

**AGROMYZID LEAFMINER INFESTATION OF DIFFERENT TOMATO (*Lycopersicon lycopersicum*) VARIETIES AND AN ASSESSMENT OF THE EFFECTIVENESS OF *Azadirachta indica* EXTRACTS AS A MANAGEMENT TECHNIQUE**

**BY**

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**DECLARATION**

I hereby declare that the thesis is my own original work and that, to the best of my knowledge, it contains no material previously published by another person for the award of a degree in any other university, except where acknowledgment has been made in the text.

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**Vimbai L. Tarusikirwa**

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**Date**

I, as supervisor confirm that the work reported in this thesis was carried out by the candidate under my supervision. The thesis was examined and I approved it for final submission.

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**Dr. P. Chinwada**

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**Date**

## **DEDICATION**

This thesis is dedicated to my family and friends. Their love and support knows no bounds; because of this, I have succeeded.

## **ACKNOWLEDGEMENTS**

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## ABSTRACT

The relative susceptibilities of 14 tomato varieties (“Rodade”, “Nemonetta”, “Rio Grande”, “Season Red”, “Little Wonder”, “Money Maker”, “RVF”, “Cal-J”, “RVFN”, “Star 9065”, “Floradade”, “Rodade Plus”, “Heinz 1370” and “Campbell”) to leafminer (*Liriomyza* spp.) (Diptera: Agromyzidae) infestation were studied during the months May-June (cold-dry season) and September-November (hot-dry season) in 2015 at the University of Zimbabwe, Harare. Tomato plants were transplanted into pots four weeks after sowing and placed in an open field in a Randomized Complete Block Design. Varietal susceptibility was assessed by recording the number of mined leaves and number of mines per plant. During the cold-dry season, the varieties “Nemonetta”, “RVF”, “Rio Grande”, “Star 9065”, “Rodade plus” and “Season Red” recorded overall low levels of infestation, with no significant differences being noted among them. On the other hand, “Little Wonder”, “Rodade”, “Campbell” and “Floradade” recorded high numbers of mines/plant. Results indicated that “Nemonetta” and “Rodade” were the most susceptible varieties during the hot-dry season, while “Little Wonder”, “Cal-J”, “RVFN”, “Heinz 1370”, “Season Red”, “RVF” and “Star 9065” had the lowest number of mines/plant. Results also indicated that leafminer activity and damage were highest during the hot-dry season. Field and laboratory trials were also carried out to assess the effectiveness of three rates (20, 30 and 40 ml per 10 litres of water) of a commercial neem (*Azadirachta indica*) oil formulation (Achta<sup>®</sup> 1% EC) and the insecticide abamectin in controlling leafminers. Leaf dip bioassays were later carried out in the laboratory in order to determine the toxicity of Achta 1% EC under more controlled conditions. Results from the field and laboratory studies showed that Achta 1% EC applied at 40 ml per 10 litres of water was just as effective as the insecticide abamectin. Achta 1% EC is therefore a suitable biopesticide for inclusion in the basket of chemicals which can be used in rotation by farmers so as to slow down insecticide resistance development by *Liriomyza* spp. Further studies are, however, recommended in order to determine if Achta 1% EC has any adverse effects on leafminer parasitoids. It is also important to assess for Achta 1% EC phytotoxic effects on tomato plants under a broad range of environmental conditions.

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## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background to the Study

The dipteran family Agromyzidae is a distinguished group of small, morphologically similar flies. It is composed of about 1,800 species worldwide, and 75% of them produce mines in leaves (Bader, 2006). The leaf-mining flies are a diverse group whose larvae feed internally in leaves, stems, flowers, seeds and roots of a wide variety of plant hosts. Three highly polyphagous Agromyzid leafminers, *Liriomyza huidobrensis* (Blanchard) (the pea leafminer), *L. sativae* (Blanchard) and *L. trifolii* (Burgess) (the American serpentine leafminer), have expanded into many new areas worldwide (Rauf *et al.*, 2000). All three species are considered serious pests of tomatoes and other solanaceous crops.

The two problem species in Zimbabwe are *L. trifolii* and *L. huidobrensis*. These two species together with *L. sativae* are all tropical or temperate species that are native to South and North America (Scheffer and Lewis, 2001; Kang *et al.*, 2009). However, these insects have since spread throughout the world. Polyphagous leafminers are considered to have invaded countries via the movement of infested plants (Spencer, 1989; Myint, 1997; Capinera, 2007; Plant Health Australia, 2009).

Leafminer damage results mainly from larval feeding, which causes aesthetic damage, reduces yield, and at high larval densities, can destroy plants. This has an adverse effect on the horticultural industry. Tomato farmers in Zimbabwe many of whom are smallholder growers (Zitsanza, 2000; Saunyama and Knapp, 2003) are affected when extensive mining causes premature leaf drop which can result in lack of shading and sun scalding of fruit (Capinera, 2007). This compromises the grading and market value of the tomatoes as they will not have a good aesthetic appearance.

The management of Agromyzid leafminers has long been and continues to be a topic of extensive research and scientific debate. Both synthetic and natural insecticides have been broadly researched and are generally used by farmers and producers for leafminer control in spite of production scale and crop (Liu *et al.*, 2009). Control of leafminers is mainly chemical, predominantly translaminar insecticides (Civelek and Weintraub, 2003; Bjorksten *et al.*, 2005). The indiscriminate use of insecticides is the reason why leafminers became a pest of economic importance (Chavez and

Raman, 1987; Reitz *et al.*, 2013). Reliance on chemical control as the sole method has led to many problems such as development of insecticide resistance and death of some natural enemies.

Many studies on the effects of various plant extracts in controlling or repelling insect pests have been carried out. However, according to Civelek and Weintraub (2004), the advent of synthetic insecticides has led to botanical insecticides constituting only about 1% of the global marketplace. They attributed the lack of commercial use of botanical insecticides to poor efficacy compared with synthetic pesticides. However, due to the increasing incidences of insecticide resistance, it is felt that efficacious botanical derivatives can provide an alternative to synthetic pesticides (Addor, 1995).

Other nonchemical control methods that have been used to control leafminers include the introduction of parasitoids (Johnson, 1993), entomopathogenic nematodes (Walters *et al.*, 2000), trapping using yellow sticky traps (Price *et al.*, 1981; Bennett, 1984), resistant plant varieties (CIP, 1993) and entomopathogenic fungi that infect their host through the cuticle (Poprawski *et al.*, 2000; Inbar and Gerling, 2008). These methods have been attempted with varied levels of success.

The genus *Lycopersicon* is characterized by great diversity within and among its nine species. Arthropod resistance has been studied most intensively in *L. lycopersicum*, *L. hirsutum* and *L. pennellii* (Kennedy, 2003). This resistance has been observed to be associated with a diverse array of traits, including the physical and chemical properties of glandular trichomes and wound-induced chemical defences associated with the leaf lamella (Kennedy, 2003).

## **1.2 Statement of the Problem and Justification**

Tomato production in Zimbabwe is adversely affected by Agromyzid leafminer infestations. The quarantine status of these leafminers leads to export restrictions. Under greenhouse conditions, use of insecticides for leafminer control is often the only means of guaranteeing economic yields where the tomato insect pest complex may also include whiteflies, red spider mites, aphids and bollworms. The genus *Liriomyza* is, however, well known for its capacity to develop resistance to chemical insecticides. Besides resistance development, the use of synthetic insecticides can lead to a decline in natural enemies that control leafminers as well as those which have an important role in the regulation of other tomato pests which may have been of minor economic importance at the time leafminers needed to be controlled.

As part of an insecticide resistance management strategy, it is essential to always have several insecticide classes registered for the same pest so that they can be used in rotation. The inclusion of botanical insecticides in this basket of pesticides for use against vegetable crop pests is now being actively promoted not only to help in managing resistance but also due to residue concerns. Neem oil (active ingredient azadirachtin) is one such botanical pesticide which has been shown to be effective against a number of insect pests due to a combination of insecticidal, ovicidal, anti-feedant and repellent properties but without leaving a significant residue imprint. However, the foremost pest management technique is to grow a resistant or tolerant variety. Thus, in addition to assessing the efficacies of botanical insecticides, the resistance status of the different tomato varieties that are being grown by farmers needs to be ascertained.

### **1.3 Objectives**

#### **1.3.1 General objective**

To assess infestation of different tomato varieties by Agromyzid leafminers and the relative efficacy of a commercial formulation of neem oil (Achta® 1% EC) in controlling the pest.

#### **1.3.2 Specific objectives**

1. To determine the susceptibility of different tomato varieties to leafminer infestation.
2. To determine the relative efficacy of neem (Achta® 1% EC) for leafminer control.

### **1.4 Hypotheses**

1. All test tomato varieties are equally susceptible to leafminer infestation.
2. Neem (Achta® 1% EC) oil is effective in reducing leafminer infestation.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Importance of the Tomato Plant

The tomato plant (*Lycopersicon lycopersicum* Mill) is one of the most widely grown vegetable crops (Ruben, 1980; Osata, 2003). According to Villareal (1993), the tomato plant is indigenous to South America and probably evolved from *L. esculentum* var. *cerasiforme*, the cherry form which was first found throughout tropical and subtropical America and later in the tropics of Africa and Asia. It was domesticated and first cultivated by early Indian civilizations of Mexico. Spanish explorers are credited with its spread from Mexico to Europe and Asia (Villareal, 1980). It was widely believed that the tomato plant was poisonous and its use as a food crop was only accepted in the 18<sup>th</sup> century. Kelley and Boyhan (2014) attribute this to the fact that the tomato plant belongs to the nightshade family whose many members contain highly toxic alkaloids. Presently, tomato is one of the most popularly grown crops around the world with an estimated global production exceeding 70 million metric tonnes (Srinivasan, 2010; Kelley and Boyhan, 2014).

