BIOLOGICAL CONTROL OF WESTERN FLOWER THRIPS, (*Frankliniella occidentalis Thysanoptera: Thripidae: Frankliniella)* IN FRENCH BEANS USING PLANT AND SOIL DWELLING MITE

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as university supervisors.

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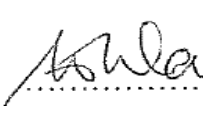
Signature:  Date 23/04/2023

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DEDICATION

I dedicate this work to my family particularly Mburathi family, who gave me unwavering support throughout my study. May the Lord God Almighty bless them and guide them in all their endeavors.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CRD	Complete Randomized Design
EPN	Entopathogenic Nematode
EPF	Entomopathogenic Fungi
HCDA	Horticultural Crops Development Authority
ICIPE	International Center of Insect Physiology and Ecology
INSV	Impatiens Necrotic Spot Virus
IPM	Integrated Pest Management
KEPHIS	Kenya Plant Health Inspectorate Services
TSWV	Tomato Spotted Wilt Virus
USA	United State of America
WFT	Western Flower Thrips

ABSTRACT

Western Flower thrips (WFT), *Frankliniella occidentalis* pergande (Thysanoptera: Thripidae), is one of the most important pests damaging a wide range of economic important crops in protected and open cultures worldwide. Its cryptic life cycle, combined with a very short generation time and the ability to rapidly develop resistance against insecticides, are characteristics that make control of this pest extremely difficult. Moreover, commonly used natural enemies often do not lead to sufficient control levels. However, it has been reported that the life cycle of *F. occidentalis* includes a soil passage, which is still neglected in biocontrol strategies. Studies were conducted from 2017 to 2018 at Real IPM Company with aim to evaluate the efficacy of Plant Predatory and Soil swelling Mite for control of pupae stage of western flower thrips in French beans (Var. Samantha) crop. A laboratory study to examine the rate of consumption of WFT pupae in potting media, by the soil dwelling mites *Hypoaspis sclerotarsa* (Costa)(Soil dwelling mite) was designed. Five *Hypoaspis sclerotarsa* predator densities were evaluated (0, 2, 6, and 8) against five densities of WFT prey (5, 10, 10 15 and 20 pupae) Pupal consumption was assessed at 2 hourly intervals over a six –hour period. The study confirmed that *H. sclerotarsa* did consume WFT pupae and that the rate of consumption increased with increasing densities of *H. sclerotarsa.* However, this was not consistent because, as the numbers of WFT pupae increased, so did the ratio of WFT pupae remaining to those consumed, increase. To evaluate the effect of combining plant and soil dwelling mite, three separate greenhouse experiments were conducted: a) Aim to evaluate different densities of the mite *Amblyseius Montdorensis* (Foliar predator; AM at 0, 5, 10 or 15 per pot); b) To assess different densities of *H. sclerotarsa* (ground predator; HS at 0. 50, 100 or 150 per pot); c) To assess a combination of the two (0AM, 0HS; 15AM, 50HS; 15AM, 100HS; 15AM, 150HS) on emergence) of WFT from soil; initial start populations of WFT were either small (10) or large (20). A complete randomized design was used and for each experiment, there were three replicates per treatment, repeated twice over time. Single applications of *A. Montdorensis*, *H. sclerotarsa* or a combination of both all had an impact on the number of WFT emerging compared with the control. There was a significant effect of *A. Montdorensis* densities on the number of WFT emerging from the soil (F=0.31, P= 0.420 df =1). There was no significant difference in the population densities of WFT emerging from soil in the control and following release of *H. sclerotarsa* when initial release densities of WFT at the two initial prey densities of 10 and 20. Combined use of *A. Montdorensis* and *H. sclerotarsa* at a density of 150 with 15 *A. Montdorensis* reduced adult WFT emergence at density of 20 WFT, by 93.35%. To determine appropriate timing and frequency application of H. *sclerotarsa*, different densities of *H. sclerotarsa* (0,50,100 and 150, Foliar *A. Montdorensis* 15), were released in the soil media solely before pre-pupation and at pupation stage of WFT development. There was significant effect in mean number of emerging thrips from the soil when compared to control in all tested densities; of 0 (54.71%), 50(41.38%), 100(24.72%) and 150(5.83%), However *H. sclerotarsa* proved better when applied as a pre-pupation rather than as a post pupation treatment with emerging thrips number recorded when *H. Sclerotarsa* was applied at different densities; 0(86.9%), 50(50.83%), 100(29.16%) and 150 (7.75%). This is the first report on the potential of *H. sclerotarsa* as a biocontrol agent of WFT in Kenya. Over all, the study confirmed

that both *H. sclerotarsa* and plant predatory mite can substantially reduce thrips population and might be important antagonists for *F. occidentalis* control in protected crops.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The western Flower thrips (WFT), *Frankliniella occidentalis* pergande (Thysanoptera: Thripidae) was found in California in orchards and on various weeds in 1923 (Frantz *et al.,* 2009). It was first seen in Europe in 1983 in *Saintpaulia ionantha* Wendl nurseries in the Netherlands (Tommasini, 2003). Since it was introduced in Europe nearly 20 years ago, it has spread rapidly throughout the continental and was valued to be one of the most important pests in protected crop production. Today in year 2020, the pest has not lost its pest status (Bale *et al.,* 2007). WFT is a serious pest on a wide range of crops throughout the world causing substantial economic losses (Bielza *et al.,* 2008).

Western Flower Thrips (WFT), *F. occidentalis* is polyphagous that feeds on a wide range of wild plants and cultivated crops, including vegetables and ornamentals. The degree of damage depends on the plant tissue that is affected, the developmental stage of the plant, susceptibility of the cultivars or species attacked and salivary toxicity (Mirnezhad, *et al.,* 2010). The feeding behaviour of *Thysanoptera* is best described as piercing-sucking. Thus, *F. occidentalis* causes direct damage to the host plant by mechanically destroying the cells and damaging the tissue of leaves, fruits or petals (Deligeorgidis, *et al.,* 2006). The surface of the leaf exhibits emptied and discolored cells, initially adopting a mother-of-pearl appearance and subsequently turning brown. If plants are massively infested, the leaves may wither and fall (Tommasini & Maini, 1995). The result of the feeding can vary from a deformation to the total destruction of the developing leaves or flower organs and subsequently scarring and deformation of fruits. Streaking, browning and distortion of leaves and/or petals or even buds of various flowering ornamentals (e.g., roses, gerberas, chrysanthemums, carnations, geraniums, pansies and marigolds) are frequent symptoms of thrips damage (Oetting *et al.,* 1993, Childers, 1997). Thrips feeding e.g., on immature cucumber fruits can result in silvery scarring, or even malformation of the fruit (Rosenheim *et al.,* 1990, Shipp e*t al.,* 2000) and feeding on

immature nectarine fruits causes minute scarring, which can develop into serious surface setting on the mature fruit. Such fruit is generally downgraded at sale, but if damage is serious enough, the fruit is culled (Pearsall, 2000). The feeding on the foliage may also have an adverse impact on leaf size and photosynthesis, and eventually results in significant yield loss (Welter *et al.*, 1990, Shipp et al., 1998, Shipp *et al.,* 2000).

In Kenya, the WFT was first reported in 1989 and thereafter it has become a serious problem in the horticulture industries (Nair, 2009). For instance, the production of French beans, which contribute to about 35-40% of foreign exchange, has been declining over the recent years due to damage associated with thrips and other pests and diseases (HCDA, 2012). Thrips (WFT) damage plants through oviposition and feeding that makes the leaves or flower petal appear silvery or flecked. Leaves and flowers become deformed due to an even tissue growth around the dead portions. The flecking and silvery damage becomes visible within one week after the thrips feeding. In addition, infested plants are often speckled black with thrips excrement. WFT are known to be vectors for viruses such as the *Tomato spotted wilt virus* (TSWV)). However, several groups of synthetic chemical pesticides used to control thrips in crops, due to consistent exposure of similar chemistries of the synthetic pesticides, *F. occidentalis* has often developed resistant to many of these pesticides. Thrips pupate in the soil thus are not easily controlled with foliar applied synthetic chemicals pesticides (Gao *et al.,* 2012). However, an ideal control strategy would target both the above ground and soil dwelling stages of the pest should be implemented. Currently, various plant and soil predatory mites have been used to manage WFT in greenhouses.

In general, the release of two natural enemies to combat a single pest species can carry divers' impacts on pest population development. Biological control can be more effective if antagonists perform in a synergistic or additive mode. But even a non-additive effect can improve control efficacy if pest mortality is still higher compared to the mortality caused by a single natural enemy species. Plants Predatory mites such as bugs, *Orius spp*. (*Hemiptera: Anthocoridae*) as well as plant extracts, are prove to be suitable and effective methods for *F. occidentalis* control (Arthurs *et*

al., 2009; Dogramaci *et al.,* 2011). Several species of the family Phytoseiidae (Acari: Mesostigmata) are important predatory mites on many crops. *Numerous phytoseiid* species had been reported as effective predators of WFT such as *Neoseiulus cucumeris* (*Oudemans), Amblyseius swirskii* (Athias-Henriot), and *Amblydromalus limonicus* (Garman and McGregor) (Messelink *et al.,* 2006; Knapp *et al.,* 2013). *Neoseiulus barkeri* (Hughes) is commonly used as a biological control agent for thrips in pepper (Ramakers, 1988). In Kenya, *Amblyseius cucumeris; Amblyseius Montdorensis* and other species of plant predatory mites are commonly used to control *Frankliniella occidentalis* at first and second larvae instar, however it's sometimes limited since the adults are only able to feed on available thrips eggs and first instar larvae (Sabelis *et.al.,* 2008).To date augmentative release of this predatory mites have not always provided sufficient control of western flower especially in crops with low economic threshold levels such as ornamentals (Bale, *et al.,* 2007). In addition, one third of western flower thrips life cycle mainly pre and pupae stage pupate in the soil (Reitz, 2009). So far only three species of soil dwelling mites research studies of *Hypoaspis* (*stratiolaelaps*) miles (Berlese), *Hypoaspisacuifer* (Canestrini) and *Macrocheles robustulus* have been done and found to be promising as biological control agent against pre and pupae stage of Western Flower Thrips (Navarro-Campos *et al.,* 2012). However, more attention has largely focused on the control of adults in the crop canopy while few attempts have been made to control soil dwelling stages of the WFT. Therefore, the objective of present study was to evaluate the efficacy of plant and soil dwelling mites as biological control of western flower thrips in French bean.

1.2 Problem statement

The Western Flower Thrips (WFT), *Frankliniella occidentalis* pergande (Thysanoptera: Thripidae) has become one of the serious pest problems facing horticultural industries in Kenya today. It causes crop losses in cucumber, sweet pepper and flowers (Yue, 2013). It is such a problem due to its wide host range, their small size making them hard to detect, their cryptic, thigmatoic behavior and high reproduction rate (Morse and Hoddle, 2006). Synthetic chemicals have been used as control strategies. Nevertheless, WFT. have become resistant to most of the

frequently used pesticides Furthermore, the control of this thrips with synthetic chemical pesticides still available in the market are unsatisfactory (Naranjo, 2013). In Kenya the market export of French bean products is below the yield potential that can be achieved under good agricultural management practices. The current situation of low quality is due to both biotic and abiotic constraints with thrips infestation being a major biotic production constraint. They cause silvery patches on fingers and this condition makes the beans to be of low quality hence rejected or fetch low prices at the market level therefore there is need to determine other ways of managing WFT thus use of soil dwelling mites (*Hypoaspis sclerotarsa)* and plant predatory mites *(Amblyseius Montdorensis)* as a biological agent which will offer a sustainable, effective and environmentally friendly solution on control of WFT at first and second larval stage and at soil dwelling development stages of *Frankliniella occidentalis*.

1.3 Justification

Horticultural crops are important to small-scale farmers since they generate income contributing to the growth of our economy. Pests and diseases, poor agronomical practices have been a production constraint to both large and small scales farmers, pest resistance development and environmental pollution caused by overuse of pesticides are also matters of concern. According to KEPHIS (2006), farmers have increasingly used chemicals for increased crop production at the expense of food safety and environment. The synthetic chemical pesticides have been linked with negative effects on non – target organism and the environment. Over the past era there has been an increase in the development of pesticides to target a broad spectrum of pests. The increased quantity and frequency of pesticide applications have posed a major challenge to the targeted pests causing them to either disperse to new environment and/or adapt to the novel conditions (Meyers & Bull, 2002; Cothran *et al.,* 2013). The adaptation of the pest to the new environment could be attributed to the several mechanisms such as gene mutation, change in population growth rates, and increase in number of generations etc. This has ultimately resulted in increased incidence of pest resurgence and appearance of pest species that are resistant to pesticides. Resistance is the most serious bottleneck in the successful use of pesticides nowadays. The intensive use of pesticides has led to the development of

resistance in many targeted pest species around the globe (Tabashnik *et al.,* 2009), that eventually contributes to low yields to bean growers among other crops , However, there is need to search for selective biological methods to solve problems of pests on farms and long-term toxicity to non-targeted organism and environment that when used properly it would control the pest and diseases that will contribute to increase in yields to beans growers among other crops. Various biological Control Agents have been alternatively used as an alternative to conventional pesticides for controlling pests and diseases hence reducing, pesticides resistance, negative impacts to human health and Environment. Some examples of biological control agents or beneficial microorganisms that can feed or parasitize a large number of pest species, some are effective against only one or two species of pests. For example, Predacious bugs**.** Several species of *Orius* bugs have been tested for control of WFT on sweet pepper and cucumber, including *O. Tristcolor* (Gilkeson et al., 1990, Steiner & Tellier 1990), *O. insidiosus* (van den Meiracker & Ramakers 1991), and *O. laevigatus* (Chambers *et al.,* 1993) and on strawberry (Frescata & Mexia 1996), Several plant Predatory mite species of *Amblyseius*, example. The predatory mite *Amblyseius (Neoseiulus) cucumeris* that was introduced in 1980, that was the first commercially used biological control agent against *F. occidentalis* in sweet pepper (Messelink *et al.,* 2005) and therein). Other mite species *Amblyseius (Typhlodromalus) limonicus* (Gillespie, 2010; Houten *et al.,* 1995), *Amblyseius (Typhlodromips) swirskii* and *Euseius ovalis* were reported to be more effective in thrips control in cucumber (Messelink *et al.,* 2005) and *A. andersoni* in pepper (Van Houten *et al.,* 2005). *Amblyseius degenerans* has been shown to suppress infestations of *F. occidentalis* on cucumber and pepper (Messelink *et al.,* 2006). The soil-dwelling predatory mites *M. robustulus* (Messelink & van Holstein-Saj, 2008a), *Hypoaspis (Gaeolaelaps) aculeifer, Hypoaspis miles (Stratiolaelaps scimitus*) and the rove beetle *Atheta coriaria* are used as commercially available biological control agents against thrips in Europe.

