ASPECTS OF BIOLOGY AND ECOLOGY OF CITRUS LEAFMINERS (LEPIDOPTERA: GRACILARIIDAE) IN MAJOR CITRUS GROWING REGIONS OF TANZANIA

GIOVANNI NGUVU

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2015

EXTENDED ABSTRACT

Field investigations on the occurrence, incidence and damage severity of the Citrus Leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on Citrus crops were conducted in Morogoro Rural, Muheza and Kinondoni districts from December 2011 to September 2012. Fifty citrus fields were randomly selected in each of Morogoro Rural and Muheza districts from which 10 000 trees (>5 years old) were examined for leafminer damage signs. A total of 25 seedling nurseries (plants <5 years) were assessed in Kinondoni district. Occurrence of the pest was noted in almost all fields albeit at low incidence (2%). Kruskal Wallis analysis of the incidence data showed neither spatial nor temporal variations between the locations and time of survey. Damage severity in Kinondoni showed an increasing trend from December 2011 to June 2012. Based on the observable morphological features of the collected specimen and damage signs, the pest was suggested to belong to the genus *Phyllocnistis* and the species *citrella*. Total development time was longer at 20^0C and shorter at 30^0C with 28 days and 10 days respectively. Oviposition period decreased with increase in temperature, from 9.26 days at 20^0C to 6.21 days at 30^0C. Female insects lived longer than males across all tested temperatures.

DECLARATION

I, Giovanni Nguvu, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

______________________ ______________________

Giovanni Nguvu Date

(MSc. Candidate)

The above declaration is confirmed

______________________ ______________________

Dr. G. M. Rwegasira Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

I express my deep sense of gratefulness to my supervisor, Dr. G. M. Rwegasira of the Department of Crop Science and Production in the Faculty of Agriculture of Sokoine University of Agriculture (SUA) for his priceless supervision, consistent help, and encouragement all the way through the course of this study.

I am also gratified by the Royal Museum for Central Africa (RMCA), Belgium, for awarding me a scholarship to undertake MSc. studies under the fruit flies Integrated Pest Management Project. I also express my sincere appreciation to Prof. M. W. Mwatawala of Sokoine University of Agriculture who was the project manager.

Special thanks go to Dr. Mark De Meyer and Dr. Jurate De Prins of Royal Museum for Central Africa for their constructive remarks, guidance and counsel, which enabled me to comprehend this study.

I am deeply indebted to my wife Lillian George Mbaga and my kids Rashidi, Nehemiah and Karsten for their devoted and diligent moral support and decent encouragement in every point of this study. In their absence, this work would have been very hard to complete.

My sincere thanks are due to my parents; my father Mr. Hippolity Mbalasi and my mother Mrs. Emmy Nyabasega Matigula for their encouragement and moral support. My friends who offered precious contributions that led to the completion of this dissertation are highly appreciated.

Above all, I submit and praise the Almighty God in the name of Jesus Christ, the name above all, who blessed me with life and strength to carry out this study. Praises, thankfulness and glory are all his, Amen!

DEDICATION

This work is dedicated to my lovely wife Lillian George Mbaga, for her patience, love and muse during my study, I deeply love you. Also to my lovely mother Emmy Nyabasega Matigula who has opened up my way to this academic life success.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
<	Less than
>	Greater than
^0C	Degree-Celsius
ANOVA	Analysis of variance
cm	Centimetre
CV	Coefficient of variation
E	East
Fig	Figure
GPS	Geographical Positioning System
H-B scale	Horsfall-Barratt scale
km	Kilometre
LSD	Least Significant Difference
m	meter
m.a.s.l	Meters above sea level
P	Probability
RH	Relative humidity
RMCA	Royal Museum for Central Africa
S	South
SE	Standard error
StDev	Standard Deviation
SUA	Sokoine University of Agriculture

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Citrus are flowering plants in the family, *Rutaceae* that are believed to originate from the tropical and subtropical regions of South East Asia, particularly India and China (Gmitter and Hu, 1990). The generic name originated in Latin, where it specifically referred to the plant now known as Citron (*Citrus medica*) (Scora, 1975). It was derived from the ancient Greek word for cedar; *kedros* (Kimball, 1999). It is comprised of a number of species including; sweet orange, lime, lemon, grapefruit and mandarin. The taxonomy and systematics of the genus are complex and the precise number of natural species is unclear, as many of the named species are clonally propagated hybrids, and there is genetic evidence that even some wild, true-breeding species are of hybrid origin. Cultivated Citrus may be derived from as few as four ancestral species. Natural and cultivated origin hybrids include commercially important fruit such as the oranges, grapefruits, lemons, some limes, and some tangerines (Pinchas and Goldschmidt, 1996).

1.2 Global Citrus Production

From an economic point of view, citrus fruits rank first in terms of world fruit production and international trade value (Norberg, 2008; UNCTAD, 2009). Citrus production is estimated at around 100 million tons produced annually. Citrus fruits are cultivated in 140 countries around the world (FAOSTAT, 2012). Production shows geographical concentration in certain areas, mainly in the Northern Hemisphere, accounting for about 70% of total citrus production. Major citrus producers worldwide are Brazil, USA, China and the Mediterranean basin. Of all citrus produced, sweet oranges are the major ones contributing to approximately 70% of the citrus output. Worldwide statistics indicate that five countries account for about 60% of the orange production. These are Brazil (29%), United States (11%), India (8%), China (8%) and Mexico (6%) (FAOSTAT, 2012).

As a result of trade liberalization and technological advances in fruit transport and storage, the citrus fruit industry is becoming more global in scope. During the last decades, production and trade in citrus fruits have increased steadily; although the intensity of growth has been different according to the type of fruit (it has been stronger in small fruits and orange juice, particularly in not-from-concentrated orange juice during the nineties, while there is a certain stagnation of other citrus fruits consumption in developed countries) (Kimball, 1999). The citrus fruit sector is evolving in a context of highly competitive global markets. The market is assisting to a change in consumption patterns, particularly in the form of an increasing focus on the quality and the value-added aspects of the product (UNCTAD, 1996). In addition, there is an increasing power of global retail chains in fruit distribution as a consequence of the process of concentration and consolidation that they are undertaking. As a result, the citrus fruit market is evolving from a producer driven form to a more consumer oriented market (FAOSTAT, 2012).

1.3 Economic Importance of Citrus to Tanzania

Tanzania is one of the countries known to have significant levels of citrus production in Africa although not among the worldwide major producers. Based on FAOSTAT (2012) the quantity produced is 46 840 tons (from 13 312 ha) per annum which ranked Tanzania sixth after Morocco, South Africa, Egypt, Nigeria and the Democratic Republic of Congo. The crop is mainly produced in Tanga, Coast and Morogoro Regions but Dar es Salaam Region is popular for citrus seedling nurseries. In other regions, citrus is grown as one to a few door yard trees and not in plantations and relatively large sized orchards as in the three mentioned regions (SCF, 2008).

According to the Tanzania National Sample Census of Agriculture, Tanga region has a total planted area of 9342.34 ha and productivity of 22 tons per ha (Agriculture Census, 2002/03). Tanga is regarded as the most significant producer of citrus fruits particularly in Muheza and Korogwe districts which are considered as home to a variety of citrus fruits such as oranges, limes, lemon, mandarin and grapefruits.

Table 1: Overview of orange production and productivity in Tanzania

Region	Area planted with orange (ha)	Productivity (Tons/ha)
Coast	7 635.31	18.31
Dar es salaam	2 022.27	4.9
Morogoro	4 548.80	10.9
Tanga	9 342.34	22.41

Source: Agriculture Census, 2002/2003

Only a small portion of the produced oranges is locally consumed. Much of the oranges are transported and sold in other towns and cities within and outside the country. In Tanzania, oranges represent the third most fruits basket after mangoes and pineapples (Verschoor and Nyambo, 2005).

Orange farming employs over 40% of household both as sole and complement economic activity in Muheza District (Lazaro, 2008). Recent expansion and/or new investments in fruit processing industries for which oranges are among the crucial raw materials, their demands have sparingly increased (Moininche, 2008). Consequently, the need to intensify production of citrus fruits to cope with the market demand became paramount. This led to expansion of number of actors along the citrus supply chain. Increased number of citrus seedlings vendors, establishment of new nurseries and orchards, expansion of the existing orchards, increased number of middlemen, adoption of new marketing strategies and increased number of transporters have all been realized as an outcome of investments in fruit processing (Haggblade and Gamser, 1991). Unfortunately, the need to intensify and increase citrus production is hampered by a series of constraints, particularly diseases and insect pests.

1.4 Citrus Production Constraints

Diseases such as Citrus tristeza have been reported to exists wherever citrus is grown in Tanzania (Rwegasira *et al*., 2010; Swai, 1988) and *Xanthomonas campestris* pv. *citri* have been observed in a few locations (Swai, 1988). Mwatawala *et al*., in 2004 reported that insect pests such as *Bactrocera invadens* and codling moths have been causing enormous damage to fruits with high economic loss. Recent observation of citrus leaf miner suspected to be micro-lepidoptera of the species *Phyllocnistis citrella* in some orchards in Morogoro could be an added threat to the citrus industry. With such big investments in the fruit industry, the need to undertake research on this new pest to determine its pest status that would lead to designing possible intervention could not be overemphasized.

1.5 Justification

Citrus production in Tanzania is comparatively very low with reference to the world statistics. According to FAOSTAT (2012) citrus production increased in quantity from about 6000 tons in 1970 to 52 054 tons in 1999, the highest ever attained in Tanzania. Thereafter, the yield continuously declined to 35 706 tons in 2009 despite the new orchards and plantations established since early 2000. Largest area ever recorded under citrus was 42 475 ha in the year 2001. Abiotic stress (drought and declined soil fertility) and biotic constraints (insect pests and diseases) are believed to have contributed to the decline in yield (Srivastava and Singh, 2005). While some efforts have been initiated to address the disease problems (*Citrus tristeza* and *Xanthomonas campestris pv. citri*) and a few insect pests such as fruit flies (*Bactrocera invadens* and other species) little has been done to address the citrus leafminer problem. De Prins and De Prins (2010) observed the mines and the moth (*Phyllocnistis spp*) in the SUA orchards in Morogoro. The extent of infestation was 1-3 moths per leaf. As the practice in other tropical countries shows, this cosmopolitan pest species rapidly becomes a significant problem and can reach infestation rates of up to 90% (Hoy, 1996; Diez *et al*., 2006). Apart from the direct injury caused by the pest, it also acts as a mediator for the citrus bacterial canker disease. Larvae of *Phyllocnistis spp* during severe infestations attack tender twigs which might end up causing die back and consequently reducing tree vigour and productivity (Kawahara *et al*., 2009). The economic impact of leaf miner feeding on leaves must be determined through measurement of reduced leaf area for photosynthesis and resulting reduced canopy development for fruit production. This could result in an increase in time (years) for young trees to reach an adequate fruit bearing size or in a reduction in reproductive wood for next year's crops in bearing trees (Achor *et al*., 1997).

Although the leafminer was observed and suspected to be *Phyllocnistis citrella*, the species was not yet scientifically studied and reported in Tanzania (De Prins and De Prins, 2010). Since SUA is among main producer and distributor of citrus seedlings in Tanzania and the pest has been observed in SUA orchards, the probability of the pest being spread in other parts of the country was high and its consequences would be detrimental to the citrus industry. The current study intended to precisely identify the species responsible for citrus leaf mining, develop some of the critical data on biology and ecology, and determine the pest status of the species in Tanzanian citrus industry. The study outcome created basis into understanding the bionomics of the pest that would be used to devise ways of controlling or minimizing damages caused by the citrus leafminer.

1.6 Objectives

1.6.1 Overall objective

To establish the pest status of the Citrus leafminer in selected major citrus growing regions in Tanzania for developing management strategies.

1.6.2 Specific objectives

To examine the biology of the leafminer

To determine the incidence and severity of citrus leafminer in selected citrus growing regions of Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

Agriculture is the leading sector in the Tanzanian economy, accounting for nearly half of the Gross Domestic Product. More than 85% of the population is involved in farming and agriculture is almost completely rain fed. Farming is predominantly low-tech with 70% of all farms cultivated using hand hoe (Irish Aid, 2008).

Tanzania's climate and growing conditions are favourable for a wide range of fruits, vegetables and flowers. The major fruit potential is in pineapples, passion fruit, citrus fruit, mangoes, peaches, pears and bananas, while vegetables include tomatoes, spinach, cabbage and okra (Irish Aid, 2008).

Tanga region is the most significant producer of citrus fruits in Tanzania, although Coast, Morogoro and Geita regions are also known for citrus fruits production. It is generally considered that orange production in Tanzania grew to major economic importance during the late 1970's (Lazaro, 2008).

2.1 Requirements for Citrus Production

Citrus trees are grown throughout the world in tropical and subtropical areas, but they achieve the best quality under subtropical conditions, with an optimum temperature ranges between 20°C and 28°C (Chang and Petersen, 2003). Low rainfall and plenty of sunshine is good for citrus trees as it promotes good flower differentiation, flower and fruits development as well as fruit quality. Citrus is well adapted to deep, well-drained soils. Loamy soils are preferred to heavy clays and poorly-drained soils which often result in poor growth and production as well as shorter life. Citrus trees can't perform well under light and sandy soil conditions. In general most citrus trees prefer the soil pH of between 5.5 and 6.5 (FFTC, 2003).

While the orange will often come from seed because of nuclear embryos, the common means of assuring the reproduction of cultivars of known quality is by budding onto appropriate rootstocks. Rough lemon became the dominant rootstock, as it induces more rapid and vigorous growth and earlier bearing (Helgi *et al*., 2005). Budded oranges are spaced at a range of 8cm x 8cm to 10cm x 10cm. In Tanga the plant density is about 250 orange trees per ha.

Fertilizer application is necessary for high yield and fruits quality. Chang and Petersen (2003) indicated that a basal application of fertilizer should be applied after harvest, in order to restore tree vigour after fruit production. It is advisable to improve soil conditions by applying organic manure or liming material. While the fruits are developing, additional doses of potassium helps to improve both quality and size of oranges. Nitrogen should be reduced or completely avoided (Sinclair, 1984).