Tomato is grown for its fruits which are used in a variety of ways. Ripe tomatoes are consumed fresh as salads or cooked with relish, or processed into various end products such as puree, paste or canned as whole fruits (Naika *et al.*, 2005). Medicinal uses of tomatoes have also been documented. Rao *et al.* (1998) reported that tomatoes and tomato products have numerous health benefits and also contribute to a well-balanced diet. Tomatoes are known to be an important source of lycopene, which is a powerful antioxidant that acts as an anticarcinogen and reduces the risks of getting certain neurodegenerative diseases (Sies *et al.*, 1992; Shi and Manguer, 2000; Srinivasan, 2010; Kelley and Boyhan, 2014).

There are several economic benefits of growing tomatoes. Growing the crop can potentially generate rural employment, stimulate urban employment, increase farmers' income and expand exports as compared to other vegetables (Villareal, 1993). In rural areas, production of tomatoes can increase employment and improve farmers' livelihoods (Kennedy (2008).

## 2.2 Diversity of Agromyzid Leafminers

Agromyzidae is one of the major dipteran families, with more than 3,000 species belonging to 30 genera worldwide (Spencer, 1989; Sasakawa, 1997; Shahreki *et al.*, 2012). About 1,165 species have been recorded from the Palaearctic region (Civelek, 2003; Çikman and Sasakawa, 2008; Dursun *et al.*, 2010). The genus *Liriomyza* contains more than 300 species (Parrella, 1987; Kang *et al.*, 2009). Within this genus 23 species are of economic importance to agricultural and ornamental plants due to their leaf-mining activity (Spencer, 1973; Parrella, 1987; Dempewolf, 2006). The key species comprise *L. trifolii*, *L. huidobrensis*, *L. sativae*, *L. bryoniae* and *Chromatomyia horticola* (Plant Health Australia, 2009; Reitz *et al.*, 2013). "Serpentine leafminer" was proposed as a common name for members of this genus because of the broad distribution, polyphagous nature and morphological similarity of many of the species (Steyskal, 1973; Parrella, 1987).

In a study of specific sequences in mitochondrial and nuclear genomes, *L. huidobrensis* was shown to be actually a complex of two cryptic species (Scheffer, 2000; Scheffer and Lewis, 2001). The existence of multiple subspecies was also deduced from the fact that populations in different areas appeared to prefer different plant hosts and exhibited varying levels of insecticide resistance. The name *L. langei* has been applied to North American and Ocenian populations while the name *L. huidobrensis* is applied to all other populations (Scheffer *et al.*, 2001).

## 2.3 Agromyzid Leafminers as Important Pests of Tomato

The economic impact of *Liriomyza* leafminers can be devastating to vegetables and ornamentals as was experienced when the chrysanthemum industry lost approximately 93 million dollars to *L. trifolii* in the United States in a span of four years in the early 1980s (Parrella, 1987). These horticultural products contribute significantly to the economic development of Africa because of the high economic returns they generate, the nutritive value of vegetables and the ability to generate significant employment through farming (Weinberger and Lumpkin, 2007; Foba, 2015).

*Liriomyza huidobrensis* is a major quarantine pest and is officially listed as such in many areas (Hara, 2002; Plant Health Australia, 2009). In South and Central America, *L. huidobrensis* was under natural biological control until the 1970s when it was secondarily subjected to massive amounts of insecticides that were directed at the leaf-mining moth *Tuta absoluta* (Meyrick)



(Lepidoptera: Gelechiidae) in potatoes (Chavez and Raman, 1987). This exposure to insecticides bred resistance, causing the leafminer to develop into an economically important pest.

The larvae leave winding trails (mines) as they feed inside leaves and other plant parts. The mines are easily visible and when the larvae are in large numbers this feeding damage can cause substantial economic losses (Reitz *et al.*, 2013). The pest status of *Liriomyza* spp. is closely tied to their biology. Losses are mostly attained from the tunnelling nature of their feeding (Capinera, 2007). Extensive mining also causes premature leaf drop which can result in sun scalding of fruit. In part, their pest status results from the ability of populations of these flies to build up rapidly (Reitz *et al.*, 2013).

Apart from internal mining by the larvae (CABI, 2006), damage to the plant is also caused in numerous ways such as the stippling that results from punctures made by females when feeding on sap and laying eggs (Parrella *et al.*, 1985; Riley *et al.*, 2007), and by allowing pathogenic fungi to enter the leaf through the feeding punctures (Deadman *et al.*, 2000). Typically, these polyphagous leafminers are considered to have invaded countries via movement of infested plants (generally ornamentals such as chrysanthemum) (Minkenberg, 1988; Spencer, 1989; Myint, 1997; Capinera, 2007; Plant Health Australia, 2009). While fully-formed mines should be readily visible to quarantine officials, signs of early infestations are much less obvious and are easily overlooked (Spencer, 1989). From 1990 to 2000, *L. huidobrensis* became globally invasive and can now be found in many greenhouses and vegetable and flower-growing areas of Europe, Asia, Africa, and the Middle East (Scheffer and Lewis, 2001). It is highly likely that the initial incursion of these species was in horticultural or urban areas, with subsequent spread into rural cropping regions.

## 2.4 Geographic distribution

Agromyzid leafminers are distributed widely but are most commonly found in temperate areas while relatively few species are found in the tropics (Parrella, 1987). Most species are cosmopolitan (Spencer, 1973; Dempewolf, 2006). Due to unintentional spread by man, Agromyzid leafminers of economic importance have a broad geographical distribution and are present in both temperate and tropical regions. From 1990 to date, *L. huidobrensis* became globally invasive and can now be found in many greenhouses and vegetable and flower-growing areas of Europe, Asia, Africa and the Middle East (Scheffer and Lewis, 2001).

According to CABI (2006), *L. huidobrensis* is spread widely throughout Africa (Kenya, Mauritius, Morocco, Seychelles, South Africa, Zimbabwe) (Figure 1), Asia (China, India, Indonesia, Jordan, Korea, Lebanon, Malaysia, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Vietnam), Central America (Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua, Panama), Europe (Austria, Belgium, Croatia, Cyprus, Czech Republic, Finland, France, Germany, Greece, Hungary, Italy, Netherlands, Norway, Poland, Portugal, Spain, Switzerland, Turkey, United Kingdom), North America (Canada, mainland USA) (*L. langei*); Oceania (Guam, Hawaii) (*L. langei*), and South America (Argentina, Brazil, Chile, Colombia, Ecuador, Peru, Uruguay, Venezuela), just to mention a few. This is a clear indication of the cosmopolitan nature of some of the *Liriomyza* species.



Figure 1. Geographical distribution of leafminers in Africa (Foba, 2015)

## 2.5 Biology and Ecology of Agromyzid Leafminers

### 2.5.1 Life cycle

Leafminers have a moderately short life cycle therefore several generations may be produced during the year (Capinera, 2007; Plant Health Australia, 2009). The time required for a complete life cycle in warm environments is often 21-28 days, so numerous generations can occur annually in tropical climates (Capinera, 2007). Eggs are laid singly, but frequently in close proximity to each other (Parrella, 1987). The female deposits the eggs on the lower surface of the leaf, but they are inserted just below the epidermis. Eggs are oval in shape and small in size, measuring about 1.0 mm long and 0.2 mm wide (Capinera, 2007). The eggs increase in size after oviposition, possibly through the imbibition of fluids from plant tissue (Parrella, 1987). Primarily they are clear but soon change into a creamy white colour. The period of egg development varies with temperature and ranges from 2-5 days (Plant Health Australia, 2009). In some species, the larva may eat the eggshell before moving into the leaf mesophyll.

There are three larval stages and all feed within the leaf or stem tissue (Riley *et al.*, 2007; Plant Health Australia, 2009). A fourth instar occurs between puparium formation and pupation; however, this is a non-feeding stage and is generally ignored by authors (Capinera, 2007). The larva begins feeding immediately after eclosion and feeds incessantly until it is ready to emerge from the leaf (Parrella, 1987). The larva is cylindrical and maggot-like and moves via peristaltic action of its hydrostatic skeleton. As the larva develops, both the diameter of the mine and the rate of mine formation increase (Parrella, 1987).

The larvae leave the plant to pupate (Parrella and Bethke, 1984), with pupae found in the soil, crop debris or occasionally on the leaf surface. Pupation is negatively affected by high humidity or drought (PHA, 2007). The puparium is initially golden brown in colour, but turns darker brown with time (Capinera, 2007). Pupal duration varies inversely with temperature but at least 50% of the total development time of a *Liriomyza* individual is spent in this stage. Total development time of the pupa at greenhouse/field temperatures is about 8-11 days (Parrella, 1987).

