Effect of temperature on the life history of *Liriomyza trifolii* Burgess (Diptera: Agromyzidae)

By

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DECLARATION

This thesis is my original work, and has not been presented for degree in any other university or any other award

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DEDICATION

This work is dedicated to my wife Pet, and daughter Tabby, both of whom stood with me through the upheavals of being a student.

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LIST OF ABBREVIATIONS

CIP : International Potato Center

EPPO: European and Mediterranean Plant Protection Organization

ILCYM: Insect life Cycle Modeling

IPCC: Intergovernmental Panel on Climate change

OEPP: Organisation Européenne et Méditerranéenne pour la Protection des Plantes

ABSTRACT

Liromyza trifolii Burgess (Diptera: Agromyzidae) belongs to a group of phytophagous insects whose larvae feed inside the leaf of a wide variety of horticultural crops like snow peas (*Pisum sativum* L.), french beans (*Phaseolus vulgaris* L.), potatoes (*Solanum tuberosum* L.) and a variety of flowers. Studies were carried out at seven constant temperatures of 10, 15, 18, 20, 25, 30 and 35^0C to determine the effect of temperature on life history traits to determine the intrinsic rate of increase and to determine the potential geographic distribution range. The study entailed rearing of a pair of newly hatched *L. trifolii* fly in a cage under constant temperature, while providing for them with one potted *Phaseolus vulgaris* plant as a source of food and oviposition site. The plant was replaced after every 24 hours. Upon removal, potted plant was placed in a holding cage within the same temperature. Egg, larvae and pupae were observed and on reaching adult stage were paired to determine daily oviposition. Staining on leaves was done to count number of non-viable eggs. Data obtained from the study was analyzed using One-way Analysis of variance (ANOVA) to determine if there was significant difference in development at different temperature regimes. The data was later analyzed using Insect Life Cycle Model (ILCYM) to compare development rate, time, mortality, senescence and oviposition rate to determine influence of temperature on these development traits. Egg, larval and pupal development did not take place at 10^0C, development time was shortest at 35^0C (Egg 1.27 ± 0.00: larvae 2.43 ± 0.00 and pupa 6.82 ± 0.01 days), longest at 15^0C (Eggs11.4 ± 0.0014 days while larvae and pupa development did not take place), lowest mortality was at 25^0C (0.070, 0.449, 0.384 for egg, larvae and pupa respectively) and highest total oviposition was recorded at 27^0C at 60 eggs per female. Development rate was inversely proportional to temperature; male senescence was highest at 30^0C (0.56 1/days) while female senescence was highest at 35^0C (8.66 1/days). Analysis of Variance (ANOVA) showed a significant difference in development time at various temperature regimes (Egg $F_{5,\ 2634=}$2806.84 p.value=0.00; Larvae $F_{4,988=}$762.32 p.value=3.8E-300 and Pupa $F_{4,448=}$2395.3948 p.value=8.5E-301). Development time of all life stages (egg, larvae and pupa) varied in temperature regimes tested. Data was further used to develop potential world distribution maps of *L. trifolii* for the year 2000 and 2050. The two maps indicated an increase in world temperatures as projected by Intergovernmental Panel on Climate Change by 3.2 to 4.0^0C would lead to colonization of new areas that previously were not affected by the fly.

CHAPTER ONE

1.0: INTRODUCTION

1.1 Background of the study

The leaf-mining fly, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), is an important pest of vegetables and ornamental plants (Minkenberg and van Lenteren, 1986; Parrella, 1987). It was accidentally introduced from the United States of America into the Afrotropical region in 1976 by cut flowers and currently causes considerable economic loss to the production of vegetable and ornamental plants (Stegmaier, 1966; Spenser, 1973; Neuenschwander *et al*., 1987; Kotzee and Dennill, 1996; Musundire *et al.,* 2011). The *Liriomyza* genus larvae feed on the internal tissues of living plants and exhibit different feeding habits such as leaf-mining, stem-mining, stem-tunnelling and cambium-mining (Dempewolf, 2004). Out of all feeding habits, leaf mining is generally the most widespread feeding behavior (Spencer, 1973). *Liriomyza trifolii* are multivoltine and are thus able to rapidly develop resistance against insecticides (Reitz and Trumble, 2002).

In Kenya, *L. trifolii* is one of the leading pests causing serious damage to snow pea (*Pisum sativum* L.), french beans (*Phaseolus vulgaris* L.), tomatoes (*Lycospersicon esculentum* Miller), runner beans (*Phaseolus coccineus* L.), potatoes (*Solanum tuberosum* L.) and a variety of cut flowers (Musundire *et al*., 2011). Leaf mining *Liriomyza* is one of the pests in the European Union (EU) list of quarantine pests, which has led to rejection of many export products to the European market (Gitonga, 2010). Currently, *L. trifolii* is an important pest in low and mid altitude areas with yield loss ranging from 10 to 100% (European and Mediterranean Plant

protection organization, 1984; Gitonga, 2010). Studies show that distribution of this insect is dependent on ecological suitability and favorable temperature (Parella, 1987). However, distribution as observed today may be attributed to changes in global average temperatures, which have increased by about 0.10-0.16^{0}C per decade for the past 50 years (Alali *et al.*, 2007). *Liriomyza trifolii* may change its distribution range and colonize areas projected to experience increased temperature and reduction of frost (Alali *et al.*, 2007).

Temperature is one of the parameters that are projected to increase with change in climate. The third assessment report of the Inter-governmental Panel on Climate Change (Houghton *et al.*, 2001) indicates that global temperatures have risen by 0.6^{0}C in the last 100 years. The fourth assessment report (Alali *et al.*, 2007), states that the twelve years between 1995 and 2006 rank among the warmest in instrumental record of global surface temperature since 1850. In many places, minimum temperatures have increased twice as much as maximum temperature.

Temperature has been identified as a critical abiotic factor influencing the dynamics of insects as it sets the limits of biological activities (Walthers *et al.,* 2002). Knowledge of the influence of temperature on insect dynamics can thus be used to understand low and high temperature thresholds as well as the optimal temperature for all major life processes (Parella, 1987). Thermal tolerance of insects varies between species, populations and developmental stages (Gilbert and Raworth, 1996). Even though other ecological factors such as food source and quality will influence the growth rate of an individual insect species, the growth rate may be impaired by temperatures above the physiological limit. Though *L. trifolii* is a cosmopolitan species, there is no knowledge on its thermal tolerance which could form the basis of predicting

the effect of climate change on its geographical range. It is on this background that this study was initiated to assess the effect of temperature on life history traits of *L. trifolii*.

1.2 Statement of the problem

The serpentine leaf miner, *L. trifolii*, is a worldwide pest of numerous vegetable and ornamental crops (Gitonga, 2010). Its infestation causes premature drop of leaves which in turn causes a drop in the yield. The pest is difficult to control due to its ability to develop resistance to pesticides. This insect may expand its distributional range with the projected climate change. Though many insects show a strong correlation between reproductive rate and temperature, limited studies have been carried out on the effect of temperature on its life history. It is important to establish the upper and lower temperature thresholds in a bid to estimate the potential distribution range under different climate conditions.

1.3 Hypotheses

a) Temperature does not affect the life history parameters of *L. trifolii.*

b) Intrinsic rate of increase of *L. trifolii* is independent of temperature.

c) Distribution of *L. trifolii* is independent of temperature variations

1.4 Research questions

a) Does temperature affect the life history traits of *L. trifolii*

b) Does temperature influence the intrinsic rate of development of *L. trifolii*

c) Will current and projected global temperatures influence distribution of *L. trifolii*

1.5 Objectives

1.5.1. General objective

To determine the effect of temperature on the life history traits, intrinsic rate of increase and the general distribution of *L. trifolii*.

1.5.2. Specific objectives

a) To determine the effect of temperature on life history parameters of *L. trifolii.*
b) Determine the intrinsic rate of increase of *L. trifolii* at selected constant temperature regimes
c) Determine potential distribution range of *L. trifolii* under current and projected world temperatures in 2050

1.5 Justification

Liriomyza trifolii is an invasive pest attacking vegetables and ornamental plants in Kenya. Being a cosmopolitan pest, it is capable of expanding its distribution beyond the current range. With the projected global climate change (Houghton *et al.*, 2001; Alali *et al.*, 2007), temperatures in areas where this species is currently found are likely to change. However, there is no information on how temperature changes will affect its life history patterns and the general distribution. This study was thus undertaken to compare fecundity, development time, longevity, mortality, sex ratio and the mean number of eggs of *L. trifolii* at different temperature regimes.
This information was used in estimating distribution of *L. trifolii* under the two climatic conditions, namely currently (2010) and the year 2050.

