plots. The greatest difference was in the number of pods per plant (Table 5.9).

Table 5.9. Effects of cypermethrin application on groundnut yield at Vaalharts.

Yield parameter	Unsprayed plants	Sprayed plants	Mean	$LSD_{0.05}$
Ovarian cavities with seeds (%)	85.2	92.5	88.9	5.36
Pod number per plant	56.5	88.9	72.7	13.48
Kernel number per plant	17.3	29.2	23.2	4.15
Pod weight per plant (g)	10.34	15.97	13.16	2.097
Kernel weight per plant (g)	7.53	11.62	9.57	1.532

5.4.3 Survey for wild hosts of GLM

An inspection of wild plants for infestation by *B. subsecivella* at the experimental sites revealed a number of plant species on which *B. subsecivella* larvae or pupae were found. This was apparent at Nelspruit and Bhekabantu, whereas at Manguzi, Vaalharts and Brits no such plants were found. At Bhekabantu, pupae, actively feeding larvae and signs of mines were

observed on *Ipomoea wightii* (Wall) Choisy (Convolvulaceae), young but not old plants of African basil *Ocinum canum* (Sims) (Lamiaceae), *Indigofera hirsuta* (Linn.) (Leguminosae) and *Pavonia burchellii* (DC.) (Dyer) (Malvaceae), all of which were growing in the vicinity (within 5 m) of heavily infested groundnut and soya bean. In addition, pupae were observed on starbur *Acanthospermum hispidum* DC. (Asteraceae) and *Malvastrum coromandelianum* subsp. *coromandelianum* (L.) (Garcke) (Malvaceae), but without the accompanying signs of feeding. *Ipomoea wightii* and *O. canum* were the most infested, carrying from 5 to 13 *B. subsecivella* larvae per plant.

The infestation of *B. subsecivella* on the wild plants at Bhekabantu occurred in April, towards the end of the growing season, and was not observed on the same species at any other time. With the exception of *I. hirsuta*, infestation by *B. subsecivella* was not observed on plants at distances beyond 5 m of the heavily infested groundnut and soya bean crops. Present at Bhekabantu, but not infested by *B. subsecivella*, was *Psoralea corylifolia* L. **(**Leguminosae); a known host for *B. subsecivella* (Shanower *et al.* 1993). At Nelspruit, *B. subsecivella* larvae, pupae and signs of larval feeding were found on *Desmodium tortuosum* (Sw.) DC. (Leguminosae), wild soya bean *Glycine wightii* L. Merr. **(**Leguminosae) and *Ipomoea sinensis* (Desr.) Choisy subsp**.** *blepharosepala* Hochst. ex A. Rich. (Convolvulaceae).With the exception of *G. wightii*, the infestedplants were within 5 m of heavily infested groundnut and soya bean. The infestations on these plant species were observed only in the months of March and April, although actively growing plants of the same species were present throughout the year. Also present at Nelspruit was *P. corylifolia* but it remained free of *B. subsecivella* infestation throughout the study period.

5.5 Discussion

Crop host utilization

The issue of host preference by the *B. subsecivella* population occurring in South Africa, among the crops known to be hosts for *B. subsecivella* in India, has previously been examined by Van der Walt (2007), who used a Y-tube olfactometer to evaluate adult orienatation towards groundnut, soya bean and lucerne. In our study, *B. subsecivella* was allowed to interact naturally with groundnut, soya bean, pigeon pea, lucerne and lablab (five of the known crop hosts for this pest). The findings from this study indicated that soya bean and groundnut were the most infested hosts (Tables 5.2 to 5.6). However, soya bean appeared to be more infested than groundnut, since in most of the cases soya bean had a higher

infestation in terms of the proportion of plants and leaflets per plant infested. This was in contrast to results obtained by Van der Walt (2007), who concluded from the Y-tube olfactometer study that the *B. subsecivella* occurring in South Africa preferred groundnut to soya bean. The discrepancy between this study's results and those of Van der Walt (2007) could be due to the difference in the methods used. Although moths can discriminate between plants for laying eggs, in favour of those hosts that are suitable as food for their larvae, it is usually only the females that can do so (Ozaki *et al*. 2011). In this respect, the major weakness with the experiment of Van der Walt was that the moths used were of unknown sex. If males were involved, the results may not have accurately reflected the oviposition preferences of *B. subsecivella*. Städler (2002) stated that during host plant location, phytophagous insects employ a specific 'host plant search image' which is based on representative chemical and visual characteristics (such as leaf shape or colour) of their host plants (Prokopy & Owens 1983). In this study, *B. subsecivella* interacted naturally with the host crops and the plant volatiles that were emitted into the air. This exposure enables the pest to link host presence to specific odours and thereby increases the chances of distinguishing between reliable and unreliable hosts (Dolch & Tscharntke 2000). Hence, the results of this study reflected more accurately the moth's preference for ovipositing as well as the suitability of the crops as food for the GLM larvae.

Although lablab is reported to be a host for *B. subsecivella* in India (Shanower *et al.* 1993), none of the plants were infested at any of the five sites where it was grown in the present study. This suggested that lablab is not at present a host for the *B. subsecivella* occurring in South Africa. The host plant status of lucerne for *B. subsecivella* is ambiguous. Du Plessis (2003) reported that in the Northern Cape Province of South Africa, lucerne served as a refuge host for *B. subsecivella* in winter, when groundnut is not grown. However, Buthelezi *et al.* (2012) failed to detect *B. subsecivella* infestation on lucerne at Vaalharts, Brits and Nelspruit, even though the crop was growing adjacent to *B. subsecivella*-infested groundnut. In the present study, *B. subsecivella* was observed on lucerne only at Brits. Even then, the infestation of lucerne by *B. subsecivella* was very low (only three infested plants in the entire experimental crop) and its infestation by *B. subsecivella* depended on the time of the year; occurring only in March 2012, towards the end of the summer growing season. During the winter months, the lucerne crops in the experimental plots, as well as those on the farms surrounding the experimental sites, were free of *B. subsecivella*. Pigeon pea was infested by *B. subsecivella* at all the test sites, but crop damage was also low since generally only a few

leaflets (one to three) per plant were infested. The host status of pigeon pea was also dependent on the time of the year. As with lucerne, most of the infestation of *B. subsecivella* on pigeon pea occurred only towards the end of the summer season (March to April), and both these perennial plants were free of the pest in the absence of groundnut and soya bean, from May 2012 until the monitoring of *B. subsecivella* infestation on them was terminated in mid-December 2012. Thus, lucerne and pigeon pea do not appear to be major hosts for the *B. subsecivella* occurring in South Africa. Also, these two perennial crops seem unlikely to act as bridging hosts in winter when groundnut and soya bean crops are absent.

Wild hosts range for *B. subsecivella*

The current literature on the *B. subsecivella* population in India gives the impression that *B. subsecivella* larvae feed largely on legume plants, with 14 species listed, and only one non-legume host, *Boreria hispida* K. Sch. (Rubiaceae), recorded (Shanower *et al.* 1993). It was, therefore, the expectation at the start of the present study that the wild hosts of the *B. subsecivella* occurring in South Africa would largely comprise legume species. Contrary to this expectation, larvae of *B. subsecivella* were found feeding and pupating on a number of non-legume species, in addition to legume species, in South Africa. The non-legume species, which included *I. wightii, I. sinensis* subsp. *blepharosepala*, *O. canum, P. burchellii*, *A. hispidum* and *M. coromandelianum* subsp. *coromandelianum*, belong to five plant families. Only three wild legume species, namely *I. hirsuta*, *D. tortuosum* and *G. wightii*, were identified as hosts for *B. subsecivella*, in contrast to seven non-legume species.

With the exception of *I. wightii*, the wild plant species that were identified as hosts for *B. subsecivella* in the present study are additional to the list that was provided by Van der Walt (2007) of wild plant hosts of *B. subsecivella* identified at Tshiombo irrigation scheme in South Africa. The list provided by Van der Walt (2007) included three species in the Malvaceae, four species in the Fabaceae, and one species each in the Convolvulaceae, Tiliaceae, Pedaliaceae and Capparoceae. Interestingly, plants of *Crotalaria vasculosa* Wall. ex Benth. (Fabaceae), *Corchorus tridens* L. (Tiliaceae), and *Cleome monophylla* L. (Capparaceae) that were included in the host list of *B. subsecivella* by Van der Walt (2007) were abundant at Bhekabantu, but no *B. subsecivella* infestation was observed on any of the plants inspected. This suggests that the host plant range of *B. subsecivella* may vary with locality. Altogether, the information obtained from the present study and that of Van der Walt

(2007) indicates that the South African *B. subsecivella* population has a broad host range that includes several plant families, and this may partly explain why it has been successful in colonizing most parts of South Africa (Du Plessis 2003; Buthelezi *et al.* 2012) and probably the rest of the African continent (Buthelezi *et al.* 2012).

Usually, the term 'plant host' (in relation to insect pests) is used when a plant is used by insect larvae as a place to feed and grow (Panizzi 1997), but may also include a plant that is used for refuge during certain stages of development, *e.g.* pupation (Panizzi 1997). Of the wild plants that were infested by *B. subsecivella* in the present study, no signs of feeding were found on *A. hispidum* and *M. coromandelianum*, which indicated that these species are probably only used for pupation, a common phenomenon among insects (Panizzi 1997). However, in the present study it appeared that the necessity for *B. subsecivella* to use *A. hispidum* and *M. coromandelianum* for pupation may have been due to high larval loads on heavily infested groundnut plants nearby, since the pupae were observed only on those plants of *A. hispidum* and *M. coromandelianum* that were very close to the infested groundnut or soya bean plants.

Amongst the wild plant hosts of the South African *B. subsecivella* identified in this study, *I. hirsuta* and *G. wightii* are also known as hosts for *B. subsecivella* in India (Shanower *et al.* 1993). Present at Bhekabantu and Nelspruit was *P. corylifolia* which has been listed as a host for *B. subsecivella* in India (Shanower *et al.* 1993), but the plants remained free of *B. subsecivella* infestation for the entire duration of the study. Based on the observations of this study and the information provided in the literature, it can be concluded that *B. subsecivella* populations in India, South Africa and Australia do share hosts, but not all crop and wild plant hosts. One puzzling aspect concerning the relationship of the South African *B. subsecivella* with its plant hosts was that infestations occurred at specific times of the season or year. At Manguzi, where farmers start to grow groundnut from the end of June, *B. subsecivella* does not infest the crop until towards the end of December. Also noted in the present study was that pigeon pea and lucerne (both perennial crops) were infested only in March and April. Previously, Buthelezi *et al.* (2012) failed to find *B. subsecivella* infestation on lucerne crops in winter at Vaalharts and Brits, where the pest readily infests groundnut and soya bean in summer. Furthermore, wild plant hosts for *B. subsecivella* that were identified at Bhekabantu and Nelspruit were only infested in the months of March and April. Surprisingly,

no infestation of the plant hosts, including wild hosts, was observed from the end of May to mid-December, the reasons of which are unclear at present.

In the current study, several wild hosts for *B. subsecivella* were identified in different locations; however, the pest's infestation on them was only detected during summer months in the presence of the main crops (groundnut and soya bean) and no infestation was observed during off-season or winter periods. Therefore, their (wild hosts) status as to whether they serve as off-season or alternative hosts still needs to be further investigated, as such information might be useful in developing control strategies against *B. subsecivella*, namely the manipulation of the crop environment, e.g. trap crops.

Effects of cypermethrin

Bilobata subsecivella is known to be a minor pest of soya bean in Australia but there is no published information on the insecticides used to control the pest in that country. In South Africa, the pest has not yet been reported by farmers to be a serious pest of soya bean; perhaps because they have regular insecticide spraying regimes. In this study, *B. subsecivella* infestation occurred on the sprayed soya bean plants, but the infestation was lower than on the unsprayed ones. The presence of the pest on the sprayed plants might be due to the combination of a short active life of cypermethrin on the crop and having infested plants in unsprayed plots nearby, which served as a reservoir of the pest between the spraying intervals. In the unsprayed plots, the high infestation levels reduced grain yields by lowering the number of seeds produced per plant and increasing the proportion of rotten seeds (Tables 5.8 and 5.9). Even though the use of pesticides reduces the infestation levels of the crops by the pest, there is still a need to determine the spraying regime that will provide the spraying intervals which will reduce the infestation of the pest on crops with minimal usage of pesticides.

5.6 Conclusion

Of the five crop hosts that are known to be infested by *B. subsecivella* in India, groundnut and soya bean were the most heavily infested by the *B. subsecivella* occurring in South Africa, with soya bean suffering the most damage under field conditions. This is consistent with *B. subsecivella's* host utilization pattern in Australia. Pigeon pea was infested by *B. subsecivella* at all South African sites tested, but crop damage was very low and infestation

was confined mostly to the months of March and April. Lucerne was scarcely attacked by the pest, as only three *B. subsecivella* larvae were found in April at only one of the five sites tested. There was no infestation observed on lablab at all sites, indicating that it is not a host for *B. subsecivella* in South Africa. Identification of wild plant hosts constituting a range of plant families indicated that the South African *B. subsecivella* population has a broad host range. However, infestations on wild plant hosts was only observed in summer, which suggested that wild plant hosts may not play a role in the carryover of the pest from one summer season to another. The current study also revealed that the South African, Australian and Indian *B. subsecivella* populations do share some, but not all, host plants (both crops and wild plants). Spraying cypermethrin on groundnut and soya bean significantly reduced the infestation level of the South African *B. subsecivella* compared to unsprayed plots, where the high infestation levels reduced grain yield by lowering the number of seeds produced per plant and increasing the proportion of rotten seeds.