Adults emerge through the dorsal anterior end of the puparium. Newly emerged adults exhibit a positive phototactic response and climb up the stalk of a plant, where they remain quiescent for a

period of approximately 20 minutes while expanding their wings and body. Adult females are usually larger than males and emerge from larger puparia (Parrella, 1987).

Adults are small, measuring less than 2mm in length, with a wing length of 1.25-1.9 mm. The head is yellow with red eyes. The thorax and abdomen are mostly grey and black although the ventral surface and legs are yellow. The wings are transparent (Capinera, 2007). Female flies use their ovipositors to puncture the leaves of the host plants causing wounds which serve as sites for feeding (by both male and female flies) or oviposition (Plant Health Australia, 2009). The males live only 2-3 days possibly because they cannot puncture foliage and therefore feed less than females. Females usually survive for about a week; however, the life span of leafminer adults ranges from 13-20 days (Capinera, 2007). Adults are primarily active during early morning, shortly after sunrise, and again just before sunset (Weintraub and Horowitz, 1995).

### **2.5.2 Climatic requirements**

Several factors, both biotic and abiotic, play a role in successful establishment and survival of insects (Danks, 1996; Hodkinson, 1999; Bale *et al.*, 2002). Abiotic factors that may affect leafminer population densities include temperature, light intensity and moisture (Shepard and Braun, 1998; Saito *et al.*, 2008).

Larval development varies with temperature and host plant (Parrella, 1987). Leibe (1984) noted that high temperatures (35°C) decreased *L. trifolii* pupal viability. Total development time of the pupa at greenhouse/field temperatures is about 8-11 days (Parrella, 1987). Another important effect of temperature is to decrease fecundity (Huang *et al.*, 2007). High daily temperatures (30°C) were shown to reduce *L. huidobrensis* adult density on cucumber (Abou-Fakhr-Hammad and Nemer, 2000). Adults are able to withstand freezing temperatures for only short periods (Parrella, 1987). Development rates increase with temperature up to about 30°C; temperatures above 30°C are usually unfavourable and larvae experience high mortality. The temperature threshold for normal development of the various stages is 6-10°C except for the egg-laying stage which requires about 12°C (Capinera, 2007).

Shepard and Braun (1998) reported that moisture level might be another factor affecting leafminer density with infestation on potato by *L. huidobrensis* being more severe during the wet season. A relative humidity between 30 and 70% is optimum for pupation (Parrella, 1987). In addition, light

conditions could also affect leafminer density levels, and the pupation of emergent *L. trifolii* larvae can be delayed for a short time by continuous lighting conditions (Leibee, 1986).

### 2.5.3 Dispersal

Leafminer adults are not regarded as strong fliers and tend to remain close to their target crops, only moving very short distances from one host to another (Zehnder and Trumble, 1984; Wang *et al.*, 1998). Natural dispersion within the crop is by flight. Instances of dispersal over long distances have been reported (Plant Health Australia, 2009) with wind being credited as the dispersal agent. However, the most notable dispersal agent over long distances is through human activities of trade (Hara, 2002). Movement of infected plants or plant parts, soil or potting media, as well as packaging can introduce the pest to uninfected territories. *Liriomyza sativae* was probably introduced to Europe through imports for glasshouse cultivation (CABI, 2006).

### 2.5.4 Host range

The host range of Agromyzid leafminers encompasses over 400 species of plants in 28 families (Reitz and Trumble, 2002). Three species that are particularly important are *L. huidobrensis*, *L. sativae* and *L. trifolii*. These three highly polyphagous species cause extensive damage to a wide range of high value vegetable and floriculture crops (Reitz *et al.*, 2013). For example, *L. huidobrensis* has been recorded on plants from 14 families (Spencer, 1990, 1973) and can be classified as truly polyphagous.

The most economically important crops attacked are beans, beet, spinach, peas, potatoes and cut flowers (most commonly gypsophila, more rarely carnations and chrysanthemum) (Spencer, 1989), as well as field peas and faba beans. Cultivated herbs, such as basil (*Ocimum basilicum*), cilantro (*Coriandrum sativum*), and mint (*Mentha spicata*), are also hosts of leafminers (Jovicich, 2009). Stegmaier (1966) reported 55 hosts from Florida, including carrot, melon, cucumber, eggplant, lettuce, celery, onion, pea, pepper, squash, and tomato. Common ornamental plant species that are hosts of leafminers are in the genera *Alstroemeria* (Alstroemeriaceae), *Dianthus* (Caryophyllaceae), *Chrysanthemum* (Asteraceae) and *Gerbera* (Asteraceae) (Jovicich, 2009).

Examples of non-commercial plants affected by *Liriomyza* include broadleaf weeds and senescent crops that might serve as alternative hosts (Plant Health Australia, 2009). Common weeds that

harbour leafminers include *Bidens pilosa* (black jack), *Solanum nigrum* (black nightshade), *Chenopodium album* (fat hen) and *Galinsoga* spp. (Jovicich, 2009).

### **2.5.5 Host selection and oviposition**

The basis for host-plant preference is thought to be genetic (Parrella, 1987). A comparison of laboratory-selected and wild populations of *L. brassicae* was carried out by Tavormina (1982) and the results suggested that there is an increased tendency to oviposit in the host that this species develops on. Factors that influence host selection include the distribution and density of plant trichomes, the phenolic content, and the nutritional value of the host (Fagoonee and Toory, 1983; Ipe and Sadaruddin, 1984). These factors distinctly affect feeding, survival and fecundity (Parrella, 1987). Various studies have shown that leafminers respond positively to high nitrogen content in leaves (Harbaugh *et al.*, 1983; Parrella, 1987). Adult females express distinct preferences for host plants, even though their feeding and oviposition behaviour remains stereotypic regardless of host (Bethke and Parrella, 1985).

Due to the fact that the larvae of leafminers are unable to move between plants, host selection by *Liriomyza* species differs from many other herbivorous insects because the choice of host is made solely by adult females (Parrella *et al.*, 1985). It has been suggested that food, temperature, and relative humidity influence the pre-oviposition period (Parrella, 1987) which may extend up to five days after adult emergence. Under greenhouse and laboratory conditions, most females begin oviposition within 24-48 hours after emergence (Parrella *et al.*, 1983).

## **2.6 Management of Agromyzid Leafminers**

### **2.6.1 Biological control**

In a broad sense, biological control is a natural way of controlling insect pests involving the use of pathogens and some insects as beneficial organisms, as well as some plant extracts (Fusire, 2008). However, in its strictest sense, biological control is only defined in terms of the regulatory role of natural enemies.

### 2.6.1.1 Natural enemies

Natural enemies may provide effective control in field crops (Johnson and Hara, 1987; Johnson, 1993; Purcell *et al.*, 1995; Liu *et al.*, 2009). It has been acknowledged that Agromyzid leafminers have a diverse range of natural enemies that periodically repress their populations (Spencer, 1973). A wide range of predators have been observed attacking Agromyzids (Parrella *et al.*, 1982), however, hymenopteran parasitoids are considered to be the most effective natural agent in the control of these leafminers (Parrella, 1987; Liu *et al.*, 2009). In a review of the biological control of *L. huidobrensis*, Waterhouse and Norris (1987) reported that over 40 species of parasitoids have been recovered globally from the leafminers. Noyes (2004) listed more than 300 species of Agromyzid parasitoids of which over 80 species are known to attack *Liriomyza* species. Typically, Agromyzid leafminers are attacked as eggs, larvae or pupae by various parasitic wasps (Spencer, 1973; Murphy and LaSalle, 1999).

Hymenopterous parasitoids in four different families which include Braconidae, Figitidae, Pteromalidae and Eulophidae have been identified as the key agents of biological control of *Liriomyza* leafminers (Liu *et al.*, 2009). In a survey carried out in the Lower Rio Grande Valley of Texas, Hernandez *et al.* (2010) reported 20 species of parasitoids belonging to the families Eulophidae, Pteromalidae, Figitidae and Braconidae as being associated with *L. trifolii*. In pre-emptive control strategies that were carried out in the Virginia horticulture area on the Northern Adelaide Plains in Australia, 10 species of Hymenopteran parasitoids were observed emerging from *R. candolleana* mines (Wood *et al.*, 2010).

Natural enemies include the following parasitoids: *Agrostocynips clavatus*, *Diglyphus* spp., *Ganaspidium utilis*, *Halticoptera circulus*, *Chrysocharis* spp., *Oenogastra* and *Opius* spp. (Plant Health Australia, 2009). In a study that was done by Asadi *et al.* (2006) in Varamin, Iran, parasitoid species that were found to attack *Liriomyza* leafminers included *Cirrospilus vittatus*, *Closterocerus formosus*, *Diglyphus isaea*, *Diglyphus crassinervis*, *Hemiptarsenus zilahisebessi* and *Pnigalio* sp. nr. *pectinicornis*. The most common species were *D. isaea*, followed by *C. formosus* and *D. crassinervis*. *Dacnusa sibirica* is used as a biological control agent in glasshouses in Germany, but three to four releases are required per week (Leuprecht, 1992).

### 2.6.1.2 Use of entomopathogens

A better alternative method for controlling sap-sucking insects is the use of entomopathogenic fungi that infect their host through the cuticle (Poprawski *et al.*, 2000; Inbar and Gerling, 2008). Various strains of the hyphomycetous fungi, *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, have been reported to be virulent to other dipteran pests such as the house fly (*Musca domestica*) (Watson *et al.*, 1995; Renn *et al.*, 1999) and Tephritid fruit flies (Dimbi *et al.*, 2003).