CHAPTER TWO:

2.0: LITERATURE REVIEW

2.1 Biology of *Liriomyza trifolii*

The leaf mining flies (Diptera: Agromyzidae) are small flies having phytophagous larvae that feed within leaves, stems, roots, flowers or seeds (Scheffer, 2007). These flies are distributed worldwide with the highest diversity occurring between the Tropic of Cancer and the Arctic Circle (Spenser, 1973). According to Scheffer (2007), Agromyzidae contain two subfamilies namely, Agromizinae and Phytomizinae. *Liriomyza* genus belongs to the subfamily Phytomizinae. The genus *Liriomyza* was erected in 1894 and contains more than 300 species. Within the genus, 23 species are economically important, causing damage to agricultural and ornamental plants by their leaf mining activities (Scheffer, 2007).

Eggs of *Liriomyza* are laid singly but in close proximity to each other inside punctures made by the female ovipositor on the surface of the leaves. Their period of development ranges from 2 to 8 days depending on prevailing temperature conditions. When the egg is ready to hatch, pressure exerted by the larvae causes the eggshell to become extended and eventually splits releasing the larvae (Parella, 1987). The larvae feed immediately after eclosion and continue to do so until they pupate. *Liriomyza trifolii* feed in the palisade mesophyll layer (these are the cylindrical cells found beneath the upper epidermis cell which contain chloroplast), *L. huidobrensis* (Blanchard) in the spongy mesophyll layer (these are irregular cells found above the lower epidermis and contain a lot of intercellular spaces) and *Liriomyza brassicae* (Riley) in the palisade and spongy mesophyll layers (Parella, 1987).

The larvae have four moults and four larval instars. When the larva is ready to pupate, it cuts a slit on the leaf surface which enables it to emerge onto the leaf surface. The slit may be on the upper or lower surface depending on the species. A pupal stage varies inversely with increase in temperature within its optimal range and may vary from 8 to 11 days (Parella, 1987).

Adults emerge through the dorsal anterior end of the puparium, the whole process taking between 5 minutes to more than 1 hour (Parella, 1987). Newly emerged adults exhibit a positive photo tactic response and climb up the side of the cage or up the stalk of the plant. Majority of adults mate soon after emergence with premating interval being inversely related to temperature. Multiple mating is often needed for maximum egg production.

2.2 Distribution of *L. trifolii*

The leaf miner *L. trifolii* is native to Florida, USA (Sujay *et al.*, 2010). It was introduced along with cut chrysanthemum (*Chrysanthemum morifolium* Ramat) flowers in early 1970 in California (Parella, 1987). The fly was accidentally introduced to Kenya around the same time (Sujay *et al*, 2010). The United Kingdom (UK) was invaded by *L. trifolii* in 1977 (Cannon *et al.*, 2012). According to Iwasaki *et al* (2000), the fly was detected in Japan, Aichi prefecture in 1990 on tomatoes and chrysanthemum.

The fly has extended to Africa, Europe, America, Oceania and Asia (Minkenberg and van Lenteren, 1986). *Liriomyza trifolii* has been intercepted but not established in Australia (European and Mediterranean Plant protection organization, 1987). *Liriomyza trifolii* is established in fields in tropics, sub tropics and warmer temperate areas while it occurs in green houses in colder climates (Sujay *et al.*, 2010).

2.3 Economic Importance

Liriomyza trifolii attacks over 400 species of plants in 28 families (Manipalil *et al.*, 2005). Host plants are found in the families' Basellaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Convulvulaceae, Cruciferae, Cucurbitaceae, Eurphobiaceae, Gramineae, Iridaceae, Labiatae, Leguminosae, Liliaceae, Malvaceae, Polemomiaceae, Primulaceae, Rosacea, Scrophulariaceae, Solanaceae, Tropaeolaceae, Typhaceae, Umbelliferae, Verbenaceae and Zygophyllaceae.

Damage to the plant is caused by the stippling that results from punctures made by the female fly, internal mining by the larvae, allowing pathogenic fungi to enter the leaf via the feeding punctures and mechanical transmission of some plant virus (Manipalil *et al.*, 2005). Mining leads to depressed levels of photosynthesis in the plant. Extensive mining may also lead to premature leaf drop which can lead to sun scalding of fruits or reduced tuber filling of potatoes.

Studies carried out by Johnson *et al.* (1983) showed that *L. trifolii* larvae caused reduced photosynthetic capacity in *Chrysanthemum*, Celery (*Alpium graveolens*), tomatoes (*Lycopersicon esculentum*) and Lima beans (*Phaseolus lunatus*). However many economically important host plants of *Liriomyza* species sustain only indirect damage because their marketable produce is non-foliar. Henz and Parella (1992) found a correlation between reduced photosynthesis caused by leaf miner larvae and ovule fertilization and seed development.

2.4 Monitoring of *L. trifolii* in fields and green houses

The European Union monitors imports of flowers and vegetables since this is the most likely pathway for dispersal of *L. trifolii* (Andersen *et al.*, 2010).In addition, soil growing media

originating from infested areas are monitored since soil has potential to be contaminated with pupae which can survive for a significantly longer period of time.

In green houses populations of adult *L. trifolii* are monitored using the Mark-release-recapture method using yellow traps (Parella *et al.,* 1989). The traps are spaced at a minimum distance of 26 m for gathering information on population trends and 47 m for evaluating treatment effects. Larvae are counted from randomly selected leaves 2.5-7.5cm of the plant height.

2.5 Control and Management of *L. trifolii*

According to Gitonga (2010), many farmers in Kenya use pesticides to control leaf miners. The main pesticides in use are Dimethoate, Abamectin, Deltamethrin, Cyclone, Tubeconazone, Propineb/Cymoxanil, petroleum oil, Lambda-cyhalothrin, Azadirachtin and alpha-Cypermethrin. Despite the use of pesticides yield loss due to leaf miner ranged from 13 to 90% (Gitonga, 2010). A study in California among chrysanthemum growers between 1982 and 1985 showed that each farmer spent an average of $ 14800 per hectare per year on insecticides for leaf miner control and still lost 23% of their crop (Sanderson *et al.*, 1989). This was mainly attributed to use of sub lethal dosage during application which eventually led to pesticide resistance by *Liriomyza.* In addition to development of resistance, most pest outbreaks have been occasioned by pesticide applications which disrupt the natural enemy complex (Abe *et al.*, 2005).

More recent studies have focused on the use of predators and parasitoids in the control of leaf miners (Ho and Ueno, 2002; Abe *et al.*, 2005; Haghani *et al.,* 2006). Studies involving the use of natural enemies to control leaf miners include the parasitoids *Dacnusa sibirica* Mimusa (Hymenoptera: Braconidae) (Abe *et al.*, 2005), *Opuis dissitus* Muesebeck (Hymenoptera:

Braconidae) *Hemiptarsenus varicornis* Girault (Hymenoptera: Eulophidae) (Ho and Ueno, 2002) and *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) (Haghani *et al*, 2006).

Though not considered to be important in biological control of *L. trifolii* predators include *Cyrtopeltis modestus* Dist (Hemiptera:Miridae), *Dicyphus cerasti* Wagner (Heteroptera: Miridae) and *Aphidoletes aphidimyza* Rondani (Diptera:Cecidomyiidae) (Liu *et al*., 2008).

2.6 Effect of climate change on arthropod populations

The earth's climate has warmed up by approximately 0.6 ^{0}C over the past 100 years (Walther *et al.,* 2002). This phenomenon has had the effect of lengthening the freeze free period in mid and high altitude regions. Satellite data reveals that there has been a 10% decrease in snow cover. The earth has also experienced changes in precipitation regime with the Northern hemisphere showing marked increase while in the subtropics rainfall has decreased.

According to Karuppaiah *et al*. (2012) global warming changes may result in extension of geographical range of insect pests. There could also be an increase in over-wintering and rapid population growth of many economically important insects.

Global warming may also cause changes in insect-host interactions thereby changing the synchrony between insect pests and their crop host or introduction of alternative host as green bridges leading to invasion by migrant pests.