5.7 References

BAILEY, P.T. 2007. Pests of Field Crops and Pastures: Identification and Control. CSIRO Publishing, Collingwood, Victoria 3066, Australia.

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2012.The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soya bean moth (*Aproaerema simplixella* PS 1) (Walker) (Lepidoptera: Gelechiidae). *African Journalof Agricultural Research* **7**(38): 5285–5292.

COMMON, I.F.B. 1990. Moths of Australia. Melbourne University Press, Carlton, Victoria, Australia.

DOLCH, R. & TSCHARNTKE, T. 2000. Insect communities of Malagasy legumes (Fabaceae): patterns of species richness, resource fragmentation, and tropical-temperate differences. In: Lourenço, W. R. & Goodman, S. M. (Eds.) Diversity and Endemism in Madagascar. Mémoires de la Société de Biogéographie, Paris.

DU PLESSIS, H. 2002. Groundnut leaf miner *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48–49.

DU PLESSIS, H. 2003. First report of groundnut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut, soybean and lucerne in South Africa. *South African Journal of Plant and Soil* **20**:48.

EPIERU, G. 2004. Participatory evaluation of the distribution, status and management of the groundnut leaf miner in the Teso and Lango farming systems. Final Technical Report of a project supported by NARO/DFID, Serere Agriculture & Animal Production Research Institute (SAARI), Kampala, Uganda.

GHEWANDE, M.P. & NANDAGOPAL, V. 1997. Integrated pest management in groundnut (*Arachis hypogaea* L.) in India. *Integrated Pest Management Reviews* **2**:1–15.

GOMULKIEWICZ, R., HOLT, R.D, BARFIELD, M. & NUISMER, S.L. 2010. Genetics, adaptation and invasion in harsh environments. *Evolutionary Applications* **3**: 97–108.

HOLT, R.D., BARFIELD, M. & GOMULKIEWICZ, R. 2005. Theories of niche conservatism and evolution: Could exotic species be potential tests? In: Sax, D.F., Stachowicz, J.J. & Gaines, S.D. (Eds.) *Species Invasions: Insights into Ecology, Evolution and Biogeography* 259–290. Sinauer Associates, Sunderland, MA, U.S.A.

JONES, W.A. Jr. 1979. The distribution and ecology of pentatomid pests of soybeans in South Carolina. Ph.D. thesis. Clemson University, Clemson, SC, U.S.A.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**: 19.

KOLAR, C.S. & LODGE, D.M. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* **4**: 199–204.

MUNYULI, T.M.B., LUTHER, G.C., KYAMANYWA, S. & HAMMOND, R. 2003. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda and Democratic Republic of Congo. *African Crop Science Conference Proceedings* **6**: 231-237.

OZAKI, K., RYUDA, M., YAMADA, A., UTOGUCHI, A., ISHIMOTO, H., CALAS, D., MARION-POLL, F., TANIMURA, T. &YOSHIKAWA, H. 2011. A gustatory receptor involved in host plant recognition for oviposition of a swallowtail butterfly. *Nature Communication* **2**: 542.

PAGE, W.W., EPIERU, G., KIMMINS, F.M., BUSOLOBULAFU, C. & NALYONGO, P.W. 2000. Groundnut leaf miner *Aproaerema modicella*: A new pest in eastern districts of Uganda. *International Arachis Newsletter* **20**: 64–66.

PANIZZI, A.R. 1992. Performance of *Piezodorus guildinii* on four species of *Indigofera* legumes. *Entomologia Experimentalis et Applicata* **63**: 221–228.

PANIZZI, A.R. 1997.Wild hosts of pentatomids: Ecological significance and role in their pest status on crops. *Annual Review of Entomology* **42**: 99–122.

PANIZZI, A.R. & NIVA, C.C. 1994. Overwintering strategy of the brown stink bug in northern Paraná. *Pesquisa Agropecuária Brasileira* **29**: 509–611.

PROKOPY, R.J. & OWENS, E.D. 1983.Visual detection of plants by herbivorous insects. *Annual Review of Entomology* **28**: 337–364.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993. Biology and control of the groundnut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**: 3–10.

SUBRAHMANYAM, P., CHIYEMBEKEZA, A.J. & RAO, G.V.R. 2000. Occurrence of groundnut leaf miner in northern Malawi. *International Arachis Newsletter* **20**: 66–67.

SUJAY, Y.H., SATTAGI, H.N. & PATIL, R.K. 2010. Invasive alien insects and their impact on agroecosystems. *Karnataka Journal of Agricultural Sciences* **23**: 26–34

STÄDLER, E. 2002. Plant chemical cues important for egg deposition by herbivorous insects. In: Hilker, M. & Meiners, T. (Eds.) Chemoecology of insect eggs and egg deposition. 171-197. Berlin, Blackwell Publishing.

TRAVIS. J.M., HAMMERSHØJ, J.M., STEPHENSON, C. 2005. Adaptation and propagule pressure determine invasion dynamics: Insights from a spatially explicit model for sexually reproducing species. *Evolutionary Ecology Research* **7**: 37–51.

VAN DER WALT, A. 2007. Small holder farmers' perceptions, host plant suitability and natural enemies of the groundnut leafminer, *Aproaerema modicella* (Lepidoptera: Gelechiidae) in South Africa. M.Env. Sci. dissertation, North-West University, Potchefstroom, South Africa.

Chapter Six

Seasonal monitoring of the incidence and flight activity of *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) at five sites in South Africa

Abstract

Bilobata subsecivella (Zeller) (Lepidoptera: Gellechidae) has recently emerged as a major pest of groundnut (*Arachis hypogaea* L.) in Africa. The origin of this new pest is uncertain and there is also not much information on its ecology to facilitate the development of control strategies against it. The aim of this study was to monitor the infestation and flight activity of *B. subsecivella* in order to understand its dispersal and off-season survival tactics and to predict its initial occurrence. The study was conducted at five localities: Vaalharts (Northern Cape), Manguzi (KwaZulu-Natal), Brits (North West), Bhekabantu (KwaZulu-Natal) and Nelspruit (Mpumalanga) in South Africa, from November 2010 to December 2012. Pheromone traps were used to monitor the moth's flight activity. In the 2010/2011 season, the incidence of damaged plants/leaves was monitored by scouting in groundnut crops grown at two planting dates (November 2010 and January 2011). In the 2011/2012 season, the incidence of damaged plants/leaves was monitored by scouting in groundnut, soya bean, pigeon pea, lucerne and lablab bean crops grown at two planting dates (November 2011 and January 2012). Wild plant hosts were inspected for damage symptoms and the presence of larvae. Information collected included climate data (rainfall, temperature and humidity) that were obtained from weather stations of the Agricultural Research Council (ARC) at four planting sites. At all locations, *B. subsecivella* moths were caught in traps before crop planting. Though low in numbers, *B. subsecivella* moths were caught during winter at all locations other than Brits. Infestations on pigeon pea and lucerne were observed in March and April 2012. Larval infestation on wild plant hosts was observed during the growing period of the main crops, despite catches of *B. subsecivella* moths in pheromone traps throughout the year at four sites. No infestations were observed on lablab beans at any of the sites for the duration of the study. At Nelspruit, there was a significant negative association between temperature and *B. subsecivella* moth catches in pheromone traps, whereas at Vaalharts, there was a significant positive association between humidity and *B. subsecivella* moth catches. There was no significant correlation between any of the recorded environmental factors and *B. subsecivella* moth catches at Manguzi and Brits.

***Key words*:** alternative hosts**,** flight activity, pheromone traps, planting dates, scouting

6.1 Introduction

Bilobata subsecivella (Zeller) (Lepidoptera: Gelechiidae) is the most important pest of groundnut (*Arachis hypogaea* L.) and soya bean (*Glycine maxima* (L.) Merr.) in Indo-Asia (Shanower *et al.* 1993). The pest was first reported in Africa in 1998 from Uganda, East Africa (Epieru 2004) after which it spread rapidly throughout several eastern, central and southern African countries, including Malawi, Democratic Republic of the Congo, Egypt, Mozambique and South Africa (Page *et al.* 2000; Subrahmanyam *et al.* 2000; Du Plessis 2002; Munyuli *et al.* 2003; Kenis & Cugala 2006).There is not much information available on the ecology of this fairly recent pest to facilitate the development of control strategies against it in South Africa. Surveys on potential *B. subsecivella* plant hosts that were conducted in five locations in South Africa during the 2011/2012 season revealed that *B. subsecivella* has a broad host range including crops and wild hosts from different plant families (Chapter 5). In Chapter 5, it was also reported that *B. subsecivella* infestation on wild plant hosts and perennial crops (pigeon pea and lucerne) was only observed in summer, which suggested that these plants may not play an important role in the carryover of the pest from one summer season to another. From these observations, it could be hypothesized that *B. subsecivella* diapauses during off-season/winter periods (June to September). Nevertheless, the relationship between *B. subsecivella* and its plant hosts needs to be investigated further. Stastny *et al*. (2006) reported that outbreaks of pest species in areas previously uninhabited by them can be favoured by the presence of secondary hosts.

Insect pests have numerous behavioural adaptations and ecological requirements which include adaptations to prevalent abiotic conditions during their different life stages (Arun & Vijayan 2004; Holt *et al.* 2005; Travis *et al*. 2005; Gomulkiewicz *et al.* 2010). One of the major factors on which their success and survival strategies depends on is how efficiently they time their growth stages, in order to utilize the maximum resources and surrounding environmental conditions (Arun & Vijayan 2004). According to Prasad & Prabhakar (2012), insect species that carry their populations over from one season to another generally have resting phases (diapause or aestivation) in their life cycles. It was reported (Jagtap *et al.* 1985 in Shanower *et al.* 1993) that *B. subsecivella* may survive the extremely hot, dry Indian summers in pupal diapause or aestivation. The severity of the pest's occurrence, however,

appears to differ between localities and years (Kenis & Cugala 2006). Smilar observations were also reported in South Africa (Van der Walt 2007). Abiotic factors, principally rainfall, humidity, and temperature are frequently suggested as causes of seasonal *B. subsecivella* population fluctuations (Muthiah & Abdul Kareem 2002). Cultural control methods such as the manipulation of planting dates have thus been suggested for reducing *B. subsecivella* infestation on crops (Kenis & Cugala 2006; ARC 2007).

Pest monitoring is a fundamental first step in the establishment of an effective integrated pest management (IPM) programme (Prasad & Prabhakar 2012). Pest monitoring uses various monitoring tools such as pheromone traps, light traps, coloured sticky traps, pitfall traps and suction traps (Prasad & Prabhakar 2012). The data obtained from trap monitoring serves several purposes that include ecological studies (Hirao *et al.* 2008), tracking insect migration (Drake *et al.* 2002), timing of pest arrivals into agro-ecosystems (Klueken *et al.* 2009), timing of pesticide applications (Lewis 1981; Merril *et al.* 2010) and prediction of later generations based on the size of earlier generations (Zalucki & Furlong 2005). Cannon *et al.* (2004) stated that pheromone traps are useful for population monitoring, particularly of lepidopteran pests, to determine the extent of an outbreak area and the effectiveness of eradication campaigns. The timing of adult male catches in the traps indicates the start of the pest's flight activity in the area (Prasad & Prabhakar 2012). In India, pheromone trapping of *B. subsecivella* was used by Das (1999) to monitor the seasonal activity of the pest in groundnut fields in the western Nimer Valley, and proved to be an adequate tool for monitoring the pest in groundnut fields. In South Africa, pheromone trapping of *B. subsecivella* was used by Du Plessis (2011) to monitor *B. subsecivella* flight activity at the border of groundnut fields in four localities.

In order to test the hypothesis that *B. subsecivella* diapause during off-season/winter periods (June to September), the study reported in this chapter involved (a) the monitoring of *B. subsecivella* flight activity using pheromone traps, (b) an experiment (with two planting dates) that examined *B. subsecivella* infestation on groundnut, (c) an experiment (with two planting dates) that examined *B. subsecivella* infestation on soya bean, groundnut, lucerne (*Medicago sativa* L.), pigeon pea (*Cajanus cajan* L.) and lablab bean (*Lablab purpureus* L.), (d) scouting of *B. subsecivella* infestation on wild plant hosts, and (e) monitoring of *B. subsecivella* infestation on groundnut at Manguzi (KwaZulu- Natal province) where farmers generally plant groundnut from the end of June to August. Information collected also

included climatic data (rainfall, temperature and humidity) which was obtained from the ARC weather stations located at the planting sites.

6.2 Materials and methods

The study was conducted at five sites in four provinces of South Africa (KwaZulu-Natal, North West, Northern Cape and Mpumalanga) which differ in climate (see climatic characteristics and geographic locations of the sites in Chapter 2, Table 2.1). In the North West province, the site was the Agricultural Research Council (ARC) research station at Brits. In the Northern Cape, the site was the ARC research station at Vaalharts Irrigation Scheme. In Mpumalanga, the site was the Department of Agriculture Lowveld Agricultural Research Station near Nelspruit. In KwaZulu-Natal, the two sites were Bhekabantu and Manguzi. The sites were chosen for their wide variation in climatic conditions, from a warm coastal subtropical climate at Manguzi to one characterized by hot summers and cold winters at Brits (Buthelezi *et al.* 2012).