Seventeen isolates of *M. anisopliae* and three isolates of *B. bassiana* were evaluated for their pathogenicity to adult *L. huidobrensis* by Migiro *et al.* (2010). Results indicated that some isolates of *B. bassiana* and *M. anisopliae* are highly pathogenic to *L. huidobrensis*, signifying a potential for their use in the control of this pest. They also suggested the possibility of *L. huidobrensis* suppression with fungi using an autoinoculation device. The main challenge for using mitosporic entomopathogenic fungi in pest control is their application in the field (Migiro *et al.*, 2010).

### 2.6.2 Use of botanicals

Plant extracts have minimal toxicity to non-targeted organisms and do not persist in the environment (Munyima *et al.*, 2004). This fact addresses environmental degradations that are associated with the use of synthetic pesticides; their use is therefore, increasingly becoming recognized as the best alternative method of insect pest management (Kopondo, 2004).

In a study to determine the insecticidal action of extracts from two plants, *Euphorbia myrsinites* L. (Euphorbiaceae) and *Urginea maritima* L. (Liliaceae) against *L. trifolii* on tomatoes, both plant extracts caused significant control of the leafminer larvae (Civelek and Weintraub, 2004). The plant extracts exhibited both translaminar and systemic activity, while higher dilutions of the extracts controlled leafminers in a statistically similar manner as the insecticide cyromazine (Civelek and Weintraub, 2004).

### 2.6.3 Cultural control

Abundant weeds on cultivated land often encourage rapid increase in pests (Fusire, 2008). Since broadleaf weeds and senescent crops are alternate hosts to the leafminer, they may serve as sources of infestation (Plant Health Australia, 2009). Destruction of weeds and deep ploughing of crop



residues is, therefore, recommended (Capinera, 2007). Constant and regular weeding is a good and effective means of controlling pests as it reduces breeding places (Fusire, 2008). Deep ploughing is an effective means of control because adults experience difficulties in emerging if they are buried deeply in soil (Capinera, 2007).

Cultural practices such as mulching and staking of vegetables may influence both leafminers and their natural enemies. Price and Poe (1976) reported that leafminer numbers were higher when tomatoes were grown with plastic mulch or tied to stakes.

#### **2.6.4 Phytosanitary measures**

Global trade in the marketing of agricultural crops has led to an increase in the invasion of alien species in several parts of the world. The spread of *L. trifolii*, initially a major pest of chrysanthemums in Florida is a key example (Hara, 2002). They may be quarantine pests on any marketed commodity that contains foliage or other parts of the plant that may be infested, especially cut flowers and foliage (Plant Health Australia, 2009). Agromyzid leafminers are quarantine pests of a variety of flowers, vegetables and ornamental foliage (Hallman *et al.*, 2011).

To curb the spread of alien species, governments have put in place strict quarantine regulations and consistently reject contaminated incoming shipments (Hara, 2002). A phytosanitary certificate is essential for cut flowers and for vegetables with leaves that are to be either imported or exported (Plant Health Australia, 2009).

Methyl bromide fumigation was often used to disinfest cut flowers of Agromyzids. Alternatives to the fumigant have been put in place as it is a stratospheric ozone-depleting substance (Heather and Hallman, 2008). Among these alternatives there is cold storage (Webb and Smith, 1970), controlled atmospheres (Kader, 1992; Cantwell and Mitcham, 1995), heat treatment (Sharp *et al.*, 1988; Heard *et al.*, 1991; Nascimento *et al.*, 1992), irradiation (APHIS, 1998; Hallman *et al.*, 2011) and combinations of treatments also called a systems approach (Moffitt, 1989; Jang and Moffitt, 1994). A systems approach includes pre-harvest crop pest management, postharvest culling, and final product inspection for successful control against several quarantine pests on various fruit crops (Moffitt, 1989; Jang and Moffitt, 1994).

Cold storage (0°C) treatment is not a feasible option for the importation of fresh vegetables and cut flowers and as a result produce inspections are required. To avoid the introduction of *L. huidobrensis* (and other leafminer species) into further European countries, OEPP/EPPO (1990) recommended that propagating material (except seeds) of capsicum, celery, carnations, chrysanthemums, *Gerbera*, *Cucumis*, *Gypsophila*, lettuces, *Senecio hybridus* and tomatoes, from countries where the pests occur, must have been inspected at least every month during the previous three months and found to be pest-free. A phytosanitary certificate is mandatory for cut flowers and for vegetables with leaves.

Even though there are phytosanitary measures in place, intercepts of insects and other invertebrates sometimes occur. These occur mostly on cut flower imports from African countries such as South Africa, Zimbabwe and Kenya; Asian countries such as India, Singapore and to a lesser extent, China. Zimbabwe had 77 interceptions from 1993 to 2012 (Table 1) (EFSA, 2012). These interceptions are a cause of concern to countries in which the pest has not yet spread to, as the climate and wide host range might provide ideal conditions for establishment of *L. huidobrensis* (PHA, 2009).

Table 1. Total number of interceptions at EU borders of *L. huidobrensis*, *L. trifolii* and other *Liriomyza* spp. from the main exporting countries from 1993 to August 2012 (only species with more than 10 interceptions are shown) (EFSA, 2012).

Country of origin	No. of interceptions	Country of origin	No. of interceptions
Israel	862	Morocco	26
Thailand	685	Egypt	24
Europe	624	India	21
Kenya	308	Tunisia	21
Vietnam	208	South Africa	17
Ecuador	202	Costa Rico	14
Zimbabwe	77	Mexico	14
Guatemala	43	Zambia	14
Colombia	40	Cote d'Ivoire	13
Ethiopia	30	Malaysia	13
United States	27	Togo	13

Once an invasive leafminer species becomes established, the economic impact would be high, and spread rapid, due to the frequent movement of produce between nurseries and markets in the cut flower and other horticultural industries (Western Australia, 2003). The action protocols for the UK provide an example of the contingency plans that should be put in place in countries not yet invaded by this pest (Cheek *et al.*, 1993). Plant Health Australia (2009) outlined various measures to prevent entry and establishment of the pest in a threat-specific contingency plan, e.g., quarantine, zoning and destruction protocols.

### 2.6.5 Chemical control

At present, the only effective insecticides used for *Liriomyza* control are translaminar insecticides (e.g. abamectin, cyromazine, neem and spinosad) which penetrate the leaves to affect the leafminer larvae (Weintraub, 2002). Vegetable growers are recommended to treat fields with such translaminar insecticides for effective leafminer control (Civelek and Weintraub, 2003; Bjorksten *et al.*, 2005). Control of leafminers can be problematic for several reasons. Leafminer larvae are inaccessible to many pesticides because they develop inside the leaf and pupate in the soil (Bjorksten *et al.*, 2005). Abamectin and cyromazine are some of the main larvicides used to control the leafminer on tomatoes. Cyromazine is effective and widely used for control of *L. trifolii* (Foster and Sanchez, 1988; Saito *et al.*, 1992) and is thought to be less harmful to parasitoids (Schuster, 1994).

No effective insecticides for use against adults have been recorded thus growers continue to use whatever is available (e.g. chlorfenapyr, chlorfluazuron, chlorpyrifos-ethyl, deltamethrin, diazinon, endosulfan and malathion), and a few effective larvicides (Civelek and Weintraub, 2003). Adults, particularly *L. trifolii*, may develop pesticide resistance rapidly (Parrella and Keil, 1984; MacDonald, 1991). Neem-based insecticides, although effective against *L. trifolii*, are expensive for non-organic agriculture (Civelek and Weintraub, 2003).

In the search for additional active ingredients to control Agromyzid pests, an old group of insecticides, based on secretions of marine annelids (*Lumbrineris* spp.), was examined for new applications. These synthetic insecticides are derivatives of nereistoxin and include cartap, bensultap and thiocyclam (Perry *et al.*, 1997). Due to the harmful effects posed by use of chemical pesticides, various less harmful compounds have been tested for their insecticidal properties. These include

low-dose, efficient synthetic agrochemicals such as neonicotinoids e.g., thiamethoxam (Karmakar and Kulshrestha, 2009) and other compounds such as avermectins (Lasota and Dybas, 1991).

Although the use of pesticides of traditional groups such as organochlorines, organophosphates, carbamates and synthetic pyrethroids are generally effective against various species of insect pests, their use is often associated with environmental contamination and pest resistance (Karmakar and Kulshrestha, 2009). One of the important factors that led to *Liriomyza* spp. becoming pests is their ability to develop resistance to insecticides (Parrella and Keil, 1984). Pesticides that kill leafminer parasitoids may also cause or aggravate leafminer outbreaks (Johnson *et al.*, 1980). Therefore, an integrated pest management (IPM) approach that seeks to conserve natural enemies wherever possible is the most sensible approach for leafminer control (Bjorksten *et al.*, 2005).