2.7 Studies on insect life tables

Calandra oryzae Demerec (Coleoptera: Curculionidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) were among the first insects whose life tables were developed (Birch, 1948; Huey, 1991). Huey (1991) compared development time of *D. melanogaster* at temperatures of 16 and 25^{0}C and found optimal development a 25^{0}C. Birch

(1948) estimated the optimal development temperature for *C. oryzae* to be 29^0C while egg laying ceased at 33.5^0C

Recently, Ju *et al*. (2010) determined the effect temperature on development and population growth of sycamore lace bug *Corythucha ciliata* Say (Hemiptera: Tingidae) at 16, 19, 22, 26, 30, 33, and 36^0C. Female and male longevity was shortest at 33^0C and longest at 16^0C while highest fecundity was recorded at 30^0C.

2.8 Temperature driven models to study insect development

Mathematical models represent a language for formalizing the knowledge on the live system obtained after experimental observation and hypothesis testing (Damos and Savopoulou-Soultani, 2012). Most of these approaches are based on the empirical detection of relationships and construction of relative models that capture all the information about response variable in relationship to temperature. The relationship maybe judged as deterministic or empirical. According to the law of total effective temperature, it is possible to estimate emergence and number of generations for a given duration of organism of interest, according to the following equation: $K=D\ (T-T_0)$ where K is the species (or stage specific) thermal constant of the poikilothermic organism, T is the temperature and T_0 the development zero temperature (Birch, 1948).

Since linear models are inadequate in describing the full range of development rate of insects, several non-linear models, for example Logan, Sharp and DeMichelle (Worner, 1992), Brier 1 and 2 (Keena, 2006), Stinner and Lactin (Damos and Savopoulou-Soultani , 2012), have been proposed to describe development rate response curve over the full range of temperature. These non-linear models are aimed either to build phenology models, or to be used as forecasting tools for pest management (Sporleder *et al.*, 2011).International Potato Center (CIP) has

developed an Insect Life Cycle Modeling Software (ILCYM) to facilitate development of further pest insect phenology models and provide analytical tools for studying pest population ecology (Sporleder *et al.*, 2011). The software consists of a model builder which facilitates development of insect phenology and a second model that allows for spatial-global or regional simulation of pest activity (pest risk mapping)

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1 Rearing of *L. trifolii*

The study entailed rearing of a pair of newly hatched *Liriomyza trifolii* fly in a cage under constant temperature of 10, 15, 18, 20, 25, 30 and 35^0C while providing one potted *Phaseolus vulgaris* plant as a source of food and oviposition site. The plant was replaced every 24 hours and on removal was placed in a holding cage within the same temperature. Egg, larvae and pupa of *L trifolii* were observed and on reaching adult stage were paired to determine daily oviposition. Staining on leaves was done to count number of non-viable eggs.

3.2 Potted *Phaseolus vulgaris* as source of food and oviposition site of *L. trifolii*

Phaseolus vulgaris plants were grown in pots and used as a source of food and oviposition site for *L. trifolii.* The potted plants were grown in a greenhouse, and only plants without any puncture were selected for the experiment. A pair of newly emerged flies (a male and a female) was introduced in a cage with a fourteen (14) day old plant (with at least 5 leaves), where the plant remained for a period of 24 hours. After 24 hours, the plant was transferred to the holding cage within the same temperature regime where the eggs, larvae, pupae and adults were allowed to mature. Egg, larva, pupa and adult were observed after every 24 hours.

3.3: Establishment of *L. trifolii* colony

Test insect, *L. trifolii*, was taken from a colony originally collected from farms in Makindu, Kenya and maintained at the ICIPE insectary at temperatures ranging from 25 to 27^0C and relative humidity of 60-80%. From this colony, 200 pupae were obtained and used to

establish an experimental sub-colony from which individual insects were randomly selected for the experiments.

3.4: Maintenance of the *L. trifolii* sub-colony

Liriomyza trifolii experimental sub-colony was reared on potted *Phaseolus vulgaris* plants. To initiate the sub-colony, 200 flies were exposed to eight potted *P. vulgaris* plants for a period of 48 hours. The plants were then transferred to a holding cage for another 48 hours and this enabled the eggs to hatch. The larvae were allowed to feed inside the leaves' mesophyll until ready for pupation. The leaves were later cut off from the rest of the plant and placed in a plastic container with a tight lid from which the pupae were collected for two consecutive days. After collection, the pupae were placed in a vial with a net at the top to allow for air circulation until they emerged. Newly emerged adults were then randomly selected for the experiments while the rest were returned to the experimental sub-colony. In each temperature regime, a total of 10 females were randomly selected while the number of males varied since every male that died during the experiment was immediately replaced with another male from the sub-colony.

3.5 Insect cages for holding a pair of *L. trifolii* flies and one potted *Phaseolus vulgaris* exposed to the pair of insects.

Two types of cages made of transparent Perspex were designed for the study. The first cage measuring 15cm x 15cm x 20cm (L x W x H) was used to hold one pair of newly emerged female and male where the flies remained to the end of the study (Figure 1). A cloth netting that allows aeration was glued on the upper face while a 4 inches (diameter) opening was placed on the front face. A total of 20 such cages were constructed

Once a potted plant was removed from the exposure cage, it was transferred into the holding cage measuring; 50cm x 50cm x 25cm (L x W x H). To allow aeration, the holding cage was designed with cloth netting placed on the two opposite vertical faces. An opening of 10 inches (diameter) was placed on the front face. Each potted plant placed in this cage was clearly labeled with the temperature, the representative female and date of exposure to the female. For example 1/1/15 mean 1= 1st 24 hours of exposure; 1=1st female representative out of a total of 10; 15= Temperature in ^{0}C

Figure 1: Insect cage for holding a pair of flies with one potted plant

3.6 Incubators used for study set at temperatures of 10, 15, 18, 20, 25, 30 and 35^{0}C

Three different incubator models were used during the experiments. They included; Sanyo Incubator (MIR-533), RUMED (Rubarth Apparate GMbH) and Elbanton Incubator. Lighting systems in the incubators were modified with two sets of light bulbs installed in each incubator; a fluorescent light bulb, and Agro/Fluoro light bulb. The fluorescent light enabled *L.*

trifolii to see the host plant inside the incubator while the Agro/Fluoro light provided light energy for plant photosynthesis. The incubators were programmed to have a photoperiod of 12L and 12D. The experiments were performed under seven different temperature regimes (10, 15, 18 20, 25, 30 and 35^0C) with relative humidity maintained at a range of 60% to 80% in all the incubators. Each incubator was equipped with a thermo hygrometer to monitor the temperature and humidity; the parameters were recorded after every 24 hours during the sample observations.

3.7 Random selection of insects for experiments

All the cages were labelled from 1 to 20 as follows: F1/15, where F1 was the cage number in the temperature of 150C. This was repeated for all temperature, namely 10, 15, 18 20, 25, 30 and 350C. All the newly emerged flies were placed inside transparent glycogen capsules and their sex determined. Sex determination was undertaken by either the presence (Female) or absence (Male) of an ovipositor. While still in individual capsules, the flies were placed on a bench and numbered 1 to 4. Every fourth fly was selected for the experiment while the rest were returned to the colony

3.8 Development time and mortality of *L. trifolii*

A pair of *L. trifolii* (a male and a female) was placed into one cage and immediately one potted plant was introduced into the cage. The cage, potted plant and pair of insect were introduced into an incubator (for example 25^0C) for a period of 24 hours. After 24 hours the potted plant was removed from the cage and replaced with a fresh potted plant. The first potted plant was marked as 1/1/25 (1st female replicate, 1st 24 hours at 25^0C) was put in the holding cage and returned to the same incubator. . A second potted plant was introduced to the pair of insects for another period of 24 hours and subsequently transferred into the holding cage. The

second potted plant was labeled as 1/2/25 (meaning 1st female replicate, 2nd 24 hours at 25^0C). The process was repeated after every 24 hours until the female died.

The leaves of each plant within the holding cage were observed after every 24 hours under a light microscope or a light box to count the number of larvae alive and the time taken for each individual to complete each stage of development. Total number of dead progeny was recorded during each observation. Following, the pupa(e) were collected using a camel hair brush from the leaf and placed in separate vial each measuring 2.5cm in diameter and 6.5cm high. The information recorded on the side of each vial included parent identification number, dates of oviposition and pupation. The time taken for adult to emerge from pupa was recorded. The total number of adults and their sex was also recorded. When adults emerged from the puparium, they were sexed, paired (a male and a female) and placed in an exposure cage. A potted plant was introduced into the cage and immediately placed in the incubator. After every 24 hours, a fresh potted plant was introduced while the previous one was labeled and placed in the holding cage within the incubator. A dead male was replaced by another male.