Water requirement in orange production is critical at reproduction stage. Flowering, fruit set and new flush development periods require optimum soil moisture (Davies and Albrigo, 1994). Water deficiency leads to smaller leaves and shortens the flush. Thus, irrigation must be performed to maintain soil moisture, when there is inadequate rain. During fruit development period, plant water requirement is high, due to high transpiration rate especially when temperature is high. During fruit maturing stage soil should be kept fairly dry. While after harvest period, a small amount of irrigation to restore growth is required. The aim is to increase photosynthesis, promote flower differentiation and avoid water and nutrients stress (Chang and Petersen, 2003).

Training and pruning are essential for three main objectives: The first one is to increase total effective leaf area and promote photosynthesis by exposing the leaves to light and air. Secondly, proper training and pruning keep the tree in the right size for ease management and trend of trees. It increases also the vigour of trees, enhance their tolerance to various stresses and help to maintain the most efficient balance between vegetative and fruiting. Thirdly, a good training and pruning help to control pests and diseases (Davies and Albrigo, 1994).

2.2 Citrus Production in the Selected Regions

2.2.1 Citrus production in Muheza

Orange trees were first planted in Muheza District in the early 1900s by Anglican Missionaries. Propagation of oranges was achieved during the period 1930 to 1940 due to the presence of a nursery run by Mlingano Sisal Research Station near Muheza. The general opinion among scholars is that orange production in Muheza District grew to be of major economic importance during the late 1970's (Kikuu, 2002). In addition, it is important to note that the orange industry within Muheza is still experiencing tremendous growth to date.

From the dialogue with Muheza Agricultural Field Officers, it was observed that most orange producing farmers in Muheza do not use fertilizers and pesticides in their production. Kwebada, Mtindiro, Kicheba and Lusanga are the major orange producing villages. According to Møiniche (2008) around 60% of the oranges produced in Tanga Region are exported to Kenya, mainly during the peak production season (June to September).

Oranges can be produced throughout the year in Muheza District. Every four months oranges flower and two months later the fruits ripen which gives two seasons per year (Lazaro, 2008). The production season is between April and October with peak season between June and August. The period from November to February is low season, while March to May is regarded as a period of fruit scarcity with a small number of farmers producing very low volumes of oranges (Table 2). It is estimated that about 65% of oranges produced in Tanga is recorded during the main production season (June to August), at least 25% in the minor production season (November to February) and less than ten percent in the low production season (March to May). Several orange varieties are grown in Muheza district. These were Early Valencia and Late Valencia (Msasa), Pineapple, Mediterranean sweet (Nairobi), Jaffa, Washington navel, and Matombo sweet.

2.3 Citrus Production in Morogoro

Morogoro Rural is the major fruit growing district in Morogoro region mainly in Matombo division. Major villages involved in orange production in Matombo include Ngong'oro, Lundi, Maputo and Kiuruka. Production statistics for orange production in Matombo have not been well studied and published but generally the production is low compared to Muheza district.

Morogoro region has a shorter production season, but has the advantage of earlier harvest, which enables them to produce higher volumes of oranges during the very low supply period between April and May when prices are highest. It is estimated that between 60-70% of oranges are harvested during the main production season (June to August) with the remainder of crop being harvested before and after the main season (Table 2).

Table 2: Seasonality of orange production in Muheza and Morogoro Rural

Location	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Muheza												
Morogoro												

Source: Explorative Study on Citrus Farming Systems (2008)

Key:

Red Fruit scarcity

Yellow Low season

Green Main season (Peak)

2.4 Citrus Leafminers and Their Economic Importance

2.4.1 Effect of leafminer in citrus production

One of the recent major constraints in citrus fields in many countries is the infestations of the pest that causes mines on citrus leaves (Knapp, 1995). Apart from reducing the photosynthetic efficiency; it causes unsightly appearance of leaves and fruits and paves way for secondary pathogens.

Economic losses due to citrus leafminers presence in orchards include: i) increased costs of insecticides for protecting seedlings and young Citrus trees (De Prins, 2005), ii) reduced citrus quality due to presence of sepertine mines and lesions on citrus fruit skin caused by leafminers and *Xanthomonas campestris pv citri* respectively which eventually reduces the crop sales (Ando *et al*., 1985; Hill, 1918), and iii) increased direct and indirect orchard production costs (Heppner, 1994).

Several studies have been conducted to correlate citrus leafminer damage with economic loss. Knapp *et al*. (1995) reported that a 10% leaf area loss did not affect citrus yield. In Florida, control of citrus leafminer increased yield of 3 to 5 year old orange trees by 13.1% to 16.9% (Stansly *et al*., 1996). In another Florida study, a 16% to 23% leaf area loss caused significant yield reduction of 15 year-old Tahiti lime trees, and 18% to 85% leaf area loss caused significant yield reduction of 5 year-old lime trees (Pena *et al*., 2000).

2.4.2 Description of citrus leafminer

Adults of the *Phyllocnistis spp* are minute moths with a 4 mm wingspread. It has white and silvery iridescent scales on the forewings, with several black and tan markings, plus a black spot on each wingtip (De Prins and De Prins, 2009). The hind wings and body are white, with long fringe scales extending from the hind wing margins. In resting pose with wings folded, the moth is much smaller in appearance (about 2.4 mm). The head is very smooth-scaled and white and the haustellum has no basal scales (Kawahara, 2009).

Plate 1: Adult moth of *Phyllocnistis citrella* Stainton
Source: Museum d'histoirenaturelle, Geneva

Phyllocnistis spp is most easily detected by its meandering serpentine larval mine, usually on the ventral side of the leaf (Ba-Angood, 1977). Larvae are minute (3 mm), translucent greenish-yellow, and located inside the leaf mine. The pupa is characteristically in a pupal cell at the leaf margin. Adults are generally too minute to be easily noticed, and are active diurnally and in the evenings (De Prins and De Prins, 2009).

2.5 Taxonomy of the Pest

The leaf-mining genus *Phyllocnistis* (Stainton, 1856) has been an exemplar of a poorly studied genus whose taxonomic placement has vacillated between many different families. It includes some of the smallest Lepidoptera known with forewing length of 2 mm. From 87 species of *Phyllocnistis* described worldwide, only five are known to occur in the Afro-tropical region (De Prins and De Prins, 2005; 2009). This global economic pest was initially placed in different lepidoptera families (De Prins and Kawahara, 2009) while some authors still treat the genus *Phyllocnistis* as a separate family (Emmet, 1985; Opler and Powell, 2009). However, most authors attribute *Phyllocnistis* to the family *Gracillariidae*: subfamily *Phyllocnistinae* (Heppner, 2004; De Prins and De Prins, 2005).

2.6 Biology of the Pest

Female citrus leafminers lay their eggs singly on newly emerging leaves near midribs or veins. Under low population densities, females prefer to lay eggs on the lower leaf surface, although eggs and mines can be found on both upper and lower leaf surfaces (Ba-Angood, 1977). Eggs are usually laid along the leaf's mid-vein usually towards the petiole of the leaf. Each female produces about 50 eggs in her lifetime which spans from 2 to 12 days (Argov and Rössler, 1998). Immediately following oviposition, the eggs are translucent and whitish in colour, and resemble tiny water droplets. After several days they become yellowish and opaque. They are extremely small measuring 0.2 X 0.3 mm

(Kawahara, 2009). Eggs hatch in 4 to 10 days later depending on the temperature (De Prins, 2005).

The citrus leafminer produces four larval instars, with a total development time of 5 to 20 days (Ujiye, 2000). The first three instars are sap-feeding forms, while the fourth instar is a non-feeding spinning form (Ba-Angood, 1977). The hatching larva immediately begins mining beneath the epidermal cell layer of the leaf leaving nearly invisible mines (Heppner, 1993). These larvae are translucent, light green in colour and very difficult to detect. The second and third instars are translucent, yellowish-green and can reach a length of 3 mm. The larger larvae are clearly visible and the mines are easily seen due to increased size and the presence of larval faeces within the mine. The mines will meander along the underside of the leaf in a serpentine pattern. The mines resemble a translucent trail of silicone with darkened areas of excrement. The larvae will rarely cross the mid-vein or to the upper side of the leaf unless overcrowded. The third instar will make its way towards the leaf margin where it will moult into the fourth instar which forms a silken cocoon within the mine (Boughdad *et al*., 1999). As the silk dries it curls the leaf edge over the cocoon forming a protective pupal cell. The pupa is yellowish-brown and darkens with age. It can easily be revealed by peeling open the pupal cell (Patel *et al*., 2012). The pupation stage requires 8 to 20 days to complete depending on the temperature (Kawahara, 2009).

The entire life cycle requires 14 to 50 days depending on temperature. Adults emerge about dawn and are active in the morning; other activity is at dusk or night. Females lay eggs evenings and at night (Beattie, 1989).

2.7 Damage Caused by the Pest

Phyllocnistis citrella attacks all varieties of citrus, other *Rutaceae* plants, and several ornamental species (Heppner, 1993; Legaspi *et al*., 1999). The citrus leafminers attacks young foliage (flush) and thus is particularly harmful to nursery seedlings and young groves. The insect also attacks mature trees, damaging new growth which could have a detrimental impact on growth and yield of producing orchards. During its larval stages, *Phyllocnistis citrella* mines the adaxial and abaxial surfaces of new leaves. A newly hatched larva immediately chews into the leaf from its egg and start feeding on the internal parts of the leaf by shearing the plant tissue, resulting in a serpentine mine. Leaf mining results in leaf deformation, partial leaf chlorosis, necrosis, and some leaf drop which ultimately results in a reduction in the tree's photosynthetic capacity (Pena *et al*., 1996; Belasque *et al*, 2005; Ujiye, 2000; Gottwald *et al*., 2001).

Plate 2: Leaf damage by the larvae of citrus leafminer

Source: Gruett Tree Health

A citrus leaf can support more than one larva, and commonly two to three larvae can be found on both the upper and the lower surfaces of the leaf. Up to nine larvae per leaf have been reported in Florida (Heppner, 1993). As the larva emerges from its mine and rolls the edge of the leaf over, curling of the leaf margin is caused. A single larva can consume 1 to 7 cm2 of leaf area and leave a 15 to 30 cm mine (Gotwald *et al*., 2002). Leaves with only one mine will often drop from the tree. Leaves with 2 to 3 larvae may receive damage to 50% of the leaf surface, and will often remain on the tree. Consequently heavily infested leaves (>4 mines per leaf) are frequently distorted and may abscise (Pena and Duncan, 1994). As the leaves mature, they are not liable to attack. In cases of massive infestation, citrus leafminers attacks even young fruits (Heppner, 1995). In Mauritius, citrus leafminers mines have once been observed on a young local Pamplemouses fruit at Pointe aux Piments. Larvae of *P. citrella* during severe infestations also attack tender twigs. Such injury can be severe causing die back and consequently reducing tree vigour and productivity. Studies in Florida have shown that significant stunting can occur if trees are not protected from citrus leafminers with insecticides from the time of planting until they are about 4 years of age (Rodgers and Stansly, 2007). Yield losses generally do not occur in mature citrus trees.

Infection by citrus canker has been observed on leaves damaged by *P. citrella* in Mauritius. The pathogen in form of pustules is more abundant along the mines on the damaged leaves. There could also be an association of *P. citrella* with citrus canker. (Cook, 1988) stated that *P. citrella* can disseminate the citrus canker bacterium. In Florida, citrus leafminers exacerbates the citrus bacterial canker situation. Citrus bacterial canker is a serious disease of citrus that initially causes lesions on leaves, stem, and fruits. If the disease is allowed to progress, defoliation, severely blemished fruits, premature fruit drop, twig dieback, and tree decline will occur (Diez *et al*., 2006).

Although citrus leafminer does not vector bacterial canker, feeding by larvae provides an opening to the mesophyll of the leaf for citrus canker, and the larva may carry the bacteria throughout the mine, increasing the amount of canker inoculums produced in a leaf (Gottwald *et al*., 2002; Belasque *et al*., 2009)

2.8 Seasonal Phenology of Citrus Leafminers

The phenology of *Phyllocnistis spp* has been extensively studied throughout the world. In Asia, *Phyllocnistis spp* completes five to six generations per year (Kawahara, 2009). In Australia, *Phyllocnistis spp* is found throughout the year but in greatest abundance during periods of new flush in citrus (Wilson, 1991). In Florida and Texas, infestations of leaves are the lowest in winter and early spring, increasing significantly through the late spring, summer and early fall (Pena *et al*., 1996; Legaspi *et al*., 1991). During the colder months of the year, *Phyllocnistis spp* does not enter diapauses, but instead development slows and adults live longer (Lim and Hoy, 2006).

2.9 Global Distribution of Citrus Leafminer

The citrus leafminer, *Phyllocnistis citrella* Stainton, was widely distributed in Southeast Asia, from India to China, Korea, and Japan, the Philippines, Indonesia, Papua New Guinea, and the northern tip of Australia (Hill, 1983; Argov and Rössler, 1998). It was also known from the Sudan in Africa (Heppner, 1995). Within the past few years, this pest has moved into many new citrus-growing regions of the world, creating major concern and disrupting integrated pest management (IPM) programs developed for citrus in these locations. After recording this pest species in South Africa at the beginning of last century *P. citrella* was mentioned as having reached East Africa in 1967 and West Africa in 1970 without indication of any particular locality. It was presumed that under the term “East Africa” probably the present country Tanzania was meant (De Prins and

De Prins, 2005). However, such a general record needed a confirmation. De Prins and De Prins in 2010 reported the suspected presence of *P. citrella* in Tanzania, in the SUA Horticulture unit (06°50'S 37°38'E). This pest has also been observed and reported by De Prins; in Kenya (Watamu, 03°21'S 40°01'E, mines observed in 2004), Cameroon (Faro riverside, 08°23'N 12°48'E, mines observed in 2003, 2005), and Congo Democratic Republic (Luki-Mayumbe Biosphere Reserve, 05°27'S 13°05'E, mines observed 2006, 2007, 2008). *P. citrella* has also been reported from Côte d'Ivoire (De Prins and De Prins, 2005), La Réunion (Quilici *et al*., 1995), Mauritius (Malausa *et al*. 1996), Mozambique (De Prins and De Prins, 2005), Nigeria (Medler, 1980), Sudan (Ba-Angood, 1977), Swaziland (Schauff *et al*., 1998), Western Sahara and Zimbabwe (Schauff *et al*., 1998).