1.4 Objectives

1.4.1 General Objective

To evaluate the efficacy of plant and soil dwelling mite to control Western Flower Thrips (*F. occidentalis)* in French beans

1.4.2 Specific Objectives

1. To evaluate the rate of consumption of Western flower thrips *(F. occidentalis*) pupae by the soil dwelling mite (*Hypoaspis sclerotarsa*)
2. To determine the effectiveness of combining plant (*Amblyseius Montdorensis*) and soil dwelling mite (*Hypoaspis sclerotarsa*) for control of Western flower thrips (*F. occidentalis*)
3. To assess the appropriate time of application for *H. sclerotarsa* for control of Western flower thrips (*F. occidentalis*)

1.4.3. Research Hypotheses

1. *Hypoaspis sclerotarsa* (Soil dwelling mite) does not consume the pupae of Western flower thrip (*F. occidentalis*) at sufficient rate in French beans.
2. Combining Plant (*Amblyseius Montdorensis*) and soil dwelling mite (*Hypoaspis sclerotarsa*) does not effectively control the western flower thrips (*F. occidentalis)* in French beans.
3. Timely application of *Hypoaspis sclerotarsa* does not have influence in control of western flower thrips (*F. occidentalis*)

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of *F. occidentalis*

Frankliniella occidentalis is known to be a native of Pacific states of North America (Kasina, 2003) and has spread to colonize almost the entire world. The full length of a female *F. occidentalis* is about 1.5 mm and that of a male about 1.0 mm (Steiner, 2004). In addition, they have many color forms and similarities with other pestiferous thrips (Morse & Hoddle, 2006; Steiner, 2004). The female colour ranges from light yellow to yellow with brown blotches. The colour of the blotches ranges from brown to dark brown (Faun Sarmiento, 2014). In contrast to the what was reported by Cheung, (2011) that females, males are light yellow (Plate 1.1). Larvae are white or yellow in colour and are wingless (Steiner, 2004). The WFTs also have reddish-orange occelar pigments (Steiner, 2004).

Plate 2.1: Adult of Western flower thrips *(Frankliniella occidentalis)*

Source; http:/nfrec.ifas.ufas.ufl.edu/programs/tomato_spotted_wilt_management.s

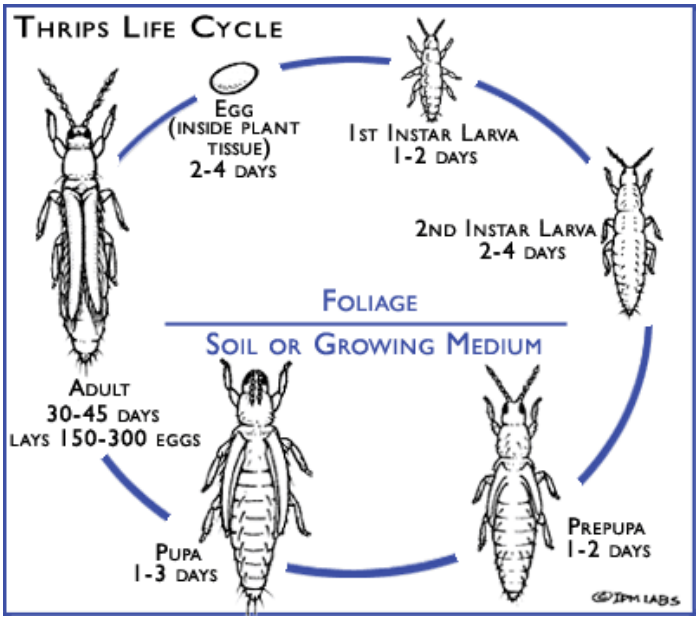

Plate 2.2: Western flower thrips *(Frankliniella occidentalis)* life cycle

Source; http://www.discoverlife.org/ Copyright Mark S. Hoddle All rights reserved.

The life cycle of *F. occidentalis* consist of four developmental stage (Plate *2*.2): egg, two larval instars and two pupal instars namely prepupa and pupa and adult (Schneweis, 2017). Depending on the thrips species and their food quality, about 30 to 300 eggs are laid by the females (Cao *et al.*, 2018). Moreover, females oviposit throughout their life after the first oviposition (Reitz, 2009). The eggs are also large relative to the female body and smoothly shelled. The two larval instars are active feeders and the following two stages pre-pupa and pupa do not feed (Facun Sarmiento, 2014) The first larval instar is not as active in feeding as the second larval instar (Reitz, 2009).The metamorphosis from the first to the second instar takes 1-3 days and is temperature dependent (Jeger *et al.*, 2015) The switch to pupation can take place in the soil or within flower structures and this takes about 2-9 days again depending on the temperature (Nothnagl *et al.*, 2007). The life cycle from egg to adult of *F. occidentalis* can be as short as 9-13 days at favourable temperatures of 25-30°C (Reitz, 2009).

F. occidentalis also reproduces parthenogenetically by arrhenotoky whereby unfertilized eggs develop into haploid males and fertilized eggs turn into diploid

females (Moritz *et al.*, 2004). After emergence adult females are somewhat inactive but after 24 hours, they become extremely active. Under laboratory conditions, females live about 40 days but can survive as long as 90 days (Forbes, 2019). Males on the other hand live half as long as the females. *F. occidentalis* has a fast reproductive rate not only because unfertilized eggs can develop into males but also because oviposition begins right after emergence and continues intermittently throughout almost all adult life. Furthermore, since females live longer than the respective males, they may switch from parthenogenesis to sexual reproduction by mating with their sons (Nothnagl *et al.*, 2007) Both sexes also lack diapause making them present all year round in the crops (Morse & Hoddle, 2006; Steiner, 2004).

2.1.1 Problems concerning *Frankliniella occidentalis*

For the last 36 years. *occidentalis* has been one of the most invasive insects and thus has developed into a worldwide pest (Reitz *et al.,* 2011; Kirk & Terry, 2003). There are many factors of its biology to why it is so troublesome. First, they are very small insects of a few millimeters size and, therefore, hard to notice on site. Secondly, they are thigmotactic scurrying away from movements and they show a cryptic life style living in complex and obscure plant parts such as inside floral buds, folded tissues, within leaf bases and along leaf veins (McKellar *et al.,* 2005; Morse & Hoddle, 2006). Their eggs are deposited in sub-epidermal tissue of floral buds and leaves. This affinity for enclosed spaces makes it hard to control *F. occidentalis* with chemicals. Third,

F. occidentalis is polyphagous meaning they feed on various kinds of plants. Within the cultivated plants thrips is a main pest in greenhouse agriculture and horticulture. Mainly vegetables such as cucurbits and peppers, (Bosco, *et al.,* 2008) are host plants. For the ornamentals they are mainly roses and carnations in America (EPPO, 1989) and chrysanthemum in Europe (Van der Linden *et al.,* 2013). Lastly, *F. occidentalis* is difficult to identify, due to their small size and similarity with other thrips (Brunner *et al.,* 2002). In addition, their high phenotypic plasticity for morphological traits makes determination even harder (Morse & Hoddle, 2006).

2.1.2 Crop damage by Western Flower Thrips

WFT was first recorded in: Europe and Netherlands in 1983 (Kirk, 2002), South Africa in 1987 (Allsopp, 2016) and Kenya in 1989 (Hondelmann *et al.,* 2017). Since its arrival, the WFT has demonstrated feeding activity against some of Kenya's most important horticultural crops (Gholam, & Sadeghi, 2016). The species feeds primarily on flowers and leaves through means of piercing mouthparts, which are highly damaging to crop plants (Cloyd, 2009; Kirk, 2002). Common damage symptoms such as streaking, scarring and deformation, result directly from feeding activity (Bethke *et al.,* 2014a; Skinner *et al.,* 2014; Cloyd, 2009; Malais & Ravensberg, 2003) Such symptoms might render flower crops unsaleable or where extensive on the leaves, reduce photosynthetic capability and thus productivity. In addition to direct symptoms, WFT acts as vector for some extremely damaging plant viruses, most notably the tomato spotted wilt virus (TSWV) and the impatiens necrotic spot virus (INSV) (Bethke *et al.,* 2014a; Skinner *et al.,* 2014; Malais & Ravensberg, 2003).

Past estimations in the USA have placed economic damage to crop plants from TSWV alone, at over $1 billion lost annually (Reitz, 2009). In Finland, the immediate future financial implications had the horticultural industry chosen not to actively confront their WFT problem were calculated as being somewhere in the region of $7 million loss (Lewis, 1997b). Lewis (1997b) states that in the case of thrips damage, higher economic losses will generally be associated with crops in which visual appearance is of vital importance.

2.2 Management of Western Flower Thrips

2.2.1 Scouting

Scouting is important to determine the number of WFT present in the greenhouse, and detect seasonal trends in the population throughout the year and assess the effectiveness of management strategies implemented (Romenh, 2018). Blue and yellow sticky card are mainly used by most farmers to scout for WFT adults although there is still disagreement on which color, they are most attracted to (Mwangi, 2015).

Visual inspection such as looking into flowers and shaking onto a white paper are the additional methods that may be used to scout WFT nymphs and adults. However, the relationship between the number of WFT captured on the colored sticky cards and the abundance of WFT present in flower has not been established (Shipp *et al.,* 2000; Jacobson 1997; Hsu & Quarles 1995) According to a study carried out by Clody and Sado (2003) in a greenhouse to establish an action threshold of 20 WFT adult per blue sticky card per week in carnation to determine the need for insecticide application and the number of WFT adult counts resulted below the action threshold indicated to have no insecticide being applied. However, threshold may vary from 10 to 40 WFT per sticky card per week depending on crop susceptibility to the viruses vectored by WFT (Van Dijken et al.1994; Frey 1993). Moreover, there are factors that may impact action thresholds through misleading sticky cards counts including Plant attractive, presence of flowers, placement of sticky cards, age structure of WFT population, migration of WFT into greenhouse, and crop growth stage. The use of action thresholds may not be reliable in greenhouse production systems. Additionally, the use of this method is only effective on control of western flower thrips at 1st and 2nd stage of WFT development.

2.2.2 Cultural Control

Sanitation practices such as removing weeds, old plant material and growing medium debris is important to minimizing WFT problem in the greenhouse. Certain weeds particularly those in the Composite and Solanaceae families and those with yellow flowers, attract WFT adults (Pilkington, 2011) and also serves as reservoirs for the viruses transmitted by WFT adults (Kahn et al.2005; Chatzivassiliou *et al*., 2001; Yudin *et al*., 1988; 1988; Bautista & mau 1994; Duffus 1971).

Bethke *et al.* (1994) reported screening greenhouse openings such as vents and sidewalls reduce populations of WFT entering greenhouse from outside into another greenhouse this may alleviate problems with WFT possibly moving from field grown crops this technique will not be effective if doors are continuously opened.

2.2.3 Chemical Control

Chemical control is the use of chemical insecticides to kill, deter or influence pests for control purposes. Use of conventional insecticides for pest management is the most common method to suppress insect pests on French beans in Kenya (Pest Control Products Board, 2014; Wambua, 2004). Some of the commonly used insecticides to control thrips, aphids and whiteflies include synthetic pyrethrin's, carbamates, neonicotinoids and benzo urea-based insecticides (Pest Control Products Board, 2014; Misheck, 2011; Kasina, 2003). In Kenya, pesticides use for the control of thrips on French beans has been evaluated and recommendations made (Wambua, 2004 Kibata & Onyango, 1996; Muriuki, 1988). The introduction of maximum residue levels in export produce by the European Union posed and still poses a challenge in the horticulture industry (Lohr, 1996). Although use of chemical pesticides is the most common method of pest management, the chemicals are expensive for small-scale farmers, thereby increasing cost of production and reducing the farmers' income (Mishek, 2011).

2.2.4 Biological control

Biological control makes use of natural predators, parasitoids or pathogenic microbes to reduce the population density of the pest. It is a "natural control" considering that some human interventions are still required to take care of the organisms applied. Predators are organisms that feed on one or more prey species to support their development and/or reproduction. Parasitoids oviposit and develop in or on a single host leading to death of the host (Din & Donchev, 2013). Pathogens are microorganisms that cause disease in a host. Mites and bugs are among the different predators preying on thrips. Parasitoids such as wasps and pathogens such as nematodes and fungi infect thrips.