2010/2011 season

The level of *B. subsecivella* infestation on groundnut was assessed through field scouting on the crop planted at two dates (Table 6.1). November planting was only done at Bhekabantu and Manguzi. For the other three sites, scouting for November planting was carried out on the groundnut crop which was already planted in mid-November 2010 at the research stations' farms. At Brits, there was only one planting, which was carried out in mid-November 2010. One groundnut variety (Natal Common) was planted in one block of 20m x 30m at each of the sites. The inter-row spacing was 45 cm, the intra-row spacing was 15-20 cm and the planting depth was 5 cm. In addition to monitoring *B. subsecivella* infestation on the experimental crop at Manguzi, infestation on the farmers' groundnut crop that was planted in July 2011 was also monitored for *B. subsecivella* infestation.

2011/2012 season

Bilobata subsecivella infestation was monitored on soya bean, groundnut, lablab bean, pigeon pea and lucerne (five of the known crop hosts for *B. subsecivella* in India). In addition, *B. subsecivella* infestation was monitored in the experiment which examined the effect of

cypermethrin application on groundnut and soya bean at Vaalharts and Nelspruit (Chapter 5 section 5.2). Planting and scouting dates are presented in Table 6.1. Experimental layout, crop management and scouting procedures are described in Chapter 5 (sections 5.2.1 and 5.2.2).

Table 6.1. Sites, planting dates and *B. subsecivella* scouting dates for the 2010/2011 and 2011/2012 groundnut seasons.

Site	Season	Planting dates	Scouting dates
Vaalharts 27º 95' 761'' S; 24º 83' 991'' E	2010/2011	04 November 2010 06 January 2011	19 January 2011 19 January 2011, 02 May 2011
	2011/2012	17 November 2011 13 January 2012	26 January 2012, 05 March 2012, 02 April 2012 05 March 2012, 02 April 2012, 02 May 2012
Nelspruit 25º 45' 452'' S; 30º 97' 154'' E	2010/2011	18 November 2010 25 January 2011	21 January 2011 18 April 2011
	2011/2012	29 November 2012	27 January 2012, 07 March 2012, 04 April 2012
Manguzi 26º 95' 532'' S; 32º 82' 356'' E	2010/2011	17 November 2010 27 January 2011	27 January 2011 23 March 2011, 27 April 2011
	2011/2012	19 January 2012	24 February 2012, 05 April 2012, 26 April 2012
Bhekabantu 27º01'12.38''S; 32º19'18.29'' E	2010/2011	18 November 2010 28 January 2011	28 January 2011 23 March 2011, 26 April 2011
	2011/2012	19 January 2012	09 February 2012, 23 February 2012, 05 April 2012, 27 April 2012
Brits 25º 59' 135'' S; 27º 76' 875'' E	2010/2011	15 November 2010	20 January 2011
	2011/2012	28 November 2011	25 January 2012, 06 March 2012, 03 April 2012

6.2.1. Land preparation and crop management

Procedures for land preparation and crop management are the same as the ones described in Chapter 5 (section 5.2.1).

6.2.2. Assessment for *B. subsecivella* infestation on the crops

The level of larval infestation on the crops was assessed through field scouting. The frequency and dates of scouting varied between the sites (Table 6.1). At the first scouting for the 2010/2011 season, the infestations were very low; hence 30 plants per row in 10 rows per site were chosen at random and inspected for infestation by *B. subsecivella*. The number of infested plants and infested leaves per plant was recorded. In subsequent scoutings, where infestations were higher, 10 plants per row in 10 rows per site were chosen at random and inspected for infestation by the pest. For each of the 10 plants chosen per row, the number of infested leaflets and the total number of leaflets were counted, and the percentage of leaflets that were infested was determined. In the farmers' groundnut crop that was planted in July 2011 at Manguzi, scouting was carried out in three farms on 2 December 2011 within a 1.27 m x 1.27 m quadrat made of wooden poles. Groundnut plants ranged between 13 and 26 within the quadrat. There were four replications on each farm. Scouting procedures for the 2011/2012 season are described in Chapter 5 (section 5.2.1).

6.2.3. Assessment for *B. subsecivella* infestation on wild plant hosts

Scouting for *B. subsecivella* infestation on wild plant hosts (identified in Chapter 5) during the crop growing season, as well as the off-season, was carried out in the vicinity of the groundnut fields at Bhekabantu and Nelspruit. At Bhekabantu, the wild plant hosts scouted included *Ipomoea wightii* (Wall.) Choisy (Convolvulaceae), *Ocinum canum* (Sims) (Lamiaceae), *Indigofera hirsuta* (Linn.) (Leguminosae), *Pavonia burchellii* (DC.) (Dyer) (Malvaceae), *Acanthospermum hispidum* DC. (Asteraceae), *Malvastrum coromandelianum* subsp. *coromandelianum*, (L.) (Garcke) (Malvaceae). At Nelspruit, the wild plant hosts surveyed included *Desmodium tortuosum* (Sw.) DC. (Leguminosae), *Glycine wightii* L. Merr. (Leguminosae) and *Ipomoea sinensis* (Desr.) Choisy subsp**.** *blepharosepala* Hochst. ex A. Rich. (Convolvulaceae).

6.2.4. Monitoring *B. subsecivella* flight activity using pheromone traps

At each site, yellow triangular delta traps (28 cm x 20 cm x 14 cm) purchased from Insect Science SA[4] were placed 1 m above the ground on wooden stakes, secured with wire and

[4]Insect Science (Pty) Ltd, P O Box 4019, Tzaneen, Limpopo 0850, South Africa. Tel: +2715 307 1391.

spaced more than 30 m apart. Five traps per site were used for monitoring during the groundnut growing season and two/three traps per site were used for off-season monitoring. Inside each of these traps were placed pheromone lures baited with a sex pheromone blend of *A. modicella* [(Z)-7, 9-decadienyl acetate, (E)-7-decenyl acetate and (Z)-7-decenyl acetate in the ratio10:2:1.4] as described by Hall *et al.* (1994). The sex pheromone was impregnated into white polyethylene vials (20 mm x 5 mm) and was supplied by the Natural Resources Institute (NRI) University of Greenwich, United Kingdom. The same sex pheromone blend was used successfully in India by Das (1999) to monitor the seasonal activity of *B. subsecivella* in groundnut fields in the western Nimer Valley. In South Africa it was used by Du Plessis (2011) to monitor *B. subsecivella* flight activity in the borders of four groundnut fields.

Sticky liners, comprising white paper with glue (18 cm x 20 cm), purchased from Insect Science SA were placed inside the traps, on the trap base. One white polyethylene vial containing the female sex pheromone blend was placed on top and in the middle of the sticky liners inside the traps. Both sticky liners and pheromone lures were replaced every two weeks. Moths (trapped on sticky liners) were stored on the sticky liners in the laboratory at room temperature until counted and moth numbers were recorded for each trap at each location. Pheromone monitoring was conducted continuously from November 2010 to December 2012. At Manguzi, three farmers' fields were chosen for monitoring *B. subsecivella* flight activity on the groundnut crop planted in July 2011 and one pheromone trap was placed per site.

6.2.5. Data for temperature, rainfall and humidity

Data for rainfall, humidity and temperature were obtained from ARC weather stations located at four planting sites (i.e. all except Bhekabantu). Due to missing data for both climate and *B. subsecivella* moth catches during some months at all sites, data used for analyses included only months where both climate and catch data were available.

6.3. Data analysis

Monthly means for *B. subsecivella* counts per trap, rainfall, humidity and temperature were calculated using MS Excel and graphs were plotted using SigmaPlot version 12.0. Pearson's tests for correlation were performed to determine the relationship between *B. subsecivella*

moth catches and the environmental factors (temperature, rainfall and humidity). Growing season data (December to March) for two seasons were considered for the correlation analyses, because it was deemed that the *B. subsecivella* populations would be advanced by the presence of the crops so it would be feasible to examine the association between moth catches and environmental factors. Data were analysed statistically using IBM® SPSS® Statistics 22.0. Data were tested for normality using a one-sample Kolmogorov-Smirnov test and non-parametric data were log-transformed (IBM® SPSS® Statistics 22.0). Differences in GLM moth catches between the study sites were evaluated by one-way analysis of variance (ANOVA) and Tukey HSD tests were applied to determine significant differences.

6.4 Results

Fluctuations in *B. subsecivella* populations between locations, seasons and years were observed in the current study (Figure 6.1). High numbers of moths were trapped during the 2010/2011 season at Vaalharts, Nelspruit, Bhekabantu and Brits whereas at Manguzi, the highest peak in *B. subsecivella* populations was obtained in the 2011/2012 season. At all locations, the highest numbers of moths were trapped between January and March (Figure 6.1) which coincides with the establishment of the crop until harvesting. Very low numbers of *B. subsecivella* moths were trapped during the winter months at the other four sites, with the exception of Brits where there were no moths trapped during that period.

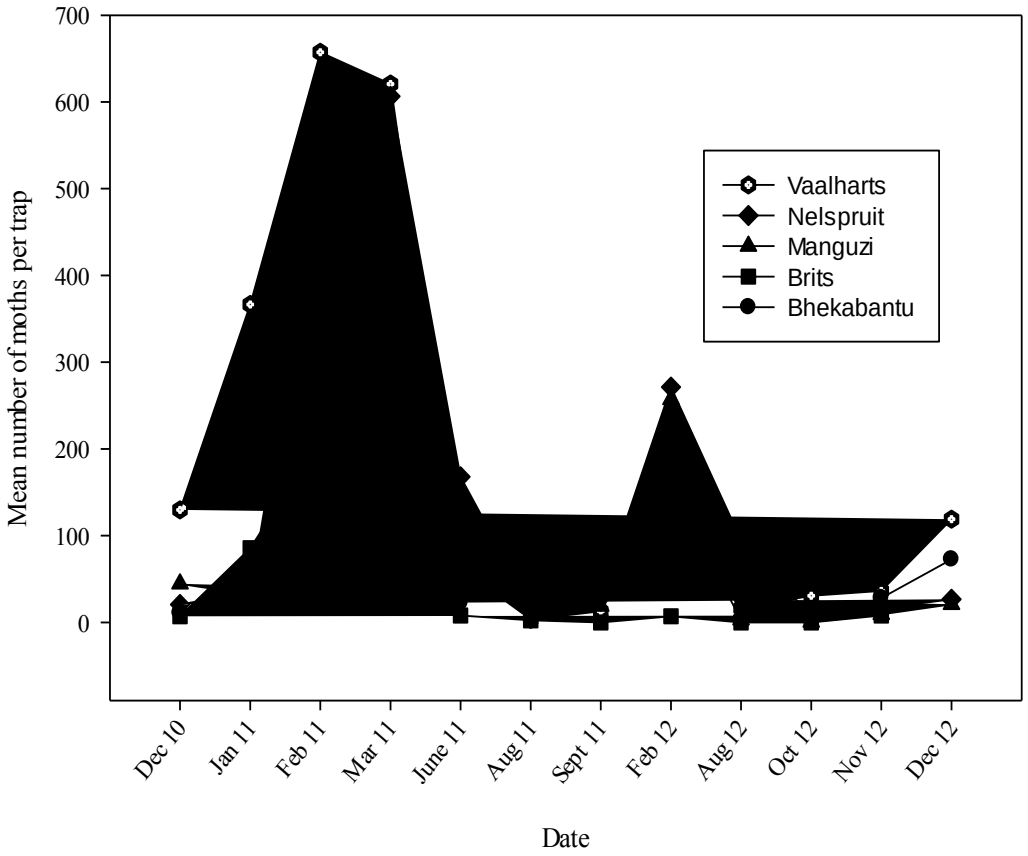


Figure 6.1. *Bilobata subsecivella* flight activity recorded monthly at the Vaalharts, Nelspruit, Bhekabantu, Manguzi and Brits groundnut sites from December 2010 to December 2012.

6.4.1 *Bilobata subsecivella* flight activity in relation to rainfall, temperature and atmospheric humidity

Vaalharts

The numbers of *B. subsecivella* males caught over the duration of the study were highest at Vaalharts compared to the other four sites (See figures 6.2 to 6.6). Results from this two-year monitoring study revealed that high numbers of moths were caught from January to April during both seasons (Figure 6.2). This peak flight activity period coincided with the establishment of the second crop and the harvesting of the first crop. The numbers of moths caught between May and December remained very low in both seasons. Two peaks in flight activity were obtained in February 2011 and April 2011 in the 2010/2011 season from the pheromone traps (Figure 6.2). For the duration of this study, Vaalharts received an average monthly rainfall ranging between 0 and 8 mm (Figure 6.2) which fell in June 2011, August

2011, November 2011, August 2012 and October 2012 (Figure 6.2). This period coincided with very low numbers of *B. subsecivella* moth catches. There was very little or no rainfall at Vaalharts from November 2010 to June 2011, December 2011 and July 2012 and this period corresponded with an increase in moth catches. In contrast, in both years moth catches continued to increase at high temperatures and declined at the time when temperatures fell to a minimum. This coincided with periods of higher humidity. During the course of the study, generally in the summer months, mean temperatures rose to 26 ^{o}C and thereafter declined, reaching their lowest values (10 ^{o}C) in the winter months. Relative humidity roughly followed a similar pattern, varying between 30% in the spring months and 65% in the autumn months. A Pearson's test for correlation indicated that humidity had a significant positive association with *B. subsecivella* moth catches ($r = 0.803$; $p < 0.05$), while there was no association between moth catches and either temperature or rainfall (Table 6.2).