For temperatures where females did not readily oviposit (10, 15 and 18^0C), 6 potted plants were placed inside the sub-colony for a period of 6 hours to enable insects oviposit on the leaves. The plants were then transferred into the holding cages placed in the respective incubators (10, 15 and 18^0C). Observations on emergence, development and mortality were made after every 24 hours.

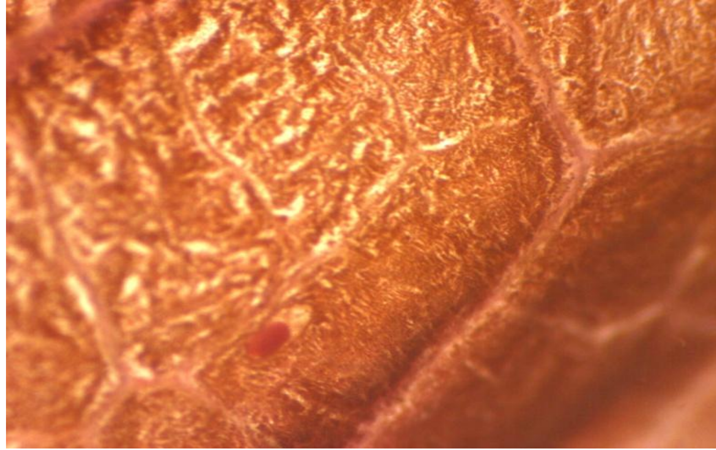


Figure 2: *L. trifoli* egg stained red inside a puncture on the leaf

Figure 3: *L. trifolii* larvae inside a leaf, behind is a trail of mines, while the dark areas are the waste products

Figure 4: *L. trifolii* pupa attached to the upper surface of a leaf

3.9 Pre-oviposition period and total number of eggs laid by female per day

A pair of *L. trifolii* fly (male and female) was placed in a cage and fresh potted plant (*P. vulgaris*) introduced into the cage after every 24 hours. Each plant represented a day in the life of the female. The number of days females took before they begun to lay eggs was recorded. Total number of egg, larvae and pupa (live and dead) on the leaf were counted. After all individuals were removed from the plant after pupation or death, the leaves were stained using Lacto phenol acid fuchsin to count the number of eggs in the leaf that did not hatch. The total number of eggs laid by each female per day was estimated from the sum of the pupae, dead larvae and eggs that failed to hatch.

3.10 Preparation of stain to view unhatched eggs in *Phaseolus vulgaris* leaves.

Lacto phenol acid fuchsin staining reagent was prepared using distilled water, fuchsin stain, phenol crystals, glycerine and acetic acid. The stain was prepared in the ratio of 1:1:1:2 (Water: Lactic acid: Phenol: Glycerin) and 0.1% of solution for fuchsin. 10 ml of Lactic acid was added to 10ml of water, phenol crystals were gently warmed to liquefy and then 10 ml added to

the solution of water and acid. 20 ml of glycerine was further added and lastly fuchsin stain was calculated at 0.1% (0.05 grams) (Nuessly *et al.*, 1995)

3.11 Differential staining to view unhatched eggs

Lacto phenol acid fuchsin staining solution was boiled on a Bunsen burner for 2 minutes and each leaf samples dipped inside the boiling solution for 2 minutes. The leaves were then placed on a petri dish and soaked in the stain solution for 8 hours. The leaves were then rinsed in warm water followed by cold water on a petri dish. The leaves were then viewed under a light microscope to count the total number of eggs that failed to hatch.

3.12 Data management

Data obtained from the study was tested for normality and homogeneity of variance prior to performing ANOVA using Levene Test with the help of SPSS version 16. Using the same statistical program (SPSS), data on the effect of temperature on egg, larvae and pupa development was then subjected to ANOVA. This was done to find out whether there were significance differences in development at various temperature regimes. Once significant differences were identified, data was subjected to post hoc analysis using Least Significance Difference Test (LSD). Similarly, stage specific mortality and generation mortality was analyzed by ANOVA and post hoc ANOVA/separation of means was done using LSD test. Developmental time values were reported as the mean ± SE and presented graphically.

3.13 The *Liriomyza trifolii* phenology model

The *L. trifolii* phenology model was implemented using Insect Life Cycle Modeling software (ILCYM version 3.0) (Kroschel *et al.*, 2013). The software has the tools for building process-based population models for the insect species. The model builder uses the same shape

distribution approach combined with a rate summation and cohort up-dating for simulation of the population dynamic model. ILCYM has several non-linear functions that describe the temperature–dependency of different processes in the insect species life history. These include the development time and its variation between individuals in a population, mortality in each immature life-stage, senescence time and reproduction frequencies of adults according to temperatures. The model builder facilitates choosing the best fitting functions for describing these temperature-driven processes in overall generic phenology model.

3.14: Temperature-dependent processes models and statistical analysis

The relationship between temperature-dependence of different processes in the *L. trifolii* life history and different temperature regimes, was analyzed by various non-linear models using ILCYM software (Sporleder, *et al*, 2011. The statistical analysis implemented in this software selected the best-fitting model to quantify the effect of temperature on the development rate, mortality, senescence and reproduction according to inbuilt model selections criteria. These included the Akaike's information criterion (AIC) and the coefficient of determination R^2, which explains how the models capture the variability within the data. in all temperatures under study, the female: male ratio was maintained at 1:1

Using this criterion ILCYM selected the following non-linear models for the study.

Table 1: Selected non-linear models

Phenology	Stage	Model selected
Development rate	Egg	Sharpe and DeMichele 1
	Larvae	Sharpe and DeMichele 13
	Pupa	Tb Model (logan)
Senescence	Male	Stinner 4
	Female	Stinner 4
Mortality	Egg	Marquardtr
	Larvae	Newton
	Pupa	Newton
Total oviposition		Marquardtr
Relative oviposition		Newton

CHAPTER FOUR:

4.0: RESULTS

4.1 Survival of eggs

Survival of eggs under different temperature regimes ranged from 40.13% (15^{0}C) to 95.32% (35^{0}C) except at 10^{0}C, where the experiment had to be terminated after 20 days as none of the eggs hatched.

Table 2: No. of eggs at the beginning of experiment

Temp ^{0}C	No. of eggs that develop to the next stage		Survival (% of eggs)
	Beginning	Emerged	
10	27	0	0.00
15	157	63	40.13
18	397	281	70.78
20	580	500	86.21
25	596	554	92.95
30	720	600	83.33
35	577	550	95.32

4.2 Development time of *L. trifolii*

The developmental times of various *L. trifolii* stages at various temperature regimes showed that temperature affected the development time (Table 3). The eggs took significantly longer time to hatch at 15^{0}C (11.4 ± 0.11) and significantly shorter time at 35^{0}C (1.27 ± 0.02). Though eggs took long to hatch at 15^{0}C than other temperature regimes, the larvae and pupae did not survive to adult stage. Significant variations were also observed in larval and pupal stages in other temperature regimes (Table 3).Both larval and pupal developmental time gradually reduced with an increase in temperature except at 35^{0}C where there was a slight increase in larval

development time. The longest development time (7.37 ± 0.11) was recorded at 18^0Cwith the minimum time (2.18 ± 0.04) observed at 30^0C. Lowest pupal development time (6.82 ± 0.07) was recorded at 35^0C with a rise in development time with reduction in temperature.