2.2.4.1 Plant Predatory Mite

The predatory mite *Amblyseius (Neoseiulus) cucumeris*, introduced in 1980, was the first commercially used biological control agent against *F. occidentalis* in sweet pepper (Messelink *et al.,* 2005). However, it was shown by Brodsgaard and Hansen

(1992) that without presence of *F. occidentalis* the mite *N. cucumeris* did not survive. In addition, it was only effective in controlling *F. occidentalis* under high densities (Messelink *et al.,* 2005). Therefore, other mite species were tested as biological control agents. *Amblyseius (Typhlodromalus) limonicus* (Gillespie, 2010; Houten *et al.,* 1995), *Amblyseius (Typhlodromips) swirskii* and *Euseius ovalis* were reported to be more effective compared to *N. cucumeris* in cucumber (Messelink *et al.,* 2005) and *A. andersoni* in pepper (Van Houten *et al.,* 2005). *Amblyseius degenerans* has been shown to suppress infestations of *F. occidentalis* on cucumber and pepper (Messelink *et al.,* 2006; Van Houten *et al.,* 2005; Houten *et al.,* 1995). For *Amblyseius (Neoseiulus) californicus* it was reported to be effective against *F. occidentalis* in pepper and in hot climates such as in Israel (Weintraub & Palevsky, 2008). *Amblyseius swirskii*, introduced in 2005, is now used worldwide for thrips as well as whitefly control in some vegetable crops as well as chrysanthemum (Messelink & Kogel, 2013; Messelink *et al.,* 2005). *A. swirskii* was more effective compared to A. *cucumeris* since the females are more aggressive towards thrips larvae. Furthermore, *A. cucumeris* is able to survive when food is scarce and able to cope with hot and dry greenhouse climates (Van Houten *et al.,* 2005; Wimmer *et al.,* 2008). Recently, in the past 3 to 4 years, *Amblyseius montdorensis* and *A. limonicus* are increasingly used for biological thrips control in some vegetable crops as well as chrysanthemum (Messelink & Kogel, 2013)

Orius species (minute pirate bugs) are also used to biologically control *F. occidentalis* in pepper (Bosco *et al.,* 2008), starting in the 1990s. *O. insidious* was first used in North America and later on more species were tested (Messelink and Kogel, 2013). A field experiment in peppers showed that *O. insidiosus* could suppress both adults and larvae of *F. occidentalis* to almost extinction (Funderburk *et al.,* 2000). In contrast, *O. laevigatus* has gained worldwide importance in biological thrips control because it is easier to rear and develops quite quickly. However, during fall and winter both *O. insidiosus* and *O. laevigatus* control *F. occidentalis* inadequately because they enter diapause due to the short-day length and thrips population would increase again (Tommasini *et al.,* 2002). *O. albidipennis* does not to enter diapause, and is thus used for biological control of WFT during fall and winter (Blaeser *et al.,* 2004).

2.2.4.2 Soil-dwelling Predators

The soil-dwelling predatory mites *M. robustulus* (Messelink & van Holstein-Saj, 2008a), *Hypoaspis (Gaeolaelaps) aculeifer*, *Hypoaspis miles (Stratiolaelaps scimitus*) and the rove beetle *Atheta coriaria* are commercially available biological control agents against thrips in Europe. These, however, only target the soil stages, i.e., the pupae of *F. occidentalis*. A great deal of *F. occidentalis* would also hide within flower buds and pupate therein (Buitenhuis & Shipp, 2008) so this is not an all-rounded control. In addition, at high relative humidity most thrips pupate within the plant and not in the soil (Steiner *et al.,* 2011).

2.2.4.3 Parasitoids

Ceranisus menes and *C. americensis* are two parasitoid wasps shown to suppress WFT in chrysanthemum (Loomans, 2006). However, developing time is slow and they failed in effectively controlling WFT (Arthurs & Heinz, 2006).

2.2.4.4 Entomopathogens

Entomopathogenic nematodes (EPN) are another type of biological control agent that only target soil-dwelling stages of *F. occidentals* commonly used nematode *Steinernema feltiae* is marketed in the UK and North America against *F. occidentalis* and leaf miners that insect ornamental and bedding plants (Arthurs & Heinz, 2006). Other EPN strains also reported such as *Heterorhabditis bacteriophora* and *Steinerma carpocapsae* which are primarily used for combating, the two strain are reported to be effective compared to *S. feltiae* due to its ability to infect both on mobile and non-mobile larvae of *F. occidentalis* (Ebssa *et al.,* 2001). It is also reported that early and regularly repeated application of these two species can control *F. occidentalis* (Belay *et al.,* 2005). *Thripinema nicklewoodi* is specialized in infecting parasite thrips during their feeding stages within the flower buds and foliar terminals. It is not commercialized because it is difficult to culture in mass production and only showed effectiveness in small scale (Arthurs & Heinz, 2006).

The uses of EPN show potential in controlling WFT but only when applied at high concentrations and at favorable conditions. In addition, studies like the one from Arthur and Heinz (2006a) show variation in effectiveness and only for a few species. Entomopathogenic fungi (EPF) are also applied to control *F. occidentalis*. They can infect all life stages of *F. occidentals* but their effects on the different stages differ (Ugine *et al.,* 2005). It seemed that adult thrips were more susceptible than immature ones (Messelink & Kogel, 2013). One possible explanation may be that as larvae pupate, they shed their skin and lose the infection. The Entomopathogenic fungi (EPFs) that control Western Flower Thrips (WFT) in vegetables in Netherlands. One of them is *Metarhizium anisopliae* (Metschnikoff) which not only regulates *F. occidentalis* but also another pest *Otiorhynchus sulcatus* (black vine weevil) (Ansari *et al.,* 2008). The fungus, *Beauveria bassiana* has been proved to be effective in the control *F. occidentalis* in agricultural crops (Mukawa *et al.,* 2011). *B. Bassiana* is a broad range pathogen of insects and it was reported not to show negative effect with *A. cucumeris* and has the potential to be combined when *A. cucumeris* cannot control WFT on its own (Jacobson *et al.,* 2001). A study on evaluating Entomopathogenic Fungi (EPF) showed that *Isaria fumosorosea* was able to cause higher mortality in WFT than the insecticide fipronil (Ansari *et al.,* 2008). *Lecanicillium lecanii* was found to be affective in all stadiums of *F. occidentalis* and the highest was observed for the adults (Vestergaard *et al.,* 1995). These last two were only studied in a laboratory setting and are commercially available to control other thrips (Ansari *et al.,* 2008).Similar to Entomopathogenic Nematodes **(**EPNs) Entomopathogenic Fungi (EPFs) require a certain degree of humidity and the right time of application to get enough infection (Shipp *et al*., 2003a) although Entomopathogenic Nematodes (EPNS) can still be effective under low humidity (Mukawa *et al.*, 2011).

2.2.4.5 Use of Combined Biological Control Agents

A combination of foliar and soil-dwelling predators would be an ideal biological control strategy. This is because soil-dwelling stages of the pest depend on relative humidity (Steiner *et al*., 2011) when this is high, they drop to the soil. In addition, host plant species also affect the dropping behaviour because in a close-leaf crop like lettuce they would not drop off to the soil (Steiner *et al.,* 2011). To increase efficacy

of biological control different predators may be combined to achieve synergistic effects. As such as *O. insidiosus* and *A. degenerans* were combined on cut roses but the control levels were similar to those of using only *O. insidiosus* (Chow *et al.,* 2008). Although it was found that *O. insidious* not only preyed on *F. occidentalis* but also *A. degenerans* making *O. insidious* an intraguild predator. Another is the study on a combination of a predatory bug and mite in Dutch ornamental sector showing that both were successful in controlling thrips (Beerling and van der Linden, Retrieved March 2014). Research on interactions between two predatory mites' *A. swirskii* and *E. ovalis* against *F. occidentalis* and the greenhouse whitefly *Trialeurodes vaporariorum* showed that *A. swirskii* reduced the number of thrips stronger than *E. Ovalis.* However, the control of the white fly was better by both predators when thrips were present than when they weren't. A mixed diet of *A. swirskii* indicated a positive effect on its predation behaviour (Messelink *et al.,* 2008b).

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study Description

A study was carried out at Kichozi Farm of RealIPM Company LTD in Thika Kenya from September 2016- April 2017 under Laboratory Conditions.

3.2 Rearing of *F. occidentalis*

The rearing procedure was based on a protocol of Bailey and Smith (1956) which was slightly modified. *F. occidentalis* colonies were collected from International Center of Insect Physiology and Ecology (ICIPE) and they were reared on ventilated plastic jar containers (20cm length × 5cm width). For ventilation a hole was cut in the lid (diameter 5 cm) and covered with Nylon mesh tissue (mesh width 63 μm, Sefar, Switzerland). One hundred sampled WFT specimens both female and male were put on the plastic jars containing 5 pieces of fresh French bean (*Phaseoulus vulgaris*) pods (7-10cm) for 48hrs. The pods were coated with sugar solution to provide energy for the thrips to lay eggs. On the bottom of the jars, two layers of folded filters were spread as hiding place. Bean pods (*Phaseolus vulgaris*, var. 'Samantha') served as food and oviposition site. The bottles were kept under controlled conditions (photoperiod 16:8 L: D, 24±1°C, 60±10% RH). For two days, the adult thrips were allowed to feed on the beans pods and to lay eggs in the tissue. After two days, the bean pods were taken out and the adults were transferred into new glass jars. Adult thrips were provided with fresh bean pods. Two days' later larvae started to emerge, another one to two days later the larvae developed to the second stage larvae. At an age of about 9 days, the larvae started to crawl around in the jars; this is the stage the larvae search for a hiding place for pupation. Thrips mount to adults from the 13th day onwards after leaving the eggs. Between the 9th and the 13th day they pass the pre-pupa and the pupa stage. The WFT pupa were then brushed and used for the experiment.

3.3 Rearing of *Hypoaspis sclerotarsa*

Hypoaspis sclerotarsa was collected from a rice-processing factory in the Thika region of Central Kenya (altitude 1,500m) in April -May 2014 (Mwangi and Wainwright, 2015). Farid Faraji, Mitox Consultants/Eurofins, and Amsterdam, based on mounted specimens (eight females and five males), identified the species morphologically. Colonies of *H. sclerotarsa* were reared on the prey mite *Thyreophagus entomophagus* (Laboulbéne & Robin), at 20±1℃ and >70% RH in small vials (7cm diameter, 7cm high); to ensure a sufficiently high humidity but avoid condensation, a layer of moistened plaster was placed in the base of the vial, and the lid was pierced with pin holes. A cover of mite-proof gauze beneath the lid prevented escape. Adults of *H. sclerotarsa* were collected and counted under a binocular microscope to ensure they were at the correct stage of development prior to use in experiments (plant 3.1).

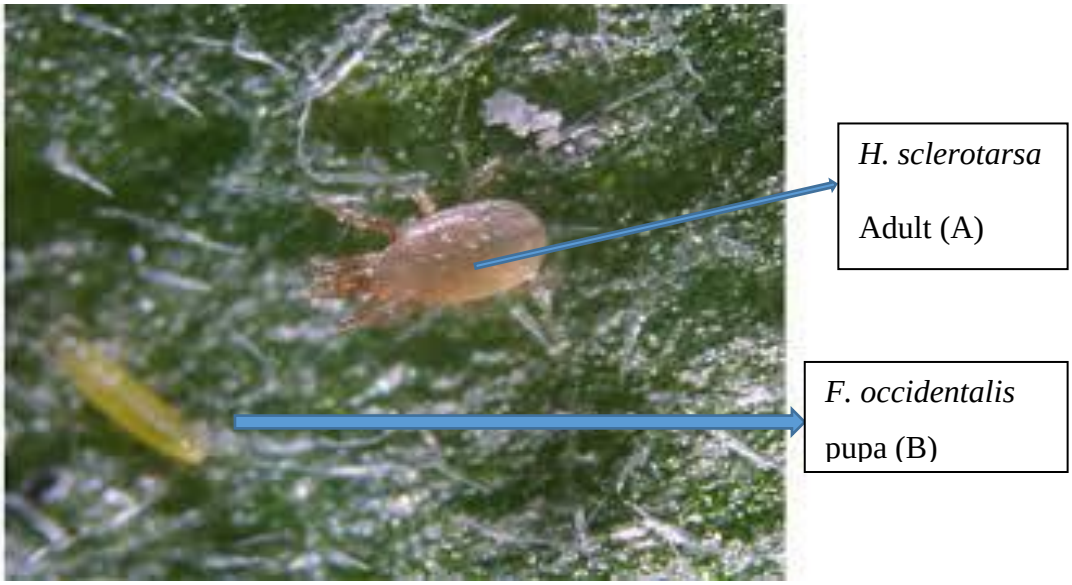

Plate 3.1: Showing an Adult of *Hypoaspis sclerotarsa* (A) and *F. occidentalis* pupa (B)

CHAPTER FOUR

RESEARCH FINDINGS AND DISCUSSION

The Rate of Consumption of Western Flower Thrips *(Frankliniella occidentalis)* pupae by the Soil Dwelling mite (*Hypoaspis sclerotarsa (Acari: Laelapidae*)

Abstract

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), are one of the most serious pests in horticultural production worldwide. One third of its lifecycle being in the soil, the potential for biological control of WFT in the soil is poorly understood and requires further elucidation. A number of studies report that polyphagous predatory mites' prey on pupal stages of WFT in the soil, but little research has been done on consumption rates of predatory mites. Therefore, a laboratory study was designed to examine the rate of consumption of WFT pupae in potting media, by the soil dwelling mite *Hypoaspis sclerotarsa* (Costa). The rate of consumption was determined by dividing the total number of pupae consumed and unit time. Five densities of predator were evaluated (0, 2, 4, 6, 8) against four WFT prey densities (5, 10, 15, and 20 pupae). Pupal consumption was assessed at 2 hourly intervals over a six-hour period. The results shows that the rate of consumption of WFT pupae by *H. Sclerotarsa* varied significantly ($P \leq 0.05$) with time after predatory mite introduction. At all predator densities, a higher rate of consumption of WFT pupae was observed at 2 hours compared with after 2 – 4 and after 4 - 6 hours. Consumption of WFT pupae was lowest at 4 – 6 hours after introduction. (Figure 1A, B, C, D). There was a significant different at an initial density of five WFT pupae ($P \leq 0.05$), the total number of pupae consumed after 6 hours was larger when either six or eight *H. sclerotarsa* were introduced compared with the introduction of two or four *H. sclerotarsa* with value (1.7±1.91,1.5±1.81) and (1.0±1.51, 1.5±1.71) respectively. The rate of consumption also increased with the density of WFT pupae but was not consistent because as the numbers of WFT pupae increased so the ratio of WFT pupae remaining to those that were consumed also increased. Therefore, the study showed that one individual could consume an average of 0-4 pupae in 24 hours.