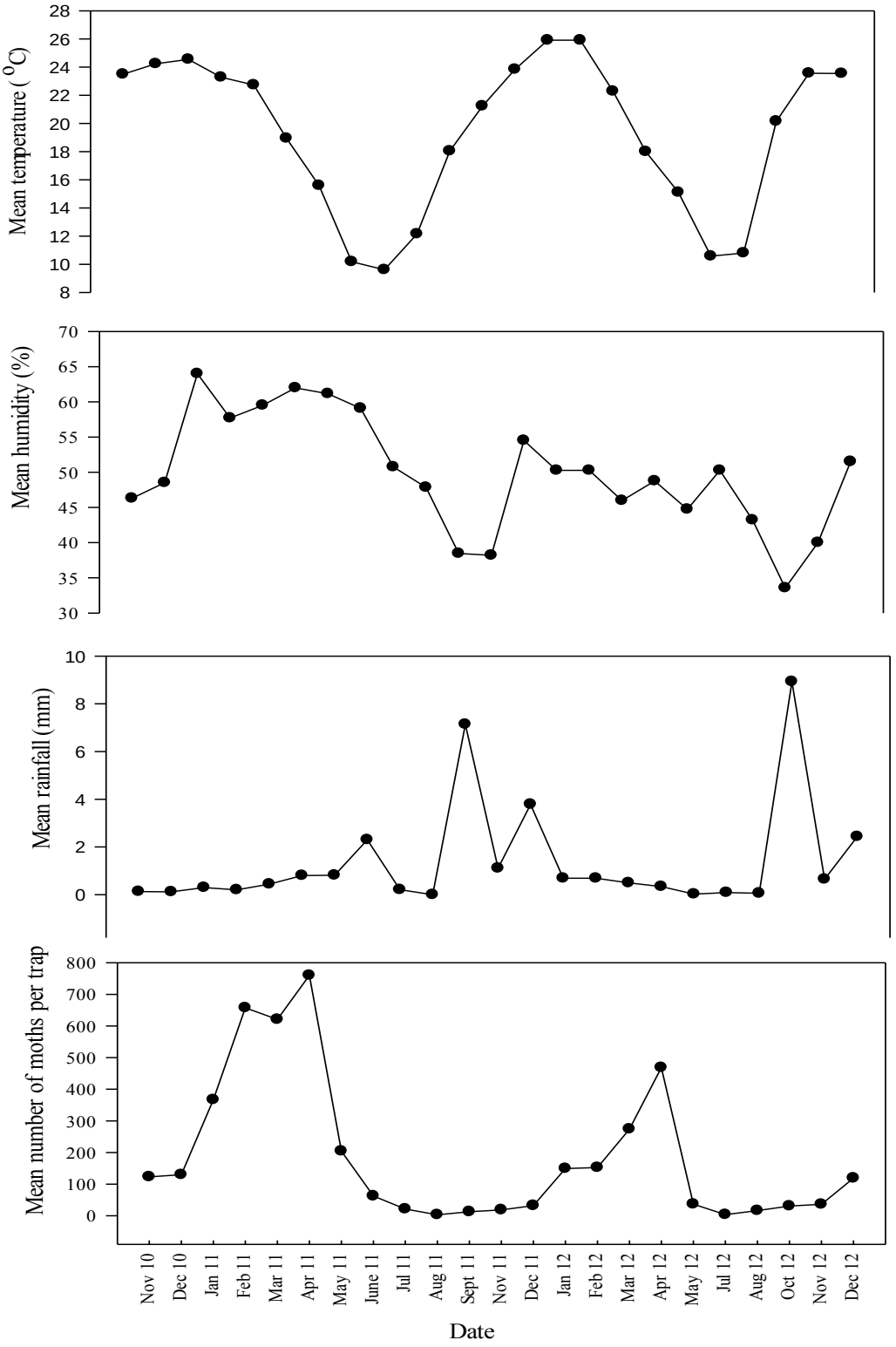


Figure 6.2 *Bilobata subsecivella* flight activity, measured as the mean number of moths caught in pheromone traps on a monthly basis, in relation to the average monthly rainfall, humidity and temperature at the Vaalharts groundnut site.

Nelspruit

At Nelspruit, the flight activity of *B. subsecivella* in the 2010/2011 season had two peaks. The first peak occurred in March 2011 and the second peak, which was smaller than the first, occurred in May 2011 (Figure 6.3). These peaks coincided with the establishment of the crop until harvesting. The numbers of moths caught from June 2011 to December 2011 were very low (Figure 6.3). Moth populations started to pick up again from January 2012 to July 2012; thereafter, they declined reaching their lowest values in December 2012 (Figure 6.3). During the 2011/2012 season, the numbers caught were not as prominent as in the 2010/2011 season. At Nelspruit, the average monthly rainfall over the duration of the study ranged between 0 and 10 mm per month and fell in December 2010, March 2011, September 2011 and December 2011 in the 2010/2011 season (Figure 6.3). This period corresponded with low numbers of moth catches. During the 2011/2012 season, rainfall remained very low (between 0 to 2 mm) from December 2011 to July 2012 and thereafter increased again up to 8 mm in December 2012. Average maximum temperatures of 26 ^{o}C, minimum temperatures below 14 ^{o}C (for summer and winter months, respectively) and relative humidity between 75% and 45% were recorded during the study period (Figure 6.3). Table 6.2 indicates a negative correlation between *B. subsecivella* moth catches and temperature which was statistically significant ($r = -0.773$; $p < 0.05$). There was no association between moth catches and either rainfall or humidity (Table 6.2).

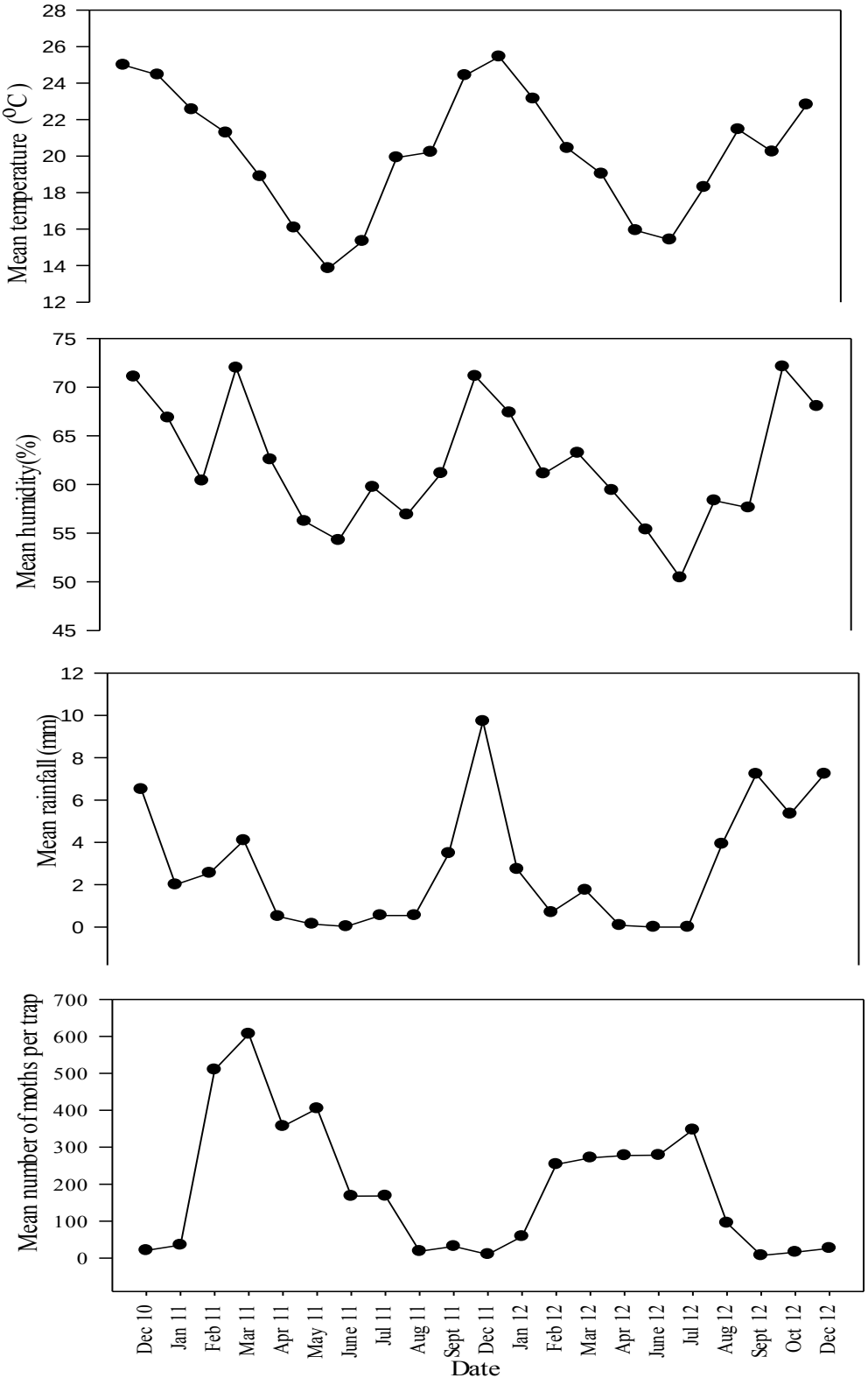


Figure 6.3 *Bilobata subsecivella* flight activity, measured as the mean number of moths caught in pheromone traps on a monthly basis, in relation to the average monthly rainfall, humidity and temperature at the Nelspruit groundnut site.

Manguzi

Manguzi reflected low numbers of *B. subsecivella* moths caught in traps compared to catches at Vaalharts and Nelspruit. This was despite the fact that groundnut is planted in the area by local farmers for longer periods, extending from June to December. From November 2010, the numbers of moths caught in traps increased from planting until February 2011 and then decreased to a minimum in April 2011. There were two peaks of moth flight activity noted at Manguzi during the 2010/2011 season. The first peak occurred in February, coinciding with the establishment of the second crop. The second peak occurred in May, corresponding with the harvesting of the second crop. The numbers of moths caught increased again from September 2011 to May 2012 and thereafter dropped from June to October 2012. The highest peak in moth flight activity was obtained between January and April in 2012, corresponding with the reproductive phase of the crops until harvesting (Figure 6.4). The numbers of moths caught in traps placed in farmers' fields of groundnut crops, which were planted in July 2011, were very low (numbers ranging between 1 and 5 per trap); hence, the data are not presented in this chapter. Average monthly rainfall received at Manguzi for the duration of the study ranged between 0 and 12 mm (Figure 6.4). For the 2010/2011 season, most rainfall (up to 12 mm) fell in December 2010 and January 2011 corresponding with very low *B. subsecivella* moth catches. Thereafter, rainfall remained low from February 2011 to October 2011. During the 2011/2012 season, an average of 4 mm fell between October 2011 and March 2012. Average minimum temperatures of 16 ^{o}C, maximum temperatures of 26 ^{o}C (for winter and summer months, respectively) and average relative humidity between 84% and 66% were recorded during the study period (Figure 6.4). A Pearson's test for correlation indicated that there was no association between moth catches and any of the environmental factors (rainfall, temperature and humidity) (Table 6.2).

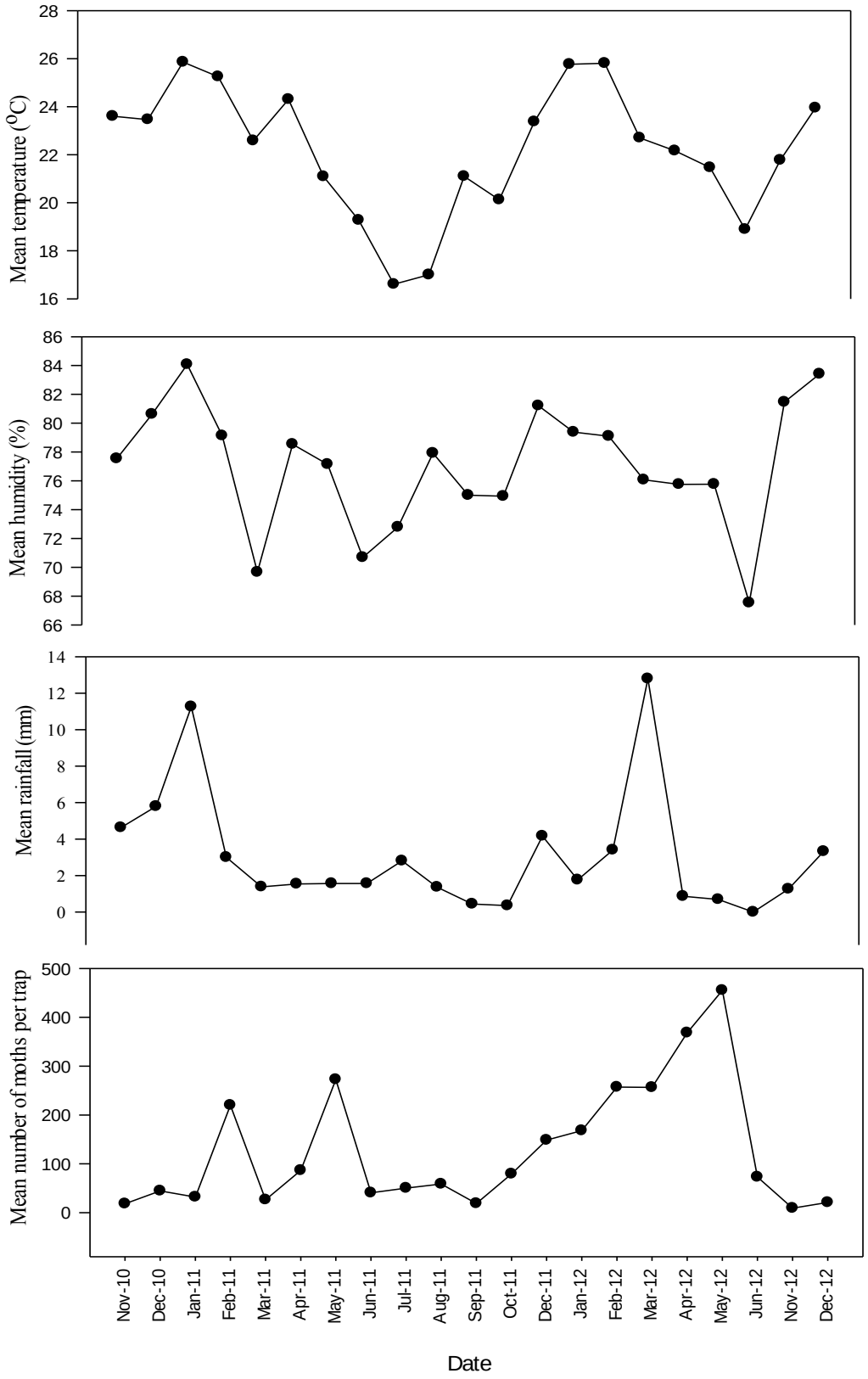


Figure 6.4 *Bilobata subsecivella* flight activity, measured as the mean number of moths caught in pheromone traps on a monthly basis, in relation to average monthly rainfall, humidity and temperature at the Manguzi groundnut site.