Table 3: Mean developmental time of various life stages of *L. trifolii* at six constant temperature regimes

Temp	Mean ± SE (n)		
^{0}C	Eggs (days)	Larvae (days)	Pupa (days)
15	11.4 ± 0.0011 [a] (n=157)	-	-
18	5.28 ± 0.0008 [b] (n=397)	7.37 ± 0.0012[a] (n=281)	20.31 ± 0.0302[a] (n=87)
20	3.43 ± 0.007 [c] (n=580)	4.10 ± 0.0044[b] (n=500)	12.23 ± 0.0072[b] (n=211)
25	2.06 ± 0.0004 [d] (n=596)	3.42 ± 0.0027[c] (n=554)	9.16 ± 0.0046[b] (n=305)
30	1.68 ± 0.0005[e] (n=720)	2.18 ± 0.0027[e] (n=692)	6.87 ± 0.0046[d] (n=212)
35	1.27 ± 0.0007 [f] (n=577)	2.43 ± 0.0035[d] (n=550)	6.82 ± 0.0120[e] (n=178)
Df	$F_{5, 2634}$	$F_{4, 988}$	$F_{4, 448}$
F	2806.84712	762.3206	2395.3948
p. value	0.00	3.8E-300	8.5E-301
F critical	2.217494	2.380939	2.391847

4.3 Development rate

. Non-linear regression was then used to analyze the data to establish the relationship between development rate and temperature

4.3.1 Egg development rate

Development rates varied from 1.23, 0.87, 0.62, 0.28, 0.21 and 0.02 at 35, 30, 25, 20, 18 and 15^{0}C respectively. Direct relationship between temperature and development rate was estimated using Sharp and DeMichele 1 non-linear regression model (Figure 5)

4.3.2 Larval development rate

Larval development rate was estimated as 0.433, 0.611, 0.433, 0.266 and 0.222 at 35, 30, 25, 20 and 18^{0}C respectively. The model estimated development rate at the lowest temperature (18^{0}C) to be 0.222 and the highest temperature (30^{0}C) to be 0.611 (Figure 6).

4.3.3 Pupae development rate

The model estimated development rate to be 0.0518, 0.089, 0.109, 0.154 and 0.183 at 35, 30, 25, 20 and 18^{0}C respectively (Figure 7). The model indicated a direct relationship between temperature and development.

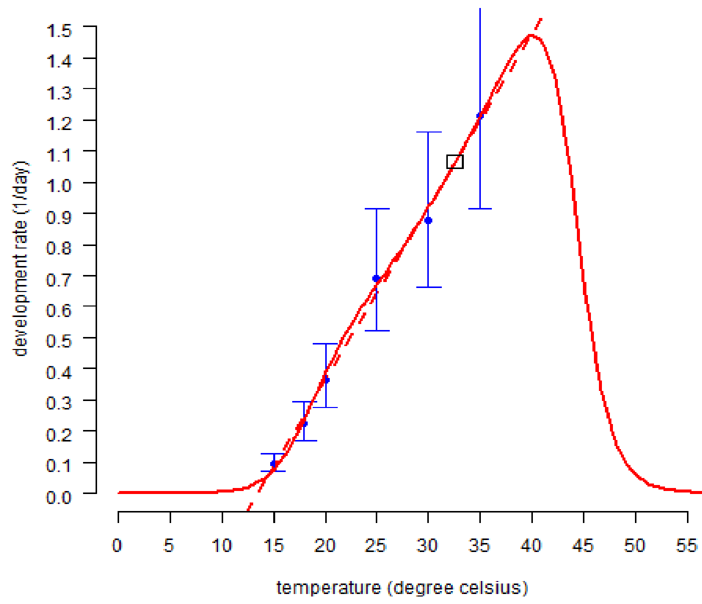


Figure 5: Rate of egg development at various temperatures

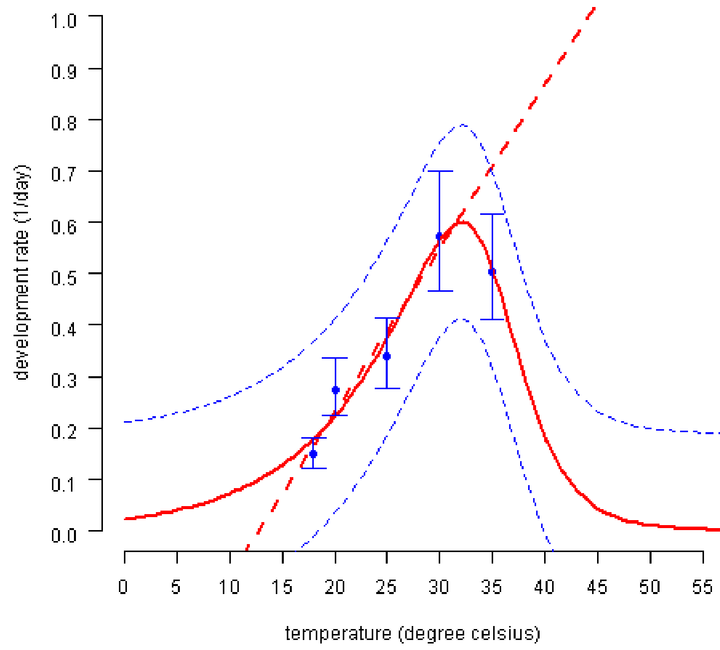


Figure 6: Rate of larvae development at various temperatures

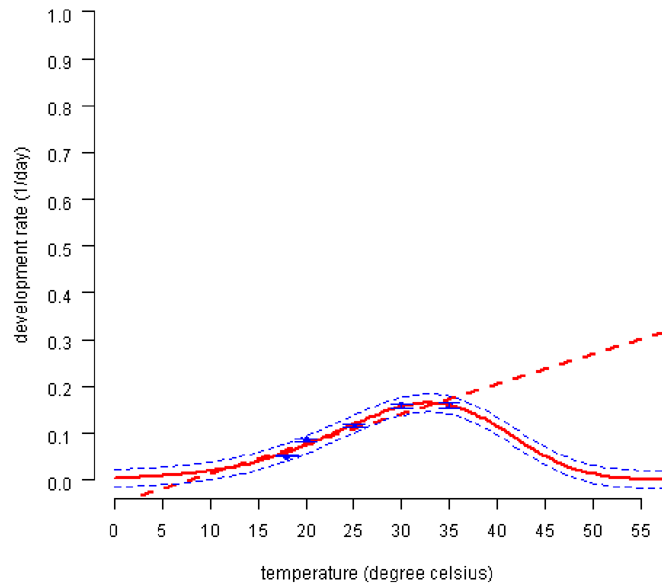


Figure 7: Pupa development rate at various temperatures

4.1.3 Male and female Senescence

Male senescence rate was 2.0, 8.66, 2.33, 0.66, 0.44 and 0.33 at 35, 30, 25, 20, 18 and 15^{0}C respectively (Figure 8) while female senescence rate was 0.56, 0.35, 0.28, 0.26, 0.24 and 0.17 at 35, 30, 25, 20, 18 and 15^{0}C respectively (Figure 9). Using non-linear model the highest rate of male senescence was estimated to be at 33^{0}C while the lowest rate was estimated at 15^{0}C (0.33). On the other hand, the highest rate of female senescence (0.56) was estimated at 35^{0}C while the lowest rate of senescence (0.17) was estimated at 15^{0}C.

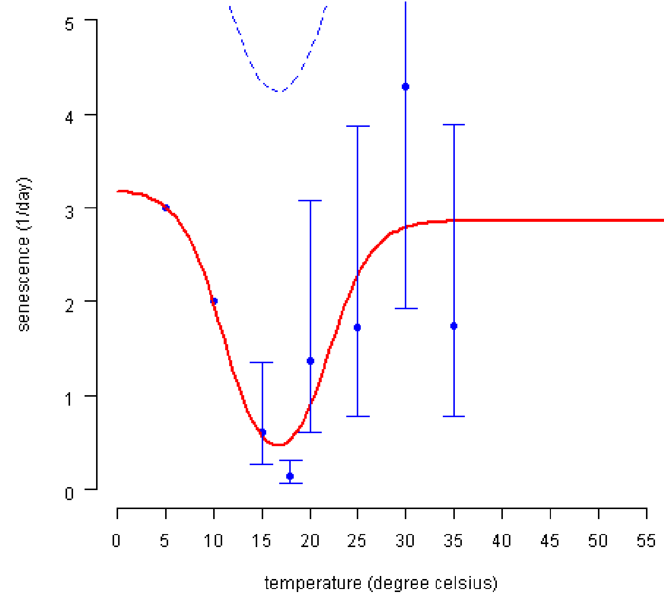


Figure 8: Rate of male senescence at various temperatures

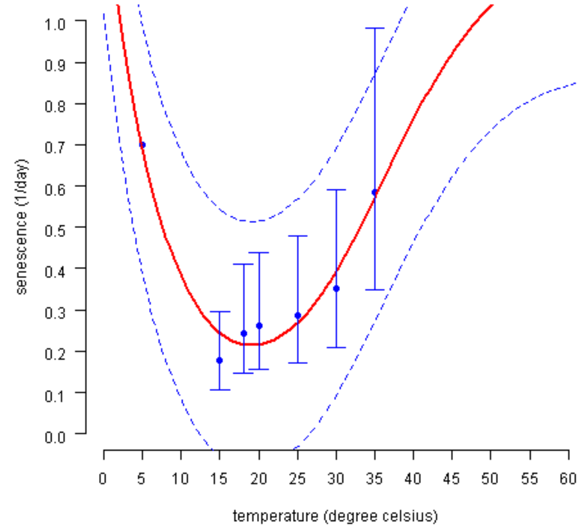


Figure 9: Rate of female senescence at various temperatures

4.4 Mortality of egg, larvae and pupa *L. trifolii*

Lowest egg mortality was recorded at 30^0C at 0.039 while the highest mortality occurred at 15^0C at 0.599 (Figure 10). Lowest larval mortality was recorded at 25^0C (0.499) while the highest mortality occurred at 18^0C (0.690) (Figure 11). Lowest pupal mortality occurred at 25^0C (0.384) while high mortality occurred at 35^0C (0.787) (Figure 12)