4.1 Introduction

The Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a pest of global economic importance on a wide range of crops (Kirk & Terry, 2003). It is thought that WFT originated in south-western North America (Kirk, 1997) but has now spread and established itself globally (Reitz 2009). Predominantly, this international spread of the WFT was facilitated by the movement of horticultural plant material, such as cuttings, seedlings and potted plants (Kirk & Terry, 2003). Adult and larval stages of WFT cause mechanical damage of plants during oviposition and feeding, making leaves and flower petals appear silvery or flecked. Leaves and flowers become deformed due to uneven tissue growth around the sections killed by WFT activity. Furthermore, WFT also transmits plant viruses (Kirk, 1997). The development time of WFT (from egg, larval stage 1, larval stage 2, prepupa, pupa to adult), depends on temperature, food resources and crop type (Malais & Ravensberg, 2003). WFT is difficult to control due to its high reproductive rate and cryptic mode of life (Lewis, 1997); one third of the WFT life cycle is in the soil (Loomans and Lenteren 1995) and therefore difficult to target by conventional control strategies targeted at the crop canopy.

Several groups of synthetic chemical pesticides have been used to control WFT. However, due to the short generation time, high fecundity and haplodiploid reproductive system of WFT, resistance to these chemicals has developed quickly in many populations (Jensen, 2000; Zhao et al., 1995). Alternative control strategies have been sought and in a recent review, Mouden, et al, (2017) suggested that Integrated Pest Management (IPM) that focused on biological control and host plant resistance had the greatest potential for successfully regulating WFT populations.

A number of predatory mite species (Acari) have also been used for control of WFT larvae and pupae (Gerson et al., 2003). Predatory mite species that have been used for control of the larval stages of WFT within the crop canopy include *Neoseiulus cucumeris* (Oudemans), *Typhlodromalus limonicus* (Garman and McGregor), *Typhlodromips swirskii* (Athias-Henriot), *Euseius finlandicus* (Oudemans), *Euseius ovalis* (Evans), *Iphiseius degenerans* (Berlese) and *Neoseiulus barkeri* (Hughes)

(Messelink et al., 2006).There are three polyphagous predatory mites that are active in the soil and known to prey on the soil-dwelling stages of thrips: *Stratiolaelaps scimitus* (Womersley) (previously placed in the genus *Hypoaspis*), *Hypoaspis* (*Gaeolaelaps*) *aculeifer* (Canestrini) and *Macrocheles robustulus*(Messelink & Holstein-Saj, 2008; Berndt et al., 2004).However, compared with canopy-active predators, there has been little research on soil-active predators or their effectiveness

With increasing concerns about the import and release of exotic natural enemies against invasive pests (classical biological control), which is associated with increased evaluation and registration demands, a trend has developed to look first for indigenous natural enemies that also have the potential to regulate invasive pest species populations. In the last decade, and for the first time, there have been more indigenous natural enemy species commercialized (18) than exotic natural enemy species (six) (Lenteren, 2012). As part of the search for indigenous predators for use against invasive pests in Kenya, *Typhlodromalus spinosus* (Meyer and Rodrigues) was evaluated and showed some potential for control of larval WFT on French beans (Mwangi et al., 2015). Despite this, the natural enemy species being used commercially in Kenya remain primarily of a non-indigenous origin (Pest Control Products Board, 2018) hence challenges in effective pest control. As part of a bioprospecting programme, individuals of the predatory mite were collected, *Hypoaspis sclerotarsa* (Costa), from the dry storage area of a rice processing plant in Thika, Region Central Kenya (Mwangi & Wainwright, 2015). While this species had been previously reported in Europe (Latvia) and the Middle East (Israel and Iran), its region of origin is currently unknown; our collection was the first report of this species in Kenya. Previously, this species had only been found in primary sand dunes characterized by fine to medium grained sandy soils with minimal organic content (Salmane, 2001). Interest in evaluating *H. sclerotarsa* as a potential biocontrol agent in Kenya arose because of our report of its presence in Kenya, its high reproductive rate and its ease of mass production (Wainwright, 2015); this species is more tolerant to dry conditions than the commercially available predatory mite species such as *S. scimitus* and *H. aculeifer* (Mwangi & Wainwright, 2015).This study therefore aimed at evaluating the rate of Consumption of Western Flower Thrips *(Frankliniella*

occidentalis) pupae by the Soil Dwelling mite (*Hypoaspis sclerotarsa* (Acari: Laelapidae).

4.2 Experiment Setup

To determine the rate of consumption of WFT pupae by *H. sclerotarsa* assays were set up in ventilated plastic Petri dishes (100mm diameter x 15mm depth) each containing 2g of sterilized sieved soil (through a 2 x 2.5mm mesh). A small hole (7mm diameter) was cut in the center of the Petri dish lid and covered with thrips-proof cloth (64μm pore size) to retain thrips but allow ventilation. A Factorial design was used with five treatment densities of the predator (0 [T1], 2 [T2], 4 [T3], 6 [T4], 8 [T5]) and four WFT pupal densities (5, 10, 15, 20) in all predator: prey density combinations. Each predator: prey density combination was replicated three times to give a total of 60 experimental Petri dish units. The experiment was repeated three times for consistency

WFT pupae were collected from stock culture and individually examined under the microscope to ensure they were at the 2nd stage of reproduction development. A camel hair brush was used to gently transfer pupae to the top of the soil in each Petri dish to achieve the required densities. Individual adults of *H. sclerotarsa* were separated from the rearing substrate using a Pasteur Pipette and introduced into the Petri dishes to achieve the required predator: prey densities. Each Petri dish was sealed with parafilm to prevent escape. The Petri dish experimental units were incubated at 23℃ for 6 hours during which time the number of livings WFT pupae remaining was assessed every two hours (i.e., after 2, 4, 6 hours).

4.3 Statistical analysis

Cumulative WFT pupal mortality was calculated by subtracting the number of WFT pupae surviving after 6 hours from the initial number of preys introduced. For each WFT density, data on rates of consumption by different predator densities over time were normalized using the log transformation and subjected to repeated measures Analysis (RM-ANOVA) using the statistical program R (R Core Team, 2013). Differences in rates of consumption of WFT at different predator densities and at

each time point were compared using the Tukey's mean separation test, using $p<0.05$ as the cut off for statistical significance.

4.4 Results

4.3.1 Rate of consumption of Western Flower Thrips *(Frankliniella occidentalis)* pupae by Soil Dwelling mite *(Hypoaspis sclerotarsa (Acari: Laelapidae)*

The rate of consumption of WFT pupae by *H. sclerotarsa* varied significantly ($P\leq 0.05$) with time after predatory mite introduction. At all predator densities, a higher rate of consumption of WFT pupae was observed at 2 hours compared with after 2 - 4 and after 4 - 6 hours. Consumption of WFT pupae was lowest at 4 – 6 hours after introduction.(Figure 4.1: A, B, C, D).There was a significant different between the mean number of WFT pupae consumed at 2hours interval when different densities of 2Hs; 4Hs and 4Hs; 8 Hs were introduced, an initial density of five WFT pupae, the total number of pupae consumed after 6 hours was larger when either six or eight *H. sclerotarsa* were introduced compared with the introduction of two or four *H. sclerotarsa* (Figure 4.1A, $P\leq 0.05$). A similar pattern was observed at an initial density of ten (Figure 4.1 B), 15 (Figure 4.1C) and 20 WFT pupae (Figure 4.1D). The higher the density of *H. sclerotarsa* introduced, the greater the number of pupae consumed. The total number of preys consumed by *H. sclerotarsa*, at a prey density of 20 WFT pupae, was significantly larger than at the lower prey densities ($P\leq 0.05$).

When data for all predator densities were combined, we found that at prey density of five/ replicate, that the total mean number of WFT pupae consumed was 16.7 out of a mean total of 20 available pupae (Figure 2). This increased to 25.9 out of a mean total of 80 available pupae when the initial prey density was 20/ replicate (Figure 2). The mean number of pupae consumed per predator was calculated and ranged from 0.83 at the lowest initial density of prey available (five), to 1.29 at the highest initial density of prey available (20) (Figure 4. 2). The ratio of available pupal number to the number actually consumed was inconsistent, increasing from 1.2 when the initial prey density was five, to 3.09 when the initial prey density was 20 (Figure 4.2).

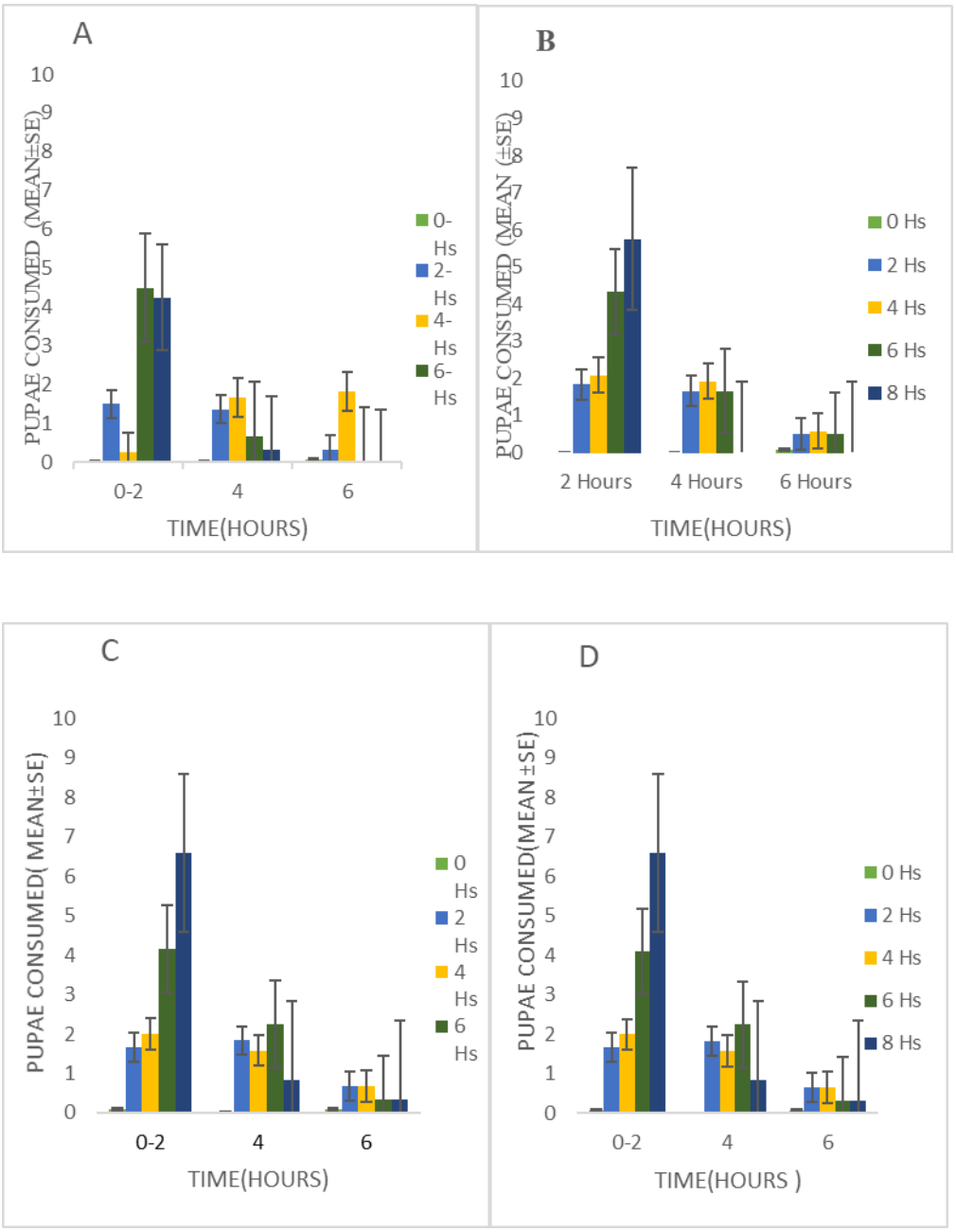

Figure 4.1: Mean number of Western Flower Thrips *(Frankliniella occidentalis)* pupae consumed by different densities of the predatory mite *(Hypoaspis sclerotarsa).* A) Initial density of WFT Pupae Introduced =5; B) Initial density of WFT pupae Introduced = 10; C) Initial density of WFT Introduced =15; D) initial density of WFT Introduced =20. Error bars represent standard error