2.10 Host Plants

Phyllocnistis citrella is very successful in its ability to exploit a wide range of citrus. Grapefruit, lemon and lime are most susceptible to damage, but the leafminer attacks many different plants and varieties mostly belonging to the citrus family Rutaceae: *Citrus sp.* (Stainton, 1856); *Citrus aurantifolia* (Christm.) Swingle, *C. aurantium* L., *C. histrix* D.C., *C. limon* (L.) Burm.f., *C. maxima* (Burm. ex Rumph) Merr., *C. medica* L., *C. paradise* Macfad., *C. reticulata* Blanco, *C. sinensis* (L.) Osbeck, *C. unshiu* Marc, *Aegle marmelos* (L.) Corr. Serv., *Atalantia sp*, *Citrofortunella microcarpa* (Bungel) D.O., *Fortunella crassifolia* Swingle, *F. marginata* (Lour.) Swingle, *Limonia sp*, *Murrayakoenigii* (L.) Spreng., *M. paniculata* (L.) Jack, *Poncirus trifoliata* (L.) Raf.,*Severinia buxifolia* (Poir.) Ten. (De Prins and De Prins 2005, 2009). Records from *Garcinia mangostana* L. [Clusiaceae], *Jasminum sambac* (L.) Aiton, *J. simplicifolium* Forst.f. [Oleaceae], *Loranthus sp* [Loranthaceae], *Alseodaphnese mecarpifolia* Nees, *Cinnamomum zeylanicum* Blume [Lauraceae], *Pongamia pinnata* (L.) Pierre [*Fabaceae*] needs confirmation (De Prins and De Prins, 2005).

2.11 Management of the Pest

In many sites in Florida, growers tried applying insecticides as an effort to mitigate the impact of the citrus leafminer, although they soon recognized that the tactic was expensive and often ineffective (Heppner, 1993). Chemical control alone was not a viable management strategy for the citrus leafminer over the long term. This was due to concerns about resistance to pesticides, disruption of biological control of other citrus pests, concerns about pesticide residues on food and in the ground water, negative impacts on worker safety, and impacts on non-target organisms in the environment (Tan and Huang, 1996).

Classical biological control was expected to provide substantial control of the citrus leafminer in Florida, particularly in mature orchards. However, they soon learned that surprisingly little was known about many of the species reported as natural enemies of the citrus leafminer in Southeast Asia (Mafi and Ohbayashi, 2006). For many species, there is not even a specific name. Few details were found about the host range of these parasitoids or their ability to be facultative hyperparasitoids, information which was crucial for classical biological control programs (Heppner, 1993). Furthermore, in most new environments, the citrus leafminer has also accrued new natural enemies, including parasitoids that moved onto the citrus leafminer from unknown hosts (Ujiye, 2000). The long term impact of these natural enemies remains to be resolved.

Management of citrus leafminer populations could be enhanced if effective grove management can be enhanced. These management practices might include managing flush patterns by altering irrigation and fertilization practices (Pena *et al*., 1996). Unfortunately, management of the citrus leafminer is hampered by a lack of clear guidelines on the economic injury caused by the citrus leafminer in different climatic zones and under different cultural practices (Heppner, 1993).

References

Achor, D. S., Browning, H. Y. and Albrigo, L. G. (1996). Anatomical and histological modification in citrus leaves caused by larval feeding of citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) In: *Proceedings of International Conference on Citrus Leafminer.* University of Florida, Orlando. 69pp.

Achor, D. S., Browning, H. Y. and Albrigo, L. G. (1997). Anatomical and histochemical effects of feeding by citrus leafminer larvae (*Phyllocnistis citrella* Stainton) in citrus leaves. *Journal of American Society of Horticultural Science* 122: 829 – 836.

Ando, T., Taguchi, K. Y., Uchiyama, M., Ujiye, T. and Kuroko, H. (1985). Hexadecadienal sex attractant of the citrus leafminer moth, *Phyllocnistis citrella* Stainton (Lepidoptera, Phyllocnistinae). *Journal of Agricultural and Biological Chemistry* 49: 3633 – 3653.

Argov Y. and Rössler, Y. (1998). Rearing methods for the citrus leafminer *Phyllocnistis citrella* Stainton and its parasitoids in Israel. *Biological Control* 11(1): 18 – 21.

Ba-Angood, S. A. S. (1977) A contribution to the biology and occurrence of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the Sudan. *Journal of Agricultural Entomology* 83: 106 – 111.

Beattie, G. A. C. (1989). *Citrus leafminer.* Report No. 4. Agriculture and Fisheries Department of Primary Industries, New South Wales, Sydney. 44pp.

Belasque, J. Jr., Para-Pedrazzoli, A. L., Neto, J. R., Yamamoto, P. T., Chagas, M. C. M., Parra, J. R. P., Vinyard, B. T. and Hartung, J. S. (2005). Adult citrus leafminers (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis pv. citri*. *Journal of Plant Disease* 89: 590 – 594.

Boughdad, A., Bouazzaoi, Y. and Abdelkhallek, I. (1999). Pest status and biology of citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) in Morocco. In: *Proceedings of the Fifth International Conference on pests in Agriculture.* Morocco. pp. 251 – 259.

Bureau of Agricultural Consultancy and Advisory Service and Sokoine University of Agriculture (2008). Establishment of Integrated Market Centre at Makambako and Segera. [www.foa.suanet.ac.tz/daea/recentconsultancies.html] site visited on 20/4/2011.

Chang, W. N. and Petersen, J. B. (2003). *Citrus Production: A Manual for Asian Farmers.* Food and Fertilizer Technology Centre. Taipei, Taiwan. 106pp.

Cook, A. A. (1988). Association of citrus canker pustules with citrus leafminer tunnels in North Yemen. *Journal of Plant Disease* 72: 541 – 546.

Davies, F. S. and Albrigo, A. G. (1994). Environmental constraints on growth, development and physiology of citrus. In: *Citrus*. (Edited by Davies, F. S. and Albrigo, A. G.), Commonwealth for Agriculture Bureau International, Wallingford, United Kingdom. pp. 51 – 82.

De Prins, W. and De Prins, J. (2005). Gracillariidae (Lepidoptera). In: *World Catalogue of Insects.* (Landry, B.), Apollo Books, Stenstrup, pp. 1–502.

De Prins, J. and De Prins, W. (2009). Global taxonomic database of Gracillariidae (Lepidoptera). [http://gc.bebif.be] site visited on 13/11/2010.

De Prins, J. and Kawahara, A. Y. (2009). On the taxonomic history of *Phyllocnistis* Zeller (Gracillariidae). *Nota lepidopterologica* 32: 27 – 35.

Diez, P. A., Pena, J. E. andFidalgo, P. (2006). Population dynamics of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in Tafi Viejo, Tucuman, Argentina. *Florida Entomologist* 89: 328 – 335.

Emmet, A. M. (1985) Phyllocnistidae. In: *Moths and butterflies of Great Britain and Ireland.* (Edited by Heath, J. and Emmet, A. M.), Harley Books, Colchester. pp. 363 – 368.

FAOSTAT (2012). Food and agricultural commodities production: Countries by commodity: Oranges. [faostat.fao.org/site/339/default.aspx]site visited on 12/6/2013.

Gmitter, F. and Hu, X. (1990). The possible role of Yunnan, China, in the origin of contemporary Citrus species (Rutaceae). *Economic Botany* 44(2): 267 – 277.

Gottwald, T. R., Reynolds, K. M., Campbell, C. L. and Timmer, L. W. (1992). Spatial and spatiotemporal autocorrelation analysis of citrus canker epidemics in citrus nurseries and groves in Argentina. *Journal of Phytopathology* 82: 843 – 851.

Haggblade, S. J. and Gamser, M. S. (1991). *A Field Manual for Sub-Sector Practitioners. GEMINI Tools for Microenterprise Programs: Nonfinancial Assistance Section*. Development Alternatives Incorporation, Washington DC. 56pp.

Helgi, Ö., Rolfe, S. A., Willis, A. J. and Street, H. E. (2005). *The Physiology of Flowering Plants.* Cambridge University Press, Cambridge. 309pp.

Heppner, J. B. (1993). Citrus leafminer, *Phyllocnistis citrella*, in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). *Journal of Tropical Lepidoptera* 4: 49 – 64.

Heppner, J. B. (1995). Citrus leafminer (Lepidoptera: Gracillariidae) on fruits in Florida. *Florida Entomologist* 78(1): 183 – 186.

Hill, G. F. (1918). History of citrus canker in the Northern Territory (with notes of its occurrence elsewhere). *Northern Territory Australia Bulletin* 18:1 – 8.

Hoy, M. A. (1996). Identifying pesticides compatible with parasites of the citrus leafminer." Hoy, M. A. (Ed.) (1996) Managing the citrus leaf miner. *Proceedings from an International Conference.* 22 – 25 April, 1996.Orlando, Florida. pp. 1 – 119.

Huang, M. D., Cheng, D. X., Li, S. X., Mai, X. H., Tan, W. C. and Szetu, J. (1989a). Studies on population dynamics and control strategy of the citrus leaf miner. *Acta Entomologica Sinica* 32: 58 – 67.

Huang, M. D., Cheng, D. X., Li, S. X., Mai, X. H., Tan, W. C. and Szetu, J. (1989b). *Studies on Annual Population Dynamics and Control Strategy of the Citrus Leaf Miner.* In: *Studies on the Integrated Management of Citrus Insect Pests*. Academical Book and Periodical Projects. Guangzhou. pp. 63 – 75.

Kawahara, A. Y., Nishida, K. and Davis, D. R. (2009). Systematics, host plants, and life histories of three new *Phyllocnistis* species from the central highlands of Costa Rica (Lepidoptera, *Gracillariidae, Phyllocnistinae*). *ZooKeys* 27: 7 – 30.

Kimball, D. A. (1999). *Citrus Processing: A Complete Guide*. (2nd Ed.). Aspen Publisher, Gaithersburg, Maryland. 99pp.

Knapp, J. L., Albrigo, L. G., Browing, H. W., Bullock, R. C., Heppner, J. B., Hall, D. G., Hoy, M. A., Nguyen, R., Pen˜a, J. E. and Stansly. P. A. (1995). *Citrus Leafminer, Phyllocnistis Citrella Stainton: Current Status in Florida.* Florida Cooperative Extension Service, Gainesville. 293pp.

Lazaro, A. E. (2008). Value chain analysis for oranges in Muheza District, Tanga Region, Tanzania. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania, 96pp.

Legaspi, J. C., French, J. V. and Bendixen, R. (1997). The citrus leafminer and its natural enemies. [http://insects.tamu.edu/extension/ bulletins/ l2471.html] site visited on 5/4/2011.

Lim, U. T. and Hoy, M. A. (2006). Overwintering of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) without diapause in Florida. *Florida Entomologist* 89: 361 – 366.

Mafi, A. S. and. Ohbayashi, N. (2004). Seasonal prevalence of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its parasitoids in controlled and uncontrolled Citrus groves in Ehime Prefecture. *Journal of Applied Entomology* 39: 597 – 601.

Malausa, J. C., Quilici, S. and Brun, P. (1996). Status and first studies on the citrus leafminer in France: Azur coast, Corsica and Reunion Island. In: Hoy, M. A. (Ed.), *Managing the Citrus Leafminer. Proceedings from an International Conference*. 22 – 25 April, 1996, Orlando, Florida. 88pp.

Medler, J. T. (1980). Insects of Nigeria — check list and bibliography. *Memoirs of the American Entomological Institute* 30(7):1 – 919.

Ministry of Agriculture, Food Security and Cooperative (2004). Environment, Floriculture, Horticulture and Irrigation: [http:// www.kilimo.go.tz/agr-Industry/Environment.htm] site visited on 11/6/2011.

Møiniche, J. M. (2008).*Citrus For Local and Regional Market. Subsector Quick Scan Tanzania.* Match Maker Associate Limited, Dar es Salaam, Tanzania. 5pp.

Mwatawala, M. W., White, I. M., Maerere, A. P., Senkondo, F. J. and De Meyer, M. (2004). A new invasive *Bactrocera* species (Diptera: Tephritidae) in Tanzania. *African Journal of Entomology* 12(1): 154 – 156.

Norberg, R. P. (2008). Economic importance of Florida citrus. [http://www.flcitrusmutual.com/files/e47fe5d8-ef81-4c15-9.pdf] site visited on 11/5/2011.

Opler, P. A. and Powell, J. A. (2009). *Moths of North West America*. University of California Press. Berkeley, California. 383pp.

Patel, R. K., Swapnil, P. and Jadhav, K. M. (2012). Biology of Citrus Leaf Miner, *Phyllocnistis citrella* Stainton on Kagzi Lime. *An International E-Journal* 1: 76 – 82.

Pena, J. E. and Duncan, R. (1994). Control of the citrus leafminer in South Florida. In: *Proceedings of the Florida State Horticultural Society 106*: 47 – 51.

Pena, J.E., Duncan, R. and Browning, H. (1996). Seasonal abundance of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in South Florida citrus. *Environmental Entomology* 25(3): 698 – 702.

Pinchas, S. and Goldschmidt, E. E. (1996). *Biology of Citrus.* Cambridge University Press, Cambridge. 248pp.

Quilici, S., Franck, A., Vincenot, D. and Montagneux, B. (1995). A new citrus fruit insect pest in Reunion *Phyllocnistis citrella* Stainton. *PHYTOMA, la Défense Des Cultures* 11(4): 37 – 40.