### **2.6.6 Host plant resistance**

The most intensively studied *Lycopersicon* species, *L. pennellii*, *L. lycopersicum*, *L. hirsutum f. typicum* and *L. hirsutum f. glabratum*, express a varied array of defence traits against insects (Kennedy, 2003). Three mechanisms are mostly involved in the resistance to arthropod pests: antixenosis, antibiosis and tolerance (Fernandes *et al.*, 2011). Plant signals responsible for inducing resistance are highly preserved among plant species and appear to be efficient in many crop and pest situations (Constable and Ryan, 1998). Fernandes *et al.* (2011) found that some tomato varieties were resistant against *L. trifolii* through antixenosis.

Presence of trichomes can induce resistance (Kennedy, 2003). Type IV trichomes have a short, multicellular stalk on a monocellular base and produce a droplet of exudate at their tip. High densities of type IV glandular trichomes and the presence of high levels of toxic acylsugars in their exudate play a major role in the resistance of *L. pennellii* to a number of arthropods, including aphids and *L. trifolii* (Ponti *et al.*, 1983; Nombela *et al.*, 2000).

Numerous types of molecular markers have been used to assess DNA polymorphism and for developing genetic linkage maps of different crops so as to identify quantitative trait loci (QTLs) associated with resistance to insects (Dhillon and Sharma, 2012). The QTLs for resistance to *L. trifolii* have also been identified in tomato (Moreira *et al.*, 1999).

Constitutive and induced defences other than those associated with glandular trichomes have been well documented in *Lycopersicon* spp. and these include glycoalkaloid  $\alpha$ -tomatine, the growth-inhibiting phenolics rutin and chlorogenic acid, and the jasmonic acid-inducible defences (Kennedy, 2003). Jasmonic acid sprays were tested by Black *et al.* (2003) as elicitors of resistance against leafminers. The results showed that jasmonic acid was effective. In two of three trials, significantly fewer leafminer adults emerged from the plants sprayed with jasmonic acid than the plants that were left untreated.

### **2.6.7 Integrated Pest Management**

Cultural, chemical and biological control measures are all preventive and curative methods of controlling pests in crops. A combination of these aforementioned methods is termed Integrated Pest Management (IPM). Precisely, IPM refers to the holistic approach for managing pests in production systems (Fusire, 2008). This is more effective than using any one sole method.

Control of leafminers can be challenging for a number of reasons. Notably, adults particularly *L. trifolii*, may develop pesticide resistance rapidly (Parrella and Keil, 1984; MacDonald, 1991). Since leafminer larvae develop inside the leaf and pupate in the soil, they are therefore inaccessible to many pesticides. IPM is the most viable approach to counter the problems related with resistance as well as to conserve natural enemies (Bjorksten *et al.*, 2005). The IPM approach has been successfully used to manage leafminers in both sugar peas (Myint, 1997) and chrysanthemums (Sivapragasam *et al.*, 1999).

## CHAPTER THREE

### GENERAL MATERIALS AND METHODS

#### 3.1 Study Site

The study was conducted at the University of Zimbabwe. Potted plant experiments were conducted at the Biological Sciences Department while field assessments on natural infestation levels and pesticide efficacy were conducted using a tomato crop which was planted at the Crop Science Department.

#### 3.2 General Materials

##### 3.2.1 Tomato varieties

Fourteen tomato varieties were used for a pot trial; namely, “Rodade”, “Nemonetta”, “Rio Grande”, “Season Red”, “Little Wonder”, “Money Maker”, “RVF”, “Cal-J”, “RVFN”, “Star 9065”, “Floradade”, “Rodade Plus”, “Heinz 1370”, and “Campbell”. Seeds of these varieties were purchased locally. For the field trial, the variety “Tengeru” was planted. The trial was established using seedlings which were purchased from a commercial nursery in Ruwa, 24 km east of Harare.

##### 3.2.2 Chemical treatments

Three rates of Achara<sup>®</sup> 1% EC (neem oil) (i.e., 20, 30 and 40ml each per 10litres of water) were assessed against Abamectin<sup>®</sup> 1.8% EC (abamectin, 1.8 g a.i./litre) at a rate of 6ml/10 litres of water and an untreated control.

#### 3.3 General Methods

The study was divided into three parts: (i) potted plant experiments to determine leafminer incidence and relative susceptibility of different tomato varieties to leafminer infestation, (ii) field trial to determine the efficacies of various chemical treatments in controlling leafminers, and (iii) leaf dip bioassays to accurately assess the effects of various chemical treatments and rates on leafminer larval mortalities. Larval mortality was confirmed by examination under a dissecting microscope.

### 3.4 Data Analysis

All collected data were subjected to analysis of variance (ANOVA). Appropriate transformations were done prior to analysis. Where the  $F$ -ratio was significant (i.e.  $P < 0.05$ ), means for the different treatments were separated by either Fisher's protected LSD test or the Student's  $t$ -test.

## CHAPTER FOUR

### DETERMINATION OF THE RELATIVE SUSCEPTIBILITY OF DIFFERENT TOMATO VARIETIES TO LEAFMINER INFESTATION

#### 4.1 Introduction

The Agromyzid leafminers, *L. huidobrensis*, *L. sativae* and *L. trifolii* are polyphagous pests which cause substantial damage to an extensive range of economically important host plants (Valladares *et al.*, 1999; Rauf *et al.*, 2000; Head *et al.*, 2003). These three species have expanded into many new areas worldwide mainly through international trade in horticultural produce. Due to the feeding nature of leafminers which involves the tunnelling of leaves, they affect the photosynthetic ability of plants as well as their aesthetic value (Reitz *et al.*, 2013).

Control of leafminers is mostly chemical (Bjorksten *et al.*, 2005). However, owing to various problems associated with chemical use such as the development of insecticide resistance and adverse effects on natural enemies, there has been some interest in the development of crop cultivars that are resistant to insect pests (Dhillon and Sharma, 2012). The use of tomato varieties that are resistant to leafminers is a viable alternative due to the high cost of spraying and the problems cited above (Fernandes *et al.*, 2011). Extensive research has been aimed at identifying and developing a mechanistic understanding of traits that confer pest resistance within the genus *Lycopersicon* (Kennedy, 2003).

Field experiments have shown significant differences in varietal susceptibility of some tomato varieties and breeding lines to *Liriomyza* spp. (Faiza-Salah *et al.*, 2012). Host plant species can affect the behaviour and other attributes of leafminers such as host searching, oviposition, and offspring fitness (Parrella, 1987). These factors result in differences in leafminer incidence among the different tomato varieties with some being more susceptible than others. The objective of this study was to determine the relative susceptibilities of different tomato varieties to leafminer infestation as measured by number of mined leaves per plant and number of mines per plant.

#### 4.2 Materials and Methods

Tomato plants were established in pots (two plants per pot) which were placed in an open space at the Biological Sciences Department. Fourteen varieties were used: “Rodade”, “Nemonetta”, “Rio



Grande”, “Season Red”, “Little Wonder”, “Money Maker”, “RVF”, “Cal-J”, “RVFN”, “Star 9065”, “Floradade”, “Rodade Plus”, “Heinz 1370” and “Campbell”). Seeds for the varieties were purchased locally in Ruwa. The tomato varieties were transplanted four weeks after sowing. Basal application of Compound D was made at a rate of 16g per pot; this was achieved by mixing the fertilizer in via shallow hoeing. The experiment was conducted in 2015 at two different times: May-July (cold-dry season) and September-November (hot-dry season). The plants were arranged in a Randomized Complete Block Design of 14 treatments in five blocks. For each treatment or variety, an experimental unit consisted of the two plants in a pot.

Sampling commenced four weeks after transplanting and was continued at a weekly interval for three weeks. Leaves were examined for presence of mines (Plate 1). Parameters that were measured were the number of mined leaves/plant and number of mines/plant. Mined leaves were also harvested and taken to the laboratory for the purpose of ‘harvesting’ adult leafminers.

Mined leaves were cleaved from each plant by a pair of scissors and placed in an A4 size khaki bag. They were then incubated in the laboratory at room temperature to allow for pupation of leafminer larvae. Pupae that had developed thereafter were collected and held singly in vials for incubation to adults. Each vial was stoppered using cotton wool. Emerged adults were then left to die inside the vials and afterwards preserved in 70% alcohol for identification to species.

### **4.3 Data Analysis**

Data on number of mined leaves per plant and total number of mines per plant were subjected to analysis of variance (ANOVA) using GenStat 14 edition statistical package. A two-way ANOVA with interaction term to determine if season and time affected the relationship between treatment i.e. varieties and measured parameters was conducted. Count data for all measured variables was transformed using  $\sqrt{(x+1)}$ . Where the *F*-ratio was significant ( $P < 0.05$ ), Student’s *t*-test was used to separate treatment means.



Plate 1. Plant showing leaf mining activity

#### 4.4 Results

##### 4.4.1 Number of mined leaves per plant

At 4 and 5 weeks after transplanting (WAT) in the cold-dry season, the number of mined leaves per plant was generally low across all the 14 varieties (0-2.5 mined leaves per plant) and there were no significant differences among them (Table 2). However, at 6 WAT, “Nemonetta”, “Rio Grande”, “RVF”, “Star 9065” and “Rodade Plus” had the least number of mined leaves per plant (0.4-1.6) while “Rodade”, “Little Wonder”, “Floradade” and “Campbell” had the highest (2.6-4.2). An entirely different picture emerged during the hot-dry season. At each of the three assessment periods (i.e. 4, 5 and 6 WAT), there were significant varietal effects. At both 4 and 5 WAT, “Rodade” (14.4 and 28.6, respectively) and “Nemonetta” (12.9 and 30.9, respectively) had the highest number of mined leaves per plant. On termination of the assessments at 6 WAT, “Nemonetta” was the only variety with the highest number of mined leaves per plant (86.6) while “Star 9065” had the lowest (20.2).