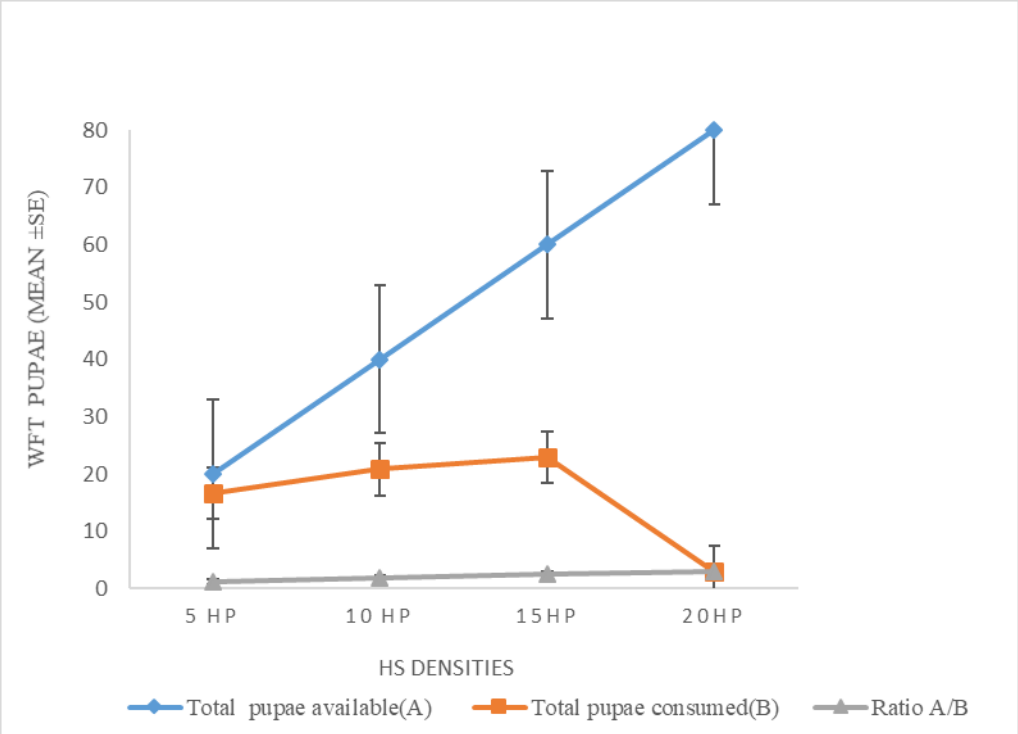

Figure 4.2: Calculated mean number of western flower thrips (WFT) pupae consumed by one individual Hypoaspis sclerotarsa (Hs) as determined 6 hours after predator introduction. Error bars represent standard error.

4.5 Discussion

Most of the published research on biological control of WFT using predators has focused on the adults and larval stages. The predatory mite *Amblyseius (Neoseiulus) cucumeris*, introduce in 1980, was the first commercially used biological control agent against *F. occidentalis* in sweet pepper (Messelink *et al.*, 2005). However, it was shown by Brodsgaard and Hansen (1992) that without presence of *F. occidentalis* the mite *N. cucumeris* did not survive. In addition, it was only effective in controlling *F. occidentalis* under high densities (Messelink *et al.*, 2005). Therefore, other mite species were tested as biological control agents. *Amblyseius (Typhlodromalus) limonicus* (Gillespie, 2010; Houten *et al.*, 1995), *Amblyseius (Typhlodromips) swirskii* and *Euseius ovalis* were reported to be more effective compared to *N. cucumeris* in cucumber (Messelink *et al.*, 2005) and *A. andersoni* in

pepper (Van Houten *et al.,* 2005). *Amblyseius degenerans* has been shown to suppress infestations of *F. occidentalis* on cucumber and pepper (Messelink *et al.,* 2006; Van Houten *et al.,* 2005; Houten *et al.,* 1995). The soil-dwelling predatory mites *M. robustulus* (Messelink & van Holstein-Saj, 2008a), *Hypoaspis (Gaeolaelaps) aculeifer*, *Hypoaspis miles (Stratiolaelaps scimitus*) and the rove beetle *Atheta coriaria* are commercially available biological control agents against thrips in Europe. Despite of reported study of soil dwelling in Europe little is known about the third phase of the life cycle where thrips pupate in the soil (Mouden et al., 2017). This research quantifies the number of thrips pupae that single *H. sclerotarsa* consume. Most research on different soil-dwelling predatory mites, such as *Stratiolaelaps scimitus* (formerly *Hypoaspis miles*), have focused on the net effect of biocontrol and assessed the impact of thrips numbers in a crop after application of biocontrol. However, Wu *et al.* (2014) reported that when offered 25 preys, one female *Stratiolaelaps scimitus* mite could consume four to six pupae within 24 hours. Our results show a similar magnitude of WFT pupae being consumed in six hours, though most of these are consumed within the first two hours. Wu *et al.* (2014) also reports that predation rates of both female and male mites increased with prey density. This could not be observed in this experiment, results from Wu *et al.* (2014) appears to be more pronounced than with our predatory mite. Despite the fact that efficacy testing was done under controlled laboratory condition, the results obtained in this study clearly showed that *H. sclerotarsa* is a potential biocontrol agent that could be commercially used for management of thrips in Horticultural crops (Figure 4.1A; B; C; D). These results indicated that an increase of prey density did not cause significant difference as one individual *H. sclerotarsa* could consume 0-1 pupae within 6 hours, therefore we conclude that one individual could consume an average of 0-4 pupae in 24 hours (Figure 4. 2).

To determine the effectiveness of Combining Plant (*Amblyseius Montdorensis* and soil-active predatory mites (*Hypoaspis sclerotarsa*) for control of Western Flower Thrips *(Frankliniella occidentalis)* in beans.

Abstract

Three separate greenhouse experiments were evaluated the effect of: a) different densities of the mites *Amblyseius Montdorensis* (foliar predator; AM at 0, 5, 10 or 15 per pot); b) different densities of *Hypoaspis sclerotarsa* (ground predator; HS at 0. 50, 100 or 150 per pot); c) a combination of the two (0AM, 0HS; 15AM, 50HS; 15AM, 100HS; 15AM, 150HS) on emergence of western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) from soil. Initial start populations of WFT were either small (10) or large (20). A completely randomized design was used and for each experiment. There were three replicates per treatment and the experiment was repeated on two occasions. Single applications of *A. Montdorensis*, *H. sclerotarsa* or a combination of both all had a significant effect on the number of WFT emerging compared with the control. There was a significant effect of *A. Montdorensis* density on the number of WFT emerging from the soil (F=0.31, P= 0.420 df =1). There was no significant difference in the population densities of WFT emerging from soil in the control and following release of *H. sclerotarsa* when initial release densities of WFT at the two initial prey densities of 10 and 20. Combined use of *A. Montdorensis* and *H. sclerotarsa* at a density of 150 with 15 *A. Montdorensis* reduced adult WFT emergence at density of 20 WFT. These findings highlight the potential for a combined use of *A. Montdorensis* with *H. sclerotarsa* for the control of soil-dwelling stages of thrips.

4.6 Introduction

Western Flower Thrips (*F.occidentalis*, Pergande) (Thysanoptera: Thripidae), are pests of economic importance on a wide range of crops throughout the world (Kirk & Terry, 2003). WFT are generally difficult to control as a result of their cryptic mode of life (Lewis, 1997; Michelakis & Amri, 1997), the development of insecticide resistance (van Lenteren & Loomans, 1998) and because pupation occurs in the soil

and not on the crop (Berndt *et al.,* 2004; Lewis, 1997). A wide range of soil-dwelling predatory mites have potential to prey on the pupae of WFT in the soil (Karg, 1993). However, *Amblyseius* species (Acarina: *Phytoseiidae)*and *Orius* species (Heteroptera: Anthocoridae) of predatory mites and bugs are most commonly used for biological control of WFT; Both these groups of predators prey on the foliar-feeding life stages of WFT, i.e. the 1st (L1) and early 2nd (L2) larval instars and the adults, but not late L2 larvae that leave the canopy to pupate in the soil, or the prepupae and pupae which form in the soil (Sabelis & van Rijn, 1997; Ramakers, 1995; Riudavets, 1995).To date, augmentative releases of foliar-active predatory mites and bugs have had variable success and not always provided sufficient control of WFT, particularly on crops with low economic damage thresholds, such as ornamentals (Frescata & Mexia, 1996; Gillespie & Ramey, 1988; Bakker & Sabelis, 1989). Thus, additional biological control agents are urgently needed for reliable management of WFT, particularly agents that target the predominantly soil-dwelling life stages.

While the majority of late L2 WFT leave the canopy to pupate in the soil (Tommasini & Maini, 1995; Varatharajan & Daniel, 1984), the actual proportion of thrips successfully pupating in the soil is influenced by host plant species (Berndt, 2002). In general, thrips spend about one-third of their life cycle (mainly as prepupae and pupae) in the soil (Loomans and van Lenteren, 1995). One option for WFT control is the use of soil-inhabiting oligophagous predatory mites of the genus *Hypoaspis* (Acarina: Laelapidae). Recent studies have shown that *Hypoaspis aculeifer* Canestrini and *Hypoaspis miles* (Berlese) are promising predators against soil-dwelling stages of WFT (Berndt, 2002; Gillespie & Quiring, 1990; Glockemann, 1992). At present, these two *Hypoaspis* species are commercially available for control of mushroom flies (Diptera: Sciaridae) (Jess & Kilpatrick, 2000; Wright & Chambers, 1994). The objective of the study was to assess the effect of single applications of either the soil-active predatory mite, *Hypoaspis sclerotarsa* (Costa), the plant-active predatory mite, *Amblyseius Montdorensis (Schicha)* or a combination of the two species together on WFT population development in the soil.

4.7 Material and Methods

4.7.1 Greenhouse condition

The greenhouse experiments were conducted using French beans crops at RealIPM from January to March in 2017. The greenhouses were 65 m long, 8.5 m wide and 5 m high, with the roof and sides covered with polyethylene sheets. The top and bottom of the sides were covered with fine-mesh screening for ventilation and insect proofing. To allow air circulation and prevent excessive heat build-up, six windows with fine-mesh screening along one side of the greenhouse were opened during the day.

4.7.2. Rearing of Western Flower Thrips

The method used to rear Western Flower Thrips (*Frankliniella occidentalis)* for this study is described in Chapter 3; Section 3.2.

4.7.3. Rearing of *Hypoaspis Sclerotarsa*

Method used to rear the *Hypoaspis Sclerotarsa* for this study is described in Chapter 3; Section 3.3.

4.7.4 Plant Predatory mite *(Amblyseius Montdorensis)*

Amblyseius Montdorensis were obtained from Real IPM, a commercial supplier of natural enemies. Adults of the same age, were separated from substrate by sucking them into Pasteur Pipettes where they were held briefly prior to release in defined numbers to meet the different treatment requirements. To prevent individuals from escaping one end of the pipette was covered with mite-proof gauze and the other sealed with plasticine.

4.7.5 French Bean Crop

A Plastic planting pots (measuring 23cm in diameter) were each filled with sterilized soil into which French beans seeds (var. Samantha) obtained from Amiran Kenya were sown separately for three experiments. Two seeds were sown in each replicate

pot at a depth of 5cm and each pot was then placed into a cage and left to grow in the greenhouse. The cages had a transparent frame with fine insect gauze (diameter 30 cm, height 40 cm). Cages were placed in a greenhouse in a 12L:12D light regime at a mean temperature of 21℃.Thinning was done 1week after germination, leaving one plant per pot. The plants were left to grow to the 2-3 leaf stage.

4.7.6. Experimental Set-up

To determine the effectiveness of combining plant (*Amblyseius Montdorensis)* and soil dwelling mite (*Hypoaspis sclerotarsa*) for control of western Flower Thrips *(F. occidentalis)* three separate experiment were conducted as described in section (4.2.6.1;4.2.6.2;4.2.6.3). Factorial Design was used to lay out the experiment, each experiment was conducted separately and for the purpose of consistency each experiment was repeated three times.

4.7.6.1. Single Application of Plant Predatory Mite (*Amblyseius Montdorensis)*

Different adult female of western flower thrips and *Amblyseius Montdorensis* were released to the crop at different densities as shown below (Table 4.1).

Table 4.1: Showing different WFT and A. *Montdorensis (Mont)* (Plant Predatory) densities released

	***Amblyseius Montdorensis* densities Released**			
Introduced WFT densities				
10 Adults	0	5	10	15
20 Adults	0	5	10	15

WFT collected in a laboratory from thrips culture were introduced at initial densities (Shown Table 4.1**)** individually on French beans plants in each potted plants that were placed in a cage, a Factorial design experiment was used, with a total of four treatments i.e. (T1 -0 *A. Mont*, T2-5 *A. Mont*, T3-10 *A. Mont T*4-15 *A. Mont)* were released in each level of WFT density in each replicate cages. On 6th day after

introducing thrips into the French beans' plants on 15th day after introducing the thrips the foliage plants were chopped off. At the same time, blue sticky cards were placed to each cage for capturing thrips adults emerging from the soil. Cages were placed in a greenhouse condition with light of 12 h/day and a mean temperature of 21℃.

4.7.6.2. Single Application of *Hypoaspis sclerotarsa*

A total of thirty two French beans plants were infested with two densities of adult female of western flower thrips (10 and 20 in each replicate cage, the thrips culture were collected from the laboratory at 10th day after introducing the thrips on the foliage plants predatory mites *Hypoaspis sclerotarsa* were released at different density (0*Hs*, 50*Hs*, 100*Hs*, and 150*Hs*) at the base of plant roots and mixed with the media and three replicate cages for each treatment ,in a Factorial Design , experiment was repeated on two occasions. On 15th day all, the French beans plants in each cage were chopped off. At the same time, blue sticky plate was added to each cage for capturing thrips adults emerging from the soil. Cages were placed in a greenhouse condition with light of 12 h/day and a mean temperature of 21℃.The experiment lasted for 21 days. Control treatments without predatory mites allowed the quantification of the proportion of WFT entering the soil and estimates of the intrinsic mortality rates.