Rwegasira G. M., Kahwa, G. K. and Herron, C. M. (2010). Survey for citrus tristeza virus and citrus aphids in Tanzania. *Proceedings of the 17th Conference*. pp. 58 – 66.

SCF (2008).*Citrus For Local and Regional Market. Subsector Quick Scan Tanzania.* Match Maker Associate Limited, Dar es Salaam, Tanzania. 5pp.

Schauff, M. E., LaSalle, J. and Wijesekara, G. A. (1998). The genera of chalcid parasitoids (Hymenoptera: Chalcidoidea) of citrus leafminer *Phyllocnistis citrella* Stainton (*Lepidoptera: Gracillariidae*). *Journal of Natural History* 32: 1001–1056.

Scora, R. W. (1975). On the history and origin of citrus. *Bulletin of the Torrey Botanical Club* 102(6): 369 – 375.

Sinclair, W. B. (1984). *Biochemistry and Physiology of Lemon and Other Citrus Fruits. Division of Agricultural Sciences*. University of California, Oakland, California. 256pp.

Srivastava, A. K. and Singh, S. (2005). Diagnosis of nutrient constraints in citrus orchards of humid tropical India. *Journal of Plant Nutrition* 29(6): 1061 – 1076.

Stainton, H. T. (1856). Description of three species of Indian Micro-Lepidoptera. *Transition Entomological Society* 3: 301 – 304.

Stansly, P. A., Albrigo, L. G. and Rouse, R. E. (1996). Impact of citrus leafminer on growth and yield of orange and grapefruit. In: Hoy, M. (Ed.). *Managing the citrus leafminer. Proceedings of the International Conference*, 23–25 April 1996, Orlando, University of Florida, Gainsville. pp. 47–48.

Swai, I. S. (1988), Citrus diseases in Tanzania. *Acta Horticulture* 218: 329 – 332.

Tan, B. and Hang, M. (1996). Managing the citrus in China. In: Hoy, M. A. (Ed), Managing the citrus leafminer. *Proceeding International Conference, University of Florida,* Orlando, Florida. pp. 49 – 52.

Tanga Regional Commissioner Office (2006). *Socio-Economic Profile, Tanga.* Final Report, Tanzania. 22pp.

Ujiye, T. (2000). Biology and control of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Japan. *Agricultural Research Quarter* 34(3): 167 – 173.

UNCTAD (2008). United Nations Conference on Trade and Development. [http://www.unctad. org/infocomm/anglais/orange/market.htm] site visited on 9/6/2011.

Verschoor, R. and Nyambo, B. (2005). *Partnership for Market Access; Towards a Sustainable Market-Oriented Horticultural Sector in Tanzania.* Wageningen, Netherlands, 54pp.

Wilson, C. G. (1991). Notes on *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) attacking four citrus varieties in Darwin. *Journal of the Australian Entomological Society* 30: 77 – 78.

CHAPTER THREE

3.0 BIOLOGY OF CITRUS LEAFMINER (Lepidoptera: Gracilariidae) IN TANZANIA

Nguvu, G and Rwegasira, G. M.

3.1 Abstract

Studies on the biology of citrus leafminer, *Phyllocnistis citrella* Stainton were carried out at the Entomology laboratory and citrus orchards at Sokoine University of Agriculture during December 2011 to September 2012. Results showed that the total development time from egg to adult was longer at 20^0C and shorter at 30^0C for 28 days and 10 days respectively. Total mortality was 26.67% at 20^0C and 6.67% at 30^0C. There was high mortality rate at larvae stage than egg stage and no mortality at pupa stage. Oviposition period decreased with increase in temperature, from 9.26 days at 20^0C to 6.21 days at 30^0C. The pest lived longer at 30^0C, but females lived longer than males at all temperatures. Oviposition rate was observed to increase with temperature; 15.2 eggs per female per day at 30^0C and 6.8 eggs per female per day at 20^0C. The overall fecundity was observed to be 28.2 eggs per female at 20^0C and 57.1 eggs per female at 30^0C. It was concluded that the optimal temperature was at 30^0C where the female: male ratio was the highest and mortality was the lowest.

3.2 Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of citrus and related Rutaceae and ornamental plants almost worldwide (Achor *et al*., 1996). Adults of *Phyllocnistis spp* are minute moths of 4 mm wingspread, with white and silvery iridescent scales, plus a black dot on each wingtip. It has 4 larval stages characteristically living by sap feeding in the new foliage (flush) leaving meandering serpentine mines (Ba-Angood, 1977). At the end of fourth (resting) instar, the pest forms a cocoon at the edge of a leaf which causes a leaf to roll (Heppner, 1995).

Temperature has been observed to have high influence on development and fecundity of citrus leafminer (Elekcioglu and Uygun, 2004). Heppner (1993) observed that the life cycle of citrus leafminer from egg to adult lasted for 13 to 52 days depending on weather conditions. Development was often continuous, resulting in up to 10 or more generations per year. Generations per year appear to be nearly continuous: six in Southern Japan (Clausen, 1931), 9 to 13 in North Central India (Lal, 1950); 10 in Southern India (Pandey and Pandey, 1964). Depending on foliage flushing cycles and weather conditions, as many as 6 to 13 generations can be expected (Jones, 2001). Adults live for only a few days. In Florida, a new generation was produced about every three weeks (Pandey and Pandey, 1964).

The citrus leafminer mines leaves, surface tissue of young shoots and stems, and less frequently the fruit (Sponagel and Diaz, 1994). The attacks of the citrus leafminer on young trees may result in their death, but older trees survive even if their young leaves are attacked. Fruit development is not directly affected (Heppner, 1993). It retards the growth of nursery citrus stock and newly planted citrus trees. It reduces yields in bearing trees.

Beside these, the citrus leafminers may also help spread of *Xanthomonas axonopodis pv. citri* leading to the citrus canker disease (Ando *et al*., 1985; Hill, 1918). This bacterium causes one of the most severe diseases of citrus worldwide and its attempted eradication has been a major source of tree loss in Florida (Smith and Hoy, 1995). The bacterium is dispersed in windblown water, and leaf wounding increases the likelihood of establishment (Belasque *et al*., 2005).

Citrus leafminer has a long history of resistance to many insecticides and development of resistance against the chemicals sometimes makes it difficult to obtain adequate control effect (Tan and Huang, 1996). Biological control is the best option for long-term control; however the effectiveness of control of citrus leafminer is complicated by its high migration ability from orchards, high fertility, and protection of the larvae inside the citrus leaf making it difficult for the chemical to contact with the pest (Mafi and Ohbayashi, 2006).

Despite of the wide distribution and apparent economic importance of citrus leafminer, there is limited information on its developmental time, mortality rate, longevity and fecundity of citrus leafminer at different temperatures under laboratory conditions (Yumruktepe *et al*., 1996). This knowledge would prove useful in improvement of control programs directed against the pest. Therefore, the present study was designed to examine the effect of different temperatures on biology of citrus leafminer under controlled laboratory conditions.

3.3 Materials and Methods

3.3.1 Identification of the pest

A survey was conducted in major citrus growing Districts of Tanzania; Morogoro, Muheza and Kinondoni. A total 12 500 trees were assessed. Leaf samples considered to contain larvae in serpentine mines were collected. A total of 1985 leaf samples were collected and individually incubated at room temperature in perforated plastic containers at the SUA entomology laboratory. Some emerging adults were wet preserved in 70% ethanol and some were dry preserved by using special micro pins under microscope for future identification. To supplement sample sources, one Monterey LG8920 pheromone trap was set in every 10 m^2 at SUA citrus orchard to trap adult moths. Citrus leafminers species collected were identified following descriptions provided by De Prins (De Prins, 2009) and by similarities in morphological features and characteristics of the damages caused by the pest.

3.3.2 Development and mortality rate of immature stages

Insects samples collected from citrus orchards during surveys and those emerged from leaf samples collected were reared on *Citrus sinensis* seedlings at 27±3°C, 80±5 % RH and a photoperiod of 16 hours artificial light in a Caron's (Model 6045) insect growth chamber for four weeks as per protocol by Kalaitzaki *et al*. (1999). New citrus plants, suitable for the pest to lay eggs upon, were replaced once every week whilst those exhibiting hatched pupae were removed.

The saplings with fresh leaves were covered with tulle cages and 20-30 leafminer adults were released into the cages. Every other day, the adults were suctioned and the leaves were examined under the hand magnification lens. The duration and the mortality at different developmental stages were recorded until eclosion by daily observations at controlled temperatures 20, 25, 30 and 35°C.

3.3.3 Longevity and fecundity of the pest

At eclosion, the newly emerged adults were kept at controlled temperatures as during their immature stages and remained under those conditions in the same tulle cages. Adults were supplied with water soaked in sponge and honey stripe for feeding. To determine the daily number of eggs laid the adults were taken from the cages with a suction trap and released to new saplings every day. The number of eggs laid per female was calculated by dividing the number of eggs per cage by the number of females. The number of dead adults was recorded daily. The survival rate was established as the proportion of adults alive within pre-established time intervals. A life table for each specific temperature was generated using formulae presented by Carey (1989).

3.3.4 Data analysis

The data obtained from daily observation were used to construct developmental, mortality and fecundity, and insect life expectancy tables according to Carey (1989). Differences in developmental/generation time, mortality and fecundity were tested by analysis of variance (ANOVA) and Fisher's Least Significant Difference test at 5 % probability.

3.4 Results and Discussion

3.4.1 Characteristic features of the specimen

The specimen that emerged from collected mined leaf samples when examined under magnification lens had a number of features which resembles *Phyllocnistis spp*. One of them is a slender body of about 2 (+/-0.4) mm long in resting position and about 4 (+/-0.2) mm wing span.

The adult has a tarnished silver forewing with broadened longitudinal yellow fascia with a wing expanse of only 5-7 mm. Colour of the collected pest specimen was faint white with silvery scales on forewings. Each wingtip had a black spot. The observed descriptions were similar to Badawy (1967), Beattie (1989) and Hill *et al*. (1985) who studied the pest in different ecological zones around the globe. The larva of *Phyllocnistis citrella* is known to be restricted to the plant family *Rutaceae*. Therefore, with these remarks it was concluded that the pest collected was *Phyllocnistis citrella* on the basis of the morphological features stipulated above.

Further reference made to De Prins and De Prins (2009) suggested the specimen to belong to genus *Phyllocnistis*. *Phyllocnistis* Zeller includes 87 described species, many of which are very small, with silvery vestiture, and similar in appearance (De Prins and De Prins 2005, 2009). The genus has been generally poorly studied because of its small size and difficulty to identify species. The precise taxonomic placement of the genus has also remained questionable because of a lack of shared adult morphological characters with other micro lepidoptera (De Prins and Kawahara, 2009). So far, it's only one species that is known to attack citrus; *Phyllocnistis citrella* Stainton (1856).

3.4.2 Leaf damage

The leaf damage caused by the pest resembles that of *P. citrella* as shown in the Figures 3a and b below:

(a) (b)

Figure 1: Sepertine mines caused by *P. citrella* (a) observed in Dar es salaam (b) reported by De Prins in Morogoro

Phyllocnistis spp is most easily detected by its meandering serpentine larval mine, usually on the ventral side of the leaf. Larvae are minute (3 mm), translucent greenish-yellow, and located inside the leaf mine. The larvae of *Phyllocnistis* usually have three or more sap-feeding instars and one non-feeding, highly specialized cocoon-spinning instar (Davis, 1987). During its larval stages, *P. citrella* mines the adaxial and abaxial surfaces of new leaves (Beattie, 2004). A newly hatched larva immediately chews into the leaf from its egg and start feeding on the internal parts of the leaf by shearing the plant tissue (Heppner, 1993). The larva creates a long, slender, sub-epidermal serpentine mine with a characteristic median frass line at the terminus of which a pupal chamber (pupal cocoon fold) is constructed, usually from the curled edge of the leaf (Davis, 1987).

Same observations were seen in this study as their similarities shown in the above diagrams. Therefore it is likely on the basis of damage caused by the pest to the citrus leaves that the pest responsible for citrus leaf mining in Tanzania is *Phyllocnistis citrella* Stainton (1856) that belongs to the order Lepidoptera and the family Gracileriidae.

3.4.3 Biological features of the pest

3.4.3.1 Development and mortality rate of immature stages

The length of egg, larval and pupal periods of *P. citrella* decreased with increasing temperatures (Table 3). Similar trend was observed by Uygun *et al*. (2004). The total developmental time from egg to adult ranged from 28.0 days at 20°C to 10.0 days at 35°C and the differences found were statistically significant at all temperatures studied.

Table 3: Development of immature stages

Temp.	Duration of immature stages (days)							
^{0}C	n	Egg	n	Larva	n	Pupa	n	Total
20	60	5.2±0.10	50	6.8 ±1.56	46	13.7±0.16	46	28.0±0.21
		(4-7)		(5-9)		(12-18)		(22-28)
25	78	2.9±0.08	72	5.5±1.45	68	7.5±0.08	68	15.4±0.29
		(2-4)		(3-6)		(7-9)		(13-15)
30	93	2.7±0.03	90	4.6±1.04	86	4.4±0.09	86	12.0±0.06
		(2-3)		(3-6)		(3-7)		(11-13)
35	125	1.8±0.03	117	3.9±0.95	113	3.7±0.18	113	10.0±0.08
		(1-7)		(4-7)		(4-8)		(10-13)

Wilson (1991) reported the egg, larval and pupal periods and the total life cycle as 2-6, 6-7, 6-7 and 14-18 days, respectively at 33^{0}C. Similar results have been recorded in this study at temperatures 30 and 35^{0}C. Pandey and Pandey (1964) recorded an incubation period of 2-10 days, a larval period of 5-10 days, a pupal period of 6-20 days and a total life cycle of 13-52 days in the field during November-January in India with no indication of the temperature. Although these results describe studies in the field, they nearly match well with the results that were recorded in the laboratory in this study. Radke and Kandalkar (1987) observed the egg, larval and pupal periods as 2.00 (1-3), 5.19 (5-6) and 6.24 (6-7) days, respectively. Ba-Angood (1977), found these periods as 2-6, 7-8, 8-9 and 18 days, respectively in the field with a changing temperature of 22-27°C. Huang *et al*. (1989) reported these periods as 0.5-10.5, 3.0-49.5 and 3.5-17.0 days, whereas Beattie and Smith (1993) reported 1-10, 5-6, and 6-22 days, respectively. Although the temperatures were not given in these studies the differences might be due to the fact that these studies were done in the field at different ecological conditions. Total mortality was high at 20°C (26.67%), but relatively low at the other temperatures indicating that the lower the temperature the higher the mortality rate. This means that the pest can survive well in warmer rather than in cooler climates.