Table 2. Mean number of mined leaves per plant at 4, 5 and 6 weeks after transplanting during the cold-dry and hot-dry seasons

Variety	Cold-Dry Season			Hot-Dry Season		
	4 WAT	5 WAT	6 WAT	4 WAT	5 WAT	6 WAT
Rodade	0.6	1.9	2.6 abc	14.4 a	28.6 a	60.4 bc
Nemonetta	0.0	0.1	0.4 e	12.9 a	30.9 a	86.6 a
Rio Grande	0.4	0.9	1.1 cde	4.0 cd	16.1 b	48.3 bcd
Season Red	0.6	0.8	1.8 bcd	1.1 e	8.0 d	33.4 d
Little Wonder	0.3	0.6	3.6 ab	6.1 bcd	10.2 cd	31.9 d
Money Maker	0.3	1.0	2.2 bcd	4.2 bcd	18.4 b	56.8 bc
RVF	0.0	0.6	0.9 de	5.1 bcd	14.8 bc	35.6 d
RFVN	0.5	1.2	1.7 bcd	3.1 de	9.0 cd	44.4 bcd
Cal-J	0.5	1.0	2.2 bcd	5.1 bcd	15.2 bc	41.4 cd
Star 9065	0.2	1.0	1.6 cde	1.5 e	4.2 e	20.2 e
Floradade	0.4	1.4	4.2 a	4.0 cd	17.9 b	49.4 bcd
Rodade Plus	0.1	1.7	1.4 cde	1.0 e	15.8 b	63.3 b
Heinz 1370	0.6	0.7	2.3 bcd	6.6 bc	16.4 b	31.9 d
Campbell	0.8	2.5	2.6 abc	7.7 b	12.1 bcd	45.7 bcd
<i>F</i> <sub>13,126</sub>	1.3760	1.6882	2.7987	10.2968	10.3032	7.4526
<i>P</i> -value	0.1799	0.0711	0.0015	< 0.0001	< 0.0001	< 0.0001

Means within a column followed by the same letters are not significantly different (Student's t-test;  $P \geq 0.05$ )

#### 4.4.2 Number of mines per plant

At 4 and 5 weeks after transplanting (WAT) in the cold-dry season, the number of mines per plant was generally low across all the 14 varieties (0-2.7 mines per plant) and there were no significant differences among them (Table 3). However, at 6 WAT, a progressive increase in number of mines per plant was noted. At 6 WAT, “Nemonetta”, “Rio Grande”, “RVF”, “Star 9065” and “Rodade Plus” had the least number of mines per plant (0.4-1.9) while “Little Wonder” and “Floradade” had the highest (4.0-5.3). Conversely, during the hot-dry season, the number of mines per plant varied widely (1-142.6). At 4 WAT, “Season Red”, “RVFN” “Star 9065” and “Rodade Plus” had the least number of mines per plant (1-3.5). “Rodade” and “Nemonetta” had the highest number of mines at both 4 (18-25.2) and 5 WAT (50.8-51.1). On termination of the assessments at 6 WAT, “Nemonetta” was the only variety with the highest number of mines per plant (142.6) while “Star 9065” had the lowest (26.8). Other varieties had values in between.

Table 3. Mean number of mines per plant at 4, 5 and 6 weeks after transplanting during the cold-dry and hot-dry seasons

Variety	Cold-Dry Season			Hot-Dry Season		
	4 WAT	5 WAT	6 WAT	4 WAT	5 WAT	6 WAT
Rodade	0.7	2.3	2.6 bc	25.2 a	50.8 a	105.0 b
Nemonetta	0.0	0.1	0.4 d	18.0 a	51.1 a	142.6 a
Rio Grande	0.4	1.0	1.2 cd	5.1 cd	21.3 b	77.9 bcde
Season Red	0.6	0.8	2.1 bc	1.5 ef	9.9 e	55.4 ef
Little Wonder	0.5	0.9	4.0 ab	8.1 bcd	12.3 cde	39.6 f
Money Maker	0.3	1.0	2.9 bc	4.2 cde	23.6 b	80.2 bcd
RVF	0.0	0.7	1.0 cd	6.1 bcd	20.1 bc	55.6 def
RFVN	0.5	1.3	2.1 bc	3.5 def	10.3 de	58.9 cdef
Cal-J	0.6	1.0	2.5 bc	5.8 bcd	18.1 bcd	61.8 cdef
Star 9065	0.2	1.0	1.9 bcd	1.5 f	5.6 f	26.8 g
Floradade	0.5	1.7	5.3 a	4.5 cd	26.4 b	70.6 bcdef
Rodade Plus	0.1	1.9	1.5 cd	1.0 f	21.1 b	92.4 bc
Heinz 1370	0.6	0.7	2.3 bc	7.7 bc	20.8 b	40.5 f
Campbell	0.8	2.7	2.8 bc	8.7 b	15.1 bcde	71.1 bcde
<i>F</i> <sub>13,126</sub>	1.1952	1.5312	2.6820	13.9556	13.4965	7.6408
<i>P</i> -value	0.2903	0.1150	0.0023	< 0.0001	< 0.0001	< 0.0001

Means within a column followed by the same letters are not significantly different (Student's t-test;  $P \geq 0.05$ )

#### 4.5 Discussion

On termination of the study, leafminer incidence, i.e., number of mined leaves per plant, was highest in the variety “Nemonetta”. Notably, high incidence rates were also noted in the varieties “Rodade”, “Rio Grande”, “Money Maker”, “RFVN” “Floradade” and “Campbell”. One outstanding characteristic about most of these varieties is their fast growth rate. According to Mujica and Cisneros (1997), leafminers are known to prefer full grown plants. The initial larval infestation and corresponding damage occur in the lower third of the plant, moving upwards to the top of the plant (Plant Health Australia, 2009). Coincidentally, fast-growing varieties have a larger proportion of mature leaves; therefore, it would seem most probable that they would be more prone to attack. Larval damage is consistently less severe during vegetative growth stages than when the plant is full grown (Plant Health Australia, 2009).

In a study carried out by Faiza-Salah *et al.* (2012), the variety “Floradade” was found to be highly susceptible to leafminer attack. These results were in line with the current research findings because although “Floradade” was not the most attacked variety, it proved to be prone to attack. The hybrid variety that proved to be the least prone to attack was “Star 9065” as it consistently had the lowest

number of mined leaves per plant as well as the lowest number of mines per plant throughout the 6 weeks of observation during both the cold-dry and hot-dry seasons.

Various studies have shown that leafminers respond positively to high nitrogen content in leaves (Parrella, 1987). However, for this study, the nutrition of the plants may not have been the leading cause for the high incidence in susceptible varieties. All plants were given the same type and amount of fertilizers. The degree of high susceptibility, or, in some cases, resistance is not fully understood; however, according to Kelsheimer (1963), it is quite a reasonable assumption to make that morphological, physiological and environmental factors contribute to the response of a given variety to leafminer tolerance.

“Nemonetta” had a very low leafminer incidence level in the cold-dry season months but a sharp rise was recorded during the warm season months. This acute rise could coincide with the absence of red spider mite infestation during the September-November period. In the cold season months, “Nemonetta” was heavily infested with red spider mites. The presence of competition might have discouraged the leafminers from heavily attacking the variety.

There were no significant differences in leafminer incidence among the varieties at 4 and 5 WAT during the cold-dry season. All the varieties generally experienced low levels of infestation during the cold-dry season but the reverse was true for the hot-dry season. This is an indication of the effect that temperature has on the development of leafminers. Leafminers generally thrive in warm climates. This result is in agreement with Head *et al.* (2002) who carried out studies on the developmental rates of leafminers in lettuce at different temperatures (11-28°C). His study revealed a linear increase in developmental rates with temperature. As with all insects, the rate of immature development of *Liriomyza* spp. is dependent on temperature.

At a consistent temperature of 28°C, one generation can be completed in 14-15 days, but an increasingly longer time will be taken at lower temperatures. Miller and Isgar (1985) observed that the pupae of *L. trifolii* could remain viable outdoors for several months and were able to withstand freezing temperatures. That is one of the reasons why the leafminers were present during the cold season though in small numbers. According to Kang *et al.* (2009), thermal stress tolerance, particularly cold stress tolerance, is essential for the completion of the life cycle, successful overwintering, and habitat exploration of insects. This appears to be the case for *Liriomyza* spp. as

quite a lot of studies have revealed that commonly sympatric *Liriomyza* species have variable distributions that are strongly influenced within a given region by temperature extremes. The incidence and relative abundance of leafminers in relation to host plants and seasons may show the impact of climate and their diverse preference for host plants (Johansen *et al.*, 2003; Foba, 2015).