4.7.6.3 Combination Application of *Hypoaspis sclerotarsa and Amblyseius Montdorensis*

An Initial WFT density adult female of western flower thrips (10 and 20) was collected from thrips culture in the laboratory and infested in a French bean crop individually. *A. Montdorensis* (at a constant density of 15) were introduced on day 6 and the different densities of *H. sclerotarsa* were introduced on day 9 i.e. *A. Montdorensis* (*AM*) and *H. sclerotarsa* (*HS*) at four densities: 0 *AM* and 0 *HS* (control); 15 *AM* and 50 *HS*; 15 *AM* and 100 *HS*; 15 *AM* and 150 *HS*) and three replicate cages for each treatment in a Factorial Design, experiment was repeated on two occasions. At day 15, in all three experiments, all the bean foliage was removed assuming enough time was given for pupation period to take place, blue sticky traps

was placed in each cage for 7 days to capture and enumerating the WFT adults emerging from the soil. Cages were placed in a greenhouse condition with light of 12 h/day and a mean temperature of 21℃.In each experiment, there were two initial start densities of WFT (10 or 20) and three replicate cages for each treatment combination and control in each experiment and each experiment was repeated on two occasions.

The cages had a transparent frame with fine insect gauze (diameter 30 cm, height 40 cm).

4.8 Statistical Analysis

Data from each experiment were analyzed separately. Raw data on the number of WFT adults emerging from the soil in each cage were square root transformed to meet the assumption of normality and homogeneity of variance. In all experiments a repeated measures analysis of variance (PROC MIXED SAS institute 1999) using maximum likelihood estimation was done to test for differences amongst treatments on each sampling day. For pair-wise comparisons between treatments and to test the effects caused by the combined use of *A. Montdorensis* and *H. sclerotarsa* a Tukey T-test was used. Significant differences between thrips population densities in the combined *A. Montdorensis* and *H. sclerotarsa* treatment compared with the sum of the mean thrips population densities when *H. sclerotarsa* and *A. Montdorensis* were introduced separately indicated whether there was an effect. Differences amongst treatment means were compared using Tukey's test at $p<0.05$ level of significance.

4.9. Results

4.9.1 Single Application of Plant Predatory Mite (*Amblyseius Montdorensis)*

Overall, there was a significant effect of *A. Montdorensis* density on the number of WFT emerging from the soil (F=0.31, P= 0.420 df =1). In small WFT populations, significantly fewer WFT adults emerged following release of 15 *A. Montdorensis* than in the control (F = 0.42, P= 0.52 df =1). There was no significant difference in the number of WFT emerging following release of either ten or 15 *A. Montdorensis*

(P<0.05) with mean values of 3.6 and 1.1 respectively (Figure 4.3A). In large WFT populations, significantly fewer WFT emerged following release of 15 *A. Montdorensis* (3.5) compared with the control (13.1) (P<0.05). Also, significantly more WFT emerged following release of five or ten *A. Montdorensis* than following release of 15 *A. Montdorensis* (P<0.05) (Figure 4.3B).

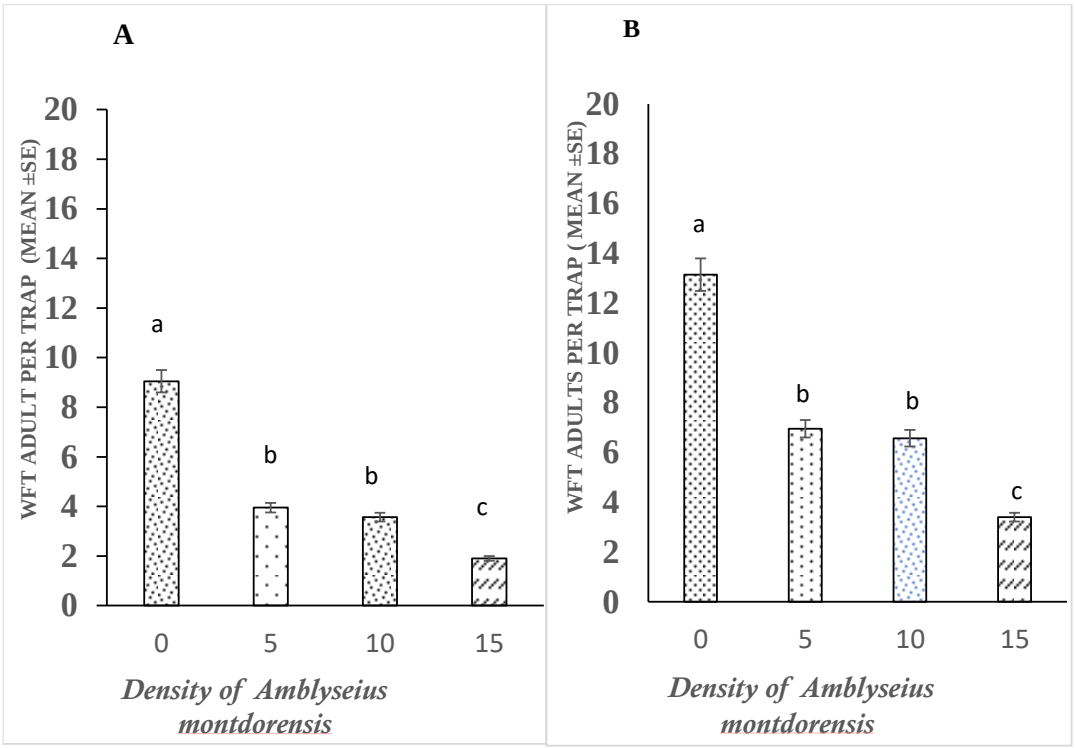

Figure 4.3: Mean (±SE) number of emerging western flower thrips Adult (*F. occidentalis*) captured in blue sticky traps placed above bean plants between the 15th and 22nd sampling day after release of the plant active predators *(Amblyseius Montdorensis)*. A) introduced 10 Adults of WFT; B) Introduced 20 Adults of WFT. Vertical bars followed by the same letter are not significantly different from each other (P<0.05).

4.9.2 Single Application of Soil Dwelling Mite (*Hypoaspis sclerotarsa)* with different densities of WFT

In small WFT populations there was no significant difference in the number of thrips

emerging from soil in the control and following release of any density of the *H. sclerotarsa* (P<0.01) (Figure. 4.4A, 4.4B). In contrast, in large WFT populations the numbers emerging were significantly reduced in the treatment with 150 *H. sclerotarsa* (1.37 emerging).

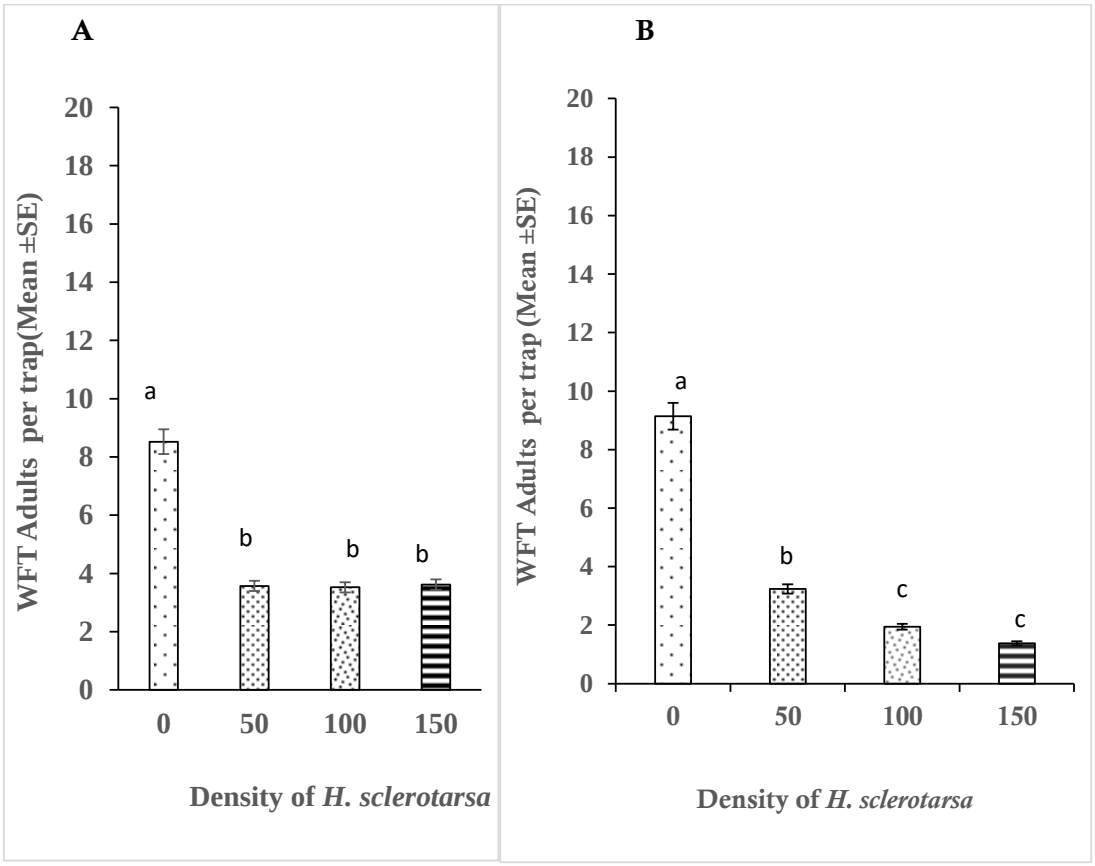

Figure 4.4: Mean (±SE) number of emerging Adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants between the 15th and 22nd sampling day after release of the Soil dwelling mite *(Hypoaspis Sclerotarsa).* A) introduced 10 Adults of WFT; B) Introduced 20 Adults of WFT. Vertical bars followed by the same letter are not significantly different from each other (P<0.05)

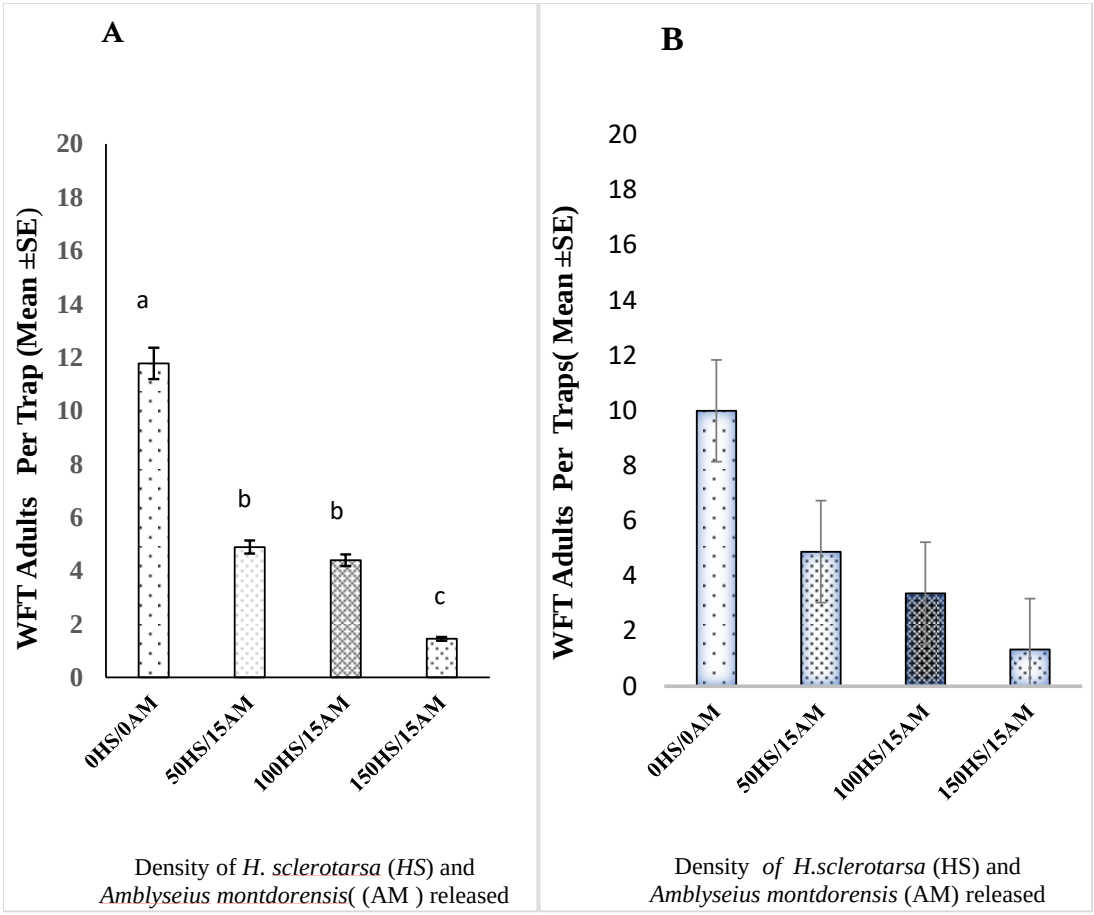

Figure 4.5: Mean (±SE) number of emerging Adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants between the 15th and 22nd sampling day after release of Foliar active predators and Soil dwelling mite *(Hypoaspis Sclerotarsa).* A) introduced 10 Adults of WFT; B) Introduced 20 Adults of WFT. Vertical bars followed by the same letter are not significantly different from each other (P<0.05).