The mortality rate of eggs ranged from 10% at 20°C to 3.33% at 30°C (Table 4). During the larval stages the mortality was higher than in the egg stage. No mortality was conducted at the pupal stage. Ba-Angood (1977) observed that in the field the mortality was higher when the temperature was high. Radke and Kandalkar (1987) observed that 19% of the eggs laid by the pest in the field died. Wilson (1991) recorded that only 5.2% of the larvae developed into pupa. The total mortality during development amounted to 96%. Mari *et al*. (1996) found a 60-80% mortality of the larval instars. These figures are much higher than the values we recorded in the laboratory in this study. The direct impact of temperature seems to be not the only mortality factor, and so other factors could be responsible for the high mortality in the field. Possible factors that could contribute to high mortality rate in the field might be the presence of natural enemies who could as predators and feed on larvae, pesticides and other agrochemicals uses which might be harming the pest and/or unpleasant weather conditions to the pest, like strong winds and rainfalls that might physically harm the pest and hence increase pest mortality rate.

Table 4: Mortality of eggs and immature stages

Temp.	Mortality of eggs and immature stages					Total mortality (egg to adult)	Mortality (%) (egg to adult)
	Egg		Larva		Pupa		
	n	(%)	n	(%)	n		
20	30	10.0	27	18.52	22	8	26.67
25	30	6.67	28	7.14	26	4	13.33
30	30	3.33	29	3.45	28	2	6.67
35	30	3.33	29	6.9	27	3	10.00

3.4.3.2 Longevity and fecundity

The period of oviposition was shortened with an increase in temperature. It was observed to be 9.26 days at 20°C and 6.21 days at 30°C (Table 5). Both adult females and males lived longer at 25°C whereby females lived for an average of about 15.2 days and males lived for an average of 12.3 days. At 30°C adult female lived 6.3 days and males 4.9 days. It was also observed that at all temperatures females lived longer than males. The highest oviposition rate was recorded at 30°C with an average of 15.2 eggs per female per day, while the lowest was at 20°C with an average of 6.8 eggs per female per day.

The overall fecundity was lower at 20°C with an average of 28.2 eggs per female and higher at 30°C with an average of 57.1 eggs per female. Overall fecundity for all temperature levels was found to be not significantly different.

Table 5: Mean duration of oviposition, longevity and fecundity

Temp.		Oviposition		Longevity (days)			No. of eggs per female	
^{0}C	n		n	Female	n	Male	per day	Total
20	30	9.26±1.48 (5-14)	30	14.8±0.16 (12-16)	30	11.81±0.41 (9-12)	6.8±1.24 (0-10)	28.2±4.22 (18-38)
25	30	7.62±1.82 (3-11)	30	15.2±0.18 (16-18)	30	12.3±0.29 (11-15)	12.4±2.18 (0-16)	49.62±6.88 (20-96)
30	30	6.21±1.61 (2-9)	30	12.32±0.09 (11-17)	30	9.22±0.06 (7-13)	15.2±2.98 (0-22)	57.1±12.76 (28-89)
35	30	5.86±1.12 (2-7)	30	6.3±0.18 (5-8)	30	4.9±0.08 (3-7)	13.6±2.4 (0-20)	47.2±10.85 (0-98)

Radke and Kandalkar (1987) found longevity of 2.37 and 3.75 days for males and females, respectively and 24.87 eggs per female. The longevity of both sexes and the number of eggs were lower in Radke and Kandalkar's study than in the present one. Huang *et al*. (1989) reported without temperature, the longevity of 1.0-22.5 days and 1.0-7.5 days for females and males, respectively and 7-108 eggs per female. Beattie and Smith (1993) observed that females could live 5-10 days and lay 20 eggs per night and more than 50 eggs per its life which matches, more or less, similar to our results with the constant temperatures of 25°C and 30°C. Knapp (1995) observed an adult longevity of 2-12 days which could increase to 20 days and a fecundity of 48 (36-76) eggs during its life which is close to the rate observed in our data. At all temperatures the females predominated over the males in number and the highest ratio was at 30°C with 1.5:1.0 (female: male). Huang *et al*. (1989) stated a female: male ratio of 1.1:1.0. Ba-Angood (1977) observed that the females slightly surpassed the males in number which is similar to our results at all temperatures studied. The results of the life tables showed that *P. citrella* displayed that at 30°C the female: male ratio was highest and mortality was lowest compared to the other temperatures studied and therefore concluded that 30°C was the optimal temperature for the pest to grow.

3.4.3.3 Mortality rate and life expectancy of mature stage

Adult pests were reared under different temperatures (20, 25, 30 and 35^{o}C) and their mortality rate and survivorship determined, and hence their respective life expectancies calculated (Appendix 1). Life expectancy was very low at temperatures 20 and 35^{o}C, 4.50 and 3.77 respectively. This was revealed with the number of days that pests spent until they were all dead also being very low (9 days). These temperatures were considered extreme low (20^{o}C) and high (35^{o}C) and hence being unfavourable for the pest. Similar results were also recorded by Radke and Kandalkar (1987) whereby mortality rate increased when temperature was either extremely lowered or increased. The optimum temperature range for pest to flourish was observed to be 25 – 30^{o}C where life expectancy was calculated to be 14.4 and 16.83 respectively and time lapsed for all pests to die was 21 and 23 days respectively. Since most of the orange growing regions have annual average temperature of about 27^{o}C there is high possibility of pest to multiply and infest large area which will result in lowered orange production in quantity and quality.

3.5 Conclusion

The study showed that an optimal temperature range for the pest growth is at 25 and 30^{0}C. At this temperature range is when the pest has low mortality rate and high fecundity, therefore having the ability to multiply fast. It is the same temperature that is found in the Coastal belt of Tanzania where there is a significant production of citrus fruits. Therefore, there is a great potential of rapid pest multiplication in the regions. Generation time of the pest ranged from 14-51 days compared to temperature, which tells that it is likely to obtain up to 20 generations per year if there will be a good distribution of rainfall and optimum temperature (25-30^{0}C). These results place Tanzania in a great risk of being severely invaded as the weather in citrus production regions highly favours *Phyllocnistis* rapid multiplication.

References

Achor, D. S., Browning, H. W. and Albrigo, L. G. (1996). Anatomical and histological modification in citrus leaves caused by larval feeding of citrus leaf-miner (*Phyllocnistis citrella* Stainton). In: *Proceedings of International Conference of Citrus Leafminer.* Orlando, Florida. 23 - 25 April 1996. University of Florida, Gainesville.pp. 1 – 69.

Ando, T., Taguchi, K.Y., Uchiyama, M., Ujiye, T. and Kuroko, H. (1985). Hexadecadienal sex attractant of the citrus leafminer moth, *Phyllocnistis citrella* Stainton (Lepidoptera, Phyllocnistinae). *Journal of Agricultural and Biological Chemistry* 49: 3633 – 3653.

Ba-Angood, S. A. S. (1977). A contribution to the biology and occurrence of the citrus leafminer, *Phyllocnistis citrella* Stainton (*Lepidoptera: Gracillariidae*) in the Sudan. *Journal of Agricultural Entomology* 83: 106 – 111.

Ba Angood, S. A. S. (1978). On the biology and food preference of the citrus leaf miner, *Phyllocnistis citrella* Stainton (Gracilariidae, Lepidoptera) in Peoples' Democratic Republic of Yemen. *Journal of Agricultural Entomology* 86(1): 53 – 57.

Badawy, A. (1967). The morphology and biology of *Phyllocnistis citrella* Stainton, a citrus leafminer in Sudan (Lepidoptera: Tineidae). *Bulletin of Entomological Society in Egypt* 51: 95 – 103.

Beattie, G. A. C. (1989). *Citrus Leafminer.* Report No. 4. Agriculture and Fisheries Department of Primary Industries, New South Wales, Sydney. 44pp.

Beattie, G. A. C. and Smith, D. (1993). *Citrus leafminer.* New South Wales Agriculture and Fisheries Department of Primary Industries, Sydney, Australia.12pp.

Beattie, G. A. C., Liu, Z. M., Watson, D. M., Clift, A. D. and Jiang, L. (1995). Evaluation of petroleum spray oils and polysaccharides for control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *Journal of the Australian Entomological Society* 34: 349 – 353.

Belasque, J. Jr., Para-Pedrazzoli, A. L., Neto, J. R., Yamamoto, P. T., Chagas, M. C. M., Parra, J. R. P., Vinyard, B. T. and Hartung, J. S. (2005). Adult citrus leafminers (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis* pv. c*itri*. *Journal of Plant Disease* 89: 590 – 594.

Carey, J. R. (1989). The multiple decrement life table: a unifying framework for cause-of-death analysis in ecology. *Oecologia* 78: 131 – 137.

Clausen, C. P. (1931), Two Citrus Leafminers of the Far East. *Agriculture Technical Bulletin* 252: 1 – 13.

Davis, D. R. (1987). Gracillariidae (Tineoidea). *Immature Insects* 1: 372 – 378.

Davies, F. S. and Albrigo, A. G. (1994). Environmental constraints on growth, development and physiology of citrus. In: *Citrus*. (Edited by Davies, F. S. and Albrigo, A. G.), Commonwealth for Agriculture Bureau International, Wallingford, United Kingdom. pp. 51 – 82.

De Prins, W. and De Prins, J. (2005). Gracillariidae (Lepidoptera). In: *World Catalogue of Insects*. (Edited by Landry, B.), Apollo Books, Stenstrup, pp. 1–502.

De Prins, J. and De Prins, W. (2009). Global taxonomic database of Gracillariidae (Lepidoptera). [http://gc.bebif.be] site visited on 13/11/2011.

De Prins, J. and Kawahara, A. Y. (2009). On the taxonomic history of *Phyllocnistis* Zeller (Gracillariidae). *Nota Lepidopterologica* 32: 27 – 35.

Elekçioğlu, N. Z. and Uygun, N. (2006). The parasitoid complex of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae) in the East Mediterranean region of Turkey and their role in biological control. *Turkish Journal of Zoology* 30: 155 – 160.

Heppner, J. B. (1993). Citrus leafminer, *Phyllocnistis citrella*, in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). *Tropical Lepidoptera* 4: 49 – 64.

Heppner, J. B. (1995). Citrus leafminer (Lepidoptera: Gracillariidae) on fruits in Florida. *Florida Entomologist* 78(1): 183 – 186.

Hill, G.F. (1918). History of citrus canker in the Northern Territory (with notes of its occurrence elsewhere). *Northern Territory Australia Bulletin* 18: 1 – 8.

Huang, M. D., Cheng, D. X., Li, S. X., Mai, X. H., Tan, W. C. and Szetu, J. (1989a). Studies on population dynamics and control strategy of the citrus leaf miner. *Acta Entomologica Sinica* 32: 58 – 67.

Huang, M. D., Cheng, D. X., Li, S. X., Mai, X. H., Tan, W. C. and Szetu, J. (1989b). *Studies on Annual Population Dynamics and Control Strategy of the Citrus Leaf Miner. In Studies on the Integrated Management of Citrus Insect Pests*. Academical Book and Periodical Projects, Guangzhou. pp. 63 – 75.

Kalaitzaki, A. P., Michelakis, S. E., Alex-andrakis, B. Z. and Lykouressis, D. P. (1999). Rearing parasitoids of *Phyllocnistis citrella* Stainton. In: *Proceedings of the 7thPanhellenic Entomological Congress of Hellenic Entomological Society* 21 – 24 October 1997, Kavala, Greece. 99pp.

Knapp, J. (1995). *Citrus Leafminer, a New Pest of Citrus in Florida, Citrus Leafminer Workshop. Institute of Food and Agricultural Sciences,*University of Florida, 26pp.

Knapp, J. L., Albrigo, L. G., Browing, H. W., Bullock, R. C., Heppner, J. B., Hall, D. G., Hoy, M. A., Nguyen, R., Pena, J. E. and Stansly, P. A. (1995). *Citrus Leafminer, Phyllocnistis citrella Stainton: Current Status.* Florida Cooperative Extension Service, Gainesville, Florida.26pp.

Lal, K. B. (1950). Insect-pests of fruit trees grown in the plains in India. *Agriculture-Animal Husbandry* 1: 30 – 45.

Mafi, A. S. and. Ohbayashi, N. (2004). Seasonal prevalence of the citrus leafminer, *Phyllocnistiscitrella*Stainton (Lepidoptera: Gracillariidae) and its parasitoids in controlled and uncontrolled Citrus groves in Ehime Prefecture. *Japan Applied Entomology* 39: 597 – 601.

Mari, F. G., Costa-Comelles, J., Vercher, R., Verdu, M. J. and A1iaga, J. L. (1996). Population trends and native parasitoids of the Citrus leafminer in Valencia (Spain). Managing the Citrus Leafminer. In: M. A. Hoy (Ed.), *Proceedings from an International Conference.* Orlando, Florida, pp. 1 – 81.

Pandey, N. D. and Pandey, Y. D. (1964). Bionomics of *Phyllocnistis citrella* (Lepidoptera: Gracillaridae). *Indian Journal of Entomology* 26: 417 – 423.

Radke, K. and Kandalkar, M. (1987). Bionomics of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae). P. K. V. *Research Journal* 11: 91 – 92.