## CHAPTER FIVE

### RELATIVE EFFICACIES OF DIFFERENT TREATMENTS IN CONTROLLING LEAFMINERS IN TOMATO

#### 5.1 Introduction

The use of conventional synthetic insecticides for controlling pests in the genus *Liriomyza* has led to the development of resistant leafminer strains (Banchio *et al.*, 2003) and rapid elimination of their natural enemies, resulting in an increase in leafminer populations (Suenaga *et al.*, 1995). Abamectin and cyzomazine are two main larvicides that are used to control Agromyzid leafminers (Civelek and Weintraub, 2003). However, there is evidence that pesticides that kill leafminer parasitoids may cause or exacerbate leafminer outbreaks (Johnson *et al.*, 1980; Bjorksten *et al.*, 2005).

Alternative means of leafminer pest control have received attention for quite some time now, including host plant resistance (Suenaga *et al.*, 1995; Dogimont *et al.*, 1999), selective translaminar insecticides (Bjorksten *et al.*, 2005), and botanical pesticides, mainly from neem (*Azadirachta indica*) extracts (Banchio *et al.*, 2003). The rising accumulation of knowledge proves that neem products work by intervening at several stages of an insect's life (Mordue and Nisbet, 2000). By 1990, researchers had shown that neem extracts could influence almost 200 insect species including many that are resistant to or inherently difficult to control with conventional insecticides such as the sweet potato white fly, green peach aphid, diamond back moth and several leafminer species (National Research Council [NRC], 1992).

The neem tree has many compounds that have pesticidal effects; however, the most popular is azadirachtin (Mordue and Nisbet, 2000). Azadirachtin has been found to have various effects on insects. These include sterilizing adults, inhibiting chitin formation (NRC, 1992), anti-feedant properties, insect growth regulator ((Mordue and Nisbet, 2000; Boadu *et al.*, 2011) and, repellent properties (Panhwa, 2005). The objective of this study was to determine the effectiveness of neem oil (Achta<sup>®</sup> 1% EC, Tagros Bioscience, India) in controlling leafminers in tomato.

## 5.2 Materials and Methods

### 5.2.1 Insecticides

Three rates of Achara<sup>®</sup> 1% EC (20, 30 and 40 ml/10 litres of water) were tested against an untreated control and Abamectin<sup>®</sup> 1.8% EC (abamectin, 1.8 g a.i./litre) (the standard) applied at 6 ml/10 litres of water. All insecticide mixes were applied using a 16 litre Jacto<sup>®</sup> knapsacker sprayer. The sprayer and nozzle were thoroughly rinsed in between each chemical change.

### 5.2.2 Field trials

#### *Crop establishment and experimental design*

The trial (Plate 2) was established at the University of Zimbabwe's Crop Science Department. The tomato variety 'Tengeru' was used throughout the trial. Hardened seedlings were obtained from a commercial nursery in Ruwa, 20 km east of Harare, and transplanted at the end of August 2015. The experimental design adopted was a randomized complete block design (RCBD) of five treatments in four blocks. A treatment plot comprised five rows with 20 plants in each giving a plant population of 100 per plot. Intra- and inter-row spacing of the tomatoes were 40 and 50 cm, respectively. Adjacent plots were separated by a 1 m path. Basal application of Compound S fertilizer (NPK = 7:21:7) was made two days prior to transplanting at the rate of 1,000-1,500 kg/ha. Two split applications of top dressing were made with ammonium nitrate (34.5% N) three weeks after transplanting and at the onset of flowering and fruit formation at the rate of 50 kg/ha. Weeding was done by hoeing whenever necessary. At 4-5 weeks after transplanting, plants were supported by being tied to wooden stakes driven into the soil alongside each plant. Overhead irrigation was applied to the crop twice weekly.





Plate 2. Field plot for spray assessments

*Insecticide applications, sampling and assessments*

A pre-spray assessment was carried out on 4 October 2015 in order to obtain baseline information on leafminer infestation levels across treatments. A total of 12 plants were randomly selected from the three middle rows in each plot and examined for presence of leaf mines. The outer two rows were used as guard rows and were thus not assessed. A day later, the first sprays were conducted.

Two days after spraying, 12 plants were again randomly selected from each plot and leafminer infestation levels assessed. From each of the selected plants, three mined leaves were destructively-sampled from the bottom, middle and upper canopies of the plant. Sampled leaves were then brought to the laboratory and examined under a stereomicroscope. Live and dead larvae were each counted and recorded and percentage live larvae per plot determined. Live larvae were easily discernible from the movement of their black mouth hooks. However, all dead leafminer larvae that were brownish or already rotting were considered as having died prior to treatment and were thus excluded from the determination of % live larvae post-treatment. Their deaths were regarded to be due to parasitism whose effect was quite evident and confirmed through leafminers sampled from

some rows which were left unsprayed on the eastern side of the field so as to be a source of leafminer infestation to the treatment plots.

### *Leaf dip bioassays*

In order to have an appreciation of the toxic effects of neem oil without the confounding effects of parasitism and disease-induced mortalities, leaf dip bioassays were conducted in the laboratory. Mined leaves were collected from the unsprayed portion of the tomato field described in 5.2.2. At least 250 mined leaves were detached and taken to the laboratory for examination under a stereomicroscope. If the larva within the mine was noted to be alive, the leaf was placed in a tray. If there were dead larvae on the same leaf, the leaf sections in which they resided were cut off and discarded. Where there was more than one live larva on the same leaf, their number were noted. All sampled leaves were handled in the same manner until a total of 250 actively-mining larvae had been obtained. Where the number fell short of the required 250, more mined leaves were brought in from the field for examination and handled as already described. The leaves containing live larvae were then divided into five groups (representing five treatments) to give a total of 50 larvae per treatment. The 50 in each lot were further subdivided into five groups of 10 each (representing five replicates per treatment).

Half a litre of water-pesticide mixes were prepared in beakers to give dosage rates equivalent to 0 ml/10 litres water (Untreated control), 6 ml/10 litres of water of Abamectin 1.8% EC (standard), and 20, 30 and 40 ml/10 litres of water of Achta 1% EC. Using a pair of forceps, leaves containing mining larvae were dipped briefly into the pesticide mix or plain water (Untreated control). After dipping, each leaf was briefly held over the rim of the beaker to allow excess liquid to drain off and then placed on a paper towel to drain off the remaining liquid. When the liquid had been drained off, each leaf was transferred to a clean and appropriately-labelled Petri dish. The Petri dishes were incubated at room temperature conditions and leaves checked for larval mortality after two days.

Percentage leafminer larval mortality for each pesticide treatment was then calculated. Mortality data were corrected for control mortality (Abbott, 1925; WHO, 2009):

$$\% \text{ Corrected mortality} = \frac{X - Y}{100 - Y} \times 100$$

Where  $X$  = percentage observed mortality in the treated sample, and

$Y$  = percentage mortality in the untreated control.

### 5.3 Data Analysis

Percentage data were transformed by arcsine square-root transformation then subjected to analysis of variance (ANOVA) using GenStat 14 edition statistical package. Where there were significant treatment effects (i.e.  $P < 0.05$ ), means for the different treatments were separated by the Student's  $t$ -test.

### 5.4 Results

#### 5.4.1 Pre-spray assessments

With the exception of the untreated control, all treatment plots recorded very high (above 90%) levels of leafminer infestation (Table 4). However, examination of the sampled leaves revealed that most of the larvae within the mines were already dead most likely as a result of parasitism (Table 5).

Table 4. Pre-spray leafminer infestation levels per plot

Product	Application rate (/10 L)	Mean % leafminer infestation
Untreated control	-	100 a
Abamectin 1.8% EC	6 ml	91.7 a
Achta 1% EC	20 ml	91.7 a
Achta 1% EC	30 ml	100 a
Achta 1% EC	40 ml	75.0 b

Means within a column followed by the same letter are not significantly different at  $P = 0.05$

Table 5. Percentage of live leafminer larvae per plot prior to spraying

Product	Application rate (/10 L)	N	Mean $\pm$ SE
Untreated control	-	35	31.2 $\pm$ 7.7 a
Abamectin 1.8% EC	6 ml	36	27.6 $\pm$ 9.9 a
Achta 1% EC	20 ml	62	39.6 $\pm$ 9.1 a
Achta 1% EC	30 ml	38	42.1 $\pm$ 15.8 a
Achta 1% EC	40 ml	24	31.1 $\pm$ 2.0 a

Means within a column followed by the same letter are not significantly different at  $P = 0.05$

### 5.4.2 Post-spray leafminer larval survival

At 2 days after the first spray, there were no significant differences ( $F_{4,15} = 0.4981$ ;  $P = 0.7375$ ) among the five treatments with larval survivorship being in the range 35-44% (Table 6). At the second spray, the percentage of larvae that were alive in the abamectin and Achta-treated plots were significantly lower (7.3-14.1%) than those recorded in the untreated control (46.5%) (Table 7). However, mean separation also showed that there were no significant differences between abamectin and any of the three Achta rates. At the third spray, larval survivorship was significantly much higher in the untreated control (76%) compared to abamectin (16.7%), Achta-30 ml (15.7%) and Achta-40 ml (9.2%) (Table 8). However, the lowest Achta rate performed poorly and had larval survivorship that was not significantly different from that recorded in the untreated control. Also noted, particularly at the 3<sup>rd</sup> spray when irrigation water was insufficient in the face of sustained hot weather, was evidence of phytotoxicity-induced leaf wilting in the Achta-treated plots.