4.10 Discussion

The results show that, in general, release of predatory mites (either *A. montdorensis* or *H. sclerotarsa*) has potential as a biological control strategy for WFT on French beans. A single application of *A. montdorensis* (at different densities) resulted in a reliable reduction in both small (initial release rate of 10) and large (initial release rate of 20) WFT populations (Figure 4.3A; B). This is likely to be because *A. montdorensis* reduced the number of WFT larvae on foliage and thus the number

entering the soil to pupate and ultimately the number emerging as adults. A similar study by Messelink and Kogel, (2013) also reported that *A. montdorensis* (and *A. limonicus*) had potential for biological control of thrips in some vegetable and ornamental crops. A single release of *H. sclerotarsa* (at different densities) also reduced both small and large WFT populations (Figure 4.4A; B). This is likely to be because *H. sclerotarsa* consumed WFT pupae in the soil, thereby reducing the number emerging as adults. Combination of *H. sclerotarsa* and *A. montdorensis* (at different densities) reduced both small and large WFT population (Figure 4.5A; B). However, when all the tested treatments were compared with control there was no significant different among the treatment at small and large WFT population. It is hypothesized that this was because, when applied together, 1st instar WFT were consumed by *A. montdorensis* on the foliage stage and pupal stages of WFT were consumed by *H. sclerotarsa* in the soil; there was no competition between the two predators because they were spatially separated. Overall, results indicate that while single applications of the foliar-active predatory mite *A. montdorensis* can deliver adequate control of WFT on bean, if it is combined with *H. sclerotarsa* it would have a greater impact on WFT populations. Other studies of the soil-dwelling predatory mite *H. aculeifer* showed that it was neither additive nor synergistic in suppressing soil-inhabiting thrips developmental stages when applied in combination with *Amblyseius cucumeris* (Berndt, 2004).

Additive/ synergistic effects have been seen in other systems when foliar-active and ground-active predators are used together. For example, pea aphids (*Acyrthosiphon pisum* Harris) preyed on by foliar-active predators release alarm pheromones that make surrounding aphids attempt to escape by dropping off the plants and on to the ground where they become susceptible to ground-active carabid beetles (Losey and Denno 1998a, b). Although alarm pheromones have been identified for WFT, compared with aphid alarm pheromones, they only illicit weak behavioral responses in other WFT (Teerling *et al*. 1993; Teerling 1995). Only a small percentage of L2 WFT drop off the plants in response to alarm pheromones. Nevertheless, a large proportion of L2 WFT naturally move to the soil for pupation (Berndt *et al*., 2004; Bennison *et al*., 2002).

Combinations of predators that, together, are active in all the habitats that different life stages of the target prey occupy could be an ideal biological control strategy because there is potential for synergy to be achieved. However, host plant canopy density can influence the dropping rate of mites (Steiner *et al.*, 2011). Also, in some cases additive or synergistic effects have not been achieved. For example, when the two predators *Orius insidiosus* Say and *Amblyseius degenerans* (Berlese) were released together against WFT on cut roses, control levels were similar to those achieved using *O. insidiosus* alone (Chow *et al.*, 2008).

To assess the appropriate time of application for *H. sclerotarsa* for control of Western Flower Thrips (*F. occidentalis)* in beans.

Abstract

The efficiency of a natural enemy combination compared to a single species release before pupation and after pupation stages for the control of western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) on French plants was investigated. Since a large part of *F. occidentalis* seems to enter the soil passage, a joint release of the plant-inhabiting predatory mite *Amblyseius montdorensis* that feeds on thrips first-instar larvae and the soil-dwelling predatory mite *Hypoaspis sclerotarsa* that preys on thrips pupae in the ground might offer a promising approach for a holistic control strategy. Therefore, two sets of experiments were conducted in cages where French beans plants were infested with twenty adults of western flower thrips. In both experiments, the predatory mites were released on 9th day (Pre-Pupation) and 10th day (Post-Pupation) at *H. Sclerotarsa* density of (0,50,100,150).

The results showed that when *H. sclerotarsa* at different density was applied alone at pre- pupation stage (8th day) it had a significant effect on the mean number of emerging thrips from the soil when compared to Control which value (54.71%,) 50(41.38%) 100(24.72%) and 150 (5.83%), However at post-pupation stage more number of emerged thrips were recorded when *H. sclerotarsa* was applied at different density control 0(86.9%),50(50.83),100(29.16) and 150 (7.75%).The combined impact of *A. montdorensis* and *H. sclerotarsa* was more effective as compared to sole application of *H. sclerotarsa* when released at densities (0, 50,100,150) lower compared to the other antagonist treatments

4.11 Introduction

WFT (*Frankliniella occidentalis*, Pergande) (Thysanoptera: Thripidae), is one of the world's major pests causing damage to a wide range of economically important crops directly through feeding and indirectly through the transmission of harmful plant virus diseases (Kirk & Terry, 2003; van Lenteren et al., 1992). Thrips are difficult to control because of their high reproductive rate, cryptic habit (larvae hide in closed

buds and pupate in soil) and resistance to commonly used insecticides (Herron & James, 2005; Jensen, 2000; van Lenteren & Loomans, 1998). In addition to chemical insecticides, a range of biological agents are available for thrips control including arthropod predators and parasitoids, and insect pathogenic nematodes and fungi (Georgis et al., 2006; Xu et al., 2006; Blaeser et al., 2004 Jacobsonet al., 2001). Whereas most attention has focused on the control of adults and larvae in the crop canopy little effort has been made to interrupt the life cycle by controlling the pupae .The entomogenous, hyphomycete fungus *Metarhizium anisopliae* (Metsch) Sorokin has been studied extensively for the control of a wide range of pests, including WFT(Maniania et al., 2002; Butt et al., 2001; Vestergaard et al., 1995;) and shows much promise for the control of subterranean pests (Ansari et al.,2004; Zimmermann, 1992). Unlike the canopy layer, the soil environment is less prone to dramatic fluctuations in temperature and humidity which can check fungal development. Indeed, Helyer et al. (1995) showed that *M. anisopliae* applied to peat-based media was effective in killing WFT pupae and helped reduce thrips populations. Therefore, the aim of this study was to determine the appropriate time and frequency of application for *H. sclerotarsa* as a control agent against western flower thrips (*F. occidentalis*)

4.12 Material and Methods

4.12.1 Rearing of Western Flower Thrips

The Method used to rear Western Flower Thrips for this study is described in Chapter 3; Section 3.2.

4.12.2 Rearing of *Hypoaspis Sclerotarsa*

The Method used to rear the *Hypoaspis Sclerotarsa* for this study is described in Chapter 3; Section 3.3

4.12.3 Rearing of *Amblyseius Montdorensis*

The Method used to rear *Amblyseius Montdorensis for* this study is described in Chapter 4; Section 4.2.

4.12.4 Experiment Setup

To investigate the effect of application time of *Hypoaspis sclerotarsa* at pre-pupation stage and post -pupation stage in reducing WFT population, two separate microcosm experiments on potted French bean plants (*P. vulgaris*) were conducted. Single application of *H. sclerotarsa* and combined application of *H. sclerotarsa* and *Amblyseius Montdorensis application* at pre- pupation (8^{th} day) and post- pupation at (10^{th} day) was examined. Bean plants were cultivated in sterilized soil in cages that were made of transparent material with fine insect gauze (diameter 30 cm, height 40 cm). Ten days old single plants (two-leaf stage), only one hole was left open and later closed with a piece of paper fixed by sticky tape and served as a window to transfer thrips and mites onto the enclosed plants or the soil, respectively. Twenty adults of western flower thrips were introduced in each pot. 18-day old adult predatory mites directly taken from the stock culture were released at pre pupation (8^{th} day) and post pupation (10^{th} day) in separate experiment at density of (0, 50,100,150) replicated 3 times, directly on the soil surface. Combined release of plant predatory mites (*Amblyseius montdorensis* into French beans was done at 6^{th} day after introducing WFT adults (at density of 0, 15) and *Hypoaspis sclerotarsa* application (at density of 0,50,100,150) was done at pre- pupation stage (8^{th} day) and post -pupation stage (10^{th} day) replicated 3 times 20 WFT adults were introduced in all cages. Both experiments lasted for 21 days. Control treatments without predatory mites allowed the quantification of the proportion of WFT entering the soil and estimates of the intrinsic mortality rates. In all experiments the thrips population density in the soil was recorded plants were cut at ground level at 15^{th} day assuming all the trips introduced had finish pupating.

Plate 4.1: Experiment set up in Thrips cages measuring (diameter 30 cm, height 40 cm) under greenhouse condition

4.13 Statistical analyses

To determine application time a greenhouse trials were analyzed independently using a generalized linear mixed model using procedure GLIMMIX of SAS® (SAS Institute, 2009). Differences among treatment means were separated using Fisher's LSD test ($\alpha = 0.05$) in the repeated measures model. One-way ANOVA (PROC GLM) was used to compare pre and post pupation stage of *H. sclerotarsa* application in different treatment plots.

4.14. Results

4.14.1 Effect of Single Application of *Hypoaspis sclerotarsa* at pre -pupation stage (8th day) and at post- pupation stage (10thday)

There was a significant difference when *H. Sclerotarsa* was applied at different densities (0, 50,100,150) pre pupation and post pupation stage (8^{th} and 10^{th} day respectively) at $P<0.05$. Less mean number of thrips emerged from the soil when *H. Sclerotarsa* at different density was applied at pre- pupation stage (8^{th} day) (Figure

4.6)**.** However,150 density of *H. sclerotarsa* had the lowest mean number of emerged when compared to control (1.16 and 10.9) respectively (Figure 4.6). Application of *H. Sclerotarsa* at different density at post - pupation stage (10^{th}) resulted to highest mean number of emerging thrips from the soil (Figure 4.6) as compared to pre-pupation stage (8^{th} day) in different density. However, *H. Sclerotarsa* at density of 100 and 150 did not differ significantly at $p<0.005$ with mean number (4.94,5.83 and 1.16,1.55) when applied pre and post pupation (Figure 4.6).

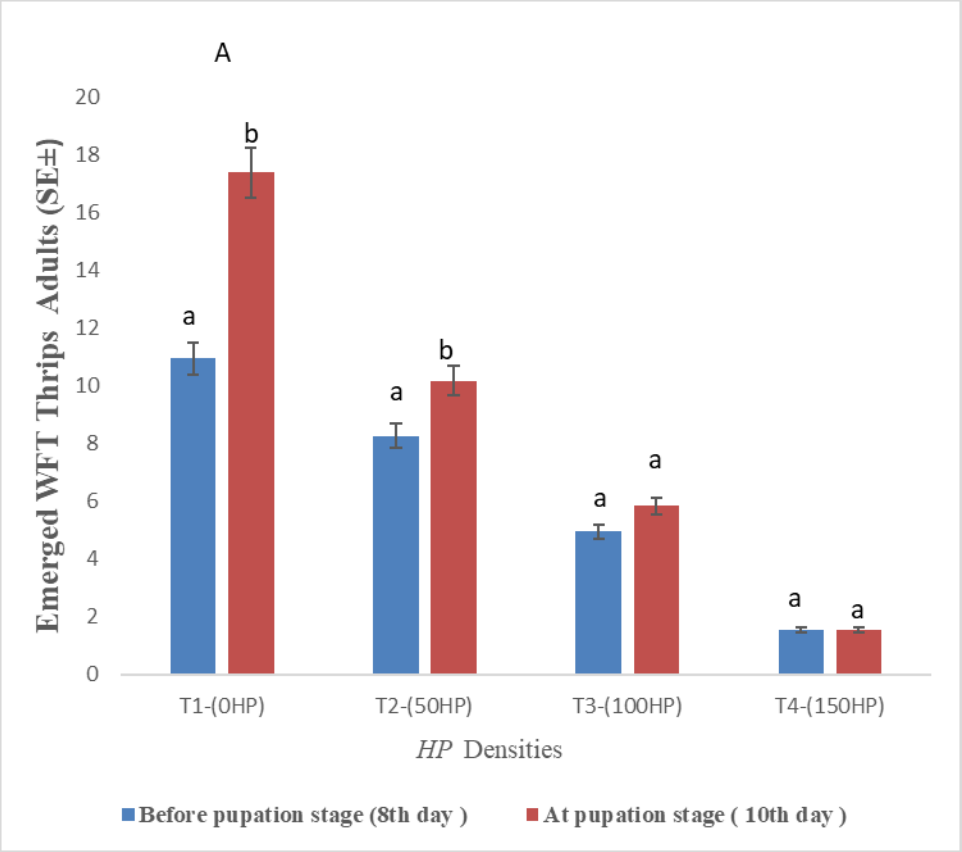

Figure 4.6: Mean (±SE) number of F. occidentalis captured in blue sticky plates hanging over French bean's plants in cages from 15th- 22nd sampling day after sole release Hypoaspis sclerotarsa before pupation stage (8th day) and pupation stage (10th day) when 20 WFT in number were introduced. Vertical bars followed by the same letter are not significantly different ($P < 0.05$). Error bar represent Standard error in percentage.

4.12.2 Effect of combined release of *Amblyseius Montdorensis and Hypoaspis sclerotarsa* application pre- pupation (8th day) and at post- pupation stage (10thday)

There was a significant different among the tested density of *Hypoaspis sclerotarsa* at P<0.05. Application of *Hypoaspis sclerotarsa* pre- pupation stage reduced the thrips population significantly in all the treatments at P<0.05with value as compared to when the application was done post - pupation stage (Figure 4.7) respectively. There was a significant different between *Hypoaspis sclerotarsa* at different densities when applied at pupation stage with value (50 Hp – 7.611±1.91),100Hp-5.1±1.51,150Hp-2.5±1. 41respectively.However, when the different densities were compared with control (0Hp) - high number of emerged adult thrips were recorded.