Sponagel, K. W. and Díaz. F. J (1994). *El Minador De La Hoja De Los Cítricos Phyllocnistis Citrella: Uninsectoplaga De Importanciaeconómica En La Citricultura De Honduras.* Fundación Hondureña de Investigación Agrícola, La Lima Cortes. 31pp.

Stainton, H. T. (1856). Descriptions of three species of Indian Micro-Lepidoptera. *Transactions of the Royal Entomological Society of London* 3: 301 – 304.

Tan, B. and Huang, M. (1996). Managing the citrus in China. In: Hoy, M. A. (Ed.) Managing the citrus leafminer. *Proceeding International Conference,* University of Florida, Orlando, Florida. pp. 49 – 52.

Yumruktepe, R., Aytas, M., Erkl1, L., Yigit, A., Canhilal, R., Uygun, N., Karaca, I., Elekcioglu, N. Z. and Kersting, U. (1996). Chemical control of the citrus leafminer and side effects of effective pesticides on natural enemies in Turkey. In: Hayed, M. A. (Ed.). Managing the Citrus Leafminer. *Proceedings from an International Conference.* University of Florida, Gainesville, 23 – 25 April 1996. Orlando, Florida. pp. 98 – 103.

Wilson, C. G. (1991). Notes on *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) attacking four citrus varieties in Darwin. *Journal of Australia Entomology Society* 30: 77 – 78.

CHAPTER FOUR

4.0 INCIDENCE AND SEVERITY OF CITRUS LEAFMINERS (Phyllocnistis spp.) IN NURSERY SEEDLINGS AND ORCHARDS IN MAJOR CITRUS GROWING AREAS OF TANZANIA

Nguvu, G. and Rwegasira, G. M.

4.1 Abstract

Field survey on the occurrence, incidence and damage severity of the Citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae) were conducted on a total of 10 000 orchard plants (>5 years) in fifty randomly selected citrus fields in Morogoro Rural (Morogoro Region) and Muheza Districts (Tanga Region) from December 2011 to September 2012.In addition, a total of 4725 nursery seedlings (<5 years) in Kinondoni Districts (Dar es Salaam Region) were assessed. A total of 100 trees per each field were examined for leaf damage signs caused by leafminers. Number of mined trees was recorded and incidence calculated. Damage severity was assessed by using Horsfall-Barratt scale. Results were graphically presented. Leafminers were recorded in nearly all fields albeit at very low incidence (2%). Kruskal Wallis analysis suggested minimal spatial and temporal variations of pest incidence among the surveyed locations. Despite the low incidence, the damage data in nursery seedlings at Kinondoni suggested an increasing trend from December 2011 through June 2012.

4.2 Introduction

Phyllocnistis citrella Stainton originates from South East Asia and has been dispersed widely on all continents and many islands where citrus trees are grown (De Prins and De Prins 2005). It is found in many citrus growing areas of the world, including Southeast Asia, Japan, Taiwan, Australia, East Africa, Mediterranean countries, and the Middle East (Hill, 1983; Heppner, 1995 and Argov and Rössler, 1998).

Citrus trees produce several flushes in a year and this is directly associated with rainfall and temperature. Young trees often appear to flush almost continually during favourable weather conditions (Hall, 2007). These new flushes have been observed to harbour larvae of citrus leafminers. Trees of four years of age and less are particularly susceptible to leafminer infestation (Gottwald *et al*., 2001). In severe infestation it may result to delayed maturity of young trees from 1 to 2 years. The mines also serve as a source for establishment of a bacterium causing citrus canker disease (Ujiye, 2000).

Citrus achieve the best quality under subtropical conditions with optimum temperature ranges between 20°C and 28°C. Low rainfall and plenty of sunshine is good for citrus trees as it promotes good flower differentiation, flower and fruits development as well as fruit quality. Different studies on citrus leafminer population dynamics have been conducted worldwide and results obtained linked it with low rainfall and warm temperature (Ba-Angood, 1977). Opler and Powell (2009) observed the leafminer populations to be highest during the warmer months and lowest during the cooler months and concluded that temperature appeared to play a major role in population change. Typically, this pest declined to very low densities during the winter and the first spring flush lacked significant populations of this pest (Knapp *et al*., 1995). Maximum, minimum and average temperature had positive relationship with citrus leafminer incidence. This indicated that citrus leafminer infestation level would increase with a concomitant increase in any or all of these factors (Ahmed *et al*., 2013).

Symptoms of infestation include; leaves with serpentine mines usually on ventral surfaces (epidermis appearing as a silvery film over leaf mines). Also curling of leaves that may harbour mealy bugs and pupation chamber near leaf margin (the edge of which is rolled over and exposed portion of chamber with a distinct orange colour). In heavy infestations succulent branches of green shoots and fruits may also be attacked (Beattie, 1989, Pandey and Pandey, 1964). The objective of the current study was to establish the pest status of citrus leafminer in major citrus growing regions of Tanzania. Specifically the study aimed at establishing the incidence levels and severity of pest attack in citrus of varied age.

4.3 Materials and Methods

4.3.1 Study locations

A study was conducted in three regions in Tanzania; the two major citrus growing regions of Tanga and Morogoro, and Dar es Salaam, the region known for a large number of citrus seedling vendors. Tanga and Morogoro were used for orchards-based studies of the incidence and distribution of the citrus leafminers while Dar es Salaam was used for studying the spatial distribution and severity of the pest damage to citrus seedlings in the nurseries. One major citrus producing district was covered in each region of Tanga (Muheza) and Morogoro (Morogoro Rural), while in Dar es Salaam, one district (Kinondoni) known to have a huge number of seedling vendors was selected.

Tanga is located between 5^o00’S and 5^o04’S and 38^o15’E and 39^o06’E. It is bordered by the country of Kenya and Kilimanjaro region to the north, Manyara region to the west and Morogoro and Pwani regions to the south. Its eastern border is formed by the Indian

Ocean. Tanga experience moderate temperature and rainfall climate (warm and wet). The coastal nature of the large part of the Region, characterized by high atmospheric humidity, which lies between 100 % maximum and 65 % minimum, affects the patterns of temperature and rainfall. Average annual temperature varies between 24°C in highlands to 32°C in low lands. Tanga region experiences moderate temperature of around 23°C almost throughout the year (TRCO, 2008). The average annual rainfall varies between 200 mm and 2000 mm. However, the average varies from year to year and between ecological zones. The coastal plain which includes part of Muheza District (275 meters above sea level) experience moderate high annual rainfall of 800 - 1400 mm, and other parts of the Region receive low rainfall ranging from 200 to 600 mm annually. Tanga region has fairly well distributed bimodal rainfall (average 1223 mm/year) except for Handeni District (875 mm/year). The long rains period is between March and May and short rains period between October and December. Some variations occur with however a weak amplitude. Morogoro region lies between 5^{o} 58"S and 10^{o} 0"S and 35^{o} 25"E and 35^{o} 30"E at 338 meters above sea level (Morogoro Rural). It is bordered by seven other regions; Arusha and Tanga regions to the North, the Coast region to the East, Dodoma and Iringa to the West, and Ruvuma and Lindi to the South. The annual rainfall ranges from 600 mm in lowlands to 1200 mm in the highland plateau. The average annual temperature varies between 18^{o}C on the mountains to 30^{o}C in river valleys. In most parts of the region, the average temperatures are almost uniform at 25^{o}C. In general the hot season runs from July to September.

4.3.2 Field survey and data collection on pest incidence

A field survey was conducted through transect across all passable roads in the selected districts (Muheza and Morogoro Rural). During field survey citrus crop fields aged 5-15 years were randomly selected for assessment after each five kilometre interval. In each field a total of 100 citrus plants were examined and 50 orchards were covered per district. Out of 100 trees picked at random, citrus plants with leaf mines were counted in each field and the percentage incidence calculated by;

$$\frac{\text{Number of plants with leaf mines}}{\text{Total number of assessed plants}} \times 100 = \text{incidence (\%)} \ldots\ldots\ldots\ldots (1)$$

Pre-established data collection sheet was used to capture field information. Recorded information included the name for region, district, ward and village, geographical reference (GPS coordinates), field size, crop age, type of citrus species assessed, general health status, other pests (fruit flies, codling moths, parasitic plants) and their respective damage signs and bacterial canker symptoms. Field owners were interviewed for the productivity of the crop, field management history and problems affecting the crop. Field surveys were conducted at three month intervals for 12 months starting from December, 2011.

4.3.3 Pest severity in nursery seedlings

A total of 25 citrus seedling vendors were randomly selected in Kinondoni district in Dar es Salaam. A total of 4725 seedlings were assessed. All of the citrus seedlings available in their nurseries were assessed for citrus leafminer damage signs. Citrus seedlings with leaf mines were counted and computed against total number of seedlings and the proportion value multiplied by 100 to obtain the percentage damage (incidence) of the pest on nursery plants;

$$\frac{\text{Number of citrus seedlings with leaf mines}}{\text{Total number of citrus seedlings assessed}} \times 100 = \text{severity (\%)} \ldots\ldots\ (2)$$

The severity of pest damage was assessed by examining each seedling possessing leaf mines by giving scores following Horsfall Barratt scale (Table 6) as follows:

Table 6: Horsfall-Barratt scale

Horsfall-Barratt category	Percent ranges	True range	Midpoint for conversion
	0	0	0
1	0±3	3	1.5
2	3±6	3	4.5
3	6±12	6	9
4	12±25	13	18.5
5	25±50	25	37.5
6	50±75	25	62.5
7	75±87	13	81.5
8	87±94	6	91
9	94±97	3	96.5
10	97±100	3	98.5
11	100	0	100

Source: European Journal of Plant Pathology, 2009

Surveys were conducted at a three months interval starting from December, 2011 to September, 2012 and GPS coordinates for surveyed locations were recorded. Seedling vendors were interviewed for problems affecting seedlings and their management strategies employed.

4.4 Data Analysis

4.4.1 Spatial and temporal variations of pest status

Data on the incidence of the pest (Appendix 2) were Z-test analysed by Microsoft Excel (Microsoft, 2007) to determine the status of the pest from each location separately. Later they were subjected to Kruskal Wallis analysis to quantify variations amongst locations and periods of assessment. The null and alternative hypotheses were set for being tested as follows;

H_0: There is no variation in pest incidence between survey dates in the locations

H_1: There is variation in pest incidence between survey dates in the locations

Probability (α level of significance) was set at 5% (0.05), degrees of freedom (df): k-1; 4–1=3 and decision rule from $\chi 2$ table at α=0.05 is 7.815 and therefore if the calculated $\chi 2>7.815$ then the null hypothesis was to be rejected.

4.4.2 Damage severity

Data on the scores of severity of pest damage between survey dates were subjected to Kruskal Wallis analysis to deduce their temporal variations. Means of pest damage were plotted to show severity progress curve so as to deduce pest build up.

4.5 Results and Discussion

4.5.1 Pest incidence

The recorded means of incidences of the pest are presented (Figure 1) to show the average levels of infestation throughout the year for each survey date in both locations. In Muheza and Morogoro rural districts the percentage incidence of trees with mines were relatively low at 3.74 % and 4.26 % respectively. This could be attributed to crop age in the surveyed sites which was above 5 years, averaged at 7.98 and 10.28 years respectively. Therefore the pest relied only on new flushes of leaves for they are known to prefer tender leaves in citrus crops of below 5 years of age. In all cases the infestation levels tended to increase soon after rainy seasons (March). This is a period when the crop produce new flushes. During the rainy season, the levels decreased because of rain drops acting as pest control measure by physically harming the pest. Pest incidence reached its peak during the month of June and started to decrease by the end of the year. This is when the crop produced no more flushes and the foliage had majority of matured leaves. This is a period during which there was no rainfall.

In Kinondoni district whereby citrus seedlings were surveyed a slightly higher level of infestation was observed compared to Muheza and Morogoro Rural. Kinondoni had an average percentage incidence of 34.11 %. This was obvious led by seedlings having young fresh leaves for the pest to rely on. The comparison of average incidence percentage between the locations is indicated in Table 7.

Table 7: Comparison of incidence between locations

Parameter	Kinondoni	Muheza	Matombo
Crop age	1.7	7.98	10.28
Mean Incidence (%)	34.11	3.74	4.26
StDev	0.69	0.19	0.26
H-B scale	4.96	1.12	1.22

The incidence trend increased from December 2011 to June 2012. This was due to the fact that seedling vendors use to irrigate their nurseries throughout and not relying on rainfall. Therefore new flushes of leaves were present throughout the year. However, these numbers could be lower than was expected because of regular application of pesticides and pruning of infested leaves by vendors.

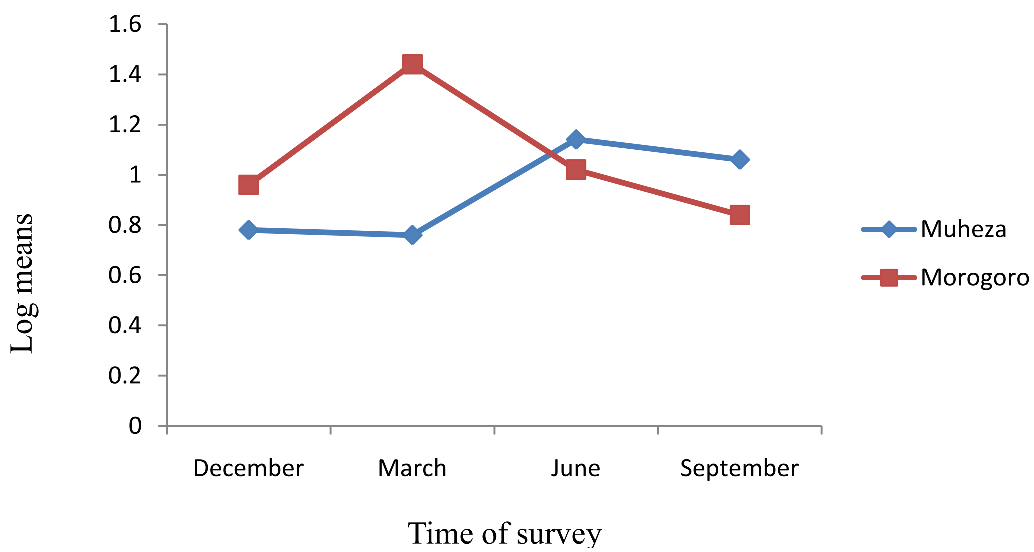

Figure 2: Log means of pest incidence against time of survey

Amongst 100 citrus fields surveyed in Morogoro Rural and Muheza districts, 74 had citrus trees of between 5-10 years of age and 26 fields had trees with more than 10 years of age (Table 8). All the 25 nurseries surveyed in Kinondoni district had citrus seedlings with less than 5 years of age. Citrus leafminers were recorded on about 16 plants in each field with less than 5 years old citrus trees. This number was large compared to an average of 1 plant per fields in citrus trees aged above five years. Hoy (1996) and Diez *et al.* (2006) observed similar results when studying leafminers' life cycle and revealed their dependency on young tender leaves for laying eggs and feeding during their larvae stage. Same reason could explain the observed trend in the current study.