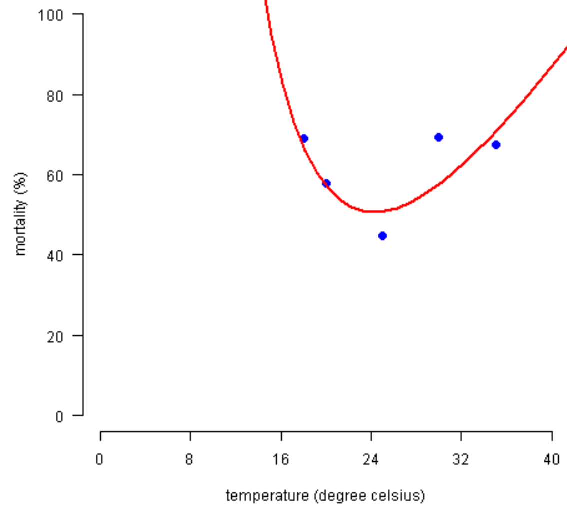


Figure 10: Rate of egg mortality at various temperatures

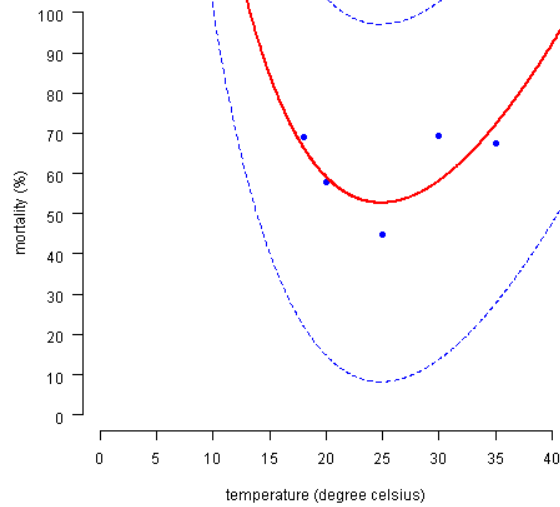


Figure 11: Rate of larvae mortality at various temperatures

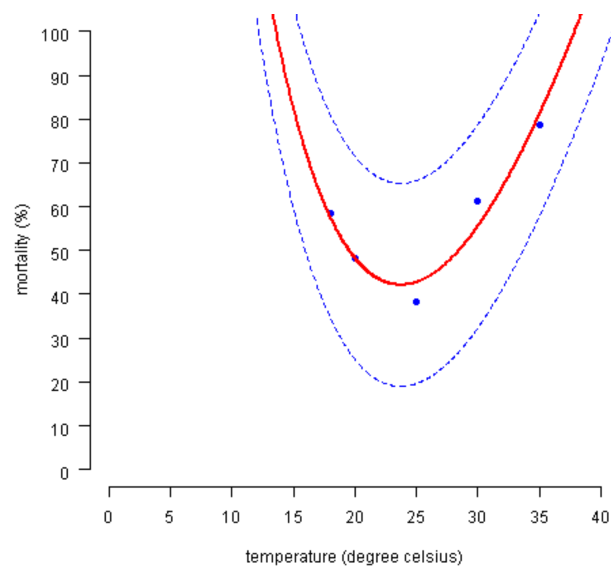


Figure 12 Rate of pupae mortality at various temperatures

Levene test was further used to test the mortality data for normality. Sig. level obtained was 0.551 which is > 0.05. Hence egg, larvae and mortality data was normally distributed.

4.5 Oviposition of female *L. trifolii*

Based on the model, the highest total oviposition was estimated at 27^0C (60 eggs per female) while the insect did not lay eggs at 15 and 35^0C.

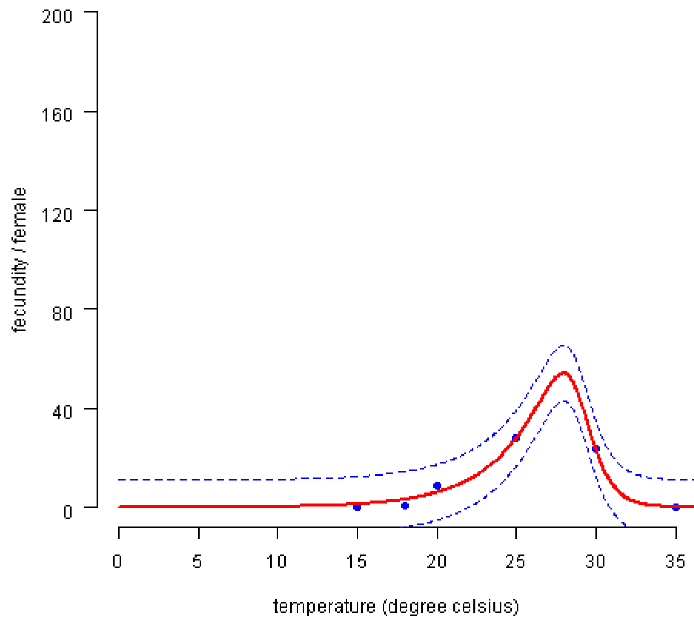


Figure 15: Temperature dependent total oviposition

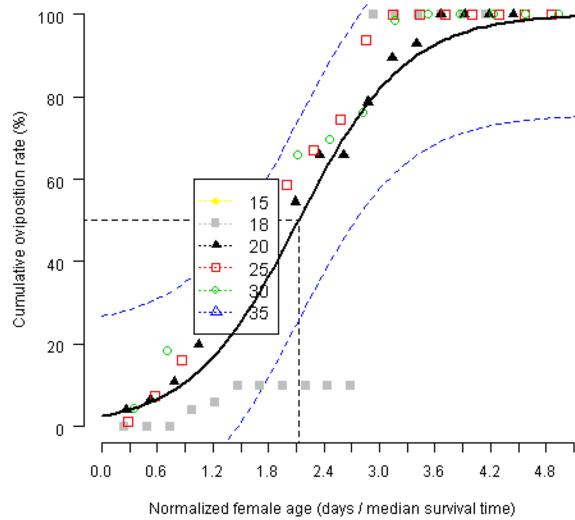


Figure 14: Temperature dependent relative oviposition

4.6 Intrinsic rate of increase

The optimal net reproduction rate (R_0) estimated from the data was at 28^0C (0.4729) while optimal intrinsic rate of reproduction (R_m) was estimated at 29^0C (-0.06601729). The highest Gross Reproduction rate (GRR) was estimated at 28^0C (6.2631) and lowest at 30^0C (2.047387). The shortest generation length (days) was estimated at 30^0C (13.75979) and longest at 24^0C (19.60261). The highest finite rate of increase was estimated at 26^0C (0.9526122) and was lowest at 30^0C (0.8873144). The doubling time was longest at 30^0C (-5.79768) and shortest at 26^0C (-14.27773) (Table 4 Figure 15).