Brits

Brits had the lowest numbers of *B. subsecivella* moths caught in traps throughout the monitoring period, compared to catches at the other four sites. The numbers of moths caught in traps increased from December 2010 to March 2011, coinciding with the reproductive phase of the groundnut crop until harvesting; thereafter, the numbers decreased to a minimum in February 2012. No catches were recorded from June to November 2011. Moth catches started to pick up again from February 2012 to June 2012. No catches were recorded from July to November in 2012. At Brits, there were two peaks of moth flight activity. The first peak occurred in February 2011 whereas the second peak occurred in June 2012. The highest peak in numbers of moths caught was obtained in 2010/2011(Figure 6.5). At Brits, the average monthly rainfall ranged between 0 and 6 mm and fell between December 2010 and June 2011, September 2011 and April 2012, and August to October 2012. Brits did not receive any rainfall for the period between April 2012 and July 2012; this period corresponded with the second peak of *B. subsecivella* moth flight activity. Average maximum summer temperatures of 25 ^{o}C, minimum winter temperatures below 10^{o} C and relative humidity between 70% and 45% were recorded for the duration of the study (Figure 6.5). A Pearson's test for correlation indicated that there was no association between moth catches and any of the environmental factors (rainfall, temperature and humidity) (Table 6.2).

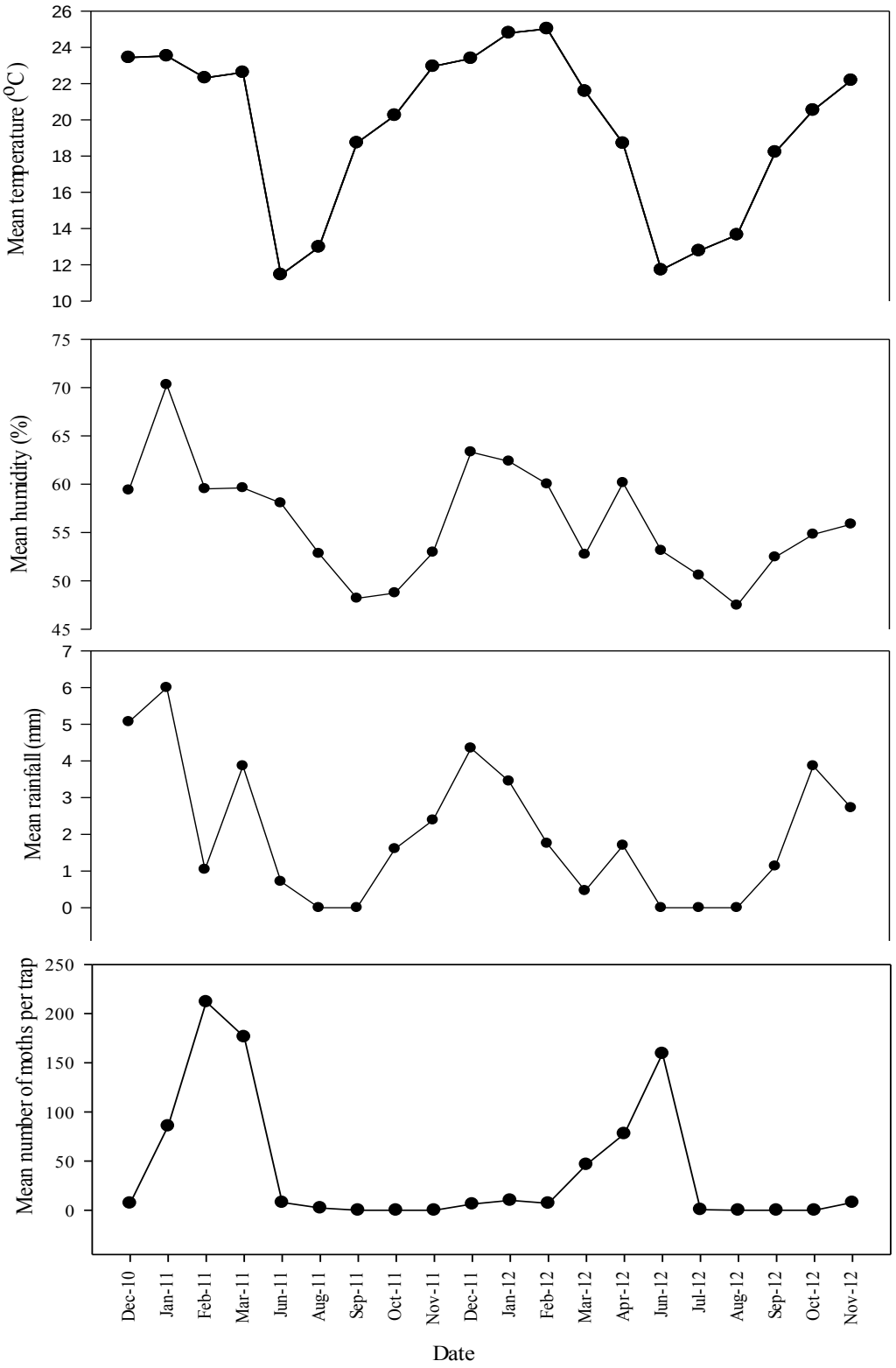


Figure 6.5 *Bilobata subsecivella* flight activity, measured as the mean number of moths caught in pheromone traps on a monthly basis, in relation to average monthly rainfall, humidity and temperature at the Brits groundnut site.

Bhekabantu

At Bhekabantu, the numbers of *B. subsecivella* moths caught in traps were lower than at Vaalharts and Nelspruit, but higher than at Manguzi and Brits. The numbers of moths caught increased from November 2010 until February 2011 and then decreased to a minimum in April 2011. This period corresponded with the reproductive phase of the crops until harvesting. The numbers of moths caught in traps remained low from April 2011 to September 2011. Thereafter, the number started to increase again from September 2011 to May 2012. A decreasing trend in numbers of moths caught was observed again from May 2012 till November 2012. There were two distinct peaks of *B. subsecivella* moth flight activity noted at Bhekabantu during the 2010/2011 season. The first peak occurred in February 2011 whereas the second peak occurred in May 2011. In the 2011/2012 season, there was one peak of moth flight activity which occurred in April/May (Figure 6.6).

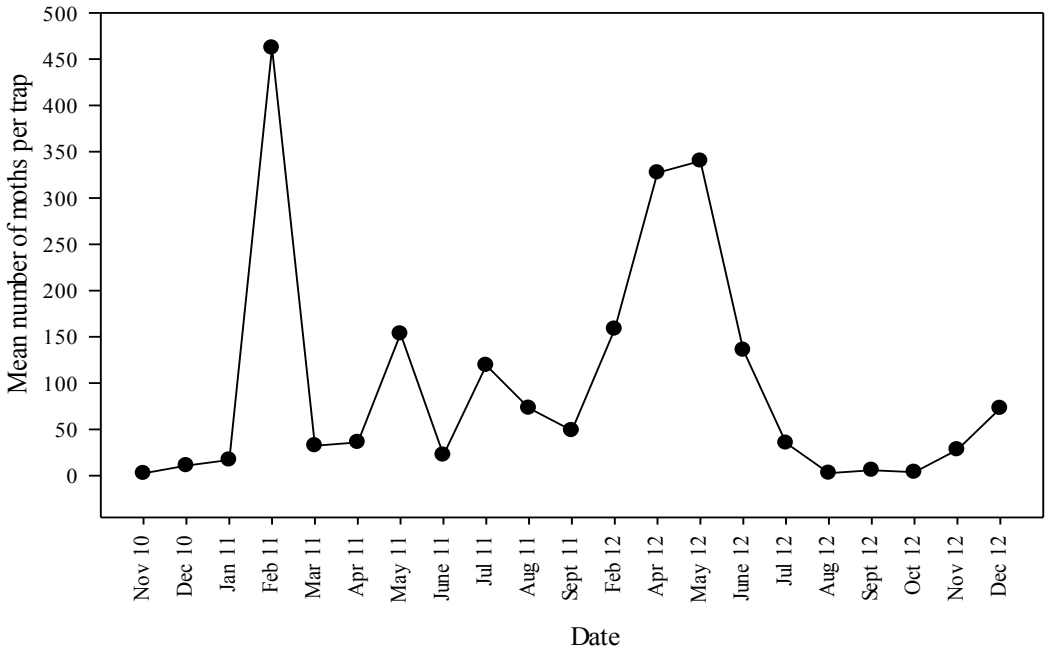


Figure 6.6 *Bilobata subsecivella* flight activity, measured as the mean number of moths caught in pheromone traps on a monthly basis, at the Bhekabantu groundnut site (environmental factors not recorded).

Table 6.2. Pearson's correlation between *B. subsecivella* moth catches and environmental factors (temperature, rainfall and humidity) at four sites where these factors were recorded.

Site	Statistical values	Temperature	Rainfall	Humidity
Nelspruit	r	-.773*	-.357	-.276
	p	.025	.386	.508
	n	8	8	8
Vaalharts	r	.232	-.371	.803*
	p	.580	.366	.016
	n	8	8	8
Manguzi	r	-.394	.344	-454
	p	.335	.405	.258
	n	8	8	8
Brits	r	-.559	-.210	-.030
	p	.150	.618	.944
	n	8	8	8

*Correlation is significant at the 0.05 level (2-tailed).

6.4.2 *Bilobata subsecivella* infestation on crops

2010/2011 season

Bilobata subsecivella infestation on groundnut was observed in both early (January) and late-planted (November) groundnut crops at all locations (Table 6.3). At all locations, the infestation rate was higher in the late-planted crop compared to the early-planted crop. However, larval infestation was observed on groundnut crops at five or more weeks after crop emergence. This was evident by the plant damage which was confined to the top young leaves during the first scouting. At all locations, especially Vaalharts, high numbers of *B. subsecivella* moths caught in traps matched the high larval infestation rates observed on groundnut crops. Leaf symptoms in early-planted crops were different from those found in late plantings. In the former, symptoms included a small brown 'bubble' formed in the midrib region, whereas in the latter, there was extensive damage to the leaf, including the folding of two leaves together with the larvae/pupae sandwiched in between. In the late-planted crops, infestations occurred at all leaf levels indicating that there had been more than one generation of moths. Dead portions of leaves that had supported older generations were darker and carried pupae. Towards the end of the growing season in all locations, most of the mines were empty, which corresponded with the low numbers of males caught in the traps.

At Vaalharts, the proportion of infested groundnut plants in the early-planted crop was 99.1% and the average number of infested leaflets per plant was 11.42. In the late-planted crop, the proportion of infested groundnut plants (27.8%) was low during the first scouting (January 2011) but increased substantially to 100% in May 2011 (Table 6.3). At Bhekabantu, the proportion of infested groundnut plants in the late-planted crop was low (30.3%) during the first scouting (January 2011) but increased significantly to 100% in April 2011 (Table 6.3).

In scouting carried out at 70 days after planting at Manguzi, 100% of groundnut plants in the early-planted crop were infested and the average number of infested leaflets was 12.6. The late-planted groundnut crop also had 100% of plants infested and 4.2 infested leaflets per plant at the first scouting carried out at 54 days after planting (March 2011). At the second scouting conducted in April 2011(91 days after planting), the proportion of infested groundnut plants was 100% and the proportion of infested leaflets was 26.2%. At Nelspruit, the proportion of infested plants was 100% and the proportion of infested leaflets was 42% in the late-planted crop (scouting at 65 days after planting) compared to 7% plant infestation in the early-planted crop (scouting at 83 days after planting). At Brits, 18.7% of plants were infested (Table 6.3).