Table 8: Correlation between plant age and incidence and severity of leaf miners

Crop Age (years)	Number of fields	Average mined trees
<5	25	16
5-10	74	1
>10	26	1

Kruskal Wallis analysis results from SPSS 16.0 edition (SPSS Incorporation, 2007) the outputs were as shown in Table 9.

Table 9: Kruskal Wallis analysis of surveyed areas

Test Statistics	Kinondoni	Muheza	Morogoro
Chi-Square	5.684	4.441	2.818
Degrees of freedom	3	3	3
Asymp. Sig.	0.13	0.22	0.42

a. Kruskal Wallis Test

Results show that in both sites the probability is less than 5 % ($p<0.05$). Therefore, we do not reject the null hypothesis hence concluded that there was no temporal variation between sampling dates within the location. Generally, the pest level was very low to almost negligible in Morogoro Rural and Muheza which was due to crop age amongst other factors. Crop in Morogoro Rural was 10.82 years on average hence not supporting pest colonisation. The crop in Muheza averaged 7.98 years of age and was literally still young but did not favour pest growth. These observations are in line with the report by Diez *et al*. (2006) that citrus leaf miner is favoured by crop that are less than five years of age.

4.5.2 Pest severity

In Kinondoni where the severity of the pest attack was assessed on young seedlings, it was established that severity increased with time despite the control measures (trimming damaged leaves, applying pesticides) that were being employed by seedling vendors. The percentage severity of the plots assessed by Horsfall-Barratt scale was plotted against the time of assessment and showed an increase from December 2011 to September 2012 as shown in Figure 3 below.

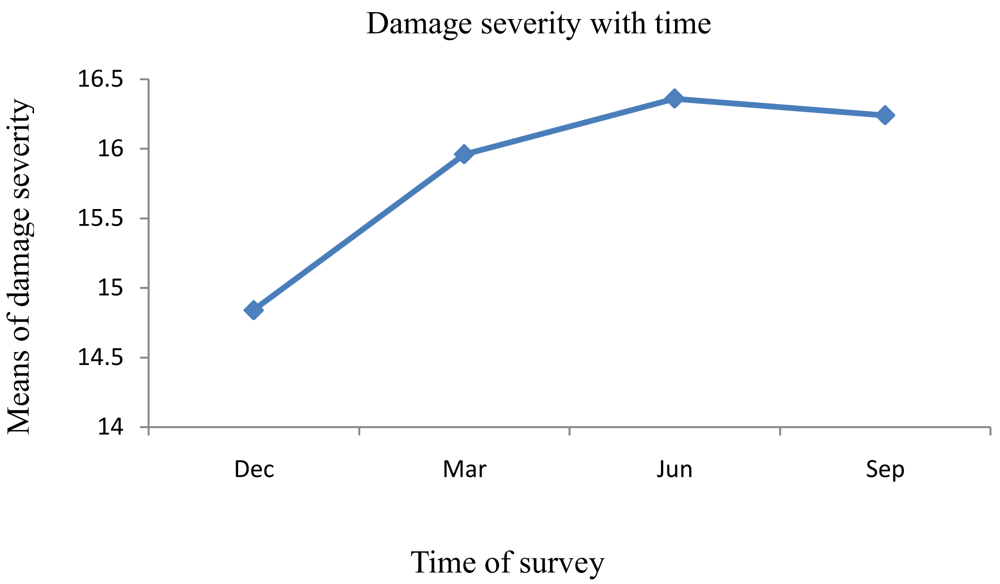

Figure 3: Means of damage severity against time of survey at Kinondoni in 2012

However, the increasing trend stopped in June and between June and September there was a decrease in damage severity. Based on weather parameters, June is usually onset of dry season characterized by low moisture, low relative humidity and low temperatures. These conditions are not suitable for reproduction and perpetuation of leafminers (Ahmed *et al*., 2013).

Rainfall seems to have greatly influenced the population of *Phyllocnistis spp*. The general trend of the populations of the species seems to be associated with rainfall pattern. High populations were recorded during and soon after rainy seasons. This period is when trees produced new flushes of leaves. Another abiotic factor that had an influence on observed population trend is humidity. A study by Duyck *et al*. (2006) has shown that atmospheric humidity strongly influences the survival of the leafminer pupae and therefore concluded that increased population of citrus leafminer is directly related to increase in relative humidity at the beginning of rainy season.

Temperature also might have influenced distribution of the pest. High numbers were recorded in the low and medium altitude areas of Kinondoni and Muheza districts compared to Morogoro Rural where it has a relatively cool temperature in some occasions. There is an inverse relationship between the infestation rate of *Phyllocnistis spp*. and the elevation at which fruits are found (Ekesi *et al*., 2006). Similarly Wong *et al.* (1985) considered the pest as lowland pest. Distribution of *Phyllocnistis spp*. might be limited by temperature as it has been reported by Duyck *et al*. (2006).

4.6 Conclusion

Ecology forms the basis of pest management by providing explanations of how the environment affects abundance, timing and diversity of insects (Pedigo, 1996). The status of an insect as a pest is determined by, among other things, the value of commodity attacked and its population numbers (Hill, 1993). A change in population numbers in most cases is directly proportional to crop losses. Insect pests occurring in large numbers are likely to cause heavy losses to crop and are therefore classified as key pests. The management of an insect pest species in a cropping system can be achieved if the techniques used is able to reduce both initial numbers and the rate of population growth (Dent, 1991). Distribution and abundance of leafminers are markedly structured by various biotic and abiotic factors which include temperature, humidity, host fruit and natural enemies. These have direct effect on species themselves as well as an indirect effect by modulating interspecific competition.

The study showed presence of citrus leafminers in all study locations, albeit at very low levels. This indicated that the pest is widely spread to major citrus growing regions. Since citrus grows in warm regions (25 - 30^{o}C), the weather that has been seen to favour pest development. The pest is also known to have up to 20 cycles per year when in conducive environment therefore it is likely that the infestations level will increase soon.

The widespread abundance of preferred hosts, the availability of major hosts throughout the year as well as the presence of a large number of fruits could result into large number of leafminers populations. Therefore, since both of the factors that favour the pest development are available in Tanzania, I propose that *Phyllocnistis spp*. be declared as present and it is of economic importance in Tanzania. However, further studies need to be undertaken for a much longer time and wider area so that more data can be collected and expand on this information so as to generate database of this pest and possible alternatives of controlling it.

References

Ahmed, S., Khan, M. A., Hassan, B., Haider, H. and Ahmad, S. F. (2013). Studies on citrus leafminer in relation to abiotic factors on different host plants in Punjab, Pakistan. *Pakistan Entomologist* 35(1): 5 – 10.

Argov, Y. and Rössler, Y. (1998). Rearing methods for the citrus leafminer *Phyllocnistis citrella* Stainton and its parasitoids in Israel. *Biological Control* 11(1): 18 – 21.

Ba-Angood, S. A. S. (1977). A contribution to the biology and occurrence of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the Sudan. Journal *of Applied Entomology* 83: 106 – 111.

Beattie, G.A.C. (1989). Citrus leafminer. New South Wales Agriculture and Fisheries Department of Primary Industries. *Sydney* 4: 41 – 44.

Dent, D. (1991). *Insect Pest Management.* Commonwealth for Agriculture Bureau International, Wallingford, London. 640pp.

De Prins, J. and De Prins, W. (2005). *Gracillariidae (Lepidoptera). World Catalogue of Insects.* Apollo Books, Stenstrup. 502pp.

Diez, P. A., Pena, J. E. and Fidalgo, P. (2006). Population dynamics of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in Tafi Viejo, Tucuman, Argentina. *Florida Entomologist* 89: 328 – 335.

Duyck, P., David, P. and Quilici, S. (2006). Climatic niche partitioning following successive invasions by fruit flies in La Reunion. *Journal of Animal Ecology* 75: 518 – 526.

Ekesi, S., Nderitu, P. W. and Chang, C. L. (2007). Adaptation to and small-scale rearing of the invasive fruit fly *Bactrocerainvadens* (Diptera: Tephritidae) on artificial diet. *Entomological Society of America* 100(4): 562 – 567.

Gottwald, T. R., Sun, X., Riley, T., Graham, J. H., Ferrandino, F. and Taylor, E. L. (2002). Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. *Journal of Phytopathology* 92: 362 – 377.

Hall, D. G. (2007). Estimating relative abundance of flush shoots in citrus with implication on monitoring insects associated with citrus. *Journal of Horticultural Science* 42: 364 – 368.

Heppner, J. B. (1993). Citrus Leafminer, *Phyllocnistis citrella*, in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). Tropical Lepidoptera 4: 49 – 64.

Hill, D. (1983). *Insect Pests of the Tropics and Their Control.* Longman Publishers, London. 746pp.

Hoy, M. A. (1996). Identifying pesticides compatible with parasites of the citrus leafminer. Hoy, M. A. (Ed.) Managing the Citrus Leaf Miner. *Proceedings from an International Conference.* April 22–25, 1996, Orlando, Florida. pp. 1 – 119.

Knapp, J. L., Albrigo, L. G., Browing, H. W., Bullock, R. C., Heppner, J. B., Hall, D. G., Hoy, M. A., Nguyen, R., Pena, J. E. and Stansly, P. A. (1995). *Citrus leafminer, Phyllocnistis citrella Stainton: Current status in Florida.* Florida Cooperative Extension Service, Gainesville, Florida. 26pp.

Microsoft Office Excel (2010). Microsoft Corporation Limited.

Opler, P. A. and Powell, J. A. (2009). *Moths of North West America.* University of California Press, Berkeley, California. 383pp.

Pandey, N. D. and Pandey, Y. D. (1964). Bionomics of *Phyllocnistiscitrella* (Lepidoptera: Gracillaridae). *Indian Journal of Entomology* 26: 417 – 423.

Pedigo, L.P. (1996). *Entomology and Pest Management*. (2nd Edition), Prentice Hall, New Jersey. 679pp.

Statistical Package for Social Sciences (2007). Software Groups Business Analytics Portfolio. IBM Corporation.

Ujiye, T. (2000). Biology and control of the citrus leafminer, *Phyllocnistiscitrella* (Lepidoptera: Gracillariidae) in Japan. *Japan Agricultural Research Quarter* 34(3): 167 – 173.

Wong, T.Y.M., McInnis, D.O. and Mochizuki, N. (1985). Seasonal distribution and abundance of adult male oriental fruit flies (Diptera: Tephritidae) in Kula, Maui, Hawaii. *Journal of Economic Entomology* 78: 1267 – 1271.

APPENDICES

Appendix 1: Life tables for *P. citrella* at different temperatures

20°C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1) / 2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.13	0.13	14	4.50
2	13	0.87	0.07	0.08	12.5	
3	12	0.80	0.13	0.17	11	
4	10	0.67	0.07	0.10	9.5	
5	9	0.60	0.13	0.22	8	
6	7	0.47	0.13	0.29	6	
7	5	0.33	0.13	0.40	4	
8	3	0.20	0.13	0.67	2	
9	1	0.07	0.07	1.00	0.5	

25^0C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/ No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1) / 2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.00	0.00	15	14.4
2	15	1.00	0.00	0.00	15	
3	15	1.00	0.00	0.00	15	
4	15	1.00	0.07	0.07	14.5	
5	14	0.93	0.00	0.00	14	
6	14	0.93	0.07	0.07	13.5	
7	13	0.87	0.00	0.00	13	
8	13	0.87	0.00	0.00	13	
9	13	0.87	0.00	0.00	13	
10	13	0.87	0.07	0.08	12.5	
11	12	0.80	0.00	0.00	12	
12	12	0.80	0.07	0.08	11.5	
13	11	0.73	0.07	0.09	10.5	
14	10	0.67	0.00	0.00	10	
15	10	0.67	0.07	0.10	9.5	
16	9	0.60	0.07	0.11	8.5	
17	8	0.53	0.20	0.38	6.5	
18	5	0.33	0.07	0.20	4.5	
19	4	0.27	0.13	0.50	3	
20	2	0.13	0.07	0.50	1.5	
21	1	0.07	0.07	1.00	0.5	

30^0C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1)/2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.00	0.00	15	16.83
2	15	1.00	0.00	0.00	15	
3	15	1.00	0.00	0.00	15	
4	15	1.00	0.00	0.00	15	
5	15	1.00	0.00	0.00	15	
6	15	1.00	0.07	0.07	14.5	
7	14	0.93	0.00	0.00	14	
8	14	0.93	0.00	0.00	14	
9	14	0.93	0.00	0.00	14	
10	14	0.93	0.07	0.07	13.5	
11	13	0.87	0.00	0.00	13	
12	13	0.87	0.00	0.00	13	
13	13	0.87	0.07	0.08	12.5	
14	12	0.80	0.00	0.00	12	
15	12	0.80	0.07	0.08	11.5	
16	11	0.73	0.00	0.00	11	
17	11	0.73	0.00	0.00	11	
18	11	0.73	0.20	0.27	9.5	
19	8	0.53	0.27	0.50	6	
20	4	0.27	0.07	0.25	3.5	
21	3	0.20	0.07	0.33	2.5	
22	2	0.13	0.07	0.50	1.5	
23	1	0.07	0.07	1.00	0.5	