Table 4: Stochastic simulation of *L. trifolii*

Intrinsic rate (r_m)	r_m=-1.595209+0.1167341T+-0.002245446T^2 R^2 =NaN R^2*Adj*=NaN AIC= -35.74 *Deviance*=0.018
Net reproduction rate (R_0)	R_0=-3.411125+0.2824107T+-0.005334821T^2 R^2 =0.444 R^2*Adj*=0.378 AIC= -7.784 *Deviance*=0.532
Gross reproduction rate (GRR)	GRR=-92.24953+7.710581T+-0.1467365T^2 R^2 =0.428 R^2*Adj*=0.0.361 AIC= 125.549 *Deviance*=417.918
Generation length in days (GL)	GL=545.6987+7.169738T+-220.0695log(T) R^2 =NaN R^2*Adj*=NaN AIC= 35.599 *Deviance*=7.007
Finite rate of increase (λ)	Λαμβδα =-0.4948817+0.002100172T^2 R^2 =NaN R^2*Adj*=NaN AIC= -37.866 *Deviance*=0.015
Doubling time (Dt)	Dt=97.27863+-4.701935T+3.449306e-12$e^{(T)}$ R^2 =NaN R^2*Adj*=NaN AIC= 107.747 *Deviance*=2862.083

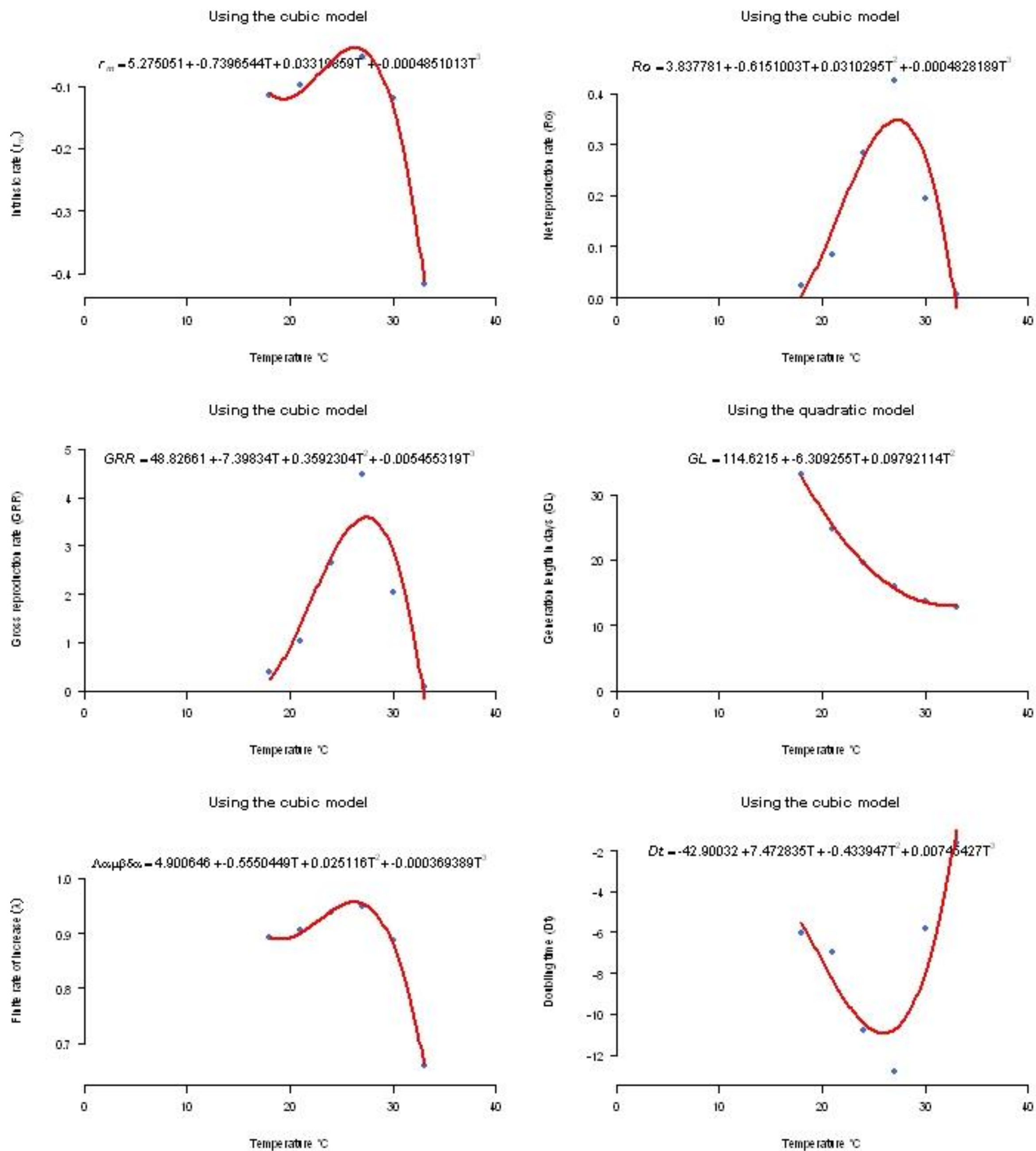


Figure 15: Describing (a) intrinsic rate of increase, (b) Net reproduction rate, (c) Gross reproduction rate, (d) Generation length in days, (e) Finite rate of increase and (f) Doubling time

4.7 Geographic distribution of *L. trifolii*

Establishment risk index (ERI) based on world temperatures of 2000 indicated the regions on the world map that *L. trifolii* is able to establish (Figure 16). These areas included America, Africa, Asia and Oceania. Figure 17 indicated the areas where *L. trifolii* potentially could survive if world temperatures in 2050 were as projected.

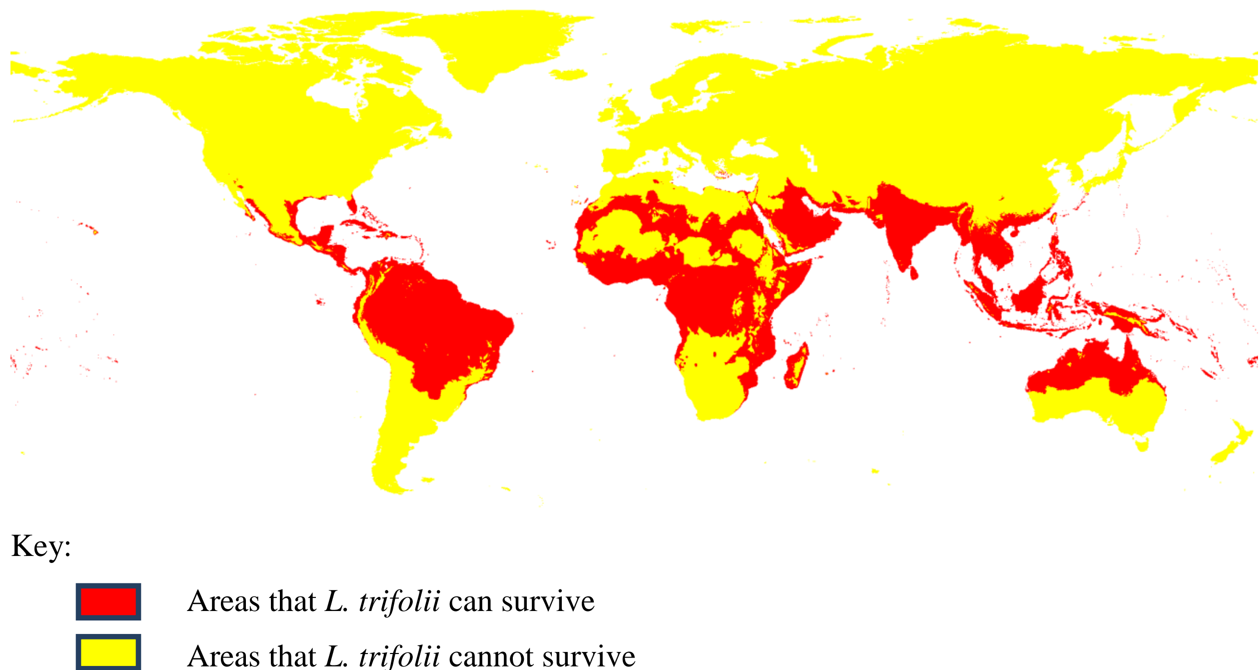
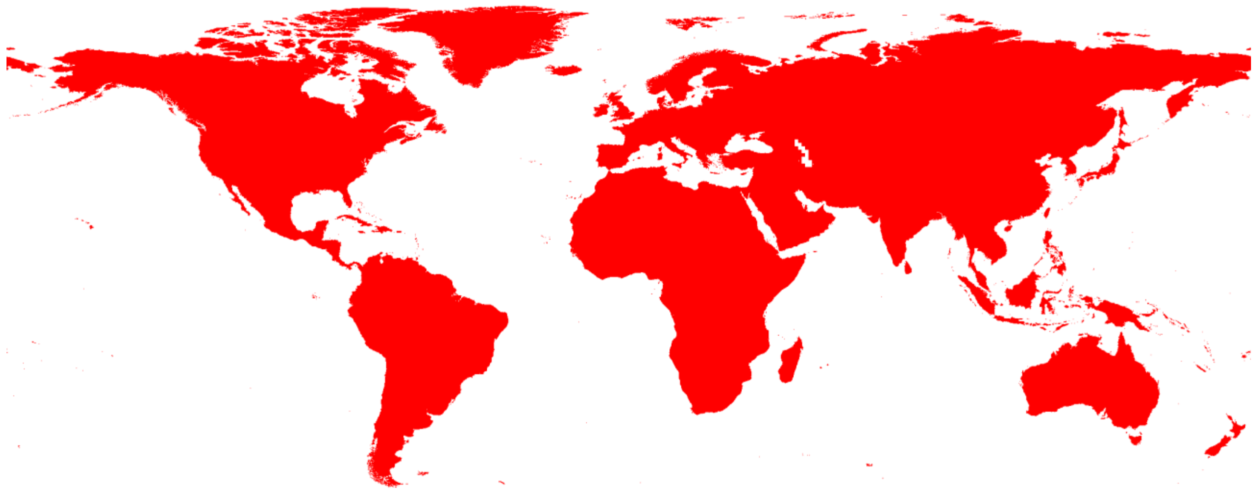