At the three sites scouted at Manguzi, where farmers' groundnut crops were planted in July 2011, *B. subsecivella* infestation was only observed in December 2011. At one of the sites, 14% of plants were infested with an average of 1.19 infested leaflets per plant. At the second site, 30.5% of plants were infested with an average of 1.45 infested leaflets per plant. At the third site, only 4% of the plants were infested with an average of 0.8 infested leaflets per plant (Table 6.4).

Table 6.3. Infestation of *B. subsecivella* on groundnut at Vaalharts, Nelspruit, Brits, Manguzi and Bhekabantu.

Site	Planting date	Scouting date	% Plants infested	Average number of infested leaflets per plant	% Infested leaflets per plant
Brits	15 November 2010	20 January 2011	18.7	1.16	-
Vaalharts	04 November 2010	19 January 2011	99.1	11.42	-
	06 January 2011	19 January 2011	27.8	2.07	-
		02 May 2011	100	-	100
Nelspruit	18 November 2010	21 January 2011	7	1.08	-
	25 January 2011	18 April 2011	100	-	42
Manguzi	17 November 2010	27 January 2011	100	12.6	-
	27 January 2011	23 March 2011	100	4.2	-
		27 April 2011	100	67.7	26.2
Bhekabantu	18 November 2010	28 January 2011	30.3	2.42	-
	28 January 2011	23 March 2011	100	4.17	-
		26 April 2011	100	-	10

-Not recorded.

Table 6.4. Infestation of *B. subsecivella* on farmers' groundnut crops that were planted in July 2011 at Manguzi.

Planting date	Scouting date	Farmer	% Plants infested	Average number of infested leaflets per plant
25-29 July 2011	02 December 2011	Farmer 1	14	1.19
		Farmer 2	30.5	1.45
		Farmer 3	4	0.8

2011/2012 season

Bilobata subsecivella infestation on crops and wild plant hosts was only observed during the crop growing season. No *B. subsecivella* infestations were detected on wild plant hosts during the off-season as well as the winter months, despite *B. subsecivella* moths being caught in traps. Detailed results on *B. subsecivella* infestation on crops for the 2011/2012 season are reported in Chapter 5 (section 5.4.)

6.5 Discussion

Results from the current study revealed fluctuations in *B. subsecivella* populations between locations, seasons and years. Similar observations were reported in India (Amin & Mohammad 1980; Logiswaran & Mohanasundaram 1986) and previously in South Africa (Van der Walt 2007). In the current study, high numbers of *B. subsecivella* moths were trapped during the 2010/2011 season at Vaalharts, Nelspruit, Bhekabantu and Brits, whereas at Manguzi the highest peak in *B. subsecivella* populations was obtained in the 2011/2012 season. At all locations, the highest numbers of moths were trapped between January and May (Figures 6.2 to 6.6) which coincides with the reproductive phase of the crop until harvesting. These observations are similar to the findings of Du Plessis (2011). During the reproductive stage, the reduction in leaf area caused by *B. subsecivella* results in yield losses (see Chapter 5) due to the reduced supply of assimilates to the developing pods (Board *et al.* 2010).

Though low in numbers, *B. subsecivella* moths were still trapped during the winter months (June and July) at all sites with the exception of Brits. This observation indicates that the pest is active throughout the year. However, off-season survival tactics of the pest still need to be investigated further as the role of secondary hosts in maintaining pest populations in the absence of the main crops is ambiguous. Du Plessis (2003) reported that lucerne crops contribute to the off-season survival of *B. subsecivella* populations on groundnut in areas where both of these crops are cultivated. In contrast, Buthelezi *et al.* (2013) failed to find the moth on lucerne during the winter months and even in the summer months, the crop was scarcely attacked and the infestation on the crop was only noticed in March 2012. *Bilobata subsecivella* infestation on pigeon pea occurred in March to April only, and both perennial crops (lucerne and pigeon pea) were pest free from May 2012 until the end of the monitoring of *B. subsecivella* infestations in mid-December 2012, despite moths being caught in traps. In the failure to obtain evidence of infestation on the host plants in winter, it is tempting to think that the low fight activity (2 to 5 moths per trap per 2 weeks) in winter could be from emergence of moths from hibernation/diapause. The re-emergence of the moth in summer after a frosty winter, during which there is no flight activity, also indicates that the pest somehow survives the winter independent of its host plants.

Field observations at all sites indicated that late-planted (January) crops seemed to be more severely infested than the early-planted (November) crops. These results are similar to those reported by the ARC (2007), but were contrary to reports from India (Lewin *et al.* (1979) in Shanower *et al.* (1993)) that early planting resulted in higher infestations of *B. subsecivella*. However, there were other reports from India (Logiswaran *et al.* (1982) in Shanower *et al.* (1993)) that later plantings were more heavily attacked than the early plantings, as occurred in our study. One of the more interesting findings from the present study was the situation at Manguzi, where farmers' groundnut crops that were planted in July remained pest free until December, even though *B. subsecivella* moths were caught in traps during that period. It is currently not known why *B. subsecivella* does not attack the crop between July and December. However, these observations indicate that timing of planting (cultural control) should be taken into consideration, together with biological and chemical control, when developing an integrated management strategy against the pest.

Another puzzling observation arising from this study was that, even though *B. subsecivella* moths were being caught in traps, larval infestations on the groundnut crop were observed some 5-6 weeks after crop emergence, which coincides with the flowering and pegging stage of the crop. This may suggest the presence of volatile compounds produced by groundnut during flowering that result in the crop being susceptible to *B. subsecivella* infestation.

Shanower *et al.* (1995) challenged the issue of climatic factors causing fluctuations in *B. subsecivella* populations between the seasons, on the basis that there is no consensus on which factors are involved. One study (Lewin *et al.* (1979) in Shanower *et al.* (1995)) reported that temperature was positively and rainfall negatively correlated with *B. subsecivella* incidence, while another (Logiswaran *et al.* (1982) in Shanower *et al.* (1995)) reported a significant negative correlation between maximum and minimum temperatures and *B. subsecivella* infestation levels, but no correlation with rainfall. Khan & Raodeo (1987) considered rainfall to be the key factor involved in regulating *B. subsecivella* populations in groundnut. Wheatley *et al.* (1989) reported that *B. subsecivella* infestation is more severe on drought stressed plants. Muthiah & Abdul Kareem (2002), in their study on the effects of climate on *B. subsecivella* catches in light traps in India, reported that relative humidity alone exerted a significant positive influence, whereas the maximum and minimum temperatures as

well as rainfall exerted a non-significant negative influence on *B. subsecivella* moth emergence.

In the current study, there was a significant negative correlation between *B. subsecivella* moth catches and temperature at Nelspruit, whereas humidity had a significant positive association with moth catches at Vaalharts. However, it was noted from the graphs (Figures 6.2 to 6.5) that high moth catches coincided with low rainfall periods, whereas low moth catches coincided with the rainy periods. Studies in India have reported that heavy rainfall reduced *B. subsecivella* populations (Amin 1987) or that rainfall was negatively correlated with *B. subsecivella* incidence (Lewin *et al.* (1979) in Shanower *et al.* (1995)). However, Shanower *et al.* (1995) reported that rainfall has no effect on the survival of both *B. subsecivella* eggs and larvae, but suggested that it may influence *B. subsecivella* populations in elusive ways. For example, heavy and persistent rainfall may interfere with adult oviposition while fungal and other pathogens that affect the moth's immature stages may be favoured by rainfall (Shanower *et al.* 1995).

6.6 Conclusions

Bilobata subsecivella moths were caught in traps at all sites for the entire duration of the study, with the exception of Brits where moths were not trapped during the winter months. This is indicative of the pest's year-round activity in areas where winter temperatures are mild. *Bilobata subsecivella* population fluctuations during the crop growing period varied between the different localities, indicating modulation by the local environment. *Bilobata subsecivella* infestation on groundnut was observed at five/six weeks after crop emergence. Based on this observation, there is a need to determine whether there are volatile compounds produced by groundnut during flowering which cause the crop to be infested by the pest. Late-planted crops were more severely infested by the moth compared to the early-planted crops. Therefore, the timing of planting should be considered when developing integrated control strategies against the pest. Moreover, there is also a need to determine the pest's between-season survival tactics in Africa for the same purpose. Given the lack of significant correlations between moth catches and climatic factors at most of the study sites, it is clear that it will be very difficult to predict *B. subsecivella* incidence in South Africa based on environmental factors alone. It could be hypothesized that there might well be other factors

that play a role in regulating *B. subsecivella* populations in South Africa. In the current study, monitoring *B. subsecivella* adult numbers with pheromone traps during the growing season, as well as the off-season, provided some useful ecological information regarding the pest. The study revealed that the pest is already present before the crops are planted and also assisted in establishing the pest's peak and off-peak periods. This information should be useful in determining control strategies such as pesticide application schedules. Therefore, the monitoring of *B. subsecivella* populations with pheromone traps should be incorporated into approaches aimed at reducing their infestation on crops.

6.7 References

AMIN, P.W. 1987. Insect pests of groundnut in India and their management. In: Veerabhadra Rao, M. & Sithanantham, S. (Eds.) *Plant Protection in Field Crops*. 219-333. Plant Protection Association of India, Hyderabad.

AMIN, P.W. & MOHAMMAD, A.B. 1980. Groundnut pest research at ICRISAT. 158-166. In: Gibbons, R.W. (Ed.) *Proceedings of the International Workshop on Groundnut, 13-17 October 1980*. ICRISAT, Patancheru, Andhra Pradesh, India.

ARC-GRAIN CROPS INSTITUTE. 2007. Development of a control strategy for groundnut leaf miner on groundnut. Online at: http://www.arc.agric.za (Accessed on 15 March 2014).

ARUN, P.R. & VIJAYAN, V.S. 2004. Patterns in abundance and seasonality of insects in the Siruvani Forest of Western Ghats, Nilgiri Biosphere Reserve, Southern India. *The Scientific World Journal* **4**: 381-392.

BOARD, J.E., KUMUDINI, S., OMIELAN, J. PRIOR, E. & KAHLON, C S. 2010. Yield response of soybean to partial and total defoliation during the seed filling period. *Crop Science* **50**:703-712.

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2012.The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soybean moth (*Aproaerema simplixella* PS 1) (Walker) (Lepidoptera: Gelechiidae). *African Journal of Agricultural Research* **7**(38): 5285–5292.

BUTHELEZI, N.M, CONLONG, D.E. & ZHARARE, G.E. 2013. A comparison of the infestation of *Aproaerema simplexella* on groundnut and other known hosts for *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *African Entomology* **21**(2): 183-195.

CANNON, R.J.C., KOERPER, D. & ASHBY, S. 2004. Gypsy moth, *Lymantria dispar*, outbreak in northeast London, 1995–2003. *International Journal of Pest Management* **50**: 259-273.

DAS, S.B. 1999. Seasonal activity of groundnut leaf miner, *Aproaerema modicella* in west Nimer Valley, Madhya Pradesh. *Insect Environment* **5**: 69.

DRAKE, V.A., WANG, H.K. & HARMAN, I.T. 2002. Insect monitoring radar: remote and network operation. *Computers and Electronics in Agriculture* **35**: 77-94.

DU PLESSIS, H. 2002. Groundnut leaf miner, *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48-49.

DU PLESSIS, H. 2003. First report of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut, soybean and lucerne in South Africa. *South African Journal of Plant and Soil* **20**:48.

DU PLESSIS, H. 2011. Flight activity of the groundnut leaf miner, *Aproaerema modicella* (Deventer) in the groundnut production areas of South Africa. *South African Journal of Plant and Soil* **28:** 239-243.

EPIERU, G. 2004. Participatory evaluation of the distribution, status and management of the groundnut leaf miner in the Teso and Lango farming systems. Final Technical Report of a Project Supported by NARO/DFID, Saari Kampala, Uganda.

GOMULKIEWICZ, R., HOLT, R.D., BARFIELD, M. & NUISMER, S.L. 2010. Genetics, adaptation and invasion in harsh environments. *Evolutionary Applications* **3**: 97-108.

HALL, D.R., BEEVOR, D.G., CAMPION, D.J., CHAMBERLAIN, D.J., CORK, A., WHITE, R., ALMESTRE, A., HENNEBERRY, T.J., NANDAGOPAL,V., WIGHTMAN, J.A. & RANGA RAO, G.V. 1994. Identification and synthesis of new pheromones. pp.1-9. In: McVeigh, L.J., Hall, D.R. & Beevor, P.S. (Eds.) *Proceedings of OILB meeting, NRI, Chathman Maritime, Kent, UK*.

HIRAO, T.M., MURAKAMI, M. & KASHIZAKI, A. 2008. Effects of mobility on daily attraction to light traps: comparison between lepidopteran and coleopteran communities. *Insect Conservation and Diversity* **1:** 32-39.

HOLT, R.D., BARFIELD, M. & GOMULKIEWICZ, R. 2005. Theories of niche conservatism and evolution: Could exotic species be potential tests? In: Sax, D.F., Stachowicz, J.J. & Gaines, S.D. (Eds) *Species Invasions: Insights into Ecology, Evolution and Biogeography* 259–290. Sinauer Associates, Sunderland, MA, U.S.A.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**: 1-9.

KHAN, M.I. & RAODEO, A.K. 1987. Seasonal incidence of groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Punjabrao Krishi Vidyapeeth Research Journal* **11**: 93-96.