Table 6. Percentage of live leafminer larvae per plot at 2 days after the 1<sup>st</sup> spray

Product	Application rate (/10 L)	N	Mean $\pm$ SE
Untreated control	-	32	37.6 $\pm$ 14.4 a
Abamectin 1.8% EC	6 ml	41	37.0 $\pm$ 7.5 a
Achta 1% EC	20 ml	41	35.4 $\pm$ 10.9 a
Achta 1% EC	30 ml	53	37.6 $\pm$ 1.6 a
Achta 1% EC	40 ml	41	43.6 $\pm$ 12.8 a

Means within a column followed by the same letter are not significantly different at  $P = 0.05$

Table 7. Percentage live leafminer larvae per plot at 2 days after the 2<sup>nd</sup> spray

Product	Application rate (/10 L)	N	Mean $\pm$ SE
Untreated control	-	54	46.5 $\pm$ 2.9 a
Abamectin 1.8% EC	6 ml	30	10.7 $\pm$ 10.7 b
Achta 1% EC	20 ml	44	14.1 $\pm$ 6.5 b
Achta 1% EC	30 ml	60	7.3 $\pm$ 3.3 b
Achta 1% EC	40 ml	50	12.2 $\pm$ 4.6 b

Means within a column followed by the same letter are not significantly different at  $P = 0.05$

Table 8. Percentage live leafminer larvae per plot at 2 days after the 3<sup>rd</sup> spray

Product	Application rate (/10 L)	N	Mean $\pm$ SE
Untreated control	-	22	76.0 $\pm$ 4.3 a
Abamectin 1.8% EC	6 ml	11	16.7 $\pm$ 16.7 c
Achta 1% EC	20 ml	7	58.3 $\pm$ 25.0 ab
Achta 1% EC	30 ml	17	15.7 $\pm$ 10.2 bc
Achta 1% EC	40 ml	19	9.2 $\pm$ 5.3 c

Means within a column followed by the same letter are not significantly different at  $P = 0.05$

### 5.4.3 Leaf dip bioassays

There were significant differences ( $F = 5.774$ ;  $P < 0.05$ ) in the mortality rates observed in the different leaf dip treatments. The highest leafminer mortalities were noted in the abamectin treatment as compared to the three rates of the botanical pesticide (Table 9). The lowest mortalities were observed in the Achta-20 ml and Achta-30 ml treatments. Although the highest Achta rate gave mortality that was higher (55.8%) than in the two lower rates (25.6-32.6%), it still could not match that given by abamectin (79.1%).

Table 9. Corrected percentage leafminer larval mortalities at 2 days after infested leaves were dipped into chemical treatment

Product	Application rate (/10 L)	n	% Mortality (mean $\pm$ SE)
Abamectin 1.8% EC	6 ml	10	79.1 $\pm$ 4.4 a
Achta 1% EC	20 ml	10	25.6 $\pm$ 5.9 c
Achta 1% EC	30 ml	10	32.6 $\pm$ 7.7 c
Achta 1% EC	40 ml	10	55.8 $\pm$ 4.4 b

Means within a column followed by the same letter are not significantly different at  $P = 0.05$

## 5.5 Discussion

According to various authors (e.g. Panhwa, 2005; Boadu *et al.*, 2011), azadirachtin does not kill insects, but works by disrupting various vital processes that take place within the insects. This could explain the low leafminer larval mortalities generally observed in the Achta treatments. The results are in agreement with NRC (1992) who stated that the slow action of neem can cause insects to continue damaging plants even after spraying. When birch trees were sprayed to control the birch leafminer (*Fenusa pusilla*), neem extract seemed to perform as well as the registered commercial pesticide Diazinon<sup>®</sup>; however, it was slower acting, and the insects continued to damage trees before

they died. The delayed action of neem extracts, which allows leafminers to pupate and slowly die, could be beneficial in conserving parasitoid populations, since fast-acting compounds might kill the hosts before parasitoids had a chance to develop (Banchio *et al.*, 2003).

Leafminer larval mortalities were highest in plots treated with abamectin and Achta 40 ml/10 litres of water. Abamectin has long since been shown to be very efficient in leafminer control (Seal *et al.*, 2007). Achta also proved to be equally efficacious when applied in sufficiently high concentrations. This shows that although Achta is made from plant derivatives it can be equally lethal to leaf-mining larvae. Use of Achta as a substitute for abamectin is advantageous because most plant derivatives do not have detrimental effects on natural enemies. However, different studies provide inconsistent results for the effect of abamectin on leafminer parasitoids (Kaspi and Parrella, 2005). In particular, abamectin has been found to be lethal to parasitoid adults. One characteristic that makes Achta efficient as a pesticide is its repellent odour. Application of the pesticide should ideally be done before the onset of infestation in order to repel adult females from ovipositing on the plants. The control plots seemed to have lower than expected numbers of live leafminers. This could be attributed to biotic factors such as the effect of parasitoids and other natural enemies.

The leaf dip assay results showed that abamectin was the most efficient pesticide, followed by Achta 40 ml. The two lower rates of Achta gave the same level of toxicity. Leaf dip bioassays are more accurate in assessing relative toxicities of different treatments because distortions brought about by the various external factors will be excluded. External factors include the effects of drift and intervention by parasitoids and other natural enemies. In conclusion, Achta 1.8% EC has the potential to provide effective control of *Liriomyza* infestation in tomatoes particularly considering the need for an insecticide rotational programme so as to slow down the development of resistance as well as address world-wide concerns about pesticide residues in the horticultural industry.

## CHAPTER SIX

### GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 General Discussion

An assessment on the relative susceptibilities of 14 different tomato varieties, i.e., “Rodade”, “Nemonetta”, “Rio Grande”, “Season Red”, “Little Wonder”, “Money Maker”, “RVF”, “Cal-J”, “RVFN”, “Star 9065”, “Floradade”, “Rodade Plus”, “Heinz 1370” and “Campbell” was conducted during the cold-dry and hot-dry seasons. Leafminer incidence varied throughout the year. In the cold-dry season, the number of mined leaves per plant was generally low across all the 14 varieties unlike in the hot-dry season. This was largely dependent on the prevailing temperatures within the different seasons. According to Head *et al.* (2002), leafminers generally do well in warmer climates.

Of all the varieties and in both seasons, Star 9065 was consistent in having the lowest levels of susceptibility to leafminer attack while “Nemonetta” was the most susceptible during the hot-dry season. Host characteristics can play a huge role in attracting leafminers. Various authors have discussed some of these characteristics at length. Kennedy (2003) revealed the constitutive and induced defences other than those associated with glandular trichomes that affect insects. Some of the plant characteristics can be repellent to the insects, such as large surface hairs. The only variety to exhibit some inconsistency was “Nemonetta”. While it exhibited very low leafminer incidence level in the cold-dry season, there was a spike in leafminer numbers during the hot-dry season.

Notwithstanding poor leafminer control by the two lower rates of Achta and indications of phytotoxicity during the hot-dry season, Achta showed promise as an effective leafminer insecticide. This is quite useful in situations where rotations of pesticides are essential so as to delay the onset of insecticide resistance as well as where biological control agents need to be conserved.

#### 6.2 Conclusions

The study revealed that different tomato varieties differ in their susceptibilities to *Agromyzid* leafminer infestations. Plant breeders should therefore look for heritable resistant traits in varieties which consistently show low infestation levels and pest incidence so as to make host plant resistance an integral part of an IPM strategy against the pests. The study showed that although there were variations in leafminer incidence between the cold-dry and the hot-dry seasons, the pests were

present throughout the year. Anyone going into commercial production of tomatoes therefore needs to plan for leafminer control in addition to several other arthropod pests that damage the crop (e.g. *Helicoverpa armigera*, whiteflies and red spider mites). The study showed that Achta 1% EC is a very efficacious agent that can be used as an alternative to conventional pesticides for leafminer control. At higher dosages, it gave a high level of leafminer control which approximated that given by the pesticide abamectin. Perhaps one drawback with the use of Achta 1.8% EC is the likelihood of plant phytotoxicity. When spraying was conducted on water-stressed tomato plants in extremely hot and dry conditions, wilting was observed on many plants in all Achta-treated plots. This did not occur in abamectin and control plots. Although the plants recovered after some days, this can present significant problems if the crop is entirely rain-fed.

### 6.3 Recommendations

- 1) The high yielding tomato varieties such as “Nemonetta”, “Rodade”, “Money Maker” and “Floradade” unfortunately possess various attributes that are favourable to leafminers, hence experiencing high incidence levels. There is need for further studies to determine what these attributes are and how best to manipulate them in order to breed leafminer-resistant tomato varieties that are also high-yielding. An additional study to explain the semiochemical and morphological characteristics of the resistant varieties is warranted. This can also shed some light on how both the leafminer and its parasitoids are affected.
- 2) It is recommended that further studies be undertaken so as to identify the leafminers that were recorded in the study to the species taxon. The current study only identified the leafminers to genus level. This can be done through molecular work.
- 3) Studies need to be conducted in order to determine the indigenous leafminer parasitoid species complex present in tomato agroecosystems.
- 4) Pesticide use greatly affects natural enemies that help to control leafminers. There is need to conduct studies to see the effect that the pesticides which are used for controlling leafminers have on the various natural enemies. Equally important is to assess for Achta 1% EC phytotoxic effects on tomato plants under a broad range of environmental conditions.

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