Figure 16: Geographical analysis of world distribution of *L. trifolii* using world temperatures of the year 2010



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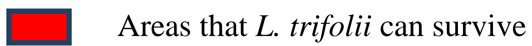

Areas that *L. trifolii* can survive

Figure 17: Establishment Risk Index of *L. trifolii* using projected temperatures of the year 2050

CHAPTER FIVE

5.0: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Results show that temperature has significant effect on development time of egg, larvae and pupa *L. trifolii*. Development time showed an inverse relationship temperature. This finding agrees with results of studies carried out by Parella (1987) and Minkenberg and van Lenteren (1988) who observed similar trends on *Liriomyza* genus (*L. huidobrensi*s, *L. sativa and L. trifolii*). However, development of this species was observed to occur within a given temperature range i.e the lowest threshold was observed at 15^{0}C while highest threshold was at 35^{0}C. These results compared favorably with Schuster's (1985) who studied development time of *Liriomyza trifolii* larvae at temperatures of 15.6, 21.1 and 26.7 and 32.2^{0}C who reported mean development time as 10.1, 7.1, 4.4 and 3.5 days respectively.

PaDIL (2012) reported that 28^{0}C was the optimum temperature and it would take 14 to 15 days for completion of a generation cycle. It was also observed that lower temperatures than this resulted in much longer development. In this particular study the shortest development time was recorded at 30^{0}C (10.73±0.14). This difference may have been brought about by intraspecific variations in insect used in the two studies. Studies were not carried out at temperatures above 35^{0}C.

The study showed an inverse relationship between development rate and temperature. For the eggs, results showed that lower temperature (10, 15 and 18^{0}C) slowed down development rate while higher temperatures (20, 25, 30 and 35^{0}C) accelerated development. This may be attributed to the effect of growth hormones which are inactive at low temperatures but are activated with increase in temperature. Similar trends were observed in the growth rates of *L.*

trifolii larvae and pupae. Low survival of larvae was observed at low temperatures of 18^0C and below. According to Kang *et al.* (2009), larvae of *L. trifolii* are more susceptible to low temperatures as compared to eggs. None of the larvae under study were able to complete development at 15^0C unlike eggs some of which were able to develop within that same temperature. Larvae development rate showed a steady acceleration from 18^0C to reach peak at 30^0C. Development rate slowed down beyond 30^0C. Reduced rate of development beyond 30^0C may be attributed to the effect of high temperatures as it is known to denature growth enzymes (Zhou, 2000).

Observed senescence rates showed that adult longevity decreased with an increase in temperature. Similar observations were made by Parella (1987) who found that longevity decreased with an increase in temperature.

At lower temperatures, females were less active and generally laid fewer eggs. They were therefore able to conserve most of their energy which they used for their survival.. Similar results were reported by Stillwell (2005) who observed that females raised at low temperatures produced eggs that had substantially reduced hatching as compared to females raised at higher temperatures. Senescence was observed to be high at low temperatures below 18^0C at very high temperatures of 35^0C.

This study revealed a strong link between mortality rates and temperature. High mortality in the egg stage may be attributed to low temperatures while less than 10% mortality was observed at temperatures above 20^0C. No eggs were observed to hatch above 35^0C. Similar results were reported by Parella (1987) who found egg mortality to be strongly influenced by temperature. Larvae and pupae on the other hand had an optimal temperature of about 25^0C.

Reitz and Trumble (2002) found optimal larval survivorship to range from 74-95% which compared to 55% (25^0C) in the present study. The difference in survivorship may be attributed to differences in rearing conditions and colony materials. Chandler (1986) carried out studies of *L. trifolii* on Bell pepper (*Capsicum annuum* L.) at 24^0C where he observed pupal mortality of 70.6%. High mortality observed by Chandler (1986) may have been caused by food type since bell pepper may not have been the most suitable food for *L. trifolii.*

Temperature influenced fecundity where optimal oviposition was estimated at 27^0C. A rise or decrease of this temperature caused a decrease in total oviposition. Female fecundity studies in India by Chandler (1986) showed a variation of 24±12 on okra (*Abelmoschus esculentus* Moench) to 136±2.5 on tomatoes. Though the current study did not focus on host plants, the results are closely related to fecundity observed on okra. According to Parella (1983), total ovipostion averaged at 200 eggs per female with an average of 35 to 39 eggs per day. Liebee (1984) observed an average of 405 eggs per female at 30^0C. When compared to the results of this study where oviposition was highest at 25^0C (33.5 average eggs per female in a lifetime) followed by 30^0C (26.35 averages eggs per female in a lifetime). Rate of oviposition was found to be lower for insects used in this study. Pre-oviposition period was longest at 18^0C and shortest at 20 and 25^0C. Proportionally, more eggs were laid at 25^0C as compared to 20^0C. Females at 30^0C laid eggs for a longer period of time than at any other temperature followed by 25^0C. According to Parella (1987), time of oviposition is strongly influenced by time and majority of eggs are laid between day 4 and 10.

As compared to other studies, the results of this study showed that insect had low rate of population increase. Takalloozade (2010), estimated the optimal rate of increase of *Aphis gossypii* Glover (Homoptera: Aphididae) at 29.862 (net reproduction rate) and 15.0317 (intrinsic

rate of increase). The optimal temperature for development for *A. gossypii* was 23^0C. Ebeid (2012) on the other hand carried out a similar study on *Tropinota squalda* Scop (Coleoptera: Scarabaeidae) with his result showing optimal net reproduction rate at 25^0C to be 1.2259 and optimal intrinsic rate of increase at 30^0C to be 1.2259. The low rate of intrinsic increase may have been caused about by comparatively low oviposition and high mortality recorded in this study.

Geographical maps of *L. trifolii* using global temperatures in 2010 showed that the fly could potentially survive in all continents and Parella (1987) describes the fly as cosmopolitan. Further analysis using projected global temperatures in 2050 projected that the fly would colonize every part of the world. Deutsch *et al.,* (2008), developed insect growth curves and predicted that at mid and high altitude regions, populations of insects are likely to dramatically increase as global temperatures rise. This information was in agreement with geographical maps based on global temperatures in 2050.

Conclusion

5.1 Like most insects, an increase in temperature led to a decrease in development and increase in L. trifolii fecundity up to an optimum of 27^0C. With a steady increase in global temperatures *L. trifolii* is likely to colonize new areas which were previously uninhabited.

5.2 Fecundity at 35^0C was recorded at 0. Thus there is need to determine whether infertility was permanent or was egg laying suppressed by the high temperature. Also was the infertility caused by either male or female or by both? Temperatures in the field vary, it is important to find out if a variation occurs, females would be able to lay eggs again.

5.3 Male senescence at 35^{0}C did not fit the pattern of female senescence at that temperature. It is important to determine the reasons for such a drop in male senescence.

Recommendations

5.4 The geographical distribution maps of *L. trifolii* are an important and simple tool that can be used in educating farmers on potential invasion of L. trifolii and methods that they can use to ensure their crops are not attacked by the insect.

5.5 Many literature speak of mechanical transmission of plants virus by *L. trifolii* however no study has been carried out of the specific plants virus and how it climate change would influence this

5.6 ILCYM is able to determine interaction between L. trifolii and parasitoids. It is important to find out how effective prospective parasitoids would be with an increase in global temperatures.

6.0 References

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