KLUEKEN, A.M., HAU, B., ULBER, B. & POEHLING, H.M. 2009. Forecasting migration of cereal aphids (Hemiptera: Aphididae) in autumn and spring. *Journal of Applied Entomology* **133**: 328-344.

LEWIS, T. 1981. Pest monitoring to aid insecticide use. *Philosophical Transactions of the Royal Society B. Biological Sciences* **295:** 153-162.

LOGISWARAN, G. & MOHANASUNDARAM, M. 1986. Influence of weather factors on the catches of moths of groundnut leaf miner *Aproaerema modicella* (Lepidoptera: Gelechiidae) in the light trap. *Entomon* **12**: 147-150.

MERRIL, S.C., GEBRE-AMLAK, A., ARMSTRONG, J.S. & PEARIRS, F.B. 2010. Nonlinear degree-day models of the sunflower weevil (Curculionidae: Coleoptera). *Journal of Economic Entomology* **103**: 303-307.

MUNYULI, T.M.B., LUTHER, G.C., KYAMANYWA, S. & HAMMOND, R. 2003. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda and Democratic Republic of Congo. *African Crop Science Conference Proceedings* **6**: 231-237.

MUTHIAH, C. & KAREEM, A.A. 2002. Correlation studies on the attraction of groundnut leaf miner *Aproaerema modicella* moths and weather factors. *International Arachis Newsletter* **22**: 51-53.

PAGE, W.W., EPIERU, G., KIMMINS, F.M., BUSOLOBULAFU, C. & NALYONGO, P.W. 2000. Groundnut leaf miner *Aproaerema modicella*: a new pest in eastern districts of Uganda. *International Arachis Newsletter* **20**: 64-66.

PRASAD, Y.G. & PRABHAKAR, M. 2012. Pest monitoring and forecasting. In: Abrol, D.B & Uma, S. (Eds.) Integrated Pest Management Principles and Practice. 41-57. CABI, UK.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993. Biology and control of the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**: 3-10.

SHANOWER, T.G., GUTIERREZ, A.P. & WIGHTMAN, J.A. 1995. Effect of simulated rainfall on eggs and larvae of the groundnut leaf miner, *Aproaerema modicella. International Arachis Newsletter* **15**: 55-56.

STASTNY, M., BATTISTI, A., PETRUCCO TOFFOLO, E., SCHLYTER, F. & LARSSON, S. 2006. Host plant use in the range expansion of the pine processionary moth, *Thaumetopoea pityocampa*. *Ecological Entomology* **31**: 481-490.

SUBRAHMANYAM, P., CHIYEMBEKEZA, A.J. & RAO, G.V.R. 2000. Occurrence of groundnut leaf miner in northern Malawi. *International Arachis Newsletter* **20**: 66-67.

TRAVIS. J.M., HAMMERSHØJ, J.M. & STEPHENSON, C. 2005. Adaptation and propagule pressure determine invasion dynamics: insights from a spatially explicit model for sexually reproducing species. *Evolutionary Ecology Research* **7**: 37-51.

VAN DER WALT, A. 2007. Small holder farmers' perceptions, host plant suitability and natural enemies of the groundnut leafminer, *Aproaerema modicella* (Lepidoptera: Gelechiidae) in South Africa. M.Env. Sci. Dissertation, North-West University, Potchefstroom, South Africa.

WHEATLEY, J.A. WHEATLEY, A.R.D, WIGHTMAN, J.A., WILLIAMS, J.H. & WHEATLEY, S.J. 1989.The influence of drought stress on the distribution of insects on four groundnut genotypes grown near Hyderabad, India. *Bulletin of Entomological Research* **79**: 567-577.

ZALUCKI, M.P. & FURLONG, M.J. 2005. Forecasting *Helicoverpa* populations in Australia: a comparison of regression based models and a bioclimatic based modelling approach. *Insect Science* **12**: 45–46.

Thesis overview

When *Bilobata subsecivella* (Zeller) (Lepidoptera: Gelechiidae) suddenly surfaced in Uganda in 1998 as a new pest of groundnut (*Arachis hypogaea* L.) in Africa (Epieru 2004), the continent was confronted with the problem of having too little information on the biology and ecology of the pest in its new environment to make informed decisions on its management. Thus, the present study was initiated to study the biology and ecology of *B. subsecivella* to generate the necessary information that would facilitate the management of this potentially devastating pest in South Africa. The major questions that guided the research were:

(i) How widespread is the problem of *B. subsecivella* in the groundnut production areas of South Africa?
(ii) What is the origin/source of the pest?
(iii) How does the pest survive throughout the year?
(iv) What are the host crops and wild plants of the pest?
(v) Can the pest be effectively controlled by chemicals such as cypermethrin?

Thus, the main activities of my research focused on determining the extent of occurrence of the pest in the groundnut producing areas of South Africa, its identity and intra- and inter-population genetic diversity in different localities in South Africa, its crop and wild plant hosts, its flight activities and the effect of chemical control on its negative impacts.

The distribution of the *B. subsecivella* in groundnut producing areas in South Africa

Before the present study, *B. subsecivella* had been detected in the Vaalharts irrigation scheme in the Northern Cape Province (Du Plessis 2002) and at other locations in the Free State, Northern Cape, North West, Mpumalanga and Limpopo provinces (Du Plessis 2003; Van der Walt 2007). The present study reconfirmed the occurrence of *B. subsecivella* in the North West province, notably at Potchefstroom, Vaalharts and Brits, and in the Mpumalanga Lowveld (see Chapter 2). Additional areas identified in this study were Manguzi and Bhekabantu in the northern coastal region of South Africa in KwaZulu-Natal (see Chapter 2). With the exception of Manguzi and Bhekabantu, which are about 60 km apart, the sites are more than 400 km apart and the locations differ considerably in climate. This indicated that *B. subsecivella* has the ability to adapt to a wide range of climates, being able to cope in

locations characterised by warm climate throughout the year (e.g. Manguzi, Limpopo) as well as in locations characterized by severe frosts in winter (e.g. Brits, Potchefstroom). Coping with such widely different climates requires different survival strategies in each of the major climatic zones. Currently, there is no information on how *B. subsecivella* survives frost conditions in localities like Brits and Potchefstroom or bridges the cropping seasons in localities like Manguzi which is warm throughout the year.

The identity and origin of *B. subsecivella* in South Africa

When groundnut leaf miner (GLM) was first noticed in Africa, the general view was that it was an invasion of *Aproaerema modicella* (Deventer) from the Indo-Asia continent (Kenis & Cugala 2006). Thus, at the initiation of the present study, the GLM occurring in South Africa was assumed to be *A. modicella* from India. Indeed, the GLM occurring in South Africa was observed in the present study to display similarities to *A. modicella* in crop damage symptoms and morphological features of the adult moths and the larvae (see Chapter 2). In addition, Van der Walt *et al.* (2008) determined by way of anatomical observations that the male larvae of the GLM occurring in South Africa have similar genitalia to those of *A. modicella* in the Indo-Asian contiment. However, subsequent analyses of sequences of mtDNA COI extracted from GLM specimens from six widely separated sites in South Africa matched 100% with those of both *A. modicella* from the Indo-Asian contiment and *A. simplexella* (Walker) from Australia (see Chapter 3). These different 'species' from the three continents were thus synonymised under the name *Bilobata subsecivella* (Zeller) based on the CO1 gene sequences (see Chapter 3).

In Australia, *B. subsecivella* (known there as *A. simplexella*) is a native moth that attacks soya bean (*Glycine max* (L.) Merr.), but not groundnut. This pest also displays similar leaf damage symptoms to those of *B. subsecivella* in South Africa on soya bean (see Chapter 2) as well as similar morphological features (see Chapter 2). Furthermore, the *B. subsecivella* moths in South Africa, India and Australia all respond similarly to the same sex pheromones (see Chapter 6). This raised questions regarding the origin and identity of the *B. subsecivella* populations occurring in South Africa (and Africa). In an attempt to determine the origin of the pest, specimens of *B. subsecivella* from India and Australia were collected from ICRISAT in India and Anstead suburb of Brisbane in Australia, respectively, for comparison of specific regions of their mitochondrial DNA and nuclear DNA (see Chapter 4). The collection of *B.*

subsecivella specimens in Brisbane was successfully accomplished using pheromone lures developed from the sex pheromones of *B. subsecivella* found in India, which further supported the hypothesis that *B. subsecivella* populations in India, Australia and Africa are either one species or are very closely related.

A more comprehensive assessment of both the mitochondrial and nuclear DNA analyses (see Chapter 4) revealed that *B. subsecivella* populations in India, Australia and Africa probably constitute a single species. According to the phylogenetic analysis of the 28S gene region, there was no genetic diversity among *B. subsecivella* populations in Australia, Africa and India, which indicates that these populations are genetically very closely related. Phylogenetic trees for COI, COII, cytb and EF-1 ALPHA revealed a lack of genetic diversity between *B. subsecivella* populations in Africa and India (see Chapter 4) indicating that they are from the same origin. The mtDNA COI gene analyses also revealed that there are *B. subsecivella* populations in Australia that are similar to the *B. subsecivella* populations in southern Africa and India. However, there is more genetic diversity within the *B. subsecivella* population in Australia, which suggests that the *B. subsecivella* populations now occurring in southern Africa and India could have originated from Australia. Therefore, at present, it could be suggested that Australia, and not India, is the origin of the *B. subsecivella* that invaded Africa. However, in support of this conclusion, there is a need for analyses of more specimens from different regions of Indo-Asia to determine the genetic diversity of the *B. subsecivella* population in India. In addition, there is a need for comprehensive anatomical comparisons to support the genetic evidence that the *B. subsecivella* populations on the three different continents (Africa, Indo-Asia and Australia) constitute a single species. Also, whatever the origin of *B. subsecivella* in South Africa, the question of how this pest found its way to Africa is still unanswered.

Inter-population and intra-population genetic diversity

The occurrence of *B. subsecivella* in South Africa, in locations that differ greatly in climatic conditions (see Chapter 2), raised the possibility of genetic segregation in *B. subsecivella* according to climatic conditions that would facilitate its adaptation to climatic extremes that characterize the different locations. Thus, I expected to observe genetic differences as well as different survival strategies between populations from the different climatic regions. Furthermore, it was noted that there was very little infestation of pigeon pea (*Cajanus cajan*

L.) and lucerne (*Medicago sativa* L.) by *B. subsecivella*, which seemed to suggest that there could be genetic-based barriers that prevented *B. subsecivella* from attacking these crops, and that the few individuals that attacked pigeon pea and lucerne could be genetically different from those that did not. To investigate this, two nuclear regions (28S and EF-1 ALPHA) and three mtDNA regions (COI, COII and cytb) of specimens that were collected from groundnut, soya bean, pigeon pea and lucerne at five sites that differed widely in climatic conditions were analysed (see Chapter 4). Both the nuclear and mtDNA analyses were unable to differentiate between the populations from the five sites and the four crop species. Thus, based on the analyses of the DNA regions in the present study, there appears to be no intra- and inter-population genetic diversity in the *B. subsecivella* occurring in South Africa. This lack of inter-population genetic variation was in spite of the wide climatic variations between the locations from which the specimens were collected. Nevertheless, caution should be taken in the interpretation of these results since the analyses were performed using a few DNA regions. It is possible that there are pertinent genes, which play an important role in the adaptation of *B. subsecivella* populations to specific climates, which were not analysed in the present study.

At the initiation of the present study, there were two hypotheses relating to the possible source of infestation and the movement of *B. subsecivella* in South Africa. The first one suggested that the pest is spread by wind (The New Vision 2009). Therefore, 'pest rain' was considered to be a possible source of infestation, with the pest being transported from distant areas and even from across oceans by wind. This hypothesis was based on the observation that the occurrence of *B. subsecivella* on groundnut was episodic. If this hypothesis was true, *B. subsecivella* populations in the major infestation hotspots would be expected to be genetically similar. The second hypothesis suggested that the pest somehow arrived in South Africa (e.g. contaminated agricultural produce) and then established resident populations. If this hypothesis was true, the pest must have alternative hosts to maintain it throughout the year. Also, regional genetic differentiation of the pest was expected due to differences in climates that would require different survival strategies. The DNA analyses showed that the *B. subsecivella* populations in South Africa were of the same origin and were genetically the same (see Chapter 4), which favoured the first hypothesis. However, flight activity data showed that in areas that are warm throughout the year, adults of *B. subsecivella* were present throughout the year although there are episodic flares of epidemic infestations in these areas (see Chapter 6). The 'pest rain' hypothesis does not seem to be likely given the great distance

between South Africa and either India or Australia. The most likely scenario is that there are resident populations in South Africa from the same unknown origin, which have not yet differentiated genetically according to the different regions of South Africa.