35^0C

Age (day)	Total No. of individuals (Nx)	Survivorship lx=Nx/No	Mortality rate dx=lx-lx+1	Probability of dying qx=dx/lx	Mean No. of moths alive Lx=(Nx+Nx+1)/2	Life expectancy Ex=ΣLx/Nx
1	15	1.00	0.20	0.20	13.5	3.77
2	12	0.80	0.20	0.25	10.5	
3	9	0.60	0.07	0.11	8.5	
4	8	0.53	0.07	0.13	7.5	
5	7	0.47	0.13	0.29	6	
6	5	0.33	0.07	0.20	4.5	
7	4	0.27	0.07	0.25	3.5	
8	3	0.20	0.13	0.67	2	
9	1	0.07	0.07	1.00	0.5	

Appendix 2: Data collected on the incidence of damage

Muheza District

Plot	FIELD SIZE (No. of trees)	CROP AGE (years)	Log average mines	Incidence (%)	STDEV	H-B scale	GPS LOCATION
1	100	6	0.40	10	1.00	1	S 05 08'55.20" E 38 52'48.40"
2	100	6	0.40	10	1.29	1	S 05 08'44.30" E 38 51'34.80"
3	100	8	-0.30	2	1.00	1	S 05 06'42.80" E 38 48'35.30"
4	100	8		0	0.00	0	S 05 06'30.30" E 38 48'50.00"
5	100	8	-0.30	2	0.58	1	S 05 04'48.40" E 38 47'25.60"
6	100	7	-0.30	2	0.58	1	S 05 04'10.70" E 38 47'54.40"
7	100	7	0.18	6	1.00	1	S 05 04'17.60" E 38 47'50.90"
8	100	7	0.00	4	0.00	1	S 05 07'46.90" E 38 47'41.60"
9	100	7	0.24	7	0.50	2	S 05 07'44.42" E 38 47'34.30"
10	100	7	0.30	8	0.00	1	S 05 07'43.10" E 38 47'30.90"
11	100	7	-0.30	2	0.58	1	S 05 13'55.90" E 38 47'47.20"
12	100	8		0	0.00	0	S 05 13'50.30" E 38 47'40.00"
13	100	7		0	0.00	0	S 05 16'16.60" E 38 46'23.30"
14	100	7	0.10	5	0.50	1	S 05 16'14.90" E 38 46'54.60"
15	100	7	-0.30	2	0.58	1	S 05 17'40.10" E 38 46'57.50"
16	100	8	0.18	6	1.00	1	S 05 16'40.60" E 38 46'59.90"
17	100	8	0.10	5	0.50	2	S 05 16'32.30" E 38 46'50.00"
18	100	8	0.18	6	0.58	2	S 05 16'54.80" E 38 46'48.20"
19	100	12		0	0.00	0	S 05 17'42.40" E 38 46'11.40"
20	100	12	-0.60	1	0.50	1	S 05 17'40.40" E 38 46'32.20"

Plot	FIELD SIZE (No. of trees)	CROP AGE (years)	Log average mines	Incidence (%)	STDEV	H-B scale	GPS LOCATION
21	100	12		0	0.00	0	S 05 19'23.10" E 38 44'22.40"
22	100	10	-0.30	2	1.00	1	S 05 19'18.70" E 38 44'54.40"
23	100	9	-0.30	2	0.58	1	S 05 19'07.70" E 38 45'11.80"
24	100	9	-0.12	3	0.50	1	S 05 19'10.30" E 38 45'44.20"
25	100	9		0	0.00	0	S 05 19'07.90" E 38 45'11.30"
26	100	9	0.00	4	0.00	1	S 05 19'07.60" E 38 45'11.70"
27	100	9		0	0.00	0	S 05 14'13.30" E 38 41'20.50"
28	100	9		0	0.00	0	S 05 14'18.00" E 38 42'19.60"
29	100	6	0.18	6	1.00	2	S 05 14'37.70" E 38 46'16.30"
30	100	10		0	0.00	0	S 05 14'30.20" E 38 46'10.10"
31	100	8	-0.30	2	0.58	1	S 05 14'18.70" E 38 46'21.10"
32	100	6	0.51	13	0.50	3	S 05 14'57.40" E 38 46'12.90"
33	100	6	0.35	9	0.50	3	S 05 14'55.00" E 38 46'08.20"
34	100	6	0.30	8	0.00	2	S 05 14'50.30" E 38 45'19.30"
35	100	6	0.35	9	0.96	3	S 05 14'35.20" E 38 46'16.50"
36	100	6	0.30	8	0.00	3	S 05 14'10.30" E 38 46'26.00"
37	100	6	0.35	9	0.50	3	S 05 12'55.30" E 38 46'39.39"
38	100	6	0.51	13	0.96	3	S 05 12'50.00" E 38 46'20.10"
39	100	12	-0.60	1	0.50	1	S 05 12'46.60" E 38 46'40.50"
40	100	12		0	0.00	0	S 05 12'44.10" E 38 46'33.70"
41	100	12	-0.60	1	0.50	1	S 05 12'36.80" E 38 43'51.10"
42	100	11	-0.30	2	0.58	1	S 05 12'18.80" E 38 45'18.90"
43	100	10		0	0.00	0	S 05 12'08.70" E 38 46'46.20"
44	100	7	-0.30	2	1.00	1	S 05 12'07.40" E 38 46'32.40"
45	100	7	-0.30	2	1.00	1	S 05 13'45.30" E 38 42'55.50"
46	100	7		0	0.00	0	S 05 13'40.80" E 38 42'32.30"
47	100	6	-0.60	1	0.50	1	S 05 13'39.80" E 38 46'27.00"
48	100	6	0.00	4	0.00	1	S 05 13'30.10" E 38 46'12.80"
49	100	6	-0.30	2	0.58	1	S 05 09'34.70" E 38 48'55.60"
50	100	6	0.18	6	0.58	2	S 05 10'28.10" E 38 50'44.20"

Morogoro

Plot	FIELD SIZE (No. of trees)	CROP AGE (years)	Log average mines	Incidence (%)	STDEV	H-B scale	GPS LOCATION
1	100	8	0.40	10	1.00	3	S 07 03'44.00" E 37 49'31.10"
2	100	8	0.35	9	0.50	3	S 07 03'46.20" E 37 49'52.20"
3	100	8	0.18	6	0.58	2	S 07 03'43.60" E 37 49'48.10"
4	100	8	0.30	8	0.00	2	S 07 03'49.00" E 37 51'01.60"
5	100	7	0.10	5	0.50	2	S 07 03'49.20" E 37 48'22.60"
6	100	8	0.10	5	0.50	1	S 07 03'42.60" E 37 49'24.50"
7	100	7	0.40	10	0.58	3	S 07 03'38.70" E 37 52'13.30"
8	100	7	0.24	7	0.50	2	S 07 03'26.10" E 37 48'68.30"
9	100	8	0.10	5	0.50	1	S 07 03'32.60" E 37 44'26.10"
10	100	7	0.10	5	1.50	1	S 07 03'15.70" E 37 47'25.10"
11	100	19	-0.30	2	0.58	1	S 07 03'16.20" E 37 46'42.10"
12	100	8	0.24	7	0.50	1	S 07 06'18.30" E 37 46'51.60"
13	100	9	0.00	4	0.00	1	S 07 06'16.30" E 37 49'18.50"
14	100	19	-0.12	3	0.50	1	S 07 06'50.30" E 37 47'13.20"
15	100	19	0.00	4	0.00	1	S 07 04'08.50" E 37 48'20.40"
16	100	8	0.10	5	0.96	1	S 07 04'11.20" E 37 46'43.00"
17	100	8	0.30	8	0.00	1	S 07 04'10.60" E 37 48'48.20"
18	100	7	0.30	8	0.00	2	S 07 04'10.20" E 37 44'30.10"

Plot	FIELD SIZE (No. of trees)	CROP AGE (years)	Log average mines	Incidence (%)	STDEV	H-B scale	GPS LOCATION
19	100	8	-0.30	2	1.00	1	S 07 04'54.70" E 37 46'17.10"
20	100	7	0.40	10	1.00	2	S 07 04'53.10" E 37 44'54.40"
21	100	7	0.24	7	0.96	2	S 07 05'20.30" E 37 44'30.60"
22	100	7	0.18	6	1.73	2	S 07 05'12.80" E 37 44'18.20"
23	100	7	0.00	4	1.41	1	S 07 06'58.20" E 37 43'20.20"
24	100	11	-0.60	1	0.50	1	S 07 05'30.80" E 37 43'22.30"
25	100	11	-0.30	2	0.58	1	S 07 05'10.50" E 37 43'10.40"
26	100	11	-0.30	2	0.58	1	S 07 04'40.20" E 37 43'30.40"
27	100	11	0.00	4	0.00	1	S 07 04'44.40" E 37 43'20.20"
28	100	11	0.00	4	0.00	1	S 07 01'56.80" E 37 44'54.70"
29	100	15	-0.60	1	0.50	1	S 07 01'54.40" E 37 44'30.20"
30	100	15	-0.12	3	0.50	1	S 07 01'40.20" E 37 44'45.40"
31	100	15	0.10	5	0.50	1	S 07 01'34.70" E 37 44'47.30"
32	100	15	-0.12	3	0.50	1	S 07 01'38.50" E 37 44'36.30"
33	100	18	0.00	4	0.00	1	S 07 01'35.60" E 37 44'18.70"
34	100	15	0.00	4	1.15	1	S 07 01'43.80" E 37 43'35.30"
35	100	20	-0.12	3	0.50	1	S 07 01'33.40" E 37 43'56.20"
36	100	20	-0.12	3	0.50	1	S 07 01'44.60" E 37 43'18.70"
37	100	6	0.00	4	0.82	1	S 07 03'18.30" E 37 43'48'20"
38	100	6	0.10	5	0.50	1	S 07 01'52.00" E 37 44'55.10"
39	100	8	-0.30	2	0.58	1	S 07 03'30.50" E 37 43'12.20"
40	100	6	0.18	6	0.58	1	S 07 03'33.30" E 37 43'40.60"
41	100	6	0.10	5	0.50	1	S 07 03'22.20" E 37 43'54.00"

Plot	FIELD SIZE (No. of trees)	CROP AGE (years)	Log average mines	Incidence (%)	STDEV	H-B scale	GPS LOCATION
42	100	10	-0.60	1	0.50	1	S 07 03'06.40" E 37 45'20.50"
43	100	6	0.00	4	0.82	1	S 07 03'13.30" E 37 45'22.20"
44	100	6	0.00	4	0.00	1	S 07 03'14.10" E 37 45'34.60"
45	100	11		0	0.00	0	S 07 03'20.50" E 37 45'40.00"
46	100	11	-0.60	1	0.50	1	S 07 03'07.60" E 37 45'38.30"
47	100	13		0	0.00	0	S 07 02'44.40" E 37 45'26.30"
48	100	10		0	0.00	0	S 07 02'37.20" E 37 44'41.60"
49	100	9	-0.60	1	0.50	1	S 07 01'16.80" E 37 44'34.80"
50	100	9	-0.60	1	0.50	1	S 07 01'17.40" E 37 44'07.60"

Kinondoni

Plot	FIELD SIZE (No. of trees)	CROP AGE (years)	Log average mines	Incidence (%)	STDEV	H-B scale	GPS LOCATION
1	188	2	1.15	29.79	1.41	5	S 06 48'15.90" E 39 12'38.70"
2	140	2	1.02	30.00	1.00	5	S 06 48'14.40" E 39 24'14.40"
3	176	2	1.09	27.84	3.30	5	S 06 48'18.20" E 39 24'18.70"
4	90	2	0.88	33.33	0.58	5	S 06 47'15.60" E 39 09'22.60"
5	190	2	1.26	38.42	1.26	5	S 06 47'26.60" E 39 08'17.90"
6	224	2	1.23	30.36	1.15	5	S 06 47'02.00" E 39 07'02.50"
7	318	2	1.33	27.04	1.91	5	S 06 47'09.90" E 39 07'45.20"
8	200	2	1.20	32.00	2.58	5	S 06 47'08.80" E 39 09'53.10"
9	302	1.5	1.41	33.77	1.91	5	S 06 46'34.20" E 39 13'09.10"
10	278	2	1.27	26.98	1.50	5	S 06 45'45.60" E 39 13'55.10"
11	122	2	1.08	39.34	1.63	5	S 06 45'33.00" E 39 13'44.30"
12	64	2	0.78	37.50	1.63	5	S 06 44'35.90" E 39 14'15.90"
13	98	2	0.85	28.57	2.00	5	S 06 44'35.40" E 39 14'16.00"
14	188	2	1.08	25.53	1.41	4	S 06 44'04.80" E 39 14'05.00"
15	230	1	1.25	30.87	1.26	5	S 06 43'48.70" E 39 12'32.40"
16	200	1	1.20	32.00	0.82	5	S 06 44'10.30" E 39 12'14.60"
17	212	1	1.28	36.32	1.50	5	S 06 43'30.60" E 39 12'28.20"
18	184	1	1.26	39.67	0.96	5	S 06 43'34.70" E 39 13'52.40"
19	170	1	1.21	38.24	0.50	5	S 06 42'50.20" E 39 13'48.10"
20	164	1	1.20	38.41	2.06	5	S 06 42'53.00" E 39 13'47.00"
21	124	2	1.18	48.39	0.82	5	S 06 42'46.90" E 39 13.36.20"
22	180	2	1.21	36.11	1.50	5	S 06 42'32.70" E 39 13.20.10"
23	200	2	1.27	37.50	1.50	5	S 06 42'06.40" E 39 13'11.60"
24	260	1	1.37	36.15	0.58	5	S 06 42'04.20" E 39 12'14.20"
25	220	2	1.33	38.64	1.50	5	S 06 42'00.40" E 39 12'24.30"