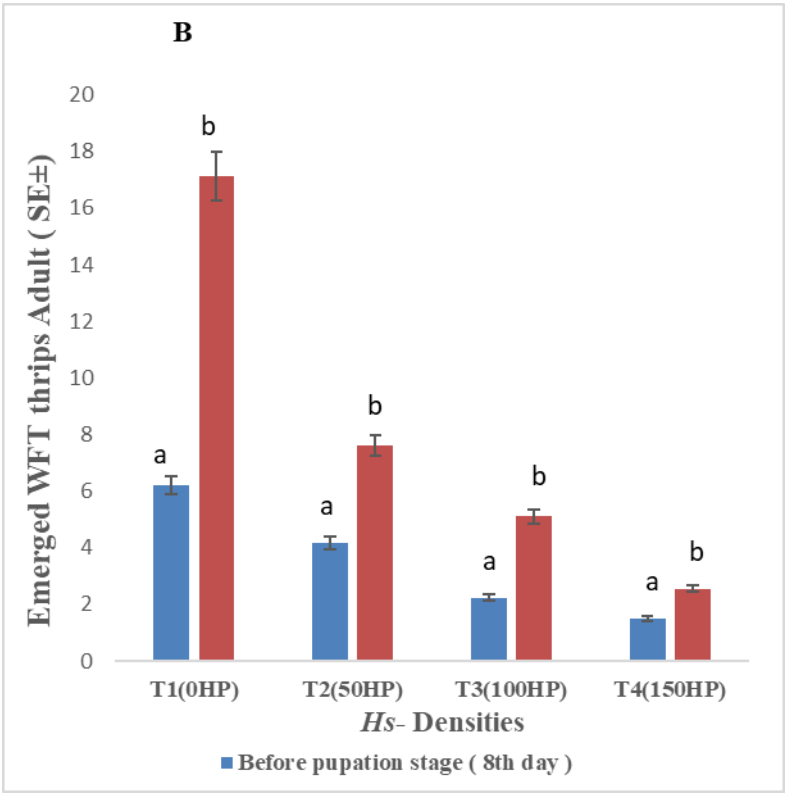

Figure 4.7: Mean (±SE) number of *F. occidentalis* captured in blue sticky plates hanging over French bean's plants in cages from 15th- 22nd sampling day after release combined *A. montdorensis and H. sclerotarsa* before pupation stage (8th

day). Vertical bars followed by the same letter are not significantly different (P <0.05). Error bar represent Standard error in percentage

4.15 Discussion

The best control of the trips was achieved when *H. sclerotarsa* and *A. montdorensis* was combined. Both application of predatory mite at pre pupation and post pupation significantly reduced thrips population, however *H. sclerotarsa* proved better when applied as a pre-pupation rather than as a post pupation treatment with emerging thrips number recorded when *H. Sclerotarsa* was applied at different densities; 0(86.9%), 50(50.83%), 100(29.16%) and 150 (7.75%). The results show that ground dwelling predators can substantially contribute to reduce a source population of thrips in the well-protected refuge soil, but they are not able to reduce the pest population below the economic threshold. A similar study reported that *M. robustulus* resulted to have a better thrips control than *H. Aculeifer* when it was applied at early stage before pupation (Berndt et al., 2004).

It is suggested that combinations of different antagonists, attacking different thrips instars living in the soil as well as on the leaves above ground, may result in a more successful strategy to optimize biological control of thrips, when applied at pre-pupation stage. Combining plant and soil-dwelling predators, desired additive effects could be expected. Compared to combinations of antagonists acting above ground, the interspecific competition (i.e., intraguild predation) should be neglectable due to the different foraging habitats. The findings presented here at first display the interrelations of soil passage and host plant in a complete artificial microcosm system and the results have to be verified in a more practical situation in the field.

CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND RECOMMEDATION

5.1 General Discussion

Most of the studies on the use of beneficial organisms such as *N. cucumeris* and *M. anisopliae* for management of WFT have been conducted on greenhouse crops. Few studies have demonstrated the efficacy of predatory mites and entomopathogenic fungi against thrips however more attention has largely focused on the control of adults in the crop canopy while few attempts have been made to control soil dwelling stages of the WFT.Therefore the study contributes to the knowledge on the potential of soil dwelling mite *H. sclerotarsa* in managing WFT at pupae stage under greenhouse conditions. Results indicate that the use of combined Plant predatory (*A. Montdorensis*) and Soil dwelling predatory mites when applied at pre pupation stage resulted to have the best result in reducing the population of thrips. The differences in thrips numbers by the different sampling methods can be explained by the fact that adult WFT prefer residing in tightly enclosed and concealed spaces of plants over plant foliage, and adult females feed on pollen to ensure stimulated oviposition, reduced larval development time, and increased female fecundity. In the greenhouse experiments, the use of *A. montdorensis* alone and use of *H. sclerotarsa* greatly reduced the numbers of WFT recovered on French bean plants, however combined use of both *A. montdorensis* and *H. sclerotarsa* gave good results in reducing thrips density. Previous studies by Messelink and Kogel, (2013) also reported that *A. montdorensis* (and *A. limonicus*) had potential for biological control of thrips in some vegetable and ornamental crops. From the second experiment a single release of *H. sclerotarsa* (at different densities) also reduced both small and large WFT populations (Figure 1A: B). This could be attributed to the fact that *H. sclerotarsa* consumed WFT pupae in the soil, thereby reducing the number emerging as adults. In addition, this suppressed both adults and larvae of *F. occidentalis.* Therefore, farmers have the option of selecting from the management options to develop an integrated pest management program against WFT. Although in this study a comparative study with chemicals used by farmers was not done, a study on

imidacloprid was reported in a similar study that was done to compare a combine *M. anisopliae,* and *N. Cucumeris* in control of Thrips in beans. The synthetic chemical pesticides has been linked with negative effects on non-target organism and the environment. There are also cases of development of resistance by WFT to imidacloprid and cases of insecticide residues in the export produce. To ensure effective management of WFT on French beans and other horticultural crops, there is a need to build capacity among smallholder farmers on the appropriate use of WFT management options such as Entomopathogenic fungi, predatory mites, and other bio-pesticides.

5.2 Conclusion

This study shows that the soils dwelling mites *H. sclerotarsa* prey on western flower thrips pupae at a rate of 0-1 pupae within 6 hours. These results suggest that H. *sclerotarsa* is a potential control agent against pupae stage of the western flower thrips.

It was found that the soil-active predatory mite *H. sclerotarsa* had a significant impact on WFT populations when released at a high density (150 *H. sclerotarsa*). At a lower density (50 *H. sclerotarsa*) it did still reduce the number of WFT emerging from the soil. According to the study, control of WFT may be enhanced by using a combination of *H. sclerotarsa* and *A. montdorensis,* although the outcomes in this study showed similar levels of control as that achieved when *A. montdorensis* was released alone.

The results clearly indicate that combining soil dwelling mites (*Hypoaspis sclerotarsa)* and plant predatory (*A. montdorensis*) improve the control of thrips, when applied at post pupation stage probably because of timely application. However, application of plant and soil dwelling mites resulted to be more effective when applied at pre- pupation stage.

5.3 Recommendation

1. *Hypoaspis Sclerotarsa* consumed Western flower thrips *(F. occidentallis)* pupae at rate of 0-1 pupae within 6 hours thus reported as potential mites for control of WFT pupae in French bean crop.
2. Single Application of *Hypoaspis Sclerotarsa; A. montdorensis* resulted to be effective in control of Western flower thrips *(F. occidentallis)* that reduced the thrips population however best result were observed when plant and soil dwelling mites were combined i.e.,150HP / 15 *A. Montdorensis* that reduced the thrips population at 93.5 % at highest thrips pressure.
3. Recommend application of soil dwelling at pre pupation stage that resulted to be more effective in controlling Western flower thrips *(F. occidentallis)* by reducing the thrip population at high pressure with 91.02%.
4. Recommend further study to be conducted on combination of plant predatory and soil dwelling mite (*Hypoaspis Sclerotarsa)* in an open field.
5. Recommend a study to be conducted to evaluate the efficacy of Soil dwelling mite (*Hypoaspis sclerotarsa*) on different soil media.
6. Recommend further study to determine whether soil temperature has any effect on the efficacy of *Hypoaspis sclerotarsa* on control of western flower thrips (*F. occidentallis*)

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APPENDICES

Appendix I: Anova tables for study results

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	62.7444	15.6861	51.34	<.001
Time	2	165.4333	82.7167	270.71	<.001
Treatment *Time	8	137.9556	17.2444	56.44	<.001
Residual	165	50.4167	0.3056		
Total	179	416.5500			

Appendix I: ANOVA of consumed number of western flower thrips pupae assessed after five pupae were introduced initially at different densities of predatory mite (Hypoaspis sclerotarsa 0, 2,4,6,8) after 0- 2, 2-4, 4-6 hours.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	14	485.4111	34.6722	181.14	<.001
Residual	165	31.5833	0.1914		
Total	179	516.9944			

Appendix II: ANOVA of consumed number of western flower thrips pupae assessed after ten pupae were introduced initially at different densities of predatory mite (Hypoaspis sclerotarsa 0, 2,4,6,8) after 0- 2, 2-4, 4-6 hours

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time	2	193.2111	96.6056	504.69	<.001
Treatment	4	99.3000	24.8250	129.69	<.001
Treatment*Time	8	192.9000	24.1125	125.97	<.001
Residual	165	31.5833	0.1914		
Total	179	516.9944			

Appendix III: ANOVA of consumed number of western flower thrips pupae assessed after fifteen pupae were introduced initially at different densities of predatory mite (Hypoaspis sclerotarsa 0, 2,4,6,8) after 0- 2, 2-4, 4-6 hours**.**

Appendix IV: ANOVA of consumed number of western flower thrips pupae assessed

	Average number of consumed pupa
	5WFT pupae
Total number of *Hypoaspis sclerotarsa* (2Hp,4Hp,6Hp,8Hp) 20	16.695
1 individual of *Hypoaspis sclerotarsa* 1	0.83

after twenty pupae were introduced initially at different densities of predatory mite (Hypoaspis sclerotarsa 0, 2,4,6,8) after 0- 2, 2-4, 4-6 hours.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	200.1889	50.0472	178.55	<.001
Time	2	200.3444	100.1722	357.37	<.001
Treatment*time	8	202.2111	25.2764	90.18	<.001
Residual	165	46.2500	0.2803		
Total	179	648.9944			

Appendix V: Table of Mean number of western flower thrips (WFT) pupae consumed by one individual *Hypoaspis sclerotarsa* as determined 6 hours after predator introduction.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	237.0938	79.0312	208.27	<.001
Residual	28	10.6250	0.3795		
Total	31	247.7188			

Appendix VI: ANOVA of number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants after release of the foliar-active predator, *Amblyseius montdorensis*. Release densities of A. montdorensis were 0 (control), 5, 10 or 15. A: Initial starting density of WFT before release of predators = 10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	476.250	158.750	69.73	<.001
Residual	28	63.750	2.277		
Total	31	540.000			

Appendix VII: ANOVA of number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants after release of the foliar-active predator, *Amblyseius montdorensis*. Release densities of A. montdorensis were 0 (control), 5, 10 or 15. A: Initial starting density of WFT before release of predators = 20

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	147.5938	49.1979	69.31	<.001
Residual	28	19.8750	0.7098		
Total	31	167.4688			

Appendix VIII: ANOVA of number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants after release of the soil-active predator, *Hypoaspis sclerotarsa*. Release densities of H. sclerotarsa were 0 (control), 50, 100 or 150. A: Initial starting density of WFT before release of predators = 10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	316.125	105.375	87.42	<.001
Residual	28	33.750	1.205		
Total	31	349.875			

Appendix IX: ANOVA of number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants after release of the soil-active predator, *Hypoaspis sclerotarsa*. Release densities of H. sclerotarsa were 0 (control), 50, 100 or 150. A: Initial starting density of WFT before release of predators = 20

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	405.7500	135.2500	213.35	<.001
Residual	28	17.7500	0.6339		
Total	31	423.5000			

Appendix X: ANOVA of number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps placed above bean plants after release of combinations of the foliar-active predator, *Amblyseius montdorensis (AM) and the soil-active predator, Hypoaspis sclerotarsa* (HS). Release densities of A. montdorensis were 0 (control),15 while release densities of H. sclerotarsa were 0 (Control), 50, 100 or 150. A: Initial starting density of WFT before release of predators = 10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	316.000	105.333	51.29	<.001
Residual	28	57.500	2.054		
Total	31	373.500			

Appendix XI: ANOVA of number of emerging, adult *F. occidentalis (WFT)* captured in blue sticky traps placed above bean plants after release of combinations of the foliar-active predator, *Amblyseius montdorensis (AM) and the soil-active predator, Hypoaspis sclerotarsa (HS).* Release densities of A. montdorensis were 0(control), 15 while release densities of H. sclerotarsa were 0 (Control), 50, 100 or 150. A: Initial starting density of WFT before release of predators = 20

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	398.250	132.750	56.97	<.001
Residual	28	65.250	2.330		
Total	31	463.500			

Appendix XII: ANOVA of number of emerging, adult *F. occidentalis* (WFT) captured in blue sticky traps after sole release *Hypoaspis sclerotarsa* before pupation stage when 20 WFT in number were introduced.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	1117.094	372.365	68.42	<.001
Residual	28	152.375	5.442		
Total	31	1269.469			

Appendix XIII: ANOVA of number of emerging, adult *F. occidentalis (WFT)* captured in blue sticky traps after sole release *Hypoaspis sclerotarsa* After pupation stage when 20 WFT in number were introduced**.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	124.844	41.615	27.99	<.001
Residual	28	41.625	1.487		
Total	31	166.469			

Appendix XIV: ANOVA of number of emerging, adult F. occidentalis (WFT) captured in blue sticky traps release of combined Amblyseius montdorensis and Hypoaspis sclerotarsa before pupation stage when 20 WFT in number were introduced .

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	969.250	323.083	100.24	<.001
Residual	28	90.250	3.223		
Total	31	1059.500			

Appendix 15: ANOVA of number of emerging, adult F. occidentalis (WFT) captured in blue sticky traps release of combined Amblyseius montdorensis and Hypoaspis sclerotarsa before pupation stage when 20 WFT in number were introduced

1