Plant host range

Crop hosts

Although the present study suggested that *B. subsecivella* populations from Africa, India and Australia constitute the same species, there is variation in the crop species that are suitable to each of the three 'species'. The crop host range identified in the present study for the *B. subsecivella* occurring in South Africa included groundnut, soya bean and, to a lesser extent, pigeon pea and lucerne (see Chapters 2 and 5). An experiment conducted at Vaalharts, Nelspruit, Brits, Manguzi and Bhekabantu to assess infestation levels on five of the known crop hosts (groundnut, soya bean, pigeon pea lucerne and lablab bean) for the *B. subsecivella* population in India (see Chapter 5) indicated that the major crop hosts of the *B. subsecivella* occurring in South Africa are groundnut and soya bean, with soya bean being infested the most when both groundnut and soya bean are grown side by side. Pigeon pea was scarcely attacked, and it was extremely rare to find *B. subsecivella* larvae feeding or pupating on lucerne even when the crop was growing next to groundnut crops that were heavily infested with *B. subsecivella*. The *B. subsecivella* occurring in South Africa did not attack lablab bean and hence differed from the *B. subsecivella* population in India which readily attacks lablab bean. *Bilobata subsecivella* in Australia is not known to attack groundnut and in this respect differs from both *B. subsecivella* populations occurring in South Africa and India, which readily attack groundnut. There is no literature in the public domain that indicates whether the *B. subsecivella* population in Australia attacks lucerne and pigeon pea, although both crops are widely grown in that country. Understanding the genetic basis of the differences in the susceptibility of these crops to *B. subsecivella* populations occurring in India, Australia and South Africa (Africa) may provide a means of genetically manipulating these crops for the effective management of the pest.

Wild plant hosts

With respect to wild hosts, *B. subsecivella* in South Africa feeds on a wide range of plants (see Chapter 5). The host plant list obtained from the present study included nine species. Eight of them were additional to the 11 species provided by Van der Walt (2007). The host range of *B. subsecivella* in South Africa, identified from the current study, incorporated five plant families which included three species in the Leguminoseae; two species each in the Convolvulaceae and Malvaceae and one species each in the Lamiaceae and Asteraceae (see Chapter 5). Surprisingly, the host list included several non-legume species, viz. *Ipomoea wightii* (Wall) Choisy (Convolvulaceae), *Ipomoea sinensis* (Desr.) Choisy subsp. *blepharosepala* Hochst. ex. A. Rich. (Convolvulaceae), *Ocinum canum* (Sims) (Lamiaceae), *Pavonia burchellii* (DC.) (Dyer) (Malvaceae), *Acanthospermum hispidum* DC. (Asteraceae) and *Malvastrum coromandelianum* subsp. *coromandelianum*, (L.) (Garcke) (Malvaceae). In the list previously compiled for *A. modicella* in India (Shanower *et al.* 1993), only one of the 14 host plant species (*Boreria hispida* K. Sch. (Rubiaceae)) was a non-legume. Nevertheless, with the exception of *Glycine wightii* L. Merr. (Leguminoseae), the host plant species recorded in the present study were only infested when they were in close proximity (within 5 m) to heavily infested groundnut plants. This suggested that these wild species may not be used as host plants under other circumstances. Indeed, the wild host plants were free of *B. subsecivella* in winter when groundnut was not growing, which indicated that they were not used as refuge crops during that time. Nevertheless, the fact that *B. subsecivella* larvae could feed and survive to pupation on them provided evidence of flexibility in their host range. This may have contributed to the success of the pest in colonizing Africa.

Flight activity and occurrence of infestations on plants

One of the major questions of the study was how the pest behaves in the different environments where it occurs in South Africa. Flight activity data obtained through pheromone traps, in conjunction with observations of infestations on suitable plants, have provided some insight into the seasonal behaviour of the pest in relation to plant infestations and to the climatic characteristics of the locations (see Chapter 6). In areas such as Brits that have severely cold winters and are subject to frost, adult *B. subsecivella* were not caught in traps during the winter months. In this location, flight activity increased quickly in early summer, but was surprisingly not accompanied by infested host plants. In locations such as

Manguzi and Bhekabantu that have a warm climate throughout the year, *B. subsecivella* adults were active throughout the year. However, moth numbers were extremely low in off-cropping seasons (winter months), averaging only one to five moths caught per two weeks in pheromone traps (see Figures 6.2 to 6.6 in Chapter 6), and increased quickly in early summer. At Manguzi and Bhekabantu, the low flight activity in the winter months occurred despite the presence of actively growing wild host plants in winter which, surprisingly, were free of the pest. A possible explanation for the absence or low activity of the adult moths in winter, and the resumption of increased activity as the temperatures increase in summer, is that *B. subsecivella* somehow enters diapause in the winter months (off-cropping season) irrespective of whether the location has a severe winter or a mild winter. If this is true, then in locations with severe winters, diapause is enforced until the temperatures have increased in early summer, while in locations with mild winters some individuals break the diapause during the winter months; hence the occasional catch in the pheromone traps. However, the most puzzling observation was that suitable host plants (including groundnut) remained free of infestation by *B. subsecivella* during the winter months while in the presence of active adults, as noted at Manguzi (see Chapter 6). Although no *B. subsecivella* flight activity was observed during the winter months at Brits, sudden increases in numbers of moths caught in traps were observed at the beginning of the growing season (summer months). This observation further strengthens the hypothesis that *B. subsecivella* could diapause during the winter months/off-season.

Deductions from the data on the numbers of moths caught in pheromone traps in groundnut crops (see Chapter 6) indicate that *B. subsecivella* accomplishes between one and two generations in the cropping season. Generally, there were two peaks per season with a generation cycle of between 28 and 30 days (see Chapter 6). This knowledge is useful in programming chemical sprays that are targeted at preventing *B. subsecivella* population explosions that could adversely affect groundnut crops. Additional knowledge gained from the present study that could facilitate the chemical control of *B. subsecivella* in groundnut production, was that the pest starts attacking groundnut crops from the flowering and pegging stage (see Chapter 6).The reasons for this behaviour are currently unknown, but the information is important in that chemical sprays against *B. subsecivella* in groundnut can be delayed until the flowering stage.

Another peculiarity of the *B. subsecivella* occurring in South Africa is that it attacks its plant hosts at specific times of the year, which has important implications for the cultural

management of the pest (i.e. crop rotations and planting dates). For example, at Manguzi where groundnut is planted in July (winter), the crop remains free of infestations until December (summer) (see Chapter 6). This was despite the activity of *B. subsecivella* moths, albeit at very low levels, between July and December. Also, wild plants and perennial crops (pigeon pea and lucerne) that are known to be hosts for *B. subsecivella* were also free of the pest during the winter period (see Chapter 5). In an experiment that compared infestation levels between groundnut, soya bean, pigeon pea and lucerne and the effects of planting dates on the infestation of these crops by *B. subsecivella* (see Chapter 5), soya bean and groundnut were susceptible to *B. subsecivella* attack from early to late in the summer season (November to March). However, it was the late-planted crops (December to January) that were more susceptible to *B. subsecivella* infestation. Pigeon pea was only susceptible late in the summer season (March to April) and the rare attack by *B. subsecivella* on lucerne was also observed late in the summer season. This information is useful in designing crop rotations and determining planting dates for these legume crops.

Effects of climatic variables on *B. subsecivella* populations

Generally, *B. subsecivella* population densities and the intensity of infestations on groundnut were observed to vary between sites and seasons (see Chapter 6), but regression analyses indicated that these variations could mostly not be accounted for by site differences in temperature, humidity and rainfall. Nonetheless, it was noted (Figures 6.2 to 6.5 in Chapter 6) that high moth catches coincided with low rainfall periods, whereas low moth catches coincided with the rainy periods. Thus, it might be expected that *B. subsecivella* infestations may be lower in wetter than in drier seasons.

Efficacy of cypermethrin in controlling *B. subsecivella* on groundnut and soya bean

Chemical control of *B. subsecivella* could be the method of choice when faced with sudden outbreaks. The efficacy of cypermethrin in controlling *B. subsecivella* was tested on groundnut and soya bean at Nelspruit and Vaalharts. Although cypermethrin is a contact insecticide, three sprays of this chemical which were four weeks apart, at a concentration of 2 ml/l of water, reduced *B. subsecivella* infestations to very low levels and increased grain yields compared to crops that were not sprayed (see Chapter 5).

Recommendations

My recommendations for future research to facilitate the management of *B. subsecivella* in southern Africa, and elsewhere on the continent, include the following:

(i) Morphological studies of specimens from *B. subsecivella* populations from Australia, India and Africa to confirm the taxonomic status of these tentatively synonymised 'species'. The correct identification of a pest species is the foundation on which good integrated pest management techniques are built.

(ii) To compliment (i) above, additional molecular and phylogenetic studies including more samples from different geographic areas where the pest has been reported, to determine the genetic relatedness of *B. subsecivella* populations in Australia, India and Africa.

(iii) Confirmation of the taxonomy of *B. subsecivella* populations in Australia, India and Africa.

(iv) Investigation of the relationships between the southern African *B. subsecivella* and its wild plant hosts and the off-season survival tactics of the pest.

(v) Chemical ecology studies to examine the volatile compounds produced by groundnut plants during their different growth stages in relation to patterns of *B. subsecivella* infestation.

(vi) Surveys for indigenous biological control agents, and subsequent studies on the biology of these and other *B. subsecivella* parasitoids reported from elsewhere on the continent and from its area of origin (once determined). The possibility of deploying them as augmented, translocated or classical biological agents (Conlong 1994) for the control of *B. subsecivella* populations in South Africa should be considered.

(vii) Adoption of methods which promote the use of cultural and biological approaches. Kenis & Cugala (2006) suggested various integrated approaches to reduce *B. subsecivella* infestation. These include: intercropping (e.g. planting groundnut with sorghum, millet or cowpea (Logiswaran & Mohanasundaram 1985, cited by Shanower *et al.* (1993)), black gram, pigeon pea, green gram and pearl millet (Muthiah 2000); the use of trap crops (e.g. suitable soya bean genotypes); trapping using *B. subsecivella* pheromone attractants (Nandagopal & Ghewande (2004); manipulation of planting dates and; the use of botanical pesticides and biological pesticides (e.g. *Bacillus thuringiensis* (Berliner)).

(viii) Use of insecticides such as cypermethrin to reduce *B. subsecivella* infestations in the short term (Cugala *et al.* 2010; Du Plessis & Van Den Berg 2011; Buthelezi *et al.* 2013). However, there is a need to determine the proper spraying schedules as well as the amount of chemicals to be applied, in order to avoid crop contamination and reduce application costs.

References

BAILEY, P.T. 2007. Pests of field crops and pastures: Identification and control. CSIRO Publishing, Australia. 221-222.

BUTHELEZI, N.M., CONLONG, D.E. & ZHARARE, G.E. 2012. The groundnut leaf miner collected from South Africa is identified by mtDNA COI gene analysis as the Australian soya bean moth (*Aproaerema simplexella*) (Walker) (Lepidoptera: Gelechiidae). *African Journal of Agricultural Research* **7**(38): 5285–5292.

BUTHELEZI, N.M, CONLONG, D.E. & ZHARARE, G.E. 2013. A comparison of the infestation of *Aproaerema simplexella* on groundnut and other known hosts for *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *African Entomology* **21**(2): 183–195.

CONLONG, D.E. 1994. A review and perspectives for the biological control of the African sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Agriculture, Ecosystems and Environment* **48**: 9–17.

CUGALA, D., SANTOS, L., BOTAO, M., SOLOMONE, A. & SIDUMO, A. 2010. Assessment of groundnut yield loss due to the groundnut leaf miner, *Aproaerema modicella* infestation in Mozambique. Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda.

DU PLESSIS, H. 2002. Groundnut leaf miner *Aproaerema modicella* in Southern Africa. *International Arachis Newsletter* **22**: 48–49.

DU PLESSIS, H. 2003. First report of groundnut leafminer, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) on groundnut, soya bean and lucerne in South Africa. *South African Journal of Plant & Soil* **20**: 48.

DU PLESSIS, H. 2011. Flight activity of the groundnut leaf miner, *Aproaerema modicella* (Deventer) in the groundnut production areas of South Africa. *South African Journal of Plant and Soil* **28:** 239–243.

DU PLESSIS, H. & VAN DEN BERG, J. 2011. Biology and management of groundnut leaf miner. *African Crop Science Conference Proceedings* **10**: 169–172.

EPIERU, G. 2004. Participatory evaluation of the distribution, status and management of the groundnut leaf miner in the Teso and Lango farming systems. Final Technical Report, Serere Agricultural and Animal Production Research Unit (SAARI), Soroti, Uganda. 30.

KENIS, M. & CUGALA, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**: 1–9.

MUNYULI, T.M.B., LUTHER, G.C., KYAMANYWA, S. & HAMMOND, R. 2003. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda and Democratic Republic of Congo. *African Crop Science Conference Proceedings* **6**: 231–237.

MUTHIAH, C. 2000. Effect of intercropping on the incidence of leaf miner (*Aproaerema modicella*) in groundnut (*Arachis hypogaea*). *Indian Journal of Agricultural Sciences* **70**: 559–561.

NANDAGOPAL, V. & GHEWANDE, M.P. 2004. Use of Neem products in groundnut pest management in Uganda. *Natural Product Radiance* **3**(3): 150–155.

SHANOWER, T.G., WIGHTMAN, J.A. & GUTIERREZ, A.P. 1993. Biology and control of the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *Crop Protection* **2**: 3–10.

THE NEW VISION. 2009. Pest devastates groundnuts. Available online at: http://www.newvision.co.ug/D/9/37/365013 (accessed on 15th May 2014)

VAN DER WALT, A. 2007. Small holder farmers' perceptions, host plant suitability and natural enemies of the groundnut leafminer, *Aproaerema modicella* (Lepidoptera: Gelechiidae) in South Africa. M.Env. Sci. dissertation, North-West University, Potchefstroom, South Africa.

VAN DER WALT, A., DU PLESSIS, H. & VAN DEN BERG, J. 2008. Using morphological characteristics to distinguish between male and female larvae and pupae of the groundnut leaf miner, *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae). *South African Journal of Plant & Soil* **25**(3): 182–184.